The quest for ingested peanut protein in human serum

A. P. Mose¹², E. Mortz³, P. S. Skov¹⁴, C. G. Mortz¹², E. Eller¹², U. Sprogøe⁵, T. Barington⁵, C. Bindslev-Jensen¹²

¹Odense Research Center for Anaphylaxis (ORCA), Odense University Hospital, Denmark
²Department of Dermatology and Allergy Center, Odense University Hospital, Denmark
³Alphalyse A/S, Odense, Denmark
⁴RefLab Aps., Copenhagen, Denmark
⁵Department of Clinical Immunology, Odense University Hospital, Denmark

ACKNOWLEDGEMENTS

The authors wish to record their gratitude to all study participants (donor and recipients) who took part in the study. Furthermore, we would like to gratefully acknowledge Janne Crawford and Rikke Raaen Lund from Alphalyse for Ara h 2 assay development and mass spectrometry analyses; Niels Peter Hell from RefLab for the Ara h 2 and 6 ELISA measurements; and Ulla Johannessen and...
Lene Albjerg from Odense University Hospital for technical assistance in the Prausnitz-Küstner tests.

Conflict of Interest
Ejvind Mortz is cofounder and COO of Alphalyse, which is a protein analysis CRO servicing a variety of biotech and pharma companies, as well as university research groups. Per S. Skov is a research consultant for Reflab ApS. Charlotte G. Mortz has served on a board for Novartis and has received payment for lectures from Novartis and Sandoz A/S. Carsten Bindslev-Jensen has received a grant from Odense University Hospital Research Foundation, has had consultant arrangements with Hal Allergy and Anergis and has received payment for lectures from Hal Allergy and Thermofisher. The rest of the authors declare that they have no relevant conflicts of interest.

Abbreviations
PK test: autologous Prausnitz-Küstner test
BHRA: basophil histamine release assay

ABSTRACT
Background: There is mounting evidence that systemic uptake of food allergens is key to triggering anaphylaxis. However, direct proof for this theory is still lacking. The purpose of this study was to quantify the absorption and to determine the absorption kinetics of immunoreactive peanut protein in relation to the allergic response in human.

Methods: Quantitative protein assays including mass spectrometry, dot blots and Western blotting were developed to determine the level of Ara h 2 absorption in human serum. The double monoclonal sandwich ELISA was applied to quantify absorbed Ara h 2 and 6, and the basophil histamine release assay and the human passive cutaneous anaphylaxis test were utilized to study the absorption kinetics of immunologically intact peanut proteins.

Results: The protein assays worked but were not sensitive enough to trace the minute amounts of absorbed Ara h 2 in human serum. The level of Ara h 6 in serum was found to be up to 0.2 ng/mL,
but Ara h 2 could not be detected with the ELISA. Both the in vivo and the in vitro methods were successful in demonstrating that: immunoreactive peanut protein was absorbed shortly after ingestion (≤ 5 min); the peanut protein concentration peaks between 1 and 4 hours; and peanut proteins can circulate for at least 48 hours in the bloodstream.

**Conclusion:** Ingested peanut protein is absorbed systemically and retains its immunoreactive capacity in human serum. However, the precise quantities and the implication for the elicitation of anaphylaxis remains to be elucidated.

**Key words:** allergen quantification, allergen absorption kinetics, Ara h 2, Ara h 6

**INTRODUCTION**

The IgE-mediated allergic reaction to dietary allergens is the result of a complex series of events; nevertheless, the allergic reaction is rapid in onset and usually occurs within one hour of food intake. It is commonly assumed that the absorption of intact proteins or large fragments thereof in the gastrointestinal tract play a key role in the development and elicitation of food-induced allergic reactions, but the present evidence is mainly indirect and suggestive and the reported levels of allergen in serum are extremely low.\(^{(1-12)}\) Certainly, a food allergen must cross-link allergen-specific IgE antibodies on the surface of effector cells for an allergic reaction to occur. However, whether systemic anaphylaxis is elicited by allergens within the gastrointestinal lumen that trigger local effector cells that spread symptoms systemically or absorbed allergens that are disseminated systemically and stimulate effector cells in the end organ(s) is still unclear.

Therefore, the aim of this work was to develop a method for the precise and specific quantification of absorbed food protein in human serum using the major peanut allergens, Ara h 2 and Ara h 6, as the model allergens. In particular, we sought to uncover when the first traces of
the allergen is absorbed into the bloodstream thus shedding light on key aspects pertaining to the elicitation of allergic reactions. To this end, we applied high-performance protein analysis techniques to quantify Ara h 2 along with two double monoclonal sandwich ELISAs to quantify Ara h 2 and 6 respectively and in vivo (the Prausnitz-Küstner test) and in vitro testing (the basophil histamine release test). In short, blood from healthy, non-allergic individuals was sampled before and after they had ingested peanut and their serum was analysed for the presence of peanut allergen up to 48-hours after ingestion.

METHODS
The following subsections outline the protein analysis techniques that we applied to detect absorbed Ara h 2 in serum. Ara h 2 was chosen for this part of the study, because it belongs to the potent conglutin family and thus is recognised as one of the most important allergens in relation to peanut allergy. In part, Ara h 2’s resistance to the proteolytic and acidic conditions in the digestive tract explains this. In addition, Ara h 2 is biochemically well characterised and make up a sizeable part of the total protein content in peanut (5.9-9.3 %).

In these protein analyses we used sera from four healthy and non-allergic individuals (1 woman and 3 men) aged 33 to 59 years (mean age 48 years). Their sera were sampled before (T=0) and after (T= 1, 2, 3, 4 hours) the individuals had ingested 100 g of dry-roasted peanuts (KiMs A/S, Søndersø, Denmark) amounting to approximately 25 g of peanut protein. For full details of these methods please visit www.onlinelibrary.wiley.com/journal/13989995.

Mass spectrometry
The aforementioned sera were analysed using three different mass spectrometry techniques and immunoassay recognition to detect Ara h 2. The blank sera spiked with purified Ara h 2 (Indoor Biotechnologies, Cardiff, UK) were used as positive controls.

First, we performed LC-MS with MRM quantification of Ara h 2 peptides on an Agilent 6410 Triple Quad mass spectrometer. The serum samples were mixed with 8 M urea and cysteines reduced and alkylated in DTT/IAA for complete denaturation and solubilisation of proteins and protein complexes. Trypsin digestion (2%) was performed overnight at room temperature and the
peptides cleaned up on MCX plates for LC MS analysis.\(^{25}\)

Next, we carried out GeLC MS with SDS PAGE and protein identification using NuPAGE 1D gels and nanoLC MS/MS protein identification on a Bruker Maxis Impact QTOF mass spectrometer. The serum samples were mixed with 1D LDS sample buffer with DTT and heated to 70°C for protein denaturation before gel electrophoresis. Gel bands were reduced/alkylated and in-gel trypsin digested. The extracted peptides were analysed by nanoLC MS/MS and proteins identified by Mascot database searching.\(^{26}\)

Last, we applied SWATH LC MS for identification and label-free protein quantification on a Sciex TripleTOF 6600 mass spectrometer using a peanut protein ion library. Peanut proteins were extracted from both roasted peanuts and defatted peanut flour by mixing at 70°C for 15 min in 0.1 M Tris pH 8 or in LDS sample buffer. Extracted peanut proteins were separated by SDS PAGE, in-gel digested and analysed by LC MS by data-dependant acquisition to generate a spectral ion library of peanut proteins. For SWATH analysis, the serum samples were digested both in-solution and in-gel and analysed by SWATH LC MS by data-independent acquisition.\(^{27}\)

**Immunosassay recognition using Ara h 2 antibodies**

We obtained polyclonal antibodies against natural Ara h 2, and purified natural Ara h 2 (Indoor Biotechnologies, Cardiff, UK) as a positive control for assay development. Polyclonal antibodies were raised (GenScript, Piscataway, USA and Cambridge Research Biochemicals, Billingham, UK) against linear epitopes using synthetic peptides in four regions of Ara h 2 recognized by IgE epitope mapping\(^{23, 28}\) and by mass spectrometric peptide mapping. The antibodies were utilized in dot blots and Western blots.

For the following parts of the study, we recruited six healthy, non-atopic individuals (all men) aged 24-65 years (mean age 35 years). Exclusion criteria were having past or present allergies to peanut and inhalant tree allergens (grass, birch and mugwort). Blood was sampled before (T= 0) and after (T= 5, 10, 20, 30, 45, 60, 90 min and 2, 4, 6, 8, 24, 48 hours) the non-allergic individuals (recipients) had ingested 100 g of partially defatted and lightly roasted peanut flour (Golden Peanut and Tree Nuts, Georgia, United States), i.e. \(=50 \text{ g of peanut protein}\), suspended in 250 ml of water for oral
administration. The challenge dose was administered as a drink to expedite the oral intake (≤ 10 seconds). This was imperative in terms of defining T= 0. The recipients fasted for at least 8 hours and kept a peanut-free diet for a minimum of 72 hours before they ingested the peanut dose. Their sera (i.e., autologous sera) were used successively in commercial Ara h 2 and Ara h 6 ELISAs, the basophil histamine release assay (BHRA) and the autologous Prausnitz-Küstner test (PK test).

In addition to the autologous sera, an allogenic (donor) serum was obtained from a person (27-year-old male) with severe peanut allergy for use in the BHRA and the PK test. The elevated specific IgE-levels to peanut, Ara h 2 and Ara h 6 in the donor serum were 51.5, 30.1 and 19.0 kU/L respectively. The donor met the specific eligibility criteria outlined by the Danish Blood Safety Directives. This includes screening for disease risk factors using a standardized health history questionnaire as well as testing for relevant transfusion-transmitted infections including HIV, viral hepatitis, human T-lymphotropic virus, and syphilis. Our local Blood Donor Centre kept the donor serum in quarantine for 2 months until a second blood-screening test of the donor for all markers was nonreactive. The donor serum were stored at −30°C until use.

Ara h 2 and 6 ELISAs
Two commercial ELISAs were used to analyse the presence and quantity of the major peanut allergens Ara h 2 and 6 in the sera from the six recipients. Both ELISAs were from Indoor Biotechnologies (Ara h 2 and 6 ELISA 2.0, Cardiff, UK) utilizing monoclonal antibodies. The assays were performed according to instructions of the manufacturer. The lower limit of detection (LLoL) was 2.0 ng/mL for Ara h 2 and 0.05 ng/mL for Ara h 6.

Basophil histamine release assay
The recipients' sera were also tested for peanut allergens, measuring histamine release from donor basophil cells passively sensitized with the serum from the person with severe allergy. The basophil cells used for the histamine release experiments were obtained from blood donor buffy coat cells, provided by the Blood Bank at the National University Hospital, Copenhagen, Denmark. Only donor basophil cells with confirmed strong reaction to anti-IgE, i.e. releasing ≥35 ng histamine/mL and no reaction to peanut, were included in the experiments. Briefly, the passive
sensitization was performed in two steps.\(29\) Firstly, the donor basophil cells were mixed with a stripping buffer at pH 3.6 (RefLab ApS, Copenhagen, Denmark) and immediately thereafter centrifuged at 11°C for 5 min. During centrifugation, the IgE was dissociated from the receptor and was afterwards removed from the cell sediment. Then the IgE stripped basophils in a volume of 500 µL were mixed with 125 µL serum from the allergic patient and incubated at 37°C for 60 min. Aliquots of 100 µL of passively sensitized donor basophil cells in the presence of IL-3 (2 ng/mL blood) were incubated at 37°C for 60 min with 100 µL serum drawn from time points before and up to 48 hours (see fig. 1) after ingestion of 50 gram of peanut protein. As positive controls the passively sensitized cells were incubated with peanut in concentrations ranging from 10 pg/mL to 10 ng/mL and anti-IgE (KPL, Gaithersburg, MD, USA) in a concentration of 5 µg/mL. Thereafter, the cell samples were centrifuged at 2000 G for 5 min at 11 °C and the supernatant was discarded in order to avoid interference from serum in the histamine analysis.

The cell sediment was lysed by adding 250 µL of 0.9 % HClO4 in Pipes buffer (RefLab, Copenhagen, Denmark) by incubation at 37 °C for 30 min. Histamine in the lysate was determined by adding 75 µL lysate in duplicate to the wells in a glass fiber coated microtiter plate (HR-Test. RefLab, Copenhagen, Denmark) and incubated for 60 min at 37 °C. After incubation, histamine was bound to the glass fiber coating and all interfering material was washed out of the plate. 75 µl of NaOH/ortho-phtaldialdehyde (OPA) mixture was added to each well allowing glass fiber bound histamine to be released and coupled to OPA. The coupling reaction between histamine and OPA was stopped after 10 min and stabilized by adding 75 µl of 0.59% HClO4. Histamine was determined fluorometrically in the Histareader™ 501 (manufacturing company, RefLab ApS, Copenhagen, Denmark). Results are given as histamine release in % of the total cellular histamine and a release > 10 % is considered significant release.

**Autologous Prausnitz-Küstner test**

In addition to the BHRA, the recipients’ sera were analysed using the PK test, which in this study was divided into three sequential steps:

**Step 1.** Recipient serum sampling after ingestion of peanut
This step is outlined previously in the section “Part two of allergen detection”.

Step 2. Passive transfer of the donor serum
Aliquots of 100 \( \mu \)L of the donor serum from the person with severe peanut allergy were used for intradermal injections (n= 14), i.e. local sensitization, in the volar aspect of the forearms in the non-allergic recipients. The safety procedures pertaining to donor eligibility and the blood donation process was carried out as described previously.\(^{30}\) Steps 1 and 2 were separated by at least 7 days.

Step 3. Autologous Prausnitz-Küstner test
Twenty-four hours after step 2, we injected aliquots of 50 \( \mu \)L of the recipients own (autologous) serum, i.e. the serum collected in step 1 as a peanut source, into the donor-sensitized skin sites. The injections were carried out in a blinded protocol, i.e. the allocation sequence of the autologous serum collected at the different time points after the ingestion of the peanut was concealed from the investigator. We recorded the sizes of the wheals by marking their borders with a pen, which was then transferred to a documentation form using a translucent adhesive tape. The measurements were undertaken when the wheal-and-flare reactions had reached their maximum size. A reaction was only considered positive if a flare was followed by the formation of a wheal with a calculated mean wheal diameter \( [(D+d)/2] \) of \( \geq 3 \) mm.

Statistics:
Results from the BHRA and the autologous Prausnitz-Küstner test are given as the median, the 25\(^{th}\) and 75\(^{th}\) percentiles and the 10\(^{th}\) and 90\(^{th}\) percentiles using Stata IC 14.0 (Stata Corporation LP\(^{\circ}\), Texas, USA).

Ethics
The Regional Scientific Ethics Committee for Southern Denmark has approved the study protocol (Project-ID: S-20130086). The donor and the recipients gave written informed consent before enrolment in the study.
RESULTS

Mass spectometry

We were able to detect Ara h 2 spiked into blank serum with all of the developed Ara h 2 assays (Figure 1), i.e. the dot-blots, the Western blotting and the three mass spectrometry techniques. The estimated detection limits for Ara h 2 were approximately 0.2-1 μg/ml, 0.5 μg/ml, 1 μg/ml and 0.5-2.5 μg/ml for the LC-MS MRM, the GeLC MS/MS, the SWATH LC MS and the dot blot protocols respectively. We were, however, unable to detect Ara h 2 in the serum samples obtained from our four non-allergic individuals who had ingested a large amount of roasted peanut.

In the LC-MS analysis of purified natural Ara h 2, we found that the region containing hydroxyproline residues showed significant heterogeneity with different levels of hydroxylation and partial missed cleavages by trypsin. The heterogeneity results in higher detection limits than are observed for non-modified peptides. This hydroxyproline rich region is of particular interest for the analysis because it is involved in the major IgE epitopes.

In the GeLC MS/MS analysis, full length Ara h2 variants and digested Ara h2 fragments were observed in the positive control samples both in the image analysis software and by the nanoLC MS/MS analysis (see Figure E3 in the Online Supplement). Analysis of the serum samples before and 4 hrs after peanut ingestion show no visual differences or differences in the LC MS/MS results that could be correlated to peanut proteins.

We established a peanut protein library containing 79 different peanut proteins, including Ara h 1–4, 6, 7 and 8, in the SWATH LC MS assay development. Multiple peptides were present for each peanut protein in the library with different isoforms and posttranslational modifications; Ara h 2 was represented with 30 peptides and a sequence coverage of 87%. The SWATH LC MS analysis was able to detect all the known peanut protein allergens with peptides covering the majority of their protein sequences.

Immunoassay recognition using Ara h 2 antibodies

In the dot blots, the detection sensitivity was limited by a high background staining due to non-specific binding to other proteins in the serum from the non-allergic individuals. Likewise, we observed background staining of proteins with a MW of 25 kDa and 50 kDa, which is above the MW region of Ara h 2 and Ara h 2 fragments (≤ 20 kDa), in the Western blotting.
Ara h 2 and 6 ELISAs

Ara h 6 was undetectable in the sera of two of the recipients while in the sera of the remaining individuals (n=4) only a very low average peak level of Ara h 6 could be detected (0.16 ng/ml, ± 0.1 ng/mL) after 45 min (Figure 2). We found no significant levels of Ara h 2 in any of the sera.

Basophil histamine release assay

We found a positive HR response in all six recipients (Figure 3). The HR response was markedly increased, i.e. positive, from 10 minutes to 8 hours after ingestion of the peanut flour in all recipients. In one recipient, a clear response was observed already after 5 minutes and in two recipients, a response was observed up to 48 hours after ingestion. The results for the dose-response to peanut and the positive control after passive sensitization in the BHRA is displayed in the online supplement (see Figures E6 and E7).

Autologous Prausnitz-Küstner test

The PK test was successful in five out of the six recipients (Figure 4). Serum sampled at 20 minutes and at 8 hours after the ingestion of peanut were the earliest and the latest sampling time points respectively that gave positive skin reactions in all of the five reacting recipients. However, three recipients developed positive skin reactions to their respective 5-minute serum sample and one recipient produced a positive skin reaction to his serum sampled as late as 48 hours after ingesting peanut flour. The recipient who was unresponsive to all autologous serum samples in the PK test had by far the highest Ara h 2-specific IgG4 serum concentration (data not shown).

DISCUSSION

The present study was designed to determine the serum concentration and absorption kinetics of peanut protein in non-allergic individuals after peanut ingestion. Though we used state-of-the-art protein analysis techniques, we could not demonstrate that Ara h 2 had been absorbed with any of these methods. This is rather surprising given the large total amount of ingested peanut protein.
(approximately 25 g). However, absorption levels of only 0.16 ng/mL (peak average) were found with the Ara h 6 ELISA and not even in the sera of all of the tested non-allergic individuals. JanssenDuijghuijsen et al. have presented similar findings in their recent study.\textsuperscript{(6)} Unfortunately, such minute amounts are below the detection limits found for the other quantitative protein analysis assays used in this study.

We were, however, able to demonstrate the time-course of peanut allergen absorption in human serum using both an in vitro and in vivo method. In this study, the presence of immunologically intact peanut protein in the blood of our non-allergic study participants was observed as early as 5 minutes after oral ingestion, which is the earliest time-point described to date.\textsuperscript{(1, 9, 12, 31)} Thus, the absorption rate in non-allergic individuals matches the time development of anaphylaxis in patients.\textsuperscript{(32)} Furthermore, the extremely rapid absorption from the gastrointestinal tract (GI) could indicate that at least the initial traces of peanut protein either partially or fully escape digestion and are absorbed immunoreactively intact. Therefore, it is plausible that the allergic reaction is triggered by systemically absorbed allergens based on these data.

Together, our results also indicate that peanut protein absorption peaks between 45 min and 4 hours after oral intake (Figure 3 and 4). Additionally, we found that the peanut proteins can persist in the circulation for several days (≥ 48 hours) which is a novel finding and was highly unexpected (Figure 4). The explanation and clinical implication of this finding is at present unknown.

Overall, our findings are in keeping with previous studies on the absorption of peanut and other dietary proteins. In the studies by Dirks \textit{et al} and Untersmayr \textit{et al}, an increase in HR within 10 minutes of either peanut or codfish ingestion was observed in persons without food allergy.\textsuperscript{(9, 12)} The authors in the latter study also reported that maximal HR was reached 1 to 2 hours after codfish ingestion. In contrast, Paganelli \textit{et al} did not detect β-lactoglobulin in the circulation using a two-site solid phase radioimmunoassay until 30 min after their non-atopic adults had ingested milk,\textsuperscript{(1)} and in a study by Husby \textit{et al}, ovalbumin was first detected in the 60-min serum samples with an enzyme-linked immunosorbent assay in non-allergic individuals who were given raw egg.\textsuperscript{(4)} The relatively broad range in these results might reflect differences in the absorption rate between various food allergens. However, such a conclusion may be confounded by substantial differences in the sensitivity, i.e. the detection limit, of the different experimental assays used in
A few additional points in our study require comment. Firstly, we have shown that the autologous PK test and the BHRA test are highly sensitive methods for the detection of absorbed peanut protein in serum. In comparison, several of the protein techniques applied in this study could not measure up. Although we cannot exclude technical reasons, we find it more probable that these protein analysis techniques were not sufficiently sensitive to detect the traces of absorbed Ara h 2 in serum. However, the strength of and thus the reason for applying e.g. the MS techniques was that the quantification, if successful, would have been specific. On a side note, it is interesting and rather surprising that we were not able to detect Ara h 2 with the ELISA given that Schocker et al. recently were able to determine the amount of absorbed Ara h 2 in breast milk using the same technique.\(^{(33)}\) Secondly, one of the weaknesses of the biologic tests is that the methods cannot distinguish between whole peanut proteins such as the Ara h 2- and 6-molecule and partially digested fragments thereof. Nevertheless, both methods provide robust evidence that the small amounts of circulating protein or fragments thereof are antigenically intact.

In conclusion, our findings confirm that immunoreactive peanut proteins can be systemically absorbed in small, but significant amounts in non-allergic individuals. It is probable that the absorption kinetics of peanut allergens in non-allergic individuals is similar to patients with peanut allergy. However, the precise quantification of peanut protein absorption in human serum awaits further studies. MS-based technologies will undoubtedly play a pivotal role in this venture.

**Authors' contributions**

C. Bindslev-Jensen, C.G. Mortz, E. Eller and A. P. Mose designed the study. A.P. Mose performed the PK-experiments and wrote the predominant part of the manuscript. E. Mortz was responsible for the protein analyses and wrote the corresponding subsections. P.S. Skov was responsible for the BHRA and the Ara h 2 and 6 ELISAs and wrote the corresponding subsection. U. Sprogøe and T. Barington contributed with patients’ sera. C. Bindslev-Jensen, C.G. Mortz and E. Eller supervised the project. All authors critically read, revised and approved the final manuscript.
REFERENCES


This article is protected by copyright. All rights reserved


FIGURE LEGENDS

Fig 1. The amino acid sequence of the Ara h 2.02 allergen (sequence variant P2). A, B and C demonstrates which Ara h 2 peptides (highlighted in grey) that were targeted by the different protein assays. N.B. The first 21 amino acids are the signal sequence not present in the mature Ara h 2.

A. The LC-MS with MRM quantification assay was setup for detection and quantification of seven specific Ara h 2 peptides (peptide 1–7) in IgE epitope rich areas\(^{(23, 28)}\) (black boxes). (N.B. The sequence variant for P1 is not shown. See Figure E1 in the Online Supporting Information). The peptide specific for the sequence variants P2 and P4 (peptide 3) contains the hydroxyproline residue (bold letter).

B. The SWATH analysis identified all the Ara h 2 peptides (n= 30, black lines) as shown in the figure.

C. The four peptides (peptide A–D) in Ara h 2 that were chosen for raising the polyclonal antibodies used in the dot blots and Western blots.

Fig 2. Ara h 6 detection (ng/ml) in serum of six non-allergic individuals after ingestion of 100 g of defatted peanut flour, i.e. 50 g of peanut protein, using the ELISA. The assay’s Lower Level of
Quantification (LLoQ) is marked in grey.

**Fig 3.** The time dependency of peanut protein absorption in non-allergic individuals based on the basophil histamine release test. Blood sampled at fixed time-points after ingestion of 100 g of defatted peanut flour, i.e. 50 g of peanut protein, were analysed for histamine release using passively sensitized basophils. Each test was done in duplicate. The results are expressed in percentage of histamine released. Unspecific histamine release induced by pre-ingestion serum was subtracted to establish allergen induced histamine in the post-peanut ingested sera. The box plot displays the distribution of the data as follows: the median, the 25th and 75th percentiles (the boundary of the box) and the 10th and 90th percentiles (the whiskers above and below the box). The response in sera after peanut ingestion is significant when above 10% corresponding to 3xsd on background fluorescence. The dotted line represents this.

**Fig 4.** The results of the autologous Prausnitz-Küstner test displaying the time dependency of peanut protein absorption in non-allergic individuals. In the experiments, the non-allergic individuals ingested 100 g of peanut flour, i.e. 50 g of peanut protein, and blood samples were drawn before and at fixed time points after the peanut meal. Serum aliquots thereof were reinjected into passively, peanut-specific-IgE-sensitized skin sites in the non-allergic individuals. Positive and negative skin reactions were recorded. The box plot shows the distribution of the data as follows: the median, the 25th and 75th percentiles (the boundary of the box) and the 10th and 90th percentiles (the whiskers above and below the box). • is the symbol for an individual result. The dotted line represents the positive cut-off for the skin response (wheal diameter ≥ 3 mm).