High-dose oral immunotherapy

in children with anaphylaxis to peanut

by

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APPENDIX
1  PREFACE

1.1  Acknowledgements

The present work is part of the “Take away food allergy: Inducing tolerance in children allergic to peanut trial” (TAKE-AWAY trial), which was conducted in February 2014. It was carried out at the Division of Paediatric and Adolescent Medicine, Oslo University Hospital, within the Oslo Research group for Asthma and Allergy in Children; the Lung and Environment (ORAACLE), and the University of Oslo, Norway. It was primarily funded by the Norwegian Extra Foundation for Health and Rehabilitation and grants were also given by the Kloster Foundation. With help from the head of the Children’s Section of Allergy and Lung Diseases, Iren Matthews, I received specialization time from clinical practice to complete the thesis in the end of this project.

I would like to express my special thanks to all the participating children, their siblings and parents: I am deeply impressed by your courage and perseverance through the time-consuming oral immunotherapy, eating distasteful daily doses of peanut. In addition, the children participating in the control group and the children who were defined as ineligible for the oral immunotherapy have contributed invaluably. This project would not have been possible without each and every one you.

Special thanks go to my main supervisor, Geir Håland. I admire your academic approach and experience, and I am grateful that you found time in a busy schedule to share some of your competence with me. I am very grateful for the personal conversations we have shared, which helped me the most throughout these years. To my co-supervisors, professor Karin Cecilie Lødrup Carlsen and professor emeritus Kai-Håkon Carlsen: I am deeply impressed by your academic achievements and really admire your ever-lasting enthusiasm in science as well as in your PhD-students. I am forever grateful for your academic advices throughout these years.

And to all co-authors: Please receive my sincere gratitude for all your contributions.
For the investigations and collection of data: Without the skills, experience, humor and help from the study nurses of this project, Liv-Julie Sørdal and Runa Kaldestad, the project would have been impossible. Not to forget Helene Lindvik who helped screening the 10 patients recruited from the Oslo Peanut Allergy Study, as well as the cook, Grete Simonsen, who baked all those ginger breads for the blinded food challenges. I will also express my special thanks to Petter Mowinckel, the statistician, who guided me through the statistical challenges and taught me a lot of funny good-to-know stories. To my dear friend and colleague, Sean Wallace, thank you so much for the time you spent reading through the last version of the thesis, correcting the language as well as asking critical academic questions (this sentence was not corrected by Sean ;)).

To the members of the ORAACLE group: It has been a great pleasure being part of a research group really engaged in each other’s projects. More important to me however, are the friendships developed from this group: It feels like I would have died without you!

To my BFFs: You are the mainstays in my life. Without you, the challenges in this project would have felt impossible to bear. I am forever grateful for having you in my life.

Finally, but most importantly, the ever-lasting love and support from my husband, Frode Reier-Nilsen, have been crucial for completing this work. However, I am most grateful that you are the one, and my companion in the life’s most important project(s): Raising our 4 children; Frida, Erik, Tuva and Martin. My leaf clover, my dearest treasures: You are the light in my world and the true meaning of my life.

Oslo, December 2018

Tonje Reier-Nilsen
1.2 Summary of the thesis

Introduction

Peanut allergy is common and the main cause of life-threatening allergic reactions. There is no available treatment, except vigilant dietary avoidance with the additional use of rescue medication like adrenaline auto injectors when needed. Accidental exposure is frequent, followed by possible life-threatening allergic reactions. Social restrictions and emotional distress result in reduced quality of life among affected children and their families.

The diagnosis of food allergy is usually based on a convincing history of an allergic reaction after exposure to the culprit allergen. The diagnosis is supported by clinical and/or immunological investigations, usually skin prick testing (SPT) and/or demonstration of specific immunoglobulin E (s-IgE). However, the oral food challenge (OFC), or preferably the double-blinded placebo-controlled food challenge (DBPCFC), is considered gold standard to determine the clinical diagnosis of allergy.

During OFC, the amount of allergen that elicits an allergic reaction can be determined and is referred to as the reactivity threshold. Determining the reactivity threshold is important for at least two reasons: Firstly, to determine the eligibility for oral immunotherapy (OIT) and secondly, to identify the level of management for the individual patient. In order to avoid the costs and the risk of systemic reactions by OFC, s-IgE to peanut and the peanut component Ara h 2, peanut SPT and basophil activation have been evaluated for prediction of the reactivity threshold. In previously published studies, an association between reactivity threshold and peanut SPT, s-IgE to peanut, s-IgE to Ara h 2 and basophil activation has been reported. However, there is little information of such associations in populations consisting solely of children highly sensitized to peanut. The lack of such information is explained by these children often being excluded from OFCs due to the risk of severe allergic reactions.
Trials with OIT have shown promising results for desensitization with acceptable safety profiles in populations of varying severity of peanut allergy. Desensitization, meaning no allergic reaction after exposure to the culprit allergen while on treatment, is the first step to sustained unresponsiveness (SU), meaning no reaction after cessation of treatment. Children with severe peanut allergy are expected to benefit the most from a successful OIT. However, it remains unclear if the promising results of desensitization and safety are transferable to this sub-group of children.

Treatment effectiveness should, in addition to biological outcomes, include patient-reported outcomes (PROs). The PROs include standardized quality of life (QoL) questionnaires and one-dimensional reports of treatment burden. The QoL has been reported to improve in children allergic to peanut after desensitization by OIT. However, previously published studies are based on parental proxy-reports and not on child self-reports, except for one study which was conducted without a control group. For one-dimensional reports of patient-perceived burden through OIT, there is little information.

The main objective of the present thesis was to determine the feasibility and effect of two years OIT in children highly allergic to peanut.

The specific research aims were:

1. To identify baseline characteristics that predicts the possibility of entering a peanut OIT and completing an up-dosing phase.
2. To determine the feasibility and identify factors associated with achieving a high maintenance dose in peanut OIT.
3. To identify patient perspective burden of peanut OIT.
4. To determine the effect of 2-years of OIT by desensitization to peanut and PROs.
Methods

The present thesis reports the results from the ongoing 4-year peanut OIT project: Take away food allergy: Inducing tolerance in children allergic to peanut trial (the TAKE-AWAY trial). The TAKE-AWAY trial is a prospective, open labelled, randomized, controlled trial with the primary aim to assess SU one year after cessation of 4 years of OIT in children with primary peanut allergy. The trial consists of four phases: a screening phase (three days of eligibility screening); an up-dosing phase (50-78 weeks); a maintenance phase (36 months) and a follow-up phase after stopping maintenance treatment (12 months). The presented results span from the screening for eligibility to participate and until two years of OIT (one year of maintenance treatment). The results obtained were categorized into three time-points: screening ($Y_0$); one year of OIT (the end of up-dosing phase) ($Y_1$) and two years of OIT (one year of maintenance treatment) ($Y_2$).

At $Y_0$, a DBPCFC with defatted peanut flour was performed in 100 5-15-year-old children with a history of systemic allergic reactions to peanut and/or sensitization to peanut ($\text{SPT} \geq 3 \text{ mm or S-IgE} \geq 0.35 \text{ kUA/L}$). Investigations preceding the DBPCFC included a general clinical examination, a structured interview, standardized QoL questionnaires (Pediatric Quality of Life Inventory Version 4.0 child self-reports and parental proxy-reports, and the Food Allergy Quality of Life – Parental Burden), SPT, lung function measurements (spirometry with reversibility testing), serological immunology assessment (IgE, IgG and IgG₄), basophil activation test (BAT) and conjunctival allergen provocation test (CAPT). International standards were used to define anaphylaxis and grade the allergic reaction during OFC.

Children randomized to OIT attended a bi-weekly up-dosing protocol until reaching the pre-defined maximum maintenance dose (MMD) of 5000 mg peanut protein or the individual maintenance dose (IMD). Adverse events (AEs) were registered and characterized by the
involved organ(s) as well as classified into subjective and mild objective, moderate or severe including anaphylaxis in line with the modified Bock’s criteria. An open OFC was performed at Y₂ to determine the level of desensitization. The QoL assessments as well as all tests from screening were repeated in all enrolled children at Y₁ and Y₂. Only children who received OIT were asked to complete a visual analogue scale (VAS) form for perceived treatment burden at Y₁ and Y₂ presented by the mean VAS-score from each of three domains: GI-related AEs, taste/amount of peanuts and time spent on OIT.

Results

Among all children referred for screening (n = 213), 36.2 % (n = 77) were enrolled in the TAKE-AWAY trial. Concern for AEs was reported by 46.5 % as the main reason for unwillingness to participate, while 17.4 % were excluded by the exclusion criteria. At the pre-trial DBPCFC, four children had no allergic reactions, while 19 children in addition to the 77 enrolled had a positive DBPCFC and reacted with anaphylaxis having objective symptoms from at least two organ systems. In the 19 children, a very low reactivity threshold \( \leq 3 \) mg of peanut protein was determined, defining them ineligible for OIT. The included 77 (median (range)) 9.6 (5.1, 15.2) year-old children were randomized to OIT (n = 57) or observation only (controls) (n = 20). All 77 enrolled children had primary sensitization to peanut with a s-IgE to Ara h 2 of geometric mean (min, max) 40.6 (27.5, 60.3) kUA/L and 81.2 % reported a history of anaphylaxis to peanut prior to enrolment. In 71.4 % of the 77 included children, the parents had a combined annual income above 850.000 NOK, and 84.4 % of the mothers and 75.3 % of the fathers had an education attainment level of at least three years of college/university. Basophil activation (CD63+ basophils \( \geq 15 \) %), peanut SPT and the ratio of peanut s-IgE/total IgE were significantly associated with reactivity threshold and lowest observed adverse events level (LOAEL) (all \( p < 0.04 \)). The basophil activation performed best in predicting very low
reactivity threshold (< 3 mg of peanut protein), with an optimal cut-off of 75.8 % giving a 93.5 % negative and a 36.8 % positive predictive value.

During OIT up-dosing, 21.1 % (n = 12) of the children reached the MMD of 5000 mg peanut protein, while 54.4 % reached the lower IMD and 24.5 % discontinued the treatment. The main reason for not reaching MMD was distaste for peanuts as reported by 66.7 % (n = 28 within IMD and 2 discontinued), followed by AEs reported by 26.7 % (n = 3 within IMD and 9 discontinued) and social reasons reported by 6.7 % (n = 3 discontinued, two found the treatment too time-consuming, while one discontinued due to family reasons). Compared with the 78.9 % children who did not reach the MMD, children who reached the MMD were significantly older, had a significantly lower s-IgE to peanut and Ara h 2, a significantly lower ratio of peanut s-IgE/total IgE, and a significantly higher ratio of peanut s-IgG₄/s-IgE. In both bivariate and multivariate regression analyses, the ratio of peanut s-IgG₄/s-IgE was the only identified factor significantly associated with achieving MMD.

Mild OIT-related AEs were reported in 13.9 % of the OIT doses, whereas moderate AEs were reported in 0.6 % of the AEs, and anaphylaxis was reported in 0.06 % of the AEs by 19.3 % of the children. The AEs were mostly related to the gastro-intestinal tract (86 %), occurred most often in the first two days of each up-dosing period (p = 0.001), as well as in the first dose-interval step (1-65 mg peanut protein) as compared with the second (66-800 mg) and the third (801-5000 mg peanut protein) dose-interval steps (overall p = 0.03).

The VAS-reported perceived treatment burden was significantly reduced from Y₁ to Y₂ for the GI-domain from (mean (95 % CI)) 2.6 (1.9, 3.3) to 1.4 (1.0, 1.8) (p = 0.001), and for the taste/amount-domain from 6.5 (5.5, 7.3) to 5.3 (4.3, 6.3) (p = 0.02)). The perceived burden of time spent on treatment was equal at Y₁ and Y₂ (2.9 (2.1, 3.7) to 2.2 (1.5, 2.9) (p = 0.06)).

The OFC at Y₂ was completed by 37 of the 39 children still receiving OIT, and demonstrated that 35/37 challenged children were desensitized to 7500 mg peanut protein independently of
maintenance doses ranging from 350 - 5000 mg with a mean (SD) dose of 3322 (1376) mg peanut protein.

In the child self-reports, the improved QoL in OIT children from Y₀ to Y₂ (mean change (95% confidence intervals (C.I.) (4.4 (0.5, 8.3)) was half of that observed in the parental proxy-reports (9.3 (4.3, 14.3) (both p<0.0001)). Controls reported no significant improvement. In contrast to the child self-report’s, the two-fold larger mean change (95% CI) in QoL observed in the parental proxy-reports of the OIT group (9.3 (4.3, 14.3)) was significantly different from that of the controls (0.4 (-7.1, 8.0)) (p = 0.02). Neither perceived treatment burden, level of desensitization, maintenance dose nor AEs significantly predicted changes in QoL.

Discussion

In a homogenous population of children highly sensitized to peanut, a large proportion refused participation prior to screening. Concern for AEs was reported as the main reason for unwillingness for participation. Furthermore, pre-trial information of a time-consuming and perhaps challenging treatment may have biased the study population towards dedicated and resourceful parents and children.

The significant associations between reactivity threshold, and basophil activation, peanut SPT and Ara h 2, are consistent with previous reports, with basophil activation being the best predictor for very low reactivity threshold and thereby eligibility for OIT. The latter may be explained by the BAT being an “in vitro OFC”, which may provide associations over a wider spectrum of reactions in the basophils, not limited to stop at a positive OFC.

The finding that no baseline characteristics were significantly associated with completion of the up-dosing phase may be explained by the non-biological finding of distaste for peanuts as the main reason for withdrawal.
The up-dosing phase was completed by 75.5 % (n = 43) of the enrolled children, while 21.1 % only reached the MMD. Distaste for peanuts was the main reason for not achieving MMD. There is little of information of peanut distaste in previous reports, which may be explained by the MMD being higher than in most other OIT trials. The high MMD was chosen based on subcutaneous immunotherapy trials of inhalant allergens reporting association between SU and maintenance dose. Nevertheless, a maintenance dose of at least 300 mg peanut protein was achieved by 73.7 % of our children in line with the previously reported 63.6 % to 86.9 % in other peanut OIT studies.

The 13.9 % prevalence of mild GI-related AEs in our children are in line with previous reports. In contrast, anaphylactic OIT-related events in 19.3 % of our children during up-dosing were higher than in most previously published reports, but in consent with the recently reported AR101 peanut OIT. In the AR101 study, 14 % of the 372 OIT children reported at least 76 anaphylactic events, a higher frequency of anaphylactic events per dose-days as compared with the TAKE-AWAY children. The high proportion of anaphylactic events may reflect a highly peanut allergic population, or maybe the high MMD. In the AR101 study, however, the maintenance dose was 300 mg of peanut protein. These observations question the safety of OIT in highly allergic patients.

The desensitization level after two years of OIT was independent of maintenance dose, similar to a recent report. The improved QoL in children after OIT as reported by the parents are consistent with previous reports mostly including such parental proxy-reports. However, the parents reported a two-fold larger improvement in child QoL as compared to their children. This parentally reported QoL score, but not the children’s, was significantly associated with OIT. The discrepancy may in part reflect the parents’ own improved QoL.
Conclusions

In conclusion, the present thesis brings important insight to the feasibility and effect of high-dose OIT in a homogeneous population of children reacting with anaphylaxis following exposure peanut. None of the baseline clinical or immunological markers were sufficient to substitute OFC in determining a reactivity threshold necessary to define eligibility for entering OIT. Nor could baseline characteristics predict the possibility to complete the up-dosing phase.

Even though the majority of the children completed up-dosing and reached a maintenance dose, high-dose oral immunotherapy was feasible only for a small proportion. Distaste for peanuts were the main limitation of reaching MMD, followed by AEs.

Mild AEs were similar in number and character with previous studies, but the proportion of OIT-related anaphylaxis was higher in this population of children exclusively highly allergic to peanut as compared with previously published studies including less sensitized peanut allergic children. This finding questions the feasibility and safety of high-dose OIT in these children.

The observed discrepancy between the extent of change in child QoL score reported by parents and children following OIT, suggests that parents may over-estimate the effect of the treatment. Hence, it may be more appropriate to use child self-reported rather than parental proxy-reported QoL when assessing patient-related outcome of OIT.
1.3 Abbreviations

AEs – adverse events

ASIT – allergen specific immunotherapy

AUC – area under curve

BAT – basophil activation test

CAPT – conjunctival allergen provocation test

% CD63+ – percentage of CD63 positive basophils – proportion of activated basophils as a measure of allergen induced basophil reactivity

CD-sens – basophil allergen sensitivity, the allergen concentration eliciting half of the maximum basophil activation

CRD – component-resolved diagnostics

DBPCFC – double-blind placebo-controlled food challenge

EAACI – the European Academy of Allergology and Clinical Immunology

EoE – eosinophil esophagitis

EPIT – epicutaneous immunotherapy

FA – food allergy

FAQL-PB – Food Allergy Quality of Life – Parental Burden

FC – food challenge

GI – gastro-intestinal

IgE-mediated – involvement of Ig-E antibodies in the pathogenesis of allergy
IMD – individual maintenance dose

LOAEL – lowest observed adverse effect level

MMD – maximum maintenance dose

NOAEL – no observed adverse effect level

OFC – oral food challenge

OAS – oral allergy syndrome

OIT – oral immunotherapy

PedsQL 4.0 – Paediatric Quality of Life Inventory Version 4.0

PPI – proton pump inhibitor

PPV – positive predictive value

PRO – patient related outcome

RCT – randomized controlled trial

SCIT – subcutaneous immunotherapy

s-IgE/G/G₄ – specific immunoglobulin E/G/G₄

ROC – receiver operating characteristic

SLIT – sublingual immunotherapy

SPT – skin prick test

SU – sustained unresponsiveness
TAKE-AWAY trial – Take away food allergy: Inducing tolerance in children allergic to peanut

\( Y_0 \) – at screening (enrolment)

\( Y_1 \) – at completed up-dosing, approximately 1 year of treatment, 1 year for controls

\( Y_2 \) – at one year of maintenance treatment, approximately 2 years of treatment

QoL – quality of life
1.4 List of papers

Paper #1:
Reier-Nilsen T, Michelsen MM, Lodrup Carlsen KC, Carlsen KH, Mowinckel P, Nygaard UC, Namork E, Borres MP, Haland G.

**Predicting reactivity threshold in children with anaphylaxis to peanut**

Paper #2:
Reier-Nilsen T, Michelsen MM, Lodrup Carlsen KC, Carlsen KH, Mowinckel P, Nygaard UC, Namork E, Borres MP, Haland G.

**Feasibility of desensitizing children highly allergic to peanut by high-dose oral immunotherapy**

Paper #3:
Reier-Nilsen T, Lodrup Carlsen KC, Michelsen MM, Drottning S, Carlsen KH, Chi Zhang, Borres MP, Haland G.

**The child’s perspective of quality of life in a randomized controlled peanut oral immunotherapy trial.**
Submitted to PAI Dec 3.
2 GENERAL INTRODUCTION

2.1 Definitions and mechanisms

2.1.1 Definitions

Allergy is defined by the European Academy of Allergology and Clinical Immunology (EAACI) nomenclature task force as a type of a hypersensitivity reaction initiated by immunologic mechanisms. Hypersensitivity reactions are reproducible with objective symptoms or signs following exposure to a defined stimulus at a dose tolerated by normal subjects, and may be either allergic or non-allergic. Allergic reactions are classified as immunoglobulin E (IgE) mediated or the rarer non-IgE mediated type, based on the involvement of IgE antibodies in the pathogenesis of reactions. The IgE-mediated allergy results from a type I hypersensitivity reaction. If the binding of antigen to IgE antibodies causes an allergic reaction, it is defined as an allergen.

Allergic reactions are categorized into four categories: Type I-IV, depending on the underlying immunologic mechanism. The type I hypersensitivity reaction is responsible for the immediate allergic reaction and the most common mechanism underlying food allergies. The type II-IV hypersensitivity reactions will therefore not be discussed in any further detail in this thesis and the term allergy will be used to equate type I hypersensitivity reaction in the remaining of the thesis.

2.1.2 Mechanisms of type I hypersensitivity reaction

To become sensitized to a food allergen, the culprit allergen that is presented to T cells must be followed by an immune response skewed towards the Th2 pathway, allowing B cells to produce s-IgE antibodies. Subsequently, the s-IgE antibodies bind to specific IgE-receptors (FεRI) on the surface of basophils in the circulating blood and mast cells in the
tissues (Figure 1). Sensitization refers to this production of allergen specific IgE antibodies only, and is not synonymous with an allergic reaction.

Figure 1. Type I hypersensitivity reaction. The first exposure causes sensitization with the production of antibodies, while the re-exposure demonstrates the mechanism for an allergic reaction. Reprinted from “onlinebiologynotes.com”, with permission Mr. Gaurab Karki.

After initial sensitization, development of a clinical allergy requires re-exposure of the culprit allergen (Figure 1). The allergen must be able to bind to the s-IgE antibodies on the surface of basophils and mast cells in sufficient numbers to cause cross-linking of the IgE antibodies. Such cross-linking causes basophils and mast cells to release mediators (cytokines and leukotrienes) contributing to the allergic reaction (3). Hence, individuals may be sensitized as determined by clinical and/or immunological sensitization tests, but without allergic symptoms at exposure to the allergen (i.e. sensitized, but tolerant patient) (3), explaining why allergy is considered a clinical diagnosis. In tolerant individuals, higher levels of s-IgG antibodies have been observed compared with that of allergic individuals, and several studies
support the hypothesis that s-IgG antibodies compete with s-IgE antibodies in allergen binding to basophils and mast cells (4-6).

2.1.3 Allergic reactions

2.1.3.1 Primary sensitization

A type I hypersensitive reaction mostly occurs within minutes of exposure, and the allergic symptoms depend on the location of allergen exposure. In primary sensitization, it is the allergen itself that causes an allergic reaction, or a primary allergy. Primary allergy may result in a life-threatening systemic hypersensitivity reaction, known as anaphylaxis (1). The European Academy of Allergy and Clinical Immunology (EAACI) task force papers use the Sampson’s clinical criteria for anaphylaxis (7) and define anaphylaxis by the occurrence of moderate allergic symptoms from at least two organ systems (8, 9) (Table 1).

<table>
<thead>
<tr>
<th>Table 1. Clinical criteria for the diagnosis of anaphylaxis.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaphylaxis is highly likely when any one of the following three criteria are fulfilled:</td>
</tr>
<tr>
<td>1. Acute onset of an illness (minutes to several hours) with involvement of the skin, mucosal tissue or both (e.g. generalized hives, pruriitus or flushing, swollen lips-tongue-larynx).</td>
</tr>
<tr>
<td>And at least one of the following:</td>
</tr>
<tr>
<td>a. Respiratory compromise (e.g. dyspnoea, bronchospasm, stridor, hypoxia).</td>
</tr>
<tr>
<td>b. Cardiovascular compromise (e.g. hypotension, collapse).</td>
</tr>
<tr>
<td>2. Two or more of the following that occur rapidly after exposure to a likely allergen for that patient (minutes to several hours):</td>
</tr>
<tr>
<td>a. Involvement of the skin or mucosal tissue (e.g. generalized hives, itch, flushing, swelling).</td>
</tr>
<tr>
<td>b. Respiratory compromise (e.g. dyspnoea, bronchospasm, stridor, hypoxia).</td>
</tr>
<tr>
<td>c. Cardiovascular compromise (e.g. hypotension, collapse).</td>
</tr>
<tr>
<td>d. Persistent gastrointestinal symptoms (e.g. crampy abdominal pain, vomiting).</td>
</tr>
<tr>
<td>3. Hypotension after exposure to known allergen for that patient (minutes to several hours).</td>
</tr>
<tr>
<td>Hypotension for children is defined as systolic blood pressure ≤70 mmHg from 1 month to 1 year, (≤70 mmHg + [2 × Age]) from 1 to 10 years, and ≤90 mmHg from 11 to 17 years.</td>
</tr>
</tbody>
</table>

When diagnosing anaphylaxis, the EAACI task force suggests classifying the anaphylactic reaction. Based on the Sampson’s Grading of Food-Induced Anaphylaxis According to the Severity of Clinical Symptoms (Table 2) with a clinical severity scale ranging from one to five (10), the EAACI task force recommends using the simplified scoring system Severity of anaphylaxis (8, 9) scoring from 1-3 (mild-moderate-severe), which later has been modified for children by Vetander et al. (11) (Table 3).

In a comprehensive review article of food-induced anaphylaxis (12), the first signs of anaphylaxis were most commonly gastro-intestinal (GI); abdominal pain and vomiting. This is probably explained by the GI-system being the location for food allergen exposure. Skin reactions were involved in 70 % to 98 % of the cases. Respiratory symptoms were the primary cause of death, especially in asthmatic patients. Cardiovascular symptoms were rare, and seldom seen in isolation from respiratory arrest, particularly in small children.

Table 2. Sampson’s Grading of Food-Induced Anaphylaxis.

<table>
<thead>
<tr>
<th>Grade</th>
<th>Skin</th>
<th>GI Tract</th>
<th>Respiratory Tract</th>
<th>Cardiovascular</th>
<th>Neurological</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Localized pruritus, flushing, urticaria, angioedema</td>
<td>Oral pruritus, oral “tingling,” mild lip swelling</td>
<td>Nasal congestion and/or sneezing</td>
<td>Change in activity level</td>
<td>Change in activity level plus anxiety</td>
</tr>
<tr>
<td>2</td>
<td>Generalized pruritus, flushing, urticaria, angioedema</td>
<td>Any of the above nausea and/or emesis x 1</td>
<td>Rhinorrhea, marked congestion, sensation of throat pruritus or tightness</td>
<td>Tachycardia (increase &gt;15 beats/min)</td>
<td>“Light headedness,” feeling of “pounding heart”</td>
</tr>
<tr>
<td>3</td>
<td>Any of the above</td>
<td>Any of the above plus repetitive vomiting</td>
<td>Any of the above, hoarseness, “barky” cough, difficulty swallowing, dyspnea, wheezing, cyanosis</td>
<td>Any of the above, dysrhythmia and/or mild hypotension</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Any of the above</td>
<td>Any of the above plus diarrhea</td>
<td>Any of the above</td>
<td>Severe bradycardia and/or hypotension or cardiac arrest</td>
<td>Loss of consciousness</td>
</tr>
<tr>
<td>5</td>
<td>Any of the above</td>
<td>Any of the above, loss of bowel control</td>
<td>Any of the above, respiratory arrest</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All symptoms are not mandatory. The severity score should be based on the organ system most affected, eg, if grade 3 respiratory symptoms are present but only grade 1 GI symptoms, then the anaphylaxis severity score would be “grade 3.” Boldface symptoms are absolute indications for the use of epinephrine; use of epinephrine with other symptoms will depend on patient’s history.

Table 3. EAACI grading of anaphylaxis.

<table>
<thead>
<tr>
<th>Grade</th>
<th>Skin</th>
<th>Gastrointestinal</th>
<th>Respiratory</th>
<th>Cardiovascular</th>
<th>Neurological</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Mild</td>
<td>Sudden itching of eyes and nose, generalized pruritus, flushing, urticaria, angioedema</td>
<td>Oral pruritus, oral ‘tingling’, mild lip swelling, nausea or orness, mild abdominal pain</td>
<td>Nasal congestion and/or sneezing, rhinorrhea, throat pruritus, throat tightness, mild wheezing, chest tightness</td>
<td>Tachycardia (increase &gt;15 beats/min)</td>
<td>Change in activity level, anxiety, tiredness</td>
</tr>
<tr>
<td>2 Moderate</td>
<td>Any of the above</td>
<td>Any of the above, crampy abdominal pain, diarhoea, recurrent vomiting</td>
<td>As above</td>
<td>As above</td>
<td>“Light headedness”, feeling of “pounding doom”, somnolence</td>
</tr>
<tr>
<td>3 Severe</td>
<td>Any of the above, loss of bowel control</td>
<td>Any of the above, loss of bowel control</td>
<td>Hypotension* and/or collapse, dysrhythmia, severe bradycardia and/or cardiac arrest</td>
<td>Confusion, loss of consciousness</td>
<td></td>
</tr>
</tbody>
</table>


2.1.3.2 Cross-reactivity

In cross-reactivity, it is proteins similar to allergens (homologues) that cause the allergic reaction. These homologues often come from closely related species or from the same protein family, are often heat-labile and often highly homologues with pollen allergens (3). The pollen-food syndrome or the oral allergy syndrome (OAS) is the most typical example of cross-reactivity. In OAS, the proteins found in fruits and vegetables are homologues to the proteins in pollen. The OAS typically results in harmless oral itching and/or swelling and sometimes a perioral rash. In rare cases, the symptoms may progress to severe throat swelling and very uncommonly, to anaphylaxis.
2.2 Epidemiology

Food allergies (FA) are common (13), affecting more than 1-2 % but less than 10 % of the population as reported in a systematic review of studies published from January 1988 to September 2009 (14). The variations between reported prevalence in the reviewed studies might be explained by lack of consistent diagnostic criteria between studies, the age of study participants or geographical variations. A North-American study (15) demonstrated that relying on self-reported FA resulted in 10 times as high prevalence compared with those who based FA on positive sensitization or challenge-proven FA. The prevalence in FA should be adjusted for age, as they generally tend to resolve before school age. In a study from the United States (16), the prevalence of doctor-diagnosed FAs decreased from 4.7 % in the first two years of life to 1.2 % in pre-school children. Food allergies also vary between geographic areas, suggesting lower prevalence in non-Westernized countries (17). Despite adjusting for age, geographic variations and different methodologies, the prevalence of FAs has been increasing in the last two to three decades (13, 18, 19) (Figure 2). In 2011, the Australian HealthNuts study reported that more than 10 % of 1-year-old children had challenge-proven IgE-mediated allergy (20).
Figure 2. Percentage of children under 18 years of age who had reported food or digestive allergy in the past 12 months, by age group: United States 1997-2007. Reprinted from NCHS Data Brief. 2008(10):1-8; Branum AM, et al.: “Food allergy among U.S. children: trends in prevalence and hospitalizations”, which appears within the public domain without further need for permission to reprint.

In children, the most commonly reported allergens are cow’s milk protein (2.2 %), peanut (1.8 %) and tree-nuts (1.7 %) are, whereas shell-fish (1.9 %), fruit (1.6 %) and vegetables (1.3 %) are most commonly reported in adults (18). However, much research has been performed on peanut allergy, as it is the main cause of life-threatening allergic reactions in the Western world (21, 22) (Figure 3). In the comprehensive review article (12), food induced anaphylaxis accounted for up to 81 % of the anaphylactic reactions in children, with nuts being the provoking food in the most severe episodes.
Figure 3. Eliciting foods in relation to age among 371 children with 381 emergency department visits due to acute reactions to foods in Stockholm, Sweden, during 2007. In (a) the five most common eliciting food items are displayed and in (b) the proportions of reactions to peanuts, specified and unspecified nuts are summated as well as the proportions of reactions to cow’s milk and hen’s egg.


Peanut allergy affects 1-2 % of the paediatric population (23), and is increasing (15, 17). In a study including three cohorts of 3- to 4-year-old children from the same geographical area in the UK, the prevalence of peanut sensitization and clinical peanut allergy increased from 1989 to 2002. In contrast to allergies to basic foods like cow’s milk, hen’s egg and soy protein, spontaneous resolution of peanut allergy is uncommon (24). A spontaneous remission rate of 20 % has been reported for clinical peanut allergy from the first two years of life to pre-school age (24, 25).
2.3 Diagnosing IgE-mediated food allergy

2.3.1 Clinical and immunological investigations

The cornerstone of diagnosing FA includes a convincing history of allergic reaction related to exposure to the culprit allergen, supported by positive relevant allergic sensitization, identified through allergy testing (26). Allergy testing includes immunological investigations by s-IgE antibodies and total IgE and more rarely the basophil activation test (BAT), as well as clinical investigations including SPT and more rarely the recently reported conjunctival allergen provocation test (CAPT) (27). Allergy testing reflects different aspects of the type I hypersensitivity mechanism, as shown in Figure 4.

Figure 4. Tests used to determine sensitization reflecting different aspects of the underlying mechanism. The skin prick test - response of skin mast cells to allergen; the basophil activation test - the response of circulating basophils to allergen; IgE tests - the concentration of circulating IgE, either total IgE or s-IgE to allergen extracts or to individual allergen components. Reprinted from The journal of allergy and clinical immunology In practice 2017;5(2):237-48; Santos AF, Brough HA.: “Making the Most of In Vitro Tests to Diagnose Food Allergy.”, with permission from Creative Commons Distributions License. https://www.ncbi.nlm.nih.gov/pubmed/28283150
The SPT reflects the response of mast cells in the skin using allergen extracts or component allergens, whereas the IgE analysis reflects the s-IgE concentration in serum samples (28). Through allergen exposure, the circulating concentration of either total IgE antibodies or s-IgE antibodies to allergen extracts or allergen components may be measured in serum samples (Figure 5). Allergen extracts contain the natural mix of allergenic and non-allergenic proteins that constitutes the allergen source (28). Sensitization given by use of allergen extracts is, therefore, considered crude and cannot distinguish between primary allergy and cross-over reactivity. Another disadvantage is the natural variation in protein composition despite using the same allergen source, as well as the variation in allergen concentration as a consequence of e.g. heating during the preparation process (28). Hence, allergen extracts may vary in composition and concentration, and the use of different allergen producers may therefore lead to different results of s-IgE levels. Four decades ago, there was a call for standardization of allergen extracts with subsequent large production of purified allergens (29). The allergen components consisting of pure allergen proteins however, are produced by purification from natural allergen sources or recombinant expression of allergen-encoding complementary DNA. Using component-resolved diagnostics (CRD) may provide more precise information about the likelihood of clinical allergy in sensitized individuals (28). Hence, CRD can give information of primary sensitization associated with primary allergy and/or cross-sensitization associated with cross-reactivity.

If a clinical history of allergic reaction together with SPT and/or s-IgE is not sufficient for a clear diagnosis of FA, further allergy testing may be warranted. Some studies suggest using ratios of s-IgE/total IgE (30) and s-IgG_{4}/s-IgE (4) when diagnosing FAs. However, there are discrepancies in the findings regarding utility of the ratio of s-IgE/total IgE for different FAs. In a study of persistent FAs (e.g. peanut, tree-nuts, shellfish) the ratio of peanut s-IgE/total IgE improved the diagnostic outcome of an oral food
challenge (OFC) when compared to s-IgE (30), while similar findings were not reported in a study focusing on transient FAs (e.g. cow’s milk, hen’s egg, wheat) (31).

For the ratio of s-IgG₄/s-IgE to peanut the diagnostic utility has not yet been established, but an association between s-IgG₄/s-IgE to peanut and the diagnostic outcome of an OFC has been reported (4).

The BAT may provide added value in allergy testing with its enhanced specificity and often conserved sensitivity as compared with the SPT and s-IgE (26). The BAT has been considered an OFC in a test tube (32), as it measures the response of basophils exposed to food allergens in a test tube. Compared with an OFC, the exposure of food allergen can continue to higher doses.

The CAPT reflects the response of mast cells in the conjunctiva (not shown in Figure 4). In the Oslo Peanut Study (27), the CAPT was able to distinguish peanut sensitized allergic vs. peanut sensitized tolerant individuals with food allergy, and in a study of immunotherapy to
cat dander (26), the CAPT reactivity threshold increased after treatment (33). Whereas SPT and CAPT are both in vivo tests, analysis of s-IgE and BAT represent vitro tests.

Up to date, the OFC is considered gold standard and the conclusive test in diagnosing FA.

2.3.2 Double-blind placebo-controlled food challenge

For the last four decades, the double-blinded placebo-controlled food challenge (DBPCFC) has been considered gold standard in the accurate diagnosis of FA (26). However, in clinical practice an open OFC may be sufficient, as food challenges are mostly used as a diagnostic tool to confirm clinical allergy. The DBPCFC is particularly useful when subjective or mild objective symptoms are considered as signs of an allergic reaction, according to the modified Bock’s criteria (Table 4) (34). The DBPCFC is also preferable to determine the reactivity threshold for allergic symptoms, i.e. the expected amount of the offending food that will elicit allergic reactions.

The starting dose of an OFC should be lower than the expected reactivity threshold and a 20 - 30 minutes interval has been recommended between each challenge dose in the PRACTALL consensus report for standardizing food challenges (34). In the absence of allergic reactions, the next challenge dose is given until the OFC is considered positive. However, there is no consensus as to when to define the food challenge as positive. In line with the PRACTALL guidelines (34), some studies record the DBPCFC positive if subjective symptoms occur in consecutive doses (35), while other studies require objective symptoms to occur (36). It has, however, been demonstrated that subjective symptoms may occur at doses 20-fold lower than the lowest dose eliciting objective symptoms (37), while some patients do not experience subjective symptoms at all prior to the occurrence of objective symptoms (38). As a solution to this problem, the PRACTALL guidelines (34) recommend observation of an objective symptom to determine a food challenge as positive, even though subjective
Table 4. The modified Bock’s criteria for classification of adverse events.

<table>
<thead>
<tr>
<th>I. SKIN</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Erythematous Rash - % area involved</td>
<td></td>
</tr>
<tr>
<td>B. Pruritus</td>
<td></td>
</tr>
<tr>
<td>0 = Absent</td>
<td></td>
</tr>
<tr>
<td>1 = Mild, occasional scratching</td>
<td></td>
</tr>
<tr>
<td>2 = Moderate - scratching continuously for &gt; 2 minutes at a time</td>
<td></td>
</tr>
<tr>
<td>3 = Severe - hard continuous scratching - excoriation</td>
<td></td>
</tr>
<tr>
<td>C. Urticaria/Angioedema</td>
<td></td>
</tr>
<tr>
<td>0 = Absent</td>
<td></td>
</tr>
<tr>
<td>1 = Mild - &lt; 3 hives, or mild lip edema</td>
<td></td>
</tr>
<tr>
<td>2 = Moderate - &lt; 10 hives but &gt;3, or significant lip or face edema</td>
<td></td>
</tr>
<tr>
<td>3 = Severe - generalized involvement</td>
<td></td>
</tr>
<tr>
<td>D. Rash</td>
<td></td>
</tr>
<tr>
<td>0 = Absent</td>
<td></td>
</tr>
<tr>
<td>1 = Mild - few areas of faint erythema</td>
<td></td>
</tr>
<tr>
<td>2 = Moderate - areas of erythema</td>
<td></td>
</tr>
<tr>
<td>3 = Severe - generalized marked erythema (&gt;50%)</td>
<td></td>
</tr>
</tbody>
</table>

| II. UPPER RESPIRATORY |  |
| A. Sneezing/itching |  |
| 0 = Absent |  |
| 1 = Mild - rare bursts, occasional sneezing |  |
| 2 = Moderate - bursts <10, intermittent rubbing of nose, and/or eyes or frequent sneezing |  |
| 3 = Severe - continuous rubbing of nose and/or eyes, paroxysmal swelling and/or long bursts of sneezing, persistent rhinorrhea |  |

| III. LOWER RESPIRATORY |  |
| A. Wheezing |  |
| 0 = Absent |  |
| 1 = Mild - expiratory wheezing to auscultation |  |
| 2 = Moderate - inspiratory and expiratory wheezing |  |
| 3 = Severe - use of accessory muscles, audible wheezing |  |
| B. Laryngeal |  |
| 0 = Absent |  |
| 1 = Mild - >3 discrete episodes of throat clearing or cough, or persistent throat tightness/pain |  |
| 2 = Moderate - hoarseness, frequent dry cough |  |
| 3 = Severe - stridor |  |

| IV. GASTROINTESTINAL |  |
| A. Subjective Complaints |  |
| 0 = Absent |  |
| 1 = Mild - complaints of nausea or abdominal pain, itchy mouth/throat |  |
| 2 = Moderate - frequent e/o nausea or pain with normal activity |  |
| 3 = Severe - notably distressed due to GI symptoms with decreased activity |  |
| B. Objective Complaints |  |
| 0 = Absent |  |
| 1 = Mild - 1 episode of emesis or diarrhea |  |
| 2 = Moderate - 2-3 episodes of emesis or diarrhea or 1 of each |  |
| 3 = Severe - >3 episodes of emesis or diarrhea or 2 of each |  |

| V. CARDIOVASCULAR/NEUROLOGIC |  |
| 0 = normal heart rate or BP for age/baseline |  |
| 1 = mild-subjective response (weak, dizzy), or tachycardia |  |
| 2 = moderate-drop in blood pressure and/or >20% from baseline, or significant change in mental status |  |
| 3 = severe-cardiovascular collapse, signs of impaired circulation (unconscious) |  |

**TABLE LEGEND:**

**GREEN:**
- Not usually an indication to alter dosing.
- Not generally sufficient to consider a challenge positive.

**Orange (scores increasing to orange):**
- Caution, dosing could proceed, be delayed, have a dose repeated rather than escalated.
- If clinically indicated, dosing is stopped.
- Symptoms that recur on 3 doses, or persist (e.g., 40 minutes) are more likely indicative of a reaction than when such symptoms are transient and not reproducible.
- 3 or more scoring areas in orange more likely represent a true response.

**RED:**
- Objective symptoms likely to indicate a true reaction
- Usually an indication to stop dosing.

symptoms in consecutive doses also are accepted. For children in particular, it is suggested that change in activity level should be considered as a very sensitive sign of a beginning clinical reaction (39).

Concerns have been raised of the possibility of evolving a rush desensitization which increases the amount of allergen required to elicit an allergic reaction during the OFC by using low starting doses, semi-logarithmic dose increases, and prolonged dose intervals (40). Even though these concerns have been contradicted by other studies (41), a final determination of a negative OFC should ideally be followed by giving a single dose with the cumulated amount of the challenged food (40).

2.3.3 Diagnosing peanut allergy

Several attempts have been made to establish highly predictive cut-off values to distinguish patients with allergic reactions from tolerant patients among sensitized subjects, in particular for the commonly used s-IgE to peanut and peanut SPT (42-44). Studies have reported that a wheal size $\geq 8$ mm (42) or s-IgE to peanut $\geq 15$ kUA/L (44) gives a 95% positive predictive value for clinical peanut allergy. These values are now the basis of many of the currently accepted predictive cut-offs in use (45). In one retrospective study of food challenges in peanut sensitized children (46), however, a history of an allergic reaction and a s-IgE to peanut $\geq 5$ kUA/L always resulted in a positive food challenge, whereas 77% with similar s-IgE without a previous allergic reaction had a negative food challenge. Hence, validated cut-off values may vary not only between populations, as well as age. For cow’s milk and hen’s egg allergies, it has been shown that the cut-off levels for a positive food challenge are lower in younger children (< 2 years old) (47).

The s-IgE to Ara h 2 are superior in predicting diagnostic outcome of an OFC (48), while the ratio of peanut s-IgE/total IgE (30) and s-IgG4/s-IgE to peanut (4) are less sensitive. The
peanut component allergen Ara h 2 has been demonstrated best in predicting severe allergic reactions (45, 49, 50). In one multicentre European study, s-IgE to Ara h 2 > 1.00 kUA/L conferred a 97 % probability of a systemic reaction (51). However, it seems like the Ara h 2 cut-off value for a 100 % positive predictive value (PPV) in predicting peanut allergy may be somewhat higher the younger the age. In adults, the Ara h 2 cut-off value with a 100 % PPV in predicting primary peanut allergy has been shown to be 1.75 kUA/L (52), whereas the 100 % PPV cut-off value in children with a median age of 6 years was 5.17 kUA/L (49). The BAT (32, 53, 54) as well as the recently reported CAPT (27) may be valuable contributors when diagnosing peanut allergy, but are until now mainly used in experimental settings.

2.3.3.1 Predicting severity of allergic reactions

Once a diagnosis of peanut allergy is made, predicting allergy severity may contribute to optimal management, including prescription of appropriate treatment. Allergy severity has been associated with basophil activation (55), and with peanut SPT and s-IgE in some (53, 56, 57), but not all (38, 58) studies. In one study of 71 patients with a median age of 16 years and various FAs (53), the allergy severity grade during a DBPCFC correlated significantly although weakly with peanut SPT \( r_s = 0.24 \) and s-IgE to Ara h 2 \( r_s = 0.31 \). In another study of 175 patients, with an age range of 1 – 26 years and a clinical history of allergic reaction to peanut, an even stronger correlation of \( r_s = 0.60 \) was reported between s-IgE to Ara h 2 and allergy severity (57).

In a study of 21 children (mean age of 60 months) with peanut allergy and 34 controls (28 tolerant and 6 non-anaphylactic reaction) (56), a peanut SPT of 11.25 mm was 33 % sensitive and 97 % specific, and a s-IgE to peanut of 7.7 kUA/L was 70 % sensitive and 97 % specific in predicting anaphylaxis. A titrated SPT (SPT_t) differentiated between Sampson severity
grade of anaphylaxis grade 3-5, grade 1-2 and negative food challenge in a study of hen’s egg allergy (59), while similar results have not been shown for peanut allergy.

In a study including SPT, s-IgE to peanut and Ara h 2, the ratio of s-IgG4/s-IgE to peanut and BAT in multivariate analyses to assess association with peanut allergy severity (55), basophil activation (% CD63+) was the marker strongest associated with severity.

2.3.3.2 Reactivity thresholds

The reactivity threshold is defined as the amount of peanut ingested at the time of a positive food challenge (60). There remains some disagreement however, as to what constitutes a positive food challenge (34). The consensus protocol for determination of threshold doses (60), however, suggested standardized threshold levels, such as the lowest observed adverse effect level (LOAEL). The LOAEL is defined as the lowest amount of food ingested eliciting mild, objective symptoms (mild urticaria, erythema, oral angioedema) according to the modified Bock’s criteria (Table 4) (34).

Determination of the reactivity threshold may be helpful to individualize treatment strategies related to peanut exposure. Previously, allergists gave precautionary advice assuming that the threshold dose of the offending food was zero (60). However, a zero-tolerance policy created huge practical problems, and increased the precautionary labelling of food by the food industry. As a consequence of the significant reduction of “allergen safe food”, the distribution model for expected LOAELs was published in 2014 (61). This distribution model was based on peanut OFCs in more than 200 peanut sensitized individuals (Figure 6) (61). The ED_{0.1} for peanut, i.e. the predicted eliciting dose for the most sensitive 1% of the population, was 0.2 mg peanut protein. The distribution model provided the basis of the revised Voluntary Incidental Trace Allergen Labelling (VITAL) 2.0 thresholds in Australia, and manufacturers were enabled to apply more appropriate precautionary labelling (61).
Determining reactivity thresholds has been complicated by reports of inconsistent reactivity thresholds, varying between different individuals with the same FA (62), as well as within the individual as demonstrated in two OFCs performed median 14 (range 7 – 126) days apart (63). Intra-individual variations may be unexplained, but augmenting factors like exercise, impaired compliance to asthma treatment, excessive tiredness, ongoing infection or menstruation are identified (35). Furthermore, different allergenic foods have been reported to have different threshold doses (60).

Determining reactivity thresholds by food challenge is resource intensive, expensive and carries the risk of a systemic reaction. Hence, attempts have been made to identify clinical or biological markers which can predict low threshold doses in individuals. In study populations heterogeneous with respect to the severity of peanut allergy, reactivity threshold has been
shown to be associated with s-IgE with Ara h 2 (57), as well as with the s-IgE to peanut, peanut SPT and basophil activation (38) in some (38, 57), but not all studies (63). However, the associations of the reactivity threshold were not sufficient to replace food challenge. The ability of these markers to predict reactivity threshold in a homogeneous population of children with severe peanut allergy is still unclear. Despite the unique ability of CRD in predicting the diagnosis of peanut allergy, OFC, and preferably a DBPCFC, is still considered gold standard to determine the severity of allergic reactions as well as the reactivity threshold (60).

2.4 Treatment strategies

2.4.1 Previous and current treatment for food allergy

Once diagnosed with primary food allergy, the doctor’s advice is to avoid the offending food. Despite vigilant dietary restrictions, accidental exposure occurs, and for peanuts, an annual accidental incidence rate of 14.3 % is reported (64). There is a need, therefore, for patients susceptible of severe allergic reactions always to carry rescue medication like adrenaline auto-injectors. Adrenaline is, however, under-used. Only one third of the children in a recent study received adrenaline (65), either before arrival or as part of the treatment at the emergency department (65). Hence, the constant risk of a potentially fatal allergic reaction results in a call for more efficacious treatment strategies.

2.4.2 Allergen specific immunotherapies

Allergen specific immunotherapy (ASIT) modifies the immune system by multiple mechanisms including desensitization of basophils and mast cells, induction of Treg and Breg cells and suppression of Th2 and Th1 cells (Figure 7) (66).
Figure 7. Immunologic changes during the course of ASIT. Within the first hours of the first dose, the activity of basophils and mast cells as well as the ability of degranulation are reduced. Subsequently, allergen-specific Treg and Breg cells are produced and the Th1 and Th2 cells are suppressed. Levels of s-IgE increase in early treatment followed by a late decrease, while s-IgG4 levels increase. The ratio of s-IgE/s-IgG4 decreases after several months.


To date, ASIT is the only therapy that has been shown closest to cure allergy. In clinical practice, ASIT has been used for more than hundred years, and the first study of ASIT was published in 1911 by Noon (67). The principle of ASIT is gradually exposure to increasing doses of a specific allergen through the oral, sublingual or subcutaneous routes until a maintenance dose is reached (68). The ASIT protocols typically consist of an up-dosing phase and a maintenance phase which is continued mostly for 3 – 5 years. The up-dosing phase is scheduled either as a conventional build-up of gradually incrementing doses administered weekly or biweekly, or as a cluster, rush or ultra-rush build-up which accelerates the schedule and shortens the up-dosing phase. The goal is to achieve post-discontinuation effectiveness
known as sustained unresponsiveness (SU) or tolerance. The first step to achieve SU is desensitization with no allergic reaction while regularly exposed to the allergen, given by the increasing threshold of allergen exposure required to elicit an allergic reaction. Desensitization and SU are the desired biological outcomes of a successful OIT. Today, subcutaneous immunotherapy (SCIT) is established as a conventional treatment for allergies to pollen, venom, mite and furry animals (69). For pollen induced allergic rhinitis, SU has been shown after three years of SCIT (70, 71). Venom SCIT has been reported to reduce life-threatening reactions (72). Higher maintenance doses have been associated with higher likelihood of SU in SCITs for inhalant and venom allergies (73, 74).

2.4.3 Allergen specific immunotherapies for food allergies including peanut

In the beginning of the 1990s, two separate studies of SCIT for peanut allergy were performed with good efficacy, but were stopped due to a high level of systemic AEs ranging from a rate of 13.3 % to 39 % (75, 76). Sublingual immunotherapies (SLITs) for food allergies have, however, reported more favorable safety profiles, but have modest success in desensitization and poor success in SU (68), as have peanut SLITs (77, 78). In one study of peanut SLIT (79), 14 of 20 patients completed 44 weeks of treatment and 60 % reported no AEs while threshold level increased from 3.5 mg to 496 mg.

Nowadays, epicutaneous immunotherapy (EPIT) for peanut is under growing investigation (80). To date, studies on EPIT report high adherence rates above 94 % with no persistent GI-symptoms and seldom AEs outside of the local patch site (81, 82). The major AEs are well-tolerated, localized patch-site reactions (80). The effect measured in increasing reactivity thresholds at OFC is associated with younger children under 11 years of age and duration of the treatment (82). The reactivity threshold is reported to increase from median 30 mg to 400 mg after one year of treatment in one study (81) and from 44 mg to 1440 mg in another (82).
sufficient to reduce allergic reactions after accidental exposure by at least 95 % on a population level (83).

The last three decades, an increasing amount of oral immunotherapies (OITs) have been commenced. Studies of OIT for cow’s milk (84) and hen’s egg (85) showed promising results for desensitization with acceptable safety profiles. Most studies, however, have been initiated for peanut OIT. The principle of OIT is outlined in Figure 8.

Figure 8. Typical protocol of OIT in clinical trials. The starting dose is lower than the threshold dose. During an initial rush build-up, doses are rapidly increased every 30 minutes to identify the highest tolerated dose. During the slow build-up phase, the daily OIT dose is increased every other week until a maintenance dose is reached. The maintenance dose is typically continued for 3- 5 years. An oral food challenge to the food is performed to assess desensitization while still receiving OIT. Sustained unresponsiveness is typically assessed 4 to 12 weeks after cessation of OIT. Reprinted from J Allergy Clin Immunol Pract 2017; 5: 250-72: Gernez Y et al.: “Immunotherapy for Food Allergy: Are We There Yet?” with permission from Elsevier. https://www.sciencedirect.com/science/article/pii/S2213219816306675?via%3Dihub#fig2
The starting dose must be lower than the reactivity threshold, preferably determined by a DBPCFC. The up-dosing phase consists of a rush build-up, a conventional build-up, or often a combination of biweekly up-dosing proceeded by a one-day escalation day. The protocol is finalized by a maintenance phase which is continued for some time. Desensitization and SU are determined by OFCs, most preferably DBPCFCs. Furthermore, the use of non-biological patient-reported outcomes (PROs) when assessing treatment effect is recommended (86). The PROs are any patient-reported health data including one-dimensional reports of AEs and multidimensional assessment of at least physical, emotional (or psychological) and social domains using standardized assessments of QoL, either generic or health-related.

Peanut OIT trials have shown promising results for desensitization with a 63.6 % to 86.9 % success rate as previously reported, with maintenance doses varying from 125 mg to 4000 mg of peanut protein (87-94). The safety profiles have been acceptable with AEs reported in up to 20 % of the dose-days (68, 87-91, 95). Evidence of SU after OIT is, however, scarce (87, 90), but two studies with maintenance doses of 4000 mg peanut protein reported SU in 50 % of the participants after four weeks cessation of treatment (87), decreasing to 15 % after six months cessation (90). Hence, the effect of OIT on SU is much smaller as compared to effect on desensitization (77). The most successful SU reported by peanut OIT, is the 78 % of 37 children (age range 9 – 36 months) who achieved a 4-weeks SU regardless of maintenance dose of 300 mg or 3000 mg peanut protein (96). However, published studies vary in design when it comes to inclusion criteria, maintenance dose, time of treatment and definition of desensitization and SU.

Adverse events reduce the feasibility of peanut OITs reflected by the relatively high drop-out rate ranging from 10 % to 32 % in studies (97). The most frequently reported AEs are GI-related (oral itching and stomach ache) (92-94, 97), while the worrying observation of OIT-related eosinophil oesophagitis (EoE) (92, 98) is estimated to develop in 2.7 % of patients.
undergoing OIT (97, 98). Anaphylactic events rarely occurred (≤ 1) in studies with maintenance doses ranging from 300 mg to 1400 mg peanut protein (88, 91, 94), except from the 14 percent of the children in the recently published AR101 who experienced anaphylaxis with the need of adrenaline. The AR101 study included children and adolescents with a reactivity threshold < 100 mg peanut protein who were treated with maintenance doses of 300 mg peanut protein (99).

A fixed starting dose and a gradual up-dosing protocol have been associated with fewer AEs and a higher rate completing the treatment (88, 100) as compared to a rush-protocol. Using anti-IgE treatment has been reported to reduce AEs in rush protocols (101). To date, it is not clear which starting dose and maintenance dose are the most appropriate with respect to efficacy by desensitization and SU, balanced against the safety of OIT. In participants with severe allergic reactions, a very low OIT starting dose may be preferable, based on the associations reported between very low reactivity thresholds and severe reactions (36, 53, 55, 102, 103).

Peanut OIT are reported to improve QoL in children (91, 104-108) despite challenging and sometimes severe AEs (92, 109). Most of these reports, however, were based on parental reports of the child’s QoL (91, 104-107), sometimes referred to as parental proxy-reports (110, 111), and rarely on the children’s self-reports (108). One study included both parental proxy-reports and child self-reports and reported improved QoL after OIT (108). However, this study did not include a control group, making it unclear whether the improvement in QoL was caused by the OIT (108). In a report of 122 children with different food allergies, parents assessed QoL in their children higher than the children themselves (112). Hence, only relying on parents’ assessment of child QoL, may be misleading. Furthermore, previous peanut OIT studies contain no information of one-dimensional patient perspectives of treatment burden including AEs, reported by, for example a visual analogue scale (VAS).
Previously published peanut OIT trials have included populations with large variations of allergy severity (87, 89-91). In one study of 23 children (88), anaphylaxis was diagnosed based solely on a peanut specific (s-) IgE of median (range) 95.6 (3 – 2071) kU/L, but clinical anaphylaxis was not verified in all by a DBPCFC. It is unclear, therefore, if the promising results of OIT desensitization are transferable to a sub-group of children with anaphylaxis to peanut, expected to benefit the most from a successful OIT (35, 89, 100).
3 OBJECTIVE AND SPECIFIC AIMS OF THE THESIS

Oral immunotherapy is promising for inducing allergen desensitization, whereas evidence that successful OIT will induce sustained unresponsiveness (SU) is scarce. Theoretically, a high allergen maintenance dose in OIT may increase the likelihood of SU. Even though children highly allergic to peanut probably would benefit the most from a successful OIT (35, 68, 89, 100), there is limited documentation of feasibility and safety of high-dose OIT in this group of patients.

Therefore, the present thesis tests the hypothesis that children who are highly allergic to peanut will benefit from a high-dose peanut OIT.

The objective of the present thesis was to determine the feasibility and effect of two-years OIT in children highly allergic to peanut.

The specific research aims of the present thesis are visualized in Figure 9 and were:

1. To identify baseline characteristics that predicts the possibility of entering (1A) and completing (1B) an up-dosing phase of peanut OIT (papers #1 and #2).
2. To determine the feasibility and identify factors associated with achieving a high maintenance dose in peanut OIT (papers #1 and #2).
3. To identify patient perceived burden of peanut OIT (papers #2 and #3).
4. To determine the effect of 2-years of OIT by desensitization to peanut and PROs (paper #3).
BAT – basophil activation test; CAPT – conjunctival allergen provocation test; DBPCFC – double-blind placebo-controlled food challenge; OFC – oral food challenge; Spiro – spirometry with reversibility testing; SPT – skin prick test; SPT_1 – titrated skin prick test; VAS*; visual analogue scale form of perceived treatment burden; QoL – standardized quality of life questionnaires

*only in the OIT children

Figure 9. End-points related to the various aims. Aim 1A is related to characteristics at baseline and outcome at Y0, whereas aim 1B is related to characteristics at baseline and outcome at Y1. Aim 2 is related to characteristics at baseline (Y0) and the up-dosing phase, Y0 to Y1. Aims 3 and 4 are related to the two years of OIT, Y0 to Y2.
4 METHODS AND SUBJECTS

4.1 Study design

The present thesis reports results from the first two years of the ongoing “Take away food allergy: Inducing tolerance in children allergic to peanut” trial (TAKE-AWAY trial), designed to assess one-year SU after four years of OIT in children with primary peanut allergy.

The TAKE-AWAY trial is a prospective, open labelled, randomized, controlled peanut OIT trial (RCT), consisting of four phases:

- screening phase (three days of eligibility screening)
- up-dosing phase (50-78 weeks)
- maintenance phase (36 months)
- follow-up phase (12 months)

Results were categorized into three time-points:

- screening (Y₀)
- one year of OIT (the end of up-dosing phase) (Y₁)
- two years of OIT (one year of maintenance treatment) (Y₂)

Children who discontinued the treatment were not assessed at subsequent time-points.

The TAKE-AWAY trial was conducted as a single centre trial at the Division of Paediatric and Adolescent Medicine, Oslo University Hospital, Ullevål, Norway. Children were recruited for screening investigations by referral from the in-house clinic, other paediatric allergy clinics in Oslo and the surrounding area, as well as from the Oslo Peanut Allergy Study (27).

Screening and subsequent enrolment were performed from February 2014 to June 2015 and all participants reached Y₂ by September 2017.
Time-points for the investigations are shown in Figure 9. Prior to screening, a telephone interview with families of referred children was performed to assess the inclusion and exclusion criteria. The 3-day investigation program included a general clinical examination, a structured interview and standardized QoL questionnaires, blood samples for serological and immunological analyses, lung function measurements (predicted forced expiratory volume in one second (FEV₁%) (113)), SPT and titrated SPT (SPTt), CAPT and BAT, followed by a DBPCFC.

The QoL assessments as well as all tests from screening were repeated in all children who attended Y₁ and Y₂. Children who received OIT filled in a visual analogue scale (VAS) form of perceived treatment burden at Y₁ and Y₂. Children who were defined ineligible for OIT had their QoL reassessed at Y₁ only. An OFC for peanut was performed at Y₂ in children undergoing OIT.

Written informed consent was obtained from both parents after detailed oral and written information. The TAKE-AWAY trial was approved by the Regional Committee for Medical and Health Research Ethics and monitored by a safety board with regular communications in case of severe or unexpected adverse events. The study was registered with ClinicalTrials.gov (number NCT02457416).

4.1.1 Inclusion and exclusion criteria and eligibility for enrolment

Inclusion criteria for screening were: Age 5-15 years with either a history of systemic allergic reactions to peanut or sensitization to peanut by a peanut skin prick test (SPT) ≥ 3 mm or s-IgE to peanut ≥ 0.35 kUA/L; living within acceptable distance from the Oslo University Hospital and willingness to participate in the peanut OIT study. Exclusion criteria were: Poorly controlled asthma; allergy or intolerance to any other ingredients of the peanut DBPCFC vehicle (ginger bread); current or previous allergen specific immunotherapy;
cardiac disease; severe atopic skin disease; diabetes mellitus or other severe diseases that might interfere with adherence to the study protocol. Participants who, during the screening visit, had a positive DBPCFC defined by at least two objective symptoms in one or more organ systems at a reactivity threshold > 3 mg peanut protein, were enrolled in the TAKE-AWAY trial.

4.1.2 Randomization
Allocation to OIT vs. observation only, followed an initial 2:1 block-size. The OIT starting dose was initially 5 mg of peanut protein based on previously published studies (27, 88, 100), but was reduced to 1 mg of peanut protein after enrolment of 26 children (17 active vs. 9 controls) due to low reactivity threshold in the referred patients.

4.2 Study population
The present thesis includes 96 children (5-15 years of age) who all had a positive DBPCFC with moderate objective symptoms in at least two organ systems, and thereby fulfilled the EAACI criteria for anaphylaxis (8, 9). None had an EAACI score of 3 or a Sampson score of 5, i.e. severe anaphylaxis. For more than half of the children, the reactivity threshold corresponded to the LOAEL.

Of the 213 children referred for screening, 95 did not wish to enter screening explained by concern for severe AEs, but also due to insufficient available time to adhere to the protocol. Another 14 children were excluded by the exclusion criteria, four withdrew during screening and four had a negative DBPCFC (Figure 10).

Fifty-seven children were randomized to OIT, 20 to observation only (controls), whereas 19 children were found ineligible for OIT due to a reactivity threshold ≤ 3 mg peanut protein (Figure 10).
Baseline characteristics were not significantly different between the OIT children, the controls and those defined ineligible for OIT (Table 5).

The baseline characteristics for children with an OIT starting dose of 5 mg and 1 mg of peanut protein (n = 17 vs. 40, respectively) were not significantly different with the exception of parental atopic disease, allowing them to be assigned as one intervention group (Table 6).
Table 5. Baseline characteristics of children randomized to peanut OIT in the TAKE-AWAY trial and children ineligible for enrolment.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Active (n = 57)</th>
<th>Controls (n = 20)</th>
<th>Ineligible (n = 19)</th>
<th>Overall p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>9.3 (5.2, 15.2)</td>
<td>9.3 (5.1, 13.3)</td>
<td>10.3 (5.6, 14.6)</td>
<td>0.08</td>
</tr>
<tr>
<td>Male</td>
<td>31 (54.4)</td>
<td>13 (65.0)</td>
<td>6 (31.6)</td>
<td>0.10</td>
</tr>
<tr>
<td>Current asthma</td>
<td>24 (42.1)</td>
<td>9 (45.0)</td>
<td>11 (57.9)</td>
<td>0.46</td>
</tr>
<tr>
<td>Allergic rhinitis</td>
<td>15 (26.3)</td>
<td>8 (40.0)</td>
<td>5 (26.3)</td>
<td>0.73</td>
</tr>
<tr>
<td>Atopic dermatitis ever</td>
<td>47 (82.5)</td>
<td>14 (73.9)</td>
<td>13 (68.4)</td>
<td>0.40</td>
</tr>
<tr>
<td>Allergy to tree-nuts</td>
<td>20 (35.1)</td>
<td>7 (36.8)</td>
<td>9 (53.0)</td>
<td>0.35</td>
</tr>
<tr>
<td>Allergy to other food than nuts</td>
<td>27 (47.4)</td>
<td>11 (57)</td>
<td>11 (61.1)</td>
<td>0.64</td>
</tr>
<tr>
<td>Parental atopic disease*</td>
<td>50 (87.7)</td>
<td>16 (80)</td>
<td>17 (89.5)</td>
<td>0.63</td>
</tr>
<tr>
<td>Parental food allergy**</td>
<td>21 (36.8)</td>
<td>6 (30)</td>
<td>11 (57.9)</td>
<td>0.16</td>
</tr>
<tr>
<td>FEV1% predicted</td>
<td>102.1 (98.7, 105.4)</td>
<td>99.3 (90.7, 107.9)</td>
<td>96.3 (88.8, 103.8)</td>
<td>0.15</td>
</tr>
<tr>
<td>Pos s-IgE (≥ 0.35 kUA/L):</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tree-nuts***</td>
<td>52 (91.2)</td>
<td>16 (80.0)</td>
<td>19 (100.0)</td>
<td>0.07</td>
</tr>
<tr>
<td>Other food****</td>
<td>54 (94.7)</td>
<td>19 (95.0)</td>
<td>19 (100.0)</td>
<td>0.47</td>
</tr>
<tr>
<td>Peanut SPT (mm)</td>
<td>10.7 (9.3, 12.2)</td>
<td>10.7 (8.3, 13.0)</td>
<td>10.4 (8.3, 12.5)</td>
<td>0.56</td>
</tr>
<tr>
<td>S-IgE (kUA/L) peanut</td>
<td>265.1 (164.9, 365.3)</td>
<td>146.8 (63.3, 230.4)</td>
<td>157.3 (83.6, 231.1)</td>
<td>0.34</td>
</tr>
<tr>
<td>Ara h 2</td>
<td>118.2 (83.3, 154.1)</td>
<td>59.8 (31.0, 88.6)</td>
<td>69.5 (38.8, 100.3)</td>
<td>0.13</td>
</tr>
<tr>
<td>Peanut s-IgE/total IgE (kUA/L)</td>
<td>0.4 (0.3, 0.5)</td>
<td>0.7 (0.3, 1.8)</td>
<td>0.3 (0.2, 0.4)</td>
<td>0.39</td>
</tr>
<tr>
<td>Peanut s-IgG₄/ s-IgE (ng/ml)</td>
<td>23.1 (10.0, 36.1)</td>
<td>62.0 (16.1, 140.1)</td>
<td>148.7 (106.1, 403.5)</td>
<td>0.12</td>
</tr>
<tr>
<td>Pre-OIT DBPCFC:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anaphylaxis severity grade:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>modified EAACI</td>
<td>1.7 (1.6, 2.0)</td>
<td>1.8 (1.6, 2.0)</td>
<td>1.8 (1.6, 2.0)</td>
<td>0.50</td>
</tr>
<tr>
<td>Sampson</td>
<td>2.7 (2.5, 2.9)</td>
<td>2.9 (2.6, 3.2)</td>
<td>2.5 (2.2, 2.9)</td>
<td>0.25</td>
</tr>
<tr>
<td>Use of adrenaline</td>
<td>30 (52.6)</td>
<td>5 (25.0)</td>
<td>8 (42.1)</td>
<td>0.10</td>
</tr>
<tr>
<td>LOAEL(mg peanut prot (ppt))</td>
<td>105.9 (28.9, 182.8)</td>
<td>96.1 (53.3, 245.4)</td>
<td>NA (31.6, 791.1)</td>
<td>0.34</td>
</tr>
<tr>
<td>Reactivity threshold (mg ppt)</td>
<td>177.3 (86.7, 268.0)</td>
<td>375.5 (40.1, 791.1)</td>
<td>4.4 (1.6, 7.3)</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Variables are given as mean (95 % CI) or n (%), except age which is given with (min, max). One-way ANOVA was applied to determine statistically significant differences between group means. Chi-square test was applied to determine statistically differences between categorical data. *Atopic disease includes asthma, allergic rhinitis, atopic dermatitis, allergic conjunctivitis. **All food allergy including peanut and tree-nut allergy.
***Hazelnut, almond, cashewnut, pistachionut, walnut, pecannut, brazilnut and macadamianut.

****Fenugreek, soybean, pea, red kidney bean, lupin seed and wheat.

Anaphylaxis severity was graded by two grading systems according to the modified EAACI position papers (8, 9) ranging from 1 to 3 and the method of Sampson (Grading of Food-Induced Anaphylaxis According to Severity of Clinical Symptoms) (10) ranging from 1 to 5. Reactivity threshold is defined as the cumulated peanut protein (mg) ingested at positive DBPCFC.

SPT, skin prick test; Ig - immunoglobulin; LOAEL - lowest observed adverse effect level; OIT – oral immunotherapy; DBPCFC – double-blind placebo controlled food challenge; LOAEL – lowest observed adverse effect level (amount of peanut eliciting mild, objective symptoms)
### Table 6. Baseline characteristic of the 57 children randomized to peanut OIT based upon their starting dose.

<table>
<thead>
<tr>
<th></th>
<th>OIT starting dose</th>
<th>OIT starting dose</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 mg peanut protein</td>
<td>5 mg peanut protein</td>
<td></td>
</tr>
<tr>
<td>(n = 40)</td>
<td>(n = 17)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>9.7 (5.4, 15.0)</td>
<td>10.4 (6.3, 15.1)</td>
<td>0.26</td>
</tr>
<tr>
<td><strong>Male sex</strong></td>
<td>23 (57.5)</td>
<td>8 (47.5)</td>
<td>0.47</td>
</tr>
<tr>
<td><strong>Ever had eczema</strong></td>
<td>32 (80.0)</td>
<td>15 (88.2)</td>
<td>0.46</td>
</tr>
<tr>
<td><strong>Current asthma</strong></td>
<td>20 (50.0)</td>
<td>11 (64.7)</td>
<td>0.56</td>
</tr>
<tr>
<td><strong>Allergic rhinitis</strong></td>
<td>10 (25.0)</td>
<td>5 (29.4)</td>
<td>0.77</td>
</tr>
<tr>
<td><strong>Parental atopic disease</strong></td>
<td>38 (90.5)</td>
<td>12 (70.6)</td>
<td><strong>0.01</strong></td>
</tr>
<tr>
<td><strong>Parental food allergy</strong></td>
<td>15 (37.5)</td>
<td>6 (35.3)</td>
<td>0.87</td>
</tr>
<tr>
<td><strong>Peanut SPT (mm)</strong></td>
<td>9.8 (4.0, 36.3)</td>
<td>9.4 (4.0, 22.9)</td>
<td>0.88</td>
</tr>
<tr>
<td><strong>Positive SPT other nuts (≥ 3 mm)</strong></td>
<td>22 (55.0)</td>
<td>6 (35.3)</td>
<td>0.17</td>
</tr>
<tr>
<td><strong>S-IgE (kUA/L) peanut</strong></td>
<td>92.8 (9.8, 2290.9)</td>
<td>102.3 (3.1, 955.0)</td>
<td>0.86</td>
</tr>
<tr>
<td><strong>Ara h 2</strong></td>
<td>46.8 (9.9, 489.8)</td>
<td>61.7 (2.5, 457.1)</td>
<td>0.60</td>
</tr>
<tr>
<td><strong>Positive s-IgE (kUA/L)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>other nuts***</td>
<td>38 (95.0)</td>
<td>14 (87.5)</td>
<td>0.33</td>
</tr>
<tr>
<td>other food****</td>
<td>38 (95.0)</td>
<td>16 (100.0)</td>
<td>0.36</td>
</tr>
<tr>
<td><strong>Peanut s-IgE/total IgE (kUA/L)</strong></td>
<td>9.5 (8.0, 10.0)</td>
<td>9.5 (9.1, 9.9)</td>
<td>0.46</td>
</tr>
<tr>
<td><strong>Peanut s-IgG4/s-IgE (ng/ml)</strong></td>
<td>4.7 (9.2, 288.4)</td>
<td>4.7 (9.9, 42.7)</td>
<td>0.72</td>
</tr>
<tr>
<td>**BAT (%CD63+), pos *******</td>
<td>63.1 (16.2, 93.3)</td>
<td>64.6 (37.2, 91.2)</td>
<td>0.80</td>
</tr>
<tr>
<td>**CAPT positive (dilution) *******</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/160</td>
<td>5 (12.5)</td>
<td>2 (11.8)</td>
<td>0.59</td>
</tr>
<tr>
<td>1/80</td>
<td>11 (27.5)</td>
<td>2 (11.8)</td>
<td></td>
</tr>
<tr>
<td>1/40</td>
<td>12 (30.0)</td>
<td>9 (52.9)</td>
<td></td>
</tr>
<tr>
<td>1/20</td>
<td>11 (27.5)</td>
<td>2 (11.8)</td>
<td></td>
</tr>
<tr>
<td>1/10</td>
<td>11 (27.5)</td>
<td>2 (11.8)</td>
<td></td>
</tr>
<tr>
<td><strong>Pre-OIT DBPCFC</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Use of adrenaline</strong></td>
<td>23 (57.5)</td>
<td>7 (41.2)</td>
<td>0.26</td>
</tr>
<tr>
<td><strong>Anaphylaxis severity</strong></td>
<td>1.7 (1.0, 2.0)</td>
<td>1.4 (1.0, 2.0)</td>
<td>0.10</td>
</tr>
<tr>
<td><strong>modified EAACI</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Reactivity threshold</strong></td>
<td>42.8 (3.0, 933.3)</td>
<td>93.7 (12.9, 1584.9)</td>
<td>0.12</td>
</tr>
</tbody>
</table>

*Atopic disease includes asthma, allergic rhinitis, atopic dermatitis, allergic conjunctivitis

**All food allergy including peanut and tree-nut allergy

***Hazelnut, almond, cashewnut, pistachionut, walnut, pecannut, brazilnut and macadamianut

****Fenugreek, soybean, pea, red kidney bean, lupin seed and wheat

*****The CAPT was recorded positive ranging from dilution level 1 (1:160) to 5 (1:1).

******N = 50. The BAT was not performed in 7 children due to technical causes (n = 5) and non-responders were excluded from the analyses (n = 2).

Anaphylaxis severity was graded according to the modified EAACI position papers (8, 9) ranging from 1 to 3.

Reprinted from Allergy 2018; Reier-Nilsen T, Michelsen MM, Lodrup Carlsen KC, Carlsen KH, Mowinckel P, Nygaard UC, et al.: «Feasibility of desensitizing children highly allergic to peanut by high-dose oral immunotherapy.”, with permission from Elsevier. 
4.3 Methods

4.3.1 The screening interview

The structured parental interview included information about history of allergic reactions including anaphylaxis, allergic co-morbidities (current asthma, allergic rhinitis, previous or current atopic dermatitis and allergy to other foods or nuts), medical history including medicine use and socio-demographic data.

4.3.2 Lung function measurements

Lung function was measured using SensorMedics to obtain maximal expiratory flow volume loops according to international standards (114), expressed as percent predicted forced expiratory volume in one second (FEV$_1$%) using the reference values of Zapletal et al. (113).

4.3.3 Skin prick test

Skin prick tests were performed according to international guidelines (115). The allergen extracts used included hazelnut, almond, soybean, birch, grass (timothy), mugwort, cat, dog, mite and mold (cladosporium herbarium) (ALK SQ extracts, ALK Abello (Hørsholm, Denmark)), pea and positive and negative controls (Allergopharma (Reinbek, Germany)). A titrated SPT ($SPT_t$) to peanut was performed with dilutions of 1:20, 1:200, 1:2000 and 1:20000 of the peanut allergen extract (ALK SQ extracts, ALK Abello (Hørsholm, Denmark)). Wheal size was recorded after 15 minutes, and regarded positive if $\geq 3$ mm larger than negative control.

4.3.4 The conjunctival allergen provocation test

Conjunctival allergen provocation test (CAPT) was performed double-blinded and placebo-controlled. Randomization was performed using Statistical Analysis System (SAS, Version
9.3, SAS Institute Inc., Chapel Hill, NC, USA), and all tests were un-blinded only after the last child had completed all investigations. A commercially available peanut extract (Greer laboratories, Lenoir, NE, USA) was diluted with NaCl 0.9 % to make test solutions of 1:160, 1:80, 1:40, 1:20, 1:10 and 1:1, as previously described by Lindvik et al. (27). The 0.9 % NaCl was used as placebo. The incremental concentrations of peanut extract and placebo were applied every 30 minutes with two independent observers recording redness, itching, chemosis and/or lacrimation. The CAPT was defined positive with the occurrence of ≥ 2 recorded symptoms (27). Photos were taken before and after the CAPT for documentation.

4.3.5 Immunological investigations

Analyses of total IgE and s-IgE to peanut and peanut component allergens (Ara h 1, Ara h 2, Ara h 3, Ara h 8, Ara h 9) as well as other common allergens including hazelnut and hazelnut allergen components (Cor a 1, Cor a 8, Cor a 9, Cor a 14), almond, cashew nut, pistachio, walnut, pecan nut, brazil nut, macadamian nut, fenugreek, soy bean, lupine seed, wheat, latex, common silver birch, timothy and mugwort were performed in fresh serum sampled in EDTA tubes. The serum was sent the same day to the Fürst Medical Laboratory (Oslo, Norway) using the Phadia CAP-System FEIA (ThermoFisher, Uppsala, Sweden) and the analyses were performed according to the manufacturer’s instruction. Specific IgE ≥ 0.35 kUA/L was considered positive.

Analyses of s-IgG and s-IgG₄ to peanut and Ara h 2 were performed in collected sera stored at - 86°C using the Phadia CAP-System FEIA (ThermoFisher, Uppsala, Sweden). Levels of IgG > 2.0 mg/L and IgG₄ of > 0.07 mg/L were considered positive, according to the manufacturer’s instructions.
The BAT was performed in fresh EDTA blood sampled prior to the food challenge and stored at room temperature (usually 1-3 hours, maximum 24 hours). The proportion of activated blood basophils (basophil activation - CD63+ basophils) was determined in whole blood aliquots incubated for 15 minutes at 37 °C. The negative control was incubated with stimulation buffer containing IL-3, calcium and heparin, and the positive control with n-Formyl-methionyl-leucyl-phenylalanine (anti-FcεR1 or fMLP). Further blood aliquots were incubated with 2.5, 5 and 10 ng/ml peanut extract (all reagents from the FlowCAST kit and CAST-allergen peanut BAG-F13, both from Buhlmann Laboratories AG, Schönenbuch, Switzerland). All aliquots were incubated with 20 µl staining solution containing a mix of anti-CD63 FITC and anti-CCR3-PE (Buhlmann), in addition to 5 µl anti-CD203c-APC monoclonal antibody (BioLegend, San Diego, CA USA). After red blood cell lysis, centrifugation and resuspension in wash buffer (Buhlman), the cells were analysed on a LSR II flow cytometer with BD FACSDIVA software (all from BD Biosciences, New Jersey, USA). Basophils were gated as side-scatter (SSC) low and CCR3+ cells, and for the present thesis, basophil activation is expressed as the % CD63 positive basophils (% CD63+) after stimulation of 5 ng/ml of the peanut extract. Because all children had strong basophil activation already to the lowest allergen concentration of 2.5 ng/ml peanut extract, the basophil allergen sensitivity (CD-sens) expressing the allergen concentration eliciting half of the maximum basophil activation could not be determined. Non-responders were defined as children responding with basophil activation < 5 % (116). In the present thesis, participants with a basophil activation of 5 – 15 % were classified as low-responders, whereas a basophil activation > 15 % was defined as positive, as suggested by Glaumann et al. (117) and the kit provider (Buhlmann Laboratories AG). In ten children, BAT was not performed due to missed sampling (n = 5) or technical causes (n = 5) (technical failure of equipment (n = 1), incorrect handling of the blood sample (n = 1) or no available allergen extract (n = 3)).
4.3.6 Double-blind placebo-controlled food challenge to peanut

The DBPCFC was performed in line with international standardized procedure (34, 41).

Peanut flour (Golden Peanut Company, Alpharetta, GA, USA) was used as the active challenge ingredient. Gingerbread was used as matrix, baked according to a recipe by Vlieg-Boestra et al. (118), allocated and blinded by the cook.

The first challenge dose contained 3 mg of peanut protein. The maximal cumulated dose of 6443 mg peanut protein was given as 7 incremental steps (3 mg, 10 mg, 30 mg, 100 mg, 300 mg, 1 000 mg, 5 000 mg of peanut protein) with 30 minute interval. In the case of persistent subjective or mild objective symptoms in line with the modified Bock’s criteria (Table 4) (34), interval was increased up to 60 minutes, in which case the next step repeated the previous dose in consent with recommendations from international work groups (119, 120). The DBPCFC was defined positive with the occurrence of two or more moderate objective symptoms (34, 39), and the DBPCFC was stopped if scored positive, even though only part of the current dose was eaten. The cumulated peanut protein (mg) intake at the time of positive DBPCFC was recorded and regarded as the reactivity threshold. The lowest amount of peanut protein that elicited the first mild, objective symptom during food challenge was recorded post hoc and is defined as the lowest observed adverse effect level (LOAEL).

The order of active vs. placebo days and block-size were unknown to all study personnel present at the challenge, provided by the statistician using Statistical Analysis System (SAS, Version 9.3, SAS Institute Inc., Chapel Hill, NC, USA), and un-blinded first at the end of the recruitment phase.
4.3.7 Oral food challenge at two-years of oral immunotherapy

An open oral food challenge at $Y_2$ was performed to determine the level of desensitization, defined as the highest cumulated amount of peanut protein ingested without eliciting allergic reactions corresponding to a positive $Y_2$ OFC.

The goal of the $Y_2$ food challenge was ingestion of a cumulated dose of 7500 mg peanut protein without allergic reactions. The first challenge dose was individualized in reflection to the patient’s maintenance dose, the second and third dose were each 25% of the maintenance dose. The cumulated dose of 7500 mg peanut protein was reached after a maximum of six doses with 30 minutes’ intervals (Table 7), in line with international standards (119, 120).

Table 7. Protocol for the open oral food challenge performed after two years of oral immunotherapy ($Y_2$) in children highly allergic to peanut.

<table>
<thead>
<tr>
<th>1. dose: peanut protein (mg)</th>
<th>2. dose</th>
<th>3. dose</th>
<th>4. dose</th>
<th>5. dose</th>
<th>6. dose</th>
<th>Cumulated dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>350</td>
<td>87</td>
<td>87</td>
<td>919</td>
<td>3000</td>
<td>3057</td>
<td>7500</td>
</tr>
<tr>
<td>450</td>
<td>112</td>
<td>112</td>
<td>769</td>
<td>3000</td>
<td>3057</td>
<td>7500</td>
</tr>
<tr>
<td>600</td>
<td>150</td>
<td>150</td>
<td>543</td>
<td>3000</td>
<td>3057</td>
<td>7500</td>
</tr>
<tr>
<td>800</td>
<td>200</td>
<td>200</td>
<td>243</td>
<td>3000</td>
<td>3057</td>
<td>7500</td>
</tr>
<tr>
<td>1000</td>
<td>250</td>
<td>250</td>
<td>2943</td>
<td>3057</td>
<td>7500</td>
<td></td>
</tr>
<tr>
<td>1250</td>
<td>312</td>
<td>312</td>
<td>2569</td>
<td>3057</td>
<td>7500</td>
<td></td>
</tr>
<tr>
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<td>375</td>
<td>375</td>
<td>2193</td>
<td>3057</td>
<td>7500</td>
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<td>450</td>
<td>1743</td>
<td>3057</td>
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</tr>
<tr>
<td>2200</td>
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<td>550</td>
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<td>3057</td>
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</tr>
<tr>
<td>2700</td>
<td>675</td>
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<td>393</td>
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<td>7500</td>
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<td>825</td>
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<td>2550</td>
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<td>7500</td>
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<td>1250</td>
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<td>7500</td>
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The 1st dose is individualized and relates to the individual maintenance dose. The 2nd and 3rd dose combined constitutes 50% of the individual maintenance dose. The last dose was equal for all individuals.
4.3.8 Up-dosing protocol of the oral immunotherapy

The biweekly step-up protocol for the peanut OIT in the TAKE-AWAY trial had a fixed starting and a high pre-defined maximum maintenance dose (MMD) of 5000 mg peanut protein (Table 8). A 50 - 100 % increase for every dose was used initially with a subsequent 20 - 44 % increase per dose.

Table 8. Long-term step-up protocol with a fixed starting dose for oral immunotherapy in the TAKE-AWAY trial.

<table>
<thead>
<tr>
<th>Dose-step</th>
<th>Peanut (mg)</th>
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<tbody>
<tr>
<td>1</td>
<td>1</td>
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<tr>
<td>2</td>
<td>3</td>
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<tr>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
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<tr>
<td>5</td>
<td>20</td>
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<td>6</td>
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<td>7</td>
<td>45</td>
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<td>8</td>
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<tr>
<td>9</td>
<td>90</td>
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<td>10</td>
<td>125</td>
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<tr>
<td>11</td>
<td>175</td>
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<tr>
<td>12</td>
<td>250</td>
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<td>13</td>
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<td>14</td>
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<td>20</td>
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<td>21</td>
<td>2200</td>
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<tr>
<td>24</td>
<td>4000</td>
</tr>
<tr>
<td>25</td>
<td>5000</td>
</tr>
</tbody>
</table>

Reprinted from Allergy 2018; Reier-Nilsen T, Michelsen MM, Lodrup Carlsen KC, Carlsen KH, Mowinckel P, Nygaard UC, et al.: «Feasibility of desensitizing children highly allergic to peanut by high-dose oral immunotherapy.”, with permission from Elsevier.

The allergen source for the lowest doses was peanut flour (Golden Peanut Company, Alpharetta, GA, USA). However, most children reported distaste for larger amounts of peanut flour, and all but one child switched to roasted peanuts at OIT doses of 65 - 500 mg peanut protein. Each up-dosing period with an increased dose-step was discussed with the patient and their parents. An up-dosing period started with an incremented OIT dose ingested under observation at the hospital with subsequent daily intake of this OIT dose at home for 14 days. Increasing the OIT dose required absence of ongoing infections, bronchial obstruction and OIT-related AEs during the five previous days. A clinical examination and peak expiratory flow rate were performed prior to, and 1-2 hours after, the ingestion of the OIT dose. If no AEs occurred, the child started a new up-dosing period with the increased OIT dose. In case of subjective or mild to moderate symptoms, participants were advised to use oral antihistamines, but if moderate objective symptoms occurred, up-dosing was postponed for one week. If the child was unwilling to increase the OIT dose or if AEs resulted in three consecutive unsuccessful attempts to increase the OIT dose, the current dose would represent the participant’s individual maintenance dose (IMD).

The child was strongly advised to avoid exercise within two hours after ingesting the OIT dose. Ingestion of other dietary peanut sources was also strongly discouraged. Menstruating girls were asked to closely monitor possible OIT-related symptoms during menstrual cycle. During the daily intake of OIT doses at home, children were advised to postpone the OIT dose to the next day in case of ongoing infections, asthma exacerbations, excessive tiredness or vaccinations. If the OIT dose was not eaten for three or fewer consecutive days, the OIT was resumed at home. However, if more than three consecutive days went by without daily ingestion of the OIT dose, the OIT was resumed under observation at the hospital. If moderate objective symptoms occurred after ingesting the OIT dose at home for > 2 - 3 consecutive days, the OIT dose was reduced to the previous dose-step in the up-dosing
protocol. In case of anaphylaxis, the OIT dose was reduced by two dose-steps for 1-2 weeks before resuming the up-dosing protocol.

The child was withdrawn from the OIT and discontinued treatment if unwilling to continue OIT, if severe or troublesome AEs occurred, or if more than two anaphylactic reactions occurred in one child. Unexpected severe AEs were reported to the independent safety board. The peanut intake, AEs, use of medication and accidental exposure to peanut were recorded in a daily symptom diary. Grading of AEs was in line with the modified Bock’s criteria (34, 120).

All participants were prescribed adrenaline auto-injectors and antihistamines, received a written treatment plan for OIT-related AEs, and had around-the-clock access to the study paediatricians.

4.3.9 Classification of allergic reactions/adverse events

Adverse events during food challenge and related to OIT were classified as mild, moderate or severe (including anaphylaxis) according to the Bock’s criteria modified by Sampson et al. (Table 4) (34, 120).

Skin symptoms classified as mild include occasional scratching, less than three hives, mild lip oedema, or a few areas of erythema, whereas moderate include scratching for more than two continuous minutes, more than three but less than ten hives, significant face oedema or areas of erythema, conjunctivitis, periocular swelling and severe symptoms include excoriations or generalised erythema or urticaria. Mild respiratory symptoms include rare bursts or mild congestion, expiratory wheezing by auscultation or less than three episodes of throat clearing, whereas moderate include less than ten bursts, frequent sniffing, inspiratory and expiratory wheezing by auscultation or hoarseness, and severe include persistent rhinorrhoea, use of accessory muscles or audible wheezing or stridor.
Mild GI-symptoms include complaints of nausea or stomach ache or one episode of emesis or diarrhoea, whereas moderate include frequent complaints of nausea and stomach ache with normal activity and two to three episodes of emesis or diarrhoea or one of each, and severe include complaints of nausea or stomach ache with change of activity level or more than three episodes of emesis or diarrhoea or two of each.

Mild cardiovascular symptoms include subjective weakness or tachycardia, whereas moderate include more than 20 % drop in baseline blood pressure, and severe include signs of impaired circulation.

Anaphylaxis was defined as objective symptoms from at least two organ systems according to the task force position papers of European Academy of Allergy and Clinical Immunology (EAACI) (8, 9), modified for children by Vetander et al. (11). The EAACI position papers (8, 9) also established a severity grading of anaphylaxis scoring from 1 - 3 (mild-moderate-severe). Additionally, anaphylaxis was graded by the method of Sampson (Grading of Food-Induced Anaphylaxis According to Severity of Clinical Symptoms) ranging from 1 - 5 (extremely severe reaction) (10).

4.3.10 Measurements of quality of life

The quality of life in participating children was assessed using the generic Paediatric Quality of Life Inventory Version 4.0 (PedsQL 4.0), which is age-adapted (age 5-7, 8-12 and 13-18 years), validated for clinical trials (110, 121) and consists of 13 items within the following four functioning domains; physical, emotional, social and school. A 5-point Likert scale (0 = never, 4 = almost always) is applied for the 8-12 and 13-18 years reports, while a simplified 3-point scale is applied for the 5-7 years child reports. Results are classified by Physical Health Score (Physical Functioning) and Psychosocial Health Summary Score (Emotional,
Social and School Functioning). Both children and parents assessed child QoL using PedsQL 4.0 self- and proxy-reports, respectively.

*The quality of life in parents of participating children* was assessed using the health-related Food Allergy Quality of Life – Parental Burden (FAQL-PB) Questionnaire (122). The FAQL-PB consists of 17 items including family/social activities (restaurant meals, social activities, child care and vacation), school, time spent for meal preparation, health concerns, and emotional issues. The FAQL-PB has a 7-point Likert scale (1 = not troubled, 7 = extremely troubled), with summated scores ranging from 17-119. The minimal important difference (MID) (the smallest change that the patient perceive as important) on a 7-point Likert scale is defined as 0.5 (123).

Translation into Norwegian has been validated for PedsQL 4.0 (124), whereas the FAQL-PB was translated into Norwegian in the OPAS-study (27) with permission from The Food Allergy and Anaphylaxis Network.

### 4.3.11 Measurement of perceived treatment burden

*A visual analogue scales (VAS) form* was developed to obtain one-dimensional perception of treatment burden during the last 12 months. The VAS form including eight VAS items ranging from 0 – 10 (0 = no burden, 10 = massive burden), and was completed by children receiving OIT together with their parents at time-points Y₁ and Y₂. One item referred to overall perception of treatment burden, whereas seven items referred to treatment burden within three domains: Gastro-intestinal (GI) related AEs (stomach ache, nausea/vomiting and oral itching) (3), taste and amount of daily peanut OIT (2), and time spent on OIT (up-dosing at hospital and ingestion of OIT doses at home) (2). Results were classified per domain and reported with mean score per domain.
4.4 Definitions, outcomes and explanatory factors

*The reactivity threshold* (paper #1) was defined as the cumulated peanut protein (mg) intake at positive DBPCFC.

*Very low reactivity threshold* (paper #1) was defined as DBPCFC reactivity threshold of ≤ 3 mg of peanut protein.

The LOAEL (60) was set post hoc and the threshold value representing the amount of peanut protein eaten at the occurrence of the first mild objective symptom. The LOAEL was set to 3 mg for children with a positive DBPCFC at the first challenge dose (paper #1).

*The feasibility of desensitization* (paper #2) was defined as the proportion of children who reached the pre-defined maximum maintenance dose (MMD) of 5000 mg peanut protein.

*The level of desensitization* (paper #3) was defined as the highest cumulated dose of peanut protein ingested without eliciting allergic reactions corresponding to a positive Y2 food challenge.

For aim #1 of the present thesis, the primary outcome was the proportion of children being enrolled in the TAKE-AWAY trial, whereas the secondary outcome was the proportion of children who still received OIT at the end of up-dosing phase (after one year of treatment).

Possible explanatory factors for enrolment and continuation of the OIT in the TAKE-AWAY trial included baseline sociodemographic characteristics, as well as immunological and clinical characteristics associated with reactivity thresholds, as reactivity threshold determined eligibility.

For aim #2, the primary outcome was the proportion of children who reached the pre-defined MMD of 5000 mg peanut protein, whereas the secondary outcome was the proportion of children who reached a lower individual maintenance dose (IMD) (< 5000 mg peanut protein). Potential explanatory factors of reaching the MMD or the lower IMDs were baseline characteristics, the treatment burden including AEs, medication for AEs and protocol
deviations (dose reduction or postponed up-dosing due to social events, AEs or infections), as well as the ability to be compliant to the OIT.

For aim #3, the primary outcome was the number AEs in the up-dosing phase, characterized by the involved organ(s) and classified into subjective and mild objective, moderate or severe (including anaphylaxis) in line with the modified Bock’s criteria (34). The secondary outcome was the reported patient perceived burden of treatment. Potential explanatory factors of treatment burden were factors known to augment AEs including exercise within two hours of a dose, ongoing infection, excessive tiredness, impaired compliance to OIT or asthma treatment, taste/amount of peanuts and time spent on OIT.

For aim #4, the primary outcome was the desensitization given by reactivity threshold at the Y2 OFC, whereas the secondary outcomes were change in QoL scores from Y0 to Y2 obtained from the PedsQL 4.0 child self-reports and the corresponding parental proxy-reports. The third outcome was the parental QoL reported by the FAQL-PB. Potential factors influencing change in QoL were OIT-related AEs, level of desensitization determined by the OFC at Y2, the maintenance dose and the child’s perception of treatment burden at Y1 and Y2 presented by the mean VAS-score from each of three domains: GI-related AEs, taste/amount of peanuts and time spent on OIT. Sub-analysis including change in QoL from Y0 to Y1 rather than Y0 to Y2 was used to determine if ineligibility for OIT influenced change in QoL.

4.5 Statistical analyses

Statistical power analyses was based upon studies reporting that a step-up peanut OIT desensitized up to 80% of children with less severe peanut allergy (89, 95) and tolerance (lasting SU) that spontaneously develops in 20% (24). A somewhat lower proportion of children with severe peanut allergy is expected to achieve desensitization, and was set to 57
%.

Consequently, a treatment group of 40 and a control group of 20 subjects would provide a statistical power of 80 % at a five percent significance level.

Due to non-normal distribution, continuous baseline characteristics are presented by geometric mean (95 % CI) and median (range), while categorical data are presented as number of cases (n) with percentage (%). To assess possible differences, the Mann-Whitney U test was used for continuous data and the Pearson’s chi-square test for categorical data.

One-way ANOVA was used to analyse the overall difference in continuous data between the three groups of children who reached the MMD, the IMD and those who discontinued OIT with the latter group as reference (paper #2). One-way ANOVA was also used to analyse the overall difference in continuous data between the three groups of children who were randomized to OIT or controls, or defined as ineligible for OIT, with controls as the reference (paper #3). Finally, one-way ANOVA was used to analyse the overall difference between AEs occurring in the three dose-intervals of the up-dosing phase (1-65, 66-800 and 801-5000 mg peanut protein) (paper #2). In the case of a significant overall p-value, the Dunnett’s post hoc test was used to confirm between which groups the statistically significant difference had occurred.

As recommended for analyses of PedsQL 4.0 (121), the items were reverse-scored (0 = 100, 1 = 75, 2 = 50, 3 = 25, 4 = 0) with the mean sum of each item reported (paper #3). Basophil activation was measured as a continuous variable (paper #1 and #2).

A paired samples t-test was used to determine if a statistically significant desensitization had occurred in the individual (paper #2), comparing the maintenance dose with the reactivity threshold at screening food challenge. Paired samples t-test was also used to determine the difference in the individual’s QoL scores at Y0 and Y2 as well as to determine the difference in VAS from Y1 to Y2 (paper #3).
Change in QoL scores from Y₀ to Y₂ were assessed using generalized repeated-measures in linear mixed models including QoL score at Y₀, age, gender, randomization group, and Y₁ and Y₂ as independent variables. The Scheffe's test was used to determine group differences (paper #3). Unpaired t-test was also used to determine significant differences in change in QoL from Y₀ to Y₁ between the ineligible children and the controls.

Bivariate unadjusted analyses were performed with Spearman correlation analyses (r_s = Spearman correlation coefficient) (paper #1 and #3).

Bivariate logistic regression analyses were used to assess the associations between explanatory factors and feasibility of desensitization (paper #2) with the proportion of children who reached the MMD (as independent variable) versus the proportion of children who reached IMD or discontinued OIT (as dependent variable). The analyses were duplicated with the proportion of children who reached MMD or IMD as the dependent variable versus the proportion who discontinued OIT.

As the underlying assumptions for the multiple linear regression analysis were not fulfilled, multivariate robust regression analyses with Huber’s M-estimator were used to assess associations between immunological parameters (as independent variables) and reactivity threshold, LOAEL as well as severity of the allergic reaction (as the dependent variables) (paper #1). Multivariate robust regression analyses were also used to assess the associations between possible factors influencing QoL (as independent variables), and change in QoL from Y₀ to Y₂ in the PedsQL child or parental reports, and the FAQL-PB (as dependent variables) (paper #3). For sub-group analyses to determine if ineligibility to OIT influenced change in QoL from Y₀ to Y₁ (paper #3), change in QoL from Y₀ to Y₁ was used as the dependent variable. Hosmer’s step down multivariate analysis (125), a priori retaining age and gender in the analysis, included all variables statistically significant at the 0.35 level in the bivariate analyses. The final model was tested for confounding with all excluded variables.
Confounding was considered significant if including the variable led to a minimum of a 25% change in the result (125).

P-value of ≤ 0.05 was considered statistically significant.


4.6 Ethical issues

The TAKE-AWAY trial was approved by the Regional Committee for Medical and Health Research Ethics and registered in the ClinicalTrials.gov (number NCT02457416). As OIT is considered an experimental treatment, the child and both parents were given thorough oral and written information prior to obtaining a written informed consent from both parents. The information was age-adjusted and included the uncertainty and probability of achieving sustained unresponsiveness, as well as the potential challenge of time expected spent on treatment as well as AEs including severe reactions (92). Severe or unexpected AEs were reported to, and consulted with, a safety board.

The prospective, open labelled, randomized controlled study design rather than placebo controlled was a result of discussions with the ethical committee, concluding that four years of blinded placebo treatment were ethically inappropriate.
5 RESULTS

5.1 Baseline characteristics predicting the possibility of entering and completing an up-dosing phase of peanut oral immunotherapy (paper #1 and #2)

5.1.1 Baseline characteristics predicting the possibility of entering peanut oral immunotherapy (paper #1)

As outlined in Figure 10, 36.2 % of the 213 children referred for screening were included in the TAKE-AWAY trial. In 71.4 % of the enrolled children, parents had a combined annual income above 850,000 NOK, whereas 84.4 % of the mothers and 75.3 % of the fathers had education attainment level of three or more years of college/university.

In unadjusted bivariate analyses, the reactivity threshold as well as the LOAEL correlated significantly with basophil activation only, with a correlation coefficient ($r_s$) for reactivity threshold of - 0.30 ($p = 0.004$) and an $r_s$ for LOAEL of - 0.02 ($p = 0.032$). Neither reactivity threshold nor LOAEL correlated significantly with any of the following: Age, gender, allergic co-morbidities, FEV$_1$,%, peanut SPT or s-IgE to peanut, Ara h 2, the ratios of peanut s-IgE/total IgE and s-IgG$_4$/s-IgE to peanut or the severity of the allergic reaction during pre-OIT DBPCFC (data not shown).

In multiple robust regression analyses including peanut SPT, s-IgE to peanut and Ara h 2, the ratios of peanut s-IgE/total IgE and s-IgG$_4$/s-IgE, and BAT, both the reactivity threshold and LOAEL were significantly associated with basophil activation, peanut SPT and the ratio of peanut s-IgE/total IgE (all $p < 0.04$) (Table 9). Furthermore, the reactivity threshold was associated with s-IgE to Ara h 2 and the ratio of s-IgG$_4$/s-IgE to peanut, while LOAEL was associated with s-IgE to peanut (Table 9). Neither reactivity threshold nor LOAEL was significantly associated with age, gender, allergic co-morbidities or lung function. Similar results were observed in subgroup analyses excluding the 23 children with a basophil activation $\leq$ 15 % CD63+ (non-responders ($n = 6$) and the low responders ($n = 17$)) (data not shown).
Table 9. Multivariate robust regression analyses for significant associations between peanut reactivity threshold and the lowest observed adverse event level (LOAEL) in children anaphylactic to peanut.

<table>
<thead>
<tr>
<th></th>
<th>Reactivity threshold</th>
<th>LOAEL</th>
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<tr>
<td></td>
<td>( \beta )-value</td>
<td>( \beta )-value</td>
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<tr>
<td></td>
<td>(95 % CI)</td>
<td>(95 % CI)</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>p-value</td>
</tr>
<tr>
<td>Peanut SPT (mm)</td>
<td>1.45 (0.08, 2.83)</td>
<td>0.87 (0.43, 1.31)</td>
</tr>
<tr>
<td></td>
<td>0.04</td>
<td>0.0001</td>
</tr>
<tr>
<td>Peanut s-IgE (kUA/L)</td>
<td>-</td>
<td>0.01 (0.002, 0.018)</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Ara h 2 s-IgE (kUA/L)</td>
<td>0.09 (0.02, 0.17)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>-</td>
</tr>
<tr>
<td>Ratio peanut s-IgE/total IgE (kUA/L)</td>
<td>29.20 (22.57, 35.82)</td>
<td>3.58 (1.44, 5.71)</td>
</tr>
<tr>
<td></td>
<td>&lt; 0.0001</td>
<td>0.001</td>
</tr>
<tr>
<td>Ratio s-IgG₄/s-IgE to peanut (ng/ml)</td>
<td>1.69 (1.61, 1.78)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>&lt; 0.0001</td>
<td>-</td>
</tr>
<tr>
<td>BAT all children (%)*</td>
<td>-0.45 (-0.73, -0.17)</td>
<td>-0.09 (-0.17, -0.002)</td>
</tr>
<tr>
<td></td>
<td>0.002</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Associations are given as the relative change (\( \beta \)) related to each mg increase in peanut threshold.

\( N = 86 \), BAT was not performed in 10 children due to missed sampling (\( n = 5 \)) or technical causes (\( n = 5 \)).

*included all children with a positive basophil activation (% CD63+ basophils)

- no significant association

SPT - skin prick test; Ig - immunoglobulin; BAT - basophil activation given as per cent activated CD63 cells test


In a receiver operating characteristic (ROC) curve to predict ineligibility for peanut OIT by a very low reactivity threshold \( \leq 3 \) mg of peanut protein, variables associated to both reactivity threshold and LOAEL were included. Among basophil activation, peanut SPT and the ratio of peanut s-IgE/total IgE, basophil activation best predicted ineligibility for peanut OIT with an area under the curve (AUC) (95% C.I) of 0.71 (0.53, 0.82), compared with the AUC for peanut s-IgE/total IgE of 0.55 (0.39, 0.67)) and peanut SPT of 0.53 (0.36, 0.66) (Figure 11). The optimal basophil activation cut-off level of 75.8 % gave a 93.5 % negative and a 36.8 % positive predictive value for predicting very low reactivity threshold.

![ROC Curve](source)

5.1.2 Baseline characteristics predicting the possibility of completing an up-dosing phase of peanut oral immunotherapy (paper #2)

The up-dosing phase was completed by 75.5 % (n = 43) of the 57 included children, while 14 children discontinued the OIT. As shown in Table 10, children who completed the up-dosing phase had significantly lower ratio of peanut s-IgE/total IgE (p = 0.009), more often allergy to tree-nuts (p = 0.002) and to other foods (p = 0.003) and more often a positive SPT to tree-nuts (p = 0.02) compared to the children who discontinued OIT.

Table 10. Baseline characteristics of children completing or discontinuing the up-dosing phase of the peanut OIT TAKE-AWAY trial.

<table>
<thead>
<tr>
<th></th>
<th>Completing (n = 43)</th>
<th>Discontinuing (n = 14)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (median, min-max)</td>
<td>9.0 (5.2, 15.2)</td>
<td>11.1 (5.4, 15.1)</td>
<td>0.13</td>
</tr>
<tr>
<td>Male</td>
<td>21 (48.8)</td>
<td>10 (71.4)</td>
<td>0.14</td>
</tr>
<tr>
<td>History of anaphylaxis</td>
<td>32 (74.4)</td>
<td>13 (92.8)</td>
<td>0.41</td>
</tr>
<tr>
<td>to peanut</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current asthma</td>
<td>16 (69.6)</td>
<td>8 (57.1)</td>
<td>0.26</td>
</tr>
<tr>
<td>Allergic rhinitis</td>
<td>13 (30.2)</td>
<td>2 (14.3)</td>
<td>0.53</td>
</tr>
<tr>
<td>Atopic dermatitis</td>
<td>36 (83.7)</td>
<td>11 (78.6)</td>
<td>0.64</td>
</tr>
<tr>
<td>Allergy to tree-nuts</td>
<td>20 (48.8)</td>
<td>0 (0.0)</td>
<td>0.002</td>
</tr>
<tr>
<td>Allergy to other food</td>
<td>25 (59.5)</td>
<td>2 (14.3)</td>
<td>0.003</td>
</tr>
<tr>
<td>than nuts</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parental atopic disease*</td>
<td>36 (83.7)</td>
<td>14 (100.0)</td>
<td>0.16</td>
</tr>
<tr>
<td>Parental food allergy**</td>
<td>14 (32.6)</td>
<td>7 (50.0)</td>
<td>0.51</td>
</tr>
<tr>
<td>FEV1% predicted</td>
<td>102.7 (98.6, 106.7)</td>
<td>99.7 (94.0, 105.8)</td>
<td>0.58</td>
</tr>
<tr>
<td>SPT tree-nuts ≥ 3 mm</td>
<td>25 (58.1)</td>
<td>3 (21.4)</td>
<td>0.02</td>
</tr>
<tr>
<td>S-IgE (≥ 0.35 kU/L):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tree-nuts***</td>
<td>40 (95.2)</td>
<td>12 (85.7)</td>
<td>0.08</td>
</tr>
<tr>
<td>other food****</td>
<td>41 (95.3)</td>
<td>13 (92.9)</td>
<td>0.63</td>
</tr>
<tr>
<td>Peanut SPT (mm)</td>
<td>10.3 (8.7, 11.9)</td>
<td>12.1 (8.3, 15.8)</td>
<td>0.48</td>
</tr>
<tr>
<td>Total IgE (kU/L)</td>
<td>570.6 (405.1, 736.2)</td>
<td>713.9 (314.1, 1113.6)</td>
<td>0.51</td>
</tr>
<tr>
<td>S-IgE peanut (kU/L)</td>
<td>197.5 (12.8, 270.1)</td>
<td>472.8 (120.2, 825.3)</td>
<td>0.09</td>
</tr>
<tr>
<td>S-IgE Ara h2 (kU/L)</td>
<td>99.0 (62.7, 135.2)</td>
<td>177.1 (85.9, 268.4)</td>
<td>0.05</td>
</tr>
<tr>
<td>Peanut s-IgE/</td>
<td>0.3 (0.3, 0.4)</td>
<td>0.5 (0.4, 0.7)</td>
<td>0.009</td>
</tr>
<tr>
<td>total IgE (kU/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peanut s-IgGd/</td>
<td>27.4 (10.5, 44.4)</td>
<td>9.7 (1.9, 21.3)</td>
<td>0.22</td>
</tr>
<tr>
<td>s-IgE (ng/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAPT pos level****</td>
<td>3.0 (2.6, 3.3)</td>
<td>2.6 (2.0, 3.2)</td>
<td>0.31</td>
</tr>
<tr>
<td>BAT (%CD63+)</td>
<td>59.5 (50.5, 68.5)</td>
<td>69.7 (54.6, 84.7)</td>
<td>0.17</td>
</tr>
</tbody>
</table>
At baseline DBPCFC:

<table>
<thead>
<tr>
<th></th>
<th>Number of anaphylaxis:</th>
<th>Anaphylaxis severity grade:</th>
<th>Use of adrenaline</th>
<th>LOAEL (mg peanut protein)</th>
<th>Reactivity threshold (mg peanut protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>43 (100.0)</td>
<td>1.7 (1.6, 1.8)</td>
<td>51.2 (22)</td>
<td>113.3 (12.4, 214.3)</td>
<td>155.4 (48.9, 261.9)</td>
</tr>
<tr>
<td></td>
<td>14 (100.0)</td>
<td>1.6 (1.4, 1.9)</td>
<td>57.1 (8)</td>
<td>82.9 (16.3, 149.5)</td>
<td>250.1 (60.9, 439.3)</td>
</tr>
</tbody>
</table>

C: Not able to compute – anaphylaxis at DBPCFC is a constant.

**Bold values are given as mean (95% CI) except age which is given as median (min, max).**

Variables are given as mean (95% CI) or n (%), except age which is given as median (min, max).

Anaphylaxis severity was graded by two grading systems according to the modified EAACI position papers (8, 9) ranging from 1 to 3 and the method of Sampson (Grading of Food-Induced Anaphylaxis According to Severity of Clinical Symptoms) (10) ranging from 1 to 5. LOAEL is defined as the cumulated peanut protein (mg) ingested eliciting mild, objective symptoms.

Reactivity threshold is defined as the cumulated peanut protein (mg) ingested at positive DBPCFC, with at least two moderate objective symptoms in one or more organ systems symptoms according to Bock’s criteria (34, 120).

SPT, skin prick test; Ig - immunoglobulin; BAT - basophil activation test; CAPT – conjunctival allergen provocation test; LOAEL - lowest observed adverse effect level; OIT – oral immunotherapy; DBPCFC – double-blind placebo-controlled food challenge.
None of the baseline characteristics were significant predictors for completing the OIT up-dosing phase until a maintenance dose was reached in bivariate (Table 11) and multivariate logistic regression analyses (not shown). Non-significant trends were, however, observed for s-IgE to peanut, s-IgE to Ara h 2, the peanut s-IgE/total IgE ratio and AEs (p = 0.06 to 0.07) (Table 11).

Table 11. Possible factors that could explain reaching any maintenance dose (≤ 5000 mg peanut protein, eq. MMD + IMD) compared to children who discontinued OIT and the pre-defined maximum maintenance dose (MMD) of 5000 mg peanut protein compared to children not reaching MMD (eq. IMD + discontinued) using bivariate logistic regression analyses.

<table>
<thead>
<tr>
<th></th>
<th>Reached MMD or IMD (n = 43)</th>
<th>p-value</th>
<th>Reached MMD (n = 12)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parent education (graded 1(low) - 5(high))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.25 (0.95, 1.66)</td>
<td>0.12</td>
<td>0.95 (0.70, 1.30)</td>
<td>0.77</td>
</tr>
<tr>
<td>Siblings</td>
<td>1.04 (0.43, 2.53)</td>
<td>0.94</td>
<td>0.83 (0.32, 2.12)</td>
<td>0.69</td>
</tr>
<tr>
<td>Male sex</td>
<td>2.55 (0.72, 9.10)</td>
<td>0.15</td>
<td>0.80 (0.23, 2.82)</td>
<td>0.73</td>
</tr>
<tr>
<td>Current asthma</td>
<td>1.89 (0.56, 6.41)</td>
<td>0.31</td>
<td>2.44 (0.66, 8.97)</td>
<td>0.18</td>
</tr>
<tr>
<td>Allergic rhinitis</td>
<td>2.14 (0.46, 10.03)</td>
<td>0.33</td>
<td>2.55 (0.67, 9.63)</td>
<td>0.17</td>
</tr>
<tr>
<td>Peanut SPT (mm)</td>
<td>0.95 (0.86, 1.05)</td>
<td>0.31</td>
<td>0.94 (0.82, 1.08)</td>
<td>0.40</td>
</tr>
<tr>
<td>S-IgE (kUA/L):</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>peanut</td>
<td>1.00 (1.00, 1.00)</td>
<td>0.07</td>
<td>1.00 (1.00, 1.00)</td>
<td>0.42</td>
</tr>
<tr>
<td>Ara h 2</td>
<td>1.00 (0.99, 1.00)</td>
<td>0.07</td>
<td>1.00 (0.99, 1.00)</td>
<td>0.57</td>
</tr>
<tr>
<td>Peanut s-IgE/total IgE (kUA/L)</td>
<td>0.10 (0.01, 1.08)</td>
<td>0.06</td>
<td>0.44 (0.03, 5.87)</td>
<td>0.53</td>
</tr>
<tr>
<td>Peanut s-IgG₄/s-IgE (ng/ml)</td>
<td>1.01 (0.99, 1.03)</td>
<td>0.45</td>
<td>1.02 (1.00, 1.04)</td>
<td><strong>0.02</strong></td>
</tr>
<tr>
<td>BAT (%CD63+)*</td>
<td>0.99 (0.96, 1.03)</td>
<td>0.98</td>
<td>0.99 (0.96, 1.03)</td>
<td>0.66</td>
</tr>
<tr>
<td>CAPT positive (dilution)</td>
<td>1.36 (0.76, 2.40)</td>
<td>0.30</td>
<td>1.55 (0.83, 2.89)</td>
<td>0.17</td>
</tr>
<tr>
<td>Anaphylaxis severity:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>modified EAACI LOAEL (mg)</td>
<td>1.35 (0.38, 4.74)</td>
<td>0.64</td>
<td>0.36 (0.10, 1.32)</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>1.00 (1.00, 1.00)</td>
<td>0.99</td>
<td>1.00 (1.00, 1.01)</td>
<td>0.08</td>
</tr>
<tr>
<td>During up-dosing:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AEs** (days/period/child)</td>
<td>0.80 (0.64, 1.01)</td>
<td>0.06</td>
<td>0.69 (0.39, 1.20)</td>
<td>0.18</td>
</tr>
<tr>
<td>Anaphylaxis (days/period/child)</td>
<td>1.83 (0.42, 79.71)</td>
<td>0.75</td>
<td>1.86 (0.25, 136.35)</td>
<td>0.78</td>
</tr>
<tr>
<td>Asthma medication (yes/no)</td>
<td>1.89 (0.56, 6.41)</td>
<td>0.31</td>
<td>2.44 (0.66, 8.97)</td>
<td>0.18</td>
</tr>
<tr>
<td>Postponements (total)</td>
<td>1.46 (0.88, 2.42)</td>
<td>0.14</td>
<td>0.87 (0.55, 1.38)</td>
<td>0.55</td>
</tr>
<tr>
<td>Dose reductions (total)</td>
<td>3.00 (0.34, 26.60)</td>
<td>0.32</td>
<td>1.40 (0.17, 11.24)</td>
<td>0.75</td>
</tr>
</tbody>
</table>

Associations are given as odds ratio (OR) (95 % CI). Bold values are statistically significant (p < 0.05).
N = 57 children randomized to active peanut OIT in the TAKE-AWAY trial.
* N = 50. The BAT was not performed in 5 children due to technical causes (n = 5) and non-responders were excluded from the analyses (n = 2).

SPT, skin prick test; IgE/G/G₄ - immunoglobulin E/G/G₄; BA - basophil activation, CAPT – conjunctival allergen provocation test; OIT – oral immunotherapy; DBPCFC – double blind placebo-controlled food challenge; LOAEL - lowest observed adverse effect level; AEs – adverse events


5.2 The feasibility of achieving a high maintenance dose in peanut oral immunotherapy (papers #1 and #2)

At end of up-dosing (Y₁), 21.1% (n = 12) reached the pre-defined MMD of 5000 mg peanut protein, while 54.4% (n = 31) reached a lower IMD and 24.5% had discontinued OIT. The median (min, max) maintenance dose reached for the IMD children was 2700 (250, 4000) mg peanut protein.

As shown in Table 12, children who reached MMD were older, had lesser allergy to other food and higher reactivity thresholds as compared with the IMD children, and had lower s-IgEs to peanut and Ara h 2, lower ratio of peanut s-IgE/total IgE and higher ratio of peanut s-IgG₄/s-IgE compared with children who discontinued OIT.
Table 12. Baseline characteristics of children randomized to peanut OIT in the TAKE-AWAY trial.

<table>
<thead>
<tr>
<th></th>
<th>Total study population (n = 57)</th>
<th>MMD (n = 12)</th>
<th>IMD (n = 31)</th>
<th>Discontinued (n = 14)</th>
<th>Overall p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (median, min-max)</td>
<td>10.1 (5.2, 15.2)</td>
<td>10.7 (7.2, 15.2)</td>
<td>8.5 (5.2, 14.4)</td>
<td>10.2 (5.4, 15.1)</td>
<td>0.02##</td>
</tr>
<tr>
<td>Male</td>
<td>31 (54.4)</td>
<td>7 (58.3)</td>
<td>14 (45.2)</td>
<td>10 (71.4)</td>
<td>0.26</td>
</tr>
<tr>
<td>History of anaphylaxis to peanut</td>
<td>45 (78.9)</td>
<td>9 (75.0)</td>
<td>23 (74.2)</td>
<td>13 (92.8)</td>
<td>0.82</td>
</tr>
<tr>
<td>Current asthma</td>
<td>24 (42.1)</td>
<td>5 (83.3)</td>
<td>11 (64.7)</td>
<td>8 (88.9)</td>
<td>0.37</td>
</tr>
<tr>
<td>Allergic rhinitis</td>
<td>15 (26.3)</td>
<td>5 (41.7)</td>
<td>8 (25.8)</td>
<td>2 (14.3)</td>
<td>0.53</td>
</tr>
<tr>
<td>Atopic dermatitis ever</td>
<td>47 (82.5)</td>
<td>11 (91.7)</td>
<td>25 (80.6)</td>
<td>11 (78.6)</td>
<td>0.64</td>
</tr>
<tr>
<td>Allergy to tree-nuts</td>
<td>20 (35.1)</td>
<td>5 (41.7)</td>
<td>15 (48.4)</td>
<td>0 (0.0)</td>
<td>0.15</td>
</tr>
<tr>
<td>Allergy to other food than nuts</td>
<td>27 (47.4)</td>
<td>6 (50.0)</td>
<td>19 (61.3)</td>
<td>2 (14.3)</td>
<td>0.02#</td>
</tr>
<tr>
<td>Parental atopic disease*</td>
<td>50 (87.7)</td>
<td>9 (75.0)</td>
<td>27 (87.1)</td>
<td>14 (100.0)</td>
<td>0.16</td>
</tr>
<tr>
<td>Parental food allergy**</td>
<td>21 (36.8)</td>
<td>4 (33.3)</td>
<td>10 (32.3)</td>
<td>7 (50.0)</td>
<td>0.51</td>
</tr>
<tr>
<td>FEV1% predicted</td>
<td>101.2 (97.6, 105.0)</td>
<td>101.0 (91.2, 112.2)</td>
<td>101.9 (97.7, 104.7)</td>
<td>99.7 (94.0, 105.8)</td>
<td>0.86</td>
</tr>
<tr>
<td>Pos s-IgE (≥ 0.35 kUA/L):</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tree-nuts***</td>
<td>52 (91.2)</td>
<td>10 (83.3)</td>
<td>30 (96.7)</td>
<td>12 (85.7)</td>
<td>0.08</td>
</tr>
<tr>
<td>other food****</td>
<td>54 (94.7)</td>
<td>12 (100.0)</td>
<td>29 (96.7)</td>
<td>13 (92.9)</td>
<td>0.63</td>
</tr>
<tr>
<td>Peanut SPT (mm)</td>
<td>9.8 (8.6, 11.0)</td>
<td>8.7 (7.0, 10.9)</td>
<td>9.7 (8.4, 11.3)</td>
<td>10.3 (7.3, 14.6)</td>
<td>0.64</td>
</tr>
<tr>
<td>S-IgE (kUA/L):</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peanut</td>
<td>110.6 (70.4, 173.8)</td>
<td>21.9 (4.9, 97.8)</td>
<td>129.3 (88.9, 188.0)</td>
<td>175.7 (55.0, 561.6)</td>
<td>0.003#</td>
</tr>
<tr>
<td>Ara h2</td>
<td>56.2 (37.2, 87.1)</td>
<td>13.5 (3.1, 59.1)</td>
<td>67.0 (47.8, 94.0)</td>
<td>89.6 (33.9, 235.9)</td>
<td>0.004#</td>
</tr>
<tr>
<td>Peanut s-IgE/total IgE (kUA/L)</td>
<td>0.4 (0.0, 1.5)</td>
<td>0.3 (0.1, 0.6)</td>
<td>0.3 (0.1, 0.6)</td>
<td>0.5 (0.4, 0.7)</td>
<td>0.002#</td>
</tr>
<tr>
<td>Peanut s-IgG4/s-IgE (ng/ml)</td>
<td>5.7 (3.7, 8.9)</td>
<td>15.5 (3.4, 60.3)</td>
<td>4.9 (2.9, 8.3)</td>
<td>3.3 (1.4, 7.8)</td>
<td>0.04#</td>
</tr>
<tr>
<td>CAPT pos level****</td>
<td>2.6 (2.3, 3.0)</td>
<td>3.0 (2.2, 4.1)</td>
<td>2.6 (2.3, 3.1)</td>
<td>2.3 (1.7, 3.1)</td>
<td>0.41</td>
</tr>
<tr>
<td>BAT (%CD63+)</td>
<td>68.0 (61.6, 75.1)</td>
<td>34.4 (15.5, 75.9)</td>
<td>49.6 (34.7, 70.8)</td>
<td>63.7 (46.0, 88.2)</td>
<td>0.28</td>
</tr>
</tbody>
</table>

To be continued on the next page.
<table>
<thead>
<tr>
<th>Total study population (n = 57)</th>
<th>MMD (n = 12)</th>
<th>IMD (n = 31)</th>
<th>Discontinued (n = 14)</th>
<th>Overall p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>At baseline DBPCFC:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of anaphylaxis</td>
<td>57 (100.0)</td>
<td>12 (100.0)</td>
<td>31 (100.0)</td>
<td>14 (100.0)</td>
</tr>
<tr>
<td>Anaphylaxis severity grade:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>modified EAACI Sampson</td>
<td>1.6 (1.4, 1.7)</td>
<td>1.4 (1.1, 1.8)</td>
<td>1.7 (1.5, 1.9)</td>
<td>1.6 (1.3, 1.9)</td>
</tr>
<tr>
<td>Sampson</td>
<td>2.6 (2.4, 2.8)</td>
<td>2.8 (2.4, 3.4)</td>
<td>2.5 (2.3, 2.8)</td>
<td>2.6 (2.3, 3.1)</td>
</tr>
<tr>
<td>Use of adrenaline</td>
<td>30 (52.6)</td>
<td>5 (41.7)</td>
<td>17 (54.8)</td>
<td>8 (57.1)</td>
</tr>
<tr>
<td>LOAEL (mg peanut protein)</td>
<td>18.4 (11.8, 28.6)</td>
<td>45.9 (10.2, 207.1)</td>
<td>15.1 (10.3, 22.1)</td>
<td>36.2 (15.4, 84.4)</td>
</tr>
<tr>
<td>Reactivity threshold (mg peanut protein)</td>
<td>46.2 (29.7, 72.0)</td>
<td>108.7 (29.3, 402.9)</td>
<td>32.1 (22.0, 46.9)</td>
<td>93.3 (40.0, 222.4)</td>
</tr>
</tbody>
</table>

Variables are given as geometric mean (95 % CI) or n (%), except age which is given as median (min, max).

Bold values are statistically significant (p < 0.05).

One-way ANOVA was applied to determine statistically significant differences between group means and the Dunnett’s post hoc test to confirm which groups differed.

# Statistically significant difference between MMD and discontinued.

## Statistically significant difference between MMD and IMD.

*Atopic disease includes asthma, allergic rhinitis, atopic dermatitis, allergic conjunctivitis

**All food allergy including peanut and tree-nut allergy

***Hazelnut, almond, cashewnut, pistachionut, walnut, pecannut, brazilnut and macadamianut

****Fenugreek, soybean, pea, red kidney bean, lupin seed and wheat

*****The CAPT was recorded positive ranging from dilution level 1 (1:160) to 5 (1:1).

Anaphylaxis severity was graded by two grading systems according to the modified EAACI position papers (8, 9) ranging from 1 to 3 and the method of Sampson (Grading of Food-Induced Anaphylaxis According to Severity of Clinical Symptoms) (10) ranging from 1 to 5. LOAEL is defined as the cumulated peanut protein (mg) ingested eliciting mild, objective symptoms.

Reactivity threshold is defined as the cumulated peanut protein (mg) ingested at positive DBPCFC, with at least two moderate objective symptoms in one or more organ systems symptoms according to Bock’s criteria (34, 120).

MMD – subjects who reached the maximum maintenance dose; IMD – subjects who reached the individual maintenance dose; SPT, skin prick test; Ig - immunoglobulin; BAT - basophil activation test; CAPT – conjunctival allergen provocation test; LOAEL - lowest observed adverse effect level; OIT – oral immunotherapy; DBPCFC – double blind placebo-controlled food challenge.

Reprinted from Allergy 2018; Reier-Nilsen T, Michelsen MM, Lodrup Carlsen KC, Carlsen KH, Mowinckel P, Nygaard UC, et al.: «Feasibility of desensitizing children highly allergic to peanut by high-dose oral immunotherapy.”, with permission from Elsevier.

Failure to reach MMD was explained by distaste for peanuts in 66.7% of the children (IMD (n) = 28 and discontinued (n) = 2), followed by AEs in 26.7% (IMD (n) = 3 and discontinued (n) = 9) and social reasons in 6.7% (discontinued (n) = 3, two found the treatment too time-consuming, while one discontinued due to family reasons). In 77.2% of the children, distaste for peanuts was reported as a daily challenge. Detailed characteristics of the children who discontinued OIT are reported in Table 13.

In the bivariate (Table 1) and the multivariate logistic regression model (not shown), the ratio of peanut s-IgG₄/s-IgE was the only identified factor significantly associated with reaching MMD.

Table 13. Characteristics of children who discontinued oral immunotherapy without reaching a maintenance dose.

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age years</th>
<th>Peanut s-IgE kUA/L</th>
<th>Ara h 2 LOAEL mg peanut protein</th>
<th>Reactivity threshold mg peanut protein</th>
<th>Dose at discontinuation mg peanut protein</th>
<th>Reason for discontinuation mg peanut protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.8</td>
<td>93.2</td>
<td>82.7</td>
<td>110.8</td>
<td>5</td>
<td>Social</td>
</tr>
<tr>
<td>2</td>
<td>11.3</td>
<td>493.0</td>
<td>221.0</td>
<td>35.8</td>
<td>5</td>
<td>AEs</td>
</tr>
<tr>
<td>3</td>
<td>14.3</td>
<td>26.2</td>
<td>14.6</td>
<td>110.8</td>
<td>243.0</td>
<td>450 AEs</td>
</tr>
<tr>
<td>4</td>
<td>15.1</td>
<td>179.0</td>
<td>77.0</td>
<td>13.0</td>
<td>13.0</td>
<td>10 AEs</td>
</tr>
<tr>
<td>5</td>
<td>14.8</td>
<td>951.0</td>
<td>457.0</td>
<td>443.0</td>
<td>943.0</td>
<td>45 AEs</td>
</tr>
<tr>
<td>6</td>
<td>6.5</td>
<td>63.9</td>
<td>32.4</td>
<td>43.0</td>
<td>43.0</td>
<td>20 Distaste</td>
</tr>
<tr>
<td>7</td>
<td>10.9</td>
<td>271.0</td>
<td>158.0</td>
<td>43.0</td>
<td>43.0</td>
<td>350 AEs</td>
</tr>
<tr>
<td>8</td>
<td>13.8</td>
<td>2311.0</td>
<td>475.0</td>
<td>43.0</td>
<td>43.0</td>
<td>45 AEs</td>
</tr>
<tr>
<td>9</td>
<td>11.7</td>
<td>114.0</td>
<td>87.4</td>
<td>3.0</td>
<td>43.0</td>
<td>1000 Distaste</td>
</tr>
<tr>
<td>10</td>
<td>10.1</td>
<td>629.0</td>
<td>179.0</td>
<td>3.0</td>
<td>13.0</td>
<td>20 AEs</td>
</tr>
<tr>
<td>11</td>
<td>9.8</td>
<td>352.0</td>
<td>210.0</td>
<td>13.0</td>
<td>143.0</td>
<td>1 Social</td>
</tr>
<tr>
<td>12</td>
<td>5.4</td>
<td>0.6</td>
<td>0.8</td>
<td>143.0</td>
<td>443.0</td>
<td>1 Social</td>
</tr>
<tr>
<td>13</td>
<td>7.3</td>
<td>92.8</td>
<td>61.6</td>
<td>143.0</td>
<td>943.0</td>
<td>65 AEs</td>
</tr>
<tr>
<td>14</td>
<td>11.6</td>
<td>285.0</td>
<td>131.0</td>
<td>13.0</td>
<td>443.0</td>
<td>5 AEs</td>
</tr>
</tbody>
</table>

5.3 The patient perspective burden of peanut oral immunotherapy (papers #2 and #3)

5.3.1 Adverse events (paper #2)

All but one child reported AEs. Mild AEs were reported in 13.9% of the OIT doses, while moderate AEs were reported in 0.6% and moderate anaphylaxis in 0.06% of the dose-days with AEs. The 11 anaphylactic events occurred in 11 children (Table 14).

Table 14. Adverse events (AEs) related to oral immunotherapy in children highly allergic to peanut.

<table>
<thead>
<tr>
<th></th>
<th>Overall OIT (n = 57)</th>
<th>MMD (n = 12)</th>
<th>IMD (n = 31)</th>
<th>Discontinued (n=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(doses = 18470)</td>
<td>(doses = 5292)</td>
<td>(doses = 11536)</td>
<td>(doses =1642)</td>
<td></td>
</tr>
<tr>
<td>Total AEs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients, n (%)</td>
<td>56 (98.2)</td>
<td>12 (100.0)</td>
<td>30 (96.8)</td>
<td>14 (100.0)</td>
</tr>
<tr>
<td>Events, n (%)</td>
<td>2560 (13.9)</td>
<td>290 (5.5)</td>
<td>1957 (17.0)</td>
<td>313 (19.1)</td>
</tr>
<tr>
<td>Mild AEs, total</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients, n (%)</td>
<td>56 (98.2)</td>
<td>12 (100.0)</td>
<td>30 (96.8)</td>
<td>14 (100.0)</td>
</tr>
<tr>
<td>Events, n (%)</td>
<td>2473 (13.4)</td>
<td>290 (5.5)</td>
<td>1725 (15.0)</td>
<td>515 (31.4)</td>
</tr>
<tr>
<td>Moderate AEs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients, n (%)</td>
<td>22 (38.6)</td>
<td>4 (33.3)</td>
<td>14 (45.2)</td>
<td>4 (28.6)</td>
</tr>
<tr>
<td>Events, n (%)</td>
<td>116 (0.6)</td>
<td>21 (0.4)</td>
<td>81 (0.7)</td>
<td>4 (0.9)</td>
</tr>
<tr>
<td>Oral itching</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients, n (%)</td>
<td>49 (86.0)</td>
<td>10 (83.3)</td>
<td>28 (90.3)</td>
<td>11 (78.6)</td>
</tr>
<tr>
<td>Events, n (%)</td>
<td>1096 (5.9)</td>
<td>173 (3.3)</td>
<td>822 (7.1)</td>
<td>79 (4.8)</td>
</tr>
<tr>
<td>GI related AEs*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients, n (%)</td>
<td>48 (84.2)</td>
<td>7 (58.3)</td>
<td>27 (87.1)</td>
<td>13 (92.9)</td>
</tr>
<tr>
<td>Events, n (%)</td>
<td>1100 (6.0)</td>
<td>31 (0.6)</td>
<td>959 (8.3)</td>
<td>110 (6.7)</td>
</tr>
<tr>
<td>Skin related AEs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients, n (%)</td>
<td>43 (75.4)</td>
<td>9 (75.0)</td>
<td>27 (87.1)</td>
<td>7 (50.0)</td>
</tr>
<tr>
<td>Events, n (%)</td>
<td>140 (0.8)</td>
<td>26 (0.5)</td>
<td>95 (0.8)</td>
<td>71 (4.3)</td>
</tr>
<tr>
<td>Respiratory related AEs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients, n (%)</td>
<td>37 (64.9)</td>
<td>10 (83.3)</td>
<td>19 (61.3)</td>
<td>8 (57.1)</td>
</tr>
<tr>
<td>Events, n (%)</td>
<td>59 (0.3)</td>
<td>10 (0.2)</td>
<td>31 (0.3)</td>
<td>18 (1.0)</td>
</tr>
<tr>
<td>Anaphylaxis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients, n (%)</td>
<td>11 (19.3)</td>
<td>2 (16.7)</td>
<td>5 (16.1)</td>
<td>4 (28.6)</td>
</tr>
<tr>
<td>Events, n (%)</td>
<td>11 (0.06)</td>
<td>2 (0.04)</td>
<td>5 (0.04)</td>
<td>4 (0.24)</td>
</tr>
<tr>
<td>Sampson severity grade median (min, max)</td>
<td>3.0 (2.0, 3.0)</td>
<td>3.0 (2.0, 3.0)</td>
<td>3.0 (2.0, 3.0)</td>
<td>3.0 (2.0, 3.0)</td>
</tr>
<tr>
<td>Used epinephrine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients, n (%)</td>
<td>6 (10.5)</td>
<td>2 (16.7)</td>
<td>2 (6.5)</td>
<td>2 (14.3)</td>
</tr>
<tr>
<td>Events, n (%)</td>
<td>6 (0.03)</td>
<td>2 (0.04)</td>
<td>2 (0.02)</td>
<td>2 (0.12)</td>
</tr>
<tr>
<td>Used acute salbutamol**</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients, n (%)</td>
<td>5 (8.8)</td>
<td>1 (8.3)</td>
<td>2 (6.5)</td>
<td>2 (7.1)</td>
</tr>
<tr>
<td>Events, n (%)</td>
<td>5 (0.03)</td>
<td>1 (0.02)</td>
<td>3 (0.03)</td>
<td>2 (0.1)</td>
</tr>
</tbody>
</table>
* Except oral itching
** In relation to OIT AEs
Percentages were based on the number of patients in each group, stratified by reaching maximum maintenance dose (MMD), a lower individual maintenance dose (IMD) or discontinuing treatment. Patients were counted once per category.

Grading of OIT-related AEs was in line with the modified Bock’s criteria by Sampson et al. (34, 119, 120).

AEs – adverse events; MMD – subjects who reached the maximum maintenance dose; IMD – subjects who reached the individual maintenance dose.


The AEs were mostly characterized as oral itching (43.5 %) or other gastro-intestinal (GI) related symptoms (42.5 %) (Figure 12). Dyspeptic symptoms were reported by eight children who either discontinued treatment (n = 2), or had symptom relief related to spontaneous resolution (n = 2) or treatment with proton pump inhibitor (PPI) (n = 4) and completed the up-dosing phase.

The 11 moderate anaphylactic events occurred in 11 different children and adrenaline was administered in six of the episodes. All but two anaphylactic reactions were preceded by known risk factors; exercise within two hours after a dose (5), ongoing infection (1), excessive tiredness (1), impaired compliance to OIT (1) or asthma treatment (1). The control group did not experience any anaphylactic events to peanut during the same time-span.

Children who discontinued OIT reported median (min, max) 2.5 (0.3, 10.5) dose-days of OIT-related AEs per dose-step, which is significantly more frequent as compared with the reported 1.0 (0.0, 12.9) (p = 0.01) as reported by children who completed the OIT up-dosing phase. On the other hand, reported dose-days of moderately graded AEs including anaphylactic events were similar in these two groups of children (p = 0.61).
The AEs occurred more often during the first two, compared with the remaining days in each up-dosing period \( (p = 0.001) \) as shown in Figure 12. Also, the AEs were more frequent in the first dose-interval step (1-65 mg peanut protein), as compared with the second (66-800 mg) and the third (801-5000 mg peanut protein) dose-interval steps (overall \( p = 0.03 \)) (Figure 12).

Figure 12. Reported doses with adverse events (AEs) per dose-day (%) in the three dose-intervals of the up-dosing phase. If there were another cycle of 14 days of the same dose-step due to AEs or vacations in the same dose-interval, this cycle would also be a part of the same dose-interval, and the Y-axis would still represent reported doses with AEs per dose-day (%). One-way ANOVA was applied to determine statistically significant differences between the intervals and the Dunnett's post hoc test to confirm which groups differed. Reprinted from Allergy 2018; Reier-Nilsen T, Michelsen MM, Lodrup Carlsen KC, Carlsen KH, Mowinckel P, Nygaard UC, et al.: «Feasibility of desensitizing children highly allergic to peanut by high-dose oral immunotherapy.”, with permission from Elsevier.

5.3.2 The patient perceived treatment burden (paper #3)

Children who completed the OIT up-dosing phase attended a median of (min, max) 25 (17, 32) hospital attendances until a maintenance dose was reached. In addition, they attended another 11 pre-set protocol-based visits until one year of maintenance treatment (Y₂); at recruitment, three months, one year and two years’ of treatment including the three-monthly visits during maintenance phase.

As shown in table 15, the treatment burden was perceived highest related to the taste-domain among children who completed the up-dosing phase as well as one year of maintenance treatment, followed by time spent related to the treatment and GI-related AEs. Although the overall score was similar at Y₁ and Y₂, the time spent on treatment remained relatively unchanged, whereas the mean (95 % CI) taste-domain burden decreased significantly from 6.5 (5.5, 7.3) to 5.3 (4.3, 6.3) (p = 0.02) and the GI-related symptom burden decreased from 2.6 (1.9, 3.3) in the up-dosing phase to 1.4 (1.0, 1.8) at Y₂ (p = 0.001) (Table 15). The four children who discontinued OIT from Y₁ to Y₂ had VAS reports at Y₁ which was not significantly different from the children who reached Y₂; for the GI-domain 2.3 (1.2, 3.5) (p = 0.64), the taste-/amount-domain 8.0 (5.8, 10.0) (p = 0.11) and the time spent-domain 3.5 (1.9, 5.0) (p = 0.29).
Table 15. The perceived treatment burden is given at $Y_1$ (after up-dosing) and $Y_2$ (after one year of maintenance treatment) as mean values with 95% confidence intervals among all children randomized to OIT.

<table>
<thead>
<tr>
<th></th>
<th>One year of treatment ($Y_1$) (N = 43)</th>
<th>Two years of treatment ($Y_2$) (N = 37)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>3.9 (3.1, 4.8)</td>
<td>3.7 (2.9, 4.6)</td>
<td>0.84</td>
</tr>
<tr>
<td>GI-domain</td>
<td>2.6 (1.9, 3.3)</td>
<td>1.4 (1.0, 1.8)</td>
<td>0.001</td>
</tr>
<tr>
<td>Oral itching</td>
<td>3.4 (2.5, 4.4)</td>
<td>2.1 (1.3, 2.9)</td>
<td>0.02</td>
</tr>
<tr>
<td>Stomach ache</td>
<td>2.6 (1.7, 3.5)</td>
<td>1.4 (0.8, 2.0)</td>
<td>0.008</td>
</tr>
<tr>
<td>Nausea or vomiting</td>
<td>1.6 (0.8, 2.5)</td>
<td>0.6 (0.3, 0.9)</td>
<td>0.02</td>
</tr>
<tr>
<td>Taste-amount-domain</td>
<td>6.5 (5.5, 7.3)</td>
<td>5.3 (4.3, 6.3)</td>
<td>0.02</td>
</tr>
<tr>
<td>Taste</td>
<td>7.0 (5.9, 8.0)</td>
<td>6.1 (4.9, 7.3)</td>
<td>0.10</td>
</tr>
<tr>
<td>Amount</td>
<td>5.8 (4.8, 6.7)</td>
<td>4.3 (3.3, 5.2)</td>
<td>0.01</td>
</tr>
<tr>
<td>Time spent-domain</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>at home</td>
<td>2.9 (2.1, 3.7)</td>
<td>2.2 (1.5, 2.9)</td>
<td>0.06</td>
</tr>
<tr>
<td>at hospital (up-dosing/visits)</td>
<td>2.5 (1.6, 3.4)</td>
<td>2.5 (1.5, 3.6)</td>
<td>0.94</td>
</tr>
<tr>
<td></td>
<td>3.0 (2.2, 3.2)</td>
<td>1.7 (1.2, 2.3)</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Unpaired t-test was applied to determine statistically significant difference between means at $Y_1$ and $Y_2$.

The GI-domain represents average of how AEs like stomach ache, nausea/vomiting and oral itching are perceived.
The taste-amount-domain represents average of perceived taste and amount of peanut eaten.
The time spent-domain represents perceived average of time spent on eating OIT doses at hospital for up-dosing visits and at ingesting OIT doses at home.
A negative correlation was observed between treatment burden at Y₁ and change in perceived treatment (range of correlation coefficient (rₛ) (–0.82, –0.44)) (p < 0.001) (Figure 13).

Figure 13. Correlation between change in perceived treatment burden from end of up-dosing (Y₁) to second year of treatment (one year of maintenance) (Y₂) and perceived burden at Y₁. Perceived treatment burden is reported as overall burden (A), and within three domains: Adverse events (stomach ache, nausea/vomiting and oral itching) (B), taste and amount of daily peanut oral immunotherapy (OIT) (C) and time spent on OIT (D).
5.4 The effect of 2-years peanut oral immunotherapy (paper #3)

After two years of OIT, 18 children had discontinued treatment, while the mean (SD) daily maintenance dose among the 39 children who still received OIT was 3322 (1376) mg peanut protein, ranging from 350 - 5000 mg. Two of the 39 refused OFC at Y2 out of concern for allergic reactions.

Desensitization to 7500 mg peanut protein was confirmed in 35/37 of the challenged children (94.6 %). Two children developed objective allergic reactions before reaching 7500 mg peanut protein; one child with a maintenance dose of 1500 mg reacted with conjunctivitis and urticaria at 7500 mg peanut protein; the other child had self-discontinued the maintenance dose of 1250 mg for three months and just resumed the OIT for the last three months prior to OFC. The latter child reacted with moderate anaphylaxis (erythema, urticaria and wheezing) at a cumulated dose of 4444 mg peanut protein.

As shown in Figure 14, the child-reported QoL was significantly higher (improved) after two years of OIT as compared baseline, with mean (95 % CI) QoL scores increasing from Y0 (82.1 (79.1, 85.2)) to Y2 (86.7 (83.6, 89.7)) (p < 0.0001). The controls reported no significant difference in QoL scores from Y0 (83.4 (75.4, 91.4)) to Y2 (82.2 (76.0, 88.4)) (p = 0.80)). However, the mean change (95 % CI) in QoL scores from Y0 to Y2 between the OIT group (4.4 (0.5, 8.3)) and the controls (-0.9 (-7.9, 6.11)) was not significantly different (p = 0.12) (Figure 14).

In the corresponding parental proxy-reports, parents of the OIT group reported significantly higher mean (95 % CI) QoL scores at Y2 as compared with Y0 with mean (95 % CI) QoL scores improving from Y0 (78.7 (73.6, 83.7)) to Y2 (88.0 (85.2, 90.8)) (p < 0.0001), with no significant difference in QoL scores for parents of the controls between Y0 (81.7 (74.6, 88.8)) and Y2 (82.1 (75.8, 88.4)) (p = 0.90) (Figure 14). However, parents of the OIT group reported a two-fold larger improvement in mean change (95 % CI) QoL scores in their child’s QoL
(9.3 (4.3, 14.3)) as compared with the children themselves (4.4 (0.5, 8.3)), and which, became significantly different from that of the controls (0.4 (-7.1, 8.0)) (p = 0.02) (Figure 14).

Figure 14. The absolute quality of life scores in children who receive oral immunotherapy (OIT) and the controls at screening (Y₀), at one year of up-dosing (Y₁) and at second year of treatment (Y₂), as reported by the child self-reports and the parental proxy-reports. Statistically significant group differences were assessed by mixed models for repeated measures. Increased value reflects improved QoL.
In unadjusted bivariate analyses, the number of AEs did not correlate significantly with change in QoL from Y₀ to Y₂, neither as reported by the children (p = 0.76) nor the parents (p = 0.90). The change in QoL from Y₀ to Y₂ was not influenced by the level of desensitization, maintenance dose or perceived treatment burden in any of the three domains at Y₁ or Y₂ (Table 16). The results did not change if children who discontinued OIT or perceived burden of time spent on OIT were included in the analyses (data not shown). During step-down procedures, the effect size of burden of peanut (dis-)taste/amount decreased by more than 25% when maintenance dose was excluded from the multivariate regression analyses model.

Table 16. Multivariate robust regression analyses for associations between factors that may influence change in quality of life from screening (Y₀) to second year of treatment (Y₂).

<table>
<thead>
<tr>
<th></th>
<th>PedsQL 4.0 child p-value</th>
<th>PedsQL 4.0 parents p-value</th>
<th>FAQLPB (parents) p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-0.49 (0.56)</td>
<td>1.50 (0.09)</td>
<td>0.02 (0.63)</td>
</tr>
<tr>
<td>Gender</td>
<td>-8.90 (0.07)</td>
<td>-5.90 (0.26)</td>
<td>0.23 (0.34)</td>
</tr>
<tr>
<td>Maintenance dose (mg)</td>
<td>-0.00 (0.48)</td>
<td>- (0.23)</td>
<td>- (0.34)</td>
</tr>
<tr>
<td>Perceived burden of:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adverse events</td>
<td>-0.22 (-0.23)</td>
<td>0.19 (-0.29)</td>
<td>-0.02 (-0.10)</td>
</tr>
<tr>
<td>Taste/amount of peanuts</td>
<td>0.15 (-0.09)</td>
<td>- (0.01)</td>
<td>0.01 (-0.09)</td>
</tr>
</tbody>
</table>

Associations are given as the relative change (β) related to each unit increase by the in QoL score.

N=37, including only children still receiving OIT at Y₂ with no missing data.

- no significant association

Change in QoL is given by the Pediatric Quality of Life Inventory Version 4.0 (PedsQL 4.0) child self-report and parental proxy-report, or the food allergy quality of life – parental burden (FAQLPB). Decreasing values of PedsQL and increasing values of FAQLPB reflects poorer QoL.

Perceived treatment burden was reported by VAS (range 0–10 (0=no burden, 10=massive burden) within the domains: Adverse events (stomach ache, nausea/vomiting and oral itching) and taste and amount of daily peanut OIT.
The parents’ QoL improved significantly (decreased score) as reported by the FAQL-PB from \( Y_0 \) to \( Y_2 \) for both the OIT group and the controls, as shown in Figure 15.

Figure 15. The absolute quality of life (QoL) scores in parents of children receiving oral immunotherapy (OIT) and the controls at screening (\( Y_0 \)), at one year of up-dosing (\( Y_1 \)) and at second year of treatment (\( Y_2 \)). Decreased value reflects improved QoL.
The QoL reported by the children who were defined ineligible for OIT was similar to that of controls at $Y_0$ and $Y_1$, with the mean change (95% CI) in QoL scores of 2.5 (-3.6, 8.6)) compared with the corresponding (-1.0 (-7.7, 5.6)) ($p = 0.37$) among the controls. Similar findings were observed for the parental proxy-reports (7.3 (0.4, 14.0) vs. 0.4 (-8.1, 9.0) ($p=0.18$)) (Figure 16).

![Graph showing QoL scores](image.png)

Figure 16. The absolute quality of life (QoL) scores in children who receive oral immunotherapy (OIT) and the controls at screening ($Y_0$) and at one year of up-dosing ($Y_1$), as reported by the child self-reports and the parental proxy-reports. Increased score reflects improved QoL.
In contrast, the QoL decreased significantly in the parents of ineligible children compared to control parents (p=0.048) one year after screening for study participation (Figure 17).

Figure 17. The absolute quality of life (QoL) scores in parents of children receiving oral immunotherapy (OIT) and the controls at screening (Y₀) and at one year of up-dosing (Y₁). Decreased score reflects improved QoL.
6 GENERAL DISCUSSION

6.1 Baseline characteristics predicting the possibility of entering and completing an up-dosing phase of peanut oral immunotherapy (papers #1 and #2)

6.1.1 Baseline characteristics predicting the possibility of entering peanut oral immunotherapy (paper #1)

All of the 96 children with a positive DBPCFC during screening for the TAKE-AWAY trial were primary sensitized to peanut and reacted with anaphylaxis. A very low reactivity threshold of less than 3 mg peanut protein was determined in 20 percent of the subjects, rendering them ineligible for peanut OIT according to our protocol. The finding that reactivity threshold as well as LOAEL was significantly associated with basophil activation, peanut SPT and the ratio of peanut s-IgE/total IgE are in line with previously published studies (38, 55, 57). The association between reactivity threshold and basophil activation given by % CD 63+, is consistent with previous reports including children with varying severity of peanut allergy (38, 55). In 52 peanut allergic children with a median age of 5.4 years, Santos et al. observed that basophil activation given by CD-sens in addition to peanut SPT, s-IgE to peanut, Ara h 2 and the ratio of s-IgG₄/s-IgE to peanut, were significantly associated with reactivity threshold (55). Basophil activation given by % CD63+ however, best predicted allergy severity grade (55). This is in contrast to the basophil activation given by % CD63+ in the TAKE-AWAY trial, which best predicted very low threshold level (< 3 mg of peanut protein). The lack of results for CD-sens in the TAKE-AWAY trial is that CD-sens could not be assessed since strong basophil activation occurred even at the lowest concentration of 2.5 ng/ml allergen extract. This probably reflects and supports the very low reactivity thresholds determined in the TAKE-AWAY cohort.

The additional significant associations observed between reactivity threshold and Ara h 2, and LOAEL and s-IgE to peanut, are consistent with previous reports (38, 55, 57). Reactivity threshold has been reported to correlate with s-IgE to Ara h 2 ($r_s = 0.30$) (57), and LOAEL
with basophil activation, peanut SPT and s-IgE to peanut and Ara h 2 ($r_s = -0.20$ to $-0.40$) (38).

The finding of a discrepancy between the associations of baseline immunologic characteristics with reactivity threshold and LOAEL may have several explanations. The larger variation observed in reactivity threshold compared with LOAEL may increase the likelihood of observing correlations. Also, LOAEL and reactivity thresholds reflect different threshold entities since reactivity threshold requires moderate objective symptoms, while LOAEL appears to be more sensitive as it reflects the lowest dose that elicits a mild objective symptom. The LOAEL was determined post-hoc to facilitate comparisons across studies with different stopping-criteria of OFC (34, 39, 41). Hence, the discrepancies may also have been caused by the LOAEL being estimated rather than exactly determined for the lowest thresholds.

The very low reactivity thresholds found in 34.4% of our study population exceeded the expected 10% with a LOAEL $\leq 3$ mg of peanut protein obtained from a data-set of OFCs in more than 200 subjects (61). This is a much higher proportion of children with low reactivity thresholds than the 4.7% reported in a study by Blumchen et al. of 63 peanut allergic children using a modified food challenge expected to result in a lower LOAEL (38). The finding that reactivity threshold was associated with baseline characteristics also reported to predict allergy severity (53, 55-57), supports the previously suggested association between allergy severity and reactivity ($p = 0.027$) (102). The may also be supported by the finding that the LOAELs were lower and the s-IgEs to peanut were higher in the homogenous cohort of highly allergic children in TAKE-AWAY trial, as compared with previous published peanut OIT trials (87-94, 99). The suggestion of an association between severity and threshold is in keeping with the previously reported correlation between LOAEL and basophil activation in
63 peanut allergic children for both % CD 63+ ($r_s = -0.32$) and CD-sens ($r_s = -0.36$) (38), since basophil activation is suggested to reflect reactivity thresholds (55).

The time-consuming protocol, with slow dose escalation to a high maintenance dose in children highly allergic to peanut required a good understanding of the study protocol and huge commitment from the participants.

6.1.2 Baseline characteristics predicting the possibility of completing an up-dosing phase of peanut oral immunotherapy (paper #2)

Among all 57 children commencing peanut OIT, 75.5 % completed the up-dosing phase and reached some level of a maintenance dose. Even though the MMD was only attained by one fifth of the children, 73.7 % reached a maintenance dose of at least 300 mg, similar to previous studies, reporting 63.6 % to 86.9 % for peanut OITs (87-94, 99, 126).

The findings of non-significant trends and associations between AEs, s-IgEs to peanut and Ara h 2, peanut s-IgE/total IgE, and completion of the OIT up-dosing phase, may be explained by the non-biological factor “distaste for peanuts” as well as by the limited sample size. Distaste for peanuts was the main reason for not reaching MMD (paper #2), which may have influenced the association between biological baseline characteristics and reaching a maintenance dose. The s-IgE to peanut (43, 44), and even more the s-IgE to Ara h 2 (45, 49, 50), have been reported to predict allergic reactions to peanut with an Ara h 2 > 1.00 kUA/L conferring a 97 % probability of a systemic reaction (51). Among the children who discontinued OIT, 64.3 % reported unacceptable AEs as the main reason for discontinuation (paper #2), in line with previously published peanut OIT trials (92).
6.2 The feasibility of achieving a high maintenance dose in peanut oral immunotherapy (papers #1 and #2)

The high pre-defined MMD was reached by 21.1 % of the children only, which is lower than the 63.6 % to 86.9 % reported for other peanut OITs (87-94, 99, 126) with lower maintenance doses ranging from 125 mg – 4000 mg peanut protein. A lower IMD was reached by 54.4 % of the children. At the onset of the TAKE-AWAY trial, there was little knowledge of what the optimal MMD was with regard to efficacy and safety. A high MMD of 5000 mg peanut protein was chosen for the TAKE-AWAY trial to reach the overall aim of SU after four years of OIT. This dosage was based upon findings from several published studies. Firstly, theoretical considerations from SCIT trials of inhalant and venom allergies suggested that a high maintenance dose were more likely to induce SU (73, 74): Secondly, an OIT study which used a bi-weekly step-up protocol to 4000 mg peanut protein reported no AEs requiring epinephrine (89).

The main reason for not reaching MMD was distaste for peanuts, followed by AEs and social reasons. An amount of 5000 mg peanut protein represents approximately 25 whole peanuts kernels, which turned out to be too challenging to eat for many of our children. Distaste for peanuts was reported as a daily challenge in almost three out of four children, a finding further discussed in an Editorial Article of paper #2 (127) in the present thesis. As suggested in the Editorial Article by Bluemchen and Eiwegger (127), distaste for peanuts may be a serious challenge when a large amount is needed, and may result in decreasing safety due to reduced compliance. Little information is found about distaste in previous reports, but in one study of 23 children (88), distaste for peanuts resulted in withdrawal of one patient and reduction of maintenance dose in two others.

Adverse events were reported by 64.3 % of the drop-out children as the main reason for withdrawal, and were second most common reason for not reaching MMD. This finding is in line with the 65.0 % reported by drop-out children in pooled data of 104 children undergoing
peanut OIT (92). Despite that our cohort included solely children with anaphylaxis to peanut, our 24.5 % drop-out rate is in line with previously published peanut OIT studies, including mainly less severe peanut allergic children, with a drop-out rate ranging from 10 – 32 % (97). The ratio of peanut s-IgG₄/s-IgE was a limited predictor of reaching MMD, as the odds ratio (OR) was almost similar to the non-significant OR for reaching any maintenance dose. This is consistent with the high ratio of peanut s-IgG₄/s-IgE reported in sensitized children who were asymptomatic on peanut exposure (128). The high ratio of peanut s-IgG₄/s-IgE (102) as well as the low s-IgE to peanut (38) and Ara h 2 (57) at Y₀, have all been reported to indicate a higher reactivity threshold. A high reactivity threshold was also determined at baseline in the MMD vs. IMD children. Furthermore, a high ratio of peanut s-IgG₄/s-IgE was associated with a higher reactivity threshold and reduced basophil activation as reported in the first paper from the TAKE-AWAY trial (paper #1). Finally, the MMD children were older than the IMD children, which may contribute to a deeper understanding of the purpose of the treatment, result in a higher degree of compliance despite troublesome AEs, and possibly facilitate the intake of a large amount of peanuts.

6.3 The patient perspective burden of peanut oral immunotherapy (papers #2 and #3)

In children with anaphylaxis to peanut, mild AEs were reported in 13.9 % of the OIT doses, 0.6 % of the AEs were reported as moderate and 0.06 % (n = 11) OIT-related moderate anaphylactic episodes occurred. The mild AE rates were higher in children who discontinued OIT, while moderate AEs plus anaphylactic events were similar in children who discontinued OIT and children who completed the up-dosing phase. The finding of 13.9 % mild OIT-related AEs is consistent with the 13.5 % AEs in the STOP II study by Anagnostou et al. including 99 children to peanut OIT, with severities of allergic reactions ranging from mild reactions in one organ system (24.2 %) to severe respiratory
symptoms (5.1%) (91). The reported 0.6% moderate AEs in the TAKE-AWAY trial was, however, lower than the 2.6% objective AEs reported by 23 highly peanut sensitized children undergoing a rush peanut OIT in another study by Blumchen et al. (88). This discrepancy may be explained by the use of a rush OIT protocol with a tailored starting in these 23 children, reported to be associated with more AEs (88, 100).

The most frequently reported AEs in the TAKE-AWAY trial were GI-related including oral itching and stomach ache, which responded well to oral antihistamines in the absence of dyspeptic symptoms. These findings are in line with previously published studies of the most common OIT-related AEs (92-94, 97), as well as reports of effectiveness of oral antihistamines in OIT (91). Eight (14%) of our children reported dyspepsia which may represent eosinophil oesophagitis (EoE) (92, 98), a much debated AE reported in 2.7% undergoing OIT (97, 98). Four of the eight children were treated with a proton pump inhibitor (PPI) with three becoming asymptomatic whereas the fourth reported a decrease in symptoms. Dyspepsia caused two children to withdraw from the study, while two children reported spontaneous symptom relief.

Anaphylactic events occurred in almost 20% of the TAKE-AWAY children, which is significantly higher than previously reported for peanut OIT studies, including study populations heterogeneous in peanut allergy severity (88, 91, 94) with AEs reported from no systemic reactions (88), only one anaphylactic episode (91) or once the use of epinephrine (94). In the TAKE-AWAY trial, 19.4% of the children experienced anaphylactic events in 11 dose-days (paper #2), most of them occurred at OIT doses above 300 mg of peanut protein. Similarly, in the AR101 study, 14% of the 372 participants with reactivity thresholds < 100 mg peanut protein and maintenance doses of 300 mg peanut protein reported anaphylactic events treated with adrenaline in at least 76 anaphylactic events (99). Hence, children highly allergic to peanut seem to run a high risk of anaphylaxis even at low-dose OIT, with possibly
increasing the risk by increasing the OIT doses. Determining the optimal maintenance dose in OIT studies are thus urgently needed, both from a safety as well as an efficacy point of view. It might be that high maintenance doses are necessary to achieve SU, and thus will outweigh the risk of anaphylaxis. However, if long term SU is achievable remains to be assessed in the TAKE-AWAY trial and other follow-up studies. For now, desensitization by low-dose peanut OIT increasing the reactivity threshold from 100 to 300 mg has been calculated shown to reduce the risk of allergic reactions from accidental exposure on a population level by 95 % (83). If SU is achievable, remains to be assessed in the TAKE-AWAY trial and other follow-up studies. If higher OIT doses prove necessary to achieve SU, the risk of potential anaphylaxis will have to be weighed up against the potential benefits of the treatment.

The finding that the number of AEs decreased as the treatment proceeded is consistent with previous reports (91), suggested resulting from a beginning desensitization (91). Mild AEs occurred significantly more often during the first two days of each up-dosing period and in the first third of the dose-steps, as previously reported (91). In contrast to the mild AEs, anaphylactic events were equally distributed between the first and second years of OIT and between children completing and not completing the OIT. Moderate AEs as well as anaphylactic episodes were similar in these two groups, suggesting that the completers and those who discontinued OIT perceived AEs differently. This is supported by the lack of association between QoL and number of AEs in the TAKE-AWAY children (paper #3), as well as in previously published OIT studies (107, 112, 129). However, the lack of association observed in the TAKE-AWAY trial, may also be explained by a potential bias caused by missing assessments of QoL and perceived treatment burden in children withdrawing from the treatment, especially if withdrawal was related to increased treatment burden.

Children who completed two years of OIT reported reduced treatment burden related to GI-symptoms and taste-/amount of the OIT-dose from the first to the second year of treatment.
We observed a negative correlation between perceived treatment burden at \( Y_1 \) and change in treatment burden from \( Y_1 \) to \( Y_2 \), as previously described for QoL at baseline and change in QoL \( (107) \) suggesting that patients with a high level of treatment burden during up-dosing experienced less AEs as they achieved maintenance dose. For children reporting increased burden, it might be that their motivation to maintain treatment and accept the related challenges decreased. Even though similar negative correlation has been reported between QoL at baseline and change in QoL after OIT \( (107) \), we could not find that perception of treatment burden influenced change in QoL \( (paper \#3) \).

### 6.4 The effect of 2-years peanut oral immunotherapy \( (paper \#3) \)

After two years of OIT, 18 children had discontinued treatment. Desensitization to 7500 mg peanut protein was confirmed in 94.6 % of the children continuing treatment. Desensitization was not associated with maintenance dose, in line with a peanut OIT report comparing the effect of maintenance doses of 300 vs. 3000 mg peanut \( (96) \).

The finding that child QoL significantly improved among OIT treated children from inclusion and until two years of OIT but not among the controls, is consistent with previously published studies mostly based on parental proxy-reports only \( (91, 104, 107, 126) \). Although child QoL improved from baseline to \( Y_2 \) as reported by both the children themselves as well as by their parents, the parents reported a two-fold larger improvement than the children. This is likely to explain why an OIT-related improvement in child QoL was observed for the parental reports and not for the children’s self-reports. The larger improvement in child QoL as reported by the parents may be explained by an influence of the improvement in parents’ own QoL, which improved similarly in the OIT group and the controls. The improvement in parental QoL for both the OIT group and the controls may result from general study participation including the pre-OIT DBPCFC \( (104, 107, 108, 130) \). Additionally, less social restrictions are reported to
have a greater impact on parents’ QoL than on children’s QoL (112), and may explain the larger improvement in child QoL as reported by the parents. For the child self-reports, the lack of significant difference in improved QoL between the OIT children and the controls may be explained by the larger variation observed in the QoL scores, as well as the limited sample size. The larger variation may indicate a more complex explanation for factors influencing change in QoL as perceived by the children themselves, like the treatment burden including distaste for peanuts and AEs (131). The limited sample size was comparable with previous studies of QoL in peanut OIT (104-107) and sufficient to identify the larger improvement in child QoL reported by parents as well as a significant difference in change in QoL for the parental proxy-reports.

The finding that QoL improved after OIT in children with anaphylaxis to peanut is in line with previously published studies of varying severities of peanut allergy (91, 104, 106, 108). In a study of 156 peanut with similar allergy severity to the TAKE-AWAY cohort, the QoL was not influenced by severity of allergic reactions allergic children (112).

Neither perception of treatment burden, level of desensitization, maintenance dose nor AEs influenced change in QoL, in line with previous studies reporting no association between change in QoL and AEs (107, 112, 129). This suggests that perceived treatment burden is disassociated from the number and severity of AEs during OIT (107, 112). The observation of maintenance dose and distaste for peanuts being confounding factors in influencing change in QoL, is probably explained by distaste for peanuts being the main reason for not reaching MMD, as it was reported as a daily challenge to 77.2 % of the children (paper #2). The negative correlation observed between perceived treatment burden at Y₁ and change in treatment burden from Y₁ to Y₂ is in line with the previously reported negative correlation between change in QoL and QoL at baseline (107) and might be explained by regression towards the mean.
Desensitization to 7500 mg peanut protein was achieved in almost all challenged children. As the level of desensitization was constant, it was less likely to influence change in QoL. Our finding that maintenance dose did not influence change in QoL has also recently been described (107). The exploratory finding that QoL deteriorated in parents of children determined ineligible for OIT after OFC, is in contrast to the improved QoL reported by parents of children undergoing OFC (130). This may be explained by the determination of a very low reactivity threshold, which may increase social restrictions and deemed the children ineligible to OIT.

6.5 Strengths and limitations

6.5.1 Strengths

To the best of our knowledge, the TAKE-AWAY trial is the first OIT study consisting of a study population in which all participants have a challenge proven anaphylaxis to peanut. The vast majority also had a previous history of anaphylaxis to peanut. Peanut OIT is currently an experimental treatment which may be implemented in clinical practice (127). The TAKE-AWAY trial provides important knowledge of OIT in children with severe peanut allergy, as there still are concerns about who will benefit the most from such treatment and what maintenance dose to apply (127). It is also unclear whether the promising results from previous published peanut OITs performed in study populations heterogeneous in peanut allergy severity, are transferable to children highly allergic to peanut (127), the subgroup of children thought to benefit the most from such treatment (35, 89, 100). The strict definitions of a positive DBPCFC in all children prior to enrolment requiring the occurrence of at least two moderate objective symptoms, confirmed the peanut allergy severity and reactivity threshold in this homogenous study population.
In line with the PRACTALL guidelines (34), some studies define a food challenge positive by milder symptoms, sometimes already by the occurrence of reproducible subjective symptoms (91). This approach may be misleading, as subjective symptoms have been demonstrated to occur at doses 20-fold lower than the LOAEL (37) one cannot rule out that an anaphylaxis would have occurred if another dose was given. However, the TAKE-AWAY children seem to have higher s-IgEs to peanut and lower LOAELs than in previously published OIT trials (87-94, 99), supporting that these children are highly allergic to peanut. Nevertheless, there is a need for a consensus of when to define a food challenge positive. While waiting for such a consensus, calculating the objective LOAEL enables comparison between studies (36).

Although not feasible for the present thesis, the TAKE-AWAY trial has been designed to assess SU one year after cessation of four years’ treatment in a group of children who are highly allergic to peanuts. Hence, the TAKE-AWAY trial may fill an important knowledge gap in terms of long term effects of OIT in children highly allergic to peanut. Immunological and clinical investigations including the novel CAPT to peanut (27) were performed at study entry as well as after one and two years of study participation. At the same time-points, PROs including standardized QoL questionnaires were also obtained and the novel VAS reports of perceived treatment burden were obtained after one and two years of treatment. Close monitoring through repeated tests at several time-points increase the ability of correct assessment of treatment efficacy. An OFC was performed after two years of treatment to determine the achieved desensitization. Hence, the combination of biological outcomes and PROs enabled complete determination of the effect of two years’ peanut OIT. The QoL assessments included two perspectives of child QoL; the child’s self-report and the parental proxy-report. These two aspects enable comparison of the assessment of QoL between children and parents in OIT. Including children ineligible for OIT in the QoL
assessment provided important insight into the effect of an unforeseen outcome of a food challenge, such as ineligibility for OIT.

The use of VAS for reporting perceived treatment burden is novel and may improve our understanding of patient perception of the burden of OIT. Although the newly developed VAS was not pre-validated for the TAKE-AWAY trial, VAS forms have previously been validated for measuring subjective psychosocial phenomena like pain and nausea and correlated strongly with numeric rating score and verbal rating scale (132).

### 6.5.2 Limitations

The TAKE-AWAY cohort including highly sensitized children in which 94.8 % had a s-IgE to Ara h 2 > 1.75 kUA/L, demonstrated to have a 100 % positive predictive value of a systemic allergic reaction (52), and a low-dose challenge would be preferred according to the consensus protocol for OFCs (60). A low-dose challenge would have enabled a more accurately determination of LOAEL for the entire study population.

Our homogeneous population of highly peanut allergic children may have limited the possibility to identify correlations between immunological and clinical characteristics on one hand, and reactivity thresholds and severity reactions on the other hand.

For the BAT, there is no consensus of diagnostic cut-off values for a positive BAT (32), and non-responding basophils (116) represent an additional limitation.

As distaste for peanuts was the main reason for not reaching the MMD, a placebo arm could theoretically have strengthened the study. However, this was not approved by the regional medical ethical committee due to the unfavourable ratio of treatment burden to expected patient benefit by placebo intervention. Furthermore, a blinded vehicle to our study’s high-dose peanut OIT appeared not feasible. Switching ingestion of defatted flour to whole roasted peanuts may have influenced efficacy of the OIT, as whole peanuts may include additional
properties and do involve larger amounts to be ingested than flour. Anyway, whole peanuts were more easily tolerated.

The allocation for observation only may represent a bias as these participants do not have an expectation for a better QoL caused by a placebo treatment, resulting in a larger difference between OIT children and the controls.

Despite a long-term step-up protocol with a fixed starting dose, and around-the-clock access to study paediatricians to increase the likelihood of a high retention rate of the OIT, every fourth child dropped out of study intervention. A small study population decreases the possibility of finding significant differences due to a type 2 statistical error. Mixed models were used to correct for the relatively high proportion of missing data following children dropping out of OIT. Nevertheless, the small study population was sufficiently large for several significant findings. These results are important, as peanut OIT still is considered to be an experimental treatment.
MAIN CONCLUSIONS

In a cohort of children with anaphylaxis to peanut, we found:

The reactivity threshold together with the objective LOAEL were associated with basophil activation, SPT and the ratio of peanut s-IgE/total IgE. Basophil activation best predicted very low reactivity threshold and thereby eligibility for OIT. None of the baseline clinical or immunological markers were sufficient to substitute OFC in determining a reactivity threshold necessary to define eligibility for entering OIT.

One fourth of the children commencing peanut OIT discontinued treatment during up-dosing. We found no significant baseline predictor for completing the up-dosing phase.

In children who were confirmed to be highly allergic to peanut, 75.5 % were able to reach a level of a maintenance dose ranging from 250 mg – 5000 mg, while 21 % only reached the pre-defined MMD of 5000 mg of peanut protein. The main reason for not reaching the MMD was distaste for peanuts, followed by AEs. The ratio of peanut s-IgG₄/s-IgE only, was significantly associated with reaching MMD.

Mild AEs occurred in 13.9 % of the dose-days most commonly reported as GI-related in line with previously published studies. In contrast, the proportion of OIT-related anaphylaxis was mostly higher in this study population, probably related to all children being highly allergic to peanut. This finding questions the feasibility and safety of high-dose OIT in these children.

Almost all children who completed two years of OIT were desensitized to 7500 mg of peanut protein. The significant improvement in child QoL with two years of OIT reported by parental proxy reports and not by the children, suggests that parents may over-estimate the effect of the treatment. Conclusively, it may be more appropriate to use child self-reported rather than the parental proxy-reported QoL when assessing patient-related outcome of OIT.
8  FUTURE PERSPECTIVES

Oral immunotherapy is promising for inducing desensitization in the majority of patients. Although still regarded an experimental treatment, it is offered in clinical practice by some allergy clinics. There are, however, several unanswered questions related to this treatment. Assessment of OIT eligibility includes determination of the reactivity threshold of the offended food by an OFC. There is, however, no consensus of when to score a food challenge positive. Although the PRACTALL guidelines (34) recommend using objective symptom(s), many OIT-studies accept recurrent subjective symptoms to define a positive food challenge. Determining the objective LOAEL may be preferable to determine reactivity threshold, enabling comparison between studies. Additionally, assessing severity of allergic reactions is a matter of current international debate (133). The need for a consensus of which scoring system to use, was recently demonstrated as severe allergy is reached more quickly in some scoring systems (134), making comparison of allergy severity between studies difficult.

The present thesis presents results from the first two years of high-dose OIT in the TAKE-AWAY trial. The study includes a homogeneous population of children with challenge proven anaphylaxis to peanut and low LOAELs as compared with previous peanut OIT trials heterogeneous in allergy severity (87-94), i.e. the patients expected to benefit the most from a successful OIT (35, 89, 100). The study raises questions and concerns about safety of peanut OIT in this group of patients, as systemic reactions occurred much more frequently than in previous published studies including children with less severe allergy (89, 95). It may be argued that this is explained by the high maintenance dose. In the large recently published AR101 study (99), low-dose OIT was commenced in a study population including mostly patients with previous anaphylaxis to peanut, and the prevalence of OIT-related anaphylaxis with use of adrenaline was similar to the TAKE-AWAY trial. Therefore, new studies optimizing the treatment with respect to starting dose, up-dosing speed, maintenance dose and
possible use of protective drugs like omalizumab are needed to improve safety of OIT in these patients. A very low starting dose with a conventional slow, gradual up-dosing may be beneficial. The maintenance dose may also be crucial, as well as the duration of the treatment. In the study of Vickery et al. (96), maintenance doses of 300 mg and 3000 mg of peanut protein were compared, with no difference in desensitization or SU four weeks after cessation of the OIT. Hence, it might be that SU depends more on the duration of the treatment. On the other hand, the Vickery study included only preschool children 9 - 36 months of age. Young children are likely to have a more “plastic” immune system, easier to modulate than in older children and adults. This is partly supported by the AR101 study reporting effect of OIT in older children and adolescents but not in adults (99).

Finally, there is a need to conclude if OIT has the potential to induce long lasting SU or only desensitization. The few studies addressing these questions report conflicting results (87, 90, 96), and the observation time is in general short, often four weeks only. Studies with longer observational time are needed to assess the potential of OIT to “cure allergy”. Assessment of potential effect should also include the child’s perspective on QoL as well as the costs for the health care system. The experience from the TAKE-AWAY trial gives the impression that these patients, understandably, need frequent contact with health personnel, suggesting that a patient coordinator is essential.

To conclude, assessing long time SU is especially important for patients highly allergic to peanut, as it is likely that one have to make a decision where the potential effect must be balanced up against safety for the patient and costs for the health care system. For some patients highly allergic to peanut, perhaps an OFC including theoretical information, practical training and subsequent follow-up, may provide similar efficacy measured in PROs as an OIT?
9 REFERENCES


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PAPERS

Paper #1
Predicting reactivity threshold in children with anaphylaxis to peanut

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Summary

Background: Peanut allergy necessitates dietary restrictions, preferably individualized by determining reactivity threshold through an oral food challenge (OFC). However, risk of systemic reactions often precludes OFC in children with severe peanut allergy.

Objective: We aimed to determine whether clinical and/or immunological characteristics were associated with reactivity threshold in children with anaphylaxis to peanut and secondarily, to investigate whether these characteristics were associated with severity of the allergic reaction during OFC.

Methods: A double-blinded placebo-controlled food challenge (DBPCFC) with peanut was performed in 96 5- to 15-year-old children with a history of severe allergic reactions to peanut and/or sensitization to peanut (skin prick test [SPT] ≥ 3 mm or specific immunoglobulin E [s-IgE] ≥ 0.35 kUA/L). Investigations preceding the DBPCFC included a structured interview, SPT, lung function measurements, serological immunology assessment (IgE, IgG and IgG4), basophil activation test (BAT) and conjunctival allergen provocation test (CAPT). International standards were used to define anaphylaxis and grade the allergic reaction during OFC.

Results: During DBPCFC, all 96 children (median age 9.3, range 5.1-15.2) reacted with anaphylaxis (moderate objective symptoms from at least two organ systems). Basophil activation (CD63+ basophils ≥ 15%), peanut SPT and the ratio of peanut s-IgE/total IgE were significantly associated with reactivity threshold and lowest observed adverse events level (LOAEL) (all P < .04). Basophil activation best predicted very low threshold level (< 3 mg of peanut protein), with an optimal cut-off of 75.8% giving a 93.5% negative predictive value. None of the characteristics were significantly associated with the severity of allergic reaction.

Conclusion and Clinical Relevance: In children with anaphylaxis to peanut, basophil activation, peanut SPT and the ratio of peanut s-IgE/total IgE were associated with reactivity threshold and LOAEL, but not with allergy reaction severity.

Keywords
anaphylaxis, basophil, double-blinded placebo-controlled food challenge, food allergy, immunoglobulin E, immunologic tests, peanut allergy, threshold levels
Peanut allergy, the most common cause of severe and fatal allergic reactions in children in the western world,\(^1\) has increased in prevalence over the last two decades\(^2,3\) currently affecting 1%-2% of the paediatric population.\(^4\) Once established, peanut allergy is likely to last a lifetime.\(^5,6\) The only established therapy is peanut avoidance and rescue medication, including intramuscular epinephrine auto injectors in case of severe systemic reactions. Nevertheless, accidental exposures frequently occur due to poorly labelled foods, manufacturing errors and peanut contamination.\(^7\) Consequently, quality of life is reduced in peanut-allergic children and their families.\(^8-10\)

Diagnosing peanut allergy requires a convincing history of allergic reaction related to peanut exposure, supported by clinical and immunological investigations and/or an oral food challenge (OFC) to peanut. Clinical investigations include skin prick tests (SPT) and possibly the recently reported clinical conjunctival allergen provocation test (CAPT),\(^11\) whereas immunological investigations include specific Immunoglobulin E (s-IgE) to peanut and its allergen components as well as the basophil activation test (BAT).\(^12-14\)

Clinical reactions to peanut have been associated with weal size of SPT and s-IgE levels to peanut,\(^15,16\) while s-IgE to the peanut component allergen Ara h 2 has been shown to be the best predictor of allergic reactions\(^17-19\) with levels exceeding 1.00 kUA/L conferring a 97% probability of a systemic reaction.\(^20\) Allergy reaction severity has been associated with basophil activation,\(^21\) and with peanut SPT and s-IgE in some\(^12,22,23\) but not all\(^24,25\) studies, whereas the ratio of s-IgG/s-IgE to peanut\(^26\) and peanut s-IgE/total IgE\(^27\) has been associated with diagnostic outcome of OFC. Finally, reactivity threshold has been associated with peanut SPT and s-IgE to peanut, Ara h 2 and basophil activation.\(^23,24\)

Reactivity threshold is necessary to personalize precautions related to peanut exposure, preferably determined by a double-blinded placebo-controlled food challenge (DBPCFC) and reported as the cumulated amount of peanut intake at the time of a positive OFC\(^28\) or as the lowest amount of peanut protein that elicited mild, objective symptoms, that is the lowest observed adverse effect level (LOAEL).\(^29\) However, children with the greatest likelihood of severe and life-threatening reactions are often excluded from DBPCFC and oral immunotherapy (OIT) due to the risk of systemic reactions.\(^30\)

Screening children with peanut allergy for enrolment in the open randomised controlled (RCT) peanut OIT trial; “Take-Away food allergy; inducing tolerance in children allergic to peanut” (the Take-Away trial), included clinical and immunological investigations as well a DBPCFC to determine reactivity threshold and severity grade of allergic reaction. The primary aim of this study was to determine whether clinical and/or immunological characteristics were associated with reactivity threshold in children with anaphylaxis to peanut, and secondarily to investigate whether these characteristics were associated with severity of the allergic reaction.
percentage predicted forced expiratory volume in one-second (FEV1%) using the reference values of Zapletal.31

Skin prick tests were performed using peanut and 11 other common food and inhalant allergens (see online supplement) according to international guidelines.32 Titrated SPT (SPTt) to peanut was performed with dilutions of 1:20, 1:200, 1:2000 and 1:20 000 of the peanut allergen extract. Weal size was recorded after 15 minutes, and regarded positive if ≥3 mm greater than negative control.

Total IgE and s-IgE to peanut and peanut component allergens (Ara h 1, Ara h 2, Ara h 3, Ara h 8, Ara h 9) as well as other common allergens (see online supplement) were analysed in whole blood sampled in EDTA tubes and sent the same day to Furst Medical Laboratory using the Phadia CAP-System FEIA (ThermoFisher, Uppsala, Sweden). Specific IgE ≥0.35 kUA/L was considered positive.

Specific IgG (s-IgG) and IgG4 (s-IgG4) to peanut and to Ara h 2 were analysed in sampled frozen sera stored at −86°C using the Phadia CAP-System FEIA (ThermoFisher). According to the manufacturer’s instructions, a positive test was defined as IgG ≥2.0 mg/L and IgG4 of >0.07 mg/L.

 Conjunctival allergen provocation test (CAPT) was performed double-blinded and placebo-controlled with commercially available extract of peanut (Greer laboratories, Lenoir, NE, USA) as described by Lindvik et al.11 The NaCl 0.9% was used as placebo and as dilution fluid to make test solutions of 1:160, 1:80, 1:40, 1:20, 1:10 and 1:11 (further details in online supplement).

 The BAT was performed in fresh EDTA blood stored at room temperature (usually 1-3 hours, maximum 24 hours). Whole blood aliquots were incubated with 20 μL staining solution as negative control, positive control, or with 2.5, 5 and 10 ng/mL peanut extract (further details in online supplement). For this study, basophil activation was only expressed as the % CD63-positive basophils (% CD63+) after stimulation of 5 ng/mL of the peanut extract, as all children had a strong basophil activation already to the lowest allergen concentration of 2.5 ng/mL peanut extract, thus CD-sens (basophil allergen sensitivity—the allergen concentration eliciting half of the maximum basophil activation) could not be determined. Non-responders were defined as children responding with 0%-5% basophil activation.33 In addition to analysing basophil activation as a continuous variable, the cut-off value for positive basophil activation in this study was set to >15% CD63+ basophils, whereas children with basophil activation of 5%-15% were classified as low responders, as suggested by Glaumann et al34 and the kit provider (Buhlmann Laboratories AG). In 10 children, BAT was not performed due to missed sampling (n = 5) or technical causes (n = 5) (technical failure of equipment [n = 1], incorrect handling of the blood sample [n = 1] or no available allergen extract [n = 3]).

 The DBPCFC was performed according to international standardized procedure35,36 using peanut flour (Golden Peanut Company, Alpharetta, GA, USA) as active challenge ingredient in baked gingerbread according to a recipe by Vlieg-Boestra et al37 (further details in online supplement). The first challenge dose was chosen to 3 mg peanut protein, and the maximal cumulated dose was 6443 mg, distributed by 7 incremental steps (3, 10, 30, 100, 300, 1000, 5000 mg of peanut protein) of 30 minutes interval. In case of persistent subjective or mild objective symptoms, the interval was increased up to 60 minutes, in line with the modified Bock’s criteria by Sampson et al,38,39 in which case the next step repeated the previous dose. The DBPCFC was regarded positive with the occurrence of moderate objective symptoms35,40 even if only part of the current dose had been eaten. Cumulated peanut protein (mg) intake at the time of positive DBPCFC was recorded. The LOAEL was recorded post hoc as the lowest cumulated amount of peanut protein that elicited the first mild, objective adverse reaction during food challenge was observed, according to the modified Bock’s criteria.
Anaphylaxis was defined as moderate symptoms from at least two organ systems in line with European Academy of Allergy and Clinical Immunology (EAACI) task force position papers\(^{41,42}\) (Table S2), modified for children by Vetander et al.\(^{43}\). Severity of anaphylaxis was also graded in line with the EAACI position papers\(^{42,43}\) scoring from 1 to 3 (mild-moderate-severe) in addition to the method of Sampson (Grading of Food-Induced Anaphylaxis According to Severity of Clinical Symptoms) ranging from one to five (extremely severe reaction).\(^{44}\)

### 2.4 Outcomes and predictors

The primary outcome was reactivity threshold, defined as the accumulated peanut protein (mg) intake at positive DBPCFC. The LOAEL\(^{28}\) was used as an additional threshold value and set to 3 mg for children with a positive DBPCFC at the first challenge dose.

Very low reactivity threshold was defined as a DBPCFC reactivity threshold of $\leq 3$ mg of peanut protein.

The secondary outcome was severity of the allergic reaction during DBPCFC.

Potential predictors were baseline characteristics (age and gender, medical history including previous history of anaphylaxis, and parental atopic disease or food allergy) allergic comorbidities (current asthma, allergic rhinitis, previous or current atopic dermatitis and allergy to other foods or nuts), FEV\(_1\%\) clinical test results (SPT and CAPT) and immunological test results (s-IgE, s-IgG and s-IgG\(_4\) to peanut and Ara h 2), the ratios of peanut s-IgE/total IgE and s-IgG\(_4\)/s-IgE to peanut, as well as basophil activation.

### 2.5 Statistical analyses

Due to non-normal distribution, continuous baseline characteristics are presented by geometric mean (95% CI) and median (range), and categorical data are presented as number of cases (n) with percentage (%). Furthermore, bivariate unadjusted analyses were performed with Spearman correlation analyses ($r_s$ – Spearman correlation coefficient). As the underlying assumptions for the standard mean regression analysis were not fulfilled, multivariate robust regression analyses with Huber's M-estimator were used to further assess associations between immunological parameters as the independent variables and both reactivity threshold and LOAEL as the dependent variables. Multivariate robust linear regression was used to assess the association between immunological parameters and severity of the allergic reaction.

A Hosmer step down multivariate analysis\(^{45}\) included all variables significant at the 0.35 level in the bivariate analyses, and the final model was tested for confounding with all excluded variables.

Statistical analyses were performed using Statistical Analysis System (SAS, Version 9.3; SAS Institute Inc., Chapel Hill, NC, USA) and the IBM Statistical Package for Social Sciences (IBM SPSS Statistics, Version 21.0.1; IBM Corp., Armonk, NY, USA).

The level of significance was set to .05.

### RESULTS

All included 96 children (median [range] age of 9.3 [5.1, 15.2] years) were sensitized to peanut with s-IgE to Ara h 2 above 1.0 kUA/L in all but 4 patients (Table 1) who had levels of 0.96, 0.82 (two children) and 0.38 kUA/L, respectively. A prior history of anaphylaxis was reported by 83.0% with unknown time interval because the last anaphylactic event, whereas all children had anaphylaxis during the DBPCFC (Table 1). None of the children had an EAACI score of 3 or a Sampson score of 5 (Table 1).

The geometric mean (95% CI) for reactivity threshold and LOAEL was 33 (22.9, 48.4) and 13.8 (9.9, 19.5) mg of peanut protein, respectively (Table 1). Reactivity threshold level was $< 3$ mg in five children, 3 mg in 14 children and $> 3$ mg in 77 children. As anaphylaxis occurred at the first challenge dose in 19 children, their LOAEL was set equal to reactivity threshold. In 14 children with reactivity threshold $> 3$ mg, the first objective symptom occurred at the first challenge dose, yet without a positive DBPCFC, resulting in a LOAEL of 3 mg. Consequently, five children were classified with a LOAEL $< 3$ mg, 28 had the LOAEL set to 3 mg, and 64 had a LOAEL $> 3$ mg.

In unadjusted bivariate analyses, basophil activation correlated significantly with reactivity threshold (correlation coefficient $r_s = -0.30$, $P = 0.04$) and with LOAEL ($r_s = -0.02$, $P = 0.032$). No significant correlations were found for reactivity threshold or LOAEL with peanut SPT or s-IgE to peanut, Ara h 2 or with the ratios of peanut s-IgE/total IgE and s-IgG\(_4\)/s-IgE to peanut (data not shown). Neither age, gender, allergic comorbidities nor FEV\(_1\)% were significantly associated with reactivity threshold, LOAEL or severity of allergic reaction in the bivariate regression analyses (data not shown).

In multiple robust regression analyses, reactivity threshold was significantly associated with basophil activation, peanut SPT, Ara h 2 and the ratios of peanut s-IgE/total IgE and s-IgG\(_4\)/s-IgE to peanut (Table 2). Neither peanut CAPT nor s-IgE or SPT, to peanut was significantly associated with reactivity threshold. Repeating the analyses for LOAEL, significant association was found with peanut SPT, s-IgE to peanut, the ratio of peanut s-IgE/total IgE and basophil activation, but not with Ara h 2, the ratio s-IgG\(_4\)/s-IgE to peanut, peanut CAPT, and peanut CAPT (Table 2).

Subgroup analyses after excluding 23 children with a basophil activation $\leq 15\%$ CD63\(^3\) (non-responders [n = 6] and the low responders [n = 17]) from the model gave similar results for reactivity threshold and for LOAEL (data not shown).

Basophil activation was significantly associated with immunological and clinical test results with closest association with peanut CAPT ($\beta = -7.60$, $P = 0.02$) (Table 3) and with age among girls ($r_s = -0.31$, $P = 0.04$), but not among boys ($r_s = -0.009$, $P = 0.95$). Additionally, basophil activation was the best predictor of very low reactivity threshold ($< 3$ mg of peanut protein) (Figure 2), with an optimal cut-off of 75.8% giving a 93.5% negative predictive value (Table S1).

Neither peanut SPT or CAPT nor CAPT to peanut nor any of the immunological parameters was significantly associated with severity of allergic reaction in the Hosmer step down multivariate robust regression analysis (data not shown).
<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Baseline characteristics from screening in the Take-Away trial</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n (%)</strong></td>
<td><strong>Geometric mean (95% CI)</strong></td>
</tr>
<tr>
<td>Age</td>
<td>9.3 (5.1, 15.2)</td>
</tr>
<tr>
<td>Male sex</td>
<td>50 (52.1)</td>
</tr>
<tr>
<td>Current asthma</td>
<td>52 (54.2)</td>
</tr>
<tr>
<td>Allergic rhinitis</td>
<td>28 (29.2)</td>
</tr>
<tr>
<td>Ever had eczema</td>
<td>74 (77.1)</td>
</tr>
<tr>
<td>Allergy to other nuts than peanut</td>
<td>36 (37.5)</td>
</tr>
<tr>
<td>Allergy to other food than nuts</td>
<td>49 (51.0)</td>
</tr>
<tr>
<td>Parental atopic disease</td>
<td>83 (86.5)</td>
</tr>
<tr>
<td>Parental food allergy</td>
<td>38 (39.6)</td>
</tr>
<tr>
<td>History of anaphylaxis</td>
<td>80 (83.3)</td>
</tr>
<tr>
<td>Mild</td>
<td>18 (18.8)</td>
</tr>
<tr>
<td>Moderate</td>
<td>36 (37.5)</td>
</tr>
<tr>
<td>Severe</td>
<td>26 (27.1)</td>
</tr>
<tr>
<td>FEV1% predicted</td>
<td>97.1 (94.8, 102.1)</td>
</tr>
<tr>
<td>Peanut SPT (mm)</td>
<td>9.8 (9.0, 10.7)</td>
</tr>
<tr>
<td>S-IgE (kUA/L)</td>
<td></td>
</tr>
<tr>
<td>Peanut</td>
<td>88.1 (62.4, 124.5)</td>
</tr>
<tr>
<td>Ara h2</td>
<td>44.6 (32.1, 61.9)</td>
</tr>
<tr>
<td>Pos s-IgE (≥0.35 kUA/L)</td>
<td></td>
</tr>
<tr>
<td>Other nuts</td>
<td>87 (91.6)</td>
</tr>
<tr>
<td>Other food</td>
<td>92 (96.8)</td>
</tr>
<tr>
<td>Ratio peanut s-IgE/total IgE (kUA/L)</td>
<td>4.0 (3.1, 5.3)</td>
</tr>
<tr>
<td>Peanut s-IgG (mgA/L)</td>
<td>13.8 (11.5, 16.6)</td>
</tr>
<tr>
<td>Peanut s-IgG4 (mgA/L)</td>
<td>2.1 (1.6, 2.6)</td>
</tr>
<tr>
<td>Ara h2 s-IgG (mgA/L)</td>
<td>6.3 (5.4, 7.3)</td>
</tr>
<tr>
<td>Ara h2 s-IgG4 (mgA/L)</td>
<td>8.9 (12.0, 6.6)</td>
</tr>
<tr>
<td>Ratio s-IgG4/s-IgE to peanut (ng/mL)</td>
<td>5.5 (3.9, 7.7)</td>
</tr>
<tr>
<td>BA (%CD63+) pos</td>
<td>69.5 (64.0, 75.4)</td>
</tr>
<tr>
<td>BA (%CD63+) all children</td>
<td>44.9 (35.0, 57.5)</td>
</tr>
<tr>
<td>CAPT positive (dilution)</td>
<td></td>
</tr>
<tr>
<td>1/160</td>
<td>9 (9.4)</td>
</tr>
<tr>
<td>1/80</td>
<td>24 (25)</td>
</tr>
<tr>
<td>1/40</td>
<td>38 (39.6)</td>
</tr>
<tr>
<td>1/20</td>
<td>20 (20.8)</td>
</tr>
<tr>
<td>1/10</td>
<td>5 (5.2)</td>
</tr>
<tr>
<td>Pre-OIT DBPCFC</td>
<td></td>
</tr>
<tr>
<td>Use of adrenaline</td>
<td>42 (43.8)</td>
</tr>
<tr>
<td>Anaphylaxis severity grade</td>
<td></td>
</tr>
<tr>
<td>Modified EAACI43</td>
<td>1.7 (1.6, 1.8)</td>
</tr>
<tr>
<td>Sampson44</td>
<td>2.6 (2.5, 2.8)</td>
</tr>
<tr>
<td>LOAEL (mg)</td>
<td>13.8 (9.9, 19.5)</td>
</tr>
<tr>
<td>Reactivity threshold (DBPCFC positive) (mg)</td>
<td>32.4 (22.1, 47.5)</td>
</tr>
</tbody>
</table>

SPT, skin prick test; Ig, immunoglobulin; BAT, basophil activation test; CAPT, conjunctival allergen provocation test; OIT, oral immunotherapy; LOAEL, lowest observed adverse effect level; reactivity threshold—cumulated peanut protein eaten at positive DBPCFC.

*Atopic disease includes asthma, allergic rhinitis, atopic dermatitis, allergic conjunctivitis.

*Food allergy includes all food allergy including peanut and tree nut allergy.

*S-IgE to other nuts ≥0.35 kUA/L includes hazelnut, almond, cashew nut, pistachio nut, walnut, pecan nut, Brazil nut and macadamia nut.

*S-IgE to other food includes fenugreek, soya bean, pea, red kidney bean, lupin seed and wheat.

*BAT results with basophil activation ≥15% CD63* basophils (positive).
**DISCUSSION**

In this novel study including 96 children who all had primary sensitization to peanut and anaphylaxis during the DBPCFC, reactivity threshold as well as LOAEL was significantly associated with basophil activation, peanut SPT and the ratio of peanut s-IgE/total IgE. Reactivity threshold was further significantly associated with Ara h 2 and the ratio of s-IgG4/s-IgE to peanut, while LOAEL was significantly associated with s-IgE to peanut. Neither reactivity threshold nor LOAEL was significantly associated with age, gender, medical history, allergic comorbidities or lung function. None of the investigated clinical or immunological characteristics were significantly associated with severity of the allergic reaction during food challenge.

The significant association between reactivity threshold as well as LOAEL with basophil activation given by % CD63+ in our children with anaphylaxis to peanut is in line with previous reports in children with varying severity of peanut allergy. In 52 of 124 children with a median age of 5.4 years with a positive peanut challenge, Santos et al. reported that basophil activation given by CD-sens as well as peanut SPT, s-IgE to peanut, Ara h 2 and the ratio of s-IgG4/s-IgE to peanut was significantly associated with reactivity threshold, whereas basophil activation given by % CD63+ was the best predictor of allergy severity grade. In 63 peanut-allergic children with a median age of 6.5 years, Blumchen et al. found similar correlation between LOAEL and basophil activation for both % CD63+ and CD-sens, which is in line with other reports of correlation between CD-sens and % CD63+.

**TABLE 2** Multivariate robust regression analyses for significant associations between peanut reactivity threshold and the lowest observed adverse event level (LOAEL) in children anaphylactic to peanut

<table>
<thead>
<tr>
<th></th>
<th>Reactivity threshold</th>
<th>LOAEL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β-value (95% CI)</td>
<td>P-value</td>
</tr>
<tr>
<td>Peanut SPT (mm)</td>
<td>1.45 (0.08, 2.83)</td>
<td>.04</td>
</tr>
<tr>
<td>Peanut s-IgE (kUA/L)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Ara h 2 s-IgE (kUA/L)</td>
<td>0.09 (0.02, 0.17)</td>
<td>.01</td>
</tr>
<tr>
<td>Ratio peanut s-IgE/total IgE (kUA/L)</td>
<td>29.20 (22.57, 35.82)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Ratio s-IgG4/s-IgE to peanut (ng/mL)</td>
<td>1.69 (1.61, 1.78)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>BAT all children (%)</td>
<td>-0.45 (−0.73, −0.17)</td>
<td>.002</td>
</tr>
</tbody>
</table>

SPT, skin prick test; Ig, immunoglobulin; BAT, basophil activation given as percentage-activated CD63 cells test; -, no significant association.

Associations are given as the relative change (β) related to each mg increase in peanut threshold.

N = 86, BAT was not performed in 10 children due to missed sampling (n = 5) or technical causes (n = 5).

*Included all children with a positive basophil activation (% CD63+ basophils).

**TABLE 3** Robust regression analyses for the associations between basophil activation and immunological and clinical parameters

<table>
<thead>
<tr>
<th></th>
<th>Bivariate</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coeff (95% CI)</td>
<td></td>
</tr>
<tr>
<td>SPT to peanut (mm)</td>
<td>0.60 (−0.34, 1.54)</td>
<td>.21</td>
</tr>
<tr>
<td>Peanut s-IgE (kUA/L)</td>
<td>0.04 (0.02, 0.06)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>S-IgE Ara h 2 (kUA/L)</td>
<td>0.08 (0.02, 0.13)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Ratio peanut s-IgE/total IgE (kUA/L)</td>
<td>58.2 (34.8, 81.7)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Ratio s-IgG4/s-IgE to peanut (ng/mL)</td>
<td>−0.14 (−0.19, −0.09)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>CAPT positivity level*</td>
<td>−7.60 (−14.06, −1.15)</td>
<td>.02</td>
</tr>
</tbody>
</table>

SPT, skin prick test; Ig, immunoglobulin; BAT, basophil activation test, CAPT, conjunctival allergen provocation test.

The coefficient represents the relative change related to percentage increase in activated basophils.

N = 80, BAT was not performed in 10 children due to missed sampling (n = 5) or technical causes (n = 5), non-responders are excluded (n = 6).

* Dilution steps at which the test was considered positive.

**FIGURE 2** Receiver operating characteristic curves predicting very low threshold (<3 mg of peanut protein). The basophil activation test used 5 ng/mL of allergen.
protein), whereas CD2-sens could not be assessed due to strong basophil activation even at the lowest concentration of 2.5 ng/mL allergen extract, reflecting a study population with very low reactivity threshold.

Our finding of significant associations between reactivity threshold and peanut SPT and Ara h 2 is in line with previous reports in children and young adults. In addition to correlation with basophil activation, Blumchen et al. found LOAEL also to correlate with peanut SPT, s-IgE to peanut and Ara h 2 (r = −.20 to −.40), in line with a Danish study reporting correlation between reactivity threshold and Ara h 2 (r = −.30) in 205 subjects 1-26 years of age, of whom 175 had a positive peanut challenge.

The associations between immunological and clinical test results and LOAEL were largely similar to those with reactivity threshold in the present study, with basophil activation, peanut SPT and peanut s-IgE being significantly associated with both, although Ara h 2 was associated with reactivity threshold only, and peanut s-IgE with LOAEL only. The reasons for the different associations for reactivity threshold and LOAEL may be related to the observed larger variation in reactivity threshold compared to the LOAEL, or they may reflect different threshold entities with reactivity threshold requiring moderate objective symptoms while LOAEL appears more sensitive reflecting the lowest cumulated dose at which a mild objective symptom was noted. Additionally, the discrepancies may be due to the estimated rather than measured LOAEL in our study, with the first challenge dose of 3 mg. The estimated LOAEL may also explain why basophil activation was less strongly correlated to LOAEL than reactivity threshold and why the correlation is weak. On the other side, such a low correlation may also be irrelevant. It has been suggested that ideally, the highest dose that does not elicit objective symptoms (NOAEL = no observed adverse effect level) should be determined for defining the exact LOAEL, but this was not possible in our study. The post hoc decision to also determine LOAEL was made to facilitate comparisons across studies with different stopping criteria of OFC. Some studies use only subjective symptoms that occur in consecutive doses as a sign of a positive OFC, although subjective symptoms may occur at doses 20-fold lower than the LOAEL, and some patients report no subjective symptoms prior to the objective symptoms. Determining LOAEL might also be helpful in identifying the peanut exposure amount that elicited the first symptom or sign related to the importance of food labelling.

The severity of allergic reactions was not associated with any of the clinical or immunological characteristics in the present study, in line with the study of Blumchen et al., but in contrast to the MIRABEL study, in which baseline characteristics like age (teenagers and adults), asthma and not having atopic dermatitis were associated with more severe allergic reactions. Also, peanut SPT and peanut and Ara h 2 have been found to correlate to severity grades of allergic reactions. Song et al. performed DBPCFC for different foods (42 of 44 positive with peanut DBPCFC) in 71 sensitized patients with a median age of 16, and found SPT (r = .24) and Ara h 2 (r = .31) to correlate with severity grade, in line with the correlation between Ara h 2 and allergy severity (r = .60) in 175 patients age of 1-26 years with a clinical history of allergic reaction. In 21 children (mean age of 60 months) with peanut allergy and 34 controls (28 tolerant and 6 non-anaphylactic reaction), Wainstein et al. found both peanut SPT and s-IgE to peanut to predict anaphylaxis, whereas Tripodi et al. reported that the end-point titration in SPT, differentiated between Sampson severity grades 3-5, grades 1-2 and negative food challenge in 20 of 47 children with OFC-proven hen's egg allergy. Methodological differences may explain some of the differences, including our reading of the SPT, after 15 minutes vs 30 minutes in the Tripodi study. Finally, after multivariate analyses, Santos et al. found that only basophil activation (%) CD63) and not SPT or other immunological test results was retained as the one marker closest associated with severity grade of allergic reaction. The lack of significant associations between biological markers and severity of allergy reaction in the present study is likely to reflect our relatively homogenous study population; all with primary peanut allergy and anaphylaxis during DBPCFC and 34.4% of the children with a LOAEL ≤ 3 mg of peanut protein, which exceeded the expected 10% with a very low reactivity threshold. It also exceeded the 4.7% of the 63 peanut-allergic children with LOAEL at 3 mg of peanut protein reported by Blumchen et al., although they suggested that their modified food challenge would result in a lower LOAEL.

Also, the DBPCFC was stopped when it was defined positive, which may have prohibited more severe reactions. Basophil activation on the other hand can be regarded as an "in vitro challenge" that can proceed to higher doses. In the present study, it was associated with immunological and clinical test results shown to predict severity grade of allergic reaction, which may suggest a relationship between allergy severity and reactivity. This finding is in line with the previously reported association between severity of allergic reaction and reactivity threshold (P = .027). To our knowledge, the association between basophil activation in the present study with the peanut CAPT dilution at which subjects experienced allergy symptoms and signs has not been shown previously, but is supported by the study of Varney et al. where conjunctival provocation threshold increased significantly after specific immunotherapy to cat dander. Finally, we found basophil activation also to correlate with female gender, a finding we are not aware has been shown previously. The clinical relevance of the latter finding is not clear.

## 4.1 Strengths and limitations

All 96 children had confirmed primary peanut allergy by DBPCFC, with moderate objective signs from at least two organ systems, excluding only skin symptoms and very mild symptoms as anaphylaxis in line with international standards, and the study included a novel CAPT to peanut to assess potential biological correlates. However, as all children had anaphylaxis this may limit the possibility to identify correlations across a wider variation of reactivity thresholds and severity reactions. Although we found immunological and clinical test results to correlate to reactivity thresholds and LOAEL within our study population, the generalizability of our study may be limited to children with similar peanut allergy severity. Further, to be
able to address biomarkers associated with very low reactivity threshold and LOAEL, the study would have benefited from setting a lower starting dose than was chosen for the study. Finally, a potential limitation to interpretation of the results may be that thresholds determined by OFC are not completely reproducible.23,24

5 | CONCLUSION

In children with anaphylaxis to peanut, basophil activation, peanut SPT and the ratio of peanut s-IgE/total IgE were associated with reactivity threshold and LOAEL. None of the clinical or immunological markers appeared sufficient to substitute oral peanut challenge to determine reactivity threshold, and none were associated with severity of the allergic reactions.

ACKNOWLEDGEMENTS

We specially thank all participating children, their siblings and parents for their participation and time. We thank the study nurses, Liv Julie Særdal and Runa Kaldestad, and the dietitian, Grete Simonsen, for their invaluable contribution to this project. We thank Helene Lindvåk for performing screening in part the patients and Havard Ove Skjerven for his help in performing part of the bivariate robust regression analyses. The study was funded by the Extra Foundation, the Kloster Foundation, South-Eastern Norway Regional Health Authority and the Norwegian Asthma and Allergy Association. We thank Thermo Fisher Scientific for supplying kits for the IgE and IgG analyses and Fürst Medical Laboratory for performing them.

CONFLICT OF INTEREST

Magnus P. Borres is employed by Thermo Fisher Scientific, and the other authors declare no conflict of interest for the present study.

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REFERENCES

Paper #2
Feasibility of desensitizing children highly allergic to peanut by high-dose oral immunotherapy

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The study was funded by the Extra Foundation, the Kloster Foundation, South-Eastern Norway Regional Health Authority and the Norwegian Asthma and Allergy Association. Thermo Fisher Scientific supplied kits for the IgE analyses, and Fürst Medical Laboratory performed the IgE analyses.

Abstract

Background: There are limited data on the feasibility, efficacy and safety of high-dose oral immunotherapy (OIT) in children highly allergic to peanuts.

Objective: In children highly allergic to peanut, we primarily aimed to determine the feasibility of reaching the maximum maintenance dose (MMD) of 5000 mg peanut protein or, alternatively, a lower individual maintenance dose (IMD), by OIT up-dosing. Secondarily, we aimed to identify adverse events (AEs) and determine factors associated with reaching a maintenance dose.

Methods: The TAKE-AWAY peanut OIT trial enrolled 77 children 5-15 years old, with a positive oral peanut challenge. Fifty-seven were randomized to OIT with biweekly dose step-up until reaching MMD or IMD and 20 to observation only. Demographic and biological characteristics, AEs, medication and protocol deviations were explored for associations with reaching maintenance dose.

Results: All children had anaphylaxis defined by objective symptoms in minimum two organ systems during baseline challenge. The MMD was reached by 21.1%, while 54.4% reached an IMD of median (minimum, maximum) 2700 (250, 4000) mg peanut protein, whereas 24.5% discontinued OIT. During up-dosing, 19.4% experienced anaphylaxis. Not reaching the MMD was caused by distaste for peanuts (66.7%), unacceptable AEs (26.7%) and social reasons (6.7%). Increased peanut s-IgG4/s-IgE ratio (OR [95% CI]: 1.02 [1.00, 1.04]) was associated with reaching MMD.

Conclusion: Although 75.5% of children with peanut anaphylaxis defined by objective symptoms in minimum two organ systems during baseline challenge. The MMD was reached by 21.1%, while 54.4% reached an IMD of median (minimum, maximum) 2700 (250, 4000) mg peanut protein, whereas 24.5% discontinued OIT. During up-dosing, 19.4% experienced anaphylaxis. Not reaching the MMD was caused by distaste for peanuts (66.7%), unacceptable AEs (26.7%) and social reasons (6.7%). Increased peanut s-IgG4/s-IgE ratio (OR [95% CI]: 1.02 [1.00, 1.04]) was associated with reaching MMD.

KEYWORDS
adverse events, desensitization, feasibility, oral immunotherapy, peanut allergy

Abbreviations: AEs, adverse events; BAT, basophil activation test; CAPT, conjunctival allergen provocation test; DBPCFC, double-blind placebo-controlled food challenge; EoE, eosinophilic oesophagitis; FC, food challenge; IMD, individual maintenance dose; LOAEL, lowest observed adverse effect level; MMD, maximum maintenance dose; OIT, oral immunotherapy; OPAS, Oslo Peanut Allergy Study; PPI, proton pump inhibitor; RCT, randomized controlled trial; SCIT, subcutaneous immunotherapy; sIgE/sIgG/sIgG₄, specific immunoglobulin E, G, G₄; SPT, skin prick test; SU, sustained unresponsiveness; TAKE-AWAY trial, The “Take-Away food allergy; inducing tolerance in children allergic to peanut” trial.

Clinical trial registration: ClinicalTrials.gov number NCT02457416.

The study was performed within the ORAACLE (Oslo Research group of Asthma and Allergy in Children; the Lung and Environment), Oslo University Hospital and the University of Oslo.
1 | INTRODUCTION

Peanut allergy affects 1%-2% of the paediatric population, is seldom resolved and is the main cause of life-threatening allergic reactions in the Western World. The only established treatment is dietary restrictions and rescue medication including epinephrine auto-injectors. However, the possibility of accidental exposure causes anxiety and reduced quality of life.

Peanut oral immunotherapy (OIT) trials are promising for inducing desensitization with acceptable safety profiles, but evidence of sustained unresponsiveness (SU) after OIT discontinuation is limited. The optimal starting dose of peanut OIT is not clear, and there is limited documentation of what maintenance dose would be safe and provide the greatest likelihood of inducing sustained unresponsiveness (SU). The ongoing “Take-Away food allergy; inducing tolerance in children allergic to peanut” trial (the TAKE-AWAY trial) is an open randomized controlled trial which primarily aims to assess SU after 4 years of peanut OIT. At the onset of the TAKE-AWAY trial, peanut OIT trials reported maintenance doses ranging from 125 to 4000 mg peanut protein, with no adverse events (AEs) requiring epinephrine reported during a biweekly step-up protocol to 4000 mg peanut protein. A high maintenance dose confers an increased likelihood of SU in subcutaneous immunotherapy (SCIIT) trials for inhalant and venom allergies, while this issue has not been adequately addressed for OIT. However, in a recent peanut OIT study, maintenance dose was not decisive for SU after 4 weeks of cessation of OIT. A fixed starting dose and a long-term step-up protocol have been associated with fewer AEs and higher retention rates. Even though a recent workshop concluded that severe reactions occur unpredictably at any dose, a possible relationship between allergen dose and the occurrence of anaphylaxis may suggest a low OIT starting dose.

The feasibility of OIT is likely to be influenced by AEs, while other factors are less well known. A low starting dose with a high maintenance dose increases the number of dose steps in an already time-consuming long-term protocol, thereby excluding patients with less time resources.

Therefore, the primary aim of the present study was to determine the feasibility of reaching the predefined maximum maintenance dose (MMD) of 5000 mg peanut protein or, alternatively, a lower individual maintenance dose (IMD), by OIT up-dosing in children highly allergic to peanut. Secondarily, we aimed to identify AEs and determine factors associated with reaching a maintenance dose, and in particular the MMD.

2 | METHODS

2.1 | Study design

The TAKE-AWAY trial, conducted at the Department of Paediatric and Adolescent Medicine, Oslo University Hospital, Ullevål, Norway, consists of four phases: the screening phase (3 days of eligibility determination), up-dosing phase (50-78 weeks), maintenance phase (36 months) and follow-up phase (12 months). The present study explored the up-dosing phase.

Children were recruited from February 2014 to June 2015 from the Oslo Peanut Allergy Study and from in-house or other paediatric allergy clinics in Oslo and the surrounding area.

Inclusion criteria for screening were age 5-15 years, a history of systemic reactions to peanut and/or sensitization to peanut by a peanut skin prick test (SPT) ≥ 3 mm or a peanut sIgE ≥ 0.35 kUA/L. Exclusion criteria were noncontrolled asthma or severe chronic disease (further details in Appendix S1).

Screening included a structured interview, blood samples for serological and immunological analyses, lung function measurements, SPT, conjunctival allergen provocation test (CAPT) and basophil activation test (BAT), followed by a DBPCFC. The DBPCFC was defined positive with at least two moderate objective symptoms in one or more organ systems according to Bock’s criteria. Cumulated peanut protein (mg) intake at positive DBPCFC was recorded as the reactivity threshold, whereas the lowest observed adverse effect level (LOAEL) was calculated post hoc and defined as the amount of peanut protein ingested eliciting mild, objective symptoms. Enrolment in the TAKE-AWAY trial required a positive DBPCFC with a reactivity threshold > 3 mg peanut protein. Of the 213 children referred for screening, 113 did not wish to enter the study, did not fulfill the screening inclusion and exclusion criteria, withdrew during

What is known on this subject

Peanut oral immunotherapy (OIT) is promising for inducing desensitization with acceptable safety profiles, but children highly allergic to peanuts and susceptible of severe systemic reactions to peanut are often excluded from OIT trials. Hence, there is limited information on the feasibility of performing OIT in this group of patients.

This study adds

The present peanut OIT study in children proven highly allergic to peanut during food challenge demonstrates that only 21% reached the predefined maintenance dose of 5000 mg peanut protein, mostly due to reported distaste for peanuts or adverse events. However, 75% of the children were able to reach an individual maintenance dose. Anaphylaxis occurred in 19.4% during up-dosing, causing discontinuation of OIT in 36.3% of these children.

Impact on current management guidelines

High-dose peanut OIT may be initiated in children highly allergic to peanut, but distaste for peanuts and adverse events may limit the likelihood of successful OIT. The high risk of anaphylaxis during treatment questions the safety of OIT in these children.
screening, and had a negative DBPCFC or a positive DBPCFC but with a reactivity threshold ≤3 mg peanut protein.22
Randomization to OIT vs observation followed an initial 2:1 block size, and restarted by approval from the Regional Committee for Medical and Health Research Ethics (the ethical committee) when the OIT starting dose was lowered (further details in Table S1).
Written informed consent was obtained from both parents after oral and written study information.
TAKE-AWAY was approved by the ethical committee (number 2013/430) with regular communications in case of severe or unexpected AEs, and registered at ClinicalTrials.gov (number NCT02457416).

2.2 | Study population
The present study includes the 57 children (5-15 years of age) randomized to peanut OIT. Anaphylaxis was defined as objective symptoms from at least two organ systems in line with European Academy of Allergy and Clinical Immunology (EAACI) task force position papers,27,28 modified for children by Vetander et al.29

2.3 | Immunological investigations
Specific IgE, IgG and IgG4 were analysed using the Phadia CAP System FEIA (Thermo Fisher, Uppsala, Sweden), with positive tests defined as sIgE ≥ 0.35 kUaL, IgG > 2.0 mg/L and IgG4 > 0.07 mg/L. The BAT is described in the Appendix S1.

2.4 | Up-dosing protocol of the oral immunotherapy
The peanut OIT followed a biweekly step-up long-term protocol with a fixed starting dose and a predefined MMD of 5000 mg peanut protein (details in the Appendix S1 and Table S2). The OIT starting dose was initially 5 mg peanut protein based on previously published studies9,18 and results from the OPAS trial,23 but lowered to 1 mg due to low reactivity thresholds in the referred patients.22 For the lowest doses, the allergen source was peanut flour (Golden Peanut Company, Alpharetta, GA, USA). Because larger amounts of peanut flour mixed with other food became too sticky to eat, all but one patient switched to roasted peanuts at OIT doses of 65-500 mg peanut protein. Each increasing OIT dose was discussed with the patient and their guardian and ingested under observation at the hospital, followed by daily intake of this dose at home for 14 days.

In case of intolerable distaste or AEs, or if AEs resulted in three consecutive unsuccessful attempts to dose step-up, the IMD was considered reached (further details in Appendix S1). Withdrawal followed self-discontinuation of OIT, intolerable or severe AEs or more than two anaphylactic reactions. All unexpected severe AEs were reported to an independent safety board. In case of ongoing infections, asthma exacerbations, excessive tiredness or vaccinations, children were advised to postpone the daily OIT dose to the next day. The OIT was resumed at home if less than three consecutive doses were missing, and in hospital if three or more doses were missed. Exercise within 2 hours after the OIT dose was discouraged.
Registration of peanut intake, AEs, use of medication and accidental exposure to peanut were based upon daily symptom diary recordings. Grading AEs followed the modified Bock’s criteria,24,25 as described in the Appendix S1.
All participants received prescriptions of epinephrine auto-injectors and antihistamines and a written treatment plan for AEs and had around-the-clock access to the study paediatricians.

2.5 | Outcomes and explanatory factors
The primary outcome was the feasibility of reaching MMD, defined by the proportion of children who reached the predefined MMD of 5000 mg peanut protein. The secondary outcome was the proportion of children who reached the IMD (<5000 mg peanut protein).
Potential explanatory factors of reaching the MMD or the lower IMDs were AEs characterized by the involved organ(s) and classified into either subjective and mild objective, moderate or severe (including anaphylaxis) in line with the modified Bock’s criteria,25 baseline characteristics, biological markers, LOAEL, severity grade of anaphylaxis at screening DBPCFC, medication for AEs and protocol deviations (dose reduction or postponed up-dosing due to social events, AEs or infections).

2.6 | Statistical analyses
The statistical power analyses at study onset were based upon studies reporting that up to 80% of peanut-allergic children were desensitized using a step-up peanut OIT12,13 and development of spontaneous tolerance in 20%.2 In children with severe peanut allergy, we expected desensitization in 57%. A treatment group of 40 and a control group of 20 subjects would provide a statistical power of 80% at a five per cent significance level.
Due to non-normal distribution, continuous baseline characteristics are presented by geometric mean with 95% confidence intervals (CI) and categorical data as number of cases (n) with percentage (%), while potential differences between groups were analysed using the Mann-Whitney U test for continuous data and the Pearson’s chi-square test for categorical data.
To determine the statistical significance of desensitization based upon the individual difference in peanut daily maintenance dose to the reactivity threshold and LOAEL at baseline, we used a paired-sample t test. The associations between explanatory factors and feasibility of desensitization were assessed using bivariate logistic regression analyses with the proportion of children who reached the MMD versus the proportion who reached either IMD or discontinued OIT as the dependent variable. The analyses were duplicated with the proportion of children who reached either IMD or IMD as the dependent variable versus the proportion who discontinued OIT.
A one-way ANOVA was used to analyse the overall difference between the three groups of children who reached the MMD, those
who reached the IMD and those who discontinued OIT with the latter group as reference. One-way ANOVA was also used to analyse the overall difference between AEs occurring in the three dose intervals of the up-dosing phase (1-65, 66-800 and 801-5000 mg peanut protein). In the case of a significant overall P-value, the Dunnett’s post hoc test was used to confirm between which groups the statistically significant difference had occurred.

Statistical analyses were performed using the Statistical Analysis System (SAS, version 9.3; SAS Institute Inc., Chapel Hill, NC, USA) and IBM Statistical Package for the Social Sciences (IBM SPSS Statistics, version 21.0.1.; IBM Corp, Armonk, NY, USA).

A 2-tailed P-value of ≤0.05 was considered statistically significant.

3 | RESULTS

All 57 children randomized to active peanut OIT were primary sensitized to peanut with geometric mean (min, max) sIgE to Ara h 2 of 56.2 (0.82, 492.0) kUA/L and had a LOAEL of 18.4 (11.8, 28.6) mg peanut protein, and 78.9% had a history of a anaphylaxis to peanut. During baseline DBPCFC, all children randomized to OIT, as well as the control children in the TAKE-AWAY trial, reacted with anaphylaxis. The baseline characteristics including grading of anaphylaxis are reported in Table 1 for children reaching MMD or IMD or those who discontinued peanut OIT, as well as for the controls.

The predefined MMD of 5000 mg peanut protein was reached by 21.1% (n = 12) of the children, while 54.4% (n = 31) reached a lower IMD and 24.5% (n = 14) discontinued (Table 2). The median (min, max) IMD reached was 2700 (250, 4000) mg of peanut protein, which was 207 (3.1, 1666.7) (P < 0.001 for both) times higher than LOAEL at screening.

The most common reasons for not reaching the MMD were distaste for peanuts in 66.7% (n = 28 within IMD and 2 discontinued) of the children and AEs in 26.7% (n = 3 within IMD and 9 discontinued) and social reasons in 6.7% (n = 3 discontinued; two found the treatment too time-consuming, while one discontinued due to parents’ divorce). Distaste for peanuts was reported as a daily challenge in 77.2% of the children.

Mild AEs were reported in relation to 13.9% of the OIT doses. One child only did not report any AEs. The AEs occurred more often in the first (1-65 mg peanut protein), compared with the second (66-800 mg) and third (801-5000 mg peanut protein) dose interval steps (overall P = 0.03), with a statistically significant difference between the first and the last dose intervals (Figure 1). The AEs, mostly oral itching (43.5%) or other gastrointestinal (GI)-related (42.5%) symptoms, occurred more frequently during the first two, compared with the remaining days in each up-dosing period (P = 0.001; Figure 1). Dyspeptic symptoms were reported as the main reason for discontinuation in two children, while six children with dyspeptic symptoms had spontaneous (n = 2) or proton pump inhibitor (PPI)-related (n = 4) symptom relief and continued treatment throughout the up-dosing phase.

Moderately graded AEs constituted 0.6% of all AEs (Table 3), and 11 anaphylactic events classified as moderate occurred in 11 children (0.06% of the doses), with epinephrine administered in six of the episodes (Table S3). All but two anaphylactic reactions were preceded by known augmenting factors: exercise within two hours of a dose, ongoing infection, excessive tiredness, impaired compliance to OIT or asthma treatment. In comparison, the control group did not experience any anaphylactic events to peanut.

Children discontinuing OIT reported significantly more AEs per dose step per child than children who reached any maintenance dose (MMD or IMD) median (min, max) 2.45 (0.27, 10.50) vs 1.04 (0, 12.90), respectively (P = 0.01), whereas moderately graded AEs were similarly reported in these two groups (P = 0.61).

The only identified significant predictor of reaching a maintenance dose was the peanut sIgG4/sIgE ratio ratio that was associated with MMD in the bivariate (Table 4) and the multivariate logistic regression model (not shown). Including Sampson’s anaphylaxis severity grading did not influence the results (not shown). We found nonsignificant trends for associations between LOAEL and MMD, and between sIgE to peanut, sIgE to Ara h 2, the peanut sIgE/total IgE ratio and AEs and any maintenance dose (MMD + IMD) (P = 0.06–0.08) (Table 4).

4 | DISCUSSION

In the randomized controlled peanut OIT TAKE-AWAY trial, desensitization to peanut was feasible for most children highly allergic to peanut and reacting with anaphylaxis at baseline food challenge. The high predefined MMD of 5000 mg peanut protein was reached by 21.1%, whereas 54.4% reached the lower IMD. Failure to reach the MMD was most often due to distaste for peanuts, whereas AEs were the main reason for discontinuation. Anaphylaxis occurred in 19.3% of the children during the up-dosing phase. Peanut sIgG4/sIgE ratio was the only significant predictor of reaching MMD, while AEs, baseline sIgE to peanut or Ara h 2 and LOAEL showed a nonsignificant tendency to be associated with the maintenance dose reached.

Desensitizing children with anaphylaxis to peanut by reaching the MMD of 5000 mg peanut protein was feasible in 21.1% only, while 73.7% reached a maintenance dose of at least 500 mg in line with the 63.6% to 86.9% previously reported. Based upon the limited experience with peanut OIT and MMD varying from 125 mg to 4000 mg of peanut protein trials, our high MMD was chosen to increase the likelihood of SU in children with severe peanut allergy. However, 5000 mg peanut protein represents approximately 25 whole peanuts, a quantity that was challenging for many children as they developed distaste for peanuts. Few reports have addressed this issue previously, except one study reporting distaste for peanuts as the reason for withdrawal of one patient and reduction of maintenance dose in two patients.

The 24.5% discontinuation of OIT in our cohort of children highly allergic to peanut is in line with previously published peanut OIT studies, ranging from 10% to 32%.
### TABLE 1  Baseline characteristics of children randomized to peanut OIT and controls in the TAKE‐AWAY trial

<table>
<thead>
<tr>
<th>Total patients receiving OIT (n = 57)</th>
<th>Patients reaching MMD (n = 12)</th>
<th>Patients reaching IMD (n = 31)</th>
<th>Patients discontinued OIT (n = 14)</th>
<th>Overall P-value between MMD, IMD and dis-continued</th>
<th>Controls (n = 20)</th>
<th>P-value between OIT patients and controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (median, min-max)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>10.1 (5.2, 15.2)</td>
<td>10.7 (7.2, 15.2)</td>
<td>8.5 (5.2, 14.4)</td>
<td>10.2 (5.4, 15.1)</td>
<td>0.02**</td>
<td>8.9 (5.1, 13.3)</td>
<td>0.28</td>
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<td>Male</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>31 (54.4)</td>
<td>7 (58.3)</td>
<td>14 (45.2)</td>
<td>10 (71.4)</td>
<td>0.26</td>
<td>13 (65.0)</td>
<td>0.41</td>
</tr>
<tr>
<td>History of anaphylaxis to peanut</td>
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</tr>
<tr>
<td>45 (78.9)</td>
<td>9 (75.0)</td>
<td>23 (74.2)</td>
<td>13 (92.8)</td>
<td>0.82</td>
<td>18 (90.0)</td>
<td>0.63</td>
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<td>Current asthma</td>
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</tr>
<tr>
<td>24 (42.1)</td>
<td>5 (41.2)</td>
<td>11 (35.4)</td>
<td>8 (57.1)</td>
<td>0.37</td>
<td>9 (45.0)</td>
<td>0.78</td>
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<td>Allergic rhinitis</td>
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<tr>
<td>15 (26.3)</td>
<td>5 (41.7)</td>
<td>8 (25.8)</td>
<td>2 (14.3)</td>
<td>0.53</td>
<td>8 (40.0)</td>
<td>0.83</td>
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<tr>
<td>47 (82.5)</td>
<td>11 (91.7)</td>
<td>25 (80.6)</td>
<td>11 (78.6)</td>
<td>0.64</td>
<td>14 (73.9)</td>
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<td>Allergy to tree-nuts</td>
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<td>101.0 (91.2, 112.2)</td>
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<td>110.6 (70.4, 173.8)</td>
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<td>129.3 (88.9, 188.0)</td>
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<td>56.2 (37.2, 87.1)</td>
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<td>67.0 (47.8, 94.0)</td>
<td>89.6 (33.9, 235.9)</td>
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<td>22.4 (8.4, 58.9)</td>
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<td>0.4 (0.0, 1.5)</td>
<td>0.3 (0.1, 0.6)</td>
<td>0.3 (0.1, 0.6)</td>
<td>0.5 (0.4, 0.7)</td>
<td>0.002*</td>
<td>0.2 (0.1, 0.6)</td>
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<td>Peanut sIgE [ng/mL]</td>
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<tr>
<td>5.7 (3.7, 8.9)</td>
<td>15.5 (3.4, 60.3)</td>
<td>4.9 (2.9, 8.3)</td>
<td>3.3 (1.4, 7.8)</td>
<td>0.04*</td>
<td>5.3 (2.6, 10.9)</td>
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<td>2.6 (2.3, 3.0)</td>
<td>3.0 (2.2, 4.1)</td>
<td>2.6 (2.3, 3.1)</td>
<td>2.3 (1.7, 3.1)</td>
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<td>3.1 (2.7, 3.4)</td>
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<td>68.0 (61.6, 75.1)</td>
<td>34.4 (15.5, 75.9)</td>
<td>49.6 (34.7, 70.8)</td>
<td>63.7 (46.0, 88.2)</td>
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<td>51.2 (32.0, 85.4)</td>
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<td>Number of anaphylaxis</td>
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<td>57 (100.0)</td>
<td>12 (100.0)</td>
<td>31 (100.0)</td>
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<td>Anaphylaxis severity grade</td>
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<tr>
<td>1.6 (1.4, 1.7)</td>
<td>1.4 (1.1, 1.8)</td>
<td>1.7 (1.5, 1.9)</td>
<td>1.6 (1.3, 1.9)</td>
<td>0.22</td>
<td>1.7 (1.5, 2.0)</td>
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<td>Sampson</td>
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<tr>
<td>2.6 (2.4, 2.8)</td>
<td>2.8 (2.4, 3.4)</td>
<td>2.5 (2.3, 2.8)</td>
<td>2.6 (2.3, 3.1)</td>
<td>0.49</td>
<td>2.8 (2.5, 3.2)</td>
<td>0.23</td>
</tr>
</tbody>
</table>

(Continues)
**TABLE 1** (Continued)

<table>
<thead>
<tr>
<th>Total patients receiving OIT (n = 57)</th>
<th>Patients reaching MMD (n = 12)</th>
<th>Patients reaching IMD (n = 31)</th>
<th>Patients discontinued OIT (n = 14)</th>
<th>Overall P-value between MMD, IMD and discontinued OIT</th>
<th>Controls (n = 20)</th>
<th>P-value between OIT patients and controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Use of adrenaline</td>
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</tr>
<tr>
<td>30 (52.6)</td>
<td>5 (41.7)</td>
<td>17 (54.8)</td>
<td>8 (57.1)</td>
<td>0.70</td>
<td>5 (25.0)</td>
<td>0.03</td>
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<tr>
<td>LOAEL (mg peanut protein)</td>
<td>18.4 (11.8, 28.6)</td>
<td>45.9 (10.2, 207.1)</td>
<td>15.1 (10.3, 22.1)</td>
<td>36.2 (15.4, 84.4)</td>
<td>0.05</td>
<td>5 (25.0)</td>
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<tr>
<td>Reactivity threshold (mg peanut protein)</td>
<td>46.2 (29.7, 72.0)</td>
<td>108.7 (29.3, 402.9)</td>
<td>32.1 (22.0, 46.9)</td>
<td>93.3 (40.0, 222.4)</td>
<td><strong>0.01</strong></td>
<td>75.9 (33.1, 173.8)</td>
</tr>
</tbody>
</table>

MMD, subjects who reached the maximum maintenance dose; IMD, subjects who reached the individual maintenance dose; SPT, skin prick test; Ig, immunoglobulin; BAT, basophil activation test; CAPT, conjunctival allergen provocation test; LOAEL, lowest observed adverse effect level; OIT, oral immunotherapy; DBPCFC, double-blind placebo-controlled food challenge. Variables are given as geometric mean (95% CI) or n (%), except age which is given as median (min, max). Bold values are statistically significant (P < 0.05).

One-way ANOVA was applied to determine statistically significant differences between group means and the Dunnett's post hoc test to confirm which groups differed.

Anaphylaxis severity was graded by two grading systems according to the modified EAACI position papers \(^\text{27,28}\) ranging from 1 to 3 and the method of Sampson \(\text{Grading of Food-Induced Anaphylaxis According to Severity of Clinical Symptoms}^\text{42}\) ranging from 1 to 5.

LOAEL is defined as the cumulated peanut protein (mg) ingested eliciting mild, objective symptoms. Reactivity threshold is defined as the cumulative peanut protein (mg) ingested at positive DBPCFC, with at least two moderate objective symptoms in one or more organ systems according to Bock's criteria.\(^\text{24-26}\)

- **Atopic disease includes asthma, allergic rhinitis, atopic dermatitis and allergic conjunctivitis.**
- All food allergy including peanut and tree nut allergy.
- Hazelnut, almond, cashew nut, pistachio nut, walnut, pecan nut, brazil nut and macadamia nut.
- Fenugreek, soya bean, pea, red kidney bean, lupin seed and wheat.
- The CAPT was recorded positive ranging from dilution level 1 (1:160) to 5 (1:1).
- Statistically significant difference between MMD and discontinued.
- Statistically significant difference between MMD and IMD.
were the cause of OIT discontinuation in 55% of our children (three with anaphylaxis and two with dyspeptic symptoms), in line with pooled data of three OIT studies including 104 children in which 20% discontinued treatment mostly due to AEs (65%) and logistic reasons (35%).

Our finding that 13.9% of the doses elicited mild AEs is in line with previously published OIT studies, including the 13.5% AEs reported in the STOP II study of 99 children with allergy severity ranging from a mild allergic reaction in one organ system (24.2%) to severe respiratory symptoms (5.1%). Our children most frequently reported GI-related AEs including oral itching and stomach ache in line with previous studies as well as effect of oral antihistamines if simultaneous dyspeptic symptoms were absent. Dyspepsia, reported by eight (14%) of our children, may be a symptom of OIT-related eosinophilic oesophagitis (EoE), estimated to develop in 2.7% undergoing OIT. In two children, OIT was discontinued due to dyspepsia, while three of the four children treated with PPI became asymptomatic and the fourth reported decreasing symptoms. Mild AEs occurred significantly more often during the first two days of each up-dosing period and in the first third of the dose steps, as previously described. The reported 0.6% of moderate AEs is somewhat lower than the 2.6% observed in previous OIT trials with more than half of them experiencing anaphylaxis already at the LOAEL. These findings are in line with previously published reports that suggest an association with high sIgE to peanut and low LOAEL, and severity of allergic reactions. All our children, older than in

4.1 | Strengths and limitations

To our knowledge, the TAKE-AWAY children had sIgE to peanut higher and LOAELs lower than in previous OIT trials with more than half of them experiencing anaphylaxis already at the LOAEL. These findings are in line with previously published reports that suggest an association with high sIgE to peanut and low LOAEL, and severity of allergic reactions. All our children, older than in

<p>| TABLE 2 | Characteristics of children who discontinued oral immunotherapy without reaching a maintenance dose |</p>
<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age years</th>
<th>Peanut sIgE kUA/L</th>
<th>Ara h 2 sIgE kUA/L</th>
<th>LOAEL mg peanut protein</th>
<th>Reactivity threshold mg peanut protein</th>
<th>Dose at discontinuation mg peanut protein</th>
<th>Reason for discontinuation mg peanut protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.8</td>
<td>93.2</td>
<td>82.7</td>
<td>110.8</td>
<td>110.8</td>
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<td>Social</td>
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<tr>
<td>2</td>
<td>11.3</td>
<td>493.0</td>
<td>221.0</td>
<td>35.8</td>
<td>35.0</td>
<td>5</td>
<td>AEs</td>
</tr>
<tr>
<td>3</td>
<td>14.3</td>
<td>26.2</td>
<td>14.6</td>
<td>110.8</td>
<td>243.0</td>
<td>450</td>
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<td>77.0</td>
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<td>6</td>
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<td>43.0</td>
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<td>10.9</td>
<td>271.0</td>
<td>158.0</td>
<td>43.0</td>
<td>43.0</td>
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<td>AEs</td>
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<tr>
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<td>475.0</td>
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<td>87.4</td>
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<td>629.0</td>
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<td>3.0</td>
<td>13.0</td>
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<td>13.0</td>
<td>143.0</td>
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<td>0.8</td>
<td>143.0</td>
<td>443.0</td>
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<td>131.0</td>
<td>13.0</td>
<td>443.0</td>
<td>5</td>
<td>AEs</td>
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</table>

AEs, adverse events; LOAEL, lowest observed adverse effect level.
some but younger than in other studies, reacted with anaphylaxis during the pre-OIT DBPCFC, which may be explained by most of our children having a history of anaphylaxis to peanut as well as not defining the food challenge positive until the occurrence of two objective symptoms. Some studies define a food challenge positive already by the occurrence of reproducible subjective symptoms as suggested in the PRACTALL guidelines, and one cannot rule out that an anaphylaxis would occur if another dose was given. Calculating the objective LOAEL enables comparison between studies.

Switching ingestion of defatted flour to whole roasted peanuts at a wide range of doses (65-500 mg) may influence the efficacy of the OIT, as whole peanuts are more aromatic and were disliked. A placebo arm could have strengthened the study as distaste was the main reason for not reaching the MMD. However, this was regarded un-ethical based on the unfavourable ratio of treatment burden to expected benefit in the placebo group. A blinded vehicle to our high-dose peanut OIT also seemed unfeasible.

An OFC after up-dosing phase would have been preferable, but was not repeated for ethical reasons.
CONCLUSION

In children highly allergic to peanut and reacting with anaphylaxis at baseline food challenge, reaching a high MMD of 5000 mg peanut protein was feasible for every fifth child. More than half of the children rather stopped at the lower IMD, mainly due to distaste for peanuts. Every fifth child experienced an anaphylactic adverse event, which questions the safety of OIT for these patients.

ACKNOWLEDGMENTS

We specially thank all participating children and parents for their participation and time. We thank the study nurses, Liv Julie Sørø.

TABLE 3

Adverse events (AEs) related to oral immunotherapy in children highly allergic to peanut

<table>
<thead>
<tr>
<th>Total patients receiving OIT (n = 57) (doses = 18 470)</th>
<th>Patients reaching MMD (n = 12) (doses = 5292)</th>
<th>Patients reaching IMD (n = 31) (doses = 11 536)</th>
<th>Patients discontinued OIT (n = 14) (doses = 1642)</th>
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</thead>
<tbody>
<tr>
<td>Total AEs</td>
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<td></td>
</tr>
<tr>
<td>Patients, n (%)</td>
<td>56 (98.2)</td>
<td>12 (100.0)</td>
<td>30 (96.8)</td>
</tr>
<tr>
<td>Events, n (%)</td>
<td>2560 (13.9)</td>
<td>290 (5.5)</td>
<td>1957 (17.0)</td>
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<td>Mild AEs, total</td>
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<tr>
<td>Patients, n (%)</td>
<td>56 (98.2)</td>
<td>12 (100.0)</td>
<td>30 (96.8)</td>
</tr>
<tr>
<td>Events, n (%)</td>
<td>2473 (13.4)</td>
<td>290 (5.5)</td>
<td>1725 (15.0)</td>
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<td>Moderate AEs</td>
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<td>Patients, n (%)</td>
<td>22 (38.6)</td>
<td>4 (33.3)</td>
<td>14 (45.2)</td>
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<td>Events, n (%)</td>
<td>116 (6.6)</td>
<td>21 (1.4)</td>
<td>81 (7.0)</td>
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<td>Oral itching</td>
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<td>Patients, n (%)</td>
<td>49 (86.0)</td>
<td>10 (83.3)</td>
<td>28 (90.3)</td>
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<tr>
<td>Events, n (%)</td>
<td>1096 (5.9)</td>
<td>173 (3.3)</td>
<td>822 (7.1)</td>
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<td>GI-related AEs a</td>
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<td>Patients, n (%)</td>
<td>48 (84.2)</td>
<td>7 (58.3)</td>
<td>27 (87.1)</td>
</tr>
<tr>
<td>Events, n (%)</td>
<td>1100 (6.0)</td>
<td>31 (0.6)</td>
<td>959 (8.3)</td>
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<td>Skin-related AEs</td>
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<td>Patients, n (%)</td>
<td>43 (75.4)</td>
<td>9 (75.0)</td>
<td>27 (87.1)</td>
</tr>
<tr>
<td>Events, n (%)</td>
<td>140 (0.8)</td>
<td>26 (0.5)</td>
<td>95 (0.8)</td>
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<td>Respiratory-related AEs</td>
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</tr>
<tr>
<td>Patients, n (%)</td>
<td>37 (64.9)</td>
<td>10 (83.3)</td>
<td>19 (61.3)</td>
</tr>
<tr>
<td>Events, n (%)</td>
<td>59 (0.3)</td>
<td>10 (0.2)</td>
<td>31 (0.3)</td>
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<tr>
<td>Anaphylaxis</td>
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</tr>
<tr>
<td>Patients, n (%)</td>
<td>11 (19.3)</td>
<td>2 (16.7)</td>
<td>5 (16.1)</td>
</tr>
<tr>
<td>Events, n (%)</td>
<td>11 (0.6)</td>
<td>2 (0.04)</td>
<td>5 (0.04)</td>
</tr>
<tr>
<td>Anaphylaxis severity grade: Sampson, median (min, max)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.0 (2.0, 3.0)</td>
<td>3.0 (2.0, 3.0)</td>
<td>3.0 (2.0, 3.0)</td>
</tr>
<tr>
<td>Used epinephrine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients, n (%)</td>
<td>6 (10.5)</td>
<td>2 (16.7)</td>
<td>2 (6.5)</td>
</tr>
<tr>
<td>Events, n (%)</td>
<td>6 (0.03)</td>
<td>2 (0.04)</td>
<td>2 (0.02)</td>
</tr>
<tr>
<td>Used acute salbutamol b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients, n (%)</td>
<td>5 (8.8)</td>
<td>1 (8.3)</td>
<td>2 (6.5)</td>
</tr>
<tr>
<td>Events, n (%)</td>
<td>5 (0.03)</td>
<td>1 (0.02)</td>
<td>3 (0.03)</td>
</tr>
</tbody>
</table>

AEs, adverse events; MMD, subjects who reached the maximum maintenance dose; IMD, subjects who reached the individual maintenance dose. Percentages were based on the number of patients in each group, stratified by reaching maximum maintenance dose (MMD), a lower individual maintenance dose (IMD) or discontinuing treatment. Patients were counted once per category.

Grading of OIT-related AEs was in line with the modified Bock’s criteria by Sampson et al. a

aExcept oral itching.

bIn relation to OIT AEs.
and Runa Kaldestad, and the cook, Grete Simonsen, for their invaluable contribution to this project. Helene Lindvik performed the screening of patients recruited from the Oslo Peanut Allergy Study, and Hege Hjertholm performed the BAT analyses. We also thank Tuva Reier-Nilsen for modelling and Frode Reier-Nilsen for taking and editing the photographs for the graphical abstract. We thank Thermo Fisher Scientific for supplying kits for the IgE, IgG and IgG4 analyses and Fürst Medical Laboratory for performing the analyses.

CONFLICT OF INTEREST

Magnus P. Borres is employed by Thermo Fisher Scientific. The other authors declare no conflict of interest for the present study.

AUTHOR CONTRIBUTIONS

G. Håland, K. C. Lødrup Carlsen, and K-H. Carlsen designed the project. M. M. Michelsen, G. Håland and T. Reier-Nilsen included patients, collected data and carried out the up-dosing protocol. U. Nygaard, E. Namork and M. P. Borres were responsible for the immunological tests. The analytic approaches were designed by G. Håland, K. C. Lødrup Carlsen, K-H. Carlsen, P. Mowinckel and T. Reier-Nilsen, while the main statistical analyses were performed by T. Reier-Nilsen in collaboration with the statistician P. Mowinckel. T. Reier-Nilsen is the lead author with significant contribution from all authors who read and approved the submitted manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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Parent and child perspective of quality of life in a randomized controlled peanut oral immunotherapy trial.

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The study was performed within the ORAACLE (Oslo Research group of Asthma and Allergy in Children; the Lung and Environment), Oslo University Hospital and the University of Oslo.

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Child, Desensitization, Double-blind placebo controlled food challenge, Oral Immunotherapy, Peanut allergy, Perceived treatment burden, Self- and proxy-reports, Quality of life, Visual analogue scale

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Conflicts of interest: Karin C. Lødrup Carlsen has received governmental and non-governmental research grants. Magnus P. Borres is employed by Thermo-Fisher Scientific. The other authors declare no conflict of interest.

Clinical trial registration: ClinicalTrials.gov number NCT02457416.

What is already known about this topic?
The improved quality of life (QoL) observed in children with peanut allergy are predominantly based on parents’ assessment. Discrepancies between the children’s and the parents’ assessments of the children’s QoL may impact the use of patient-reported outcomes (PROs) in oral immune therapy (OIT).

What does this article add to our knowledge?
The present randomized controlled peanut OIT study includes child self-reports and parental proxy-reports to assess QoL. Compared with the children’s reports, parents reported a two-fold increase in their children’s QoL from before to after two years of OIT, and the improved QoL was significantly associated with OIT for these parental proxy-reports only.

How does this study impact on current management guidelines?
Evaluation of OIT efficacy based upon PROs may be more appropriate using the child-reported rather than the parents’ proxy-reported QoL which may over-estimate improvement in QoL with this treatment.
Abbreviations:

AEs – adverse events

BAT – basophil activation test

CAPT – conjunctival allergen provocation test

%CD63+ - allergen induced basophil reactivity (proportion of activated basophils)

CI – confidence interval

DBPCFC – double blind placebo controlled food challenge

FAQL-PB - Food Allergy Quality of Life – Parental Burden

GI – gastro-intestinal

s-IgE/s-IgG/s-IgG₄ – specific Immunoglobulin E, G, G₄

OFC – oral food challenge

OIT - oral immunotherapy

PedSQL 4.0 - Pediatric Quality of Life Inventory Version 4.0

PRO – Patient-related outcome

QoL – quality of life

SPT – skin prick test

SU – sustained unresponsiveness

TAKE-AWAY trial – The “Take-Away food allergy; inducing tolerance in children allergic to peanut” trial

QoL at Y2 in the TAKE-AWAY trial 03.12.18
VAS – visual analogue scale

Y₀ – at screening (enrolment)

Y₁ – at completed up-dosing, approximately after 1 year of treatment, 1 year for controls

Y₂ – at one year of maintenance treatment/after 2 years of treatment
SUMMARY

Background

Improved quality of life (QoL) after oral immunotherapy (OIT) in peanut allergic children is often reported by their parents, while the child’s perspective is less clear.

Objective

We aimed to explore if two years of OIT improved QoL in children with peanut allergy and to identify factors influencing change in QoL.

Methods

In the open labelled randomized controlled TAKE-AWAY peanut OIT trial, QoL was assessed in 77 5-15 year-olds with anaphylaxis to peanuts. The children and their parents fulfilled the Pediatric Quality of Life Inventory Version 4.0 at enrolment (Y0) after one year (end of up-dosing) (Y1) and after two years (Y2) of OIT (n=57) or observation only (n=20). Perceived treatment burden was recorded by visual analogue scales, including adverse events (AEs). An open food challenge (OFC) was performed at Y2.

Results

At Y2, 18 children had discontinued OIT, 2/39 OIT children refused OFC, while 35/37 were desensitized to 7500 mg peanut protein. From Y0 to Y2, the child’s mean change (95% confidence intervals) in QoL was 4.4 (0.5, 8.3) by child self-report and twice as large by parental proxy-report (9.3 (4.3, 14.3)) (both p<0.0001), with no significant improvement in the 20 controls. The change in QoL was significantly associated with OIT for the parental proxy-reports only (p=0.002). Neither treatment burden nor AEs significantly predicted changes in QoL.

Conclusion
Two years of OIT improved child QoL as reported by parents, but not by the children, suggesting that parents may over-estimate improvement in child QoL by OIT.
INTRODUCTION

Peanut oral immunotherapy (OIT) appears promising for inducing desensitization (no allergic reaction while regularly exposed to the allergen) (1-5), which is the first step to sustained unresponsiveness (SU) after OIT discontinuation. In line with recommendations, measurements of treatment effect should include patient-reported outcomes (PROs) (6) such as QoL assessments including physical, emotional (or psychological) and social domains.

Improved QoL has been reported in children after OIT (1, 7-12) despite adverse events (AEs) (13). Most studies on QoL during OIT are based on parents’ assessments of their child’s QoL (1, 7-10), sometimes referred to as proxy-reports (14, 15), while a recent study showed that parents of 122 0–18 years old children with peanut, hazelnut or egg allergy assessed their children’s QoL better than did the children themselves (16), questioning the appropriateness of relying on parental proxy-reports alone. While one study demonstrated improved QoL after OIT by both parental proxy-reports and child self-reports, the lack of a control group limited the possibility to assess if this was due to the OIT (11). Information on one-dimensional PROs using e.g. a visual analogue scale (VAS) of treatment burden is largely lacking.

Peanut OIT is projected soon to become available as a treatment for peanut allergy. However, a recent editorial (17) raised concerns regarding OITs in children highly sensitized to peanuts due to high risk of systemic AEs and distaste for peanuts and low feasibility of reaching a high maintenance dose, based upon our ongoing 4-year peanut OIT; “Take-Away food allergy: Inducing tolerance in children allergic to peanut” trial (the TAKE-AWAY trial) (18).

This open labelled randomized controlled trial has an overall objective to assess if four years of OIT followed by one year without regular peanut exposure can induce SU in children with primary peanut allergy. In the present study, we explored the effect of OIT on desensitization and PROs reported by the children and their guardians. Our primary aim was to explore if peanut OIT improved child QoL as reported by the parents and/or the children themselves.
Secondarily, we aimed to explore if factors including perceived treatment burden influenced change in QoL from pre-treatment to second year of treatment. Finally, we aimed to explore if ineligibility to OIT affected QoL one year later.
METHODS

Study design

As shown in supplement Figure 1, the TAKE-AWAY trial (5, 18) has four phases; screening for eligibility (Y₀), the OIT up-dosing phase ending after 50-78 weeks (Y₁), 36 months maintenance therapy with desensitization assessed after two years OIT (Y₂) and the follow-up of 12 months with SU assessed at Y₅ (to be completed in 2020).

The present study reports QoL assessed at Y₀, Y₁ and Y₂. Children who discontinued treatment were not assessed at subsequent time-points, while QoL was reassessed at Y₁ in the 19 OIT-ineligible children.

Children 5-15 years old with sensitization to peanut by a peanut skin prick test (SPT) ≥ 3 mm and/or a specific IgE (s-IgE) to peanut ≥ 0.35 kUA/L or with a history of systemic reactions to peanut were screened for study enrolment at the Department of Pediatric and Adolescent Medicine, Oslo University Hospital, Ullevål, Norway from February 2014 to June 2015, as previously described (18).

Enrolment further required a positive double-blind placebo-controlled food challenge (DBPCFC) defined by at least two objective symptoms in one or more organ systems and a reactivity threshold > 3 mg peanut protein (18). Anaphylaxis was defined according to the European Academy of Allergy and Clinical Immunology criteria (19). Children with a reactivity threshold ≤ 3 mg peanut protein were defined ineligible for randomization. Exclusion criteria were non-controlled asthma or severe chronic disease including severe atopic eczema and diabetes mellitus.

The screening investigations, described in detail in the online supplements included a structured interview, standardized QoL questionnaires, serological and immunological analyses, lung function measurements (predicted forced expiratory volume in one second
Randomization to active peanut OIT or controls (observation only) had a block-size of 2:1, which restarted when the OIT starting dose was decreased (5) as described in the online supplements and Supplement Table 1.

The OIT started with 1 mg or 5 mg followed by bi-weekly up-dosing up to the predefined high maintenance dose of 5000 mg, as described in the online supplements (5).

The QoL assessments and all tests from the screening except the DBPCFC, were repeated in all children at Y1 and Y2. Children undergoing OIT recorded perceived treatment burden in a VAS form at Y1 and Y2.

At Y2, desensitization was assessed by an open food challenge (OFC) with a maximum cumulated dose of 7500 mg peanut protein, as described in details in the online supplement. Written parental informed consent was obtained after detailed oral and written study information.

The TAKE-AWAY trial was approved by the Regional Committee for Medical and Health Research Ethics (number 2013/430) with regular communications in case of severe or unexpected AEs, and registered at ClinicalTrials.gov (number NCT02457416).

**Study population**

As previously reported (5), 96 children (5-15 years of age) had a positive DBPCFC at Y0 with moderate objective symptoms in minimum two organ systems, and fulfilled the EAACI criteria for anaphylaxis. Fifty-seven children were randomized to OIT and 20 to controls, whereas 19 were defined ineligible for enrolment (Figure 1). The 77 children enrolled had primary sensitization to peanut with a mean (95% CI) s-IgE to Ara h 2 of 103.0 (75.8, 130.3) kUA/L. Baseline characteristics were not significantly different between children randomized to OIT (n=57) or controls (n=20), except from significantly more children randomized to OIT.
needed adrenaline during DBPCFC (Table 1) (5). Of the 57 children randomized to OIT, 43 completed Y1 and 39 Y2, while all 20 controls attended investigations at both Y1 and Y2.

**Quality of life measurements**

Children completed the validated (14, 22) age-adapted (age 5-7, 8-12 and 13-18 years) generic Pediatric Quality of Life Inventory Version 4.0 (PedsQL 4.0) consisting of 13 items within four domains of functioning; physical, emotional, social and school. The PedsQL 4.0 applies a 5-point Likert scale (0=never, 4=almost always) for the 8-12 and 13-18 years reports, and a simplified 3-point scale for the 5-7 years child reports.

Parents completed both the PedsQL 4.0 proxy-report as well as the Food Allergy Quality of Life–Parental Burden (FAQL-PB) Questionnaire (23, 24) for parents’ own QoL, both described in online supplements.

**Measurement of perceived treatment burden**

A VAS-form was developed to obtain one-dimensional reports of perceived treatment burden during the last 12 months within three domains: Gastro-intestinal (GI) related AEs (stomach ache, nausea/vomiting and oral itching), taste and amount of daily peanut dose, and time spent on OIT (up-dosing at hospital and ingestion of OIT doses at home) with details in the supplementary material.

**Outcomes and explanatory factors**

The primary outcome was change in child-QoL scores from Y0 to Y2 reported by the children themselves, the secondary outcome was the corresponding parental proxy-reported child QoL. The level of desensitization was defined as the highest cumulated dose of peanut protein eaten without eliciting allergic reactions corresponding to a positive Y2 OFC.
Factors that potentially could influence the change in QoL included level of desensitization, AEs, maintenance dose at Y1 and personal perception of treatment burden (mean of the three domains: GI-related AEs, taste/amount of peanuts and time spent on OIT) at Y1 and Y2.

**Statistical analyses**

Baseline characteristics are reported as geometric means with 95% confidence interval (95% CI) or means (95% CI) for non-normal and normal distribution for continuous data, respectively. Potential differences between groups were explored using the Mann-Whitney U test for continuous data, and the chi-square test for categorical data.

Analyses in the present study were explorative, while the TAKE-AWAY study population size was determined by statistical power analysis outlined in the online supplements, indicating that a treatment group of 40 and a control group of 20 subjects would provide a statistical power of 80% to observe a difference in SU at a five percent significance level between the two groups.

The items in the PedsQL 4.0 were reverse-scored (0=100, 1=75, 2=50, 3=25, 4=0), as recommended (22), reporting the mean sum of each item. To assess changes in QoL scores from Y0 to Y2, we applied generalized repeated-measures linear mixed models with QoL score at Y0, age, gender, randomisation group, and Y1 and Y2 as independent variables, while significant differences and changes between groups were assessed by Scheffe's test.

The changes in VAS from Y1 to Y2 were assessed using paired t-test.

As the normality assumptions for the multiple linear regression analysis not were fulfilled, multivariate robust regression analyses with Huber’s M-estimator and further description in online supplements was used to assess associations between possible factors influencing QoL, and change in QoL from Y0 to Y2.

Bivariate unadjusted analyses were performed with Spearman correlation analyses ($r_s$=Spearman correlation coefficient).
P-value of $\leq 0.05$ was considered statistically significant.

Randomization to OIT or controls was performed using Statistical Analysis System (SAS, Version 9.3, SAS Institute Inc., Chapel Hill, NC, USA) and statistical analyses were performed with the IBM Statistical Package for Social Sciences (IBM SPSS Statistics, Version 23, Armonk, NY: IBM Corp).
RESULTS

By Y2, 18/57 children had withdrawn from OIT (31.6%), with their baseline characteristics given in Supplement Table 2, while all 20 controls attended Y0, Y1 and Y2. The mean (SD) daily maintenance dose of peanut protein was 3322 (1376) mg, ranging from 350-5000 mg. The Y2 OFC was refused by 2 of the remaining 39 OIT children due to fear of allergic reactions. Among the 37 challenged children, tolerance to 7500 mg peanut protein was confirmed in 35 (94.6%), as described further in the online supplements.

As shown in Figure 2, the children’s self-reported mean (95% CI) QoL scores improved significantly from Y0 (82.1 (79.1, 85.2)) to Y2 (86.7 (83.6, 89.7)) within the OIT group (p<0.0001), whereas the QoL scores among controls did not change significantly from the 83.4 (75.4, 91.4) at Y0 to 82.2 (76.0, 88.4) at Y2 (p=0.80). However, the mean (95% CI) QoL change of 4.4 (0.5, 8.3) from Y0 to Y2 in the OIT group was not significantly different from the -0.9 (-7.9, 6.11) change observed among the controls (p=0.12) (Figure 2).

The parental proxy-reported mean (95% CI) QoL score of 88.0 (85.2, 90.8) at Y2 was significantly higher than the QoL score at Y0 (78.7 (73.6, 83.7) (p<0.0001) in the OIT group, while the corresponding QoL at Y2 of 82.1 (75.8, 88.4) was similar to the QoL at Y0 of 81.7 (74.6, 88.8) (p=0.90) among controls, as shown in Figure 2.

In contrast to the child-reports, the two-fold larger mean (95% CI) change in parents’ proxy-reported QoL of 9.3 (4.3, 14.3) in the OIT group was significantly different from the parental proxy-reported change (0.4 (-7.1, 8.0)) (p=0.02) among the controls (Figure 2).

For OIT children, the change in QoL from Y0 to Y2 was not significantly associated with the number of AEs in bivariate analyses, nor maintenance dose, any of the three perceived treatment burden domains at Y1 or Y2 (Table 2). Nor was the level of desensitization significantly associated with change in QoL in the final regression model (Table 3), as outlined in details in the online supplements.
The parents’ QoL reported by the FAQL-PB improved significantly among both the OIT group and controls from Y0 to Y2 (Supplement Figure 3).

The QoL was similar among OIT ineligible children and controls at Y0 and Y1 (Supplement Figure 4A), while the QoL of parents was significantly (p=0.048) poorer among parents of ineligible compared with parents of control children at Y1, despite being similar at Y0 (Supplement Figure 4B).
DISCUSSION

By two years of OIT, 18 children had discontinued treatment, while most children with two years of OIT were desensitized to 7500 mg peanut regardless of maintenance dose. The child-reported QoL improvement after two years OIT was not significantly different between OIT children and the controls, while the corresponding two-fold larger improvement in child-QoL reported by the parents of OIT children was significantly larger than that reported by control parents. Neither AEs nor perception of treatment burden reported by the children, level of desensitization or maintenance dose significantly influenced the change in QoL. The QoL of OIT ineligible children was similar to that of controls at enrolment and Y1 while the QoL of their parents was poorer after one year compared with control parents.

Our finding that parental proxy-reported, but not child self-reported QoL improved significantly with two years of OIT compared to that of controls, is to the best of our knowledge novel. In line with studies mostly based on parental proxy-reports (1, 7, 10, 12), we observed significant improvement in child QoL with two years of treatment in both parental proxy-report and the child self-report. However, the significant difference in child QoL improvement reported by parents only in the OIT compared with control group, is likely due to the two-fold larger improvement compared with child self-reports. In line with discrepancies shown between child self-reported and parental proxy-reported QoL in children with food allergies (16) and children undergoing OFCs (25), the QoL of our OIT parents improved more than that of controls. Parents may thus respond more positively than their children to lesser social restrictions (16) in line with improved desensitization. The larger variation observed among the child-reports as well as the limited sample size may also contribute to the non-significant differences in change in QoL between OIT children and controls. However, our study size is comparable to previous studies on QoL in peanut OIT
(7-10, 12) and sufficiently large to identify significant larger improvement in the parental proxy-reports of the OIT children compared with controls.

Neither perception of treatment burden, level of desensitization, maintenance dose nor AEs influenced change in QoL from baseline to two years OIT, supported by the lack of association between change in QoL and AEs shown previously (10, 16, 26). This suggests that perceived treatment burden may be, at least partly disassociated from the number and severity of AEs during OIT (10, 16).

**Strength and limitations**

The study strengths include the standardized QoL assessments completed by children and parents of both the OIT group and the controls, close follow-up and detailed information of AEs and a VAS scale for child-assessment of treatment burden after one and two years of OIT. Using VAS to report perceived treatment burden may improve our understanding of patient perceived burden of OIT. The VAS was not pre-validated for the TAKE-AWAY trial, but has previously been validated for pain and nausea (27).

Our results may be biased towards an overestimation of the positive effect on QoL by OIT, as we unfortunately did not have the QoL assessment of the 23% of the OIT children discontinued treatment, supporting our interpretation that peanut OIT did not significantly improve child-reported QoL.
CONCLUSION

Among the two-thirds of children who completed two years of peanut OIT, QoL improved compared with controls as reported by the parents, but not by the children themselves, indicating that parents may over-estimate improvement in QoL by OIT.
AUTHOR CONTRIBUTIONS

G. Håland, K. C. Lødrup Carlsen, K-H. Carlsen designed the project. M. M. Michelsen, G. Håland and T. Reier-Nilsen included patients, collected data and also S. Drottning carried out the up-dosing protocol. M. P. Borres contributed in carrying out immunological tests. The analytic approaches were designed by G. Håland, K. C. Lødrup Carlsen, K-H. Carlsen, C. Zhang and T. Reier-Nilsen, while the main statistical analyses were performed by T. Reier-Nilsen in collaboration with the statistician C. Zhang. T. Reier-Nilsen is the lead author with significant contribution from all authors who read and approved the submitted manuscript.
We specially thank all participating children, their siblings and parents for their participation and time. We thank the study nurses, Liv Julie Sørdal and Runa Kaldestad, and the cook, Grete Simonsen, for their invaluable contribution to this project. Helene Lindvik performed the screening of patients recruited from the Oslo Peanut Allergy Study and Håvard Ove Skjerven helped performing the multivariate robust regression analyses. The study was funded by the Extra Foundation, the Kloster Foundation, South-Eastern Norway Regional Health Authority and the Norwegian Asthma and Allergy Association. We thank Thermo Fisher Scientific for supplying kits for the IgE, IgG and IgG₄ analyses and Fürst Medical Laboratory for performing the analyses.
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QoL at Y2 in the TAKE-AWAY trial 03.12.18
Table 1. Baseline characteristics of children randomized to peanut OIT or controls, and children ineligible for enrolment.

<table>
<thead>
<tr>
<th></th>
<th>Active (n = 57)</th>
<th>Controls (n = 20)</th>
<th>Ineligible (n = 19)</th>
<th>Overall p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td>9.3 (5.2, 15.2)</td>
<td>9.3 (5.1, 13.3)</td>
<td>10.9 (5.6, 14.6)</td>
<td>0.08</td>
</tr>
<tr>
<td><strong>Male</strong></td>
<td>31 (54.4)</td>
<td>13 (65.0)</td>
<td>6 (31.6)</td>
<td>0.10</td>
</tr>
<tr>
<td><strong>History of systemic reaction to peanut</strong></td>
<td>45 (8.9)</td>
<td>18 (90.0)</td>
<td>17 (89.5)</td>
<td>0.55</td>
</tr>
<tr>
<td><strong>Current asthma</strong></td>
<td>24 (42.1)</td>
<td>9 (45.0)</td>
<td>11 (57.9)</td>
<td>0.47</td>
</tr>
<tr>
<td><strong>Allergic rhinitis</strong></td>
<td>15 (26.3)</td>
<td>8 (40.0)</td>
<td>5 (26.3)</td>
<td>0.83</td>
</tr>
<tr>
<td><strong>Atopic dermatitis</strong></td>
<td>47 (82.5)</td>
<td>14 (73.9)</td>
<td>13 (68.4)</td>
<td>0.40</td>
</tr>
<tr>
<td><strong>Allergy to tree-nuts</strong></td>
<td>20 (35.1)</td>
<td>7 (36.8)</td>
<td>9 (53.0)</td>
<td>0.37</td>
</tr>
<tr>
<td><strong>Allergy to other food than nuts</strong></td>
<td>27 (47.4)</td>
<td>11 (57.9)</td>
<td>11 (61.1)</td>
<td>0.57</td>
</tr>
<tr>
<td><strong>Parental atopic disease</strong>*</td>
<td>50 (87.7)</td>
<td>16 (80.0)</td>
<td>17 (89.5)</td>
<td>0.84</td>
</tr>
<tr>
<td><strong>Parental food allergy</strong></td>
<td>21 (36.8)</td>
<td>6 (30.0)</td>
<td>11 (57.9)</td>
<td>0.17</td>
</tr>
<tr>
<td><strong>FEV1% predicted</strong></td>
<td>101.2 (97.6, 105.0)</td>
<td>95.5 (88.3, 107.2)</td>
<td>93.3 (85.8, 102.3)</td>
<td>0.15</td>
</tr>
<tr>
<td><strong>Pos s-IgE (≥ 0.35 kUA/L):</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tree-nuts***</td>
<td>52 (91.2)</td>
<td>16 (80.0)</td>
<td>19 (100.0)</td>
<td>0.07</td>
</tr>
<tr>
<td>other food****</td>
<td>54 (94.7)</td>
<td>19 (95.0)</td>
<td>19 (100.0)</td>
<td>0.65</td>
</tr>
<tr>
<td><strong>Peanut SPT (mm)</strong></td>
<td>9.8 (8.6, 11.0)</td>
<td>9.3 (7.4, 11.7)</td>
<td>9.8 (8.2, 12.1)</td>
<td>0.97</td>
</tr>
<tr>
<td><strong>S-IgE (kUA/L):</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peanut</td>
<td>110.6 (70.4, 173.8)</td>
<td>52.2 (20.3, 134.4)</td>
<td>128.8 (74.1, 218.8)</td>
<td>0.34</td>
</tr>
<tr>
<td>Ara h 2</td>
<td>56.2 (37.2, 87.1)</td>
<td>22.4 (8.4, 58.9)</td>
<td>63.6 (38.0, 102.3)</td>
<td>0.13</td>
</tr>
<tr>
<td><strong>Peanut s-IgE/total IgE (kUA/L)</strong></td>
<td>0.3 (0.2, 0.4)</td>
<td>0.2 (0.1, 0.4)</td>
<td>0.3 (0.2, 0.5)</td>
<td>0.39</td>
</tr>
<tr>
<td><strong>Peanut s-IgG4/s-IgE (ng/ml)</strong></td>
<td>117.5 (82.9, 165.9)</td>
<td>97.7 (46.3, 208.8)</td>
<td>104.7 (42.4, 263.0)</td>
<td>0.39</td>
</tr>
<tr>
<td><strong>QoL child self-report</strong></td>
<td>82.1 (79.1, 85.2)</td>
<td>83.4 (75.4, 91.4)</td>
<td>81.8 (75.8, 87.7)</td>
<td>0.81</td>
</tr>
<tr>
<td><strong>QoL parental proxy-report</strong></td>
<td>79.8 (76.3, 83.3)</td>
<td>80.7 (73.6, 87.7)</td>
<td>80.0 (69.6, 90.4)</td>
<td>0.62</td>
</tr>
<tr>
<td><strong>Baseline DBPCFC:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anaphylaxis severity grade:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>modified EAACI</td>
<td>1.6 (1.4, 1.7)</td>
<td>1.7 (1.5, 2.0)</td>
<td>1.7 (1.5, 1.7)</td>
<td>0.50</td>
</tr>
<tr>
<td>Sampson</td>
<td>2.6 (2.4, 2.8)</td>
<td>2.8 (2.5, 3.2)</td>
<td>1.8 (1.5, 2.0)</td>
<td>0.25</td>
</tr>
<tr>
<td>Use of adrenaline</td>
<td>30 (52.6)</td>
<td>5 (25.0)</td>
<td>8 (42.1)</td>
<td>0.10</td>
</tr>
</tbody>
</table>
LOAEL (mg peanut prot (ppt)) | 18.4 | 16.6 | NA | 0.34
| (11.8, 28.6) | (6.2, 44.7) | | 
Reactivity threshold (mg ppt) | 46.2 | 75.9 | NA | 0.06
| (29.7, 72.0) | (33.1, 173.8) | |

Continuous variables are given as geometric mean (95 % CI) or n (%), except age which is given as median (min, max) and QoL which is given as mean (95 % CI).

One-way ANOVA was applied to determine statistically significant differences between group means.

Chi-square test was applied to determine statistically differences between categorical data.

Significant differences are shown in bold.

*Atopic disease includes asthma, allergic rhinitis, atopic dermatitis, allergic conjunctivitis.

**All food allergy including peanut and treenut allergy.

***Hazelnut, almond, cashewnout, pistachionut, walnut, pecannut, brazilnut and macadamianut.

****Fenugreek, soybean, pea, red kidney bean, lupin seed and wheat.

Ineligible children had a reactivity threshold of 3 mg peanut protein or less.

Anaphylaxis severity was graded by two grading systems according to the modified EAACI position papers (19, 28) ranging from 1 to 3 and the method of Sampson (Grading of Food-Induced Anaphylaxis According to Severity of Clinical Symptoms) (29) ranging from 1 to 5.

Reactivity threshold was defined as the cumulated peanut protein (mg) ingested at positive DBPCFC.

Quality of life scores was given by the generic Pediatric Quality of Life Inventory Version 4.0 (PedsQL 4.0) (14, 22).

SPT, skin prick test; Ig - immunoglobulin; LOAEL - lowest observed adverse effect level;

OIT – oral immunotherapy; DBPCFC – double blind placebo controlled food challenge;

LOAEL - lowest observed adverse effect level (amount of peanut eliciting mild, objective symptoms); QoL – quality of life
Table 2. The perceived treatment burden is given at $Y_1$ (after up-dosing) and $Y_2$ (after one year of maintenance treatment) as mean values with 95% confidence intervals among all children randomised to OIT.

<table>
<thead>
<tr>
<th></th>
<th>One year of treatment ($Y_1$)</th>
<th>Two years of treatment ($Y_2$)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(N=43)</td>
<td>(N=37)</td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>3.9 (3.1, 4.8)</td>
<td>3.7 (2.9, 4.6)</td>
<td>0.84</td>
</tr>
<tr>
<td>GI-domain</td>
<td>2.6 (1.9, 3.3)</td>
<td>1.4 (1.0, 1.8)</td>
<td>0.001</td>
</tr>
<tr>
<td>Oral itching</td>
<td>3.4 (2.5, 4.4)</td>
<td>2.1 (1.3, 2.9)</td>
<td>0.02</td>
</tr>
<tr>
<td>Stomach ache</td>
<td>2.6 (1.7, 3.5)</td>
<td>1.4 (0.8, 2.0)</td>
<td>0.008</td>
</tr>
<tr>
<td>Nausea or vomiting</td>
<td>1.6 (0.8, 2.5)</td>
<td>0.6 (0.3, 0.9)</td>
<td>0.02</td>
</tr>
<tr>
<td>Taste-/amount-domain</td>
<td>6.5 (5.5, 7.3)</td>
<td>5.3 (4.3, 6.3)</td>
<td>0.02</td>
</tr>
<tr>
<td>Taste</td>
<td>7.0 (5.9, 8.0)</td>
<td>6.1 (4.9, 7.3)</td>
<td>0.10</td>
</tr>
<tr>
<td>Amount</td>
<td>5.8 (4.8, 6.7)</td>
<td>4.3 (3.3, 5.2)</td>
<td>0.01</td>
</tr>
<tr>
<td>Time spent-domain</td>
<td>2.9 (2.1, 3.7)</td>
<td>2.2 (1.5, 2.9)</td>
<td>0.06</td>
</tr>
<tr>
<td>at home</td>
<td>2.5 (1.6, 3.4)</td>
<td>2.5 (1.5, 3.6)</td>
<td>0.94</td>
</tr>
<tr>
<td>at hospital (up-dosing/visits)</td>
<td>3.0 (2.2, 3.)</td>
<td>1.7 (1.2, 2.3)</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Unpaired t-test was applied to determine statistically significant difference between means at $Y_1$ and $Y_2$.

The GI-domain represents average of how AEs like stomach ache, nausea/vomiting and oral itching are perceived.

The taste-/amount-domain represents average of perceived taste and amount of peanut eaten.

The time spent-domain represents perceived average of time spent on eating OIT doses at hospital for up-dosing visits and at ingesting OIT doses at home.
Table 3. Multivariate robust regression analyses for associations between factors that may influence change in quality of life from screening (Y₀) to second year of treatment (Y₂).

<table>
<thead>
<tr>
<th></th>
<th>PedsQL 4.0 child</th>
<th>p-value</th>
<th>PedsQL 4.0 parents</th>
<th>p-value</th>
<th>FAQLPB (parents)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-0.49 (-2.17, 1.19)</td>
<td>0.56</td>
<td>1.50 (0.24, 3.23)</td>
<td>0.09</td>
<td>0.02 (-0.06, 0.10)</td>
<td>0.63</td>
</tr>
<tr>
<td>Gender</td>
<td>-8.90 (-18.55, 0.76)</td>
<td>0.07</td>
<td>-5.90 (-16.29, 4.50)</td>
<td>0.26</td>
<td>0.23 (-0.25, 0.71)</td>
<td>0.34</td>
</tr>
<tr>
<td>Maintenance dose (mg)</td>
<td>-0.00 (-0.01, 0.00)</td>
<td>0.48</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Perceived burden of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adverse events</td>
<td>-0.22 (-0.59, 0.15)</td>
<td>0.23</td>
<td>0.19 (-0.17, 0.56)</td>
<td>0.29</td>
<td>-0.02 (-0.04, 0.00)</td>
<td>0.10</td>
</tr>
<tr>
<td>Taste/amount of peanuts</td>
<td>0.15 (-0.02, 0.32)</td>
<td>0.09</td>
<td>-</td>
<td>-</td>
<td>0.01 (-2.02, -0.13)</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Associations are given as the relative change (β) related to each unit increase by the in QoL score. N=37, including only children still receiving OIT at Y₂ with no missing data.

- no significant association

Change in QoL is given by the Pediatric Quality of Life Inventory Version 4.0 (PedsQL 4.0) child self-report and parental proxy-report, or the food allergy quality of life – parental burden (FAQLPB). Decreasing values of PedsQL and increasing values of FAQLPB reflects poorer QoL.

Perceived treatment burden was reported by VAS (range 0–10 (0=no burden, 10=massive burden) within the domains: Adverse events (stomach ache, nausea/vomiting and oral itching) and taste and amount of daily peanut OIT.
FIGURE LEGENDS

Figure 1
Flow chart from screening to second year of oral immunotherapy in the TAKE-AWAY trial.

Figure 2
The absolute quality of life (QoL) scores in children who receive oral immunotherapy (OIT) and the controls at screening (Y₀), at one year of up-dosing (Y₁) and at second year of treatment (Y₂), as reported by the child self-reports and the parental proxy-reports. Statistically significant group differences were assessed by mixed models for repeated measures. Increased value reflects improved QoL.
Figure 1

Screening ($t_0$)

DBPCFC positive > 3 mg peanut protein (N = 96)

Yes

Eligible
N = 77

Allocation

Peanut OIT N = 57
Control group N = 20

No

Ineligible
N = 19

Withdrawal N = 14
- 9 adverse events
- 2 distaste
- 3 social reasons

End of up-dosing ($t_3$)

Peanut OIT N = 43
Control group N = 20

Withdrawal N = 4
- 2 adverse events
- 2 distaste

2 years of OIT ($t_4$)

Peanut OIT N = 39
Control group N = 20

QoL at Y2 in the TAKE-AWAY trial 03.12.18
Figure 2

QoL-score

* p < 0.0001

Child self-report

Parent proxy-report

Y0 (screening)  Y1 (end of up-dosing)  Y2 (two years of treatment)
SUPPLEMENTARY MATERIAL

METHODS

Study design

Inclusion criteria for screening in the Take-Away trial were age 5-15 years, a history of systemic reactions to peanut or sensitization to peanut by a peanut specific skin prick test (SPT) ≥ 3 mm or a specific immunoglobulin E to peanut (s-IgE) ≥ 0.35 kUA/L, and living within acceptable distance from the Oslo University Hospital to ensure feasibility of a possible OIT. Exclusion criteria were non-controlled asthma, allergy or intolerance to any other ingredients in the peanut DBPCFC vehicle (ginger bread), current or previous allergen specific immunotherapy, cardiac disease, severe atopic skin disease, diabetes mellitus or other severe diseases that might interfere with adherence to study protocol.

The OIT starting dose was initially 5 mg of peanut protein based on previously published studies (2, 21, 30), but lowered to 1 mg of peanut protein after including 26 children (17 active vs 9 controls) due to low reactivity threshold in the referred patients (18). Lowering the starting dose was approved by the ethical committee, and results from statistical analyses comparing children with a different OIT starting dose of 5 mg vs 1 mg of peanut protein (n = 17 vs 40), allowed that all children on active OIT could be assigned as one intervention group.

Immunoglobulin assessments and skin prick test

Specific IgE was analyzed for peanut and peanut allergen components (Ara h1, Ara h2, Ara h3, Ara h8, Ara h9), hazelnut and hazelnut allergen components (Cor a 1, Cor a 8, Cor a 9, Cor a 14), almond, cashew nut, pistachio, walnut, pecan nut, brazil nut, macadamian nut, fenugreek, soy bean, lupine seed, wheat, latex, common silver birch, timothy and mugwort with positive tests defined as s-IgE ≥ 0.35 kUA/L. Specific IgG and IgG4 were analyzed for peanut and Ara h 2 with positive tests defined as IgG > 2.0 mgA/L and IgG4 of > 0.07 mgA/L.
Immunoglobulins were analyzed using the Phadia CAP-System FEIA (ThermoFisher, Uppsala, Sweden).

The SPT was performed according to European guidelines (31) and included common food and inhalant allergens including peanut, hazelnut, almond, soy, birch, grass (timothy), mugwort, cat, dog, mite and mold (cladosporium herbarium) (ALK SQ extracts from ALK Abello (Hørsholm, Denmark), pea positive control (histamine) and negative control (Allergopharma (Reinbek, Germany).

Anaphylaxis definition and grading

Anaphylaxis was defined as moderate symptoms from at least two organ systems in line with European Academy of Allergy and Clinical Immunology (EAACI) task force position papers (19, 28), modified for children by Vetander et al. (32). Severity of anaphylaxis was also graded in line with the EAACI position papers (19, 28) scoring from 1-3 (mild-moderate-severe) in addition to the method of Sampson (Grading of Food-Induced Anaphylaxis According to Severity of Clinical Symptoms) ranging from one to five (extremely severe reaction) (29).

Up-dosing protocol of the oral immunotherapy

For the lowest doses, the allergen source was peanut flour (Golden Peanut Company, Alpharetta, GA, USA). Since larger amounts of flour was found hard to eat, all but one child switched to roasted peanuts at OIT doses of 65-500 mg peanut protein. Each up-dosing period started with the incremented OIT dose ingested under observation at the hospital, followed by daily intake of a specific peanut dose at home for 14 days. In case of ongoing infections, asthma exacerbations, excessive tiredness or vaccinations we advised to postpone the daily OIT dose to the next day. The OIT was resumed at home if less than three consecutive doses were missing, and in hospital if three or more doses were missed. Exercise within two hours after ingesting the OIT dose was strongly discouraged.
Registration of peanut intake, AEs, use of medication and accidental exposure to peanut were based upon daily symptom diary recordings. An independent safety board was contacted if unexpected severe AEs occurred. All participants received prescriptions of adrenaline auto-injectors and antihistamines, a written treatment plan for AEs, and had around-the-clock access to the private cell-phone numbers of the study personnel.

The AEs were classified as mild, moderate and severe (including anaphylaxis) according to the modified Bock's criteria (33, 34). Mild skin symptoms include occasional scratching, less than three hives, mild lip oedema, or a few areas of erythema, whereas moderate include scratching for more than two continuous minutes, more than three but less than ten hives, significant face oedema or areas of erythema, and severe skin symptoms include excoriations or generalized erythema or urticaria. Mild respiratory symptoms include rare bursts or sniffing, expiratory wheezing by auscultation or less than three episodes of throat clearing, whereas moderate include less than ten bursts, frequent sniffing, conjunctivitis, inspiratory and expiratory wheezing by auscultation or hoarseness, and severe respiratory symptoms include persistent rhinorrhea, use of accessory muscles or audible wheezing or stridor. Mild GI-symptoms include complaints of nausea or stomach ache or one episode of emesis or diarrhea, whereas moderate include frequent complaints of nausea and stomach ache with normal activity and two to three episodes of emesis or diarrhea or one of each, and severe GI-symptoms include complaints of nausea or stomach ache with change of activity level or more than three episodes of emesis or diarrhea or two of each. Mild cardiovascular include subjective weakness or tachycardia, whereas moderate include more than 20 % drop in baseline blood pressure, and severe cardiovascular symptoms include signs of impaired circulation.

The oral immunotherapy protocol

QoL at Y2 in the TAKE-AWAY trial 03.12.18
The peanut OIT followed a biweekly step-up protocol with a fixed starting dose and a pre-defined maximum maintenance dose (MMD) of 5000 mg peanut protein as previously reported (18). Each increment of peanut dose was based upon an agreement between the participating child, parents and the study pediatrician. In case of distaste for peanuts, intolerable AEs or three consecutive unsuccessful up-dosing attempts due to AEs, the current dose would represent the individual maintenance dose. Withdrawal was initiated by self-discontinuation, severe AEs or more than two anaphylactic reactions.

Grading of AEs was in line with the modified Bock’s criteria (33-35) (further described in online supplements).

**Quality of life measurements**

For the PedsQL 4.0, results are classified by Physical Health Score (Physical Functioning) and Psychosocial Health Summary Score (Emotional, Social and School Functioning).

Translation into Norwegian has been validated for PedsQL 4.0 (36).

In addition to fulfilling the PedsQL 4.0 parental proxy-report, parents completed the health-related Food Allergy Quality of Life – Parental Burden (FAQL-PB) Questionnaire (23, 24).

The FAQL-PB (23, 24) consists of 17 items including family/social activities (restaurant meals, social activities, child care and vacation), school, time spent for meal preparation, health concerns, and emotional issues. The FAQL-PB has a 7-point Likert scale (1 = not troubled, 7 = extremely troubled), with summated scores ranging from 17-119. The minimal important difference (MID) (the smallest change that the patient perceive as important) on a 7-point Likert scale was defined as 0.5 (37). The FAQL-PB was translated into Norwegian for use in the Oslo Peanut Allergy Study (21) with permission from The Food Allergy and Anaphylaxis Network.

**Measurement of perceived treatment burden**
The VAS developed to obtain one-dimensional reports of perceived treatment burden during the last 12 months included eight individual VAS items and was completed by OIT children together with their parents at Y1 and Y2. One item referred to overall perception of treatment burden, whereas seven items referred to treatment burden within three domains: Gastro-intestinal (GI) related AEs (stomach ache, nausea/vomiting and oral itching), taste and amount of daily peanut dose, and time spent on OIT (up-dosing at hospital and ingestion of OIT doses at home). Each VAS item ranged from 0–10 (0=no burden, 10=massive burden), reporting the mean score per domain.

**Oral food challenge at Y2**

The first challenge dose equaled the maintenance dose. A cumulated dose of 7500 mg peanut protein was reached after maximum six doses, ingested with a 30 minutes interval.

**Statistical analyses**

The statistical power analysis at onset of the TAKE-AWAY trial was based upon reports desensitization of up to 80% of peanut allergic children using a step-up peanut OIT (3, 4) and spontaneous tolerance development in up to 20% (38). Assuming that desensitization would be less successful in children with severe peanut allergy, we estimated that with a desensitization rate of 57%, a treatment group of 40 and a control group of 20 subjects would provide a statistical power of 80% at a five percent significance level. As the underlying assumptions for the multiple linear regression analysis were not fulfilled, multivariate robust regression analyses with Huber’s M-estimator was used to assess associations between possible factors influencing QoL (as independent variables), and change in QoL from Y0 to Y2 in the PedsQL 4.0 or the FAQL-PB (as dependent variables). Hosmer’s step down multivariate analysis (39) retaining age and gender in the analysis included all variables significant at the 0.35 level in the bivariate analyses and the final model was tested.
for confounding with all excluded variables. Confounding was considered significant if
including the variable caused a minimum of a 25% change in the result (39).

RESULTS

At Y2 OFC, two children had allergic reactions during challenge. One child reacted with
conjunctivitis and urticaria at 7500 mg peanut protein (maintenance dose 1500 mg), and the
other child (maintenance dose 1250 mg) with a 3-month treatment discontinuation before
resuming OIT the last three months before OFC had moderate anaphylaxis (erythema,
urticaria and wheezing) at 4444 mg peanut protein.
The change in QoL from Y0 to Y2 was not significantly associated with the number of AEs in
unadjusted bivariate analyses, neither as reported by the children (p=0.76) nor by the parental
proxy-reports (p=0.90). Among the OIT-children, perceived treatment burden was
significantly lower at Y2 compared with Y1 for the GI-domain and the taste-/amount-domain,
but not for the time spent-domain from Y1 to Y2 (Table 2). The associations between changes
in perceived treatment burden from Y1 to Y2 vs baseline VAS reports at Y1 are shown in
Supplement Figure 2). Although maintenance dose altered the effect size of the burden of
peanut taste/amount >25 % in step-down analyses, neither maintenance dose, any of the three
perceived treatment burden domains at Y1 and Y2, nor the level of desensitization were
significantly associated with change in QoL in the final regression model (Table 3). The
results were similar including children who discontinued OIT and burden of time spent on
OIT into the analyses (data not shown).
The change in perceived treatment correlated significantly with the VAS reported burden at
Y1 with mean correlation coefficients ranging from –0.82 to –0.44) (p<0.001) (Supplement
Figure 2).
The parents’ QoL improved significantly (decreased score) from Y₀ to Y₂ in both groups as shown in Supplement Figure 3, with a FAQL-PB score decreasing from 2.1 (1.8, 2.3) to 1.5 (1.3, 1.7) (p<0.0001) among the OIT parents and from 1.9 (1.7, 2.3) to 1.4 (1.2, 1.7) (p = 0.004) among the control parents. No significant difference in mean change (95% CI) among parents’ QoL was observed from Y₀ to Y₂ between the OIT group (-9.9 (-14.6, -5.3)) and the controls (-9.4 (-15.3, -3.6)) (p = 0.57) (Supplement Figure 3).
SUPPLEMENTARY REFERENCES


35. Reinfjell T, Diseth TH, Veenstra M, Vikan A. Measuring health-related quality of life in young adolescents: reliability and validity in the Norwegian version of the Pediatric Quality of Life Inventory 4.0 (PedsQL) generic core scales. Health Qual Life Outcomes. 2006;4:61.


### Supplement Table 1. Baseline characteristics in children with peanut OIT starting dose 1 mg and 5 mg peanut protein.

<table>
<thead>
<tr>
<th></th>
<th>Peanut starting dose 1 mg peanut protein (n = 40)</th>
<th>Peanut starting dose 5 mg peanut protein (n = 17)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>9.7 (5.4, 15.0)</td>
<td>10.4 (6.3, 15.1)</td>
<td>0.26</td>
</tr>
<tr>
<td>Male sex</td>
<td>23 (57.5)</td>
<td>8 (47.5)</td>
<td>0.47</td>
</tr>
<tr>
<td>Ever had eczema</td>
<td>32 (80.0)</td>
<td>15 (88.2)</td>
<td>0.46</td>
</tr>
<tr>
<td>Current asthma</td>
<td>20 (50.0)</td>
<td>11 (64.7)</td>
<td>0.56</td>
</tr>
<tr>
<td>Allergic rhinitis</td>
<td>10 (25.0)</td>
<td>5 (29.4)</td>
<td>0.77</td>
</tr>
<tr>
<td>Parental atopic disease*</td>
<td>38 (90.5)</td>
<td>12 (70.6)</td>
<td><strong>0.01</strong></td>
</tr>
<tr>
<td>Parental food allergy**</td>
<td>15 (37.5)</td>
<td>6 (35.3)</td>
<td>0.87</td>
</tr>
<tr>
<td>Peanut SPT (mm)</td>
<td>9.8 (4.0, 36.3)</td>
<td>9.4 (4.0, 22.9)</td>
<td>0.88</td>
</tr>
<tr>
<td>Positive SPT other nuts (≥ 3 mm)***</td>
<td>22 (55.0)</td>
<td>6 (35.3)</td>
<td>0.17</td>
</tr>
<tr>
<td>S-IgE (kUA/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peanut</td>
<td>92.8 (9.8, 2290.9)</td>
<td>102.3 (3.1, 955.0)</td>
<td>0.86</td>
</tr>
<tr>
<td>Ara h 2</td>
<td>46.8 (9.9, 489.8)</td>
<td>61.7 (2.5, 457.1)</td>
<td>0.60</td>
</tr>
<tr>
<td>Positive s-IgE (kUA/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>other nuts***</td>
<td>38 (95.0)</td>
<td>14 (87.5)</td>
<td>0.33</td>
</tr>
<tr>
<td>other food****</td>
<td>38 (95.0)</td>
<td>16 (100.0)</td>
<td>0.36</td>
</tr>
<tr>
<td>Peanut s-IgE/total IgE (kUA/L)</td>
<td>9.5 (8.0, 10.0)</td>
<td>9.5 (9.1, 9.9)</td>
<td>0.46</td>
</tr>
<tr>
<td>Peanut s-IgG4/s-IgE (ng/ml)</td>
<td>4.7 (9.2, 288.4)</td>
<td>4.7 (9.9, 42.7)</td>
<td>0.72</td>
</tr>
<tr>
<td>BAT (%CD63+), pos *****</td>
<td>63.1 (16.2, 93.3)</td>
<td>64.6 (37.2, 91.2)</td>
<td>0.80</td>
</tr>
<tr>
<td>CAPT positive (dilution) *****</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/160</td>
<td>5 (12.5)</td>
<td>2 (11.8)</td>
<td>0.59</td>
</tr>
<tr>
<td>1/80</td>
<td>11 (27.5)</td>
<td>2 (11.8)</td>
<td></td>
</tr>
<tr>
<td>1/40</td>
<td>12 (30.0)</td>
<td>9 (52.9)</td>
<td></td>
</tr>
<tr>
<td>1/20</td>
<td>11 (27.5)</td>
<td>2 (11.8)</td>
<td></td>
</tr>
<tr>
<td>1/10</td>
<td>11 (27.5)</td>
<td>2 (11.8)</td>
<td></td>
</tr>
<tr>
<td>Pre-OIT DBPCFC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Use of adrenaline</td>
<td>23 (57.5)</td>
<td>7 (41.2)</td>
<td>0.26</td>
</tr>
<tr>
<td>Anaphylaxis severity</td>
<td>1.7 (1.0, 2.0)</td>
<td>1.4 (1.0, 2.0)</td>
<td>0.10</td>
</tr>
<tr>
<td>modified EAACI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reactivity threshold</td>
<td>42.8 (3.0, 933.3)</td>
<td>93.7 (12.9, 1584.9)</td>
<td>0.12</td>
</tr>
</tbody>
</table>

*Atopic disease includes asthma, allergic rhinitis, atopic dermatitis, allergic conjunctivitis
**All food allergy including peanut and treenut allergy
***Hazelnut, almond, cashewnut, pistachionut, walnut, pecannut, brazilnut and macadamianut
****Fenugreek, soybean, pea, red kidney bean, lupin seed and wheat
***** The CAPT was recorded positive ranging from dilution level 1 (1:160) to 5 (1:1).
****** N=50. The BAT was not performed in 7 children due to technical causes (n=5) and non-reponders were excluded from the analyses (n=2).
Anaphylaxis severity was graded according to the modified EAACI position papers (19, 28) ranging from 1 to 3 and the method of Sampson (Grading of Food-Induced Anaphylaxis According to Severity of Clinical Symptoms) (29) ranging from 1 to 5.

SPT, skin prick test; BAT, basophil activation test; CAPT, conjunctival provocation test; Ig - immunoglobulin; LOAEL - lowest observed adverse effect level; OIT – oral immunotherapy; DBPCFC – double blind placebo controlled food challenge; LOAEL - lowest observed adverse effect level (amount of peanut eliciting mild, objective symptoms)
**Supplement Table 2.**

<table>
<thead>
<tr>
<th></th>
<th>Patients completing OIT up-dosing (n = 43)</th>
<th>Patients discontinuing OIT (n = 14)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (median, min-max)</td>
<td>9.0 (5.2, 15.2)</td>
<td>11.1 (5.4, 15.1)</td>
<td>0.13</td>
</tr>
<tr>
<td>Male</td>
<td>21 (48.8)</td>
<td>10 (71.4)</td>
<td>0.14</td>
</tr>
<tr>
<td>History of anaphylaxis to peanut</td>
<td>32 (74.4)</td>
<td>13 (92.8)</td>
<td>0.41</td>
</tr>
<tr>
<td>Current asthma</td>
<td>16 (69.6)</td>
<td>8 (57.1)</td>
<td>0.26</td>
</tr>
<tr>
<td>Allergic rhinitis</td>
<td>13 (30.2)</td>
<td>2 (14.3)</td>
<td>0.53</td>
</tr>
<tr>
<td>Atopic dermatitis</td>
<td>36 (83.7)</td>
<td>11 (78.6)</td>
<td>0.64</td>
</tr>
<tr>
<td>Allergy to tree-nuts</td>
<td>20 (48.8)</td>
<td>0 (0.0)</td>
<td>0.002</td>
</tr>
<tr>
<td>Allergy to other food than nuts</td>
<td>25 (59.5)</td>
<td>2 (14.3)</td>
<td>0.003</td>
</tr>
<tr>
<td>Parental atopic disease*</td>
<td>36 (83.7)</td>
<td>14 (100.0)</td>
<td>0.16</td>
</tr>
<tr>
<td>Parental food allergy**</td>
<td>14 (32.6)</td>
<td>7 (50.0)</td>
<td>0.51</td>
</tr>
<tr>
<td>FEV1% predicted</td>
<td>102.7 (98.6, 106.7)</td>
<td>99.7 (94.0, 105.8)</td>
<td>0.58</td>
</tr>
<tr>
<td>SPT tree-nuts ≥ 3 mm</td>
<td>25 (58.1)</td>
<td>3 (21.4)</td>
<td>0.02</td>
</tr>
<tr>
<td>Pos s-IgE (≥ 0.35 kUA/L):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tree-nuts***</td>
<td>40 (95.2)</td>
<td>12 (85.7)</td>
<td>0.08</td>
</tr>
<tr>
<td>other food****</td>
<td>41 (95.3)</td>
<td>13 (92.9)</td>
<td>0.63</td>
</tr>
<tr>
<td>Peanut SPT (mm)</td>
<td>10.3 (8.7, 11.9)</td>
<td>12.1 (8.3, 15.8)</td>
<td>0.48</td>
</tr>
<tr>
<td>Total IgE (kUA/L)</td>
<td>570.6 (405.1, 736.2)</td>
<td>713.9 (314.1, 1113.6)</td>
<td>0.51</td>
</tr>
<tr>
<td>S-IgE (kUA/L):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peanut</td>
<td>197.5 (12.8, 270.1)</td>
<td>472.8 (120.2, 825.3)</td>
<td>0.09</td>
</tr>
<tr>
<td>Ara h2</td>
<td>99.0 (62.7, 135.2)</td>
<td>177.1 (85.9, 268.4)</td>
<td>0.05</td>
</tr>
<tr>
<td>Peanut s-IgE/total IgE (kUA/L)</td>
<td>0.3 (0.3, 0.4)</td>
<td>0.5 (0.4, 0.7)</td>
<td>0.009</td>
</tr>
<tr>
<td>Peanut s-IgG/total IgE (ng/ml)</td>
<td>27.4 (10.5, 44.4)</td>
<td>9.7 (1.9, 21.3)</td>
<td>0.22</td>
</tr>
<tr>
<td>QoL child self-report</td>
<td>78.6</td>
<td>81.7</td>
<td>0.80</td>
</tr>
<tr>
<td></td>
<td>(75.6, 81.7)</td>
<td>(77.6, 86.3)</td>
<td>p</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>--------------</td>
<td>--------------</td>
<td>-----</td>
</tr>
<tr>
<td>QoL parental proxy-report</td>
<td>82.7 (80.0, 85.4)</td>
<td>82.1 (75.5, 88.8)</td>
<td>0.71</td>
</tr>
</tbody>
</table>

**At baseline DBPCFC:**

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of anaphylaxis:</td>
<td>43 (100.0)</td>
<td>14 (100.0)</td>
<td>C</td>
</tr>
<tr>
<td>Anaphylaxis severity grade:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>modified EAACI</td>
<td>1.7 (1.6, 1.8)</td>
<td>1.6 (1.4, 1.9)</td>
<td>0.70</td>
</tr>
<tr>
<td>Sampson</td>
<td>2.7 (2.3, 2.9)</td>
<td>2.7 (2.3, 3.1)</td>
<td>0.94</td>
</tr>
<tr>
<td>Use of adrenaline</td>
<td>22 (51.2)</td>
<td>8 (57.1)</td>
<td>0.70</td>
</tr>
<tr>
<td>LOAEL (mg peanut protein)</td>
<td>113.3 (12.4, 214.3)</td>
<td>82.9 (16.3, 149.5)</td>
<td>0.11</td>
</tr>
<tr>
<td>Reactivity threshold (mg peanut protein)</td>
<td>155.4 (48.9, 261.9)</td>
<td>250.1 (60.9, 439.3)</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Variables are given as mean (95% CI) or n (%), except age which is given as median (min, max).

Bold values are statistically significant (p<0.05).

C  Not able to compute – anaphylaxis at DBPCFC is a constant.

*Atopic disease includes asthma, allergic rhinitis, atopic dermatitis, allergic conjunctivitis

**All food allergy including peanut and treenut allergy

***Hazelnut, almond, cashewnut, pistachionut, walnut, pecannut, brazilnut and macadamianut

****Fenugreek, soybean, pea, red kidney bean, lupin seed and wheat

*****The CAPT was recorded positive ranging from dilution level 1 (1:160) to 5 (1:1).

Anaphylaxis severity was graded by two grading systems according to the modified EAACI position papers (19, 28) ranging from 1 to 3 and the method of Sampson (Grading of Food-Induced Anaphylaxis According to Severity of Clinical Symptoms) (29) ranging from 1 to 5.

LOAEL is defined as the cumulated peanut protein (mg) ingested eliciting mild, objective symptoms

Reactivity threshold is defined as the cumulated peanut protein (mg) ingested at positive DBPCFC, with at least two moderate objective symptoms in one or more organ systems symptoms according to Bock’s criteria (33, 34, 40).

Quality of life scores was given by the generic Pediatric Quality of Life Inventory Version 4.0 (PedsQL 4.0) (14, 22).

SPT, skin prick test; Ig - immunoglobulin; LOAEL - lowest observed adverse effect level;

OIT – oral immunotherapy; DBPCFC – double blind placebo controlled food challenge;

LOAEL - lowest observed adverse effect level (amount of peanut eliciting mild, objective symptoms); QoL – quality of life
SUPPLEMENTAL FIGURE LEGENDS

Supplement Figure 1
Overall study design. The “Take away food allergy; inducing tolerance in children allergic to peanut” trial (TAKE-AWAY trial) established in 2012 consists of four phases; the screening phase (three days of eligibility screening), up-dosing phase (50-78 weeks), maintenance phase (36 months) and follow-up phase (12 months). Defined time-points in the study was Y₀ – at screening, Y₁ - after one year of OIT (the end of up-dosing phase), Y₂, Y₃, Y₄ after two, three and four years of OIT (one, two and three years of maintenance treatment), respectively, and Y₅ - one year after cessation of 4 years of OIT, with assessments expected to be completed in 2020.

Supplement Figure 2
Correlation between change in perceived treatment burden from end of up-dosing (Y₁) to second year of treatment (one year of maintenance) (Y₂) and perceived burden at Y₁.
Perceived treatment burden is reported as overall burden (A), and within three domains: Adverse events (stomach ache, nausea/vomiting and oral itching) (B), taste and amount of daily peanut oral immunotherapy (OIT) (C) and time spent on OIT (D).

Supplement Figure 3
The absolute quality of life (QoL) scores in parents of children receiving oral immunotherapy (OIT) and the controls at screening (Y₀), at one year of up-dosing (Y₁) and at second year of treatment (Y₂). Decreased value reflects improved QoL.

Supplement Figure 4
A. The absolute quality of life (QoL) scores in children who receive oral immunotherapy (OIT) and the controls at screening (Y₀) and at one year of up-dosing (Y₁), as reported by the child self-reports and the parental proxy-reports. Increased score reflects improved QoL.
B. The absolute quality of life (QoL) scores in parents of children receiving oral immunotherapy (OIT) and the controls at screening ($Y_0$) and at one year of up-dosing ($Y_1$). Decreased score reflects improved QoL.
Supplement Figure 1
Supplement Figure 2

A: Overall

B: GI AEs

C: Taste

D: Time

$r_{s} = 0.65, p = 0.00$

$r_{s} = 0.82, p = 0.00$

$r_{s} = 0.44, p = 0.01$

$r_{s} = 0.53, p = 0.00$
**Supplement Figure 3**

![Graph showing QoL scores over time.](image)

- **Y₀ (screening)**
- **Y₁ (end og up-dosing)**
- **Y₂ (two years of treatment)**

QoL scores:
- **Active**
- **Controls**

Significance:
- *p < 0.0001*
- *p = 0.004*
Supplement Figure 4A

QoL-score

Child self-report

Parent proxy-report

Y₂ (screening)  Y₁ (end of up-dosing)
Supplement Figure 4B

QoL-score

Controls

Ineligible

* p = 0.048

Y₀ (screening)

Y₁ (end of up-dosing)
Take-away studien: Intervjuskjema

1. Kode [ ] [ ] [ ]

2. Fødselsdato
   dd [ ] mm [ ] åååå [ ]

3. Kjønn:
   1. Gutt
   2. Jente

4. Dato for intervju
   dd [ ] mm [ ] åååå [ ]

5. Alder [ ] år [ ] mnd

6. Hvem følger:
   1. Mor
   2. Far
   3. Mor og far
   4. Andre

7. Hvem svarer:
   1. Mor
   2. Far
   3. Mor og far
   4. Andre

Familie:

8. Hvor mange søsken har barnet: [ ] [ ]

9. Helsøsken: [ ]

10. Halvsøsken: [ ]

11(a-e). Alder søsken
   [ ] år [ ] år

12. Hvem bor deltager sammen med:
   □ Hos mor og far
   □ Hos far
   □ Andre

13. Hvor mange søsken bor hjemme? [ ] [ ]

14 Foreldrenes utdanning:

14a. Mors utdanning:
   □ Grunnskole
   □ Videregående
   □ Høyskole/universitet inntil 3 år
   □ Høyskole/universitet 4 år eller mer
   □ Informasjon ikke tilgjengelig

14b. Fars utdanning:
   □ Grunnskole
   □ Videregående
   □ Høyskole/universitet inntil 3 år
   □ Høyskole/universitet 4 år eller mer
   □ Informasjon ikke tilgjengelig

15 Økonomi- familiens totale brutto yrkesinntekt siste år:

15a Samlet husstand:
   □ 0-400.000
   □ 400.001-550.000
   □ 550.001-700.000
   □ 700.001-850.000
   □ Over 850.000
   □ Informasjon ikke tilgjengelig

15b. Mors husstand:
   □ 0-400.000
   □ 400.001-550.000
   □ 550.001-700.000
   □ 700.001-850.000
   □ Over 850.000
   □ Informasjon ikke tilgjengelig

15c. Fars husstand:
   □ 0-400.000
   □ 400.001-550.000
   □ 550.001-700.000
   □ 700.001-850.000
   □ Over 850.000
   □ Informasjon ikke tilgjengelig
Sykdom familie

16. Har mor, far eller søsken hatt, eller har de i dag noen av følgende sykdommer; astma, høysnue, matallergi, atopisk eksem, eller anafylaktisk reaksjon?:

17. Hvis ja på sp 16:

<table>
<thead>
<tr>
<th>Astma</th>
<th>Rhinitt</th>
<th>Konjunktivitt</th>
<th>Matareallergier, unntatt peanøtter og nøtter</th>
<th>Peanøttallergi</th>
<th>Allergi mot nøtter</th>
<th>Atopisk eksem</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>h</td>
<td>o</td>
<td>v</td>
<td>w</td>
<td>x</td>
<td>y</td>
</tr>
<tr>
<td>b</td>
<td>i</td>
<td>p</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c</td>
<td>j</td>
<td>q</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d</td>
<td>k</td>
<td>r</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

18. Andre kroniske sykdommer

Røyk/Snus

19. Røyker noen ihusstanden?

20. Hvis ja, hvem:

21a. Hvis nå, hvor hyppig?:

<table>
<thead>
<tr>
<th>Mor</th>
<th>Deltager</th>
<th>1. Daglig</th>
<th>2. Ukentlig, men ikke daglig</th>
<th>3. Av og til, men ikke ukentlig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Far</td>
<td>Andre</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

21b. Hvis tidligere, hvor hyppig?:

<table>
<thead>
<tr>
<th>Mor</th>
<th>Deltager</th>
<th>1. Daglig</th>
<th>2. Ukentlig, men ikke daglig</th>
<th>3. Av og til, men ikke ukentlig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Far</td>
<td>Andre</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

22. Hvor lenge er det siden noen i husstanden røkte hjemme, dersom det ble røykt i husstanden tidligere? (år)

23. Bruker deltagersnus?

24. Bruker mor eller far snus?

25. Røkte mor under svangerskapet?

26. Brukte mor snus under svangerskapet?
Svangerskap

Brukte mor noe av dette under svangerskapet?

1. Kode

27. Kosttilskudd

0. Nei
1. Ja, under deler
2. Ja, under hele
3. Vet ikke/husker ikke

Hvis ja, hvilke............................................
Hvor ble de kjøpt?........................................

28. Tran

0. Nei
1. Ja, under deler
2. Ja, under hele
3. Vet ikke/husker ikke

29. Vitamin D

0. Nei
1. Ja, under deler
2. Ja, under hele
3. Vet ikke/husker ikke

30. Folat

0. Nei
1. Ja, under deler
2. Ja, under hele
3. Vet ikke/husker ikke

31. Paracetamol

0. Nei
1. Ja
3. Vet ikke/husker ikke

32. Dispril, globoid eller aspirin

0. Nei
1. Ja, under deler
2. Ja, under hele
3. Vet ikke/husker ikke

33. Andre medikamenter

0. Nei
1. Ja
3. Vet ikke/husker ikke

Hvis ja, hvilke.............................................

34. Hadde mor diett under svangerskapet?

Hvis nei, gå rett til sp. 36.

0. Nei
1. Ja
3. Vet ikke/husker ikke

35. Hvis ja, hvilke matvarer ble unngått?

a. Melk og melkeprodukter
b. Egg
c. Peanøtter
d. Nøtter
e. Hjete/gluten
f. Fisk
g. Jordbær
h. Andre matvarer

36. Hvis nei på sp. 34. Hvor ofte spiste mor peanøtter under svangerskapet?

1. Daglig
2. Ukenlig
3. Av og til, men ikke ukentlig

Fødsel og amming

37. Forløsningsmetode

1. Vaginal
2. Sectio
3. Ukjent

38. Komplikasjoner i svangerskapet

0. Nei
1. Ja, under deler
2. Ja, under hele
3. Vet ikke/husker ikke

Hvilke......................................................

39. Gestasjonsalder ved fødsel (hele uker)?

0. Nei
1. Ja
3. Husker ikke/vet ikke

40. Fødselsvekt (g):

41. Fødselslengde (cm):

42. Ble barnet ammet?

0. Nei
1. Ja
3. Husker ikke/vet ikke

43. Hvor lenge ble barnet fullammet (mnd)?

44. Hvor lenge ble barnet ammet (mnd)?
1.Kode

45. Unngikk mor peanøtter under amming?  
   0. Nei  
   1. Ja  
   3. Husker ikke/vet ikke

46. Hvor ofte spiste mor peanøtter under amningen?  
   1. Daglig  
   2. Ukentlig  
   3. Av og til, men ikke ukentlig

47. Unngikk mor andre matvarer under amming?  
   0. Nei  
   1. Ja  
   3. Husker ikke/vet ikke

48. Har barnet fulgt vanlig vaksinasjonsprogram?  
   0. Nei  
   1. Ja  
   3. Husker ikke/vet ikke

Hvilke matvarer ble evt. unngått: .............................................................................................

Vaksiner

48. Har barnet fulgt vanlig vaksinasjonsprogram?  
   0. Nei  
   1. Ja  
   3. Husker ikke/vet ikke

Hvilke vaksiner har barnet ikke fått? .................................................................

Introduksjon av matvarer

49. Alder ved introduksjon av matvarer:  
   0. Husker ikke/vet ikke  
   1. 0-6 mnd.  
   2. 7-12 mnd.  
   3. 13-18 mnd.  
   4. 19-24 mnd.  
   5. 2-3 år  
   6. >3 år  
   7. Aldri

Mineral/vitamintilskudd til barnet

50. Brukte barnet mineral/vitamintilskudd  
   1. leveår:  
   0. Nei  
   1. Ja  
   2. Husker ikke/vet ikke

51. Hvis ja, hvilket tilskudd:

52. Brukte barnet mineral/vitamintilskudd etter  
   1. leveår og frem til siste år:  
   0. Nei  
   1. Ja  
   2. Husker ikke/vet ikke

53. Hvis ja, hvilket tilskudd:

54. Bruker barnet mineral/vitamintilskudd nå:  
   0. Nei  
   1. Ja  
   2. Husker ikke/vet ikke

55. Hvis ja, hvilket tilskudd:

Hvilke(t):.................................................................

Page 4 of 14
Pubertet  
56. Vekstspurt  
0. Husker ikke/vet ikke  
1. Har ikke startet  
2. Har så vidt startet  
3. Er definitivt i gang  
4. avsluttet  

57. Hårvekst(armhuler/kjønnshår)  
0. Husker ikke/vet ikke  
1. Har ikke startet  
2. Har så smått begynt å vokse  
3. Er definitivt i gang  
4. Behåringen øker ikke lenger  

58. Hudforandringer? Kviser spesielt?:  
0. Nei  
1. Ja, så vidt  
2. Ja, definitivt  
3. Avsluttet  

Pubertet- Gutter  
59. Dypere stemme?  
0. Nei  
1. Ja, så vidt  
2. Ja, definitivt  
3. Avsluttet  

60. Skjeggvekst?  
0. Nei  
1. Ja, så vidt  
2. Ja, definitivt  
3. for fullt  

Pubertet- Jenter  
61. Har hatt første menstruasjon?  
0. Nei  
1. Ja  

62. Alder ved første menstruasjon  
år  

63. Regelmessig menstruasjon?  
0. Nei  
1. Ja  

64. Antall dager i syklus?  
dager  

65. Hvor er du i syklusen nå? (Dager siden 1.dag?)  

66. Er du plaget med PMS?  
0. Nei  
1. Ja, litt  
2. Ja, mye  

Sykehistorie peanøttallergi  
67. Ble det oppbevart peanøtt i huset i l.a 1.leveår?  
0. Nei  
1. Ja  
2. Husker ikke/vet ikke  

68. Ble det oppbevart peanøtt i huset etter 1.leveår?  
0. Nei  
1. Ja  
2. Husker ikke/vet ikke  

69. Ble det oppbevart peanøtt i huset siste år  
0. Nei  
1. Ja  
2. Husker ikke/vet ikke  

70. Har barnet spist peanøtt?  
0. Nei  
1. Ja  
2. Husker ikke/vet ikke  

71. I hvilken form spiste barnet peanøtt:  
0. Ren peanøtt  
1. Peanøttsmør  
2. Bakt  
3. Annet.................................  

72. Barnets alder ved første kjente eksponering overfor peanøtt (mnd):  
år  
mnd  

73. Alder ved første allergiske reaksjon på peanøtt (mnd):  
år  
mnd
74. Ved første allergiske reaksjon. Hvilken form av peanøtt spiste barnet?

- Ren peanøtt
- Peanøttsmør
- Bakt
- Annet

75. Var første allergiske reaksjon på peanøtt i forbindelse med første kjente eksponering?

- Nei
- Ja
- Husker ikke/vet ikke

76. Hvilke symptomer fikk barnet ved første allergiske reaksjon på peanøtt:

- Elveblest/urticaria
- Angioødem
- Eksem
- Annet utslett
- Kloe i huden
- Rhinit
- Konjunktivitt
- Astma
- Annet pustebesvær/andre luftveissympt

77. Hvor alvorlig var den første allergiske reaksjonen?

- lett
- Moderat
- Alvorlig
- Svært alvorlig

78. Mengde peanøtt inntatt ved første allergiske reaksjon

- Spor
- Litt
- Mye
- Vet ikke/ukjent

79. Ble lege kontaktet ved 1. reaksjon?

- Nei
- Ja
- Vet ikke

80. Ble det gitt medikamentell behandling?

- Nei
- Ja
- Vet ikke

81. Hvis ja, hvilke medikamenter:

- Adrenalin
- Steroider inj
- Steroider p.o.
- Antihistamin inj
- Antihistamin p.o.
- Andre medikamenter
- Husker ikke hvilke medikamenter

82. Hvor raskt oppsto symptomene ved 1. reaksjon?

- inner 10 min
- 10-30 min
- 30 min - 2 timer
- 2-4 timer
- 4-8 timer
- over 8 timer
- Husker ikke/vet ikke

83. Har barnet hatt flere allergiske reaksjoner på peanøtt?

Hvis nei gå til spm 93

- kraftigere
- Lettere
- Varierende
- Uendret
- Vet ikke

84. Hvis ja, oppgi antall allergiske reaksjoner på peanøtt:

85. Hvis ja, var siste reaksjon-- enn første reaksjon:

- Elveblest/urticaria
- Angioødem
- Eksem
- Annet utslett
- Kloe i huden
- Rhinit
- Konjunktivitt
- Astma
- Annet pustebesvær/andre luftveissympt

- Kloe i munn/svelg
- Kvalme/oppkast
- Magesmerter
- Diare
- Uro/adferdsendring
- Anafylaksi
- Bevissthetstap
- Blekhet
- Annet
92. Hvor raskt oppsto symptomene ved siste reaksjon?

1. innen 10 min
2. 10-30 min
3. 30 min - 2 timer
4. 2-4 timer
5. 4-8 timer
6. over 8 timer
7. husker ikke/vet ikke

88. Mengde peanøtt inntatt ved siste allergiske reaksjon

0. Spor
1. Litt
2. Mye
3. Vet ikke/ukjent

90. Ble det gitt medikamentell behandling ved siste reaksjon?

0. Nei
1. Ja
3. Vet ikke

91. Hvis ja, hvilke medikamenter:

Adrenalin
Antihistamin inj
Steroider inj
Antihistamin p.o.
Steroider p.o.
Andre medikamenter

93. Unngår dere å ha peanøtter hjemme?

0. Nei
1. Ja
3. Vet ikke

95. Har dere peanøttsmør hjemme?

0. Nei
1. Ja
3. Vet ikke

97. Unngår barnet å være på steder hvor det serveres peanøtter?

0. Nei
1. Ja
3. Vet ikke

99. Hvilke tiltak er iverksatt ved skolen/barnehage?

0. Totalforbud
1. Andre barn kan ha med peanøtter, barnet unngår selv.
3. Kan smake på matvarer med spor av peanøtter

101. Unngår barnet matvarer som inneholder spor av nøtter?

0. Nei
1. Ja
3. Vet ikke

102. Er det andre matvarer (enn peanøtter og nøtter) som dere unngår hjemme?

0. Nei
1. Ja
3. Vet ikke

103. Hvis ja, hvilke:

Mellom
Hvete
Egg
Annet
Fisk

Pga barnet eller andre i familien?:

104. Hvordan forholder barnet seg til peanøtt, og hvordan påvirker det hverdagen?

□ Unngår å besøke andre
□ Unngår å spise hos andre
□ Spiser hos andre, men har med egen mat
□ Spiser hos andre, men tar forholdsregler
□ Unngår ferieturer en ellers ville tatt
□ Drar på ferieturer, men tar forholdsregler
□ Går på kafe/restaurant, men tar forholdsregler
□ Unngår å gå på kafe/restaurant
105. Hvor engstelig er foreldrene for at barnet skal få i seg peanøtt?

106. Hvor engstelig er du for å få i seg peanøtt?

Her spør en barnet!

0. Ikke engstelig
1. Litt engstelig
2. Veldig engstelig
3. Barnet i en slik alder at spørsmålet ikke er relevant

107. Har barnet opplevd allergisk reaksjon mot andre nøtter:

0. Nei
1. Ja
2. Vet ikke

108. Hvis ja, hvilke(n) nøtt(er)?

- Hasselnøtt
- Cashew
- Paranøtt
- Mandel
- Pecannøtt
- Valnøtt
- Pistasj
- Andre nøtter

Reaksjon på andre matvarer enn peanøtter og nøtter:

111. Har barnet reagert allergisk mot noen andre matvarer enn peanøtter og nøtter, hvis nei-gå til spm 118

109. Hvis ja, hvilke matvarer har dere opplevd at barnet noen gang har reagert allergisk mot?

- Melk
- Egg
- Hvete
- Bakevarer
- Fisk
- Soya
- Stenfrukt
- Sitrus
- Tomat
- Annet

I tilfelle hva:........................................

110. Hvis ja, hvilke matvarer er barnet allergisk mot i dag?

- Melk
- Egg
- Hvete
- Bakevarer
- Fisk
- Soya
- Stenfrukt
- Sitrus
- Tomat
- Annet

I tilfelle hva:........................................

Hvilke symptomer fikk barnet etter å ha spist matvaren(e):

112. Melk:
- Elveblest/urticaria
- Angioødem
- Eksem
- Annet utslett
- Kloe i huden
- Rhinit
- Konjunktivitt
- Astma
- Annet pustebesvær/luftveissympt

113. Egg:
- Kloe i munn/svelg
- Kvalme/oppkast
- Magesmerter
- Diare
- Uro/adfærdsendring
- Annet
- Astma
- Annet pustebesvær/luftveissympt

Page 8 of 14
114. Hvete:
- Elveblest/urticaria
- Angioødem
- Eksem
- Annet utslett
- Kløe i huden
- Rhinitt
- Konjunktivitt
- Astma
- Annet pustebesvær/ luftveissympt
- Annet

115. Fisk:
- Elveblest/urticaria
- Kvalme/oppkast
- Angioødem
- Eksem
- Magesmerter
- Annet utslett
- Diare
- Kløe i huden
- Uro/adferdsending
- Rhinitt
- Anafylaksi
- Konjunktivitt
- Bevissthetstap
- Astma
- Blekhet
- Annet pustebesvær/ luftveissympt
- Annet

116. Soya:
- Elveblest/urticaria
- Angioødem
- Eksem
- Annet utslett
- Kløe i huden
- Rhinitt
- Konjunktivitt
- Astma
- Annet pustebesvær/ luftveissympt
- Annet

117. Andre matvarer:.................................................
- Elveblest/urticaria
- Kvalme/oppkast
- Angioødem
- Eksem
- Magesmerter
- Annet utslett
- Diare
- Kløe i huden
- Uro/adferdsending
- Rhinitt
- Anafylaksi
- Konjunktivitt
- Bevissthetstap
- Astma
- Blekhet
- Annet pustebesvær/ luftveissympt
- Annet

---

Anafylaksi

118. Har barnet hatt alvorlig allergisk reaksjon med påvirkning av flere organ systemer samtidig, eller med påvirket bevissthet for andre matvarer enn peanøtt? Hvis nei eller vet ikke så gå rett til spørsmål nr. 124.

0. Nei
1. Ja
3. Vet ikke

119. Hvis ja, antall ganger:
- 1. En gang
- 2. 2-4 ganger
- 3. 5-10 ganger
- 4. Mer enn 10 ganger
- 5. Vet ikke

120. Hvis ja, hvilke(n) matvare(r) ga anafylaktisk reaksjon:
- Hassenøtt
- Melk
- Hvete
- Soya
- Annen nøtt
- Egg
- Fisk
- Annet

Type nøtt: ...........................................................

121. Hvis ja, kom reaksjonen i tilknytning med fysisk aktivitet eller 2-6 timer etter fysisk aktivitet?

0. Nei
1. Ja
3. Vet ikke

122. Hvis ja, ble barnet innlagt på sykehus?

0. Nei
1. Ja
3. Vet ikke

123. Hvis ja, barnet behandlet med adrenalin?

0. Nei
1. Ja
3. Vet ikke
Behandling ved akutt allergisk reaksjon

1.Kode

124. Har barnet EpiPen/adrenalinpenn?
0. Nei
1. Ja
3. Vet ikke
Hvis nei, gå til spm 138

126. Har dere fått opplæring i bruk av EpiPen?
0. Nei
1. Ja
3. Vet ikke
Hvor?: ................................................

127. Hvis ja, hvor ofte anskaffes ny EpiPen?
0. Aldri
1. Av og til
2. Alltid
3. Vet ikke
1. For den er gått ut på dato
2. Årlig
3. Hvert annet år
4. Annet

128. Hvis ja, hvor mange EpiPen'er har barnet?
0. Nei
1. Ja
3. Vet ikke

129. Hvis ja, hvor ofte har barnet den med seg?
0. Aldri
1. Av og til
2. Alltid
3. Vet ikke

130. Hvis ja, har foresatte eller barnet satt EpiPen i forbindelse med akutt allergisk reaksjon?
0. Nei
1. Ja
3. Vet ikke

131. Hvis ja, antall ganger:

133. Har barnet EpiPen med i barnehage/på skole?
0. Nei
1. Ja
3. Vet ikke

135. Har barnet andre medikamenter hjemme til bruk ved allergiske reaksjoner?
0. Nei
1. Ja
3. Vet ikke

137. Har barnet behandlingsskjema for akutt allergisk reaksjon?
0. Nei
1. Ja
3. Vet ikke

138. Har du fått diagnosen høysnue, allergisk rhinitt?

0. Nei
1. Ja
3. Vet ikke

139. Har du hatt nesetetthet, rennende nese eller nysing uavhengig av forkjølelse?

0. Nei
1. Ja
3. Vet ikke

Har du hatt kløende/rennende øyne uavhengig av forkjølelse?

140. Har du noen gang iløpet av de siste 12 månedene hatt nese symptomer (se over) uavhengig av forkjølelse?

0. Nei
1. Ja

Hvis nei på begge gå til spm 143.

143. Hvis ja, alder ved første gangs utskrivelse (år):

0. Nei
1. Ja
3. Vet ikke

134. Har barnehage/skole fått opplæring??

0. Nei
1. Ja
3. Vet ikke

136. Hvis ja, hvilke?

a. ☐ Antihistamin
b. ☐ Steroider
c. ☐ Adrenalin
d. ☐ Annen behandling

135. Har barnet andre medikamenter hjemme til bruk ved allergiske reaksjoner?

0. Nei
1. Ja
3. Vet ikke

137. Har barnet behandlingsskjema for akutt allergisk reaksjon?

0. Nei
1. Ja
3. Vet ikke

Rhinitt

138. Har du fått diagnosen høysnue, allergisk rhinitt?

a. ☐ 0.Nei
b. ☐ 1.Ja

139. Har du hatt nesetetthet, rennende nese eller nysing uavhengig av forkjølelse?

a. ☐ 0.Nei
b. ☐ 1.Ja

Hvis ja, alder ved debut:

c. ☐ Rennende nese
d. ☐ Nesetetthet
e. ☐ Nysing

Har du hatt kløende/rennende øyne uavhengig av forkjølelse?

f. ☐ 0.Nei
g. ☐ 1.Ja

Hvis ja, alder ved debut:

140. Har du noen gang iløpet av de siste 12 månedene hatt nese symptomer (se over) uavhengig av forkjølelse?

a. ☐ 0.Nei
b. ☐ 1.Ja

c. ☐ Rennende nese
d. ☐ Nesetetthet
e. ☐ Nysing

Har du i løpet av de siste 12 månedene hatt kløende/rennende øyne?

f. ☐ 0.Nei
g. ☐ 1.Ja

Hvis nei på begge gå til spm 143.
141. Hva er det som utløser disse øye/nese symptomer?

- a. □ vet ikke
- b. □ katt
- c. □ Hund
- d. □ Kanin
- e. □ Hest
- f. □ Andre dyr
- g. □ Bjørk
- h. □ Gress (timotei)
- i. □ Burot
- j. □ Andre pollen
- k. □ Midd
- l. □ Muggsopp
- m. □ Temperaturforandring/physisk aktivitet
- n. □ Fødemidler
- o. □ Annet

Hvilke dyr: ........................................
Hvilke pollen: ...................................

142. I hvor stor grad har disse øye/nese symptomene innvirket på de daglige aktivitetene?

- 1. Ikke i det hele tatt
- 2. Lit.
- 3. Moderat
- 4. Mye

143. Har du gjennomført en allergiologisk luftveis utredning?

- a. □ Prikktest
- b. □ Provokasjon
- c. □ Sykehistorie alene
- d. □ Blodprøver (Spesifikk IgE)
- e. □ Røntgen/CT/MR (Øvre LV)
- f. □ Røntgen/CT/MR (Nedre LV)

Hvis ja, hvordan?

Hva:

144. Hva slags behandling har du fått for dine øye/nese symptomer?

- a. □ Net
- b. □ Siste 12 måneder
- c. □ Siste 14 dager
- d. □ Kontinuerlig

- a. □ Lokale antihistaminer
- b. □ Systemiske antihistaminer
- c. □ Leukotrienantagonister
- d. □ Immunoterapi/allergivaksiner
- e. □ Topical steroid
- f. □ IgE antagonist (Omalizumab)
- g. □ Natriumkromglukat
- h. □ Annet

Atopisk eksem

145. Har barnet/har barnet hatt atopisk eksem, d.v.s. rødt, tøft og kløende eksem?

- 0. Nei
- 1. Ja
- 3. Vet ikke

Hvis nei, gå til spm 152

146. Hvis ja, alder ved symptomstart (mnd):

- 0. Nei
- 1. Ja
- 3. Vet ikke

1: Tidligere, ikke siste 12 måneder
2: Siste 12 måneder
3: Siste 14 dager
4: Kontinuerlig

147. Hvis ja, har barnet fortsatt atopisk eksem?

- 0. Nei
- 1. Ja
- 3. Vet ikke

148. Hvis nei (147), alder ved symptomslutt (mnd):

149. Faktorer som forværrer eksemet:

- a. □ Melk og melkeprodukter
- b. □ Egg
- c. □ Peanøtter
- d. □ Nøtter
- e. □ Sitrus
- f. □ Tomat
- g. □ Jordbær
- h. □ Andre matvarer
- i. □ Pollen
- j. □ Dyr
- k. □ Annet

150. Når er eksemnet tilstede:

- 1. Hele året
- 2. Kun i vinterhalvåret
- 3. Vet ikke/husker ikke

151. Hva slags behandling har barnet fått for eksemnet:

- a. □ Kun fuktighetskrem
- b. □ Steroidsalve/krem gruppe 1-2
- c. □ Steroidsalve/krem gruppe 3-4
- d. □ KP-bad/krydte badevann
- e. □ Lokal immunmodulator (protopic/elidel)
- f. □ Annet
1. Kode

0. Nei  
1. Ja  
2. Vet ikke

152. Har barnet/har barnet hatt elveblest?  
Hvis nei, gå til spm 156

153. Faktorer som forværrer/utløser elveblesten:

- a. Melk og melkeprodukter
- b. Egg
- c. Peanøtter
- d. Nøtter
- e. Sitrus
- f. Tomat
- g. Jordbær
- h. Andre matvarer
- i. Pollen
- j. Dyr
- k. Annet
- l. Fysikalske stimuli
- m. Infeksjoner

Hvis nei, gå til spm 156

154. Hva slags behandling har barnet fått for elveblest:

- a. Antihistamin
- b. Steroider
- c. Adrenalin
- d. Annen behandling
- e. Ingen behandling

155. Har barnet hatt pustevansker i forbindelse med episoder med elveblest:

- 0. Nei
- 1. Ja
- 2. Vet ikke

156. Har du noen gang hatt tung pust, tetthet eller piping/vesing i brystet?

157. Har du hatt tørr hoste om natten uten å være forkjølet eller ha andre luftveisinfeksjoner?  
Hvis nei, gå til spm 163

158. Hvor mange perioder med tung pust, tetthet eller piping/vesing i brystet har du hatt siste 12 måneder?

- 0. Ingen
- 1. 1-3
- 2. 4-12
- 3. Mer enn 12

159. Hvor mange dager med tung pust, tetthet eller piping/vesing i brystet har du hatt siste 14 dager?

- 0. Ingen
- 1. 1-3
- 2. 4-12
- 3. Mer enn 12

160. Er/var det årstids- variasjon i symptomer?

- 0. Nei
- 1. Ja

161. Hvis ja 160, hvilken/hvilke årstider er verst?

- a. Vår
- b. Sommer
- c. Host
- d. Vinter

162. Hva er/var det som utløser/forværrer symptomerene?

- a. Anstrengelse
- b. Sigarettrøyk
- c. Pollen
- d. Mat/drikke
- e. Tåke/fuktig luft
- f. Infeksjoner
- g. Sterke lukter
- h. Pelsdyr
- i. Kald luft
- j. Annet

163. Har du fått diagnosen astma noen gang?

- 0. Nei
- 1. Ja

164. Har du noen gang brukt medisin for luftveiene?

- 0. Nei
- 1. Ja

165. Hvis ja, kun hostesaft/efedrin?
166. Har du noen gang brukt (1-3): (som hjemme behandling)

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>a.</td>
<td>b.</td>
<td>c.</td>
<td>d.</td>
<td>e.</td>
<td>f.</td>
<td>g.</td>
<td>h.</td>
<td>i.</td>
<td>j.</td>
<td>k.</td>
<td>l.</td>
<td>m.</td>
<td>n.</td>
<td>o.</td>
</tr>
</tbody>
</table>

167. Hvis du har brukt β-2 agonist siste 12 mnd/14 dager, hvor stort har forbruket i gjennomsnitt vært pr. brukeruke?

<table>
<thead>
<tr>
<th>1. Daglig</th>
<th>2. 4-6 dager/uke</th>
<th>3. 1-3 dager/uke</th>
</tr>
</thead>
<tbody>
<tr>
<td>a.</td>
<td>d.</td>
<td>e.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Hvor mange uker</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dager</th>
</tr>
</thead>
<tbody>
<tr>
<td>f.</td>
</tr>
</tbody>
</table>

168. Har du brukt β-2 agonist (hurtigvirkende astmamedisin) i forbindelse med fysisk aktivitet de siste 12 måneder?

<table>
<thead>
<tr>
<th>0. Nei</th>
<th>1. Ja</th>
</tr>
</thead>
<tbody>
<tr>
<td>a.</td>
<td>b.</td>
</tr>
</tbody>
</table>

169. Hvis du har brukt inhalasjonssteroider, hva var alder ved behandlingsstart?

<table>
<thead>
<tr>
<th>Bruker du fortsatt inhalasjonssteroider?</th>
</tr>
</thead>
<tbody>
<tr>
<td>0. Nei</td>
</tr>
<tr>
<td>a.</td>
</tr>
</tbody>
</table>

Hvis nei, alder ved seponering:

<table>
<thead>
<tr>
<th>0. Nei</th>
<th>1. Ja</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.</td>
<td>d.</td>
</tr>
</tbody>
</table>

170. Hvis du har brukt inhalasjonssteroider siste 12 mnd/14 dager, hvilken type og hvor stor dose?

<table>
<thead>
<tr>
<th>1. Flutide</th>
<th>2. Pulmicort/Becotide/Aerobec</th>
</tr>
</thead>
<tbody>
<tr>
<td>a.</td>
<td>c.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dose (ug/dag)</th>
</tr>
</thead>
<tbody>
<tr>
<td>d.</td>
</tr>
</tbody>
</table>

171. Hvis du bruker inhalasjonssteroider, bruker du det hele året?

<table>
<thead>
<tr>
<th>0. Nei</th>
<th>1. Ja</th>
</tr>
</thead>
<tbody>
<tr>
<td>a.</td>
<td>b.</td>
</tr>
</tbody>
</table>

172. Hvis ja, hvor mange måneder, siste år?

<table>
<thead>
<tr>
<th>0. Vår</th>
<th>1. Høst</th>
</tr>
</thead>
<tbody>
<tr>
<td>a.</td>
<td>b.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sommer</th>
<th>Vinter</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.</td>
<td>d.</td>
</tr>
</tbody>
</table>

173. Hvis nei, hvilken/hvilke deler av året bruker du inhalasjonssteroider?

<table>
<thead>
<tr>
<th>0. Nei</th>
<th>1. Ja</th>
</tr>
</thead>
<tbody>
<tr>
<td>a.</td>
<td>b.</td>
</tr>
</tbody>
</table>

174. Hvis du bruker inhalasjonssteroider, bruker du det kun ved forverrelser?

Hvis ja, hvor mange perioder brukte du inhalasjonssteroider siste år?

<table>
<thead>
<tr>
<th>0. Nei</th>
<th>1. Ja</th>
</tr>
</thead>
<tbody>
<tr>
<td>a.</td>
<td>b.</td>
</tr>
</tbody>
</table>
175. Har du noen gang brukt systemiske steroidkurer? a. 0. Nei 1. Ja  
   Hvis ja, hva var alder ved første kur? b.  
   Antall systemiske steroidkurer siste 12 mnd? c.  

176. Hvordan vil du karakterisere din helse i forhold til astma/astmaligne symptomer?  
   Tidligere, ikke siste 12 mnd  
   a.  
   Siste 12 mnd  
   b.  
   Siste 14 dager  
   c.  
   0. Ikke syk i det hele tatt  
   1. Svært lite syk  
   2. Endel syk, men ikke særlig plagsomt  
   3. Mye syk, men tolerabelt for familien  
   4. Svært mye syk, går utover familien  

177. Hvor mye har du vært borte fra skolen pga astma? a. siste 12 mnd b. siste 14 dager  
   1. Intet fravær 2. < 5 dager 3. 5-10 dager 4. > 10 dager  

178. Føler du at astmaen hemmer din fysiske aktivitet? a. tidligere b. siste 12 mnd  
   0. Nei 1. Ja  

Andre sykdommer  

182. Har barnet annen kronisk sykdom: 0. Nei 1. Ja  
183. Hvis ja, hvilke(n): a hjertesykdom d epilepsi  
   b diabetes e Annet .................................................................  
   c revmatisk sykdom  

184. Faste medikamenter (med unntak av med. for atopisk sykdom): 0. Nei 1. Ja  
   Hvis ja, hvilke:.................................................................