CARDIOVASCULAR RESPONSES DURING IgE-MEDIATED PEANUT ALLERGIC REACTIONS.

Monica Ruiz-Garcia

Section of Paediatrics
Department of Medicine
Imperial College London

A thesis submitted for the degree of Doctor of Philosophy
31 January 2018

ABSTRACT:

INTRODUCTION:

The pathophysiology of IgE-mediated food allergy is poorly described and this impairs our ability to develop new treatments or predict reaction phenotype. Data from case series and animal models suggest there may be significant cardiovascular changes during severe reactions. The aims of this thesis were to describe the local and systemic cardiovascular (CVS) changes during IgE-mediated reactions to peanut, and evaluate whether local vascular responses to skin prick test can predict threshold or severity of reaction.

METHODS:

Fifty-seven peanut-allergic adults underwent continuous, non-invasive cardiac monitoring during double-blind placebo-controlled food challenges. CVS parameters during a 10-minute epoch at time of objective symptoms were compared to a 10-minute epoch at baseline. Comparisons were also made to equivalent data at the placebo reaction, and a further repeat open challenge in the same participants. Skin blood flow and titrated skin prick testing (SPT) were performed at each challenge.

RESULTS:

A significant increase in peripheral blood flow (median 20%, IQR [-2.2 to 46.7%]), decrease in stroke volume (mean -2.3ml/beat/m^2, 95% CI [-0.3 to -4.2]) and increase in heart rate (mean 7.7bpm, 95% CI [5.6 to 9.8]) were observed during reactions irrespective of reaction severity, which were reproduced at open challenge. Changes in heart rate variability were also noted, consistent with increased sympathetic activity, however these were not observed at repeat challenge. Titrated SPT (as a measure of local cutaneous vascular response) was found to predict reaction threshold at challenge. Time to resolution of peanut SPT wheal was associated with several measures of reaction severity at challenge.

CONCLUSION:

There is a significant reduction in stroke volume during IgE-mediated reactions to peanut. This is likely to be caused by peripheral vasodilatation leading to reduced venous return, and was seen in both mild and severe reactions. This finding highlights the importance of adequate fluid resuscitation in the management of IgE-mediated allergic reactions to food.

TABLE OF CONTENT:

DECLARATION OF ORIGINALITY:

The work contained in this thesis is my own, unless otherwise referenced. I recruited all the participants following study protocols and performed all screening visits. I conducted and supervised all the challenges performed. I acquired all the data, conducted the analyses, interpreted the data and wrote all the study text.

I was supported by the following colleagues, who made a substantial and valued contribution to the conception and design of the study, data interpretation and provided critical feedback on written texts.

Specifically, they made the following contributions:

- Supervisors:
    - Dr Robert Boyle (first supervisor, Clinical Senior Lecturer, Paediatric Department, St Mary´s Hospital, Imperial College London) provided guidance and support, and who was site PI on the TRACE study, which provided my salary costs.
    - Dr Paul Turner (second supervisor, MRC Clinician Scientist in Paediatric Allergy & Immunology, Honorary Consultant in Paediatric Allergy & Immunology at St Mary's Hospital and the Evelina Children's Hospital, Honorary Lectureship at the University of Sydney) provided guidance, support and funding (through an MRC Fellowship) for the acquisition of the necessary equipment, laboratory assessments and salary support costs.
- Collaborating researchers:
    - Dr Alex Lyon provided guidance on the cardiological data, provided the ECG Holter and computer programs need for the analyses of this data.
    - Dr Carl Hayward provided guidance on analysing the cardiovascular and ECG data.
    - Dr Andrew Clark, Dr Pamela Ewan, Dr Shelley Dua and Ian Kimber as part of TRACE peanut study setup.
    - Dr Claire Mills produced the challenge materials.
    - Dr Isabel Skypala prepared the challenge material on each challenge day.
    - Abigail Robb provided support on the laboratory work.
    - Danielle Belgrave, Sadia Haider and Jared Smith who provided statistical support.
    - Prof. Stephen Durham provided support on the clinical challenges.
    - Emily Wilson, Louise Cross and Gonçalo Abrantes, nurses giving support on challenge days.

- Alistair Tang, Zoe Tattersall, Ashna Lakhani and Shaline Patel, BSc students who collaborated in data acquisition and initial pilot data analyses.
- Dr Marta Vazquez-Ortiz and Sarah Lindsley providing punctual support on challenge days.
- Food Standard Agency sponsored TRACE study and salary support costs.
- Medical Research Council provided funding for equipment/consumables and salary.
- The NIHR Respiratory Biomedical Research Unit at the Royal Brompton Hospital provided space for food challenges and nursing support when required.
- BRC-Imperial for the grant awarded allowing for the purchase of Holter monitor.

Author´s signature: Monica Ruiz-Garcia

Date: 31 January 2018.

LIST OF ABBREVIATIONS:

ATS: American Thoracic Society.

Apen: Approximate Entropy.

ACQ: Asthma Control Questionnaire.

AHSC: Academic Health Sciences Centre.

AI: Augmentation Index.

ANS: Autonomic Nervous System.

AV: Atrio-ventricular.

APC: Antigen presenting cell.

AMP: Adenosine monophosphate.

ASA: Acetylsalycilic acid.

ACE: Angiotensin-converting-enzyme.

BAT: Basophil activation test.

BP: Blood pressure.

dBP: diastolic blood pressure.

sBP: systolic blood pressure.

BHR: Bronquial Hyperresponsiveness.

BTS: British Thoracic Society.

BSA: Body surface area.

CRF: Case record form.

CRH: Corticotropin-releasing hormone.

CVS: Cardiovascular system.

CO: Cardiac output.

CBP: Central blood pressure.

CV: Coefficient of variance.

CNS: Central nervous system.

CI: Confidence interval.

DBPCFC: Double-blind placebo control food challenge.

DFA-1: Short-term fractal scaling exponent.

ED: Eliciting dose.

EAACI: European Academy of Allergy and Clinical Immunology.

EPT: End-point titration.

ECG: Electrocardiogram.

Abbreviations.

EIA: Exercise induced asthma.

ERS: European Respiratory Society.

EoE: Eosinophilic esophagitis.

FA: Food Allergy.

FEV1: Forced expiratory volume in 1 second.

FDA: Food and Drug Administration.

FDEIA: Food dependent exercise induced anaphylaxis.

GI: gastro-intestinal.

GDFT: Goal directed fluid therapy.

HRV: Heart rate variability.

HR: Heart rate.

Hb: Haemoglobin.

HF: High Frequency.

IgE: Immunoglobulin E.

IM: intramuscular.

ICC: Intraclass correlation coefficient.

IQR: Interquartile range.

IV: intravenous.

LF: Low Frequency.

MED: Minimal eliciting dose.

MI: myocardial infarction.

MAP: Mean arterial pressure.

MC: Mast cell.

MCT: Mast cell tryptase.

NSAID: Non-steroidal anti-inflammatory drug.

NHS: National Health Service.

NIHR: National Institute of Health Research.

Nu: normalised units.

NI: non-intervention.

NIAID: National Institute of Allergy and Infectious Disease.

OCR: Objective clinical reaction.

OFC: Oral food challenge.

OAS: Oral allergy syndrome.

PAF: Platelet activating factor.

PMAX: peanut non-diluted SPT at 20 minutes.

Abbreviations.

PC 3/6: Concentration required to reach 3 or 6mm wheal.

PT 3/6: Time required to reach a 3 or 6mm wheal.

PIS: Patient information sheet.

POEM: Patient- Orientated Eczema Measure.

PAC: Pulmonary artery catheter.

PFAS: Pollen food allergy syndrome.

PEFR: Peak expiratory flow rate.

PPI: Proton pump inhibitor.

RR: R to R interval.

SDNN: Standard deviation of the NN interval.

Sampen: Sample Entropy.

SABA: Short-acting beta agonist.

SV: Stroke volume.

SPT: Skin prick test.

STE: ST elevation.

STEMI: ST elevation myocardial infarction.

SVT: Supra ventricular tachycardia.

TRACE: Threshold of Reactivity And Clinical Evaluation study.

TPR: Total peripheral resistance.

TNSS: Total Nasal Symptom Score.

VAS: Visual Analogue Scale.

VET: Ventricular ejection.

WBC: White blood count.

WAO: World Allergy Organization.

LIST OF FIGURES:

LIST OF TABLES:

ACKNOWLEDGEMENTS:

I am indebted to my supervisors, Dr Robert Boyle and Dr Paul Turner for their guidance and support over the course of my PhD. Both believed in my abilities, helped me understand the world of science, provided encouragement, advice, endless support and helped me overcome many PhD-related challenges, which made this PhD a very rich experience.
They are both excellent allergists and researchers, are an inspiration to me and I admire them greatly, they have taught me so much that I will always be grateful to them.

I would also like to express my gratitude to all the nurses that have helped me during this PhD, in supervising the hundreds of food challenges done, special mention to Emily Wilson and Louise Cross, without them this thesis would not have been possible. For always giving me a helping hand, a nice word, kindness and making me laugh on the bad moments.

Thanks to Dr Alex Lyon and Carl Hayward for their advice, patience and supervision of all the Cardiology aspects of this PhD. I have learned so much from Carl Hayward on how to focus my PhD and wise advice on where to put our efforts on this very complex part of the thesis.

I am endlessly grateful to Prof. Stephen Durham for his knowledge, his priceless advice, good ideas and believing in my capacity of doing research. He is an example for me to follow as a scientist and also as a person.

I am very thankful to Dr Isabel Skypala and Dr Parviz Habibi for critically examining the early and late stage PhD reviews.

During these 4 years I was able to supervise 4 BSc students (Alistair Tang, Zoe Tattersall, Ashna Lakhani and Shalinee Patel). I would like to thank the massive effort they made on helping with the acquisition and initial analyses of the hours of recorded data. They reminded me of why I like research and medicine and were a spark of positive energy during their 3 months with me.

One of the biggest gratitude I have is to all the participants who voluntarily underwent the multiple food challenges, the confidence they had on my abilities and myself. They made my everyday work much easier having in mind the great responsibility and risk the food challenges had.

I would like to thank Dr Joaquin Sastre who encouraged me to move abroad, to keep learning and broadening my knowledge and abilities, without his advice I would have never moved to London, which has allowed me to grow personally and professionally. I would always look back and remember my time in London and my PhD as one of the most important in my entire life. To date this PhD has been the biggest challenge in my life.

Without my friends and family none of this would have been possible and will always be thankful to them. The biggest gratitude to my mum who is my biggest support and the best example of pursuing your dreams in life, she is the wiser and kinder person I know, thank you for always having the correct words, optimism and not allowing me to quit, ever. You are the best example to follow. Thanks to my auntie who always looks after me like her own child.

My deepest gratitude to my future husband, Israel, who even knowing we would be apart for at least 4 years encouraged me to move to London to become a better researcher and allergist, for always having kind words when I had bad moments and for his endless patience. I could have not find a better partner in life.

Last, but not least I would like to thank the Respiratory CRF team at the Royal Brompton Hospital and the Paediatric Research Unit team and St Mary´s Hospital for their help and kindness along the way.

PAPERS RELEVANT TO THE CONTENT OF THIS THESIS:

PUBLISHED:

1. Rajia Bahri, Adnan Custovic, Peter Korosec, Marina Tsoumani, Martin Barron, Jiakai Wu, Rebekah Sayers, Alf Weimann, **Monica Ruiz Garcia**, Nandinee Patel, Abigail Robb, Mohamed H Shamji, Sara Fontanella, Mira Šilar, E.N. Clare Mills, Angela Simpson, Paul J Turner; Mast cell activation test in the diagnosis of allergic disease and anaphylaxis; J Allergy Clin Immunol. Accepted January 2018.

COMMUNICATIONS TO CONGRESS:

1. **Monica Ruiz-Garcia**, Carl Hayward, Alistair Tang, Andrew Clark, Isabel J. Skypala, Stephen R. Durham, Alexander R. Lyon, Robert J. Boyle, Paul J. Turner; Effects of Intramuscular Epinephrine on Cardiovascular Parameters during IgE-Mediated Allergic Reactions to Peanut; Journal of Allergy and Clinical Immunology February 2016 Volume 137, Issue 2, Supplement, Page AB50.

2. **Ruiz-Garcia M**, Clark A, Skypala I, Belgrave D, Durham S, Turner PJ, Boyle RJ; Cardiac haemodynamic changes during acute IgE-mediated peanut allergic reactions in man, EAACI 2016, Vienna.

3. **Ruiz-Garcia M**, Hayward C, Tang A, Sim M, Clark A, Wilson E, Skypala I, Durham S, Lyon A, Turner PJ, Boyle RJ; Electrocardiographic changes during acute peanut allergic reactions in adults; EAACI 2015 Barcelona, PRIZE FOR THE BEST POSTER OF ITS CATEGORY.

1.INTRODUCTION:

1.1 History of food allergy:

It has been recognised since Ancient Greece that food can cause illness, disease and health concerns. Hippocrates was perhaps the first person to describe an "idiosyncratic" reaction to food, in this case cheese, in chapter 20 of his *Corpus Hippocraticum*:

"For cheese does not prove equally injurious to all men, for there are some who can take it to satiety, without being hurt by it in the least, but, on the contrary, it is wonderful what strength it imparts to those it agrees with; but there are some who do not bear it well, their constitutions are different, they differ in this respect, that what in their body is incompatible with cheese, is roused and put in commotion by such a thing; and those in whose bodies such a humor happens to prevail in greater quantity and intensity, are likely to suffer the more from it. But if the thing had been pernicious to the whole nature of man, it would have hurt all." [1]

Lucretius, a Roman philosopher, observed in 50 B.C: "what is food to one person, may be poison to another" [2]. The study at this time in history of the four humours led to the word "idiosyncrasy" which might have included allergic reactions.

The start of the 20th century saw the publication of multiple reports supporting the fact that foods are a problem for some people and can cause multiple medical illness and diseases. This was a development of medicine as practised in the preceding centuries (and still to this day), where physicians treated their patients with dietary manipulation. Physicians in the early 1900s often made clinical observations and developed a theory to explain them, which they then tested in other patients. Despite the fact that physicians practiced independently prior to the advances of mass transportation, they often reached the same conclusions, namely that food allergies can cause illness, disease and poor health.

I will summarise the most important publications in this section:

In 1905 Dr Hare wrote The Food Factor in Disease[3] as a result to his investigation in 1889 that migraine was relieved when patients were put on a special diet excluding fat, carbohydrates and alcohol.

In 1906 Dr Clemens von Pirquet suggested the use of the word "allergy"[4] to describe inappropriate reactions to food or other substances.

In 1908 Dr Alfred Schofield, an English physician, first described a case of egg allergy in a boy and how he was successfully treated [5], probably due to the natural resolution of egg allergy which we now know is a relatively common occurrence.

The first diagnosis of food allergy by skin test was performed by Dr Oscar Schloss, an American paediatrician, by means of a scarification test to egg white in 1912[6]. He also isolated fractions of hen´s egg white and determine that ovomucoid, ovoglobulin and ovomucin were the main elicitors of skin reactions [7].

In 1931 Dr Rowe documented that food allergies can cause a wide range of symptoms and can affect people of any age[8]. Dr Warren T. Vaughan, after studying an entire population in a small town in Virginia[9], stated 3 key points in allergy[10]: 1, food allergies are the most common cause of allergy in humans; 2, a person can become sensitized to any food; and 3, it is unusual to become allergic to just one food.

Dr Coca in the 1950s described the changes in pulse after exposure to food allergen in a book "*The pulse test",* which describes the direct relationship between food allergies and some illnesses such as hives and high blood pressure[11].

1.2 What is food allergy?

It is important to acknowledge the different terminology used in order to understand the underlying immunologic mechanisms in food allergy. Hypersensitivity is defined as an exaggerated immune response to a foreign agent. There are at least 4 different types of hypersensitivity reactions, as originally described by Gell and Coombs, shown in Table 1. It has been proposed that there´s a 5^{th} type of hypersensitivity where antibodies are produced to stimulate specific cell targets (an example is Grave´s disease)[12]. The World Allergy Organization (WAO) defines hypersensitivity as an "objectively reproducible symptoms or signs initiated by exposure to a defined stimulus at a dose tolerated by normal persons"[13] and defines allergy as "a hypersensitivity reaction initiated by specific immunologic mechanisms"[13], which can be antibody or cell-mediated.

Table 1 Types of hypersensitivity:

Type	Mediator	Description	Examples
I (Allergic)	• IgE.	• Reaction occurs within minutes. • Requires previous sensitization to the allergen. • Cross-link between Ig-E and allergen in the surface on mast cells and basophils. • Degranulation.	• Atopy. • Anaphylaxis.
II (Cytotoxic)	• IgM. • IgG. • Complement.	• Cellular destruction when antibody binds to antigen on target cell wrongly perceived by immune system as foreign.	• Autoimmune hemolytic anemia. • Thrombocytopenia.
III (Immune complex)	• IgG. • Complement. • Neutrophils.	• Antibody binds to antigen forming immune complexes, which get deposited.	• Arthritis. • Systemic lupus erythematous.
IV (Delayed-type, antibody independent)	• T cells	• Memory Th1 cells activate macrophages on re-exposure to antigen causing an inflammatory response.	• Contact dermatitis. • Coeliac disease.

It is important to differentiate between allergic reactions to food, which involve the immune system and, where mediated through IgE i.e. Type 1 hypersensitivity, can result in life-threating anaphylaxis and food intolerances, which do not involve an immune-mediated process and are generally thought to be due to events in the digestive system (for example, absolute or relative enzyme deficiencies which result in an a carbohydrate load causing osmotic effects in the gut) and cannot result in life-threatening, immune-mediated reactions. The European Academy of Allergy and Clinical Immunology (EAACI) defines food allergy as “an adverse reaction to food mediated by an immunologic mechanism“[14]. Food allergy (FA) can be of 3 types, IgE-mediated (type-I hypersensitivity reaction), non-Ig mediated (eg Food protein-induced enterocolitis syndrome (FPIES)) or a combination of both (eg eosinophilic oesophagitis (EoE)). For the purpose of this thesis I will focus on Type I Ig-E mediated food allergic reactions.

1.3 IgE-mediated FA:

This type of FA is characterized by reactions of rapid onset, usually within 15-30 minutes of exposure (although later reactions up to 2 hours have also been described[15]) to the allergen and it involves binding of the allergen to a specific antibody (IgE). This is in contrast to non IgE-mediated or mixed type FA, where symptoms are typically delayed in onset. Circulating IgE becomes bound to a specific receptor (the high affinity IgE receptor)

on the surface of effector cells (such as mast cells and basophils). Exposure to allergen results in allergen-IgE complexes, which cross-link with 2 specific IgE receptors (FcεRI) resulting in activation of a downstream pathway causing cell degranulation and the release of mediators, which cause the allergic reaction. IgE-mediated FA is also the most common cause of FA in the population affecting up to 10% of children[16] whilst the true prevalence of non IgE-mediated or mixed FA is unknown.

In order to develop food allergy, prior sensitization to the allergen (resulting in the production of allergen-specific IgE) is required, although this is no longer thought to have to occur via the oro-gastric route[17]. The most common route of sensitization is probably through the skin barrier. It has been shown that skin barrier dysfunction, as seen in atopic dermatitis, allows the allergen to enter the body and activate the immune system, which can develop an altered response to the allergen producing specific IgE against it[18]. Filaggrin gene has an important role in skin barrier structure and function and has been related to an increase risk in atopic dermatitis, asthma and FA[19, 20]. Other routes of sensitization have also been described mainly the airway[21] and gastrointestinal system[22].

The process, which leads to sensitisation in Type I, IgE-mediated FA can be divided into 2 categories:

Class 1 FA, also referred to as “primary” food allergy, usually occurs in childhood with first exposure to the food allergen itself: the allergic individual is sensitised to the food allergen itself. This is in contrast to class 2 FA, also referred to as “secondary” FA, where initial sensitisation is to an aeroallergen, which has cross-reactivity to epitopes in a food protein. Class 2 allergens are classically panallergens such as profilins and PR-10, which are common both to foods and pollens, hence the derivation of the term “Pollen-food allergy syndrome” (PFAS) which is often used to describe this. For the purpose of this thesis we will refer to PFAS (also referred to as oral allergy syndrome (OAS), although the latter term describes oral symptoms which also occur in primary FA, rather than a syndrome) as “secondary FA”.

Class 1 food allergens are often resistant to heat/enzyme/acid degradation whilst Class 2 food allergens are generally heat-labile, susceptible to digestion, highly homologous with pollen allergens[23] and rarely cause anaphylaxis[24] except for lipid transfer proteins (LTP) which are also panallergens but more heat-stable and less susceptible to digestion, and are associated with a high rate of systemic reactions[25, 26].

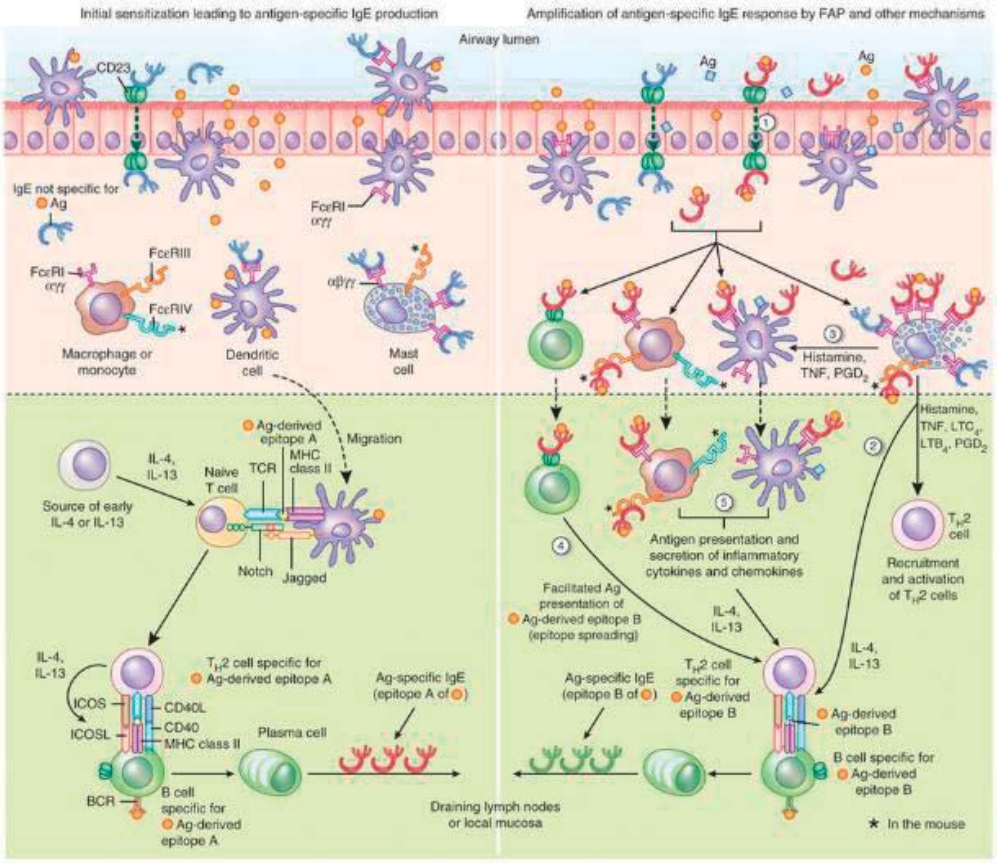
1.3.1 IgE sensitization:

The mechanisms (shown in Figure 1[27]) by which the immune system develops an undesired response to an otherwise innocuous allergen causing sensitization imply activation

of antigen presenting cells (APC) when in contact with allergen and in the presence of IL4 and IL-13 present the processed antigens to cognate naive T cells that then acquire a T helper type 2 (T_H2) cell phenotype[28], which results in the production of Th2 cytokines (IL-4, IL-5, IL-10 and IL-13) responsible for "class switching" of B cells and allowing specific IgE production[29]. IgE producing B cells can be generated in the respiratory mucosa[30] and gastrointestinal tract[31] not only in lymphoid germinal centers supporting the idea that IgE can be produced locally. In the case of food allergy, the location of B cells, which produce food allergen-specific IgE production is currently unknown.

Upon re-exposure to the allergen, specific IgE binds to the allergen forming a complex, which then binds to the high affinity receptor FcεRI receptor in the surface of the effector cells, which are mainly mast cells resident in mucosal and epithelial tissues and circulating basophils in blood. This IgE-allergen complex with the FcεRI receptor is internalized in the cell causing degranulation of mediators.

Figure 1 Mechanisms of IgE-mediated FA sensitization:



Sensitization with production of antigen-specific IgE and amplification of response on re-exposure to the allergen.
Illustration taken from paper *IgE and mast cells in allergic disease.* Nat Med, 2012[27].

The early phase response of the effector cells occurs within seconds. Mast cells contain preformed mediators, which are released, including histamine, tryptase, TNF-alpha, platelet activating factor (PAF), IL4, IL13 and leukotrienes (C4, D4 and E4)[32]. These creates an inflammatory cascade amplified by the recruitment of eosinophils, basophils and Th2 lymphocytes responsible for the late phase reaction which can take up to 8-12 hours to develop[33]. Although late phase reactions may occur in IgE-mediated food allergy, they are not commonly reported in clinical practice. Where both an early and late phase reaction occur, this is termed a biphasic reaction. However, it is now clear that the presence of IgE implies sensitization but not always clinical allergy: it is not uncommon for IgE-sensitized individuals to show no symptoms on exposure to the food allergen, therefore a diagnosis of food allergy requires the development of specific signs and symptoms on exposure to the offending allergen.

1.3.2 Epidemiology of IgE mediated FA:

IgE-mediated FA is a major public health concern, resulting in allergic reactions, which can vary in severity from mild reactions to life threatening anaphylaxis and death.

The prevalence of FA is reported to be up to 10% of children and 2-3% of adults [16, 34]. Some studies are self-reported or rely on serological data which can overestimate the true prevalence of food allergy compared to data relating to challenge-positive food allergy, thus precise estimates vary between studies [35, 36]. Data suggest that the frequency of FA is increasing in the last 20 years[37-40] although estimates of the actual incidence and prevalence are uncertain as few studies include DBPCFC performed in unselected cohorts to assess FA epidemiology.

The EuroPrevall study in 2013, using self-reported questionnaires and blood samples for specific IgE to different foods across 8 European centres, estimated a prevalence of IgE sensitization to any food in an adult population ranged between 6.6-23.6% [41]. Only a few studies include oral food challenge (OFC) at a population level. One example is the HealthNuts Study conducted in Melbourne, Australia in 2011[16], which reported FA prevalence in 12 month-old children of 3% to peanut, 8.9% to raw egg white and 0.8% to sesame. A study performed in the UK in 2010 [40] with OFC estimated a prevalence of 2% of peanut allergy in children aged 8 years old.

The most common offending foods in FA and anaphylaxis are milk, egg, peanut, tree nuts, fish, wheat and soya for young children, and peanut, tree nut and fish for adults[42, 43]. Fruits and vegetables are common causes of FA in adults, usually as part of the pollen food syndrome when eaten raw, but these foods only rarely cause anaphylaxis[24], although LTP-mediated FA is an emerging and important exception in Mediterranean regions.

The prevalence of IgE-mediated FA varies by age, being more frequent in children and young adults. IgE-mediated FA is different depending on geographical location, with lower rates in India and Russia compared with Europe, North America and Australasia, [44] and race/ethnicity with increased food sensitization in the non-Hispanic black population in US [45]. The reasons for these variations are not currently understood, although some authors have suggested that ambient humidity or vitamin D levels may be relevant, and recent data suggest that early skin care may be an important factor in the development of FA [46-48]. A recent review from Australia has suggested the 5"D´s" as possible risk factors in developing FA which include those which increase the risk of FA which include the presence of dry skin and Filaggrin mutation, and vitamin D deficiency and those which decrease the risk of FA which includes the presence of dogs in the house, early introduction of allergenic foods in the diet and dribble and shared microbial exposure [49].

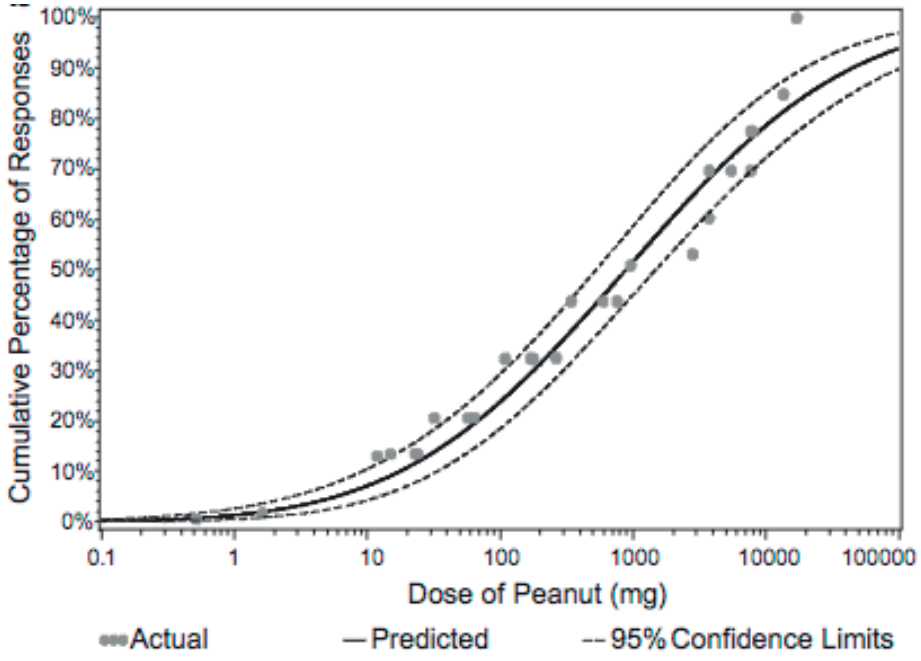
1.3.2.1 Peanut Allergy:

Peanut allergy is one of the most common food allergies affecting between 1.1-1.6% of children[16, 36, 50] with recent studies suggesting an increase in prevalence[50]. In the UK and USA, peanut allergy is the most common cause of fatal food anaphylaxis[42, 51] in children and young adults. It´s an increasing public health problem with adverse medical, psychosocial, and economic effects and which carries a high risk of severe reactions[51].

Different peanut proteins have been identified that confer allergy; seed storage proteins members of cupins (vicilin Ara h 1[52] and glycinins Ara h 3[53] and Ara h 4[54]), conglutins (Ara h 2[55], Ara h 6[56], and Ara h 7[57]), a non-specific lipid transfer protein (Ara h 9[58]), PR-10 (Ara h 8[54]), profilin (Ara h 5[59]) and oleosins (Ara h 10 and Ara h 11[57]).

Data has been published to assess the amount/threshold (or eliciting dose) needed to trigger symptoms in food-allergic patients, particularly for peanut (Figure 2). Of note, there is a great variability in peanut threshold, with often a 4–5 log-fold difference between subjects[60]. This data differs from that from aeroallergen immunotherapy studies where very little variability is seen between patients after nasal challenges[61]. The factors which might affect an individual's eliciting dose are unclear, and two of these, namely exercise and sleep deprivation, are being assessed in the TRACE study, which has formed the foundation of the data presented in this thesis.

Figure 2 Probability distribution curve for peanut:



Log-normal probability distribution models of individual peanut thresholds (expressed as whole peanut) for peanut-allergic individuals.
Graph taken from paper *Threshold dose for peanut: risk characterization based upon published results from challenges of peanut-allergic individuals.* Food Chem Toxicol, 2009[62].

1.3.3 Impact of FA:

The direct and indirect costs of childhood food allergy on affected families in the USA have been estimated at just under $25 billion per annum[63] although disparities exist in the economic impact of food allergy based on socioeconomic status[64]. The cost of food allergy in adulthood has not been reliably quantified [42].

Both children and adults suffering from food allergy, and their carers, report impaired health-related quality of life (HRQL) and increased stress and anxiety [65] due to the need for constant vigilance against accidental allergen exposure and the risk of a severe reaction. There is an increase in days off work or school compared to non-allergic population, and FA leads to social restrictions and an emotional burden [66]. Teenagers have difficulty balancing safety and impact of their FA on their quality of life [67]. Families of affected children experience a higher degree of impaired QoL compared to parents of non-allergic children, an impact which increases with younger age of onset, birth order (born second or later) and having multiple FA [68]. Families of patients affected with FA also experience lack of support in the effort to keep their children healthy and safe [69]. Quality of life in food allergy is generally improved by clearer diagnosis, by passing a food challenge[70], and may be improved by support programmes or other educational interventions [71, 72].

Improved understanding of factors mediating severity of reaction might allow to improve quality of life through better prediction, prevention and treatment of severe food allergic reactions [73].

1.3.4 Food anaphylaxis:

The EAACI defines anaphylaxis as a "severe, life-threatening generalized or systemic hypersensitivity reaction which is characterized by being rapid in onset with life threatening airway, breathing or circulatory problems and is usually associated with skin and mucosal changes"[74]. The true incidence of food anaphylaxis is difficult to ascertain, due to heterogeneity in the definitions of anaphylaxis used in different studies. The international consensus (ICON) study from the WAO for anaphylaxis showed there is no consensus on the definition of anaphylaxis[75]. Moreover a significant proportion of anaphylaxis cases remain without an identified trigger [76, 77]. This means that epidemiological data for cause-specific anaphylaxis must be interpreted with caution due to potential variations in coding/record-keeping/diagnosis.

1.3.4.1 Symptoms of anaphylaxis:

Following WAO consensus[78] the symptoms and signs of anaphylaxis include sudden respiratory symptoms (shortness of breath, wheeze, cough, stridor, hypoxemia), sudden reduced blood pressure (BP) or symptoms of end-organ dysfunction (i.e. collapse, hypotonia, incontinence).

1.3.4.2 Epidemiology of food anaphylaxis:

Food represents the most common trigger of anaphylaxis in young adults, adolescents and children, and other causes only dominate in older age groups [79, 80]. Food anaphylaxis incidence varies over the lifespan, being most common in preschool children [42] but fatal food anaphylaxis predominates in the second and third decade of life and the reason for this remains unknown[42].

Recent systematic reviews suggest that self-reported anaphylaxis is 30 times more common than medically diagnosed food anaphylaxis (Table 2)[81]. Hospital admission for food anaphylaxis occurs less often, approximately once every 250 to 1000 person years [81]. There is evidence that hospital admissions for food anaphylaxis are increasing, we found a 5% per annum increase in England and Wales between 1992 and 2012, but it is not clear whether this is due, in part, to changes in coding/awareness/behaviour or reflects a true increase in disease[42, 82, 83]. In support of a true increase in disease, there is evidence that the incidence of food anaphylaxis requiring mechanical ventilation on an intensive care unit is also increasing, our own data

show that both for food anaphylaxis (incident rate ratio 1.16, 95% CI 1.04, 1.29) and for all anaphylaxis (IRR 1.14, 95% CI 1.08, 1.20) there has been a significant increase in recent years[84]. This is consistent with other evidence that intensive care unit admissions for all-causes of anaphylaxis are increasing [85].

Table 2 Estimated risk of food anaphylaxis in food allergic people:

Anaphylaxis definition	Age group	Estimated annual incidence rate
Self-reported food anaphylaxis	All ages	Less than 1 episode every **10** person years
	Aged 0-19	Less than 1 episode every **10** person years*
Medically coded food anaphylaxis	All ages	Up to 1 episode every **70-100** person years
	Aged 0-19	Up to 1 episode every **40** person years
	Aged 0-4	Up to 1 episode every **10** person years
Hospital admissions for food anaphylaxis	All ages	Up to 1 episode every **1000** person years
	Aged 0-19	Up to 1 episode every **500** person years
	Aged 0-4	Up to 1 episode every **250** person years
Fatal food anaphylaxis	All ages	Up to 1 episode every **100,000** years
	Aged 0-19	Up to 1 episode every **100,000** years

* Higher rates have been reported in selected hospital-based populations using definitions of anaphylaxis which are more inclusive than the NIAID definition [86, 87] which both reported rates of once every ~2-4 years. Data reproduced from the systematic review of Umasunthar et al [88].

Fatal outcome in food anaphylaxis is very rare (Table 2)[81]. The most common food trigger of fatal-food anaphylaxis both for children and adults in the UK is nuts[42], data from Australia has found seafood to be the most frequent trigger for fatal food anaphylaxis[42, 89]. Despite the increases noted in hospital admissions and intensive care admissions for all-cause anaphylaxis and food anaphylaxis, fatal anaphylaxis rate did not change significantly in the UK between 1992 and 2012 remaining stable at a rate of 0.047 cases per 100,000 population [42] or in the US[90] in contrast with a recent study from Australia showing an increase in fatalities for all-causes of anaphylaxis by 6.2% per year.[89].

1.3.5 Diagnosis of IgE-mediated FA:

The clinical history is one of the most important pieces when trying to differentiate between sensitization and FA as skin prick test (SPT) and serum specific IgE (ssIgE) only determine the presence of IgE against the allergen but not necessarily FA.

SPT is one of the most common used diagnostic tests as it is minimally invasive, inexpensive, results are available within 15-20 minutes, and results can be reproducible

when performed by trained physicians. Although SPT has a high negative predictive value (NPV), its main limitation is a low specificity for food allergens which range from 20-60%[91]. Modified SPT in the form of endpoint titration (EPT) has been used in hen´s egg allergy to determine severity of reaction[92]. This has not been reproduced with other frequent food allergens like peanut[93] although the concentration of peanut extract required eliciting a positive SPT is increased in patients who respond to oral peanut immunotherapy[93, 94]. In this thesis we will look at endpoint titration for both severity and threshold of reaction, as it probably allows evaluation of threshold better than severity.

ssIgE is another possible diagnostic test now available in most Allergy Clinics. ssIgE on its own as diagnostic test has similar limitations to SPT with a specificity of 69% and a lower NPV in general compared to SPT[95]. Combining specific IgE and SPT can improve specificity up to 88%[95].

SPT and ssIgE can be useful in deferring food challenges where the value is above a particular cut-off, as they can identify patients who are highly (defined as >95%) likely to have a positive reaction[96], although diagnostic cut-offs need to be verified in the local population. Component resolved diagnostics (CRD) have helped in identifying cross-reactive specific components to other similar allergens from different pollen species or foods and has also helped in identifying specific clinical phenotypes. With some allergens CRD can help in determining the risk of severity of reaction in specific cases like peanut Ara h 2[97, 98] but this probably varies depending on geographical location and therefore needs to be used in the context of a clinical history and local data to support diagnostic cut-offs.

Data from oral immunotherapy (OIT) studies suggest that high levels of specific IgE may help to determine those patients who will respond worse to oral OIT or will fail the treatment[99] suggesting more severe allergy and ratio sIgE/sIgG4 helps to identify better those children with high probability of tolerance to egg[100].

Given the discordance between the accuracy of SPT and specific IgE levels, double-blind placebo controlled oral food challenges (DBPCFC) remain the gold standard for diagnosing food allergy, and a previous history of a severe reaction seems to be the best predictor of clinical reaction severity[101].

While current diagnostic tests are able to predict likelihood of clinical reaction, they cannot predict the severity or dose of exposure required which would help healthcare professionals to risk stratify allergic patients. Prescription of adrenaline auto injectors as a rescue medication to treat anaphylaxis is a mainstay of risk management, but provision varies according to risk assessments (often based on false assumptions) made by clinicians with influencing factors such as the allergen (more prescription if the allergen is peanut or tree

nut), trace reactions and parental anxiety amongst others showing that anaphylaxis guidelines are not always implemented appropriately[102]. This may mean that patients have unnecessarily restricted diets or unnecessary emergency management plans, for those who are at relatively low risk of a reaction or a severe reaction; conversely, patients who might require stricter allergen avoidance or strengthened emergency management plans might not even be prescribed rescue medication due to an incorrect risk assessment.

1.3.6 Prognosis of IgE mediated FA:

Depending on the food allergen, childhood FA may be transient or persist into adulthood. Milk and egg generally have a better prognosis and are expected to be outgrown by school age[103-107] although more recent studies have suggested that even these allergies commonly persist into adolescence and young adulthood [108-110] with resolution of cow´s milk allergy by 80% and almost 70% of hen´s egg allergy by the age of 16 years old. This may be due to a change in the natural history of FA, or just reflecting a different and perhaps more atopic population, or at least a population at risk of more persistent FA.

Other allergies like peanut tend to be more persistent with resolution of only 20% by adulthood[111, 112]. Some studies suggest that peanut resolution can be as much as 50% by adulthood and that recurrence of peanut allergy once reintroduced the allergen in the diet is reported, but is uncommon, affecting less than 10% of patients with resolved peanut allergy [113].

It is difficult to determine when a patient may have outgrown their allergy, accidental ingestions are infrequent as patients largely avoid the offending food and clinical and laboratory guidelines have not firmly established when oral challenges should be performed to determine tolerance. Studies suggest that low or reducing levels of SPT and specific IgE are useful predictors of challenge outcome although no cut-off points have been clearly established[114, 115].

The current available data do not allow us to determine when FA resolution occurs and what mechanisms may be at play that facilitate resolution in some people, but not in others.

1.3.7 Management of peanut allergy:

The only management strategy that is currently accepted in routine clinical use is complete dietary avoidance of peanut, together with the provision of rescue medication in the event of an accidental allergic reaction. One study assessed the benefit of anti-IgE therapy in patients with peanut allergy, and reported a significant increase in reaction threshold to peanut and gave partial protection against most unintended ingestions of peanut[116].

Peanut allergy is a lifelong condition in most patients: several studies and treatments have tried to generate modifications in the specific immune response in order to create desensitization or sustained unresponsiveness[117] to peanut, which in some cases are promising. These treatments include: OIT to peanut[118, 119] also with added probiotics[120], sublingual immunotherapy[93, 94], epicutaneous immunotherapy with less promising results[121] and treatment with anti-IgE[116] which has shown to increase the threshold for peanut in peanut allergic patients[122].

Heat treatment affects the ability of peanut to induce allergic reactions: roasting increases allergenicity, while frying or boiling decreases it[123]. The use of modified allergen may represent a safer and more effective approach to OIT.

However, there is a general consensus that OIT strategies are not ready for routine clinical use, as they are associated with a high rate of adverse events and therefore not every patient can be candidate for this type of treatment[124, 125].

1.4 Pathophysiology of symptoms during IgE-mediated food allergy:

IgE-mediated FA severity range from local symptoms like itchy mouth and throat, to anaphylaxis; anaphylaxis itself also represents a spectrum of severity, ranging from mild respiratory symptoms, to severe, life-threatening anaphylactic shock, which is the most severe presentation of IgE-mediated FA. Reactions in humans can involve the cutaneous, respiratory, gastrointestinal (GI), cardiovascular or neurological systems and usually happen within minutes up to couple of hours of ingestion of the allergen. Data from case series of fatal anaphylaxis reactions describe cardiac arrest during IgE-mediated FA at a median time of 30 minutes after ingestion of the allergen[126].

IgE-mediated FA studies involving mostly paediatric population describe abdominal symptoms as the most frequent during food anaphylaxis, however; this is less frequently described by adults[127].

Observational studies describe that most patients[128] suffering from food anaphylaxis complain of respiratory compromise involving upper and lower airway with symptoms described of wheeze, dyspnoea, cough, hoarse voice, stridor and signs of tachypnoea.

The most common finding from the largest series of post-mortem necropsies after all causes of anaphylactic reactions in the UK [129] was non-specific pulmonary congestion and oedema. Upper airway oedema was more common in deaths related to food allergens (77% of immediate deaths) than in those triggered by reactions to venom or drugs, but it is unclear whether the upper airway oedema was severe enough to be the cause of death in these cases.

Skin symptoms may be absent in up to 10% to 20% of any severity of anaphylaxis and around 80% of fatal food-induced anaphylaxis is not associated with skin findings[130].

Cardiovascular symptoms, mainly hypotension, are less common in food anaphylaxis compared to other causes of anaphylaxis and usually accompany respiratory symptoms[131]. Fatality is mostly due to respiratory compromise in children versus cardiovascular collapse in adults[131].

However, data from rigorous human studies is limited due to the risks of inducing anaphylaxis and the fact that the majority of food-induced anaphylaxis is a community event, and not occurring in a medical facility. Improving our understanding of the mechanisms of anaphylaxis might help healthcare professionals understand why some food-allergic individuals appear to be more at risk of severe reactions. Given the limitations of human studies, such research to date has focused on animal models.

1.4.1 Pathophysiology of symptoms during IgE-mediated food reactions in animals:

Data available regarding pathophysiology of food allergic reactions mainly comes from animal models but there are multiple limitations to these studies and results from animal models cannot always be extrapolated to humans, as shown in Table 3.

Table 3 Characteristics of animal and human models of allergy:

	Murine models	**Human**
FcεRI on MC and basophils[132]	Present	Present
FcεRI on APC´s[132]	Absent	Present
MC activation by IgE[132]	Present	Present
IgG mediated anaphylaxis[132, 133]	Present	No evidence
Oral induction of anaphylaxis[134, 135]	High doses of antigen needed.	ED05 1.5mg of peanut protein[62].

Key similarities and differences between animal models of food anaphylaxis and human studies. MC: Mast Cells; APC: Antigen Presenting cells.

The majority of studies assessing physiological changes in animal studies have been limited, in most cases, to study of a single organ system, so conclusions about the relative involvement of different organ systems in causing symptoms and outcome must be guarded. The available data suggest that vascular changes are prominent in some models of food allergy, with venous pooling and capillary leak in the splanchnic and hepatic circulation and/or vagal responses leading to reduce cardiac output [136]. Other studies suggest an important role for respiratory events, with severity of respiratory involvement and death related to pre-existing mast cell density in the respiratory tract [137, 138].

Table 4 summarises findings from animal studies of food anaphylaxis, documenting the physiological changes seen. The main pathological features in the respiratory tract are

reduced lung conductance and compliance, similar to those symptoms seen in observational studies, and decreased pulmonary blood pressure; in the cardiovascular system peripheral vasodilation, reduced venous return and decreased or increased blood pressure can be seen [139]. Interstitial capillary leakage and vascular fluid extravasation has been described and is correlated with reduced blood pressure[140, 141], which could explain the signs and symptoms seen in observational studies of food allergy with hypotension and cardiovascular collapse during anaphylaxis.

Table 4 Physiological changes described in animal models of food allergy:

Study	Animal	Mode of Sensitization	Trigger	Anaphylaxis Definition	Gastrointestinal Findings	Respiratory Findings	Cardiovascular Findings	Other Findings
Levine [142]	Rat	Subplantar injection	IV or SP OVA, azoalbumin, casein or mouse serum	Prostration and cyanosis.	Non-uniform congestion and haemorrhage - mainly jejunum and ileum.	Not reported	Proximity to larger blood vessels was associated with reduced GI changes.	Not reported
Zhang [143]	Rat	SC OVA	IV OVA	Nil	Not reported	Not reported	Vasodilation of the hepatic artery and splanchnic bed in early phase; with a later increase in hepatic/splanchnic/ portal resistance and portal venous pressure.	Not reported
Takano [144]	Rat	SC OVA	IV OVA	Nil	Not reported	Not reported	Hepatic pre and sinusoidal vasoconstriction; decreased systemic arterial pressure.	Not reported
Martin, Takeishi [137, 138, 145]	Mouse	IP: bovine γ-globulin	IV: bovine γ-globulin Anti-IgE	Nil	Not reported	Mast cells contribute to severity of pulmonary changes (reduced pulmonary dynamic compliance and lung conductance) during anaphylaxis but mast cell-independent mechanisms exist that can result in lung function changes	Tachycardia, mediated by mast cells, and decreased systolic BP independent of mast cells	Not reported
Karczewski [139]	Rabbit	IP OVA	IV OVA	Nil	Not reported	Reduced lung conductance, increased tidal volume due to hyperventilation	Reduced arterial BP	Not reported

Study	Animal	Mode of Sensitization	Trigger	Anaphylaxis Definition	Gastrointestinal Findings	Respiratory Findings	Cardiovascular Findings	Other Findings
Cui [146]	Rat	SC OVA	IV OVA	Nil	Not reported	Not reported	Decreased BP, central venous pressure, cardiac output; increased venous resistance and portal venous pressure.	Increased haematocrit
Davidson [147]	Rat	SC OVA	IV OVA	Nil	Not reported	Not reported	Decreased BP, carotid blood flow, cerebral cortical blood flow, and cerebral tissue oxygen pressure	Not reported
Shinomiya [148]	Mouse	SC OVA	IV OVA	Nil	Not reported	Activation of $\beta 2$ (but not $\beta 1$) adrenoreceptors and nitric oxide	Pulmonary artery vasoconstriction, increased right heart afterload, reduced venous return, reduced BP	Not reported
Faye[140]	Rat	SC OVA	IV OVA	Nil	Interstitial capillary leakage that correlated with the drop in arterial blood pressure.	Not reported	Significant decrease in MAP and HR.	Not reported
Dewachter [141]	Rat	SC OVA	IV OVA	Nil	Not reported	Rapid and sustained decrease of tissue oxygen partial pressure.	Significant decrease in MAP. No change in aortic blood flow velocity or HR.	Significant increase in plasma adrenaline and noradrenaline.

IV intravenous; SP subplantar; OVA ovalbumin; SC subcutaneous: CysL Cys Leukotrienes; FoxP3 Forkhead box protein 3–positive; MC; Mast cells; LPMC Lamina propia Mast Cells; CTMC Connective Tissue Mast Cells. MAP: Mean Arterial Pressure.

Table 5 summarises findings from animal studies of inflammatory mediators released during food anaphylaxis. In general, the mediators identified during food anaphylaxis in animal models are consistent with mast cell and/or basophil degranulation. Platelet activating factor (PAF) and histamine receptor blockade reduce anaphylaxis in animal models [149]; interleukin-9 (IL9) and IL9 receptor are needed for MC degranulation and are related to the presence of symptoms [150, 151]; and MC numbers appear to be related to anaphylaxis severity [145, 152].

Table 5 Changes in inflammatory mediators described in animal models of food allergy:

Study	Animal	Mode of Sensitization	Trigger	Anaphylaxis Definition	Mediators assessed	Mediators/receptors implicated in food anaphylaxis	Mediators/receptors not implicated in food anaphylaxis
Arias **[149]**	Mouse	Enteral cholera toxin	IP peanut	Rectal temperature/ symptoms	Leukotrienes, histamine, PAF	PAF receptor blockade decreased vascular leak	H1/ H2 receptors, CysL
Jiang **[153]**	Rat	IP egg albumin	IV OVA	Decrease in blood pressure >20 mmHg	5-HT, and histamine	5-HT(3) and H1 receptor antagonists reduce anaphylaxis	Not reported
McNaughton **[154]**	Rat	IP OVA	Anti-OVA antibody	Nil	LTB4, 5-HETE 5-HPETE, LTC4, LTD4, PAF	LTC4, LTD4 and PAF increase during anaphylaxis	LTB4, 5-HETE, 5-HPETE unchanged in anaphylaxis
Oliver **[155]**	Rat	IP OVA	ID 2.5 mg OVA	Skin reaction	Histamine, 5-HT creatinine sulfate, indomethacin, leukotriene E4 (LTE4), LTD4, prostaglandin D2 (PGD2 PGE1, PGE2, PAF, and tetrodotoxin	Ciclooxygenase, lipoxygenase enzyme inhibitors, LTD4 and PAF receptor antagonists inhibit anaphylaxis	No effect from 5-HT antagonists, atropine and tetrodotoxin
Osterfeld **[150]**	Mouse	IP OVA	Oral OVA	Rectal temperature / diarrhoea	IL9, IgE, IgG	IL9 needed for intestinal mastocytosis and MC degranulation; anti-IgE totally abrogated anaphylaxis.	Blocking with anti-FcgRII/RIII mAb had no effect on anaphylaxis.
Forbes **[151]**	Mouse	IP OVA	Oral or IV OVA	Rectal temperature / diarrhoea	IL-9, MCP-1	Reactions were IL-9 dependent. mMCP-1 levels were significantly lower in oral antigen challenged OVA-sensitized IL-9 deficient mice.	CD4 +, CD8 + T cell, B cell, regulatory T cell (CD4 + , CD25 + , CD45RB and FoxP3 +), and DC levels. IL-4 and IFN γ.
Bartnikas **[156]**	Mouse	1. EC OVA 2. Oral OVA	1.Oral OVA 2.IV OVA	Body temperature / MCP-1	IgE, IL4, MCP-1	IgE is essential in anaphylaxis and MC expansion and mMCP-1 increase is IgE dependant. Increased IL4 during anaphylaxis.	FoxP3 in the gut
Strait **[157]**	Mouse	1. IV IgE anti-TNP. 2. IP OVA	1. Oral TNP-OVA 2. Oral OVA	Rectal temperature / activity score	IgA	IgA binds to allergen, blocking anaphylaxis	Not reported

Study	Animal	Mode of Sensitization	Trigger	Anaphylaxis Definition	Mediators assessed	Mediators/receptors implicated in food anaphylaxis	Mediators/receptors not implicated in food anaphylaxis
Ahrens **[152]**	Mouse	IP OVA	Oral OVA	Rectal temperature / diarrhoea	FcεRI, IL-9, MC density	Systemic responses depend on IgE/FcεRI signalling. Increased IL-9 in anaphylaxis. MC degranulation is but MC density is not dependant on FcεRI Intestinal MC density is related to systemic reactions and may influence severity.	Not reported
Krishnamurthy **[158]**	Mouse	IP Casein	IV Casein	Temperature and symptom scoring	Peripheral leucocyte counts, in vitro splenocyte stimulation, tissue histology (lung, spleen, liver, heart, kidney).	Decreased neutrophils/ platelets; red cells; thrombi in lung/liver/heart; increased IL-4, IL-5, IFN-γ, IL-10	No change in basophil number
Perrier **[159]**	Mouse	Intra-gastric OVA	Intra-gastric OVA	Body temperature, allergy score, MMCP-1 blood level	Blood, mesenteric LN, spleen/ jejunum samples. IL-5, IL-13, IL-10, IFNγ, IL-17	OVA absorption related to allergic reaction severity; increased IL-5, IL-13, IL-17, IL-10; increased IFN-γ	LPMC, and CTMC not significantly increased. No change in TGF-β.
Vinuesa **[160]**	Rabbit	SC OVA	Rectal OVA	Nil	CD4, CD5, CD25 and RLA-II	CD 25+ and CD 5+ cells increased in ileal epithelium and lamina propia;	CD4+ cells RLA II+ cells
Sun **[161]**	Mouse	Oral peanut	IP peanut extract	Symptom score/ body temperature	Vascular permeability, MC mediator release, leukocytes	Histamine and LTC, lymphocytes and eosinophils, vascular permeability increases; increased albumin in pleural fluid.	Not reported
Shibamoto **[162]**	Mouse	SC OVA	IV OVA	Not shown	PAF, histamine	PAF is responsible for systemic hypotension and histamine causes hypertension, PAF and H1 receptor antagonists attenuated the increase in HR.	Not reported
Khodoun **[163]**	Mouse	Oral Peanut,extract	IV Peanut extract	Rectal temperature	IL-4, B and T cells, macrophages, basophils and MC, PAF and histamine, C3a and C5a	IL-4 protective against severe reactions. Macrophages, basophils and complement activation (C3a) appear to be more important than mast cells or adaptive immunity in peanut extract-induced shock. PAF receptor and PAF/histamine antagonists ameliorate peanut extract-induced shock.	Mast cell depletion did not detectably inhibit shock severity. C5a

1.4.2 Pathophysiology of IgE mediated food allergy in humans:

Real-time data on the pathophysiology are very limited given the rapid onset and relatively short duration of reactions. This is especially so for food anaphylaxis, which is typically a community event, occurring outside hospital with no detailed medical monitoring. The available data describing the physiology and mediators in human food anaphylaxis are from emergency medical settings and necropsy studies. The most common finding from the largest series of post-mortem studies of fatal anaphylaxis was non-specific pulmonary congestion and oedema, in 41% (23/56) of cases there were no specific post-mortem findings suggestive of anaphylaxis [129]. Hyperinflation of the lungs and/or mucous plugging of airways and petechial haemorrhages suggesting an asthmatic and/or asphyxial component were present in 40% of immediate (within an hour) food anaphylaxis deaths. Data from a large case series of all causes of fatal anaphylaxis documented cardiac arrest immediately following postural change in several cases, mainly during food anaphylaxis [89, 164] suggesting hypovolemia capillary leakage as seen in animal models. This and the animal data cited above suggest that venous return to the heart may be compromised during food anaphylaxis.

A case report recently published described during the course of anaphylaxis symptoms of palor, mental unresponsiveness, abdominal pain, vomit, hypotension, shortness of breath with low oxygen saturation, bowel incontinence and tachycardia. This patient quickly responded to IM adrenaline and IV fluids. An abdominal computed tomography was performed as diarrhea and abdominal pain persisted showing diffuse bowel wall edema with evidence of “shock bowel” suggesting fluid extravasation from the GI system[165].

Histamine is a difficult mediator to study as it has a peak in blood of 10 minutes after the reaction and has a half-life of 30 minutes [166] data from a paediatric study has shown increase in plasma histamine during DBPCFC[167].

Results published for mast cell tryptase (MCT) are variable regarding food allergic reactions in humans. There´s data showing increase of MCT in serum during severe food allergic reactions [168] but at the same time it can increase non-specifically after death [169] so MCT in isolation may not be a reliable parameter to assess following fatal anaphylaxis.

Platelet activating factor (PAF) and PAF-acetylhydrolase (AH) has been studied more recently in the last decade. Circulating PAF levels are increased and circulating PAF acetylhydrolase activity is decreased [170] and this is related to the severity of organ system involvement in food allergic reactions which has not been shown for histamine or

tryptase [171],although these results have not been consistently reproduced by other groups[172].

These data are summarised in Table 6. In general, the data are consistent with mast cell and/or basophil degranulation during reactions, but data on the physiological events during human food anaphylaxis are generally lacking.

Table 6 Changes in inflammatory mediators and physiology described in human food allergy studies:

Author	Population studied	Mediator or event assessed	Cardiac	Respiratory	GI	Mediators/receptors implicated in FA	Mediators/receptors not implicated in FA
van Odijk [173]	11 patients (4 with IgE mediated FA undergoing DBPCFC), 4 controls. Symptoms recorded	Fecal samples: Eosinophil protein X (EPX) and tryptase. Urinary samples: EPX and LTE4. Serum sample: eotaxin and food-specific IgE antibodies.	Not reported	Not reported	Abdominal pain, distension, flatulence and nausea were similar in the 'allergic' and control group.	Non-stat significant increase in Fecal EPX. No change in serum eotaxin	No correlation between the levels of inflammation markers with the severity of reaction.
Lewis and Hourihuane [101, 174]	40 peanut allergic patients (23 children >6yrs, 17 adults)	Score of symptoms and specific IGE Binding patterns to peanut proteins	Not reported	Not reported	Not reported	Peanut -specific IgE level correlates with challenge score. The intensity of IgE binding or number of proteins recognized are more important than recognition of specific proteins; correlation between the total number of bands bound by a patient and their challenge score.	Negative relationship between intensity of binding to Ara h 1 and symptom severity.
Caffarelli [175]	80 children with a history of reaction to foods and/or positive food specific IgE.	Brachial blood pressure.	Symptoms characteristic of anaphylaxis induced by OFCs were associated with low SBP.	Not reported	Not reported	Not reported	Not reported
Brown [168]	132 causes of food allergy	Mast cell tryptase Cytokines (IL-2, IL-6, IL-10 and TNRFI) Anaphylatoxins (C3a, C4a and C5a) PAF and PAF-AH	46% of reactions were hypotensive more frequent with older age and drug as causative agent of anaphylaxis.	69% of all reactions involved resp symptoms. 9.2% of all reactions (12% of anaphylaxis) had documented hypoxemia. 29/196 (15%) of resp	37% of Grade III reactions had GI $	Peak concentrations of every mediator tested were significantly higher in patients with severe reactions but were not significantly different in patients having delayed deteriorations or with causative trigger.	Not reported

				reactions involved hypotension. *44% of cases of anaphylaxis wheeze was present and hypoxemia was more frequent within this group.		PAF-AH activity was below normal in 26% of severe reactions.	
Vadas [170]	21 patients with food anaphylaxis of a total of 44 causes of anaphylaxis	PAF levels and PAF-AH activity.	Not reported	Not reported		PAF levels were higher in anaphylaxis and this increase was bigger with the severity of anaphylaxis. Inverse correlation between PAF levels and PAF-AH activity. PAF-AH levels were lower in those with fatal-anaphylaxis and decreases with severity of reaction.	Not reported
Sampson [167]	33 patients with AD underwent DBPCFC.	Plasma histamine levels	Not reported	Not reported		Plasma histamine was significantly elevated in active challenges compared to placebo and to levels before the challenge.	Not reported
Sampson[176]	6 fatal and 7 near-fatal anaphylaxis in children and adolescent.	Tryptase and TNF-α levels.	Not reported	All food allergic reactions caused difficulty in breathing that led to respiratory arrest in 13/16.	Not reported	Tryptase was elevated in 14/16 of all causes of fatal anaphylaxis.	Tryptase levels or TNF-α levels did not increase in fatal or near-fatal anaphylaxis compared to controls.
Pumphrey[129]	16 cases of fatal food anaphylaxis, total of 56 fatal anaphylaxis cases.	Tryptase and macroscopic post mortem findings.		Upper airway oedema was the most common post-mortem finding in fatal food anaphylais		Not reported	Not reported

1.5 Evidence for mast cell and basophil degranulation in IgE-mediated FA:

An increase in histamine can be detected after positive oral food challenges of any severity [167]. Brown et al demonstrated significant histamine release together with MCT, IL-6, IL-10, TNFRI (C3a and C5a) only in severe anaphylactic reactions, something, which may imply increased mast cell degranulation in severe reactions [168].

Basophil activation tests have been performed to diagnose peanut allergy, reflecting a functional response to the cross-linking of allergen, specific IgE and FceRI and is able to discriminate between allergic and tolerant patients, and possibly severity of reaction [177-180]. This implies that basophil degranulation mechanisms may be relevant to severity of reaction.

1.6 Cardiovascular effects of mast cell mediators:

Mast cells are found in heart tissue[181, 182] responding to IgE-mediated stimuli but can also be activated by other stimuli such as C3a, C5a, substance P and eosinophilic cationic proteins [182]. Mediators of anaphylaxis released from mast cells and possibly basophils also have a direct effect on the myocardium[183-186]. Infusion of histamine into healthy volunteers can provoke coronary arterial spasm [184], rapid decrease in mean aortic pressure, arrhythmias and atrioventricular (AV) conduction block [187]. PAF released within the systemic circulation can induce peripheral vasodilatation with relative hypovolemia and severe hypotension[188]. Mast cell mediators can therefore induce a myocardial depression and this may contribute to the severity of food anaphylaxis.

The main peripheral vascular changes during anaphylaxis are fluid extravasation and vasodilation, causing a mixed distributive hypovolemic shock pattern[189, 190].

Data from a large case series of all causes of fatal anaphylaxis documented cardiac arrest immediately following postural change in several cases, mainly during food anaphylaxis[89, 164]. This suggests that venous return to the heart may be compromised during food anaphylaxis.

In drug anaphylaxis the most common change in cardiac rhythm was supraventricular tachycardia (SVT), which was more frequent in those with no pre-existing cardiac disease, followed by SVT with ST elevation [189]. Kounis syndrome is defined as the concurrence of acute coronary syndromes such as coronary spasm, acute myocardial infarction, and stent thrombosis, with conditions associated with mast-cell and platelet activation involving inflammatory cells in the setting of allergic reactions [191]. There are very few reports of arrhythmias or Kounis syndrome during food allergic reactions [192, 193] but this may be due to the lack of food allergy studies involving cardiac monitoring and because food

allergic reactions are frequently rapid in onset and outside a medical facility. It is clear that Kounis syndrome can occur during IgE-mediated FA reactions, but the overall contribution of this and other cardiac complications to reaction severity is not clear.

The incidence of all causes of anaphylaxis with circulatory compromise has been reported to be around 7.9-9.6 per 100.000 population in the US and Switzerland [194, 195]. It is common to find cardiovascular compromise in anaphylaxis triggered by medications or insect sting, which usually occur in older population compared to food anaphylaxis and in whom pre-existing cardiac disease may be an important determinant of reaction severity. Aside from the observations made above, circulatory compromise in food anaphylaxis is less well documented [164].

Observational studies of cardiovascular and cardiac conductance changes during IgE-mediated FA reactions have not been widely reported[196, 197]. Overall it seems likely that IgE-mediated FA reactions are at least partially mediated by mast cell/basophil degranulation; and at least some of the mediators released in such reactions can have significant cardiac effects. It is important, therefore, to evaluate cardiovascular changes during IgE-mediated FA reactions, in order to better understand the pathophysiology and thereby inform future research and therapeutic strategies.

Registry data suggest that cardiovascular shock is rare in food anaphylaxis, compared with respiratory compromise, however other data presented above support a potential role for the cardiovascular system in mediating reaction severity as shown above.

1.7 Factors which may influence severity of food allergic reaction:

1.7.1 Host factors:

Proposed risk factors for severe outcome in food-allergic reactions include asthma (especially poorly controlled asthma)[198] and delayed intramuscular adrenaline administration[176, 199-201]. Asthma is the most established risk factor for fatal food anaphylaxis, and 85-96% of fatal food anaphylaxis occurs in people with a current asthma diagnosis[51, 201]. However, asthma is present in 29%-76% of all FA people whereas only ~1 in 100,000 FA people suffer fatal food anaphylaxis each year [202, 203], thus while the absence of asthma has reasonable negative predictive value for fatal food anaphylaxis, the positive predictive value of asthma for future fatal food anaphylaxis risk is low.

Most of fatal food anaphylaxis occurs in people with known asthma [51], and respiratory symptoms are prominent in case reports/series of food anaphylaxis [42, 204]. Uncontrolled asthma is related to life-threatening anaphylactic food allergic reactions [205] and asthma has been associated with a 5.2-fold increased hazard of all-cause anaphylactic shock [206]. Fatal and near fatal food triggered anaphylactic reactions predominantly have respiratory

symptoms, therefore it seems to be a target organ related to more severe reactions although there is still lack of information in this field [176].

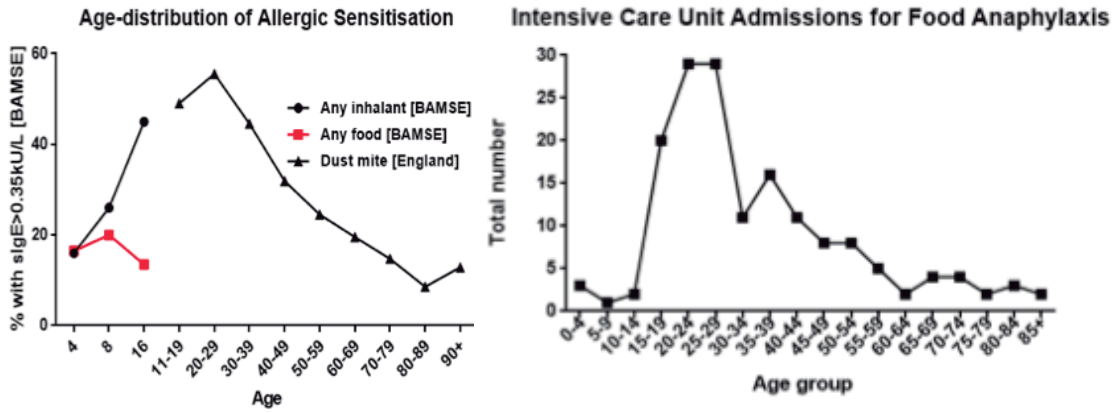
Since reduced consciousness may cause respiratory arrest, and even mild cognitive changes may impair ability to use rescue medication, neurological effects of food anaphylaxis might be considered as possible mediators of reaction severity. Direct evidence for this, however, is lacking.

Age is strongly associated with food allergic reaction severity. The distinctive age-distribution of fatal and near-fatal food anaphylaxis, different to the age distribution of less severe IgE-mediated FA reactions, suggests that age-related factor(s) modulate severity of reaction. While IgE-mediated FA and FA reactions are most common in infants and preschool children, severe reactions peak around the age of 15-25 years, although the increased risk persists into the fourth decade of life [42]. Sensitization to pollen allergens increases with age, with a peak at 25-30 years old[207, 208]. Interestingly the age-distribution of near fatal food anaphylaxis is similar to the age distribution of total and inhalant IgE levels in the general population, shown in Figure 3, suggesting a possible link between aeroallergen sensitisation and/or airway inflammation and reaction severity in food allergy.

Observational data from OIT studies suggest that pollen exposure, stress, exercise and possibly modified methods of food intake such as lying down after intake, fasting or using a straw, may trigger reactions in individuals who usually tolerate the food [209-211].

Figure **3** Age distribution of inhalant allergens and near fatal food anaphylaxis:

A B



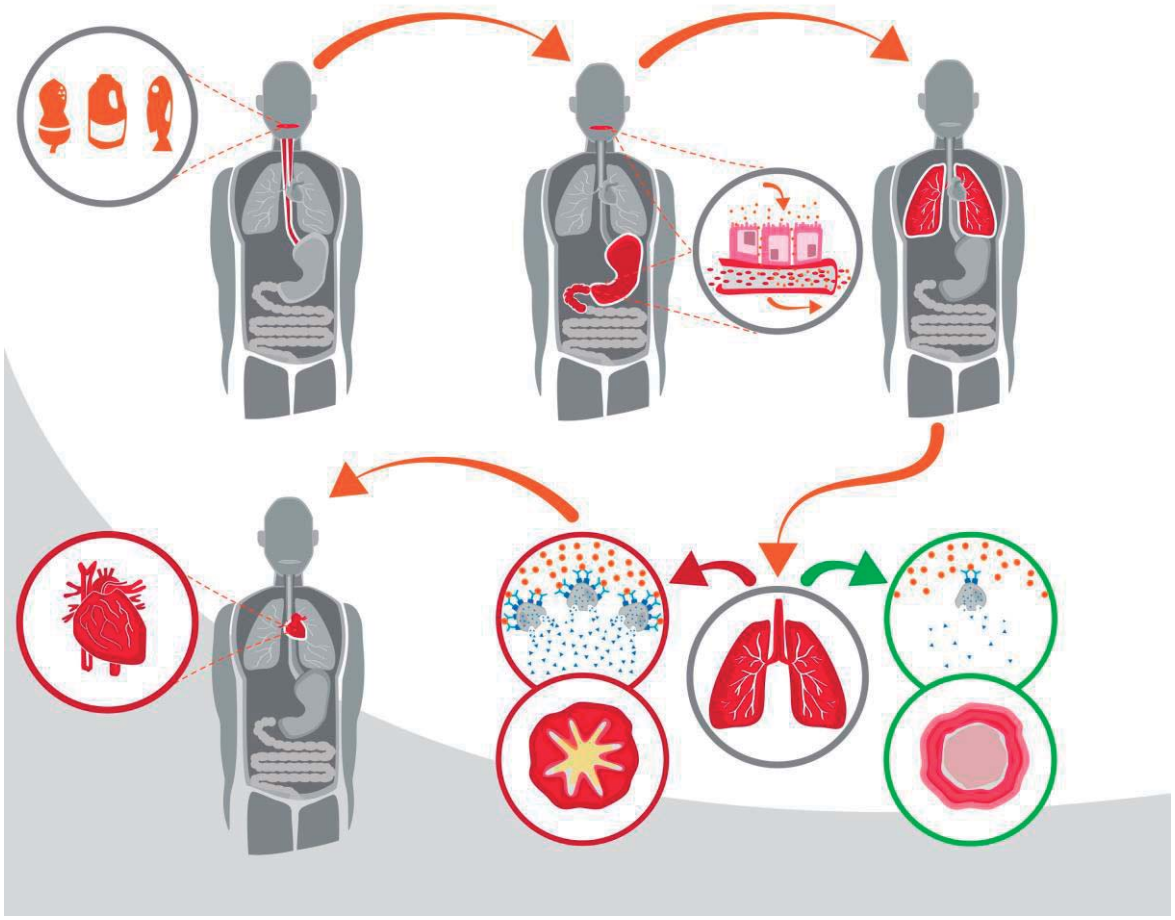
Inhalant allergen age distribution from BAMSE cohort [207](A) and age distribution of near fatal IgE-mediated food anaphylaxis from UK cohort[42] (B).

Exposure to aeroallergen via nasal or bronchial challenge, or natural exposure, leads to a further acute increase in mast cell numbers/FcεRI expression[212-214]. In food-allergic individuals with concomitant sensitisation to aeroallergens, increased exposure to aeroallergens is also likely to lead to a higher density of food-specific IgE bound to airway mast cells. Subsequent exposure to the food allergen (which can be rapidly absorbed from the oral cavity[215]) might result in rapid crosslinking of IgE on airway mast cells, resulting in mast cell degranulation and mediator release in the airways which might influence severity of reaction.

With the data already described above in animal and human models of anaphylaxis we hypothesise a model for the mechanism of severity of reaction, as shown in Figure 4.

The food allergen is absorbed in the oral mucosa[215, 216] and GI system directly reaching the blood stream, and distributed quickly through the blood stream including to the airways. Those aeroallergen-sensitised individuals with a higher MC density[137, 138, 212-214] in the airways may then experience more severe respiratory compromise due to increased release of mast cell mediators in the respiratory tract, which could lead to cardiac compromise when these inflammatory mediators reach the heart via the pulmonary venous circulation. In other individuals, perhaps with lower airway MC density, MC mediator release is less and therefore such individuals are at lower risk of severe systemic reactions with cardiac involvement.

Figure 4 Proposed mechanism for severe IgE-mediated FA:



Food is initially absorbed in the mouth and GI system and the allergen passes to the blood stream. Once in the blood the allergen quickly reaches the respiratory system, those participants who have a higher MC density in the lungs have more severe respiratory compromise. Mediators released in the lungs rapidly reach the heart through pulmonary venous circulation and responsible for the cardiac response during IgE-mediated FA.

1.7.1.2 "Switch-off" mechanism:

A balance between activation and inhibition to avoid excessive or inappropriate responsiveness and to maintain homeostasis must strictly regulate activation of cells in the immune system.

The factors that regulate the resolution of allergic inflammation are poorly understood. Some effector cells may undergo apoptosis as concentrations of cytokines that promote the survival of such cells locally diminish[217]; others (such as mast cells) may decrease the extent to which they differentiate, mature or proliferate locally[218]; and others may emigrate from the affected site[219].

In some models of allergic contact hypersensitivity, the production of IL-10 by mast cells contributes significantly to the ability of mast cells to reduce many features of inflammation in the affected sites[220]. Whether similar anti-inflammatory or immunosuppressive actions of mast cells can be elicited in the context of IgE-associated allergic inflammation remains to be determined. However, several types of innate and adaptive immune cells that infiltrate sites of allergic inflammation (including eosinophils and various populations of regulatory T cells) can produce mediators, cytokines, chemokines and growth factors that could reduce inflammation or promote repair at these sites. Such products include the resolvin and protectin lipid mediators[221], IL-4 (which can have anti-inflammatory effects[222]), TGF-β[223, 224], TGF-α[225], IL-10 [220, 224, 226] and IL-35[226]

The β chain of the FcεRI receptor has four membrane spans and an immunoreceptor tyrosine-based activation motif (ITAM), activation of tyrosine kinases is key to the ability of both FcεRI and KIT to transmit downstream signalling events needed for the regulation of mast cell activation[227]. FcεRI requires the recruitment of Src family tyrosine kinases (Lyn and Fyn) and Syk to control the early receptor-proximal signalling events, but there are other receptors in MC surface that modify or regulate its function[228].

Mast cells are unique among hematopoietic cells, they can undergo repeated rounds of degranulation and regranulation[229-232], however, the consecutive morphologic changes of the individual cell, as well as the cytokine transcript expression during degranulation and recovery processes, remain unclear. An Animal model study[233] have shown through time-lapse photography that a single mast cell can recover after the dynamic process of degranulation and release β-hexosaminidase 48 hours after the first cross-linkage of the high-affinity IgE-receptor (FcεRI) when using BMCMCs that maintained a constant cell number, confirming the recovery of an individual cell.

Cytokine induction at gene transcript levels after mast cell recovery by means of IL-13 and IL-6 levels showed peak induction at 2 hours after activation for both cytokines; the induction then returned to basal levels after 12 hours. Re-activation 48 hours after the initial activation led to upregulation of IL-13 and IL-6 transcripts, with exactly the same kinetics shown for the initial activation. After recovery, the upregulation kinetics of cytokines IL-13 and IL-6 by mast cells after a second anaphylactic activation is similar to that seen during the initial stimulation, thus suggesting recovery of the cell.

This indicates that mast cells can upregulate the production of the aforementioned mediators during their degranulation, which can be triggered repeatedly after regranulation. The capacity of mast cells to undergo several cycles of degranulation and regranulation is an important feature of these cells in the induction and perpetuation of an allergic reaction.

Human models of cultured human lung mast cells, IgE-mediated activation triggers a degranulation process, which results in some small and immature mast cells[230]. These small cells undergo morphologic changes accompanied by nuclear and cytoplasmic expansions, with the rapid development of synthetic structures such as Golgi apparatus and ribosomes. Eventually, these immature granule-containing cells further expand into mature mast cells[231].

Regulatory pathways, which lead to inhibition of mast cell degranulation, may be key in differentiating between those patients who have severe, life-threatening reactions and those with less severe reactions. In this thesis we explored this possibility, by using duration of skin prick test wheal response as a surrogate marker for the effectiveness of ‘termination’ mechanisms in the context of IgE-mediated mast cell degranulation.

1.7.2 Characteristics of the allergen:

Food processing can be beneficial in many ways towards reducing the allergenic capacity of some foods, such as boiling or baking, but it can also increase the allergenicity such as roasting (especially nuts). Studies investigating the impact of food processing on allergen structure do not show consistency in terms of the effects of food processing on resulting allergenicity from one allergen to another[234]. The matrix in which an allergen is consumed can also alter both the immunogenic capacities of the allergen and gastrointestinal absorption, leading to variations in eliciting dose, and potentially impact upon reaction severity [235]. The matrix and the cooking process by which the allergen is ingested can affect its bioavailability[235-237] potentially delaying symptom onset or minimizing initial mild oral symptoms. Part of the iFAAM study will try to explain this change in severity and threshold depending on the food matrix.

Greater IgE epitope diversity and higher IgE binding affinity were found to be associated with clinical phenotypes and severity of milk allergy using peptide microarray and IgE binding to higher numbers of milk peptides was also associated with more severe allergic reactions during food challenge [238].

Some specific peptide allergens tend to elicit more severe allergic reactions. For example allergens involved in the pollen food syndrome, such as Ara h 8, usually cause less severe reactions than primary food allergens such as Ara h 2, which is related to severe reactions[239-242]. There are however exceptions, for example, soya allergic people with pollen food syndrome due to the Bet v 1 homologue Gly m4 can have severe reactions including anaphylaxis [243-245]. Peanut epitope recognition has been shown to correlate with severity of peanut allergic reactions using peptide microarray immunoassay [246]. Peanut is one of the most common food allergens, and the majority of fatal food anaphylaxis reported in adults has been triggered by peanut or tree nuts [42, 199] suggesting that these foods may have intrinsic properties, which increase reaction severity. Systematic reviews of fatal and non-fatal anaphylaxis risk in people with food allergy did not find significantly increases in those with peanut allergy compared with all food allergic people, suggesting nut allergy may not be any more dangerous than other primary food allergies [81, 88, 176]. Dose of allergen ingested may be important factors in determining reaction severity. Lower threshold of reactivity has not been related with more severe reactions[247], but there is data supporting the theory that more severe reactions occur after ingestion of a higher dose of allergen[248] although there is not sufficient data to relate dose and severity of reaction[249].

MCT can be elevated during food allergic reactions[168, 250, 251], recent studies suggest this mediator is more useful in those experiencing severe anaphylaxis compared to mild, moderate anaphylaxis and can therefore be a useful marker in life threatening anaphylactic reactions[168] rather than to diagnose IgE mediated food allergic reactions as it´s not always above normal levels and baseline levels are not always available to determine an increase from baseline. Similar results have been published for histamine, IL-6 and IL-10[168] for severe anaphylaxis. Increased PAF and decreased PAF-AH levels have also been associated with severity of reaction[170].

Animal data suggest that gastrointestinal and respiratory MC density is related to severity of reaction[152] and MC contribute to severity of pulmonary changes during anaphylaxis[137, 138].

Any alteration in effector cells can lead to increased severity in food allergy. Mastocytosis, a mast cell disease, is a rare disorder of both children and adults caused by the presence of too many mast cells (*mastocytes*) and CD34+ mast cell precursors. It´s known that people who suffer from mastocytosis have a higher risk of suffering life threating allergic reactions given the higher number of mast cells and their potential to release mediators into the blood stream. Knock-out murine models of peanut allergy have demonstrated that the absence of mast cells, basophils or macrophage alone prevent severe reactions, implying that all three lineages are needed for severe outcome, at least in the murine model. [252].

What is unknown is how, if any, basophils contribute to severity of reaction in humans. Gene association with increased susceptibility for anaphylaxis of any cause include nucleotide-binding domain and Leucine-rich Repeat-containing (NLR) family pyrin domain containing 3 (NLRP3) [253], and PAF V279F, present in 30% of the Japanese population and the most common loss of function mutation in PAF-AH [254].

1.7.3 External non-pharmacological factors:

Experimental studies to assess the influence of external factors are difficult to design, but studies such as the UK Food Standards Agency-funded TRACE peanut study will clarify whether external factors such as stress and exercise can influence reaction severity in people with FA.

Human behaviour is affected after alcohol intake and may make the subject less aware of potential risks around them including food intake. Alcoholic drinks may thereby facilitate or trigger symptoms in patients with different allergies, particularly to foods. Clinical experience suggests that some people with FA experience a lower threshold of reactivity and/or increased severity of reaction if alcohol is consumed at or before allergen exposure. Exacerbation of food allergy in chronic alcoholics appears to be linked to catabolism of

histamine, increased gastrointestinal mucosal permeability and alcohol's potential as a histamine-liberator [255]. Total serum IgE levels are increased in relation to alcohol exposure through the life course [256-259], and chronic alcohol consumption also generates increased Th2 cytokines such as IL-6, IL-8, IL-10, IL-12, and IL-13 [258]. However, it is not clear whether these immune changes are relevant to the clinical experience of increased FA with alcohol exposure. Objective data documenting this association are lacking, and although several mechanisms are possible, there is no direct evidence for a pathway through which alcohol increases severity of FA reaction.

Exercise exerts gross effects on the immune system. During and immediately after exercise, the total number of white blood cells (leukocytes) in the circulation increases. This leukocytosis is in proportion to the intensity and duration of the exercise performed [260]. During the post-exercise period, there is a characteristic decline in numbers of circulating lymphocytes and monocytes, whereas circulating numbers of neutrophils continue to increase, peaking several hours post-exercise [260, 261]. Specific effects on allergic immune responses have not been shown, but exercise is one of the most frequent co-factors associated with exacerbating food allergic reactions in the literature, primarily in the context of food-dependent exercise induced anaphylaxis (FDEIA). People with FDEIA suffer FA reactions when they exercise during or shortly after intake of a specific food allergen, but do not suffer reactions if exercise or allergen ingestion occurs alone. Several types of exercise predispose individuals to FDEIA, this can be high or low intensity, just walking can exacerbate or predispose to symptoms after food intake. Symptoms of FDEIA can be observed at any moment during or after physical activity, however; approximately 90% of patients develop symptoms within 30 min after exercise cessation [262]. Exercise facilitates intestinal allergen absorption[263] although other data suggests absorption is reduced with mild to moderate exercise[264]. Recent data suggest that reduced gastric acid during exercise affects the allergen digestion and therefore more structurally intact allergens get absorbed[265].

Data from milk [211] and peanut [118] OIT studies suggest that infection may be an important trigger of symptoms during treatment. Anaphylaxis registries report concomitant infection in 2.5-3% of anaphylactic reactions in children [204, 266] and in 1.3-11% in adults [266, 267]. Respiratory or gastrointestinal infections may alter oropharyngeal and/or gastrointestinal mucosal permeability, allowing increased systemic absorption of allergen.

Certain bacteria, fungi and viruses can activate MC and basophils by binding to pathogen recognition receptors that can be found in these cells [268, 269] and can therefore induce degranulation and potentially exacerbate FA reactions.

Psychosocial stressors may increase the trans-epithelial passage of food antigens into the intestinal mucosa, increasing the risk of adverse reactions to foods. Most of the data comes from animal models although some evidence suggests that similar mechanisms may happen in humans. A corticotropin-releasing hormone (CRH) pathway linking stress to intestinal permeability may exist in humans as stress exposure influences the hypothalamic-pituitary-adrenal axis where CRH production in the hypothalamus occurs. There is one study investigating the effect of acute stress on epithelial permeability in the intestine of healthy and food allergic participants, showing an increased release of mast cell mediators after cold-pain stressor, with a greater increase in food allergic participants than healthy participants [270].

1.7.4 Drugs:

It has been described in single case studies [271], limited series of patients [272] and during OIT [118] that different drugs can alter the severity of a food allergic reaction or can enhance a reaction by working as a cofactor. Limited data are available regarding the reason for this and there is lack of proper in vivo or in vitro diagnostic tests. Mechanisms underlying non-immunological hypersensitivity to NSAID's may contribute to its role as cofactor of IgE mediated food allergies but this is not completely understood. Studies suggest that intestinal absorption of allergen can be upregulated by treatment with acetylsalicylic acid (ASA), possibly through dysregulation of tight junctions [263].

Inhibition of beta-adrenergic signals on effector cells of anaphylaxis, such as MC and basophils leads to the inhibition of the cyclic adenosine monophosphate (AMP) system and therefore a destabilization of these cells. The signal and the response to the allergen are amplified evidenced by an increase in mediator synthesis and mediator release. An increase in IgE production may also be seen while treatment with Beta-blockers.[273].

Adverse events of angiotensin-converting-enzyme (ACE) inhibitor are clinically relevant due to the number of subjects exposed to this drug. The mechanism underlying it's possible role as cofactor in food allergy hasn't been proven but studies [274] focusing on angioedema caused by ACE inhibitors suggest is mainly due to the decreased degradation of bradykinin which potentially dilates blood vessels, mediates inflammation and increases vascular permeability. Recent data [275] shows an increased risk of anaphylaxis when both (B-blockers and ACE-inhibitors) drugs are combined. Animal data shows this may be due to a decreased threshold of MC activation.

Preliminary data both from animals[276, 277] and human[278] suggest increased allergen sensitization whilst in treatment with proton pump inhibitors (PPI) and an increased threshold of the allergen (fish) when this is previously digested[279]. A current study as part

of the iFAAM project will yield results on how treatment with PPI can influence both threshold and severity of reaction.

1.8 Prediction of phenotype in IgE mediated food allergy:

As discussed above, in section 1.3.3, with the current diagnostic tests we are unable to predict threshold or severity of reaction accurately and safely, therefore unable to risk stratify our patients[280]. The resulting uncertainty may lead to excessive risk avoidance behaviour by some groups, and a failure to securely implement strategies to prevent serious reactions in groups who could be identified as being at increased risk for accidental reactions or severe reactions.

1.8.1 Threshold of reactivity:

Thresholds can be defined as the maximum amount of an allergenic food that can be tolerated without producing any adverse reaction, in contrast the lowest observed adverse effect level (LOAEL) is the minimum dose required to cause an allergic reaction.

In FA threshold varies between[62, 135] and within individual, this latter with exercise, stress or infections. Individual patient threshold doses have been used to generate population dose-distribution curves establishing an eliciting dose (ED)05 to peanut (dose at which 5% of the population allergic to peanut will react) of 1.4mg[135]. TRACE study will generate the first population dose-distribution curve in adults with peanut allergy in the UK.

Historically in FA studies, threshold has been referred to as the cumulative dose but recent studies suggest that this may not be the accurate dose of reaction as patients can react over 2 hours after the dose given and not just within the 20-30 minutes after intake[15] and therefore being the discrete dose of reaction different to the cumulative dose. Single dose challenge studies have also been performed to identify the most highly sensitive patients[281].

The main limitation of supervised food challenges in determining threshold is that OFC scenario is not the same as real life exposure to the allergen as, in general, larger amounts of the allergen are consumed in the community whilst incremental dose challenges may induce a transient desensitization effects.

1.8.1.1 How might threshold of reactivity be determined:

Peanut can be absorbed across the oral mucosa into the blood in non-allergic patients who had chewed peanuts for 2 minutes before spitting the peanut out[216]. Thus, peanut can be rapidly absorbed from the mouth without the need for gastric absorption[215]. Variations in oropharyngeal mucosal permeability, the oral mucosa is not uniform, highly permeable tissue and shows regional variation[282], may have an important influence on

the systemic bioavailability of food allergen. No data is available on how alcohol or treatment with non-steroidal anti-inflammatory drug (NSAID) treatment may alter oral mucosal permeability.

Previous work has found a strong relationship between aeroallergen sensitisation, dose of allergen plus non-specific bronchial hyper-responsiveness (BHR, measured as histamine/metacholine-PC20), and allergen-induced bronchospasm during allergic reactions triggered by bronchial allergen challenge [283, 284]. This suggests that the bronchial response to inhaled allergen is related to the degree of aeroallergen sensitisation and exposure, combined with non-specific BHR.

1.8.1.2 Clinical studies which have identified predictors of threshold of reactivity in IgE-mediated food allergy:

Currently the diagnostic tests available in routine clinical use for FA are unable to predict threshold accurately, although there is a preliminary report that BAT can be used to determine threshold *in vivo* when basophils are challenged *in vitro* for peanut allergy[247]. More available diagnostic tests including SPT and specific IgE show a negative correlation with threshold i.e. those patients with lower thresholds have significantly higher SPT and specific IgE levels[15, 100, 247] and those with higher thresholds had higher ratio of peanut-specific IgG_4 to IgE[100, 247]. Higher levels of IgE to Ara h 2 were associated with lower thresholds during food challenges in children and adults[241, 285]. All this suggests that the higher the level of sensitization and/or effector cell responsiveness in general the lower the threshold but there are not clear cut-off points and therefore it has very limited clinical utility.

1.8.2 Severity of reaction:

Food allergic reaction severity varies from mild to anaphylaxis, this latter can at the same time be graded from mild to severe anaphylaxis. There is no consensus on how to define anaphylaxis, which is key when trying to classify patients. EAACI definition[74] includes severe, life threatening, systemic, rapid in onset including airway or circulatory problems whilst NIAID definition[286] also includes skin, gastrointestinal symptoms and reduced blood pressure. This lack in consensus on how to define anaphylaxis translates on the lack of consensus to classify food allergic reactions and therefore results of FA studies usually only fit the population it has been designed for. There are multiple classifications of allergic reactions[172, 287, 288] approaches range from description of key symptoms, “2 or more” rule, sum of scores or physiological parameters but they all have deficiencies and therefore cannot be extrapolated to other populations. In general use of IM adrenaline as rescue

medication has not been used to grade food allergic reaction severity but recently, as part of iFAAM study, a new (as yet unpublished) grading system has been proposed which has been validated against use of IM adrenaline. Whether this represents a valid approach remains to be seen, as use of IM adrenaline is a subjective decision, and where used, is likely to be associated with more severe reactions.

1.8.2.1 Clinical studies which have identified potential predictors of severity in IgE-mediated FA reactions:

As we have already discussed previously in this chapter we are unable to risk stratify our patients, which involves our inability to predict the severity of food allergic reactions with the current available diagnostic tests. Efforts have been done to modify diagnostic tests like SPT using end point titration (EPT), which differentiate between those patients having anaphylaxis and those who have mild/moderate reactions[92].

Higher levels of specific IgE have been related to more severe reactions[289] and could be used to predict outcome severity and efficacy of OIT[290].

Basophil activation test (BAT) has also been able to predict severity outcome of food allergic reaction to peanut. This study suggests a cut-off point of 1.3 i.e basophil activation of 1.3 or greater CD63 peanut/anti-IgE increased the proportion of severe reactors by 3-fold[247].

1.9 Vascular responses to cutaneous allergen challenge as potential predictors of clinical phenotype in IgE mediated food allergy:

1.9.1 SPT to predict threshold of reactivity:

There is very limited data on skin prick test assessing threshold, data available comes from OIT studies suggesting a decrease in EPT in those participants undergoing active treatment i.e as the threshold tolerated increases there is a decrease in EPT[291-294].

1.9.2 SPT to predict severity of reaction:

To date SPT is not able to predict severity of reaction but as discussed previously a study on hen´s egg showed that EPT was higher on those patients who had anaphylaxis with no overlapping between those who had negative or mild reaction and those who had anaphylaxis[92]. In this thesis we will try to explore a different way of using SPT to predict severity of reaction by measuring non-diluted SPT at specific intervals until it disappears. The rationale behind this has to do with the "switch-off" mechanisms in IgE-mediated reactions that may be different (longer) in those suffering more severe reactions.

1.10 Rationale for this thesis:

The evidence base for current treatments for food anaphylaxis is very limited[73, 295, 296]. We know that a significant proportion of fatal food anaphylaxis cases (approximately one third in the UK Registry) received IM adrenaline promptly[201], and that fatal food anaphylaxis rates have not decreased in the past 20 years in England and Wales despite a large increase in adrenaline auto-injector prescriptions during that period[42]. In order to improve our ability to treat food anaphylaxis, we need a better understanding of the pathophysiology of this disease.

Data about the role of the cardiovascular system (CVS) in acute allergic reactions, mostly from animal models, suggest the CVS is not only a target organ but also responsible for many of the symptoms and signs present in an allergic reaction, such as angioedema, tachycardia and arrhythmia. However, the role of cardiovascular events in determining severity of food allergic reactions remains understudied. In this thesis I will explore the pathophysiology of IgE-mediated FA focussing mainly on cardiovascular events. This will be studied in adults (18-45 years) with IgE-mediated peanut allergy as a model, since this represents one of the commonest forms of food allergy, the commonest cause of fatal food anaphylaxis, and an age group at high risk of severe outcomes during food allergic reactions, in which it is possible to undertake detailed studies. I will also investigate the effects of IM adrenaline on cardiac physiology and rhythm parameters.

Previous attempts using SPT to predict threshold and severity have found inconclusive results[15, 297]. Research efforts should be made to improve the diagnostic tests that are already available or finding new ones, which will help predict both threshold and severity. In this thesis I will focus on titrated SPT done on the same day as the DBPCFC as a predictor of outcome and I will also investigate the time of resolution as a "switch off" mechanism, which may be key in moderating severity.

1.10.1 Aims of this thesis:

1. To understand the cardiovascular physiological changes that occurs during IgE-mediated peanut allergic reactions.
2. To understand the changes in cardiac electrical activity that occurs during IgE-mediated peanut allergic reactions.
3. To identify whether local vascular response to titrated SPT, measured as threshold of response, and time to resolution of SPT response, are prognostic factors for threshold and severity of reaction respectively, in peanut allergic individuals undergoing DBPCFC.

1.10.2 Hypotheses of this thesis:

1. In peanut-allergic adults undergoing DBPCFC to peanut, a change in cardiovascular physiological measurements accompanies clinical symptoms, which are not present during placebo challenges.
2. Cardiac electrical conductance disturbances can be detected in adults undergoing IgE-mediated allergic reactions to peanut, which are not present during placebo challenges. Specifically, we expect to find changes in HRV on our adult population similar to that found previously in a paediatric population.
3. In adults with IgE-mediated peanut allergy, the local vascular response to peanut SPT can predict severity of reaction by using the time taken for the SPT wheal to reduce below 3mm and can predict threshold of reactivity by taking the concentration of peanut required for a 3mm SPT wheal adjusting for relevant covariates such as age, sex, peanut-specific IgE, bronchial hyperresponsiveness (BHR), cumulative dose of peanut during DBPCFC, percentage peak of mast cell tryptase, release of endogenous adrenaline, participant´s and investigator´s VAS score.

2. METHODS:

2.1TRACE Peanut Study:

Study name: Threshold of Reactivity And Clinical Evaluation study.

TRACE study is a two-site study, which aim is to understand the changes in threshold and severity over time and with intervention of sleep deprivation and exercise.

2.1.1 Study setting:

All individuals studied for this thesis were participants in the TRACE Peanut Study, at the Imperial College London TRACE study site. TRACE has a randomized cross-over peanut-challenge design, aimed at understanding the stability of threshold and severity of reaction over time, in young adults with IgE-mediated primary peanut allergy. After an initial screening, participants underwent double-blind, placebo-controlled food challenge (DBPCFC) to confirm the presence of peanut allergy. Eligible participants were then randomized to undergo repeat peanut challenges on three separate occasions. Participants were randomly allocated to one of six arms, ABC/BCA/CAB/ACB/BAC/CBA, each letter representing a different condition under which they received peanut. The three conditions studied were ‘non-intervention’ i.e. a repeat of the baseline challenge; ‘exercise’ with intense periods of exertion on an exercise bicycle immediately after each dose of peanut or placebo was administered; and ‘sleep deprivation’ where sleep was restricted the night before the challenge. The three repeat peanut challenges were undertaken at ≥12 week intervals. The order of challenges between the six groups was balanced by employing a Latin square design for a six–by-three crossover trial. The data described in this thesis were acquired from TRACE study participants during their baseline DBPCFC, and for some analyses I also evaluated data acquired during repeat peanut challenges ‘sleep deprivation’ and ‘non-intervention’. Data from exercise challenge were not used in this thesis, due to the known effects of exercise on cardiovascular responses.

Two study sites were used to recruit patients, Cambridge University and Imperial College London (comprising Imperial College Healthcare NHS Trust and Royal Brompton and Harefield NHS Trust). Between the two sites, 100 adults aged 18-45 with a history of a typical type-1 hypersensitivity reaction to peanut and who met the eligibility criteria were recruited.

The rationale for the TRACE study is that the translation of minimal eliciting allergen doses (MED) into acceptable levels of allergen contamination for the population requires consideration of a 'safety factor', to account for individual variability in dose threshold and

severity. Data suggest that individual variability in dose threshold and severity depends in part on extrinsic factors (including, exercise and sleep restriction). Each factor may have a different effect in scale and direction.

The primary objectives of TRACE were:

- Establish a dose-distribution curve for peanut thresholds in an adult UK peanut allergic population.
- Model the variability of challenge thresholds over time within individuals, as a result of repeat challenges, and to:
    - Examine how these extrinsic factors shift the dose response curve:
        - Exercise
        - Stress through sleep restriction
- By comparison with the background variability, we can establish whether the threshold is reduced, increased or unchanged, and provide a factor for shift in MED with extrinsic factors.

The Secondary Objectives were:

- Development of an online database e-environment.
- Establish the magnitude of change in threshold induced by extrinsic factors.
- Identify which extrinsic factors (exercise or stress) are involved and, depending on the data obtained, which have the greatest effect.
- Define how other factors such as age, gender, co-existing asthma and application of repeat challenges alone, may modulate responses.
- Indicate the magnitude of an appropriate safety factor.

Parallel to the main TRACE study is the study of mechanisms underlying peanut allergic reaction in TRACE participants. This study was only conducted in those participants who attended the London site (Royal Brompton and Harefield NHS Trust), and this thesis comprises part of the TRACE mechanistic study.

The hypotheses of the TRACE mechanistic study were:

- The allergic response to peanut challenge is related to the systemic availability of peanut protein across the oro-gastrointestinal mucosal barrier.
- Systemic activation of effector cells (including basophils) occurs with both localised and generalised allergic reactions to peanut, and is related to the systemic availability of peanut protein.
- Oral allergen challenge with peanut causes vascular effects beyond the site of allergen exposure, even in the absence of clinical symptoms of a systemic reaction.

The objectives were:

- To define the key physiological and vascular events of an acute allergic reaction to peanut.
- To assess factors which may account for variations in threshold between peanut-allergic individuals.
- Investigate potential factors underlying variation in severity of reaction in peanut-allergic subjects.

Primary Objectives

- To measure the absorption of peanut protein in allergic individuals and how this correlates with downstream events and the onset of clinical symptoms.
- To assess for evidence of systemic cell (basophils, neutrophils) involvement during an allergic reaction and how this correlates with the onset of clinical symptoms.
- To determine the changes in cardiac output during acute allergic reactions to food and how these relate to clinical symptoms.

Secondary Objectives

- To assess how variations in the absorption of food proteins during an allergic reaction may account for differences in both clinical symptoms and threshold of onset of symptoms.
- To determine whether mediators, which have been proposed to be important in the pathogenesis of acute food allergic reactions in animal models of anaphylaxis are relevant to man.
- To analyse local mucosal and systemic vascular responses during the acute allergic reaction to food.
- To determine whether endogenous compensatory mechanisms may explain the wide variation seen in the allergic response between individuals.
- To obtain data on whether allergen exposure results in a state of basophil anergy and how this might impact on the individual.

DBPCFC food challenges and active intervention challenges were undertaken in the two centres using a "dessert matrix", based on that developed in the EuroPrevall project [298], the blinding of which has been shown using triangle-testing, and utility for both diagnosis of peanut allergy and determination of dose-responses[288].

TRACE Peanut Study was funded by the UK Food Standards Agency and the TRACE mechanistic study is funded through a Medical Research Council Clinician Scientist Award

to Dr Paul Turner. Additional funding for the work described herein was obtained through the National Institute for Health Research (NIHR) Biomedical Research Centre at the Imperial Academic Health Sciences Centre (AHSC), a partnership between Imperial College Healthcare NHS Trust and Imperial College London. Imperial College London is the Sponsor for the TRACE mechanistic study and Cambridge University Hospitals NHS Foundation Trust is the Sponsor for the TRACE Peanut Study.

2.1.2 Study population:

Participants were recruited using a marketing agency (Media and marketing with impact (MWI)) which used newspaper advertisements, social media and online advertising; through allergy support groups, and through letters sent to existing NHS allergy clinic patients with a diagnosis of peanut allergy (Appendix 1). All potential participants were directed to a website for registration and preliminary screening (www.tracestudy.com). Participants who passed the online screening questions received an email from the study team with the patient information sheet (PIS) (Appendix 2) and had an opportunity to discuss the study by telephone or email. Potentially eligible participants were invited for a visit to sign informed consent (Appendix 3) and if appropriate, proceed with the screening appointment.

Inclusion criteria:

- Written informed consent must be obtained before any assessment is performed.
- Male and female subjects 18-45 years of age at the time of study entry (screening visit) who have a diagnosis of acute peanut allergy as manifested by urticaria, angioedema or respiratory/gastrointestinal tract symptoms, with acute onset of symptoms after ingestion (up to 2 hours).
- Sensitisation to peanut demonstrated by skin prick test, or serum specific IgE.
- A positive peanut DBPCFC at baseline. This outcome is defined as the onset of objective allergic events after ingestion of peanut protein but not to the placebo. Eligibility to the DBPCFC requires fulfilment of all other eligibility criteria at screening visit.
- Subjects must be able to comply with the study procedures.

Exclusion criteria:

- Oral allergy syndrome to peanut (defined as a clinical history of only oral allergy symptoms on exposure to peanut and principal sensitization to only PR-10 homologues of peanut (Ara h 8), and low level (<8kU/l) serum specific IgE to Ara h 1, 2, 3).
- Mono-sensitisation to Ara h 9.
- Use of investigational drugs at the time of enrolment, or within 30 days or 5 half-lives of enrolment, whichever is longer.
- History of hypersensitivity to any of the matrix components used within the material for the oral food challenge (OFC).
- Poorly controlled asthma. The Asthma Control Questionnaire (ACQ) (Appendix 4) will assess asthma control. Patients with a score <20 won't be eligible for this study. Also, patients should have FEV1 >80% of their predicted value and a BTS score of <3 (Appendix 5).
- History of significant and repeated exercise –induced asthma attacks requiring treatment, independent of food ingestion or a drop in FEV1 of >15% during screening Vo2max exercise session.
- Musculo-skeletal disease, which in the opinion of the investigator could impair the participant's ability to perform the exercise challenge.
- A sleep or psychiatric disorder, which in the opinion of the investigator could impair the participant's ability to perform the study procedures.
- Pregnancy.
- Alcohol or drug misuse.
- Night-shift worker.
- Concomitant use of:
    - Systemic immunosuppressant.
    - Beta-blocker.
    - ACE inhibitor or other hypertensive drugs.
    - Sedative drugs.
    - Antacid medication (either proton pump inhibitors or H2-antagonists).
- History of any of the following:
    - History of severe anaphylaxis to peanut as defined by hypoxia, hypotension, neurological compromise (cyanosis or SpO2 < 92% at

any stage, hypotension, confusion, collapse, loss of consciousness, or incontinence).
    - A previous reaction to peanut that in the opinion of the investigator (or Trial Management Group) was life-threatening.
    - Mastocytosis.
    - Coronary artery disease.
    - Eosinophilic oesophagitis.
    - Gastric or duodenal ulcer.

- A past medical history of clinically significant ECG abnormalities or identified during study (screening visit).
- Recent (within the last three years) and/or recurrent history of autonomic dysfunction (e.g., recurrent episodes of fainting, palpitations, etc.).
- Haematological parameters (total WBC count or Hb level, platelet counts) that fall outside the normal reference range of the laboratory at screening and are clinically significant.

2.1.3 TRACE Study procedure:

2.1.3.1 Overall study procedures:

Participants were required to attend the hospital for a total of 6 visits (Figure 5) of which the first comprised the screening visit and the subsequent 5 visits were peanut challenges according to the protocol described at the beginning of this section. While initially all peanut challenges were designed to be DBPCFC, the study protocol was modified on 10/10/2014 at the request of the funder, due to slow study progress for the parent TRACE study. The modification involved switching to open challenges for all repeat peanut challenges, and was justified by a blinded evaluation of all DBPCFC undertaken to date showing no evidence of placebo reactions.

<u>Screening visit:</u>

After informed consent, a full clinical history was taken, guided by the Allerg-e-lab database question fields (Appendix 5). A full examination and the following procedures were undertaken:

- Draw blood for serum IgE (specific IgE to total peanut, Ara h 1,2,3,8 and 9), full blood count including eosinophil count, urea and electrolytes, DNA storage and collection of peripheral blood mononuclear (inflammatory) cells.

- Skin prick test with peanut, and a panel of inhalant and ingestant allergens from Stallergenes (London, United Kingdom).
    - Inhalants: Grass pollen, D. pteronyssinus, D. farinae, Alternaria, Aspergillus, Cladosporium, Alder, Birch, Hazel, Plane, Cat and Dog.
    - Ingestant allergens: Hazelnut, Almond, Walnut, Cashew, Pistachio, Macadamia, Pecan, Brazil Nut, Soya, Lupine flour, Shrimp, Cod, Milk, Egg, Wheat and Sesame.
- 12-lead electrocardiogram.
- Spirometry (measurement of FEV_1) with reversibility in accordance with ATS/ERS recommendations[299].
- For patients with eczema – Patient Orientated Eczema Measurement (POEM, Appendix 6).
- VO_{2max} cycling fitness test and post-test spirometry in order to exclude those participants who may have Exercise-induced asthma (EIA).
- Asthma ACQ; BTS Staging of asthma medication. (Appendix 7).
- Provide with two-week sleep diaries with instructions to complete for the two weeks prior to each challenge.
- Pregnancy test (for females).
- Bronchial Hyperresponsiveness (BHR) to histamine using dosimeter technique. The Standard Operating Procedure (SOP) is shown in Appendix 8.

If participant was eligible after screening they attended on a separate occasion for DBPCFC using a specially prepared "dessert matrix" manufactured at Manchester University, modified from that used in the EuroPrevall project[300]. After the DBPCFC challenge, eligible participants were randomised to one of six sequences and challenges were performed with a minimum 12-week interval between food challenges. Food challenges undertaken subsequent to the eligibility DBPCFC were open food challenges, due to logistical and financial constraints on the TRACE project. Data in this thesis are largely taken from the baseline, eligibility DBPCFC, although for some analyses I have used data from subsequent post-randomisation challenges under 'sleep restriction' or 'non-intervention' conditions.

Figure 5 TRACE study design:

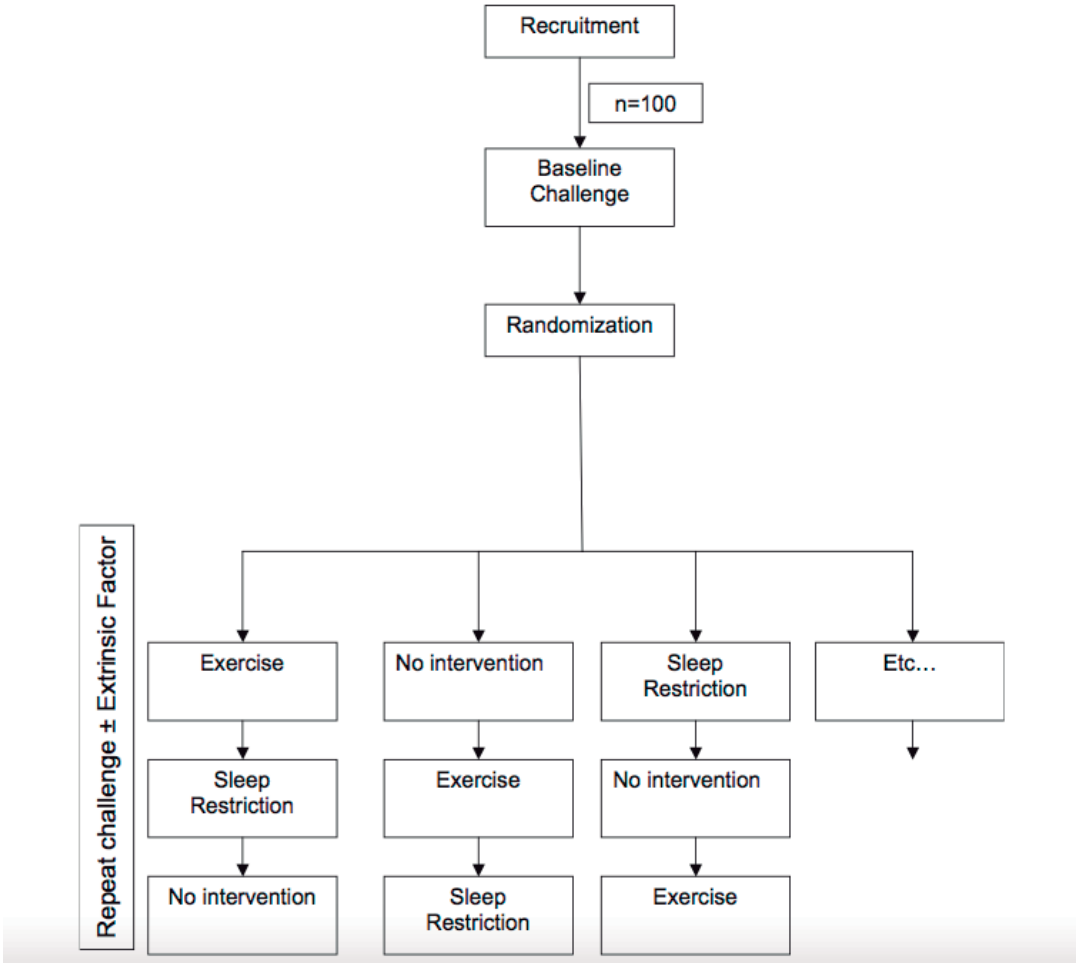


Figure taken from TRACE study protocol document.

2.1.3.2 Food Challenge procedure:

The baseline DBPCFC (SOP is shown in Appendix 9) took place on two days, separated by at least 7 days. On one day all doses were active, on another day all doses were placebo. Each challenge day involved administration of up to eight doses (3µg, 30 µg, 300 µg, 3mg, 30mg, 100mg, 300mg and 1000 mg of peanut protein) separated by 30-minute intervals.

The order of the two days was randomly assigned. The computer-generated randomisation list was created by Cambridge University. Randomization was stratified by age, centre and presence of asthma. Randomization lists were used by

University of Manchester to provide randomized, coded sets of challenge for each centre. Placebo and active challenge materials ('dessert matrix') manufactured in

Manchester University were delivered to the Imperial College London study site in batches, and made up fresh on the day by a dietician who held the randomisation list for each participant. The dessert matrix was developed such that the taste, appearance and

smell of the active and placebo doses could be identical. The dietician was independent of the team overseeing DBPCFC, and made up doses fresh for each food challenge day, leaving them in a kitchen so that the team overseeing the DBPCFC could use them without direct contact with the dietician. The team overseeing the DBPCFC remained blind to active/placebo allocation throughout the study.

Before the challenge took part, the participant underwent a series of procedures and questionnaires in order to determine if it was safe for the participant to proceed with the challenge:

- All participants underwent a thorough physical examination of general appearance, skin, lungs, heart, abdomen, back, limbs and lymph nodes.
- All participants had a cannula for blood samples and administering medication.
- Titrated Skin Prick Test (SPT) to peanut, histamine and codeine was performed on the left arm volar surface. This is explained in further detail in section 2.6.
- In order to monitor the heart and the cardiovascular system (CVS) participants were attached to a 12-lead ECG Holter monitor (SEER 12, GE Healthcare, Chicago, United States) and to a continuous non-invasive CHEETAH NICOM^{TM} monitor (Cheetah medical, Boston, Massachusetts) for CVS physiology measurements. Both techniques are explained in further detail in sections 2.5 and 2.4 respectively.
- ACQ and POEM score questionnaires were given if the participant has asthma or eczema respectively.
- Standardized questions per protocol were assessed (See Appendix 10 for Case Record Form (CRF)) in order to determine if the participant could proceed to the challenge.
- Baseline observations including central and peripheral blood pressure (BP), heart rate (HR), oxygen saturation, body temperature and spirometry with FEV1 and PEFR were performed prior to starting the OFC. These observations were repeated before each dose given and when needed throughout the challenge.
- A continuous non-invasive measure of skin blood flow was performed in every participant; this is explained in further detail in section 2.4.3.3.

2.1.3.3 Stopping criteria and treatment protocol:

The stopping criteria for the study shown in Table 7 are based on a modification of the PRACTALL consensus[288]. The study teams at both sites, to ensure consistency, agreed this stopping criteria. The stopping criteria are based on a “traffic light” system with

green, yellow and red symptoms specified by organ according to their severity. It requires at least 3 concomitant objective ("yellow") symptoms or 1 severe ("red") symptom to stop the challenge. This guideline was strictly adhered to, although, the supervising clinician for safety reasons could, in theory, override all challenge stopping decisions. Where the decision to 'stop and treat' was in doubt, the supervising clinical team sometimes delayed administration of the next dose, and/or administered a repeat of the last dose given, in order to reduce the risk of provoking a severe reaction by giving a high dose of allergen to a participant already in the process of reacting.

Table 7 PRACTALL consensus for challenge termination as modified for use in TRACE:

SKIN	
Erythematous Rash - % area involved	See body surface area diagram in Figure 2.1
Pruritus	Absent
	Green - Occasional scratching
	Green - Scratching continuously for > 2 minutes at a time
	Yellow - Hard continuous scratching ➔ excoriations
Urticaria/Angioedema	Absent
	Yellow - < 3 hives, or mild lip oedema
	Red - < 10 hives but ≥3, or significant lip or face oedema
	Red – Generalized involvement
Rash	Absent
	Green – Few areas of faint erythema
	Yellow – Areas of erythema
	Red – Generalized marked erythema (>50%)

UPPER RESPIRATORY	
Sneezing/Itching	Absent
	Green - Itching in ear canal
	Green – Rare bursts, occasional sniffing
	Green – Bursts < 10, intermittent rubbing of nose, and/or eyes or frequent sniffing
	Yellow– Continuous rubbing of nose and/or eyes
	Yellow - Periocular swelling and/or long bursts of sneezing,
	Yellow - Persistent rhinorrhoea
LOWER RESPIRATORY	
Wheezing	Absent
	Green – chest tightness without any fall in PEFR
	Green - chest tightness with a <10% fall in PEFR
	Yellow - chest tightness with a 10-20% fall in PEFR
	Red – Expiratory or inspiratory wheeze
	Red – Use of accessory muscles and/or audible wheezing (or silent lung)

UPPER RESPIRATORY	
Laryngeal	Absent
	Green – throat tingling / altered sensation in throat
	Yellow –>3 discrete episodes of throat clearing or cough, or persistent throat tightness/pain
	Red – Hoarseness, frequent dry cough
	Red – Stridor

GASTROINTESTINAL	
Nausea/pain	Absent
	Green – transient nausea
	Green – transient abdominal pain
	Yellow – persistent nausea
	Yellow –Persistent abdominal pain
Emesis/diarrhoea	Absent
	Yellow – 1 episode of emesis or diarrhoea
	Red – >1 episodes of emesis or diarrhoea or 1 of each

Cardiovascular	
	Normal heart rate or BP for age/baseline
	Yellow - Subjective response (weak, dizzy), or tachycardia
	Red - Drop in blood pressure and/or >20% from baseline, or significant change in mental status.
	Red - Cardiovascular collapse, signs of impaired circulation (unconscious)
Neurological	Altered consciousness (record GCS score)

Table taken from TRACE study protocol document.

The treatment protocol that was followed for red symptoms can be seen in Table 8, although again this was a guide and final treatment decision was at the clinical investigator´s discretion.

In case of ≥3 yellow symptoms with the exception of three yellow symptoms confined to the skin (=one organ), the use of adrenaline was encouraged, and short acting beta agonists (SABA) were used in case of involvement of the lower airways.

Table 8 Treatment protocol for red symptoms:

Signs and symptoms	Stopping criteria	Recommended treatment
Skin		
Urticaria/Angioedema	< 10 hives but ≥3, or significant lip or face oedema	In isolation: follow local procedures, consider fast acting anti-histamines (eg. cetirizine) first In combination with any symptom from a different system, consider: 0.5 mL adrenaline (1:1000) IM
	Generalized involvement	0.5 mL adrenaline (1:1000) IM
Rash	Generalized marked erythema (>50%)	0.5 mL adrenaline (1:1000) IM
Lower respiratory		
Wheezing	Expiratory wheezing on auscultation	0.5 mL adrenaline (1:1000) IM +SABA
	Mild audible (inspiratory and) expiratory wheezing	0.5 mL adrenaline (1:1000) IM +SABA
	Use of accessory muscles and/or audible wheezing (or silent lung)	0.5 mL adrenaline (1:1000) IM +SABA
Laryngeal	Hoarseness, frequent dry cough	0.5 mL adrenaline (1:1000) IM, consider nebulised adrenaline (1mg in 5ml saline).
	Stridor	0.5 mL adrenaline (1:1000) IM, consider nebulised adrenaline (1mg in 5ml saline). Notify anaesthetist / ICU.
Gastrointestinal		
Emesis/diarrhoea	2-3 episodes of emesis or diarrhoea or 1 of each	0.5 mL adrenaline (1:1000) IM)+ 1000 mL, consider 1 litre 0.9% saline bolus over 1-3 minutes
	>3 episodes of emesis or diarrhoea or 2 of each	0.5 mL adrenaline (1:1000) IM + 1000 mL 0.9% saline bolus over 1-3 minutes
Cardiovascular/neurologic		
	Drop in blood pressure and/or >20% from baseline, or significant change in mental status. Cardiovascular collapse, signs of impaired circulation (unconscious)	0.5 mL adrenaline (1:1000) IM. Inform ICU/ anaesthetist +1000 mL 0.9% saline bolus over 1-3 minutes (repeat as required) Consider IV adrenaline; diluted to at least 1:10,000, Start infusion at 5-15 µg/min. ECG /P/BP monitoring essential. Contact ICU / anaesthetist.

SABA= Short-acting Beta Agonist.
Table taken from TRACE study protocol document.

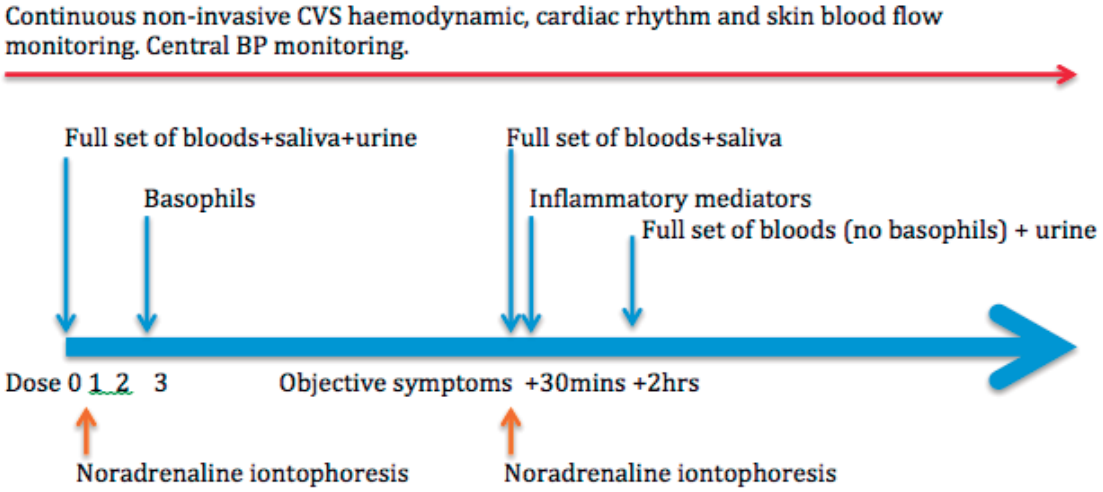
2.2 TRACE Mechanistic assessments:

As part of the TRACE mechanistic study different procedures were carried out during the OFC and these are shown in Figure 6.

- Full set of bloods were taken for analysis of inflammatory cell activation (by flow cytometry), gene expression (RNA PAXgene storage), genotype (DNA storage).

- Saliva samples for salivary IgA, and blood for basophils, mast cell tryptase (MCT) and catecholamines were taken at baseline, at time of reaction, or for placebo days at baseline and 1 hour after the last dose was given. For MCT results are shown as absolute value and as a percentage, at onset of objective clinical reaction (OCR) and the peak achieved from OCR to 2hr after OCR as MCT peaks between 15-120min after it´s released with a half-life of 1.5-2.5hrs[301].
- Participants were asked to void prior to challenge, with a urine sample collected 2hrs after the time of reaction.
- Titrated SPT was performed in the volar surface of the left arm to commercial peanut extract, histamine and codeine.
- Skin blood flow was continuously monitored by laser Doppler as described below, for the whole of the challenge.
- Continuous monitoring of CVS physiological measurements and cardiac rhythm was carried out from the arrival of the participant until discharged.
- Central BP was performed at baseline, before each dose was given, at time of reaction and at time of discharge of the participant.

Figure 6 TRACE mechanistic study design:



2.3 Ethics:

Ethical approval for all the study interventions and procedures were obtained from the NHS Health Research Authority (12/EE/0289 and 15/LO/0286). Local R&D approval was obtained at the Royal Brompton Hospital NHS Trust. The study was registered prospectively at Clinicaltrials.gov (NCT01429896) and all study participants gave informed consent. The assessments were conducted in accordance with the recommendations for physicians involved in research on human subjects adopted by the 18th World Medical Assembly, Helsinki 1964 and later revisions.

2.4. Assessment of cardiovascular system (CVS) physiology:

2.4.1 Settings and study participants.

TRACE peanut study participants undergoing baseline DBPCFC were included in this study if they reacted to peanut at challenge and didn't react to their placebo day. Exclusion criteria were non-completion of baseline DBPCFC, and inconclusive or negative outcome of DBPCFC. Reactive or non-reactive days were coded as per the modified PRACTALL criteria described in section 2.1.3.3. The point at which the TRACE study stopping criteria were fulfilled was termed the 'Objective Clinical Reaction' (OCR).

Measurements of CVS physiology were recorded using non-invasive monitoring as described below (section 2.4.2). CVS physiology was also recorded on subsequent TRACE study peanut challenges, but the key analyses in this thesis are derived from the DBPCFC, where the inclusion of a placebo challenge allows for the exclusion of diurnal or other effects when evaluation changes during allergic reactions to peanut.

2.4.2 Time-points used for measurement of CVS and electrical conductance physiology:

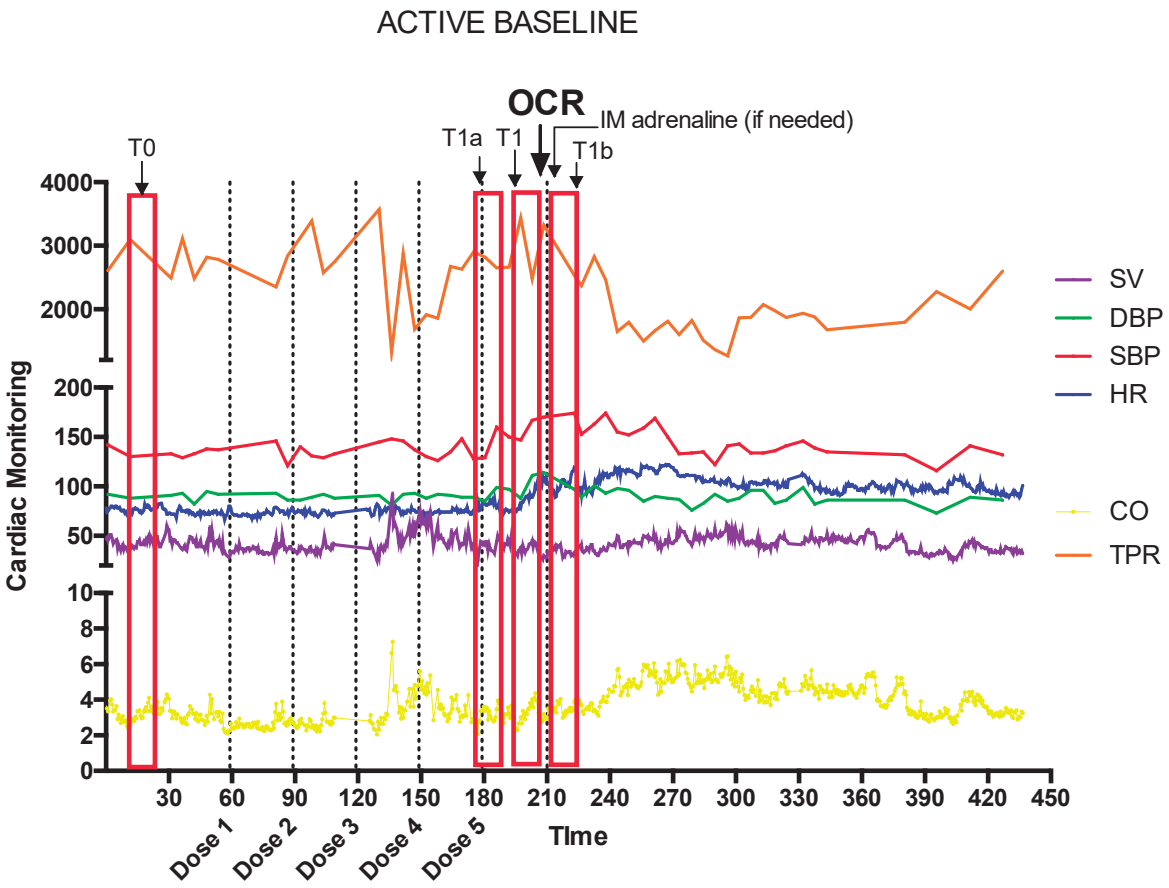
Participants were monitored for the whole of the challenge during baseline DBPCFC, the 10-minutes epochs selected for primary data analyses are shown in Table 9. These time-points were selected by consensus after discussion with cardiology collaborators (AL, CH) and review of the literature regarding exogenous influences on CVS physiology. In order to explore when these changes, if any, happened 10-minute epochs were analysed for up to 240 minutes before reaching OCR. The key period of interest was T1, since time-point T1b may be influenced by treatment given (intramuscular adrenaline when required) for an allergic reaction, and T1a may be too early to see changes in CVS physiology associated with a peanut allergic reaction. The purpose of including other time-points was to explore the kinetics of any positive changes seen, and the influence of intramuscular adrenaline on those changes, when this was administered. These time points are shown on Figure 7.

Table 9 Time-point used for measurements of CVS physiology:

Epoch	T0 (baseline)	T1a	T1	T1b
Active	10 minutes before starting challenge	20 minutes before OCR	10 minutes before OCR	10 minutes after OCR
Placebo	10 minutes before starting challenge	20 minutes before T1 on active day	10 minutes before T1 on active day	10 minutes after T1 on active day

The placebo time point for "reaction (T1)" is the time point at which the same participant had at OCR on their active challenge day. This has the benefit of reducing potential confounding of psychosomatic factors (e.g. stress, anxiety, diurnal variations), which would also affect the cardiovascular measurements.

Figure 7 Continuous non-invasive cardiovascular measurements of patient 02012:



Continuous non-invasive cardiovascular measurements during and active challenge and the different time points used for analyses.
OCR: time point when the modified PRACTALL stopping criteria were fulfilled.
T1: Time epoch 10 minutes before reaching OCR.
T1a: Time epoch 20 minutes before reaching OCR.
T1b: Time epoch 10 minutes after IM adrenaline in those participants requiring this as treatment.

2.4.3 Techniques used for CVS measurements:

2.4.3.1 Non-invasive CVS monitoring of CVS physiology:

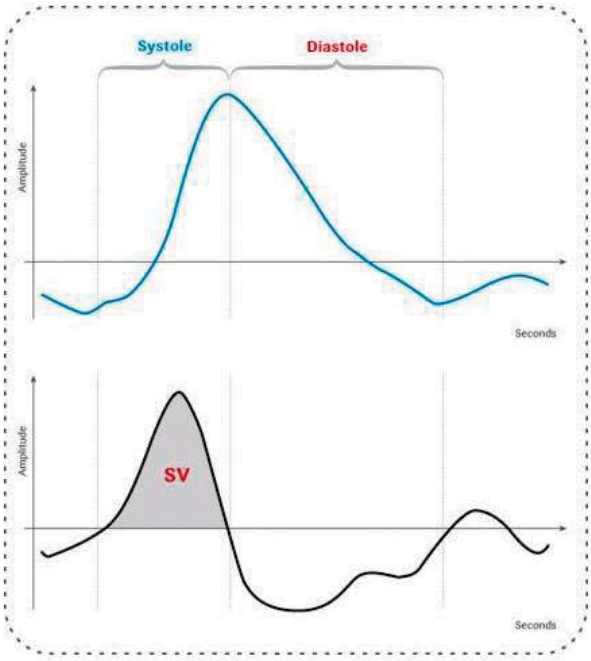
CVS physiological measurements were obtained using the non-invasive CHEETAH NICOM™ monitor (Cheetah medical, Boston, Massachusetts) (Figure 8), which measures the different parameters through thoracic bioreactance®, which depends on the blood and biological tissue electrical resistive, capacitive and inductive properties that induce phase shifts between an applied electrical current and the resulting voltage signal. The pulsatile blood flow in the large thoracic arteries, mainly the aorta, causes the amplitude of the applied thoracic voltage to change and causes a time delay between the applied current and the measured voltage. The time delay between the current and the voltage correlates with stroke volume.

During systole there is a build-up of the phase shift until a peak is reached in the end of systole, reflecting the increase in aortic blood volume during ventricular ejection; during

diastole there is a decrease in the phase shift representing reduction in blood volume (Figure 9). The maximum flow (dX/dtmax) is measured by the maximum point of the CHEETAH NICOM^{TM} signal and the Ventricular Ejection Time (VET) once this is measured the SV can be detected as SV=dX/dtmax x VET.

Figure 8 CHEETAH NICOM^{TM} monitor:

Figure 9 Heart beat waveform:



Upper graph represents a single beat on the Cheetah signal and lower graph represent a single beat of the Cheetah signal derived by time (phase shift). Stroke volume is found by computing the area under the positive part of the Cheetah Signal Derivative, or the part of the waveform that represents systole.

Four dual sensors, each containing an outer transmitting sensor and an inner sensor for receiving, were placed on the participant's thorax, above and below the heart. This non-invasive monitor measured heart rate (HR) as sensors have built in ECG leads, stroke volume (SV) and peripheral blood pressure (PBP) by oscillometry. Using these measured parameters, it derived cardiac output (CO); being HR x SV= CO, and total peripheral resistance (TPR); being TPR = change in pressure/cardiac output.

Cardiac output monitoring requires invasive technologies such as pulmonary artery catheter (PAC) for thermodilution, radial catheter for pulse contour analysis, intratracheal tube for partial CO2 rebreathing or oesophageal tube for Doppler analysis (EDM). Different validation studies have been performed in order to demonstrate that non-invasive CO monitoring correlates well with invasive CO measurements. Squara et al[302] studied 119 patients admitted to the ICU and compared CO measurements using PAC and CHEETAH NICOM^{TM} monitor showing that both measurements were highly correlated in stable periods of CO (R=0,82) precision was higher than PAC when CO changed. Precision was better with NICOM than PAC for increasing CO ($p < 0.0001$) and for decreasing CO ($p = 0.002$). Rapid increases or decreases in CO were detected earlier (3.1± 3.8min, $p<0.01$) by CHEETAH NICOM^{TM} than PAC. Sensitivity and Specificity of NICOM for detecting significant changes was 93% for both values. Similar results were found in patients in cardiac catheterization laboratories and cardiac care units[303]. NICOM monitor has also been validated for SV measurements against Esophageal Doppler Monitor (EDM) during goal directed fluid therapy (GDFT) after surgery showing consistent and significant correlation of baseline SV between monitors (Pearson Correlation Coefficient 0.48, $p=0.0002$), both monitors demonstrate about 50% of patients were "fluid responsive", this being a 10% increase in SV, this was highest at 15 min after bolus administration with no significant disagreement between measurements[304]. CHEETAH NICOM^{TM} has also been validated against FloTrac-Vigileo™[305], which uses arterial pressure signal monitoring to assess stroke volume after arterial catheterization, and pulse contour [306] for CO and SV measurements, results showed that this method has similar monitoring capabilities to NICOM using thermo dilution as the reference method.

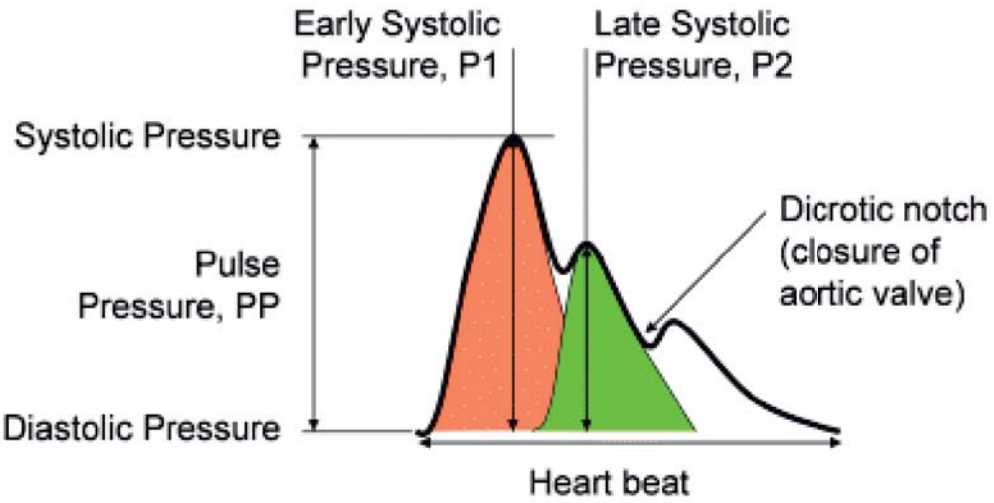
Data for CHEETAH NICOM^{TM} measurements other than blood pressure were analysed as 20 x 30-second measurements within each 10-minute epoch.

Data for sBP and dBP were analysed every 5 minutes continuously from T0 through to discharge of participant from the Clinical Research Facility.

2.4.3.2 Central blood pressure (CBP):

Systolic and diastolic CBP were measured using BP+ Pulsecor®. This monitor measures the augmentation index (AI), which is a ratio calculated from the blood pressure waveform (Figure 10), in order to estimate central blood pressure.

Figure 10 Peripheral pulse:



Arterial waveform showing DBP, PP, SBP and the early and late systolic shoulder of the peripheral pulse. The late systolic pressure determines the peripheral AI.

These measurements have been validated against invasive aortic BP[307] showing an intraclass correlation coefficient (ICC) of 0,85 ($p< 0,001$) at baseline and ICC of 0,90 ($p < 0,001$) after glycerol trinitrate, a potent vasodilator, was given in patients undergoing coronary angiography. Another study[308] showing validation of non-invasive cuff-based against invasive pressure waveforms in 6 participants showed no significant difference between the 2 measurements for systolic CBP (mean difference -5±8 mmHg, $p=0.2$) and mean arterial pressure (MAP) (mean difference -1±3 mmHg, $p=0.6$) but a significant difference for diastolic CBP (mean difference 8±3, $p<0.001$), the cuff-based pressure waveforms were similar to those acquired invasively (cross-correlation coefficient 0.93). This same study showed in 1107 participants that CBP measured by Pulsecor® was comparable to that estimated by tonometry (average difference 3±6 mmHg and ICC=0.91).

CBP was measured at T0, then every 30 minutes through to OCR or the end of the challenge day if non-reactive; following OCR CBP was measured as required for clinical monitoring according to participants' clinical status.

2.4.3.3 Peripheral blood flow:

During food allergic reactions one of the most common symptom is erythema probably due to peripheral vasodilatation. In order to determine if this can be measured during peanut allergic reactions TRACE participants undergoing DBPCFC and following the settings in section 2.2 were continuously monitored using a laser Doppler (moorVMS-LDF laser Doppler, Moor Instruments, Axminster, England) for blood flow at the back of the neck.

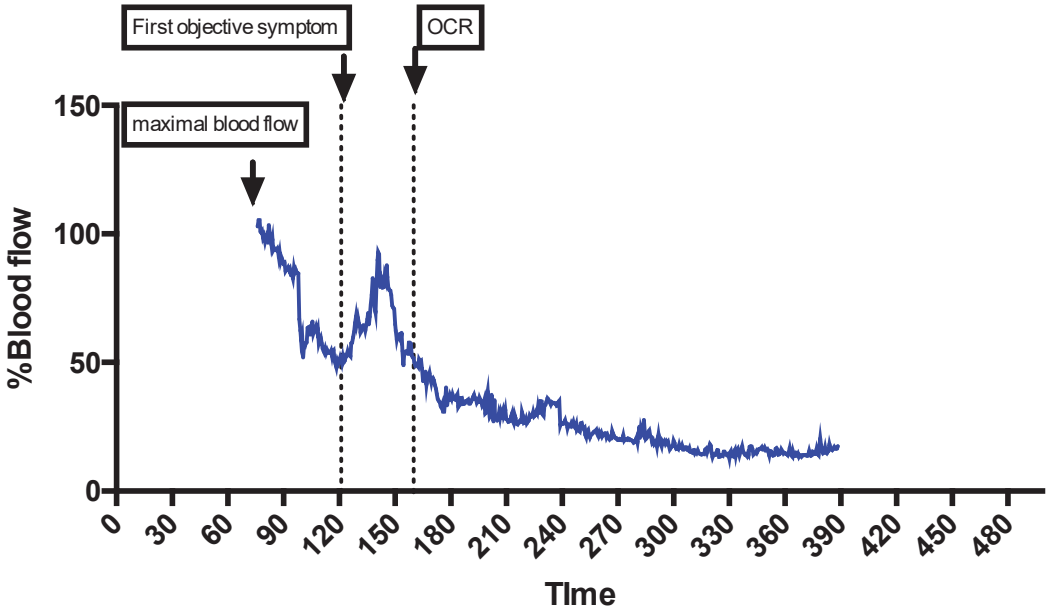
Participants were monitored for a 5-minute baseline blood flow in order to determine that this was consistent, then the measured area was heated for 30 minutes at 44ºC in order to achieve maximal blood flow, after this the measured area was continuously monitored until the participant was discharged. Data was analysed as a % change of maximal blood flow achieved after 30 minutes of heating at 44ºC.

Missing data included not reaching maximum vasodilation, no obvious fall from maximum vasodilation or frequent spikes making the data too noisy to be analysed usually because of movement.

2.4.3.3.1 Time epochs:

The change in peripheral blood flow was measured by comparing, on active days, the lowest flow rate observed between baseline and OCR, with the highest flow-rate observed from the onset of first yellow (objective) symptom through to OCR (Figure 11), yellow symptoms and OCR being defined according to modified PRACTALL consensus as described in section 2.1.3.3. For non-reactive days the flow was measured by comparing the lowest and highest flow rates seen between baseline and time of reaction (OCR), and take the highest rate during the same period, excluding spikes of less than 5 minutes. Peripheral blood flow rate was expressed as a % of the maximal blood flow achieved through pre-heating prior to baseline and prior to initiation of OFC.

Figure 11 Continuous monitoring of peripheral blood flow from patient 02048:



Blood flow represented as a % of maximal blood flow.

2.4.4 Pilot data for cardiovascular measurements:

2.4.4.1 Continuous digital BP using Finapres NOVA technology:

The minimum time between BP measurements, in order to acquire reliable peripheral BP data, using the CHEETAH NICOM™ monitor is 5 minutes due to the refractory period. Given this, continuous digital BP measurements could be a more accurate approach to evaluating changes in peripheral BP during peanut allergic reactions. In order to evaluate whether a continuous measure of blood pressure such as the Finapres® digital blood pressure system (Enschede, The Netherlands) might be preferred to intermittent brachial blood pressure measurements, we undertook a pilot study comparing these 2 methods in healthy volunteers exposed to cardiovascular stress in the form of an exercise test.

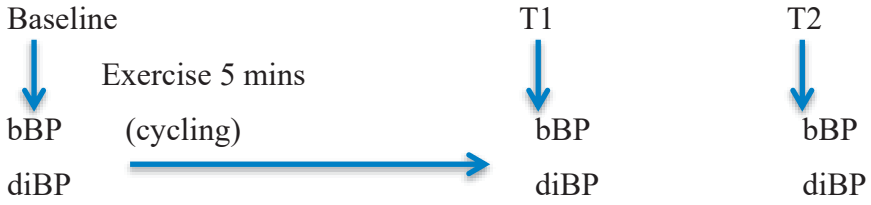
Six healthy volunteers underwent a period of 5 minutes rest during which brachial BP (bBP), using Fukuda Denshi Dynascope (Tokyo, Japan), was measured twice with an interval of 2 minutes between them and continuous digital BP (dBP) in the middle finger of the right hand was measured using Finapres equipment.

The six healthy volunteers underwent thereafter 5 minutes of cycling (DKN Technology bike, Hamme, Belgium) at a load level of 4.

After completion of the exercise period each volunteer underwent a 5-minute recovery period in which 2 measurements (time point 1(T1)) of BP were taken with an interval of 2 minutes and continuous dBP was measured during those 5-minutes; and a second 5-minute measurement of bBP during continuous dBP measurement, finishing a total of 10-minutes after the exercise (time point 2 (T2)) shown in Figure 12. During brachial and continuous digital BP measurement both hands were kept at knee level and arms at rest

for both resting and recovery periods. Results for the six volunteers are shown in Table 10.

Figure 12 Continuous digital BP study design:



T1: Recovery period, 5 minute-epoch.

T2: Five minute-epoch at 10 minutes after finishing exercise.

bBP = brachial blood pressure.

diBP = digital blood pressure.

Table 10 Pilot data for brachial and digital peripheral BP:

Pilot ID	Baseline				T1				T2			
	Brachial		Digital		Brachial		Digital		Brachial		Digital	
	sBP	dBP	sBP	dBP	sBP	dBP	sBP	dBP	sBP	dBP	sBP	dBP
1	111	71	144	81	126	79	145	77	115	80	146	78
2	134	87,5	148	90	172	78	139	67	153	84	153	80
3	125	87,5	161	104	130	83	162	90	124	88	159	96
4	124	80	150	87	138	68	153	79	127	73	144	76
5	106	59	135	78	119	59	144	66	118	58	131	61
6	112	65	151	83	133	56	144	71	121	57	140	70

sBP: Systolic blood pressure, dBP: Diastolic blood pressure. T1: 5-minute epoch immediately after completing exercise. T2: 5-minute epoch 10 minutes after completing exercise.

In order to analyse the agreement between brachial and digital BP in their ability to measure BP and to detect the differences in BP after exercise different statistical analysis were done:

1. To determine if there is a significance difference between the 2 types of measurements for systolic and diastolic BP pre and post exercise a Wilcoxon test was performed.
2. Linear regression was performed to see if there is any correlation between the change with exercise for brachial and digital BP.

RESULTS of FINAPRES assessment:

1. Wilcoxon test for the difference between pre and post exercise brachial and digital systolic and diastolic BP.

   A: Pre-systolic brachial BP vs pre-systolic digital BP: p value 0.03

   B: Pre-diastolic brachial BP vs pre-diastolic digital BP: p value 0.03

   C: Post-systolic brachial BP1 vs post-systolic digital BP1: p value 0.35

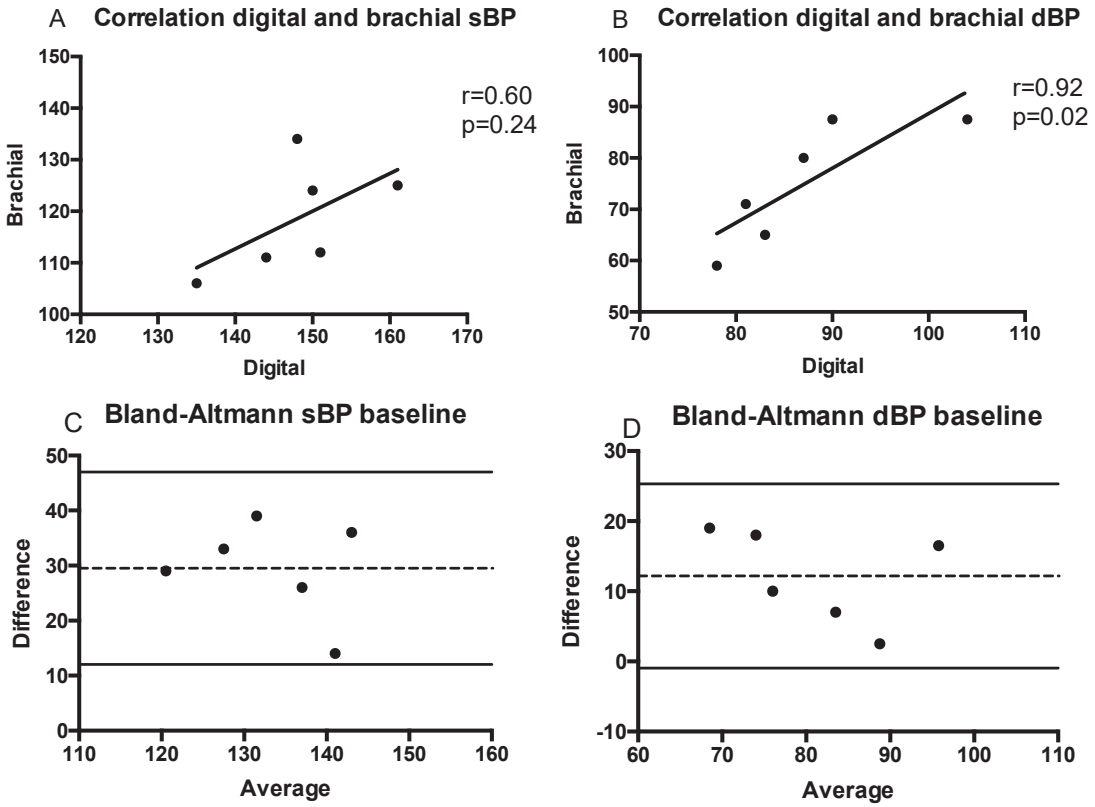
   D: Post-diastolic brachial BP1 vs post-diastolic digital BP1: p value 0.29

   E: Post-systolic brachial BP2 vs post-systolic digital BP2: p value 0.04

   F: Post-diastolic brachial BP2 vs post-diastolic digital BP2: p value 0.25

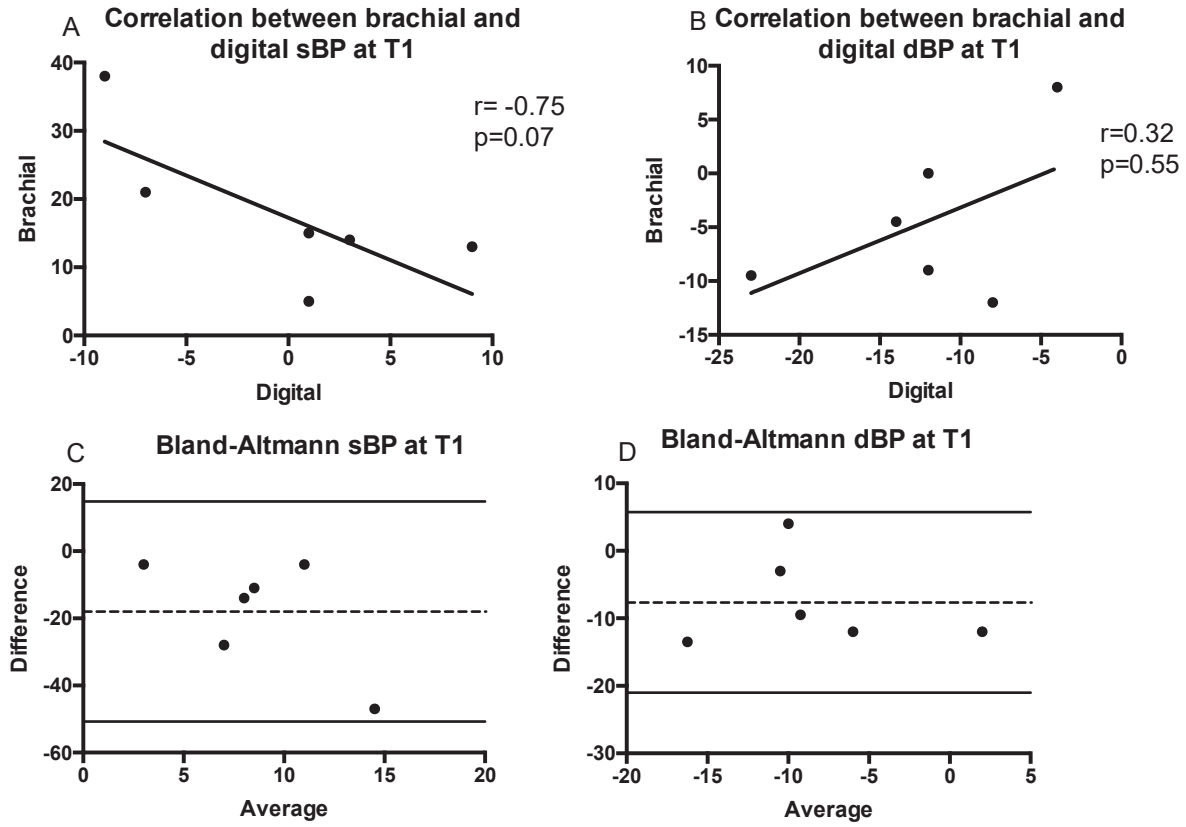
2. Level of agreement comparing BP measurements made using digital and brachial methods pre (A) and post exercise (B) (Figures 13-15).

Figure 13 Agreement between digital and brachial BP at baseline:



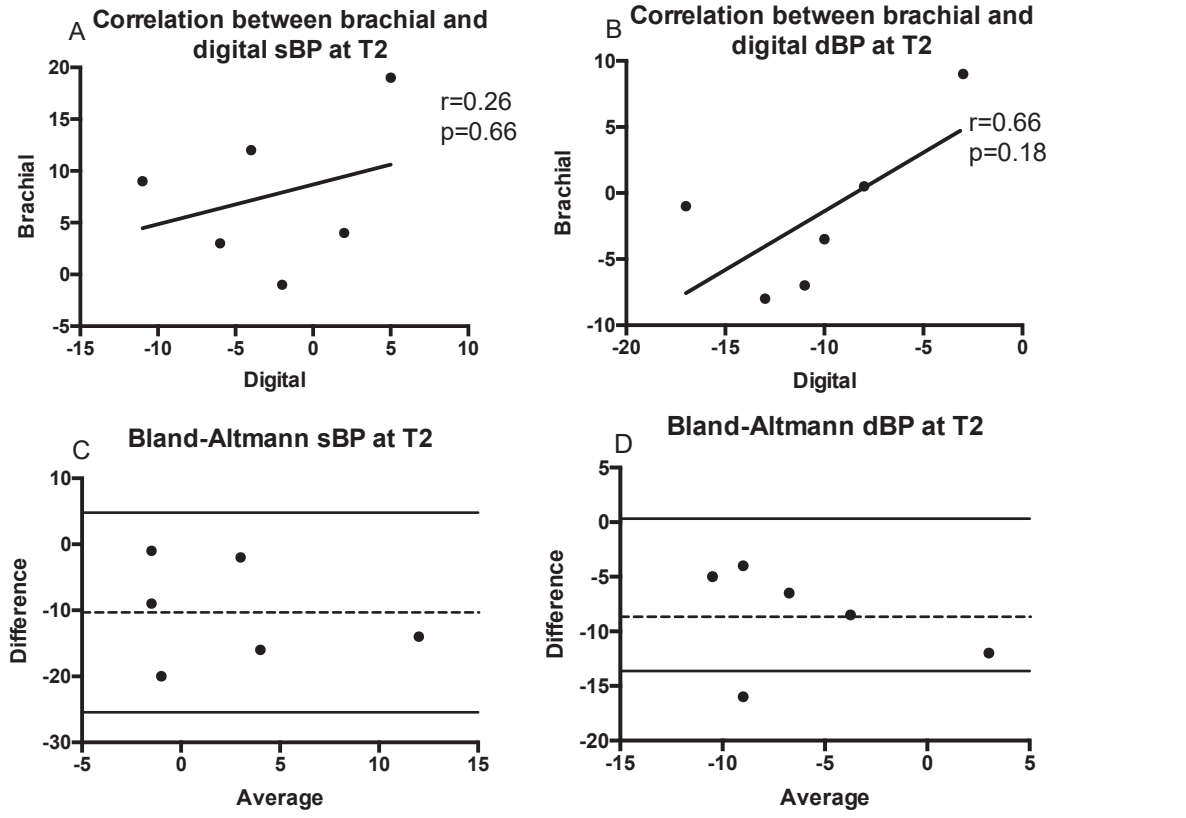
Spearman correlation between brachial and digital sBP (A) and dBP (B) and their corresponding Bland-Altmann plot (C and D).

Figure 14 Agreement between digital and brachial BP at time point 1:



Spearman correlation between brachial and digital sBP (A) and dBP (B) immediately after exercise and their corresponding Bland-Altmann plot (C and D).

Figure 15 Agreement between digital and brachial BP at time point 2:



Spearman correlation between brachial and digital sBP (A) and dBP (B) 10 minutes after exercise and their corresponding Bland-Altmann plot (C and D).

Our pilot data shows that there are significant differences between systolic and diastolic BP at rest (pre-exercise) for brachial and digital measurements. There was no significance difference immediately after exercise for systolic and diastolic BP for both measurements but this difference is significant for sBP within 10-minutes (time point 2) after exercise between digital and brachial measurements. These findings are consistent with data already published for a normotensive population showing a significant difference between oscillometric brachial BP and beat-to-beat digital BP when measuring systolic BP [309, 310].

When looking at the difference between post and pre-exercise for systolic and diastolic measurements using both devices the correlation between them is good at baseline for dBP and at T1 for sBP but this correlation is poor at all other measurements for both sBP and dBP.

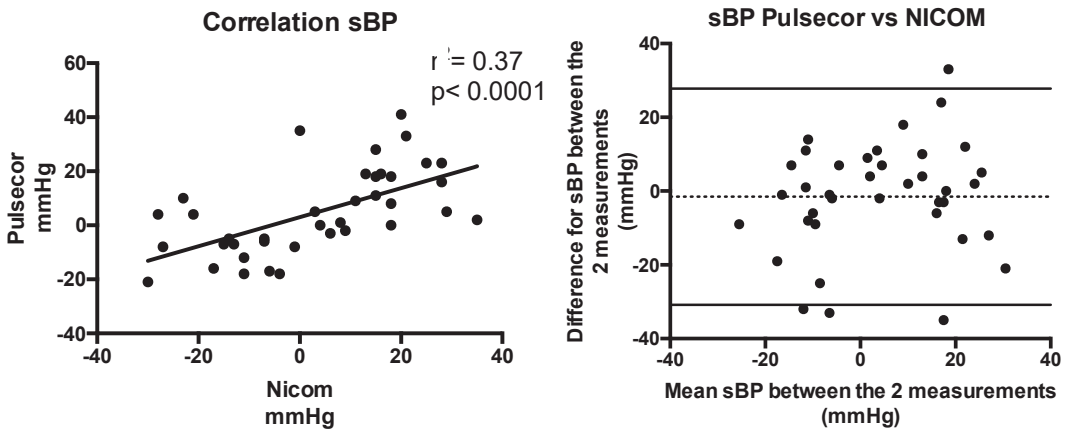
From this pilot study we concluded that beat-to-beat continuous digital BP is not better than intermittent brachial BP, in fact the two methods are poorly correlated, so we elected to use intermittent brachial BP using oscillometry.

2.4.4.2 Level of agreement for peripheral blood pressure:

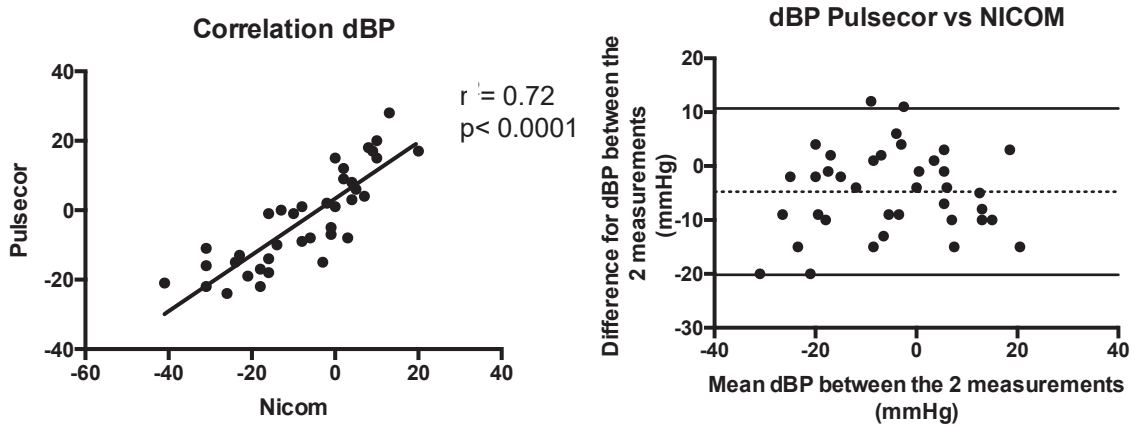
Peripheral BP was measured in our participants using 2 different monitors (NICOM CHEETAH^{TM} and Pulsecor (BP+ Cardioscope I, Pulsecor, Auckland, New Zealand). In order to determine the level of agreement between these two types of measurements for systolic and diastolic BP, I performed Bland Altman plots (Figure 16) showing that there is a moderate level of agreement between these two measurements for systolic BP (r^2=0.37, p<0.0001) but a good correlation for diastolic BP (r^2=0.72, p<0.0001). Ninety-five percentage confidence intervals for the difference between CHEETAH NICOM^{TM} and Pulsecor® for peripheral systolic BP were over 20mmHg.

Figure 16 Agreement of BP measurements between CHEETAH NICOM™ and Pulsecor®:

A.



B.

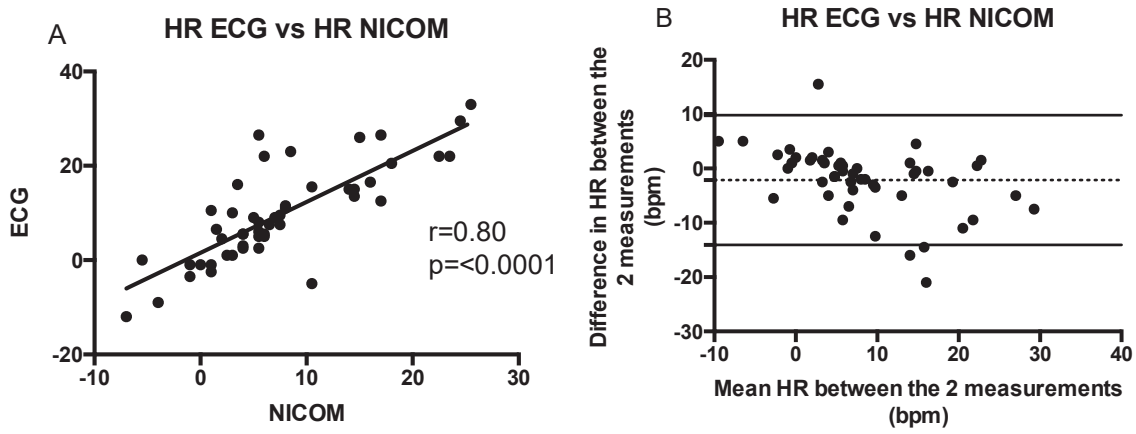


Spearman correlation and Bland-Altman (mean and 95% CI) plots for the difference from baseline for sBP (A) and dBP (B) between NICOM and Pulsecor.

2.4.4.3 Level of agreement for Heart rate:

HR was measured throughout each challenge using 2 different methods, ECG by means of a Holter monitor and bioreactance by means of CHEETAH NICOM™. Analyses of agreement between these 2 methods was performed in order to determine possible discrepancies, results on Figure 17 shows a good correlation between these two methods of measuring HR, but poor level of agreement with the 95% confidence levels for agreement being at least 10 beats per minute different. Due to this poor level of agreement, we decided to use only one method of analyses of HR for all the analyses for consistency and this was the ECG. CHEETAH NICOM™ heart rate measurements are likely to be less accurate than ECG because spike T waves, which can be common in healthy and young individuals, can be labelled as R in the QRS complex by the CHEETAH NICOM™ monitor, therefore giving a less accurate HR measurement. The ECG results have to be manually analysed and therefore only normal QRS complexes are used to determine HR.

Figure 17 Analyses of agreement for HR measurements:

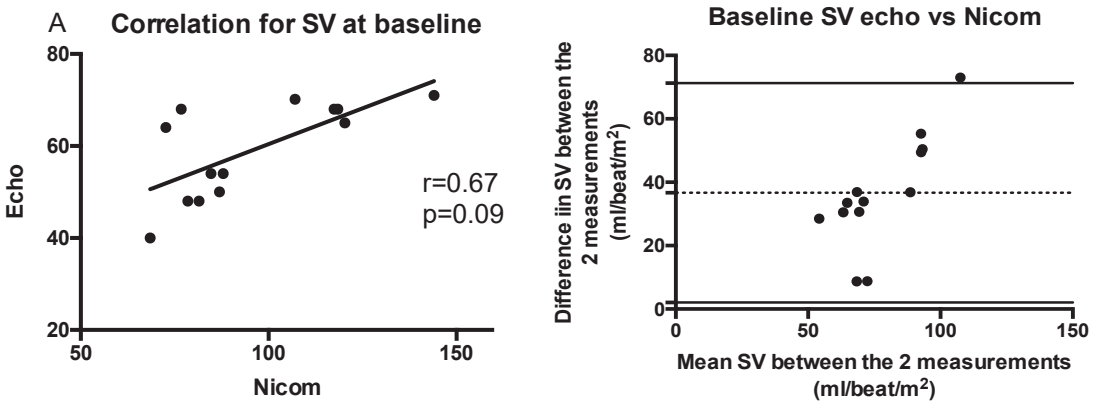


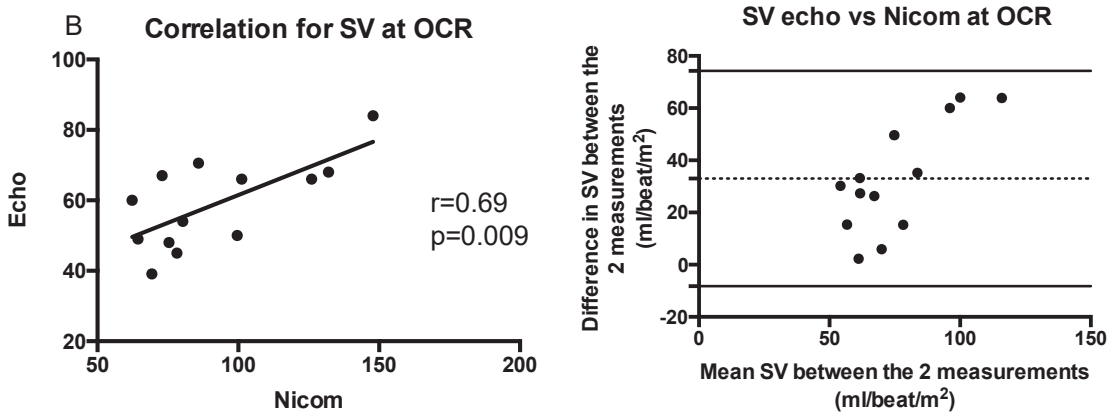
Spearman correlation (A) and Bland-Altman (mean and 95% CI) plot (B) between HR measured by the Holter monitor and NICOM monitor.

2.4.4.4 Level of agreement for Stroke volume:

Echocardiography is a non-invasive method that can measure aortic blood flow and aortic artery diameter and therefore provide measurements of SV. Echocardiography were performed in 13 participants by the same technician and analysed in a blinded way for CHEETAH NICOM^{TM} results. We compared a 10-minute epoch at baseline (T0) and at time of reaching OCR (T1) for NICOM measurements with a single measurement at the same time point for echocardiography. Results in Figure 18 show a moderate correlation between both measurements at these time points, but a poor level of agreement, suggesting that consistent and accurate measures of SV may be hard to achieve.

Figure 18 Agreement for SV between echocardiography and CHEETAH NICOM^{TM}:





Spearman correlation and Bland-Altman (mean and 95%CI) at baseline (A) and OCR (B).

2.5 Assessment of cardiac conductance changes: cardiac rhythm and Heart Rate Variability (HRV):

2.5.1 Settings:

TRACE peanut study participants undergoing baseline DBPCFC were included in this study if they reacted to peanut at challenge and didn't react to their placebo day. Exclusion criteria were non-completion of baseline DBPCFC and inconclusive or negative outcome of DBPCFC. Reactive or non-reactive days were coded as per the modified PRACTALL criteria described in section 2.1.3.3. Measurements of cardiac rhythm and HRV were performed using a 12-lead Holter monitor (GE SEER 12®). This data was analysed using MARS® program for cardiac rhythm changes and with Kubios® automated programme for changes in HRV.

2.5.2 Selection of epochs:

The time epochs used in the analysis of this data are explained in section 2.4.2.

2.5.3 Techniques used for cardiac conductance monitoring:

2.5.3.1 Holter monitor:

A Holter monitor is a non-invasive battery powered portable device that measures and records cardiac rhythm continuously for 24 or 48 hours and provides a 12-lead ECG (Figure 19). This device is used regularly in clinics to detect anomalies in cardiac rhythm such as arrhythmias.

ECG values for heart rate (HR), PR interval, QRS duration, QTc interval and ST elevation (STE) were obtained (Figure 20). Before exporting the data, manual editing of the Holter data was performed to ensure correct identification of QRS complexes, as suggested by the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology[311]. These data are then analysed

using MARS® program for ECG data and Kubios® Program for Heart Rate Variability (HRV). HRV parameters measured were time domain; standard deviation of the NN interval (SDNN), frequency-domain components; (low (LF (n.u.)) and high frequency (HF (n.u.)) normalised units, linearity is a limitation of these time and frequency domain methods, therefore non-linear dynamics are also used; (short-term fractal scaling exponent (DFA-1), approximate entropy (Apen) and sample entropy (Sampen)).

HRV is considered a measure of neuro-cardiac function, representing autonomic nervous system balance[312] and one previous publication suggests that HRV may change during oral food challenge in children[197].

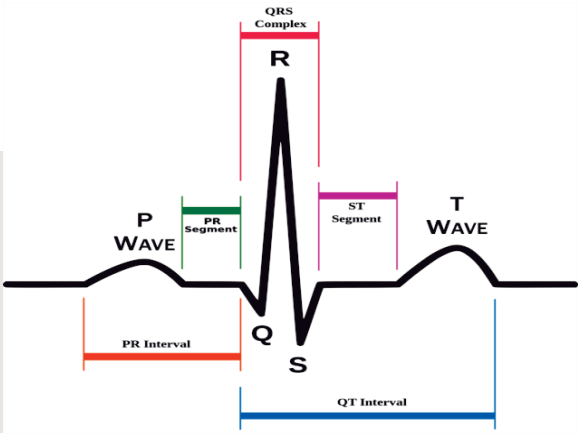
Healthy individuals present beat-to-beat variability and this is a desirable situation. Low frequency component (LF (n.u.)) is a general indicator of aggregate modulation of both the sympathetic and parasympathetic branches whilst high frequency component (HF (n.u.)) is an index of modulation of the parasympathetic branch of the autonomic nervous system (ANS) as it influences the sinoatrial node of the heart. The short-term fractal-scaling exponent (dfa1) represents the beat-to-beat regularity whilst sample entropy (sampen) represents the beat-to-beat irregularity.

TRACE peanut study participants were continuously monitored with the Holter monitor from arrival until discharge. Data from the Holter monitor was analysed as 10 x 1 minute and data for HRV were analysed as 2 x 5-minute epochs within each 10-minute epochs.

This is described in the specific Standard Operation Procedure (SOP) for CVS electrical changes (Appendix 11).

Figure19 Holter monitor:

Figure 20 ECG heart beat:



2.5.3.1.1 Pilot evaluation of methods for PR, QT interval and QRS complex:

Holter monitor data were analysed using MARS programme, which after manually selecting normal QRS complex provides automated measurements for HR, QRS complex, PR, QT and QTc intervals therefore analyses of agreement between the automated and manual measurements were performed for QRS complex, PR and QT intervals, in order to ensure that the automatically calculated measures were valid in this particular clinical setting. Data were analysed for a period of 10x1 minute for a period of 10 minutes for the same participant. Results for QRS complex, PR and QT interval in Table 11 showed that there was a very good level of agreement with coefficient of variation of 3.65%, 2.77% and 1.87% respectively between the manual and the automated measurements.

Table 11 Manual and automated measurements of PR, QRS and QT intervals:

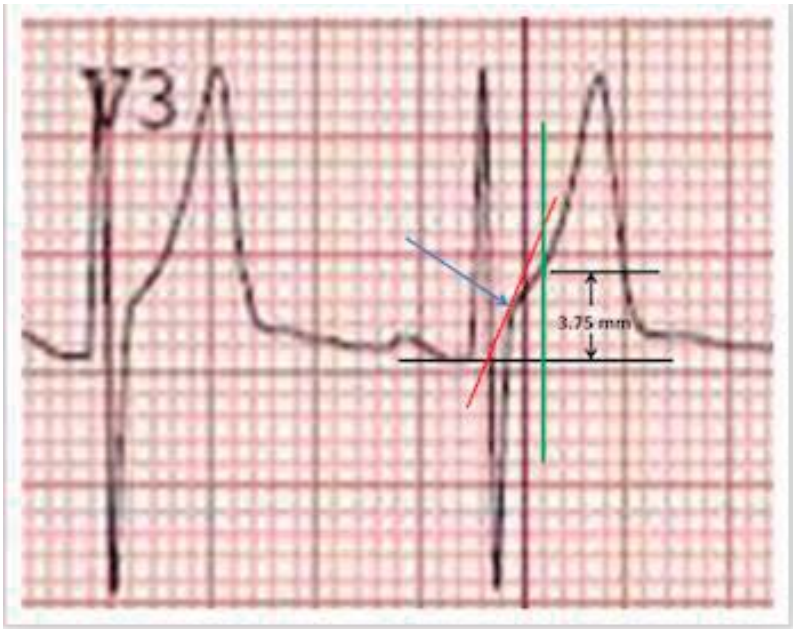
MARS PR	Measured PR	Within-subject variance (s2)	Subject mean(m)	s squared/mean squared (s2m2)	Mean s2m2	SD s2m2
134	138	8	136	0,00043	0,000765	0,000799
140	138	2	139	0,00010		
158	159	0,5	158,5	0,00002		
160	156	8	158	0,00032		
138	144	18	141	0,00091		
156	148	32	152	0,00139		
160	149	60,5	154,5	0,00253		
150	147	4,5	148,5	0,00020		
114	117	4,5	115,5	0,00034		
110	116	18	113	0,00141		
					Coefficient of Variation	2.77%
MARS QRS	**Measured QRS**	**Within-subject variance, s2**	**Subject mean, m**	**s squared/mean squared, s2m2**	**Mean s2m2**	**SD s2m2**
86	88	2	87	0,00026	0,001333	0.001686
80	88	32	84	0,00454		
92	88	8	90	0,00099		
92	91	0,5	91,5	0,00006		
82	89	24,5	85,5	0,00335		
72	78	18	75	0,00320		
70	72	2	71	0,00040		
96	97	0,5	96,5	0,00005		
94	96	2	95	0,00022		
86	88	2	87	0,00026		
					Coefficient of Variation	3.65%
MARS QT	**Measured QT**	**Within-subject variance, s2**	**Subject mean, m**	**s squared/mean squared, s2m2**	**Mean s2m2**	**SD s2m2**
386	386	0	386	0,00000	0,000188	0,000351
382	381	0,5	381,5	0,00000		
380	386	18	383	0,00012		
388	388	0	388	0,00000		
400	397	4,5	398,5	0,00003		
392	391	0,5	391,5	0,00000		
386	388	2	387	0,00001		
366	364	2	365	0,00002		
368	353	112,5	360,5	0,00087		
374	359	112,5	366,5	0,00084		
					Coefficient of Variation	1.87%

Measurements from 1 participant measuring10 x 1-minute epochs for both manual and automated measurements of PR, QT interval and QRS complex.

2.5.3.1.2 Pilot evaluation of methods for ST Elevation (STE) between 3 clinicians:

STE is measured in reference to the J point (the end of the QRS segment and the beginning of the ST segment) but the exact location where this J point is it´s not clearly stated therefore there has been considerable disagreement between clinicians when measuring STE[313, 314] especially in non-specialised clinicians. In general the ST segment is generally measured at 40 or 60 ms after the end of the QRS complex (Figure 21). STE was measured manually, blinded for challenge outcome, at 60ms from the J point (end of the QRS and beginning of the ST segment). Forty ECG´s from the same patient (02047) were measured for STE at V3 lead by 3 different people (1 medical student, 1 consultant Cardiologist and myself) in order to assess level of agreement shown in Table 12. Results showed the 95% limits of agreement for the mean was between -0.901 to 0.785mm with an intra-class correlation coefficient of 0.976 showing an acceptable level of agreement.

Figure 21 J point location:



ECG strip showing 2 QRS complexes from V3 lead to illustrate how ST elevation is measured. Lower black line shows the PR segment. Red line shows tangent to the tracing where the angle on each side of the tangent is equal. Blue arrow shows the J point and the green line represents 60ms after the J point.

Table 12 STE measurements by 3 different readers:

Time points	Minutes	1st reader MEAN	1st reader MEDIAN	2nd reader MEAN	2nd reader MEDIAN	3rd reader MEAN	3rd reader MEDIAN
02047-5 T0	1	3,44	3,00	3,17	3,00	3,10	3,00
	2	3,75	4,00	3,83	4,00	2,83	3,00
	3	3,78	4,00	3,57	3,50	3,15	3,00
	4	3,90	4,00	3,90	4,00	3,10	3,00
	5	4,75	5,00	4,50	4,50	3,44	3,50
	6	3,50	3,00	4,00	4,00	3,61	3,50
	7	3,10	3,00	3,30	3,00	3,00	3,00
	8	4,67	5,00	4,75	4,75	3,60	3,50
	9	4,00	4,00	3,83	4,00	3,67	3,50
	10	3,70	4,00	4,17	4,00	3,65	4,00
02047-5 T1	1	3,00	3,00	3,25	3,25	2,90	3,00
	2	2,86	3,00	3,00	3,00	2,60	3,00
	3	2,86	3,00	2,63	2,75	2,70	3,00
	4	3,57	4,00	4,00	4,00	3,10	3,50
	5	4,14	4,00	3,50	3,50	3,50	4,00
	6	3,67	4,00	3,67	3,50	3,12	3,00
	7	2,86	3,00	3,30	3,00	3,00	3,00
	8	3,29	3,00	3,16	3,00	2,84	3,00
	9	3,71	4,00	3,75	3,75	3,23	3,50
	10	3,50	3,50	2,83	3,00	3,10	3,00
02047-13 T0	1	5,00	5,00	5,33	5,00	6,00	6,00
	2	5,00	5,00	5,33	5,50	6,30	6,00
	3	6,00	6,00	6,00	6,67	7,42	7,00
	4	5,75	6,00	7,00	6,50	6,33	6,00
	5	6,78	7,00	6,00	7,17	6,10	6,00
	6	7,67	8,00	8,00	9,00	7,32	7,00
	7	7,78	8,00	8,30	8,00	7,01	7,00
	8	7,63	8,00	8,00	8,00	6,86	7,00
	9	7,67	8,00	8,00	8,00	6,75	7,00
	10	7,63	8,00	7,50	8,00	6,00	6,00
02047-13 T1	1	NM	NM	NM	NM	NM	NM
	2	8,00	8,00	7,50	8,00	7,16	7,00
	3	7,50	7,50	7,50	8,00	7,75	8,00
	4	7,50	7,50	8,00	8,00	7,21	7,00
	5	7,75	8,00	8,00	8,00	7,20	7,00
	6	NM	NM	NM	NM	NM	NM
	7	8,17	8,00	7,67	8,00	6,42	7,00
	8	7,13	7,00	7,67	8,00	6,74	7,00
	9	7,90	8,00	8,00	8,00	6,80	7,00
	10	8,00	8,00	7,17	8,00	6.91	7,00

Measurements for ST segment for 1 participant measuring10 x 1-minute epochs at baseline and OCR on both challenge days. NM= not measured.

2.6. Skin prick test (SPT):

2.6.1 Settings:

TRACE peanut study participants undergoing baseline DBPCFC were included in this study if they reacted to peanut at challenge and didn't react to their placebo day. Exclusion criteria were non-completion of baseline DBPCFC and inconclusive or negative outcome of DBPCFC. Reactive or non-reactive days were coded as per the modified PRACTALL criteria described in section 2.1.3.3. Titrated SPT was performed following the European guidelines[91].

2.6.2 Technique:

Using ALK lancets (ALK-Abelló, Hørsholm, Denmark), SPT was performed on the volar side of the left forearm of the patient. Duplicated, titrated SPT (1, 1:10, 1:100, 1:1000, $1:10^4$, $1:10^5$) using commercial peanut extract (Stallergenes, London, United Kingdom) and singlicate titrated SPT to 1% histamine (1, 1:10, 1:100, 1:1000) was performed on both challenge days. To reduce variability, SPT was performed and read by the same clinician. The wheal size is measured after 20 minutes for peanut extract and after 10 minutes for histamine, then the wheal to undiluted peanut extract is measured every 30 minutes, until the wheal had disappeared or the patient was discharged. SPT results were recorded by drawing around the circumference of the wheal and transferring to paper with cellophane tape. This is described in the Standard Operating Procedure (SOP) for SPT (Appendix 12).

2.6.2.1 SPT measurements:

Mean diameters of wheals at each concentration and time point (average of longest length and perpendicular width) were measured.

The mean wheal elicited by SPT using undiluted peanut extract at 20 minutes was called PMAX, and a formula was used to calculate the dilution of peanut extract required to elicit a 3 mm or 6mm wheal (PC3, PC6).

The formula used was:

R= defined wheal diameter we are investigating (3mm or 6mm).

C1= concentration that induced a wheal diameter closest to and less than R.

W1= size of wheal at C1.

C2= concentration that induced a wheal diameter closest to and greater than R.

W2= size of wheal at C2.

$$PC = C1 + (R - W1) \times \frac{C2 - C1}{W2 - W1}$$

A similar formula was used to determine the time taken for PMAX to diminish to a 6mm and 3mm wheal (PT3, PT6):

R= defined wheal diameter we are investigating (3 or 6).

T1= time when wheal diameter had shrunk to just below R.

W1= size of wheal at T1.

T2= time when wheal diameter had shrunk to just above R.

W2= size of wheal at T2.

$$PT = T1 + (R - W1) \times \frac{T2 - T1}{W2 - W1}$$

2.6.2.2 Statistical analysis:

To assess repeatability of skin reactivity values, SPSS version 23 (IBM, New York, United States) was used to calculate coefficients of variation (CV).

To determine correlation between the SPT measures (PC3, PC6, PT3, PT6, PMAX) and threshold or severity Spearman´s Rank correlation coefficient was used for unadjusted correlations, and linear regression for adjusted analyses. Multivariate analyses for predictive models of threshold and severity were performed using binary regression for binary variables and linear regression for continuous or ordinal variables.

2.6.3 Pilot evaluation of SPT methodology:

Codeine has been used previously as a positive control for SPT[315] as it is a mast cell degranulating agent, but is not commonly used due to cost and a lack of data demonstrating any benefit over using histamine as a positive control[315]. We sought to determine if codeine was of any benefit compared to histamine as a positive control for titrated SPT in our study population. Results for this show that only 4/27 participants reacted to 1:10 or further titration of codeine therefore we didn´t include codeine results in the final analysis as most participants were non-reactive to 1/10 dilutions of 9% codeine phosphate (Stallergenes, London, United Kingdom).

2.6.4 Evaluation of covariates which might impact on threshold or severity of reaction:

Different covariates were measured during the study and were used to assess if titrated SPT and covariates such as age, sex, peanut-specific IgE, bronchial hyperresponsiveness (BHR), cumulative dose of peanut during DBPCFC, percentage peak of mast cell tryptase, release of endogenous adrenaline, participant´s and investigator´s VAS score can help predict threshold and severity of reaction.

BHR (SOP is shown in Appendix 8) was performed with histamine, those participants with a PC20 less than 0.1mg/ml were excluded, as their lung hyperresponsiveness was high and therefore not safe to perform the challenges. PC_{20} was calculated using the following formula:

$$PC_{20} = C1 + (20 - R1) \times \frac{C2 - C1}{R2 - R1}$$

C1: concentration of histamine that induced a % fall in FEV1 closest to and less than 20%.
C2: concentration of histamine that induced a % fall in FEV1 bigger than or equal to 20%.
R1: % fall in FEV1 closest to and less than 20%.
R2: % fall in FEV1 bigger than or equal to 20%.

2.7 Effects of intramuscrular adrenaline on cardiovascular physiology and electrical conductance:

IM adrenaline is the treatment of choice for anaphylaxis, but little data exist on the cardiovascular effects of IM adrenaline during anaphylaxis, as such episodes typically occur outside a medical facility.

2.7.1 Settings:

TRACE peanut study participants undergoing baseline DBPCFC were included in this study if they reacted to peanut at challenge, didn't react to their placebo day and had anaphylaxis on the reactive day requiring use of IM adrenaline.
Reactive or non-reactive days were coded as per the modified PRACTALL criteria described in section 2.1.3.3.
Participants are continuously monitored for cardiac rhythm, HRV and cardiovascular physiology and the different parameters analysed for effects of IM adrenaline are the same as described in sections 2.4.3.1, 2.5.3.1 and for SPT we looked at PMAX, PT3 and PT6 as possible predictors of severe reactions.

2.7.2 Selection of epochs and control data:

To assess the effects of adrenaline in those participants who had anaphylaxis (based on UK and Australian guidelines) a 10-minute epoch at baseline was compared to a 10-minute epoch before adrenaline was given and this latter was compared to a 10-minute epoch immediately after adrenaline was given (figure 3). There were limited data for effects of IM adrenaline, since relatively small numbers of participants were treated in this way and evaluation of IM adrenaline effects was not a key outcome of the TRACE peanut study. I

evaluated the effects of IM adrenaline on cardiovascular and respiratory parameters by comparing 3 groups of participants. First those participants who required adrenaline on one challenge, but not on another one were assessed, to see whether changes during the challenge they received IM adrenaline differed from challenge reactions with no IM adrenaline. Second all reactions requiring IM adrenaline at baseline DBPCFC were compared with all reactions across the total participant population which did not require IM adrenaline. Third, a simple pre/post analysis of participants who received IM adrenaline at any TRACE study OFC (excluding exercise intervention challenges), using the 10-minute epoch before adrenaline was given, and the 10-minute epoch after administration.

2.8 Assessment of threshold and severity of reaction:

To date there is no validated classification of food allergic reactions. Those classifications previously published[287, 288] have been generated to fit a known population and cannot be generally extrapolated to other population studies. Guidelines also differ in the criteria to define anaphylaxis and the criteria necessary to establish its diagnosis it´s not globally uniform.

For this study, anaphylaxis was defined using the NIAID definition of anaphylaxis[286] as this is the most common used criteria in other studies to define anaphylaxis. The reactions experienced by the participants were classified according to; 1 Ewan & Clark[287] as this classification was performed specifically for food allergic reactions and 2 World Allergy Organization subcutaneous immunotherapy systemic reaction grading system[316] as a different way of classifying systemic allergic reactions as it has been extensively used in clinics to asses severity of reactions[128]. We also analysed the used of IM adrenaline as a measure of reaction severity.

For threshold determinations we used cumulative dose as the measurement of the amount of peanut ingested.

2.9. Approach to statistical analyses:

2.9.1 Sample size calculation:

Based on preliminary data of n=10 participants undergoing DBPCFC we have, with an anticipated sample size of n=50, an 80% power at a 2-sided alpha of 0.05 to detect a minimum change for CVS physiology parameters of 1l/min for CO, 10bpm for HR, 10ml for SV, 10mmHg for systolic and diastolic BP, 10% change in peripheral blood flow and for electrical changes of a prolongation of 10ms in QTc and PR intervals and 5ms for QRS complex.

2.9.2 Power calculation for SPT:

With an anticipated 50 participants with peanut allergy undergoing DBPCFC we have 80% power at a 2-sided alpha of 0.05, to detect a difference between high and low threshold reactors (ie greater or less than the median value of cumulative dose of peanut protein of 330mg) of 0.78 in log10 PC6 (mean logPC6 is 0.91).

Based on preliminary analysis of n=27 PT3 readings we have 80% power at 2-sided alpha 0.05 to detect a difference between adrenaline needed (estimated 25%) and not needed of 0.19 in Log10 PT3 (mean log10PT3 is 2.19).

2.9.3 Data analysis:

Statistical analysis methods varied for different outcomes and are described in detail in the relevant sections of the results chapters.

Before analysing the data, the type of distribution was explored using PRISM® in order to determine if it was normal or not normally distributed and examine the presence of outliers.

For results chapters 3 and 4 mostly are paired data as we are comparing the same participant on the 2 different challenge days, according to the data distribution paired test was used for analysis of parametric data and for non-parametric Wilcoxon matched-pairs signed rank test was used for analysis.

For results chapter 5 SPT data is mostly unpaired data, according to the data distribution unpaired t-test was used for parametric data and Mann-Whitney test was used for non-parametric data analysis. Mann-Whitney test was used to assess significance between adrenaline use and threshold dose and adrenaline use and severity was analysed using Fisher test. To assess repeatability, the SAS procedure VARCOMP was used to calculate the intraclass correlation coefficient (ICC) and SPSS was used to analyse coefficient of variation.

For analysis of pilot data, level of agreement and correlation between 2 methods or parameters we used Bland-Altmann plots and correlation coefficient, Pearson correlation coefficient was used for parametric data and Spearman correlation was used to analyse non-parametric data.

Data was explored using SPSS and PRISM in order to determine if it followed normal distribution or was otherwise skewed and if there were any possible outliers by creating histogram and boxplot with the data. No correction was done for multiple comparisons following external statistical advice, as we were not comparing the parameters between them but at different time points.

2.10 Data management:

2.10.1 Data acquisition:

All the data for the study is stored in an Excel database in anonymised form, stored and backed up in a data secure environment accessible only to clinical researchers in paediatric allergy, within Imperial College Healthcare NHS Trust.

Data for SPT and cardiac rhythm (HR, PR, QTc and QRS) are acquired manually and transferred to the excel database while data from HRV, CVS physiology changes and peripheral blood flow are automatically transferred to the excel database once the time epochs have been assigned. Prior to sending the data to the study statistician the analysis of the SPT and CVS data was blinded to placebo or active day, reactive/non-reactive outcome and adrenaline/non-adrenaline use for each participant.

Analysis of the data was done using SPSS® (IBM, New York, United States) and PRISM® (GraphPad, La Jolla, California, USA) and graphs are designed using PRISM® (GraphPad, La Jolla, California, USA). DBPCFC were unblinded to the study statistician (DB) after agreement of the statistical analysis plan, and database lock, in order to remove any placebo reactors from the dataset prior to running analyses.

Storage of data was performed following the research ethics; participant data is anonymous and only recognizable by the participants ID number.

3. CARDIOVASCULAR PHYSIOLOGY CHANGES DURING IGE-MEDIATED PEANUT ALLERGIC REACTIONS:

ABSTRACT:

Data from fatal food anaphylaxis describes cardiac arrest in patients who have suffered from anaphylaxis of any cause when these are moved to a more upright position. This phenomenon suggests that there is reduced preload causing what it´s known as the concept of "empty heart". With this rationale we decided to study de cardiovascular changes during food allergic reactions.

Fifty-seven peanut allergic participants undergoing peanut oral challenges were continuously monitored using a non-invasive bioreactance technique. The parameters analysed were stroke volume (SV), blood pressure (BP) including systolic blood pressure (sBP), diastolic blood pressure (dBP), and mean arterial pressure (MAP). Measurements for cardiac output (CO) and total peripheral resistance (TPR) were derived. Peripheral blood flow was also measured.

Results for 52 participants showed a significant reduced SV, increased in peripheral blood flow and increased HR at time of objective clinical reaction and on active days compared to placebo days. These changes are accompanied by an increase in blood pressure and CO. No significant differences were seen depending on reaction severity and no, other than an increase in HR, changes were observed in the other cardiovascular parameters after the use of IM adrenaline. Similar cardiovascular changes were observed on repeated peanut challenges performed on a smaller sample of the same group of participants.

These results suggest fluid extravasation probably both on the skin and GI system, generating a possible "third space". We believe these findings have an impact on the management of food anaphylaxis as a greater importance and earlier fluid therapy must be considered.

3.1 Introduction:

Food allergy and hospitalisations for food anaphylaxis have increased in recent decades [36, 42]. Most people who experience severe food anaphylaxis have respiratory symptoms, with cardiovascular events thought to be secondary to respiratory involvement. Results from the Network of severe Allergic ReActions (NORA) study reported that 77% of patients with food anaphylaxis had respiratory involvement, most commonly dyspnoea, compared to 60% of participants with insect anaphylaxis and 62% of patients with drug anaphylaxis. Cardiovascular involvement (most commonly dizziness and hypotension/collapse) was present in 45% of patients with food anaphylaxis, compared to 75% for insect anaphylaxis and 67% for drug anaphylaxis [317]. Oral food challenges, conducted under medical supervision, are common practice in specialist allergy centres, yet very few cardiovascular monitoring data have been reported from such subjects. Hypotension is one of the most common cardiovascular system findings in severe anaphylaxis[317]. A recent study reported prevalence for hypertension in all cases of anaphylaxis of 13%, although of this only 3% were due to food [318].

The majority of the literature regarding the role of the cardiovascular system (CVS) during acute allergic reactions is derived from animal models, and suggests that CVS involvement is responsible for many of the symptoms and signs present in an allergic reaction, such as angioedema, tachycardia and hypotension[319, 320]. A study conducted in rabbits concluded that anaphylaxis has direct actions on the pulmonary vascular bed, the distal airways and alveoli. However, changes in breathing, blood pressure and large airway calibre were mainly dependent on vagal reflex activity[139]. Findings from fatal food anaphylaxis series imply that cardiovascular physiology compromise may be important in some cases of fatal anaphylaxis: postural changes (to an upright position) appear to trigger cardiac arrest and sudden death in some individuals [89, 164]. However, it is not clear whether postural collapse in food anaphylaxis reflects a primary role for cardiovascular changes, or is secondary to respiratory failure. The cardiovascular physiology events, which occur during human food allergic reactions, remain understudied.

Circulating blood volume has been described to decrease up to 35% within 10 minutes in perioperative anaphylaxis, mainly due to plasma extravasation [189]. Severe vasodilation resistant to adrenaline, responding only to potent vasoconstrictors, has also been described [190]. We are not aware of any previous data describing a rigorous assessment of cardiovascular physiology changes during human food allergic reactions.

Intramuscular (IM) adrenaline (epinephrine) is the treatment of choice for anaphylaxis, but few data exist on the cardiovascular effects of IM adrenaline during anaphylaxis in humans,

as such episodes typically occur outside a medical facility. Some investigators have reported poor cardiovascular and clinical responses to bolus of intramuscular (IM) or intravenous (IV) adrenaline [201, 321].

We have chosen peanut allergy to study, as this is the most common persistent food allergy and the food that has been most commonly implicated in fatal food anaphylaxis[34, 51]. We studied peanut allergy in adults and young adults, first because detailed evaluations are more easily undertaken in this age group, and second because young adults appear to be more susceptible to anaphylaxis and fatal anaphylaxis than other ages[42]. We include assessment of respiratory physiological changes (forced expiratory volume in 1 second, FEV1; and peak expiratory flow rate, PEFR) in order to confirm that these changes are a prominent feature of food allergic reactions, to confirm that our physiological assessments are able to reliably detect changes during food allergic reactions, and to evaluate whether CVS changes co-exist with respiratory changes during food allergic reactions, or are independent.

The aims of this chapter are to describe:

1. Cardiovascular physiology changes during food allergic reactions in humans.
2. Whether any changes are more prominent in more severe reactions.
3. The effects of IM adrenaline on cardiovascular physiology parameters, when administered to treat food allergic reactions.

3.2 Methods:

3.2.1 Study protocol:

All participants randomised in the TRACE Peanut Study at Imperial College London were studied. The primary objectives of TRACE were to quantify the variation in peanut threshold over time in young adults with primary peanut allergy, and to assess the influence of exercise and sleep deprivation on such variation (www.tracestudy.com).

Participants were age 18-45 years, with a clinical history and laboratory tests consistent with primary peanut allergy, and no known cardiovascular abnormality. Full inclusion and exclusion criteria for TRACE peanut study are described in Methods section 2.1.2. None of the potential participants screened for eligibility to enrol in TRACE peanut study were excluded due to cardiovascular abnormalities.

3.2.2 Study procedures:

All participants underwent a double-blind placebo controlled food challenge (DBPCFC) to peanut, challenge procedures were derived from those used in EuroPrevall, incremental semi-log doses were administered orally using a validated blinding recipe and 8-dose schedule at 30-minute intervals.

Participants were continuously monitored using a non-invasive bioreactance technique (CHEETAH NICOM^{TM}, Cheetah medical, Boston, Massachusetts), which is approved by the United States Food and Drug Administration (FDA) and validated against pulmonary artery catheter for the measurement of cardiac output [302] (see methods section 2.4.3.1). The parameter analysed using bioreactance was stroke volume (SV), blood pressure (BP) including systolic blood pressure (sBP), diastolic blood pressure (dBP), and mean arterial pressure (MAP) were measured by the same monitor using oscillometry. Measurements for cardiac output (CO) and total peripheral resistance (TPR) were derived. All these parameters were indexed for body surface area (BSA). Central blood pressure (CBP) was measured using non-invasive brachial measurement (BP+ Cardioscope I, Pulsecor, Auckland, New Zealand). Heart rate was measured using a 12-lead electrocardiogram (ECG), peripheral blood flow was measured using a laser Doppler (moorVMS-LDF laser Doppler, Moor Instruments, Axminster, England).

Lung function (% of predicted FEV1 and PEFR) was measured using spirometry (Micro I, BD, New Jersey, USA) at baseline, before each dose given, at time of objective clinical reaction (OCR) and at any time during and after the challenge if required. OCR has been described in the Methods chapter section 3.3.2 and is the time at which the supervising clinician stopped the food challenge and administered treatment. Serum samples were

obtained at baseline, OCR and at 30 and 120 minutes post-OCR, for mast cell tryptase (MCT) determination. Catecholamines were measured at baseline; time of OCR and 120 minutes post-OCR. Participants were asked to void their bladder and then provide a urine sample for measurement of albumin/creatinine ratio at baseline and 2hr after OCR.

3.2.3 Selection of data for analysis:

The time points used for CVS analyses, shown in Figure 22, were 10-minute epochs prior to OCR (to ensure that measurements were taken before any medication was administered), at the onset of subjective symptoms, at the onset of objective symptoms; and at baseline prior to administration of the first dose of peanut (or placebo). To determine when any changes occurred at all during allergic reactions, 10-minute epochs at baseline and OCR were compared. Data for the 4 hours prior to OCR were analysed to understand the timing of any changes seen.

Data analyses for peripheral blood flow measured the difference between the maximum % blood flow between the onset of objective symptoms and OCR, and the lowest % blood flow before OCR, all expressed as a percentage of maximal peripheral flow during pre-challenge heating (this is shown in Figure 23).

Changes in the CVS could, to some extent, be confounded by an "order effect". That is, participants who underwent active challenge on the first challenge day may be less anxious knowing that the second challenge is likely to be a placebo, and this may influence their CVS responses on the placebo day. We therefore categorised participants according to the order of their DBPCFC, and explored the data for order effects.

3.2.4 Analysis of reaction severity:

We evaluated whether there was a relationship between the magnitude of any CVS changes seen and severity of allergic reaction, using several different measures of reaction severity, since there is no agreed and validated tool for measuring severity of food allergic reactions. Clinical measures include the requirement for IM adrenaline, definition of anaphylaxis using the National Institute of Allergy and Infectious Disease (NIAID) classification (Appendix 13), which defines a severe reaction (anaphylaxis) as the presence of respiratory and/or severe gastrointestinal symptoms. For the TRACE study population we classified severe gastrointestinal symptoms as persistent GI symptoms for at least 30 minutes and more than one episode of emesis/diarrhoea at least 20 minutes apart. Other measures of reaction severity were Ewan and Clark symptom severity score (Appendix 14), where reactions were classified as mild (1-3) or moderate/severe (4-5); World Allergy Organization Subcutaneous Immunotherapy Systemic Reaction Grading System (Appendix 15), where reactions were

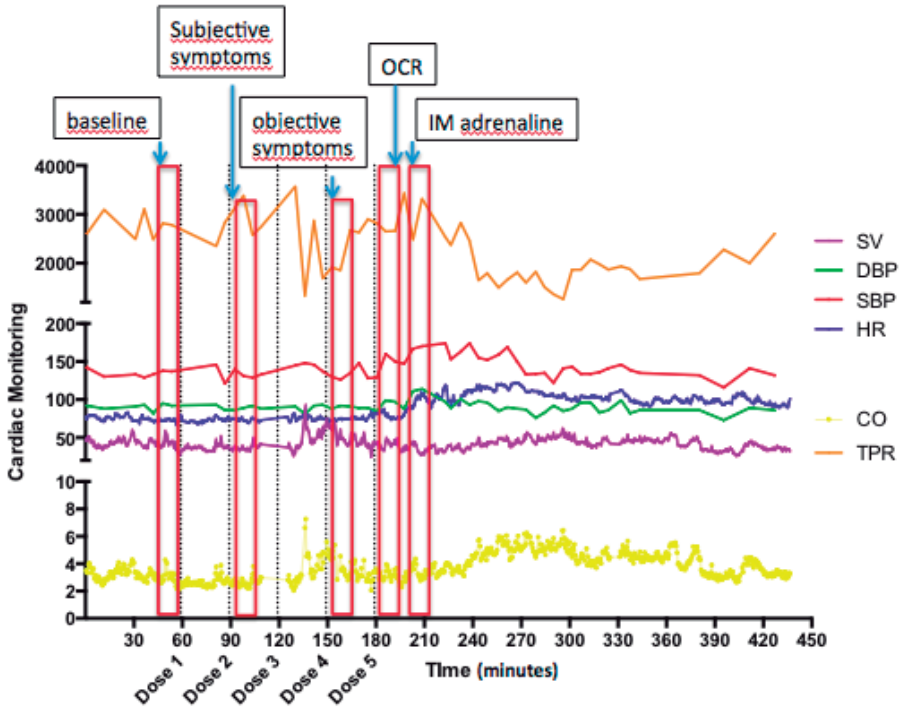
classified as mild (1-2) or moderate/severe (3-5); and visual analogue scales of reaction severity recorded by participant and investigator separately on the day of challenge (Appendix 16).

Biochemical measures of reaction severity were changes in mast cell tryptase (MCT) level (% change and absolute value), changes in serum catecholamines (adrenaline and noradrenaline) and changes in urine metabolic parameters (creatinine, microalbumin and albumin/creatinine ratio). Changes in CVS parameters were correlated with both clinical and biochemical measures of reaction severity.

3.2.5 Analysis of the physiological effects of intramuscular adrenaline:

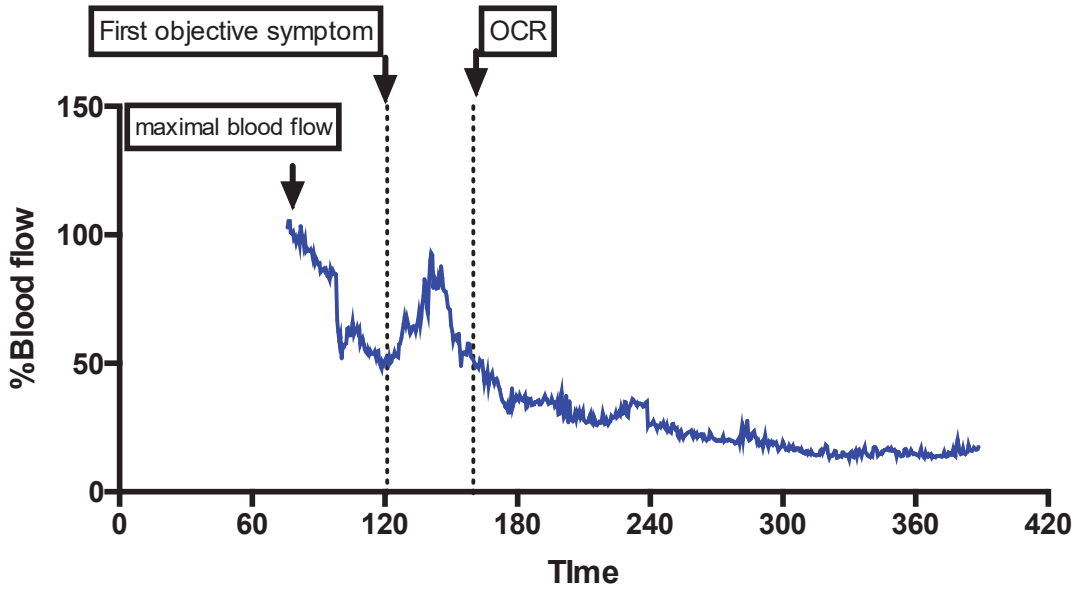
The effects of IM adrenaline on the CVS and on lung function (% of predicted and absolute volume of PEFR and FEV1) were also explored. The 10-minute epoch immediately after IM adrenaline was administered, was compared with two other 10-minute epochs, firstly, the time immediately before IM adrenaline was administered, in the same participant on the same challenge day and second, the time immediately after OCR for the same participant on a different day, for an allergic reaction where adrenaline was not required. Exercise challenges were excluded from all these analyses, in order to avoid confounding by the effects of exercise on the CVS.

Figure 22 Continuous cardiovascular monitoring of patient 02012:



Continuous monitoring of stroke volume (SV), systolic and diastolic blood pressure (sBP, dBP), heart rate (HR) and derived measurements of cardiac output (CO) and total peripheral resistance (TPR) during a peanut challenge. Dose 1, 2, 3,4 and 5 shows when the corresponding doses were given to the participant. Red bars show the 10-minute epochs used for analyses at the different time points labelled on the graph. OCR: objective clinical reaction.

Figure 23 Continuous measurement of peripheral blood flow of patient 02048:



Continuous peripheral blood flow represented as a % blood flow of maximum blood flow reached after 30 minutes of heating at 44ºC.

3.2.6. Statistical analysis:

Median values for each minute of every 10-minute epoch were used, for all analyses. For paired, parametric data, paired t test was used; for paired, non-parametric data Wilcoxon test was performed. For unpaired, non-parametric data, Mann-Whitney U test was performed. For correlation coefficients on non-parametric data, Spearman's rank correlation test was performed and Pearson correlation coefficient for parametric data.

P values of <0.05 were considered statistically significant, and formal correction for multiple comparisons was not undertaken. However, the number of separate analyses undertaken, and consistency of findings across different related analyses, was taken into consideration in interpreting findings.

3.3 Results:

3.3.1. Study population:

Complete data on both baseline challenge days for 52 out of a total of 57 participants were obtained, whose characteristics are described in Table 13. Reasons for missing data were technical failure by the monitoring equipment (n=4) or incorrect download by investigator (n=1) on one of the two challenge days. Twenty-eight participants underwent a further open non-intervention (NI) challenge at which similar data was acquired, data for 26 participants was analysed, reason for missing data was technical failure by the monitoring equipment (n=2).

Table 13 Characteristics of the study population:

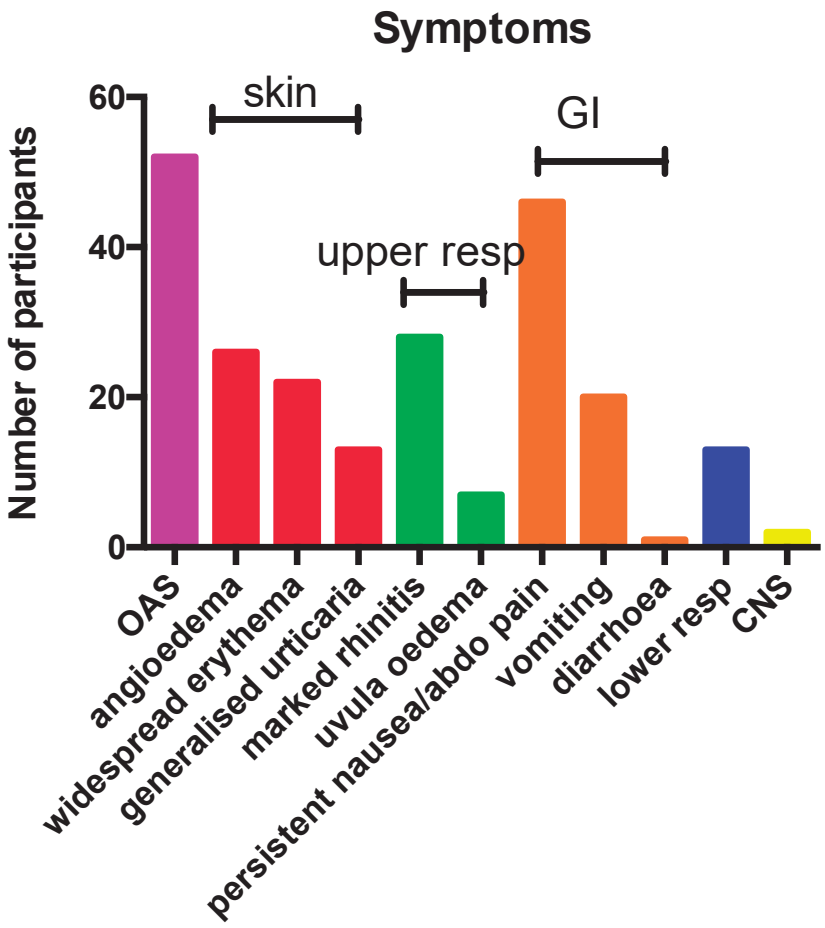
	Baseline challenge	**Repeated challenge (NI)**
Age at enrolment	24 (20, 29) years	25 (22, 29) years
Age at time of diagnosis	2 (1, 6) years	2 (1, 4) years
Sex (female)	30 (58%)	17 (65%)
Asthma	29 (56%)	14 (54%)
Rhinitis	40 (77%)	20 (77%)
Eczema	27 (52%)	14 (54%)
Total IgE	221 KU/L (107, 576)	254 KU/L (123, 617)
Specific IgE to peanuts	10 KUA/L (3.3, 31.9)	15 KUA/L (3.4, 34.5)
Specific IgE to Ara h 2	7 KUA/L (2.0, 20.4)	12 KUA/L (2.1, 23)
Wheal size SPT to peanut extract, Stallergenes®	11mm (9, 15)	11mm (9, 13)
Sensitised to other nuts	33 (63%)	17 (65%)
Sensitised to other non-nut foods	29 (56%)	15 (58%)
Cumulative dose of peanut protein ingested.	133mg (33, 433)	133mg (33, 133)
Baseline HR	69bpm (63, 76)	74bpm (68, 82)
Baseline SV	55ml/beat/m^2 (46, 60)	49ml/beat/m^2 (43, 58)
Baseline SBP	109mmHg (104, 119)	110mmHg (104, 120)
Baseline DBP	71mmHg (67, 78)	76mmHg (69, 80)
IM adrenaline during peanut challenge	11 (21%)	3 (12%)
NIAID (anaphylaxis)	13 (23%)	6 (23%)
Ewan&Clark (moderate/severe)	21 (40%)	9 (35%)
WAO (moderate/severe)	12 (25%)	4 (15%)
Mast Cell Tryptase (% peak change)	26% (7.25, 46.75)	N/A
Adrenaline (change from baseline)	0.39ug (-0.06, 1)	N/A
Noradrenaline (change from baseline)	0.01ug (-0.08, 0.11)	N/A

All data is shown as median and IQR unless specified otherwise. N/A: Data not available.

3.3.2. Summary of symptoms during peanut allergic reaction:

A modified PRACTALL consensus (shown in Methods section 2.1.3.3) was followed in order for all challenges to have the same criteria when reaching the objective clinical reaction (OCR). The symptoms experienced by the participants at their baseline challenge are summarised in Figure 24, and self-reported symptom severity is summarised in Figure 25. All participants had oral allergy symptoms followed by gastrointestinal symptoms; persistent nausea and stomach pain being the most frequent and almost 50% of participants experienced upper respiratory symptoms being marked rhinitis the most common. Symptom severity was rated by participants as most severe for throat and abdominal symptoms, although severe symptoms were reported in every domain by at least one participant (Figure 25). Eleven participants had lower respiratory symptoms at their baseline challenge and required intramuscular (IM) adrenaline as rescue medication. Two participants showed symptoms of altered level of consciousness at their baseline challenge, lasting for less than 2 minutes with no recurrence or progression to other organs.

Figure 24 Symptoms during DBPCFC to peanut for the 52 participants included in the analyses of this chapter:



Summary of the most common symptoms participants experienced during an allergic reaction separated by organ.

Figure 25 Symptom severity for the 52 participants included in the analyses of this chapter:

	Skin	Upper resp	Lower resp	GI	Anxiety	Overall
02003	8	6	0	0	4	4
02004	0	7	2	7	2	6
02008	1	2	0	1	1	5
02010	6	6	4	9	8	6
02012	7	9	2	4	8	7
02014	0	0	0	3	10	9
02016	7	6	5	7	3	5
02018	4	4	1	8	3	5
02019	8	6	6	8	7	7
02023	1	8	3	8	1	6
02024	0	6	1	5	5	5
02025	1	4	3	7	4	4
02034	3	8	7	4	4	7
02037	5	6	8	7	0	7
02040	1	6	1	7	6	7
02045	6	8	8	7	8	8
02047	0	6	6	8	6	8
02048	8	5	3	8	5	5
02049	6	7	0	3	5	4
02051	4	6	2	7	2	4
02053	2	1	0	4	5	4
02055	6	3	0	6	1	4
02056	7	5	2	8	5	6
02063	5	4	0	0	1	4
02064	3	8	1	3	0	4
02066	7	6	2	8	6	7
02073	1	3	0	6	3	3
02074	7	7	0	8	3	7
02075	0	5	0	8	9	3
02078	5	4	5	6	3	5
02079	0	8	2	10	7	7
02080	3	6	3	8	2	6
02081	4	6	4	9	2	6
02087	4	2	7	4	7	7
02090	2	3	0	3	3	3
02091	3	6	4	1	5	3
02092	7	5	4	0	4	6
02093	3	5	3	5	0	5
02094	2	6	0	7	4	5
02097	3	4	0	7	1	4
02098	0	3	0	6	10	10
02100	5	8	7	0	0	6
02103	2	3	3	6	2	4
02104	1	7	6	7	1	8
02106	8	6	6	6	9	8
02108	2	8	4	4	4	5
02109	0	6	0	8	2	7
02111	3	2	3	0	1	1
02112	3	5	2	6	6	5
02117	0	4	2	4	2	6
02120	6	5	0	0	7	6
02124	5	7	1	4	3	6

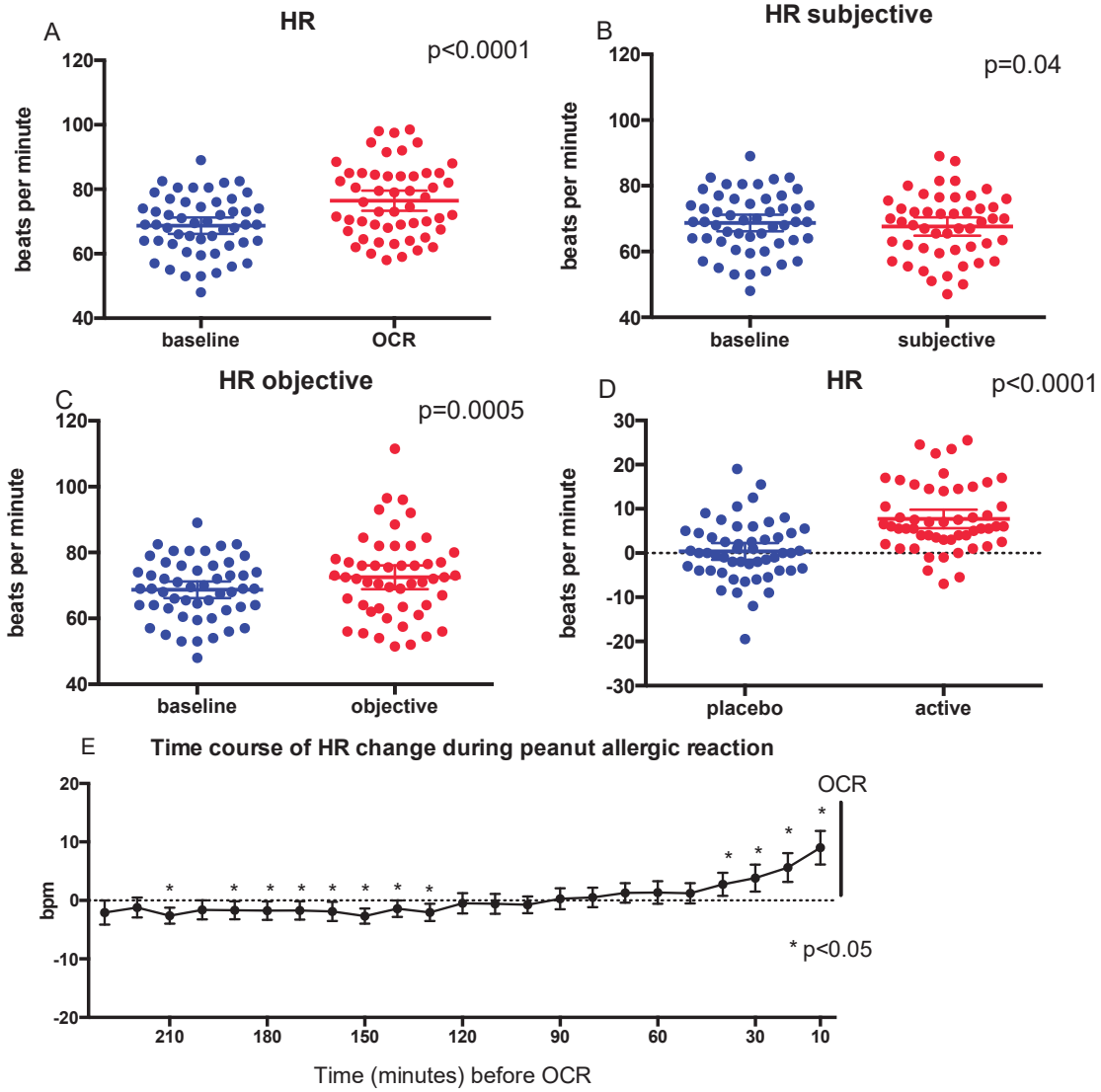
Heat map for the VAS symptom score by participants for each organ, anxiety and overall reaction score.

3.3.3 Changes in cardiac parameters:

3.3.3.1 Heart rate (HR):

Change in HR during peanut allergic reaction is shown in Figure 26. There is a significant increase in HR during peanut allergic reaction compared with placebo challenge. There is no increase in HR at onset of subjective symptoms, but HR increased significantly at onset of objective symptoms (mean increase 3.8bpm, 95% CI [1.8 to 5.9]) and at OCR (7.7bpm, [5.6 to 9.8]). The increase in HR is statistically significant from 40 minutes prior to meeting criteria for OCR.

Figure 26 Change in HR on baseline challenge:

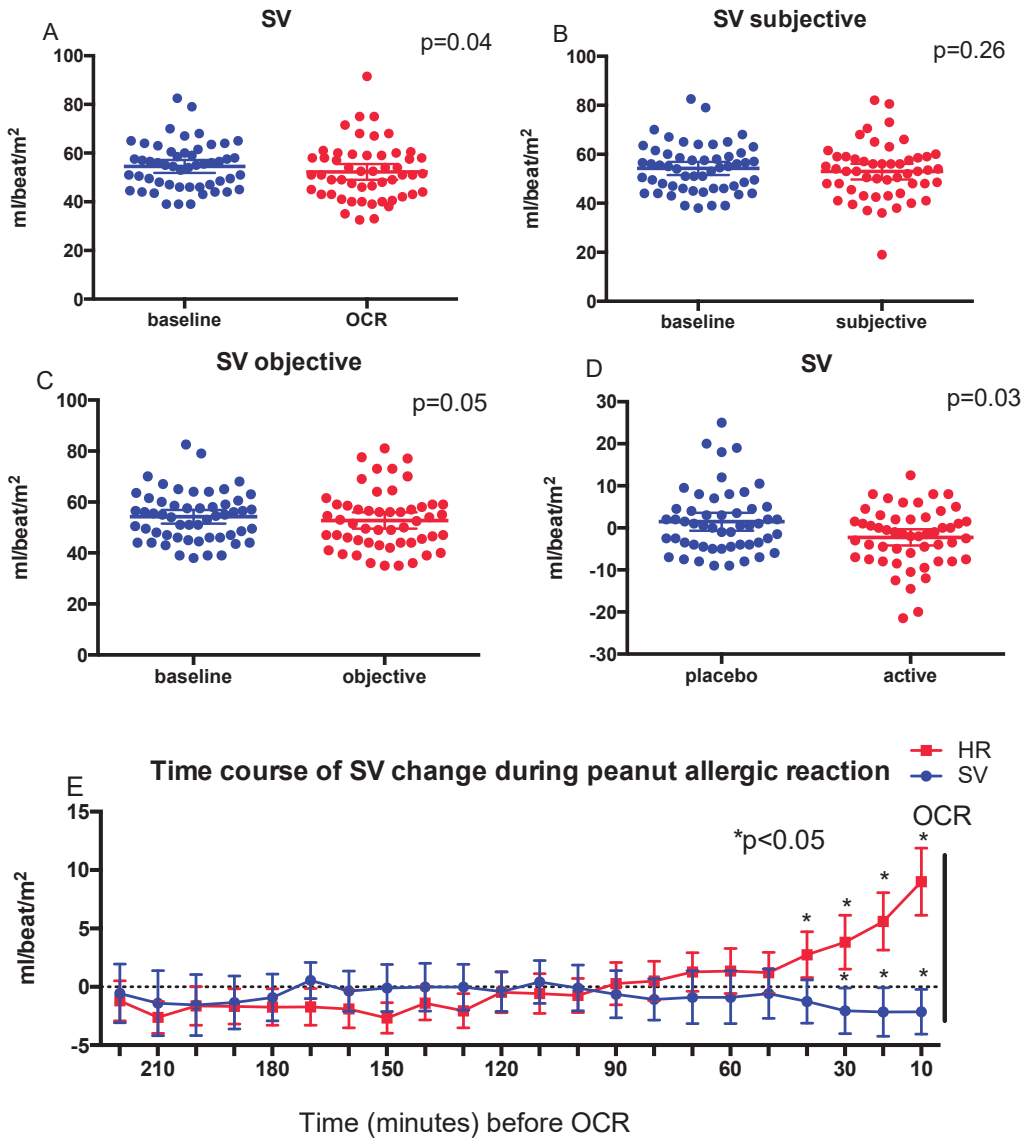


Change on active day at time of OCR (A), at onset of subjective (B) or objective (C) symptoms prior to peanut allergic reaction, (D) change on placebo and active days, and (E) the time course of change in HR during peanut allergic reaction. Shown is mean and 95% CI.

3.3.3.2 Stroke volume (SV):

Change in SV during peanut allergic reaction is shown in Figure 27. There is significant decrease in SV during peanut allergic reaction compared with placebo challenge. There is no decrease in SV at onset of subjective symptoms, but SV decreases significantly at onset of objective symptoms (mean difference -1.5ml/beat/ m^2, 95%CI [-3.5, 0.48]) and at time of OCR (-2.3ml/beat/m^2, [-0.3, -4.2]). The decrease in SV was statistically significant from 30 minutes prior to OCR.

Figure 27 Change in SV on baseline challenge:

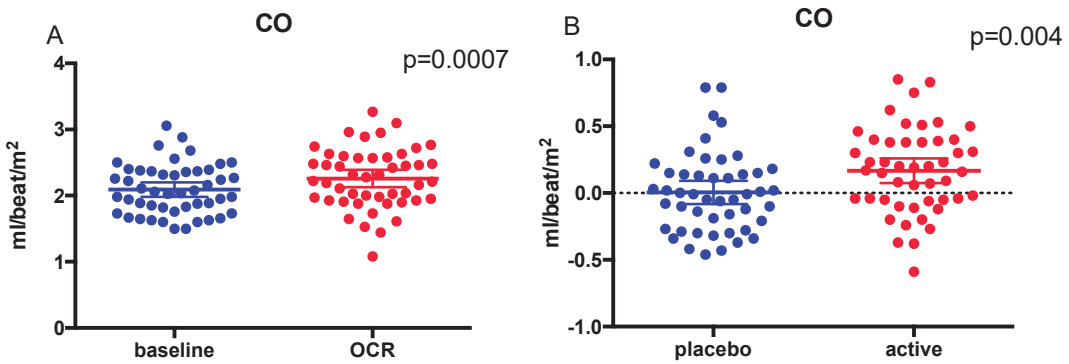


Change on active day at time of OCR (A), at onset of subjective (B) or objective (C) symptoms prior to peanut allergic reaction, (D) change on placebo and active days, and (E) the time course of change in SV and HR during peanut allergic reaction. Shown is mean and 95% CI.

3.3.3.3 Cardiac output (CO):

Cardiac output was manually measured using HR values from the 12 lead ECG Holter monitor, with which participants were monitored, and SV values from bioreactance monitoring. Results in Figure 28 show a significant increase in CO at OCR compared to baseline (mean increase 0.2 ml/beat/m^2, 95% CI [0.1, 0.3]) and on active day compared to placebo day.

Figure 28 Change in CO on baseline challenge:



Change on active day at time of OCR (A) and on active day compare to placebo day (B). Shown is mean and 95% CI.

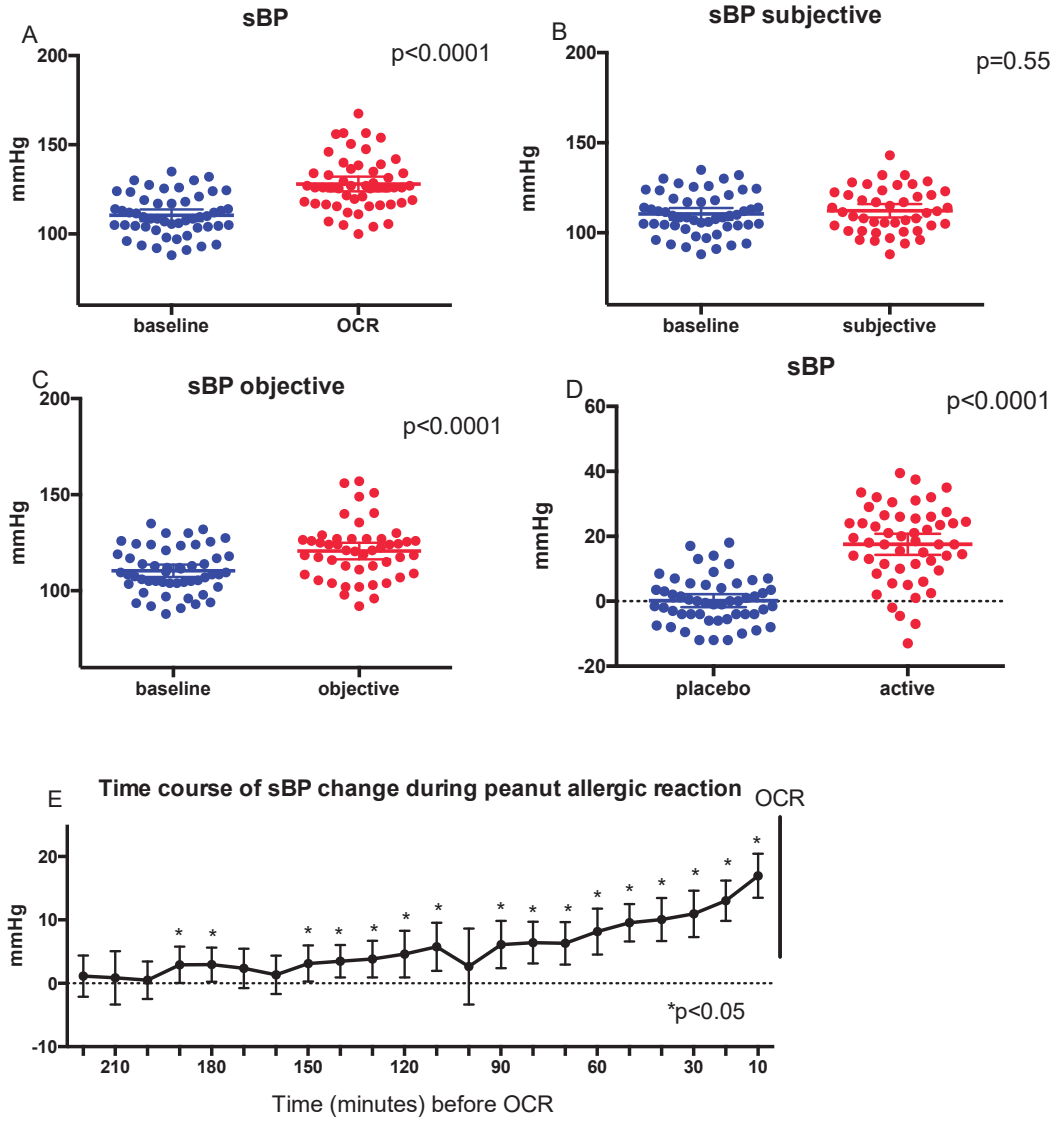
3.3.4 Changes in vascular parameters:

3.3.4.1 Peripheral blood pressure (BP):

3.3.4.1.1 sBP and dBP during peanut allergic reaction:

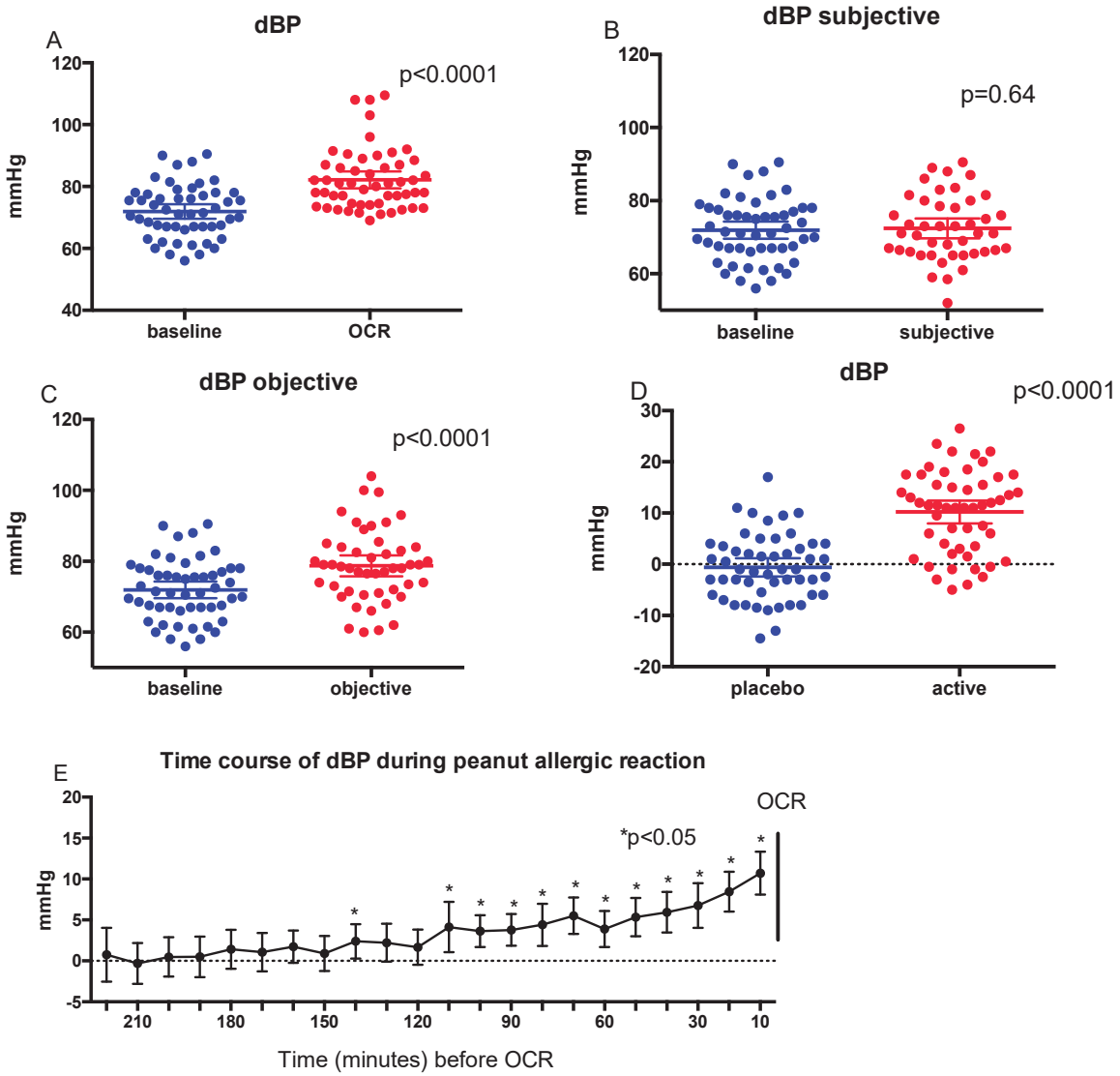
There is a significant increase in sBP and dBP during peanut allergic reaction compared to placebo challenge. There was no increase in sBP or dBP at onset of subjective symptoms but sBP increased at onset of objective symptoms (mean increase 10.7mmHg, 95% CI [7, 14.3]) and at time of OCR (17,5mmHg [14.3, 20.8]) and dBP increased at onset of objective symptoms (7.3mmHg, [4.7, 9.9]) and at time of OCR (10.2mmHg, [8, 12.5]). These changes were significant from 150 and 110 minutes, for sBP and dBP respectively, prior to meeting the OCR criteria. These changes are shown in figures 29 and 30.

Figure 29 Change in sBP on baseline challenge:



Change on active day at time of OCR (A), change in sBP at onset of subjective (B) or objective (C) symptoms prior to peanut allergic reaction, (D) change on placebo and active days, and (E) the time course of change in sBP during peanut allergic reaction. Shown is mean and 95% CI.

Figure 30 Change in dBP on baseline challenge:

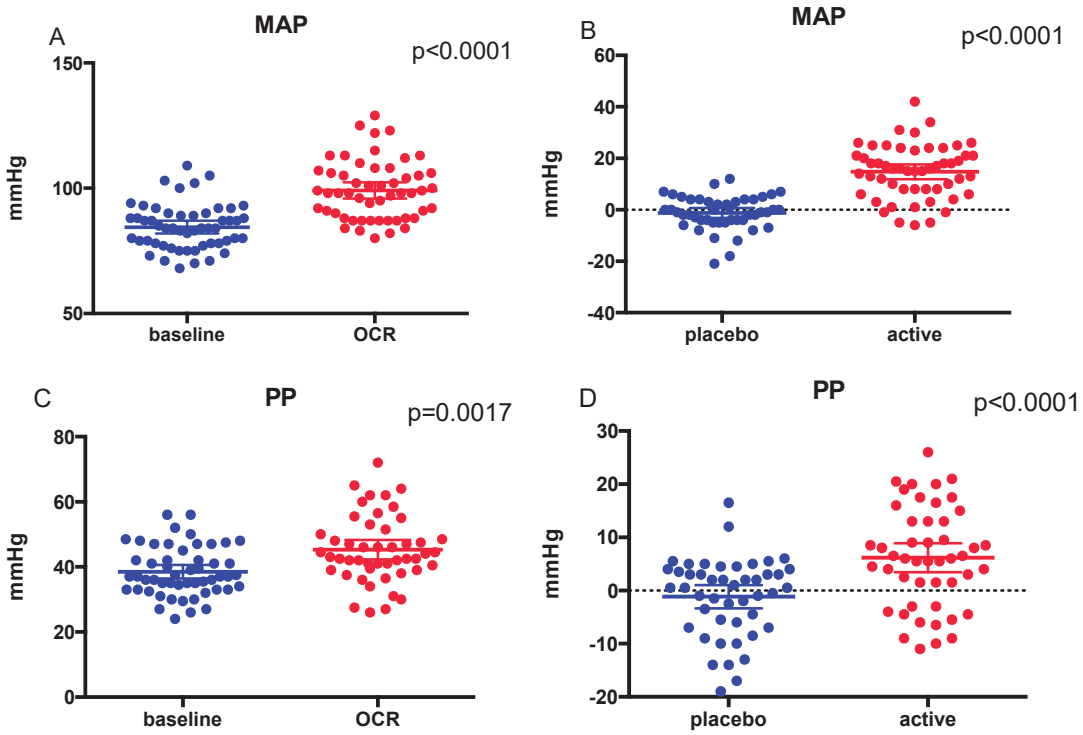


Change on active day at time of OCR (A), change in dBP at onset of subjective (B) or objective (C) symptoms prior to peanut allergic reaction, (D) change on placebo and active days, and (E) the time course of change in dBP during peanut allergic reaction. Shown is mean and 95% CI.

3.3.4.1.2 Analyses of mean arterial pressure (MAP) and pulse pressure (PP):

Results in Figure 31 show a significant increase in both MAP and PP during peanut allergic reaction compared to placebo challenge, on peanut allergic reaction at time of OCR compared to baseline and a poor correlation between SV and PP.

Figure 31 Change in MAP and PP on baseline challenge:

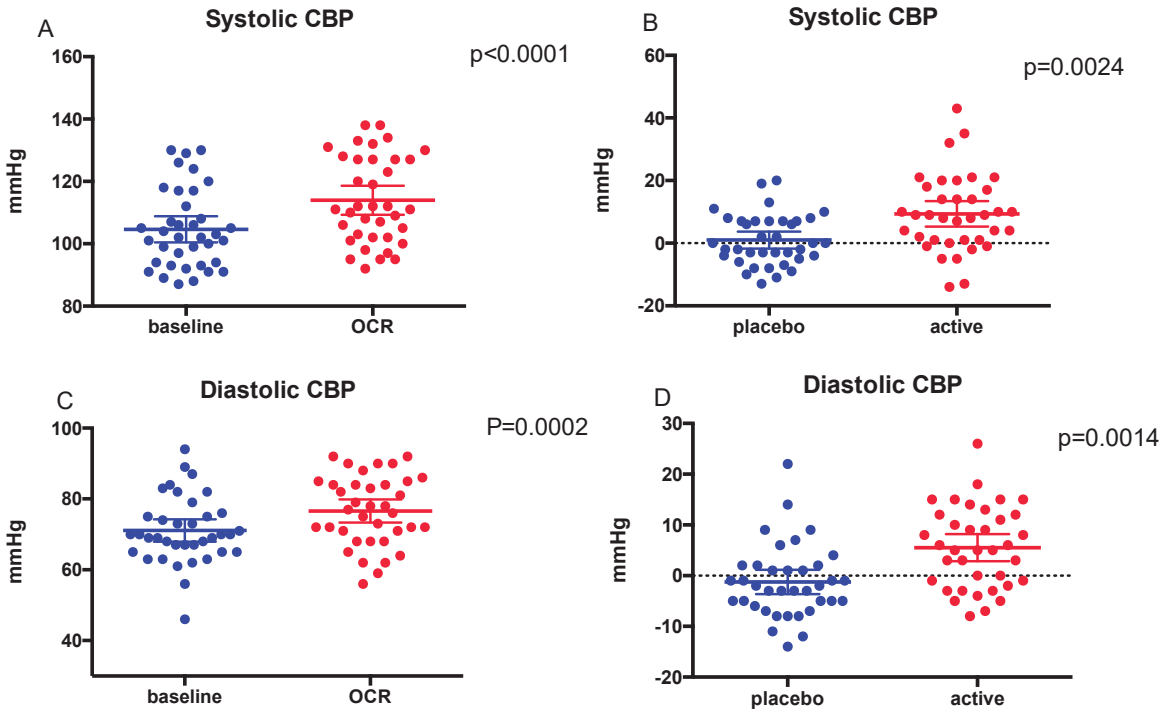


Change on active day at time of OCR (A) and (C) and on placebo and active days (B) and (D). Shown is mean and 95% CI.

3.3.4.2 Central blood pressure (CBP):

CBP data for 37 participants was analysed, missing data was due to a faulty or missing recording (n=9) or non-availability of the monitor on one of the 2 challenge days (n=11). Changes in CBP during peanut allergic reaction are shown in Figure 32. There is a significant increase in both systolic (mean increase 9,4mmHg, 95% CI [5.3, 13.4]) and diastolic (mean increase 5,5mmHg, 95% CI [2.8, 8.2]) CBP during peanut allergic reaction, at time of OCR and compared to placebo challenge.

Figure 32 Change in central sBP on baseline challenge:

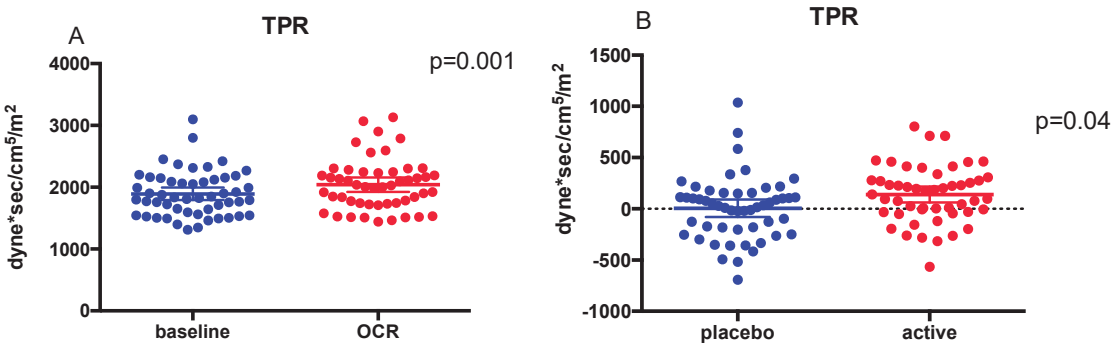


Change on active day at time of OCR (A) and on active day compared to placebo day (B) and changes in dCBP on active day (C) at time of OCR and on active day compared to placebo day (D). Shown is mean and 95% CI.

3.3.4.3 Total Peripheral Resistance (TPR):

There was a significant increase in TPR during peanut allergic reaction at time of OCR (mean increase 139 dyne*sec/cm^5/m^2, 95% CI [60, 218]) and on active day compared to placebo day shown in Figure 33.

Figure 33 Change in TPR on baseline challenge:



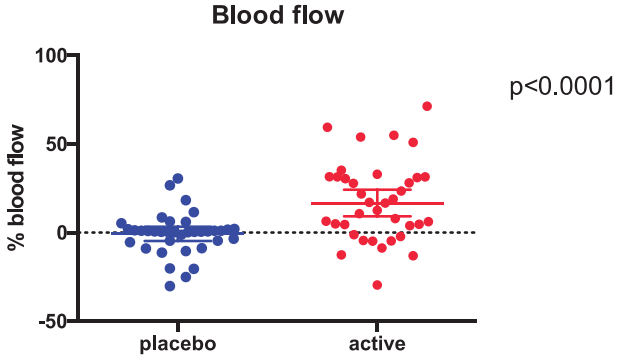
Change on active day at time of OCR (A) and on active day compared to placebo day (B). Shown is mean and 95% CI.

3.3.4.4 Peripheral blood flow:

Peripheral blood flow was measured on the neck, data from 50 participants were obtained and studied but only data from 37 participants were included in this analysis, for the following reasons: failure to reach maximum vasodilation (n=2), no obvious fall from maximum vasodilation (n=4), first objective symptom occurring prior to return to baseline blood flow (n=1), or frequent spikes (n=6) usually due to movement artefact creating a signal too noisy, in at least one of the two challenges.

Figure 34 shows a significant increase in peripheral blood flow during peanut allergic reaction (median 20%; IQR [-2.2 to 46.7%]) compared to placebo challenge.

Figure 34 Change in peripheral blood flow on baseline challenge:

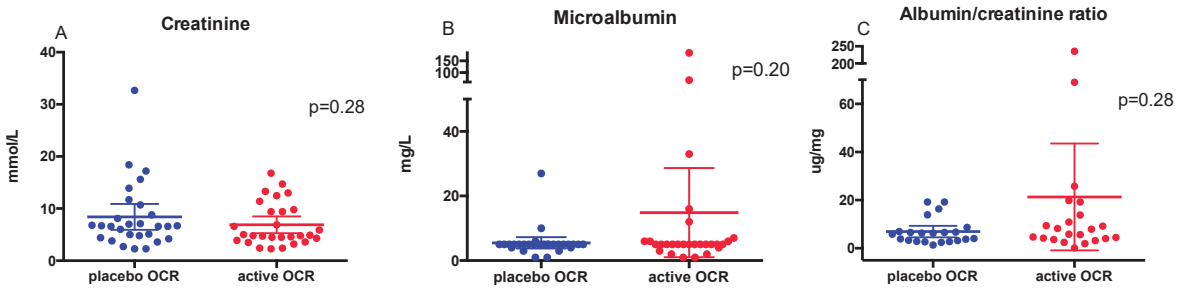


Change in blood flow at onset of objective symptoms during peanut allergic reaction and at an equivalent time during placebo challenge. Shown is mean and 95% CI.

3.3.5 Urine metabolic parameters:

Urine samples for creatinine, microalbumin and creatinine/albumin ratio were obtained from 37 participants and data was analysed for 28 participants, missing data includes sample not processed by the lab (n=6) and sample not obtained from the participant (n=3) on one of the two challenge days. Samples were obtained at arrival and 2hrs after reaching OCR. Figure 35 shows no significant difference between OCR at placebo and active day for the 3 parameters analysed.

Figure 35 Urine metabolic parameters:

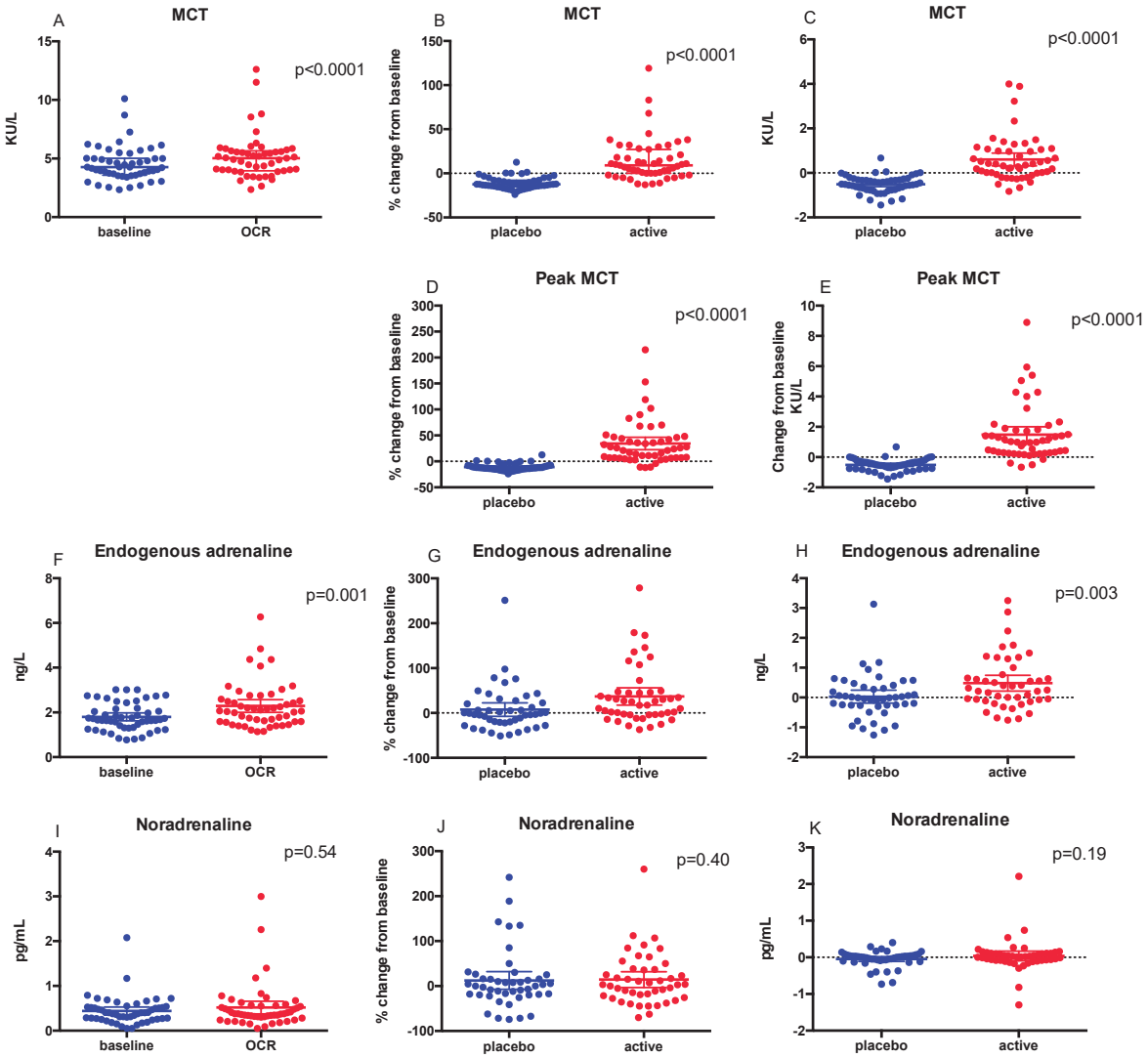


Change in (A) urine creatinine, (B) microalbumin and (C) albumin/creatinine ratio on active day compared to placebo day both at time of OCR. Shown is mean and 95% CI.

3.3.6 Mast cell tryptase and catecholamines:

Objective measures of reaction severity include change in MCT and release of catecholamine. Figure 36 shows there is a significant increase in MCT (median increase 9% IQR [0, 27]) on active day at time of OCR and on active day compared to placebo day both for absolute value and % increase. A significant decrease on MCT was found on placebo day. Similar results were found for the peak MCT, for both % change from baseline and absolute value, from OCR to 2hr after reaction. A significant increase was found for endogenous adrenaline on active day at time of OCR (median increase 17.7%, IQR [-4.2, 47.2]) and on active day compared to placebo day. No changes were found for noradrenaline release.

Figure 36 Change in MCT and catecholamines:

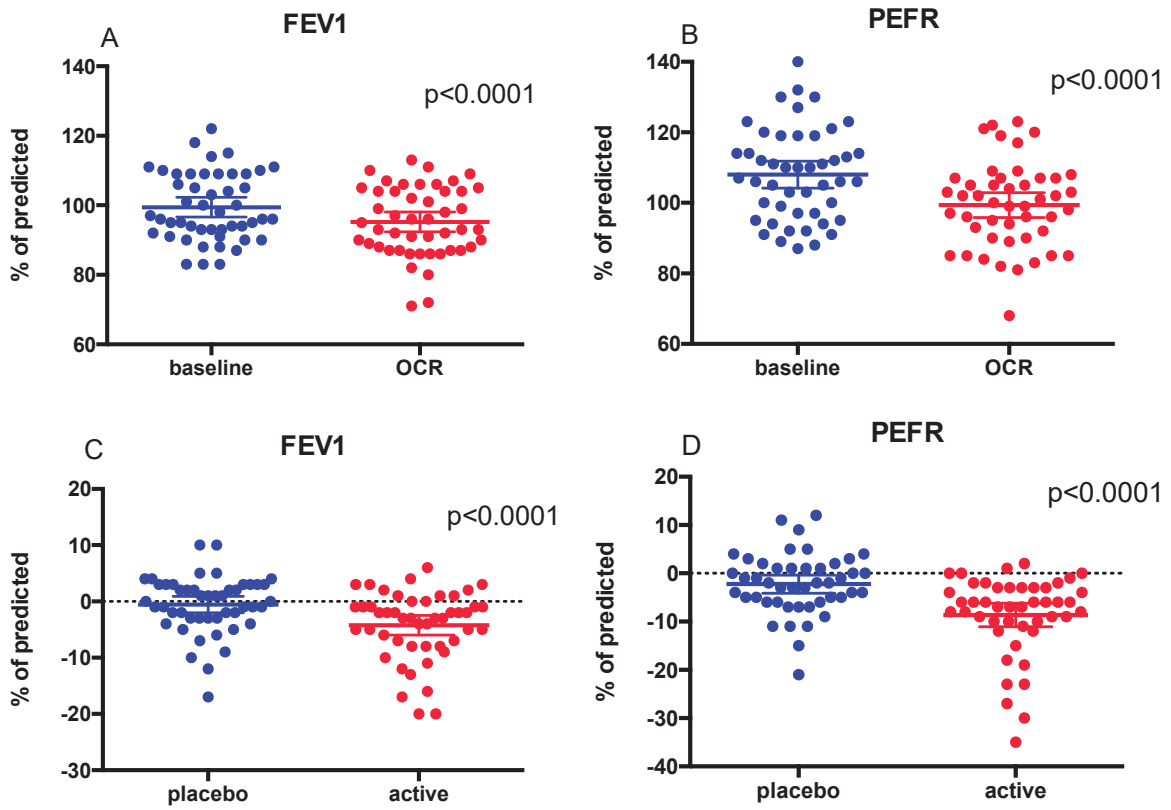


Change in (A), endogenous adrenaline (F) and noradrenaline (I) on active day at time of OCR and changes in MCT (B), endogenous adrenaline (G) and noradrenaline (J) as a % change from baseline and as absolute values (C,H,K) between active and placebo day. Change in peak MCT on active day compared to placebo (E) absolute value and (D) % change from baseline. Shown is mean and 95% CI.

3.3.7 Lung function during peanut allergic reaction:

Lung spirometry was performed in all participants, data for 49 participants was analysed, reason for missing data was due to not performing this technique at OCR as participants didn´t have any lower respiratory symptoms (n=3). Figure 37 shows a significant decrease in % of predicted FEV1 (median decrease -3%, IQR [-0.25, -7.75]) and PEFR (-6.5%, [-3, -10.25]) on active days at time of OCR and on active day compared to placebo day.

Figure 37 Change in lung function at baseline challenge:

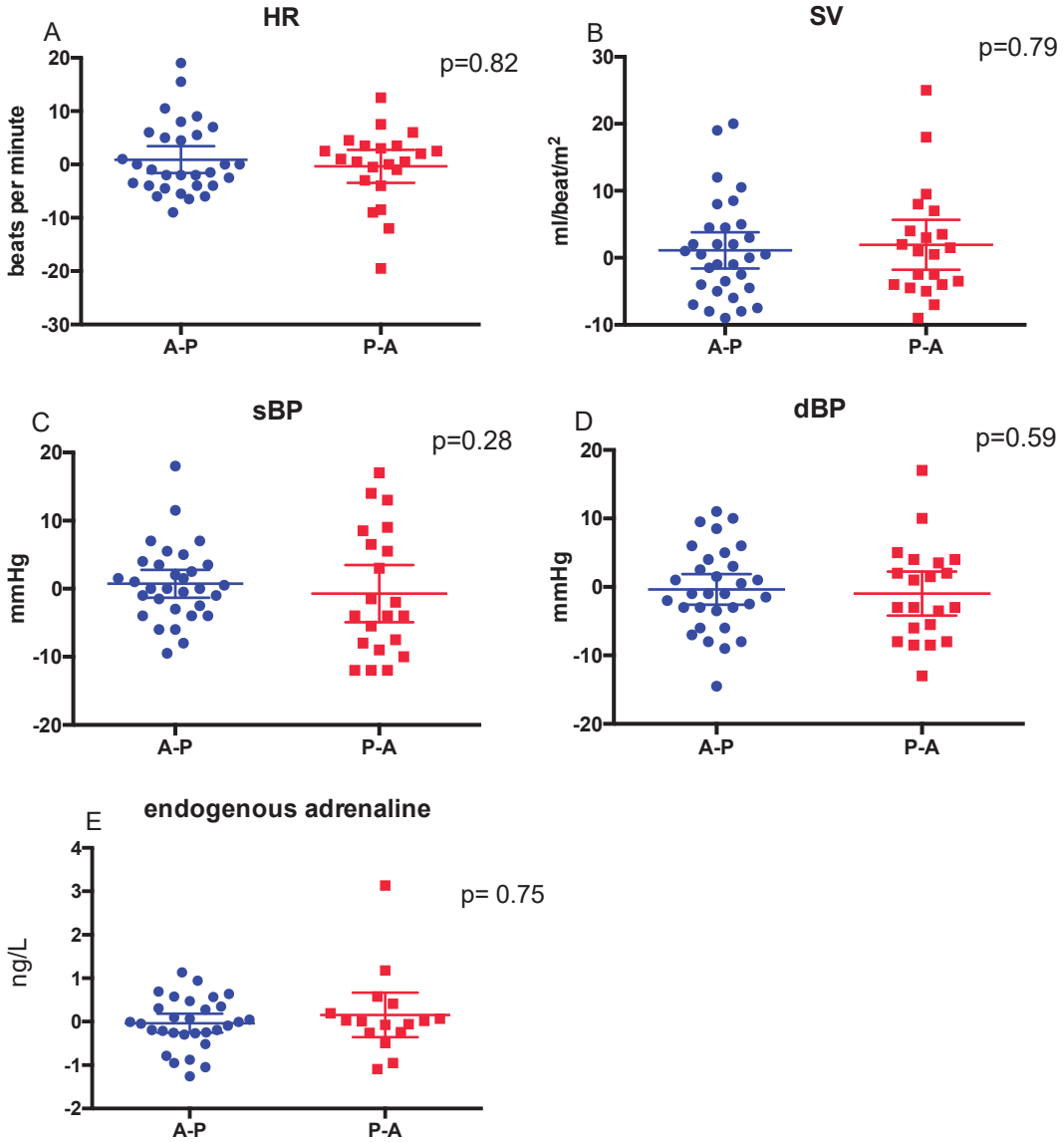


Change in (A) % of predicted FEV1 and (B) PEFR on active day at time of OCR and (C and D) on active day compared to placebo day. Shown is mean and 95% CI.

3.3.8 Impact of challenge order:

Figure 38 shows there was no significant difference in any of the parameters, which might be affected by anxiety or stress (certain CVS parameters and catecholamine) according to challenge order of the DBPCFC. We compared participants who had a placebo challenge before their active challenge, and might have been more anxious during their placebo challenge, compared with those who had a placebo challenge after their peanut allergic reaction, who might have been less anxious at the time of their placebo challenge; no significant difference was seen.

Figure 38 Difference depending on challenge order:



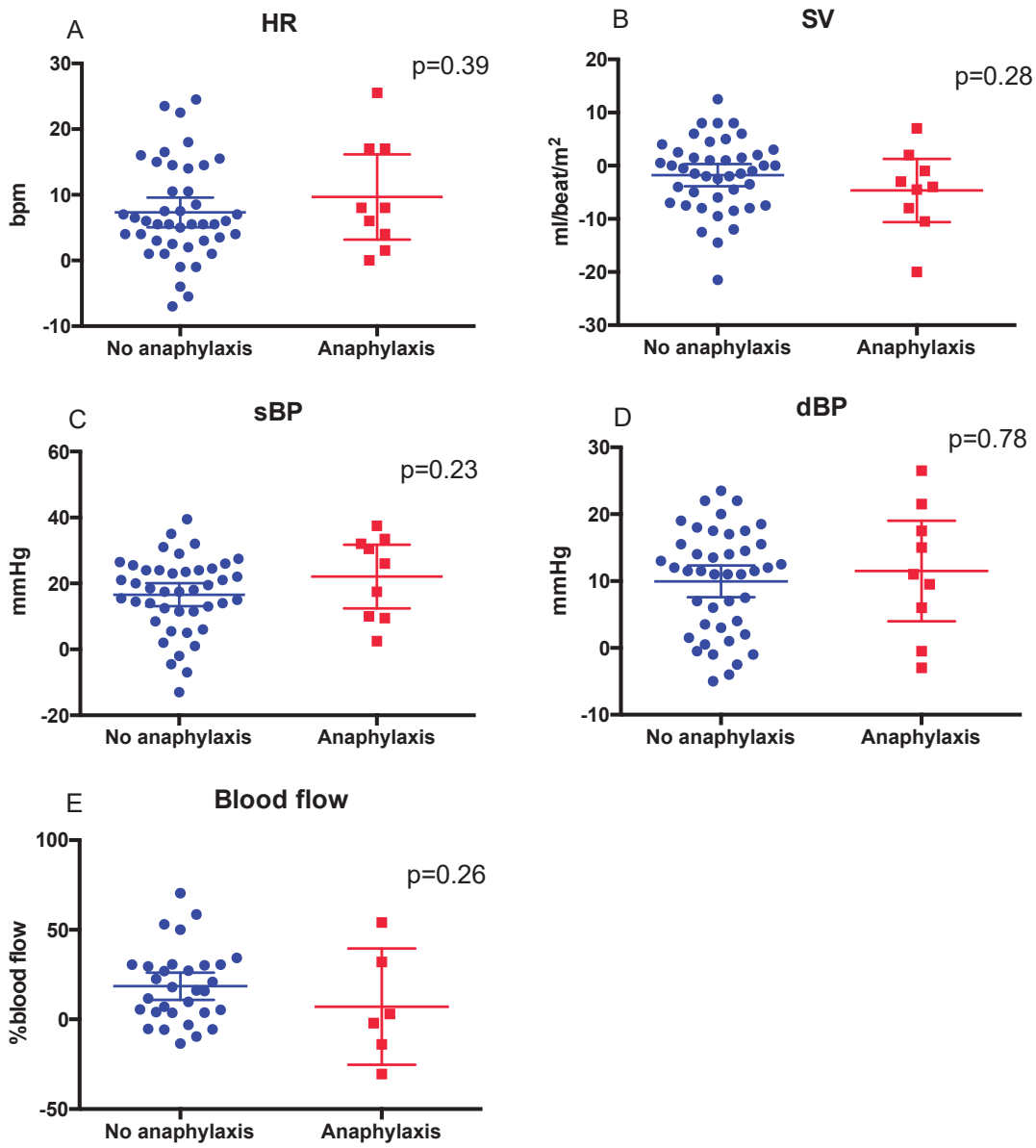
Change in (A) HR, (B) SV, (C) SBP, (D) DBP and (E) endogenous adrenaline on placebo day challenges. A-P corresponds to those participants having placebo second and P-A corresponds to those participants having placebo first as their order for DBPCFC. Shown is mean and 95% CI.

3.3.9 Association between cardiovascular changes and severity of reaction:

3.3.9.1 Comparison of changes in CVS system and different scoring systems:

Figure 39 shows there´s no significant difference in any of the CVS parameters analysed between those participants who required IM adrenaline given the severity of their reaction compared to those who did not.

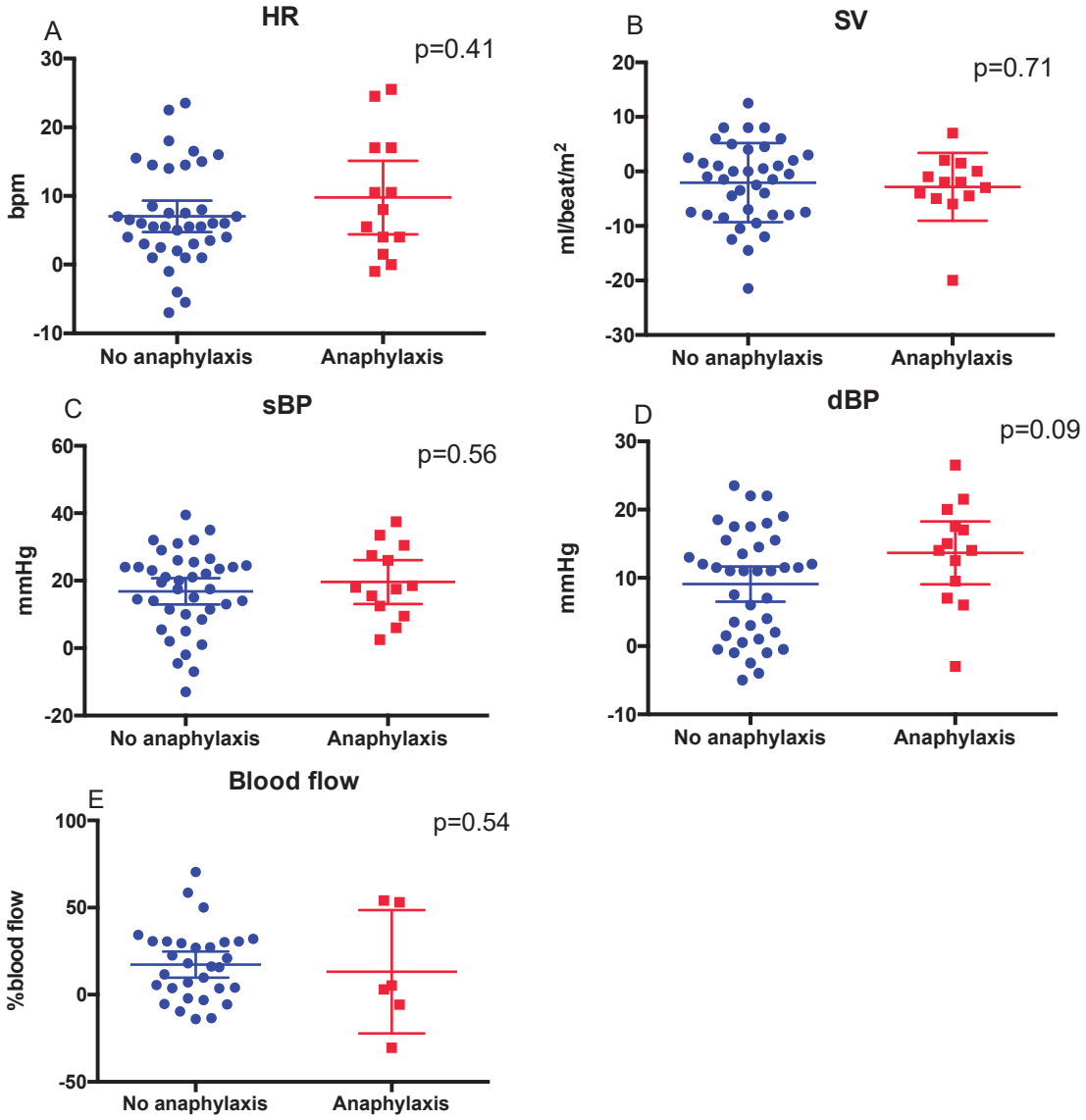
Figure 39 Differences by use of IM adrenaline at baseline challenge:



Differences for (A) HR, (B) SV, (C) sBP (D) dBP and (E) blood flow between those participants who had anaphylaxis and those who did not. Shown is mean and 95% CI.

Results in Figure 40 show no significant differences for any of the CVS parameters analysed between those participants who had anaphylaxis compared to those who didn´t according to the NIAID classification of anaphylaxis.

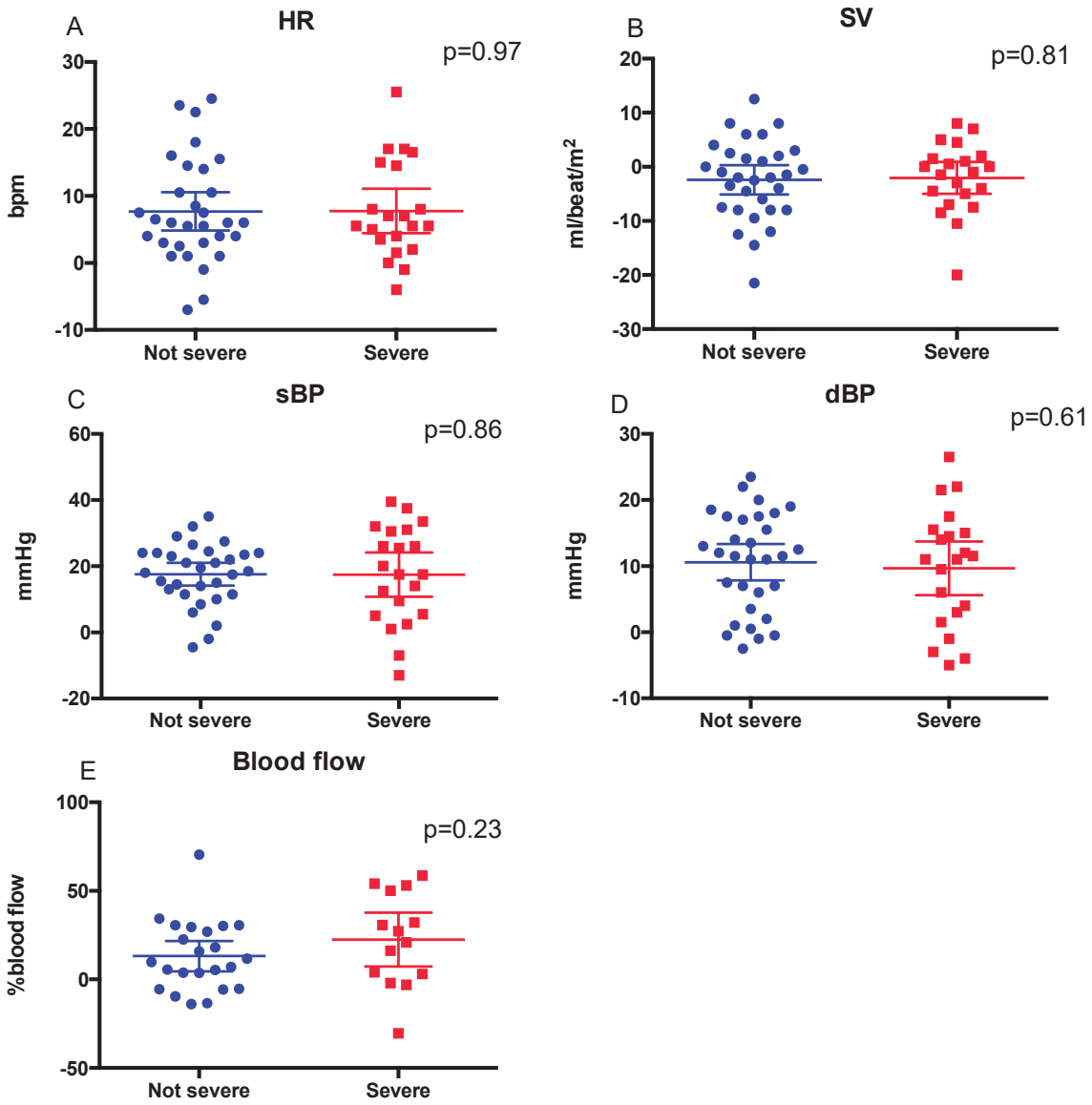
Figure 40 Differences according to NIAID classification of anaphylaxis at baseline challenge:



Difference for (A) HR, (B) SV, (C) sBP, (D) dBP and (E) blood flow. Shown is mean and 95% CI.

Results in Figure 41 show no significant differences in any of the parameters analysed between those participants classified as having a mild reaction compared to those classified as having a moderate/severe reaction according to Ewan and Clark classification.

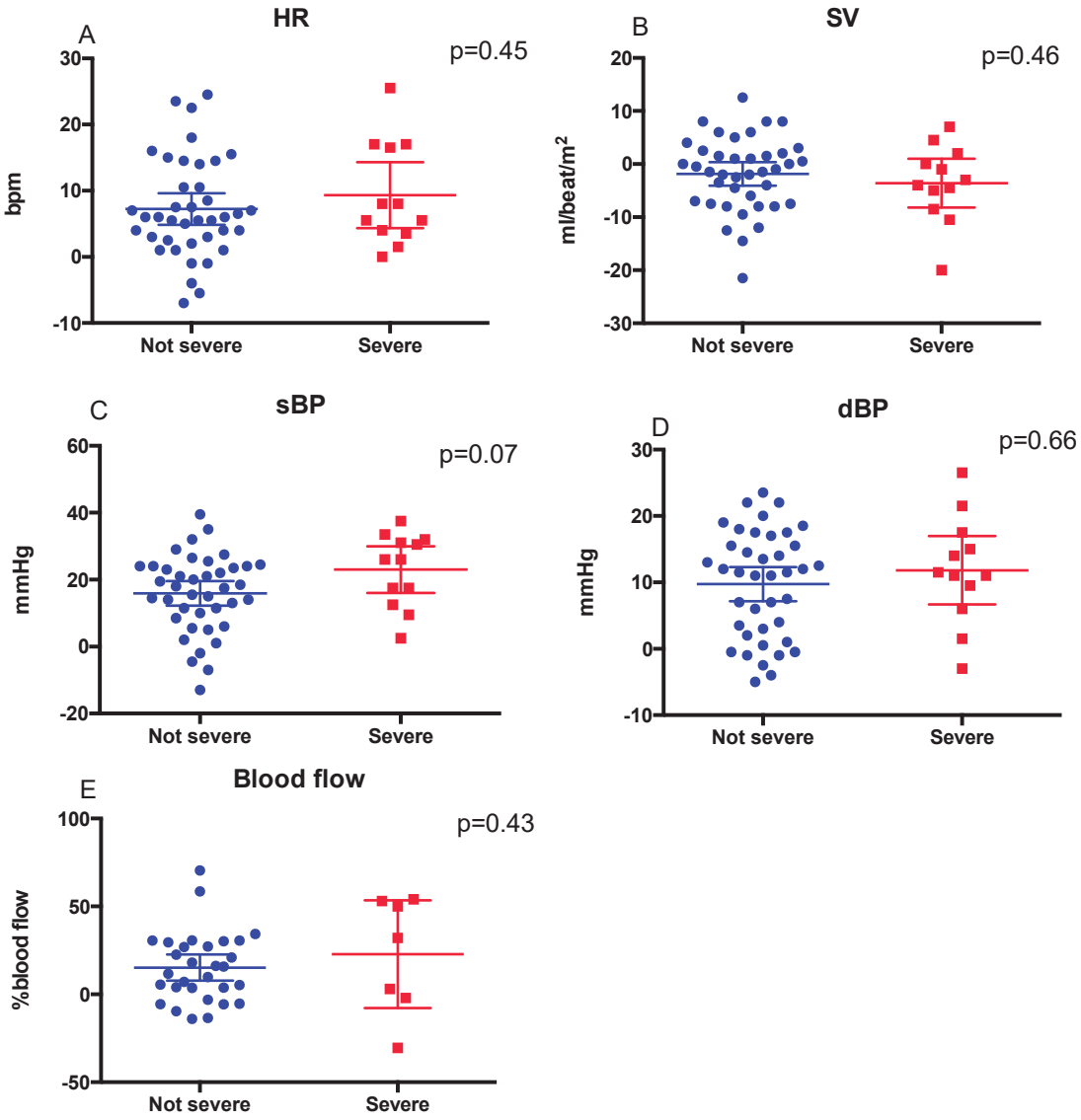
Figure 41 Differences according to Ewan & Clark classification of reaction severity at baseline challenge:



Difference for (A) HR, (B) SV, (C) sBP, (D) dBP and (E) blood flow between those participants classified as mild (1-3) and those classified as moderate/severe (4-5) reactions. Shown is mean and 95% CI.

Results in Figure 42 show no significant differences in any of the parameters analysed between those participants classified as having a mild reaction compared to those classified as having a moderate/severe reaction according to the WAO classification.

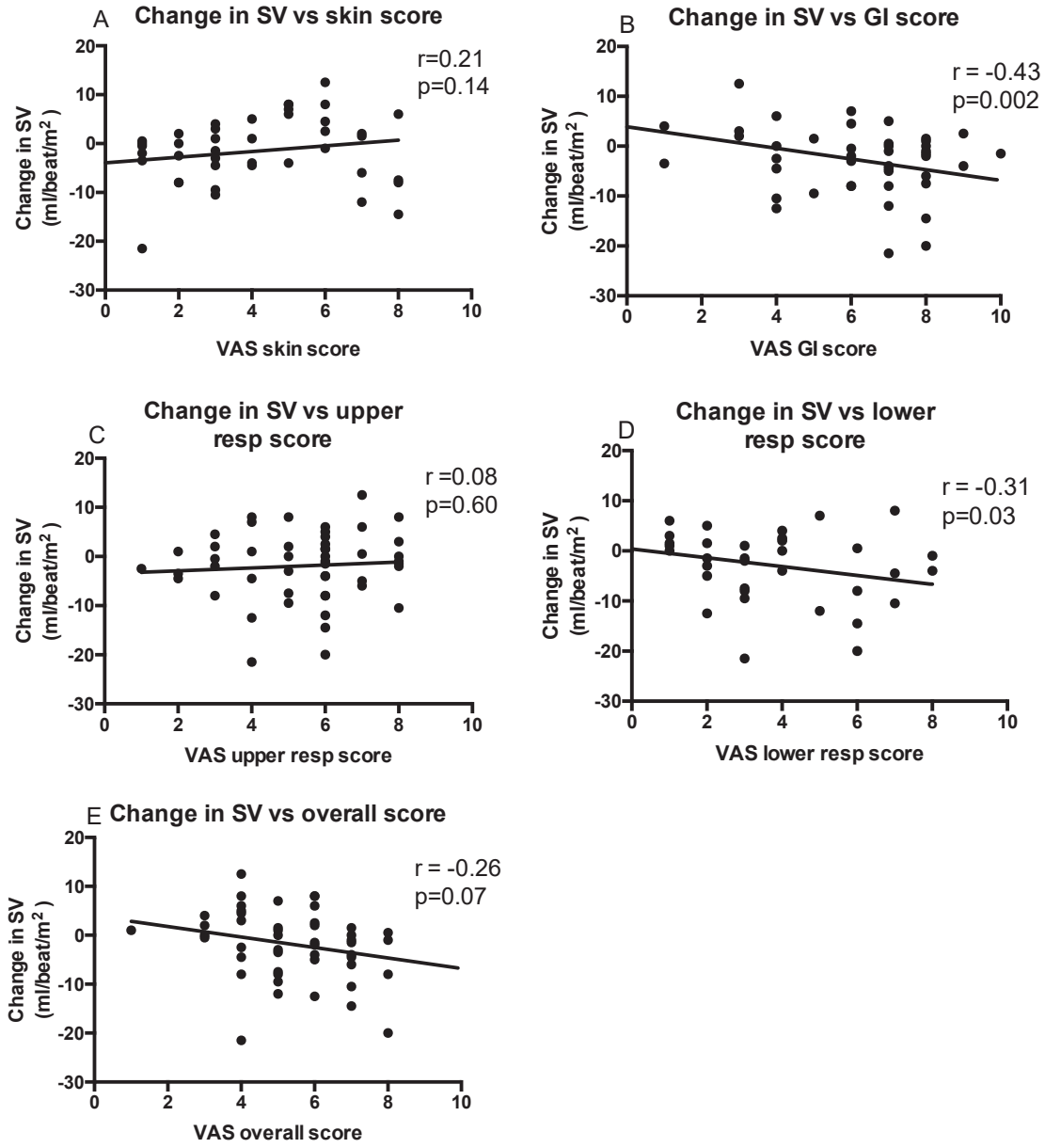
Figure 42 Differences according to the WAO classification for subcutaneous immunotherapy systemic reaction grading system at baseline challenge:



Difference for (A) HR, (B) SV, (C) sBP, (D) dBP and (E) blood flow between those participants classified as mild (1-2) and moderate/severe (3-5) reactions. Shown is mean and 95% CI.

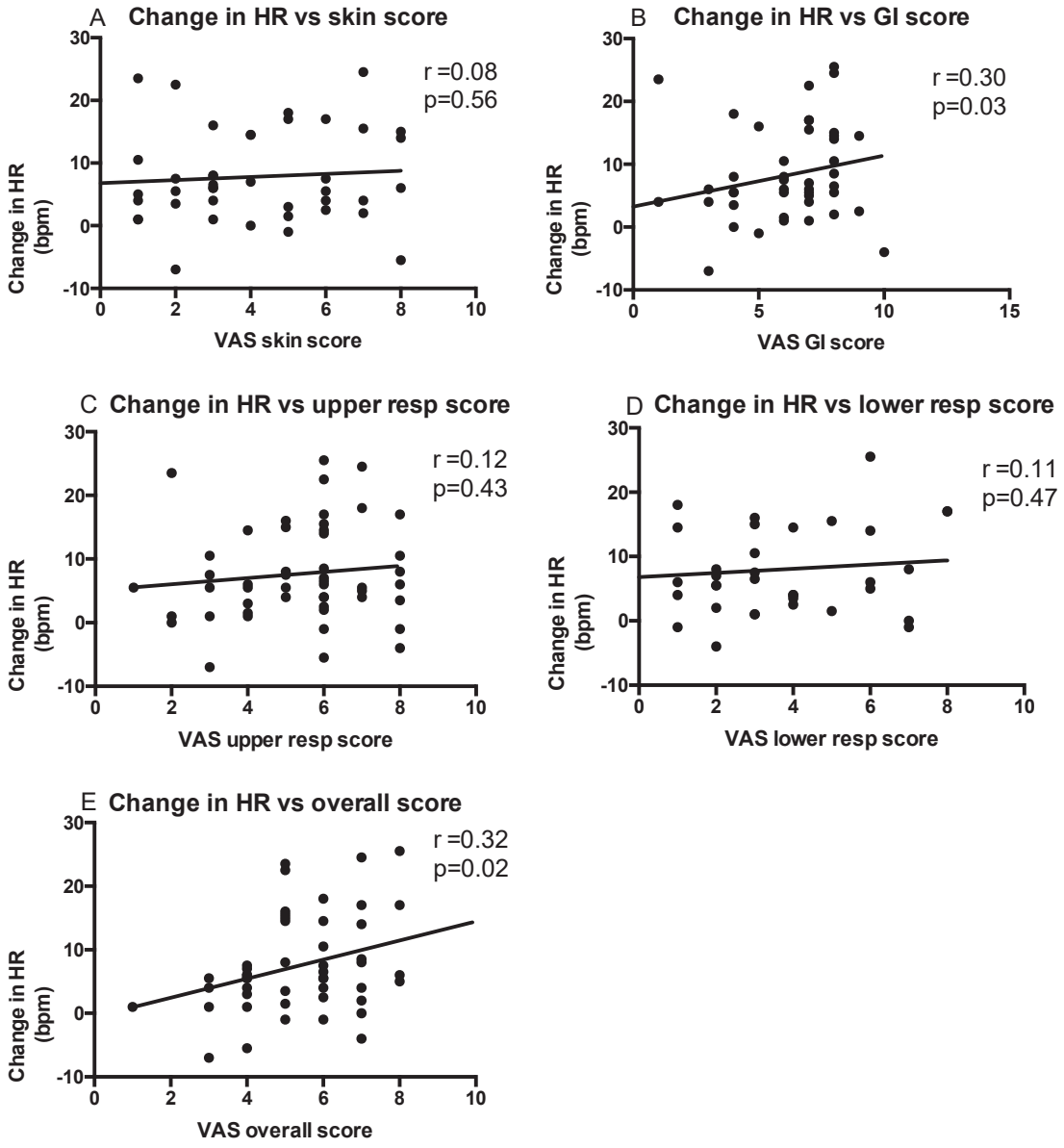
CVS parameters were correlated with a VAS score obtained by both participant´s and clinician on the day of the allergic reaction for different organ systems and overall score. Figures 43 to 47 shows, overall, a poor correlation between the change in CVS parameters and the participant´s VAS severity score. Moderate correlation was seen between changes in SV and HR and the participant´s VAS score for GI symptoms. Similar results were found for the correlation between the CVS parameters and the clinician´s VAS score (Appendix 17).

Figure 43 Relationship between SV and symptoms at baseline challenge:



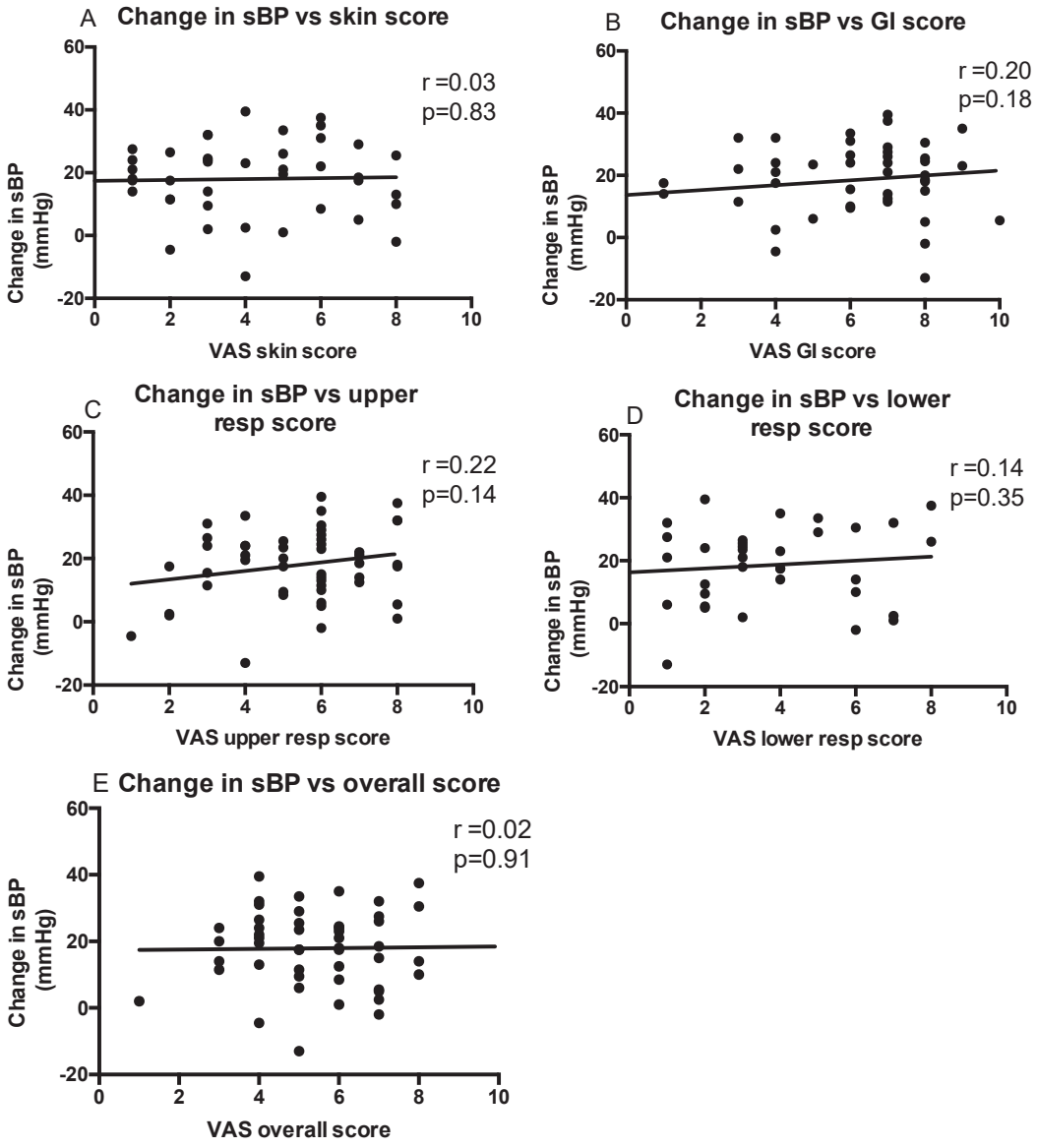
Spearman correlation between change in SV and participant´s VAS score for (A) skin severity, (B) GI severity, (C) upper respiratory severity, (D) lower respiratory severity and (E) overall reaction severity.

Figure 44 Relationship between HR and symptoms at baseline challenge:



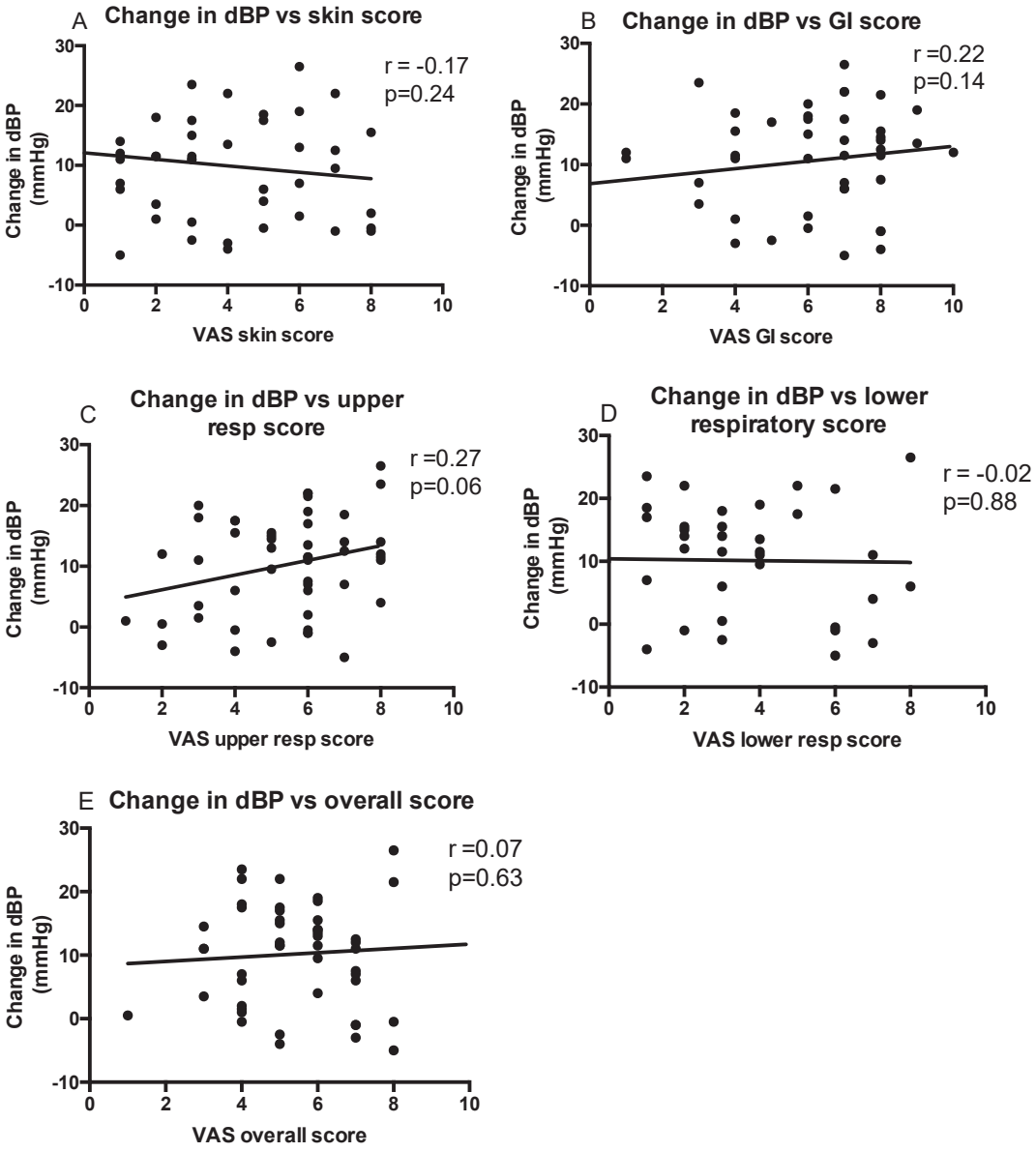
Spearman correlation between change in HR and participant´s VAS score for (A) skin severity, (B) GI severity, (C) upper respiratory severity, (D) lower respiratory severity and (E) overall reaction severity.

Figure 45 Relationship between sBP and symptoms at baseline challenge:



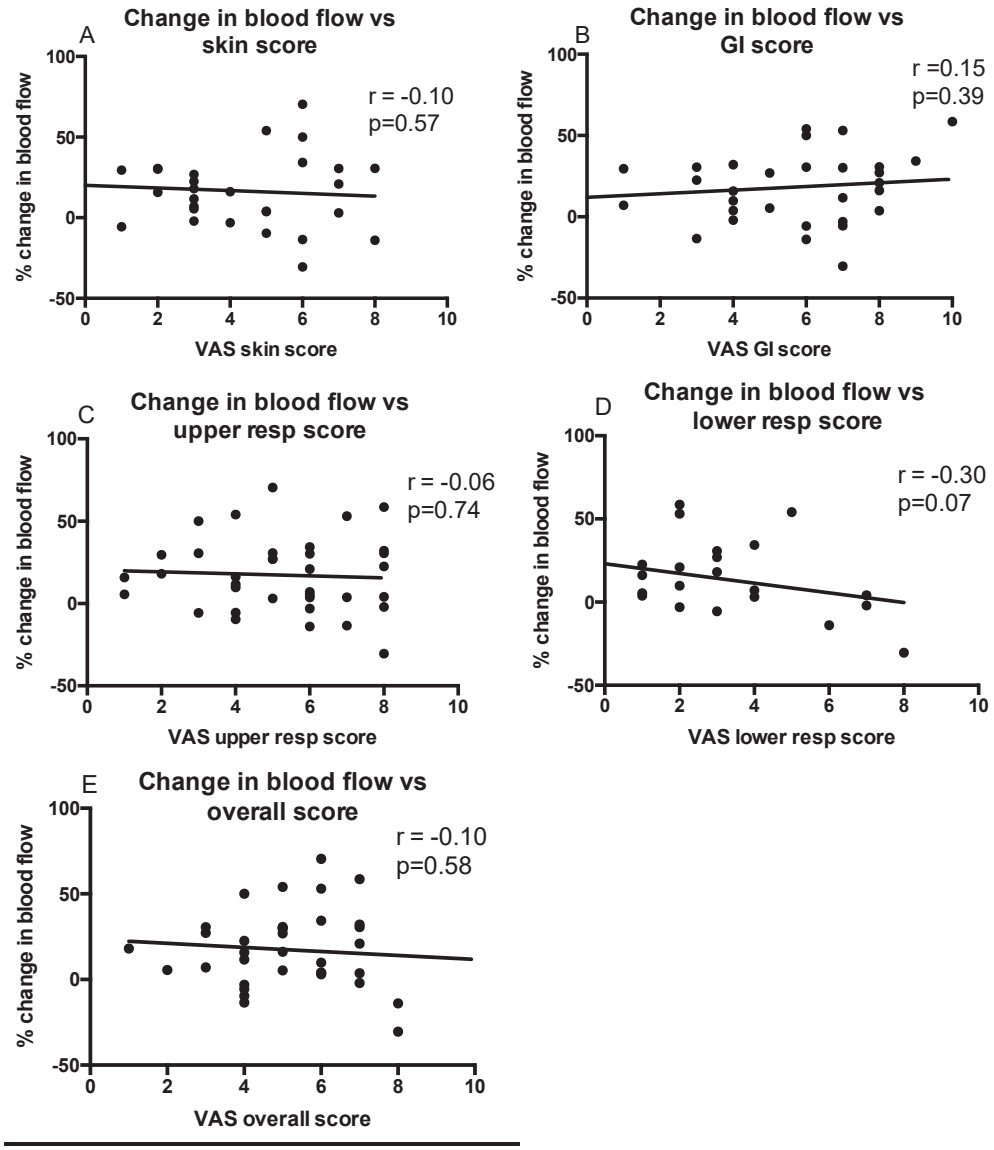
Spearman correlation between change in sBP and participant´s VAS score for (A) skin severity, (B) GI severity, (C) upper respiratory severity, (D) lower respiratory severity and (E) overall reaction severity.

Figure 46 Relationship between dBP and symptoms at baseline challenge:



Spearman correlation between change in dBP and participant´s VAS score for (A) skin severity, (B) GI severity, (C) upper respiratory severity, (D) lower respiratory severity and (E) overall reaction severity.

Figure 47 Relationship between peripheral blood flow and symptoms at baseline challenge:



Spearman correlation between change in % blood flow and participant´s VAS score for (A) skin severity, (B) GI severity, (C) upper respiratory severity, (D) lower respiratory severity and (E) overall reaction severity.

3.3.10 Correlation between CVS changes, lung function and clinical and laboratory measurements of allergic reaction:

Table 14 shows the correlations between CVS parameters, peripheral blood flow, lung function and laboratory measures of allergic reaction. Of note there is a good correlation between HR measured by means of an ECG and SV, good correlation between different measures of blood pressure, and between change in MCT and change in serum adrenaline level.

Table 14 Relationship between the different parameters analysed:

	HR	sBP	dBP	MAP	PP	sCBP	dCBP	Peripheral blood flow	% of predicted FEV1	% MCT peak	% MCT at OCR	Endogenous adrenaline
SV	**r=-0.42 (p=0.002)**	r=-0.04 (p=0.75)	r=-0.08 (p=0.59)	r=-0.15 (p=0.30)	r=-0.02 (p=0.89)	r=-0.15 (p=0.48)	r=0.08 (p=0.63)	r=0.13 (p=0.48)	r=-0.06 (p=0.70)	r=-0.03 (p=0.86)	r=-0.02 (p=0.91)	r=-0.17 (p=0.24)
HR		r=0.21 (p=0.13)	r=0.26 (p=0.06)	r=0.13 (p=0.35)	r=0.15 (p=0.32)	r=0.14 (p=0.42)	r=0.13 (p=0.44)	r=-0.09 (p=0.63)	r=0.11 (p=0.49)	r=0.10 (p=0.51)	r=0.16 (p=0.28)	r=0.19 (p=0.20)
sBP			**r=0.61 (p<0.0001)**	**r=0.53 (p<0.0001)**	**r=0.71 (p<0.0001)**	**r=0.36 (p=0.03)**	r=0.28 (p=0.18)	r=-0.11 (p=0.54)	r=0.05 (p=0.75)	r=0.18 (p=0.23)	r=0.12 (p=0.42)	r=0.17 (p=0.24)
dBP				**r=0.64 (p<0.0001)**	r=-0.04 (p=0.80)	r=-0.09 (p=0.60)	r=0.12 (p=0.49)	r=-0.04 (p=0.81)	r=0.16 (p=0.28)	r=0.02 (p=0.88)	r=0.15 (p=0.30)	r=0.16 (p=0.28)
MAP					r=0.007 (p=0.92)	r=0.29 (p=0.09)	**r=0.36 (p=0.03)**	r=0.09 (p=0.62)	r=0.03 (p=0.85)	r=0.25 (p=0.09)	r=0.27 (p=0.06)	r=0.13 (p=0.37)
PP						**r=0.36 (p=0.03)**	r=0.04 (p=0.83)	r=-0.15 (p=0.40)	r=-0.08 (p=0.63)	r=0.07 (p=0.64)	r=-0.09 (p=0.56)	r=-0.20 (p=0.18)
sCBP							**r^2=0.48 (p=0.002)**	r^2=-0.04 (p=0.85)	r^2=-0.09 (p=0.63)	r^2=0.22 (p=0.21)	r^2=0.10 (p=0.57)	r^2=0.10 (p=0.55)
dCBP								r=0.02 (p=0.91)	**r=0.42 (p=0.02)**	**r=0.35 (p=0.04)**	r=0.28 (p=0.10)	**r=0.37 (p=0.03)**
Peripheral blood flow									r=-0.08 (p=0.67)	r=0.09 (p=0.62)	r=0.25 (p=0.14)	r=-0.003 (p=0.99)
% of predicted FEV1										r=0.14 (p=0.36)	r=0.26 (p=0.08)	r=0.06 (p=0.69)
% MCT peak											**r=0.76 (p<0.0001)**	**r=0.37 (p=0.01)**
% MCT at OCR												**r=0.31 (p=0.03)**

Spearman correlation for the change from baseline at OCR on active challenge day. HR: Heart rate measured by ECG; SV: stroke volume; sBP: systolic blood pressure; dBP: diastolic blood pressure; MAP: mean arterial pressure; PP: pulse pressure; sCBP:systolic central blood pressure; dCBP: diastolic central blood pressure; % MCT peak: peak % change in MCT from OCR to 2hr after. Highlighted $p<0.05$.

3.3.11 CVS physiology on repeated NI challenges:

Similar results were obtained for changes on non-intervention repeated challenges; the data from placebo day on the baseline DBPCFC were used on these analyses, since repeat peanut challenges were undertaken as open challenges, without a placebo day.

No significant changes were seen for CO on repeated challenge (p=0.46), CO was maintained during the challenge without changes from baseline. A significant difference was observed between results for CO between baseline and repeated challenge (p=0.03), with a significant increase in CO only observed at baseline challenge. This is shown on Appendix 18.

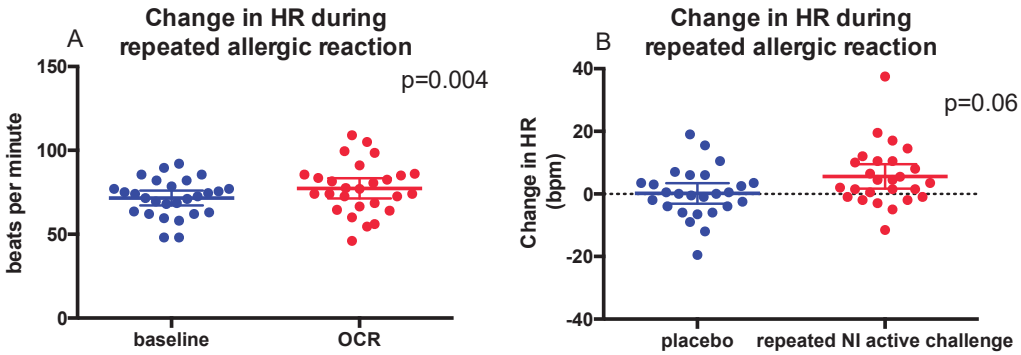
No data were obtained on repeat challenges for MCT and catecholamines. Shown below are data for key parameters which changed at initial DBPCFC, confirming that these also changed during repeat open peanut challenge.

3.3.11.1 Changes in cardiac parameters:

3.3.11.1.1 Heart rate:

Results in Figure 48 show a significant increase in HR on active day at time of OCR (mean increase 6.0bpm, 95% CI [1.7, 9.5]) but this is less clear on active day compared to placebo day. A significant difference was observed between results for HR at baseline compared to repeated challenge (p=0.03) showing a smaller increase in HR on repeated challenge compared to baseline.

Figure 48 Change in HR on repeated challenge:



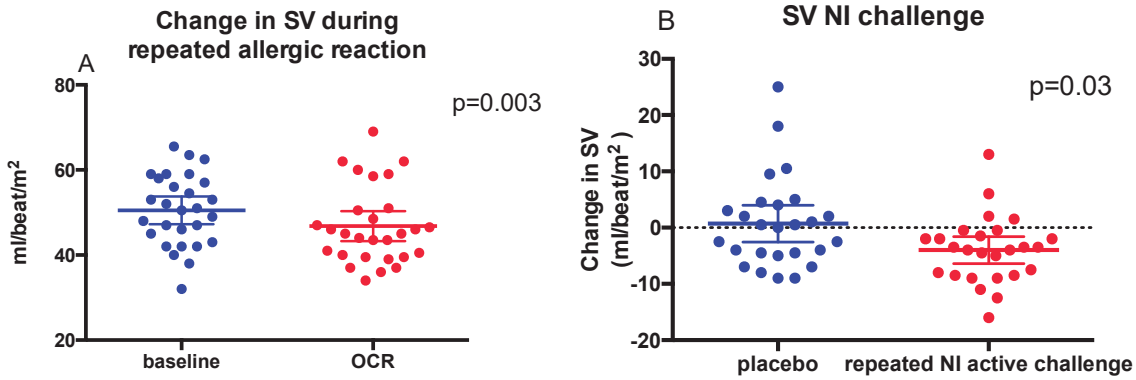
Change during repeated peanut allergic reaction at time of OCR (A), and on active day compared to placebo day (B). Shown is mean and 95% CI.

3.3.11.1.2 Stroke Volume:

Results in Figure 49 show a significant decrease in SV on active day at time of OCR (mean decrease -4.0ml/beat/m^2, 95% CI [-6.4, -1.6]) and on active day compared to placebo day. No correlation was found between changes in SV on baseline and non-intervention challenges (r=0.13 p=0.55). No significant differences were observed

between the changes in SV on baseline compared to repeated challenge day (Appendix 18).

Figure 49 Change in SV on repeated challenge:



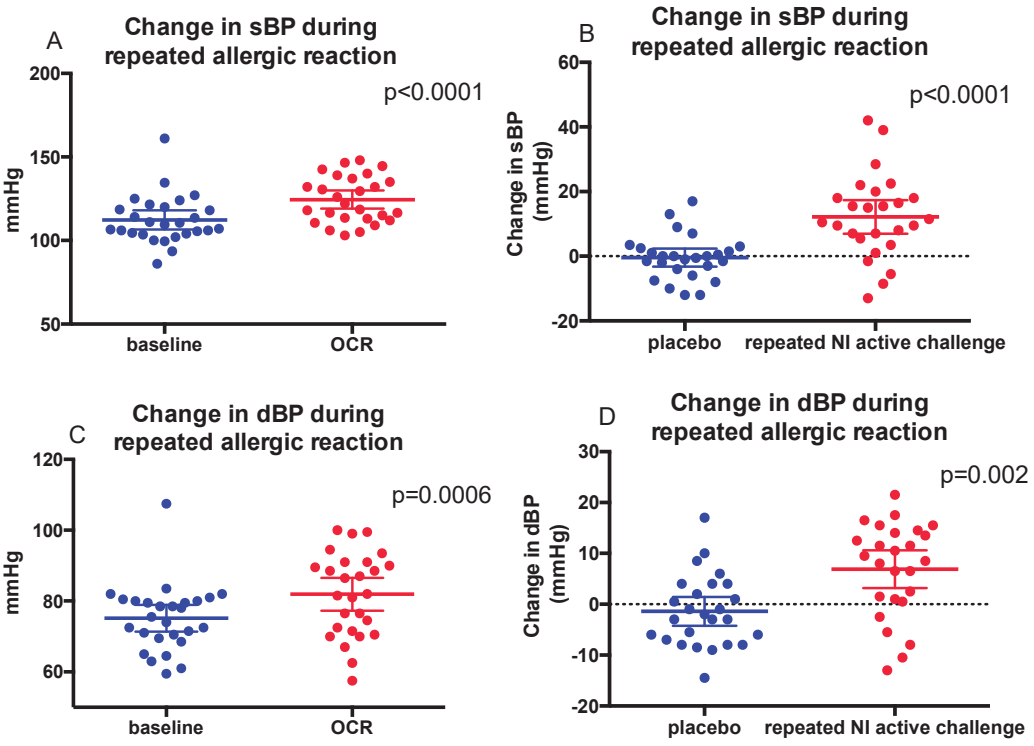
Change during repeated peanut allergic reaction at time of OCR (A), and on active day compared to placebo day (B). Shown is mean and 95% CI.

3.3.11.2 Change in vascular parameters:

3.3.11.2.1 Peripheral BP:

Results in Figure 50 show a significant increase in both sBP (mean increase 12.0mmHg, 95% CI [6.9, 17.4]) and dBP (6.9mmHg [3.2, 10.6]) on active day at time of OCR and on active day compared to placebo day.

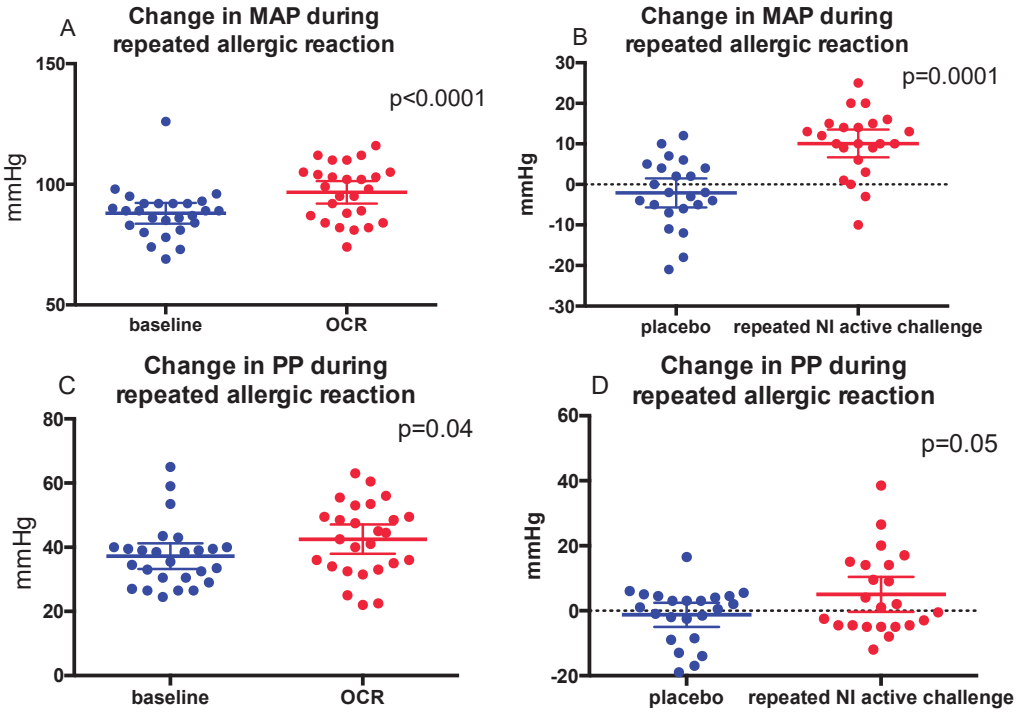
Figure 50 Change in sBP on repeated challenge:



Change during repeated peanut allergic reaction at time of OCR for sBP (A) and dBP (C), and on active day compared to placebo day for sBP (B) and dBP (D). Shown is mean and 95% CI.

Figure 51 shows a significant increase in MAP and PP on active day at time of OCR and on active day compared to placebo day.

Figure 51 Change in MAP and PP on repeated challenge:

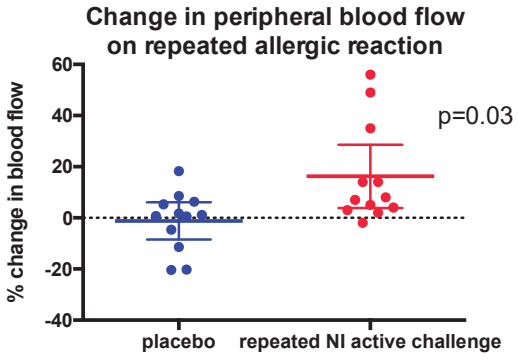


Change during repeated peanut allergic reaction at time of OCR (A and C) and compared to placebo day (B and D). Shown is mean and 95% CI.

3.3.11.2.2 Peripheral blood flow:

Results in Figure 52 show a significant increase in peripheral blood flow on active day compared to placebo day (median increase, IQR).

Figure 52 Change in peripheral blood flow on repeated challenge:

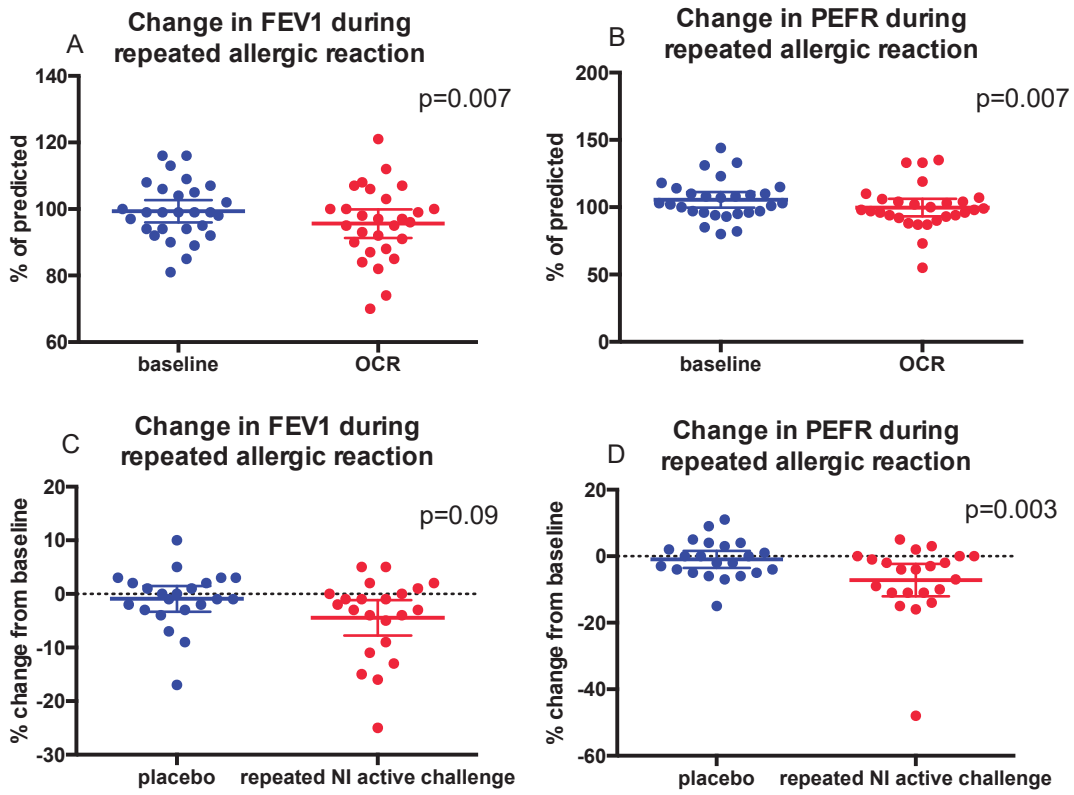


Change in blood flow at onset of objective symptoms during peanut allergic reaction and at an equivalent time during placebo challenge. Shown is mean and 95% CI.

3.3.11.3 Lung function:

Figure 53 shows a significant decrease in % of predicted FEV1 (median decrease -2.5%, IQR [-1.1, -7.7]) and PEFR (-4.0%, [-2.3, -12]) on active day at time of OCR but only a significant decrease in % of predicted PEFR is seen on active day compared to placebo day.

Figure 53 Change in lung function on repeated challenge:



Change in % of predicted FEV1 and PEFR on active day at time of OCR (A and B) and on active day compared to placebo day (C and D). Shown is mean and 95% CI.

3.3.11.4 Association between CVS changes and severity of reaction:

The reactions of those participants who went through a repeated NI challenge were classified according to the need of IM adrenaline as rescue medication, NIAID, Ewan&Clark and WAO severity scoring in the same way as for baseline challenges. Results show no significant difference for HR, SV, sBP, dBP and peripheral blood flow, between those participants who had anaphylaxis and those who did not and no significant difference between those participants who were classified as having a severe reaction with those who did not. Results are shown in Appendix 19.

We were unable to reproduce the association between SV, HR and participant´s VAS GI symptoms on these repeated NI challenges shown in Appendix 19. Correlation between HR, SV and clinician´s VAS score for repeated NI challenge is shown in Appendix 19.

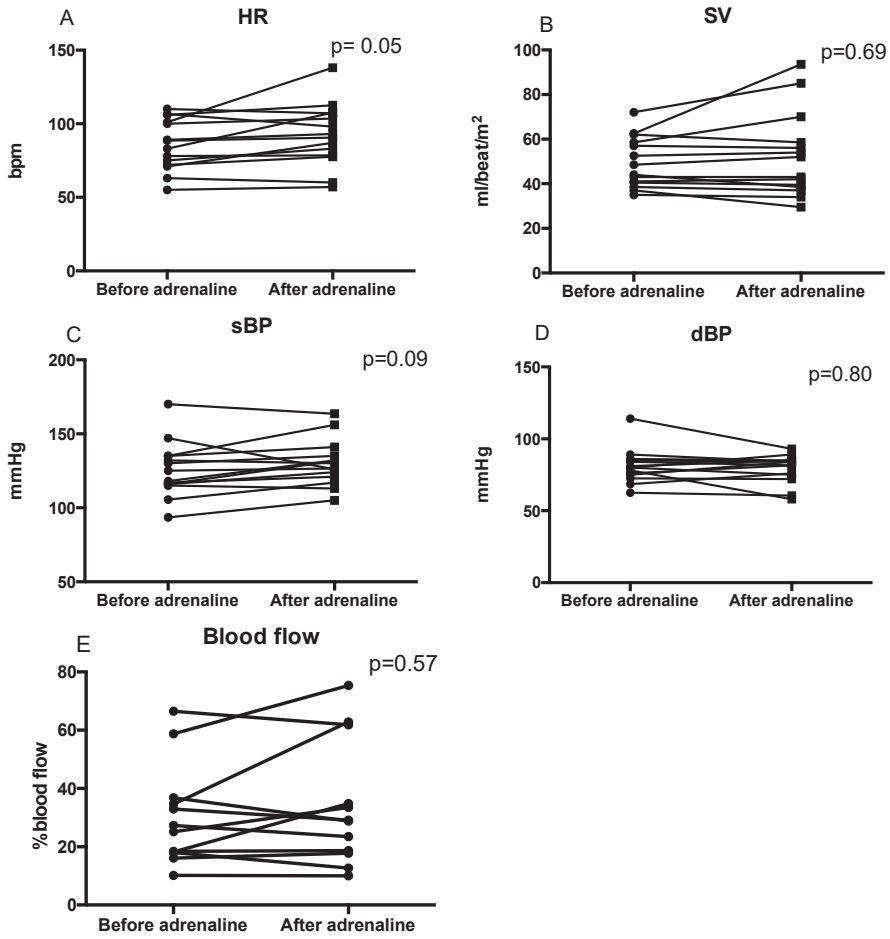
3.3.12 Effects of IM adrenaline on cardiorespiratory physiology:

3.3.12.1 Cardiovascular physiology parameters:

Seventeen participants in a total of 21 challenges required IM adrenaline as a rescue medication; data for 12 participants in 14 challenges for CVS physiology measurements and lung function were studied. Reason for missing data was monitor not available (n=1), faulty functioning of the monitor (n=3) or data from exercise challenge (n=4), which we are not including in this analysis. The data for this section of results includes baseline, sleep deprivation and non-intervention challenges as part of the TRACE study described in Methods section 2.1.

Figure 54 shows a borderline statistical increase in HR (mean increase 6.8bpm, 95% CI [-0.1, 10]) and a trend towards an increase in sBP (mean increase 4.8mmHg, 95% CI [-1.4, 10.9]), although this is not statistically significant, during the 10 minutes after administration of IM adrenaline. No significant differences were seen in any of the other parameters after IM adrenaline was administered. Of the 14 challenges analysed, 13 had nebulised salbutamol as treatment together with IM adrenaline.

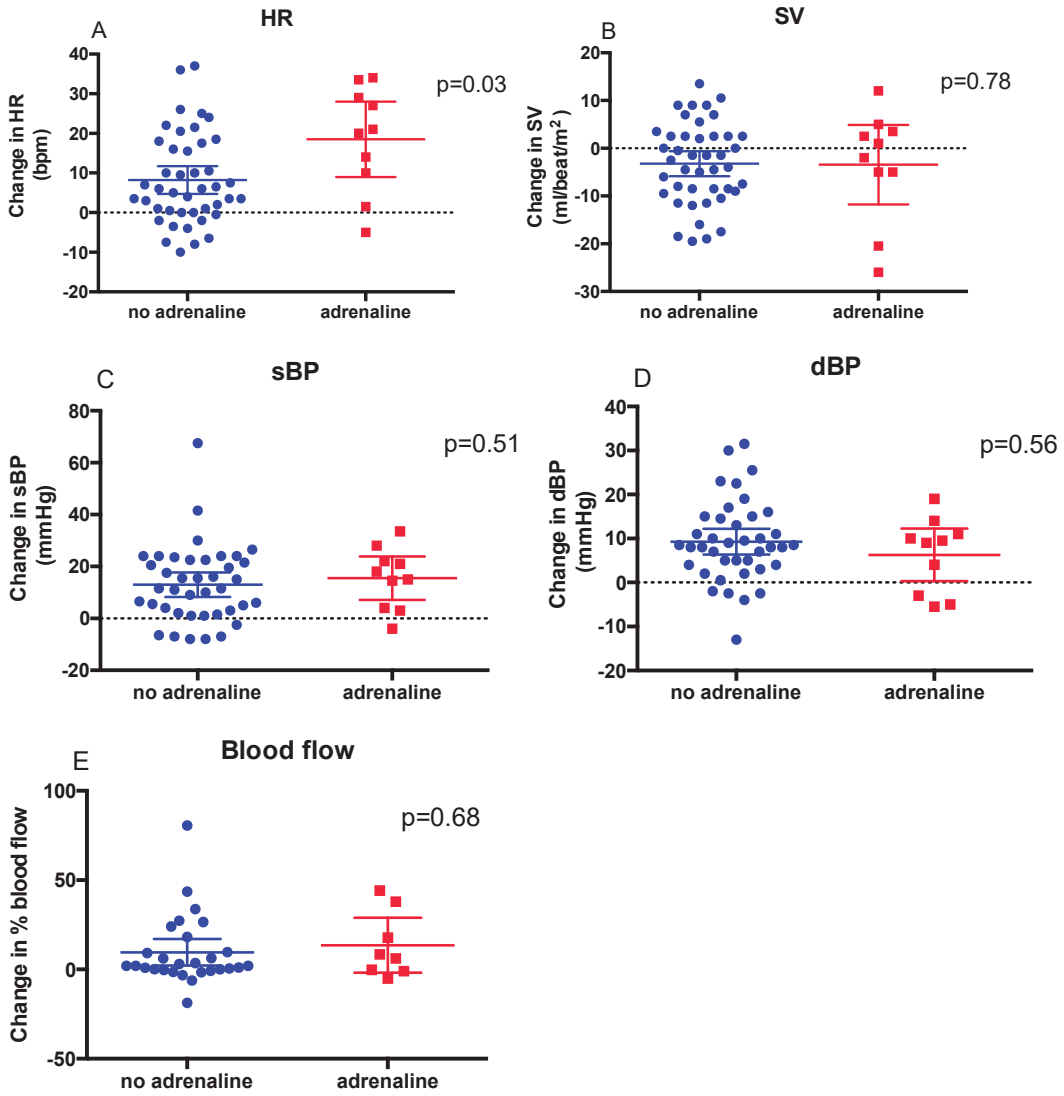
Figure 54 Changes with the use of IM adrenaline:



Change in (A) HR, (B) SV, (C) sBP, (D) dBP and (E) blood flow before and after 0.5mg of IM adrenaline were administered.

Figure 55 shows a significant increase in HR during the 10-minutes after administration IM adrenaline compared to the 10-minutes after treatment of those participants who did not require IM adrenaline as rescue medication. No differences were found for SV, sBP, dBP and blood flow.

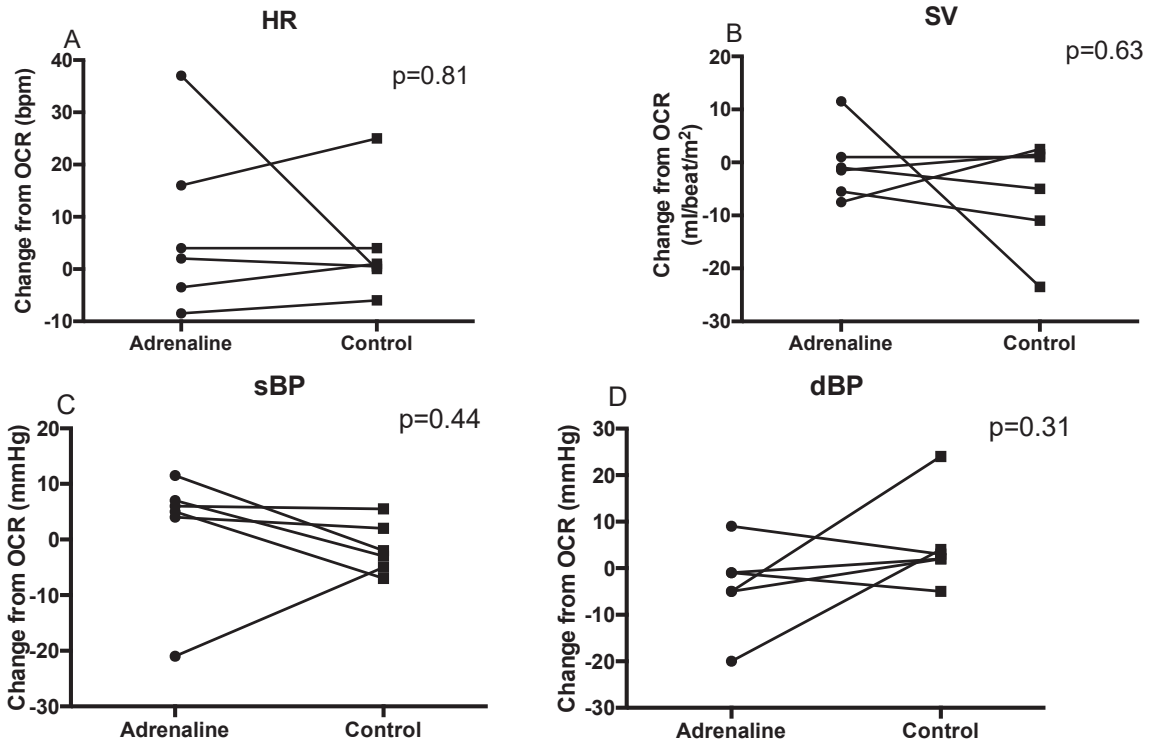
Figure 55 Differences according to the use of IM adrenaline:



Difference in (A) HR, (B) SV, (C) sBP, (D) dBP,and (E) blood flow between the 10 minutes after administration of IM adrenaline and the 10 minutes after other non IM adrenaline treatment during the baseline allergic reaction. Shown is mean and 95% CI.

Those participants who required adrenaline were compared to themselves on a repeated challenge, data for 6 participants was available and analysed; results in Figure 56 show no significant differences in any of the CVS physiology parameters studied regardless of the treatment given. This analysis was not performed for blood flow, as data was only available for 2 participants.

Figure 56 Differences in CVS parameters:



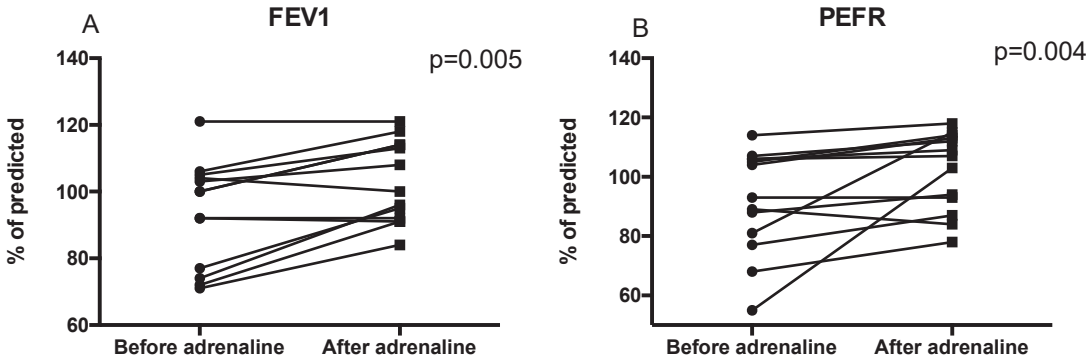
Difference in HR (A), SV (B), SBP (C) and DBP (D) between the 10-minute epoch post IM adrenaline administration and 10-minute epoch post OCR on a challenge that didn´t require adrenaline for the same participant,both compared to the 10-minute epoch before OCR.

3.3.12.2 Lung function:

Respiratory compromise and reduced lung function with a drop in % of predicted FEV1 and/or PEFR was present in most of the severe reactions and was the reason for IM adrenaline administration in 13 of the 14 challenges requiring adrenaline.

Results in Figure 57 show a significant increase in % of predicted FEV1 (median increase 10%, IQR [0,17]) and PEFR (7.5% [3.3, 10]) after IM adrenaline was administered.

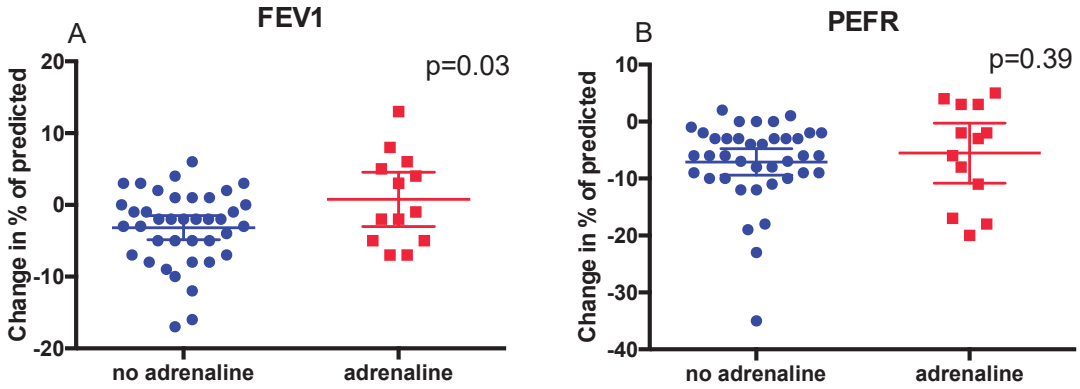
Figure 57 Change in lung function with use of adrenaline:



Change in % of predicted FEV1 (A) and PEFR (B) before and after IM adrenaline was administered.

Results in Figure 58 show a significant increase in FEV1 during the 10-minutes after administration of IM adrenaline compared to the 10-minutes after treatment of those participants who did not require IM adrenaline as rescue medication, no changes were found for PEFR.

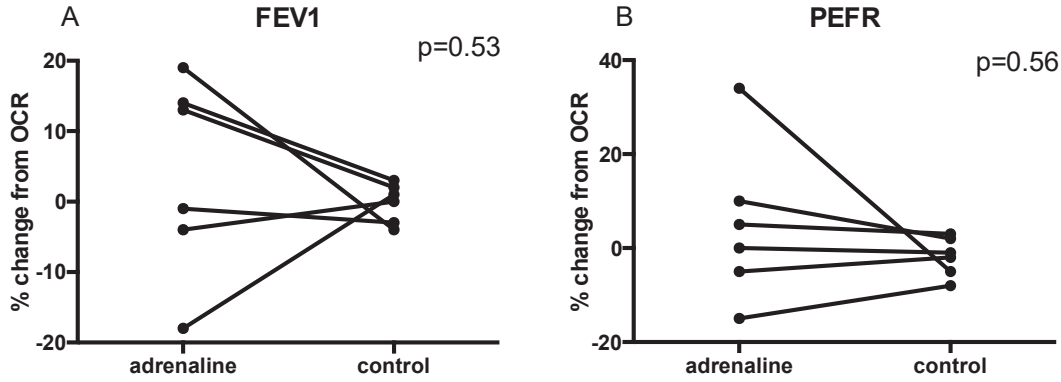
Figure 58 Difference in lung function:



Change in % of predicted (A) FEV1 and (B) PEFR between those participants who required adrenaline and those who did not. Shown is mean and 95% CI.

Figure 59 shows no significant difference for %FEV1 and PEFR from OCR after treatment between a challenge day when IM adrenaline was required as rescue medication and a day that another type of treatment was required.

Figure 59 Change in lung function:



Change in (A) FEV1 and (B) PEFR between the 10-minute epoch post IM adrenaline administration and 10-minute epoch post OCR on a challenge that didn´t require adrenaline for the same participant, both compared to the 10-minute epoch before OCR.

3.4 Discussion:

In this cross-sectional study of 52 adults undergoing peanut-induced allergic reactions under medical supervision, we observed a number of significant changes in cardiovascular parameters, including an increase in HR (mean 7.7bpm at baseline, 6.0bpm at repeat challenge) and BP (sBP: 17.5mmHg baseline, 12.0 repeat, dBP: 10.2mmHg baseline, 6.9 repeat), decreased SV (mean 2.3ml/beat/m^2 baseline, 4.0 repeat) and increased peripheral cutaneous blood flow. These changes are all apparent at around 30 minutes prior to objective clinical reaction. As expected, we also found obstructive changes in respiratory physiology with reduced FEV1 (mean decrease 3% baseline, 2.5% repeat) and PEFR at time of reaction, and biochemical changes suggesting mast cell degranulation (increased mast cell tryptase) and adrenergic hormone release (increased plasma adrenaline). There was an inverse correlation between change in SV and change in HR (r=-0.42), suggesting that at least some of the tachycardia, which accompanies reactions is a compensatory response produced by sympathetic drive to maintain cardiac output. Interestingly, although the cardiovascular changes were generally greater in participants judged as having suffered from anaphylaxis, there was significant overlap such that the magnitude of the cardiovascular changes could not be predicted from observed symptoms. While SV and HR changes were associated with increased gastrointestinal symptoms in baseline challenges, this finding was not reproduced in the repeat challenge. Finally, we observed only minor CVS changes following treatment with IM adrenaline, with increased HR 6.8bpm, which may have been due to the underlying reaction. In contrast, measures of respiratory obstruction changed significantly following IM adrenaline, FEV1 increased by 10%, and PEFR by 7.5%, again, the influence of the underlying allergic reaction or its treatment on these changes is unclear as 13 out of the 14 participant who required IM adrenaline also had nebulised salbutamol as treatment.

Overall our findings suggest that significant CVS changes occur during peanut allergic reactions, and this has important implications for clinical management. We hypothesise that release of mast cell mediators triggers fluid redistribution to the intestinal tract and skin, the latter due to peripheral vasodilation, and this results in reduced cardiac preload and therefore SV. This drives a sympathetic compensatory response (as evidenced by the increase in plasma adrenaline levels), which results in an increase in HR and BP. The resulting increase in CO is greater than that which might be expected, as CO is not just maintained but also increases during peanut allergic reactions. Result for CO on repeated challenge showed no change from baseline due to a smaller increase in HR on this repeated challenge, which could be explained by less anxiety on the repeated challenges despite still having a significant decrease in SV similar to that observed on baseline challenge. We also notice that

the rise in BP precede HR and SV suggesting the possibility that change in BP may relate to early symptoms such as stomach pain while SV and HR may relate to peripheral vasodilation happening closer to OCR. However these changes are not clinically or physiologically important in healthy subjects but they can be during food allergic reactions.

Reports from severe and fatal anaphylaxis imply that distributive shock and decreased SV can potentially lead to fatal outcome, although these changes have not been reported for more typical, non-life-threatening reactions until now. Data from fatal anaphylaxis series describe cardiac arrest after postural changes to an upright or standing position after food anaphylaxis[164], which might be due to a failure in cardiovascular compensation. Similar changes were observed during both baseline and non-intervention challenges in this study. Therefore, at least in our cohort, these changes are consistent and reproducible.

The fall in SV and rise in HR were associated with severity of abdominal symptoms as reported by both participants and investigators, however this association was not reproduced in the NI challenges. An interesting case report describes the case of anaphylactic shock with predominance of GI symptoms, showing diffuse bowel wall oedema on computed tomography scan performed as result of no improvement of GI symptoms after treatment of anaphylaxis including IM adrenaline, IV steroids, antihistamines and fluids[165]. This shows the importance of GI symptoms, which can be present in up to 40% of patients suffering anaphylaxis[322, 323]. The increase in blood pressure is harder to explain, as it does not correlate with reduced SV so may be caused by a combination of a compensatory response for peripheral vasodilation, and a pain or anxiety response. This could also be due to some degree of imprecision in these measurements due to the variability in time of reaction from participants.

Data from studies in patients with asthma have reported decreased SV due to increased intrathoracic pressure during acute asthma episodes in children[324]. In our cohort, a significant fall in FEV1 was observed at time of reaction, although the magnitude of the change is far more modest than that seen during acute asthma exacerbations. We do not therefore consider that the drop in SV was due to changes in intrathoracic pressure, and this is substantiated by the poor correlation between changes in SV and changes in lung function. The fall in SV is more likely due be due to a fall in preload.

Increases in HR during drug and venom anaphylaxis have been previously described in humans[168] and in animal models of anaphylaxis[138], consistent with our findings. A small but significant decrease in HR was observed at onset of subjective symptoms during peanut challenges, perhaps due to relaxation at the start of the challenge. HR then increased in the 30-40 minutes prior to OCR. The reasons for this may include anxiety or pain together

with compensatory mechanisms. We did not however identify obvious evidence that HR or other CVS changes differed according to challenge order, which might be expected to influence anxiety level i.e. participants who underwent placebo challenge following active challenge may be more relaxed at placebo challenge than those who underwent placebo first. However, we did not find an effect of challenge order, thus the changes seen in HR are unlikely to be related to anxiety over the expectation of symptoms prior to their occurrence. The relative contribution of pain/discomfort is harder to evaluate.

Limited data are available for blood pressure changes during food allergic reactions of any severity; the little data available from prospective studies shows that low blood pressure is not necessarily related to severe outcomes [175]. Data from reports of fatal food anaphylaxis[164] describe a drop in blood pressure but this data is usually collected when the patient arrives at the emergency department and the reaction has evolved to a more severe grade. Our data almost certainly reflect the initial cardiovascular response to an allergic reaction, with an initial tachycardia and maintained or increased blood pressure levels due to increased peripheral resistance. Safety was our first priority, with the incidence anaphylaxis limited by the challenge protocol, thus limiting the severity of reactions seen. It is therefore not unexpected that we did not observe hypotension in this cohort.

Given the nature of a food challenge, anxiety can impact significantly on the occurrence of subjective symptoms, such as oral itch. Likewise, anxiety might impact upon CVS parameters. The study design incorporated a modified version of the PRACTALL consensus[288] in order to determine the stopping criteria for the challenge in as objective manner as possible. Subjective symptoms that were classified as potential stopping criteria in the original consensus document were relegated to more mild symptoms in the modified version, therefore increasing the objecivity but also possibly the threshold for severity of reactions. The use of more objective criteria allowed better discrimination between objective symptoms and those symptoms that, to some extent, might be confounded by anxiety. Results for catecholamines show that adrenaline was significantly increased on peanut allergic reactions while noradrenaline remain unchanged this suggests that only selectively the adrenergic pathway mediated by the sympathetic nervous system was activated.

To date, there is no consensus as how to classify severity of food allergic reactions. Multiple severity scorings have been published but usually made to fit a certain population studied and therefore difficult to apply to other different scenarios. It therefore becomes challenging to evaluate whether these cardiovascular changes are associated with reaction severity. We evaluated severity using several objective and subjective measures. While changes in HR, SV and BP were generally greater in participants with anaphylaxis than in participants

without anaphylaxis, measured by IM adrenaline use, NIAID criteria or WAO criteria, the difference between anaphylaxis and non-anaphylactic reactions was generally not statistically significant, with complete overlap in the range of values for anaphylaxis and non-anaphylaxis. Thus, reliable clinical discrimination of the patient with significant cardiovascular changes during allergic reaction is not likely to be possible using standard assessment tools. However the importance of participant safety means that the study protocol did not allow the full range of reaction severity, and relatively few reactions were classified as anaphylaxis, hence there is significant uncertainty in this finding. It is likely, although not confirmed, that more significant changes might occur in fatal and near-fatal anaphylaxis[201].

Of note, aside from statistical borderline change in HR, no significant cardiovascular changes were found following IM adrenaline during anaphylaxis, which suggest that a single dose of IM adrenaline may not have a major effect on the cardiovascular system in the context of peanut anaphylaxis. Interestingly, significant changes were seen in the lung function after IM adrenaline, and in most of the cases this treatment was given due to reduced lung function with clinical symptoms. While it is tempting to speculate that IM adrenaline may therefore be targeting receptors in the lungs more effectively than in the CVS, further studies are needed to confirm this since most participants given IM adrenaline were also treated with nebulised salbutamol, which has known bronchodilator and cardiac chronotropic effects. CVS changes during peanut anaphylaxis may be relatively non-responsive to parenteral (and perhaps endogenous) adrenaline in food-induced anaphylaxis, which could explain both the lack of correlation between adrenaline use or adrenaline levels and objective CVS measures in this study, as well as the observation that one in three cases of fatal anaphylaxis occur despite timely adrenaline[201].

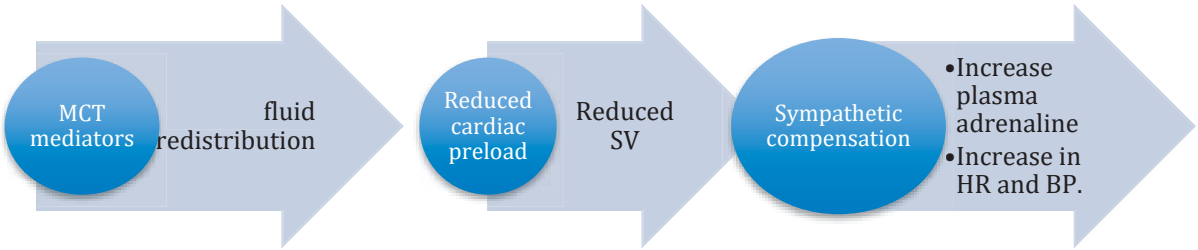
To our knowledge, there are no other reports in the literature describing cardiovascular changes during food-induced allergic reactions in humans. Patients underwent a number of supervised oral food challenges with detailed, prospective cardiovascular monitoring, providing a unique opportunity to not only study changes, but also the effect of treatment. We sought to confirm our findings by analysing CVS changes in the repeat challenges participants underwent as part of the TRACE study. Reassuringly, we observed the same CVS changes as in the first challenges undertaken.

Although we identifed clear changes in the cardiovascular system during peanut allergic reactions, it is important not to over-interpret our data. Cardiovascular monitoring was performed using non-invasive means; data on the methods section show a good correlation between bioreactance and echocardiography in measuring SV. These were very acute

changes and we may be seeing an overcompensation of HR and BP given the changes found for CO at least at baseline challenges. The findings may be limited by the size of the study cohort, and all our participants were relatively healthy and mostly with a prior history of non-severe reactions. Pre-existing cardiovascular disease has been shown to be a risk factor for fatal anaphylaxis; therefore, there is a possibility that the changes seen may be more significant in those with pre-existing cardiovascular disease.

In summary, we have observed significant changes in the cardiovascular system during food allergic reactions, which are not closely related to investigator-evaluated or patient-reported reaction severity. Peripheral vasodilation and reduced cardiac stroke volume appear to be commonplace during peanut allergic reactions, and trigger a set of compensatory mechanisms driven by sympathetic activation. Clinicians should therefore have a low threshold to take steps to maintain venous return during systemic allergic reactions to food, including postural support (lying patients flat, with the legs raised) and early intervention with fluid replacement. Further studies are required to develop a reliable, non-invasive tool to identify and monitor changes in cardiac preload or stroke output during allergic reactions, which might be used for guiding therapy. Given the lack of a valid biomarker for reaction severity, such studies are likely to impact significantly on our knowledge of the pathophysiology of anaphylaxis.

PROPOSED HYPOTHESIS:



4. CARDIAC ELECTRICAL CONDUCTANCE CHANGES DURING IGE-MEDIATED PEANUT ALLERGIC REACTION:

ABSTRACT:

Data from drug and hymenoptera venom anaphylaxis and near-fatal anaphylaxis describe myocardial damage known as Kounis syndrome, defined as the concurrence of acute coronary syndromes associated with mast-cell and platelet activation, in the setting of an allergic or anaphylactic reaction. Very little data is known about Kounis syndrome or any type of acute coronary disease in the context of food allergy or food anaphylaxis and no cases have been described with peanut allergy. Heart rate variability has been described in children undergoing controlled food challenges and the analyses of different parameters allowed the challenge to be terminated up to 17 minutes before trained physicians.

Fifty-seven participants undergoing DBPCFC to peanut were continuously monitored with a 12 lead ECG. The parameters analysed were PR, QT, corrected QT (QTc) intervals, QRS complex and ST segment. ST elevation was manually measured at J point, 40 and 60ms from J point. The parameters analysed for HRV were for time domain, standard deviation of the normal-normal R-R interval (SDNN). SDNN reflects all the cyclic components responsible for variability in the period of recording. For frequency domain, low frequency normalised unit (LF (n.u.)), which is a general indicator of aggregate modulation of both the sympathetic and parasympathetic branches of the ANS and high frequency normalised unit (HF (n.u.)), which is the index of modulation of the parasympathetic branch of the ANS.

HRV results showed significant changes at time of objective clinical reaction on active days and on active days compared to placebo days suggesting sympathetic activation. However these changes were not reproduced on repeated peanut challenges on a smaller sample of the same population. No significant changes were seen for any of the other cardiac rhythm parameters analysed or ST elevation.

These results suggest a possible anxiety component during peanut allergic reactions that may be responsible for the HRV changes however no significant correlation was found between patient´s reported anxiety and HRV changes therefore further studies are required. Cardiac rhythm does not seem to be involved or altered in food allergic reactions.

4.1.Introduction:

Food allergic reactions typically happen outside a medical facility where cardiac monitoring is usually not performed; therefore, data on the electrical conductance of the heart during an allergic reaction is limited.

Significant numbers of mast cells, comprising 0.5-1.5% of all cells, have been isolated from atrial appendages in human heart tissue[181] and these are a source of different mediators including leukotrienes (LTC4) and prostaglandins (PGD2), which cause a rapid and sustained increase in coronary vascular resistance[182]. Histamine receptors in atrial and ventricular myocardium mediate a positive chronotropic and inotropic response (H2 receptors) and coronary artery vasoconstriction (H1 receptors). Administration of intravenous histamine as an infusion into healthy, non-allergic volunteers caused PR prolongation and the occurrence of atrioventricular (AV) block[325]. The incidence and duration of the AV block was dose-related, and a shortening of PR interval along with prevention of the arrhythmias could be achieved by administration of antihistamines[325]. Platelet activating factor causes a negative inotropic effect[185] and induces arrhythmias, as it also delays A-V conduction[186]. Thus, the existing data from healthy volunteers suggest the possibility of electrical conductance changes during systemic IgE-mediated allergic reactions to peanut, which may potentially be of clinical significance.

Clinical reports document acute coronary changes during severe allergic reactions. Myocardial damage has been reported in case studies of anaphylaxis irrespective of cause, known as Kounis syndrome. This is defined as the concurrence of acute coronary syndromes associated with mast-cell and platelet activation, in the setting of an allergic or anaphylactic reaction[326]. Three types of Kounis syndrome have been reported: vasospastic allergic angina (type I, and the most common), allergic myocardial infarction (MI) (type II) and stent thrombosis with occluding thrombus infiltrated by eosinophils and/or mast cells (type III)[326]. The syndrome presents with a variety of ECG findings: ST elevation is the most common ECG change seen, typically in the inferior leads, followed by ST depression, any degree of heart block and cardiac arrhythmias. Although this syndrome has been described in all age groups, it is more common in those aged between 40-70 years old[327]. Chest pain is the most common clinical manifestation, and a past medical history of hypertension is a risk factors for Kounis syndrome[327].

Drug allergy is the most common trigger for Kounis syndrome, antibiotics being responsible for over 27% of cases[327]. Kounis syndrome is less common in food allergic reactions, perhaps due to the lack of food allergy studies involving cardiac monitoring and because food allergic reactions are frequently rapid in onset and generally occur away from a

medical facility, in the community. Food allergy-triggered Kounis syndrome has been reported during allergic reactions to seafood and kiwi fruit, but there are no existing case reports where peanut was the trigger[327].

Heart rate variability (HRV), the physiological variation in the beat-to-beat interval, is considered a measure of neuro-cardiac function and representing autonomic nervous system (ANS) balance[312]. It is increasingly being used to inform risk stratification and prognosis in diabetes[328], anesthesia[329], intensive care[329] and myocardial infarction[330], something aided by its non-invasive nature. HRV was previously reported to change during allergic reactions to food, in a paediatric population. HRV changes occurred up to 17 minutes earlier than trained clinicians were able to recognise that a definite allergic reaction was occurring, suggesting the possibility of using HRV as an early marker of allergic reaction during oral food challenges[197]. Overall, the prevalence and severity of electrical conductance changes during allergic reactions to food have not been systematically studied in a well-characterised population, either in relation to arrhythmia risk, electrical signs consistent with Kounis syndrome, or HRV changes which might potentially serve as an early diagnostic read-out during supervised food challenges.

These aims of this chapter are to:

1. Characterise the electrical conductance cardiac response to an acute peanut allergic reaction, with a focus on changes in cardiac rhythm, ST segment changes, which might be suggestive of Kounis syndrome, and HRV.
2. Describe how these changes may contribute to arrhythmias and CVS physiology dysfunction, in terms of timing and relation to the severity of the allergic reaction.
3. Assess the effects of intramuscular (IM) adrenaline on electrical conductance cardiac changes, when used as a treatment for peanut allergic reactions.

4.2 Methods:

4.2.1 Study protocol:

This has been previously described in the methods chapter section 2.1, and in the cardiovascular physiology changes section 3.2.1 and 3.2.2.

4.2.2 Study procedures:

Participants underwent a double-blind placebo controlled food challenge (DBPCFC) to peanut, in which 8 incremental doses were administered orally using a validated blinding recipe at 30-minute intervals.

Participants were continuously monitored using a 12-lead ECG Holter monitor (SEER 12, GE Healthcare, Chicago, United States). Data was downloaded, normal QRS complexes were manually selected for analysis, data was analysed using MARS ambulatory ECG analysis system (GE Healthcare, Chicago, United States) and manually copied into an excel file. The parameters analysed were PR, QT, corrected QT (QTc) intervals, QRS complex and ST segment (shown in Figure 60). Corrected QT interval was automatically measured by the MARS system using Bazett´s formula, but also manually computed using Fridericia´s [331] and Framingham´s [332] formulae (shown in Figure 61). ST elevation was manually measured (shown in Figure 62) at J point, 40 and 60ms from J point at leads II (when this lead presented artefacts lead III was measured for ST elevation), V2 and V6 in order to cover the territories of the circumflex artery, right coronary artery and left anterior descending artery respectively. As discussed in Methods section 2.5.3.1.2, ST elevation analysis was undertaken blind to participant characteristics or challenge outcome, and measurements were validated by a qualified consultant cardiologist as the “gold standard”.

The data obtained from the Holter monitor were saved as a text file and analysed using the Kubios program (version 3.0.0 Kuopio, Finland) for heart rate variability (HRV), artefact correction applied to remove excessively long/short R-R intervals, and results were automatically downloaded in 5-minute epochs as an excel file. The parameters analysed for HRV were for time domain, standard deviation of the normal-normal R-R interval (SDNN). SDNN reflects all the cyclic components responsible for variability in the period of recording[333]. For frequency domain, low frequency normalised unit (LF (n.u.)), which is a general indicator of aggregate modulation of both the sympathetic and parasympathetic branches of the ANS and high frequency normalised unit (HF (n.u.)), which is the index of modulation of the parasympathetic branch of the ANS[334]. The representation of LF and HF in normalized units (n.u.) emphasizes the controlled and balanced behaviour of the two branches of the ANS[333].

For linear regression, which is determined by interactions of CVS physiology, electrophysiological and humoral variables, as well as by autonomic and central nervous regulations; detrended fluctuation analyses-1 (DFA-1), approximate entropy (Apen) and sample entropy (Sampen) were analysed. These parameters were chosen following advice from two cardiologists (AL and CH) as being the most useful to determine neuronal influences on heart rate. The standard operation procedure (SOP) is shown in Appendix 11.

Figure 60 Components of the ECG complex between the start of the P wave and the end of the T wave:

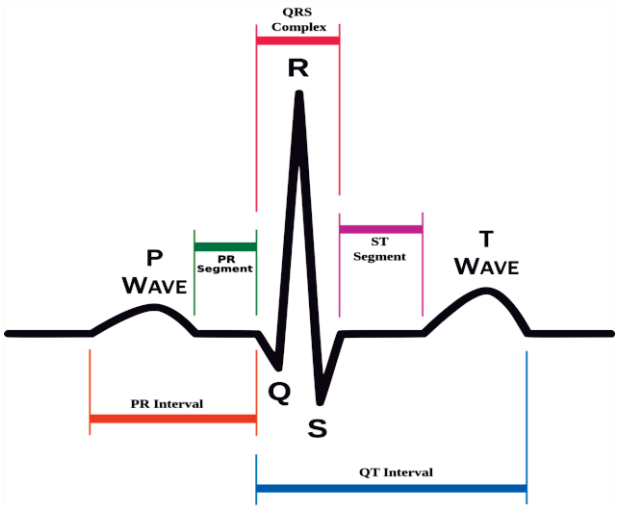


Figure 61 Formulas to manually calculate QTc:

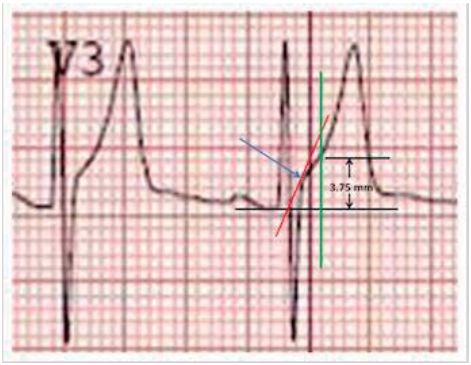
Bazett´s formula[335]: $QTc = QT/\sqrt{RR}$

Fridericia´s formula[336]: $QTc = QT/\sqrt[3]{RR}$

Framingham´s formula[332]: $QTc = QT+0.154 \times (1-RR)$

Legend: RR is the R to R interval calculated as 60/HR.

Figure 62 Location of J point:



The lower black line shows the lowest point of the PR segment; the red line shows the tangent at the beginning of the ST segment; the blue arrow shows the J point which is the junction between the termination of the QRS complex and the beginning of the ST segment. The green line shows 60ms from the J point.

4.2.3 Selection of data for analysis:

The time-epochs selected were a 10-minute epoch at baseline, prior to administration of the first dose of peanut (or placebo) and a 10-minute epoch at time of objective clinical reaction (OCR), consistent with the other cardiovascular data collected in this thesis. To determine when any observed changes began, 10-minute epochs between baseline and OCR were analysed. Data for the 4 hours prior to OCR were presented, since all changes occurred within this time-period, earlier time points contained significant missing data due to shorter duration of some food challenges. Time course data were included for the period during which data were complete for at least 20 study participants.

Each participant´s ECG was manually and individually checked minute-by-minute for the duration of the challenge for any abnormalities, such as arrhythmias, on both placebo and active day.

4.2.4 Analysis of reaction severity:

We evaluated whether there was a relationship between the magnitude of any significant ECG changes seen, and the severity of the associated allergic reaction. We used several different classifications of reaction severity, since there is no generally agreed measure of severity for food allergic reactions. These included anaphylaxis according to the National Institute of Allergy and Infectious Disease (NIAID) classification; Ewan and Clark food reaction severity score (with reactions divided into 2 groups: mild (1-3) and moderate/severe (4-5)); World Allergy Organization Subcutaneous Immunotherapy Systemic Reaction Grading System (with reactions divided into mild (1-2) and moderate/severe (3-5) (these grading systems are shown in Appendix 13-15); and the use of IM adrenaline for treatment. For the purpose of this thesis, persistent GI symptoms was defined as GI symptoms present for over 30 minutes and more than one episode of emesis/diarrhoea at least 20 minutes apart. On the day of the reaction, prior to leaving the medical facility, participants and clinician were given a visual analogue scale (0-10 shown in Appendix 16) in order to independently score the symptoms experienced throughout the food challenge. This scoring was correlated with electrical conductance changes and HRV changes as a possible measure of reaction severity.

4.2.5 Analysis of electrical conductance effects of intramuscular adrenaline:

The effect of IM adrenaline on the cardiac rhythm was explored as discussed in Methods section 2.7. Unfortunately, those participants who were given IM adrenaline on one challenge occasion but not on another repeated challenge, data was only suitable for analysis

for 2 participants, therefore this sub-analysis for the effects of IM adrenaline was not performed on this chapter.

4.2.6 Statistical analysis:

The approach used was identical to that used for analysis of cardiovascular physiology changes during IgE-mediated peanut allergic reactions, as described in chapter 3 section 3.2.6.

4.3.Results:

4.3.1 Study population:

Complete data for both challenge days was available for 52 out of a total of 57 participants, whose characteristics are described in Table 15. Reasons for missing data were: no monitor available (n=4) or technical failure (n=1) on one or both challenge days. Twenty-eight participants underwent a further open non-intervention (NI) challenge: data from this challenge was analysed for 22 participants, reason for missing data were: excessive artefact (n=4), technical failure (n=1) on the repeat NI challenge day or data not recorded (n=1).

Table 15 Characteristics of the study population:

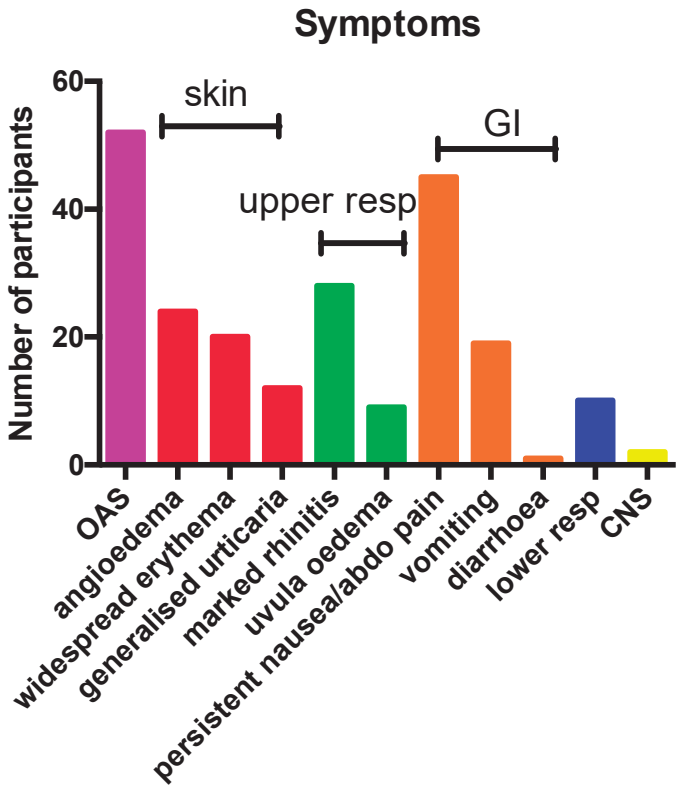
	Baseline challenge	**Repeated challenge (NI)**
Age at enrolment	24 (20,29) years	24 (21, 29)
Age at time of diagnosis	2 (1, 6) years	2 (1, 4) years
Sex (female)	29 (56%)	16 (73%)
Asthma	28 (54%)	13 (59%)
Rhinitis	39 (75%)	18 (82%)
Eczema	27 (52%)	12 (55%)
Total IgE	254 kU/L (118, 710)	239 kU/L (119, 576)
Specific IgE to peanut	14 kUA/L (3.9, 33.9)	15 kUA/L (3.7, 25.7)
Specific IgE to Ara h 2	7 kUA/L (2.1, 21.5)	10 kUA/L (2.3, 15)
SPT wheal to peanut extract	11mm (9, 15)	11mm (9, 13)
Sensitised to other nuts	31 (60%)	14 (61%)
Sensitised to other non-nut foods	30 (58%)	12 (52%)
Cumulative dose of peanut protein ingested	133mg (33, 433)	133mg (33, 133)
Baseline PR interval	144ms (133, 153)	N/A
Baseline QT interval	380ms (368, 415)	N/A
Baseline QTc interval	412ms (394, 465)	N/A
Baseline QRS complex	85ms (78, 94)	N/A
IM adrenaline during peanut challenge	10 (19%)	4 (17%)
NIAID (anaphylaxis)	14 (27%)	5 (23%)
Ewan & Clark (moderate/severe)	20 (38%)	8 (36%)
WAO (moderate/severe)	12 (23%)	3 (14%)
Mast cell tryptase (peak % change)	25% (7, 46)	N/A
Adrenaline (change from baseline)	0.32 nmol/L (-0.07, 0.89)	N/A
Noradrenaline (change from baseline)	0.01 nmol/L (-0.09, 0.11)	N/A

All data is shown as median and IQR unless specified otherwise. N/A: Data not available.

4.3.2 Summary of symptoms during peanut allergic reaction:

The symptoms experienced by participants during the challenge are summarised in Figure 63. Similar to the data presented in the previous chapter, all participants experienced oral allergy symptoms. Gastrointestinal symptoms (persistent nausea and stomach pain) were the next most common symptom. Almost 50% of participants experienced upper respiratory symptoms, marked rhinitis being the most common. Ten participants had lower respiratory symptoms and required intramuscular (IM) adrenaline as rescue medication. Two participants showed symptoms of altered level of consciousness lasting for less than 2 minutes, with no recurrence or progression to other organs. Our participants rated severity of symptoms as most severe for throat and abdominal, although severe symptoms were reported in every domain by at least one participant shown in Figure 64.

Figure 63 Symptoms during DBPCFC to peanut for the 52 participants analysed in this chapter:



Summary of the most common symptoms participants experienced during an allergic reaction separated by organ.

Figure 64 Symptom severity: for the 52 participants analysed in this chapter:

	Skin	Upper resp	Lower resp	GI	Anxiety	Overall
02003	8	6	0	0	4	4
02004	0	7	2	7	2	6
02008	1	2	0	1	1	5
02010	6	6	4	9	8	6
02018	4	4	1	8	3	5
02019	8	6	6	8	7	7
02023	1	8	3	8	1	6
02024	0	6	1	5	5	5
02025	1	4	3	7	4	4
02034	3	8	7	4	4	7
02037	5	6	8	7	0	7
02040	1	6	1	7	6	7
02045	6	8	8	7	8	8
02047	0	6	6	8	6	8
02048	8	5	3	8	5	5
02049	6	7	0	3	5	4
02051	4	6	2	7	2	4
02053	2	1	0	4	5	4
02055	6	3	0	6	1	4
02056	7	5	2	8	5	6
02063	5	4	0	0	1	4
02064	3	8	1	3	0	4
02065	0	8	0	4	1	7
02066	7	6	2	8	6	7
02067	7	8	0	6	7	7
02073	1	3	0	6	3	3
02074	7	7	0	8	3	7
02075	0	5	0	8	9	3
02078	5	4	5	6	3	5
02079	0	8	2	10	7	7
02080	3	6	3	8	2	6
02081	4	6	4	9	2	6
02087	4	2	7	4	7	7
02090	2	3	0	3	3	3
02091	3	6	4	1	5	3
02092	7	5	4	0	4	6
02093	3	5	3	5	0	5
02094	2	6	0	7	4	5
02097	3	4	0	7	1	4
02098	0	3	0	6	10	10
02100	5	8	7	0	0	6
02103	2	3	3	6	2	4
02104	1	7	6	7	1	8
02106	8	6	6	6	9	8
02108	2	8	4	4	4	5
02109	0	6	0	8	2	7
02111	3	2	3	0	1	1
02112	3	5	2	6	6	5
02117	0	4	2	4	2	6
02120	6	5	0	0	7	6
02121	3	1	0	0	2	2
02124	5	7	1	4	3	6

Heat map for the self-reported VAS symptom score for each organ, anxiety and overall reaction score.

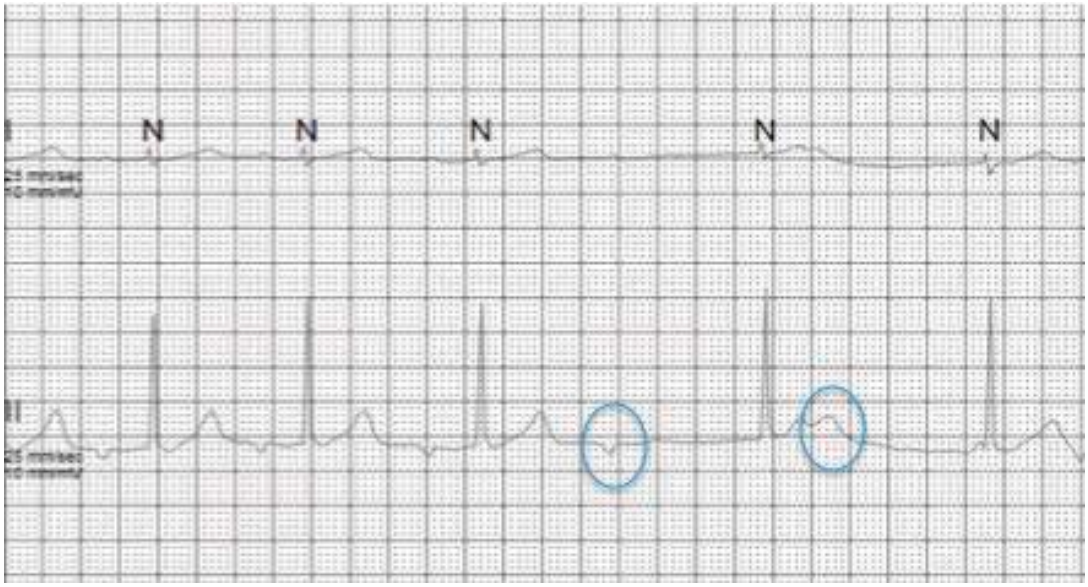
4.3.3 Electrocardiographic changes:

4.3.3.1 Case descriptions of acute ECG changes during peanut challenge:

Each participant´s ECG was manually checked minute by minute throughout the duration of the challenge for any abnormalities in electric conductance. Atrio-ventrocular (AV) block were identified in 2 participants during their active peanut challenge, which were not observed at baseline or on the placebo challenge day (consistent with exclusion criteria for the TRACE study, including a previous medical history of cardiac impairment (Methods, section 2.1.2).

Participant 02008 experienced a second-degree AV block type 2 (Figure 65) where the PR interval remains unchanged but there is a non-conducted P wave with no associated QRS complex, seen in two subsequent heartbeats (the P wave is superimposed on the T wave on the second heartbeat). No similar abnormalities were seen at any further open challenges for this participant during the TRACE study. The occurrence of the AV block coincided with objective symptoms of an allergic reaction, namely intense and persistent stomach pain and nausea, but no chest pain or other cardiac symptoms. Vital signs were within normal limits (BP 107/66 mmHg, HR 63bpm) at the time.

Figure 65 ECG strip from participant 02008:



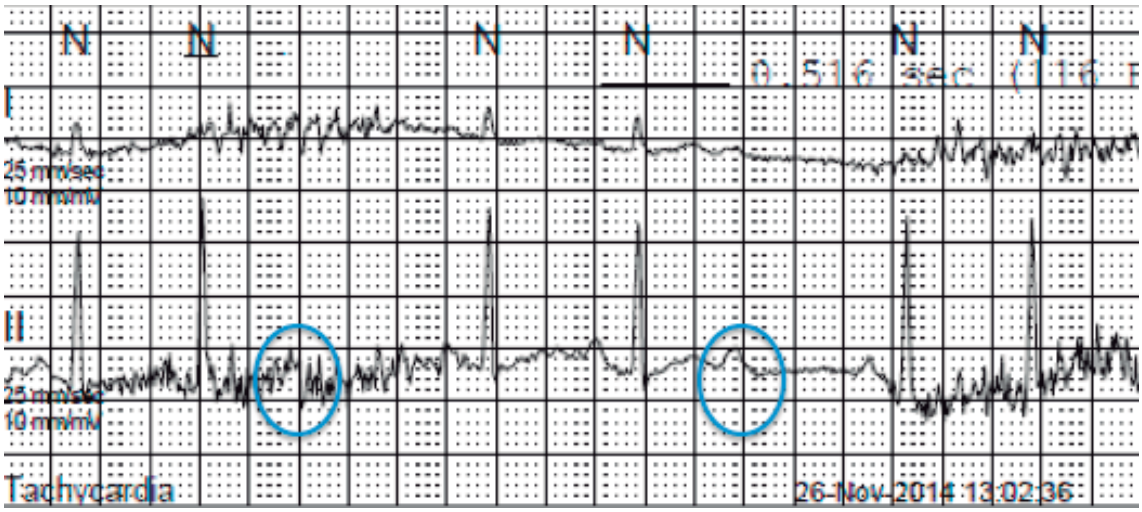
Non-conducting P waves (shown in blue circles) on the ECG strip during the active challenge of the DBPCFC.

Participant 02014 also experienced AV block type 2 (Figure 66), similar to the previous participant. At the time of the AV block the participant complained of light-headedness, and clinically presented with skin pallor and a tachycardia of 116bpm, but normal BP of 117/73 mmHg. No other symptoms were seen during the further 4 hours of supervision.

Due to concerns of a dysrhythmia, the Study Safety committee excluded this participant from the rest of TRACE study, and so no further challenges were undertaken. Of note, at the time of the AV block, the intravenous cannula was being manipulated, as it was not working, therefore a pain or vagal stimulus may have contributed to the symptoms. The AV-block occurred 10 minutes after the dose was given (dose 3) and there were no signs or symptoms of an allergic reaction at the time.

Due to the symptomatic nature of this episode, the participant had echocardiography performed subsequently, with normal results.

Figure 66 ECG strip from participant 02014:

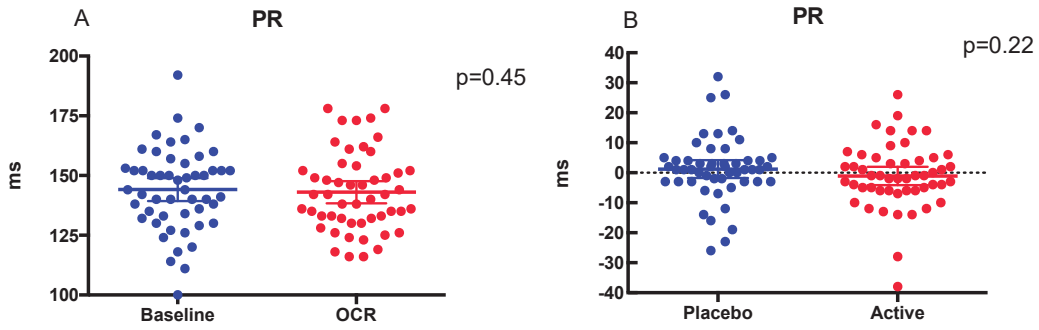


Non-conducting P waves (in blue circles) on the ECG strip during the active challenge of the DBPCFC.

4.3.3.2 PR interval:

Change in PR interval is shown in Figure 67. No significant changes were found on active day at time of OCR compared to baseline (Figure 67A) or on active day compared to placebo day (Figure 67B).

Figure 67 Change in PR interval:

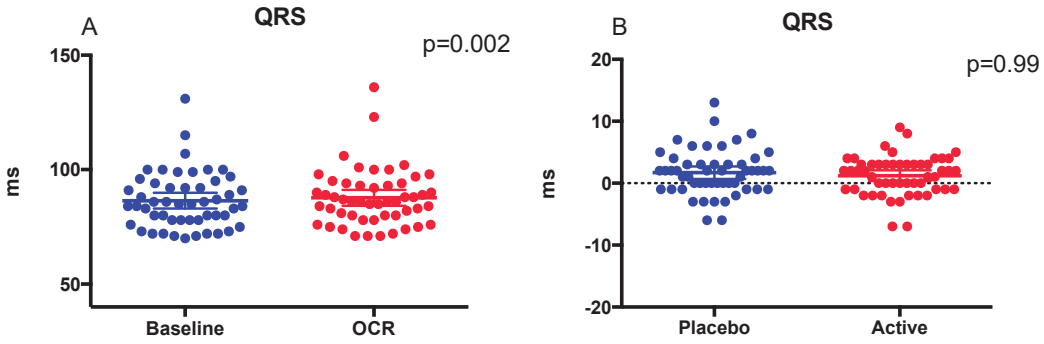


Change on active day at time of OCR (A) and on active day compared to placebo day (B). Shown is mean and 95% CI.

4.3.3.3 QRS complex:

Change in QRS complex is shown in Figure 68. There is a significant increase in the QRS complex (median increase 2ms, IQR [-1, 3]) on active day at time of OCR (Figure 68A) but no significant differences were seen between active day and placebo day (Figure 68B).

Figure 68 Change in QRS complex:



Change on active day at time of OCR (A) and on active day compared to placebo day (B). Shown is mean and 95% CI.

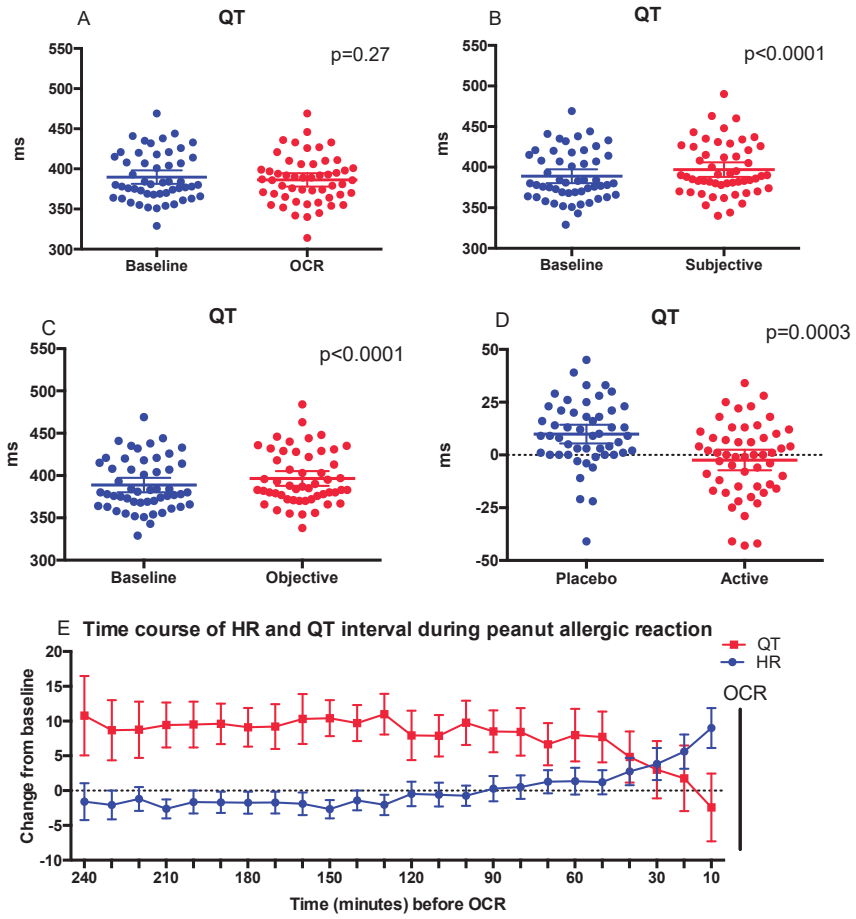
4.3.3.4 QT interval:

During active challenges, a small but significant prolongation of the QT interval was seen at onset of subjective (median increase 8.5ms, IQR [1.3, 16], Figure 69B) and objective symptoms (9.0ms, IQR [0.5, 15], Figure 69C). However, no significant changes were seen on active day at time of OCR (Figure 69A), and a significant shortening of the QT interval (mean shortening -3.8ms, 95% CI [-8.6, 1.2]) was seen on active day compared to placebo day (Figure 69D, p=0.0003).

A significant prolongation of QT interval was found on placebo challenges at time of OCR compared to baseline (mean increase 11.4ms, 95% CI [6.1, 16.8]) and a comparison of the baseline data on active and placebo days shows no significant differences between them (p=0.57).

The QT interval is dependent on the heart rate; as the latter increases, the QT interval shortens. Figure 69E shows the time course for both QT interval and HR on active days. As HR increases just prior to OCR, QT subsequently shortens, which would lead to a shortening in QT interval at OCR compared to prior time points.

Figure 69 Change in QT interval:



Change on active day at time of OCR (A), at onset of subjective (B) or objective symptoms (C) prior to peanut allergic reaction, change in QT on placebo and active days (D) and €the time course of change in HR and QT interval during peanut allergic reaction. Shown is mean and 95% CI.

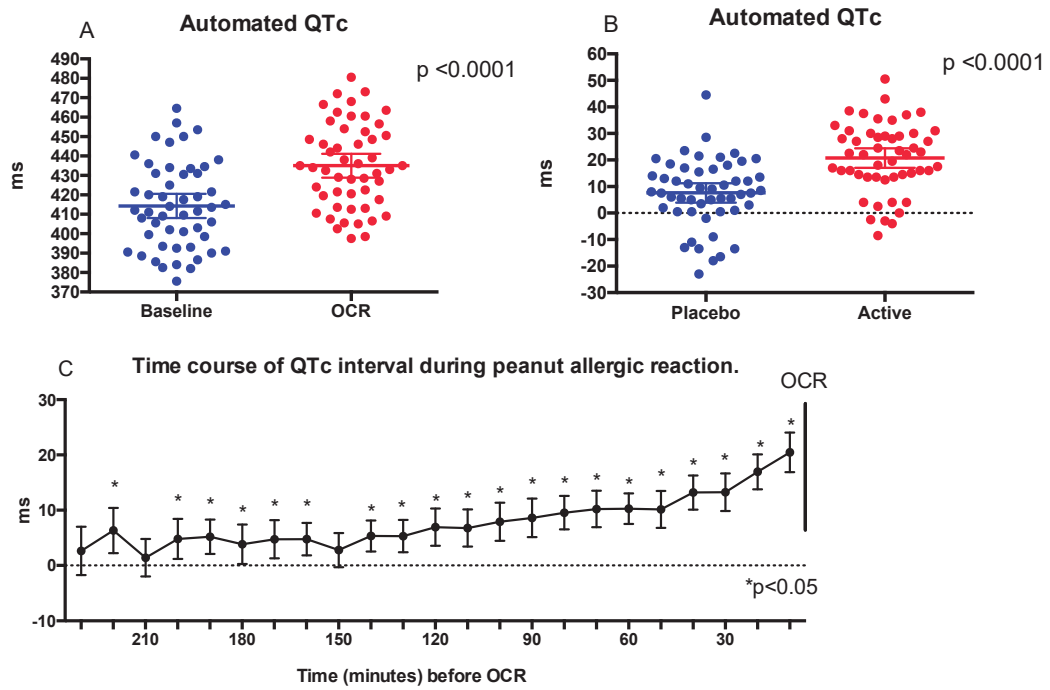
4.3.3.5 Corrected QT (QTc) interval:

Given the interdependence of QT on HR, and the results for QT, which indicates the possible confounding of changes in QT due to the increase in HR observed at time of OCR, we used a number of different methods to correct QT for heart rate.

4.3.3.5.1 QTc interval using Bazett's formula:

The change in QTc interval calculated using Bazett´s formula by the MARS system is shown in Figure 61. A significant increase in QTc was seen on active day at time of OCR (mean increase 20.8ms, 95% CI [17.1, 24.4], Figure 70A) and on active day compared to placebo day (Figure 70B).

Figure 70 Change in QTc using Bazett´s formula:



Change in automated QTc interval on active day at time of OCR (A), on active compared to placebo day (B) and (C) the time course of change in automated QTc interval during peanut allergic reaction. Shown is mean and 95% CI.

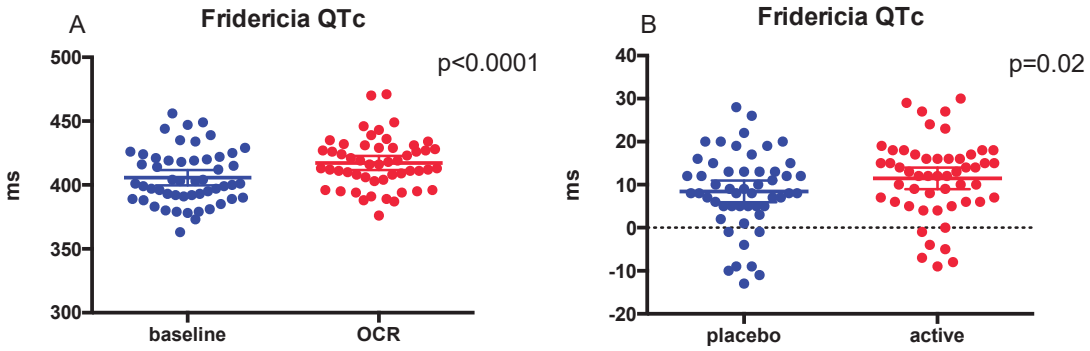
4.3.3.5.2 QTc interval using Fridericia´s and Framingham´s formulae:

Bazett´s formula tends to overestimate for QTc interval when HR is above 60bpm, and given the increase in HR both on active day at time of OCR compared to baseline, and on active day compared to placebo day, we also manually calculated QTc interval using Fridericia´s and Framingham´s formulae (shown in section 4.2.2 of this chapter), the most common alternatives to determine QTc in clinical practice.

4.3.3.5.2.1 QTc interval using Fridericia´s formula:

Change in QTc using Fridericia´s formula is shown in Figure 71. A significant increase was seen in QTc interval at time of OCR (mean increase 11.5ms, 95% CI [9, 14], Figure 71A) and on active day compared to placebo day (Figure 71B).

Figure 71 Change in QTc using Fridericia´s formula.:

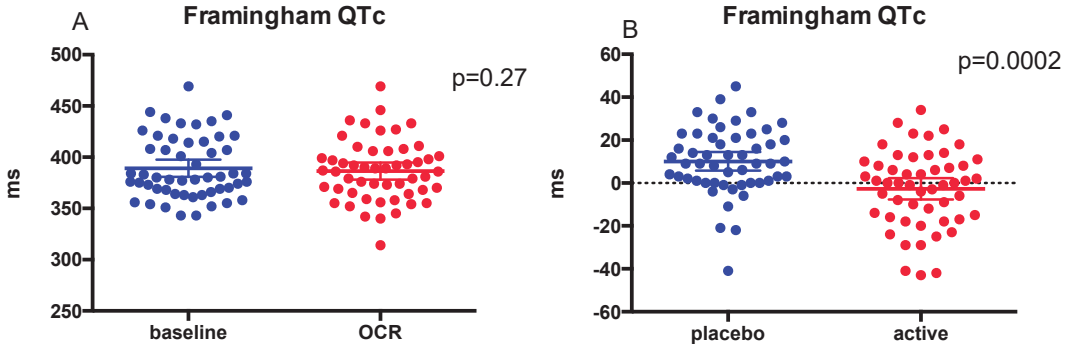


Change in manual QTc on active day at time of OCR (A) and on active day compared to placebo day (B). Shown is mean and 95% CI.

4.3.3.5.2.2 QTc interval using Framingham´s formula:

Change in manual QTc using Framingham´s formula is shown in Figure 72. No significant differences were seen on active day at time of OCR but a significant decrease was seen on active day compared to placebo day (mean decrease -2.7ms, 95% CI [-7.7, 2.2], Figure 72B)

Figure 72 Change in QTc using Framingham´s formula:



Change in manual QTc on active day at time of OCR (A) and on active day compared to placebo day (B). Shown is mean and 95% CI.

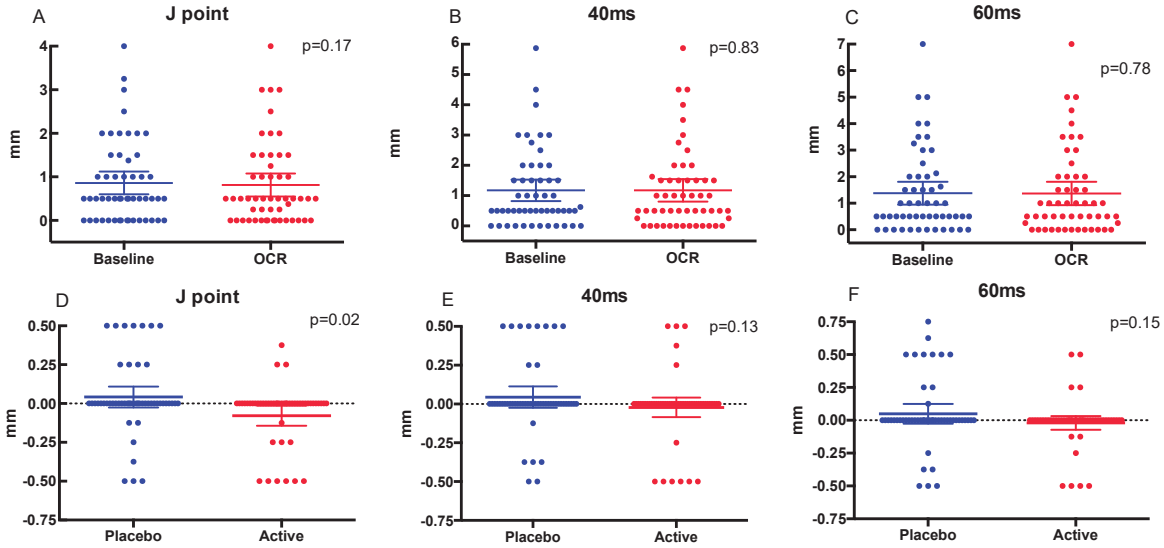
4.3.3.6 Change in ST segment during peanut allergic reaction:

We manually measured the ST segment in 3 different leads, II (inferior), V2 (anterior) and V6 (lateral) and at 3 different time points; at J point, and 40ms and 60ms from J point.

4.3.3.6.1 ST segment in lead II:

Change in ST segment on lead II is shown in Figure 73. No significant changes were seen at any of the 3-time points for active day at time of OCR, a significant decrease on the ST segment (mean decrease -0.08mm, 95% CI [-0.01, -0.14], Figure 73D) was seen at J point on active day compared to placebo day but no changes were seen at 40 or 60ms.

Figure 73 Change in ST segment on lead II:

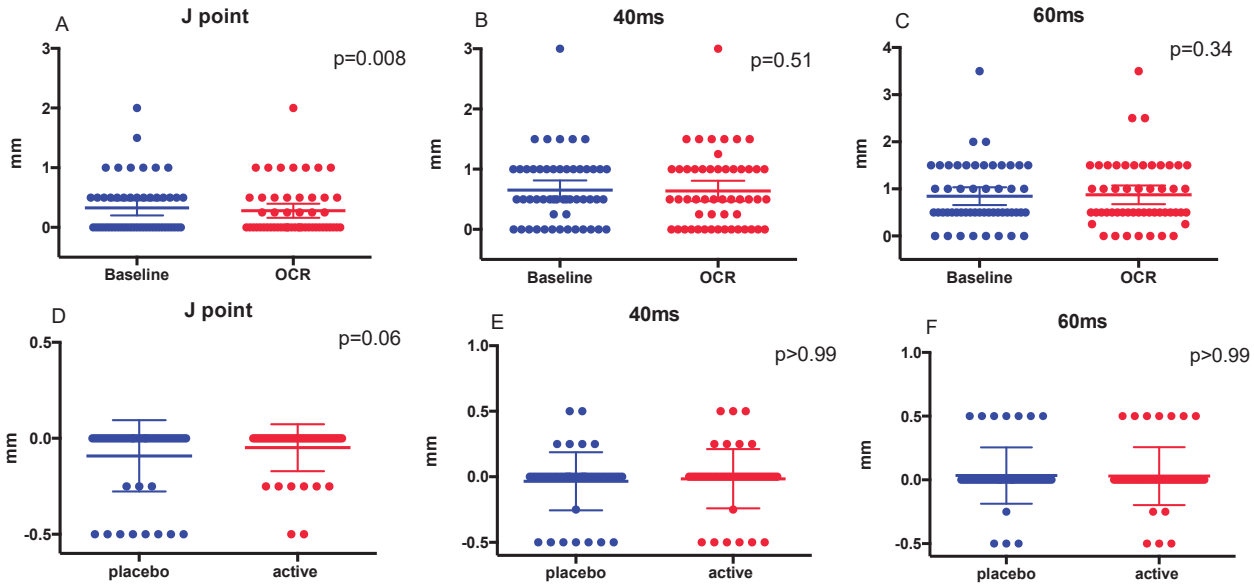


Change on active day at time of OCR at (A) J point, (B) 40ms, (C) 60ms and on active day compared to placebo day at (D) J point, (E) 40ms and (F) 60ms. Shown is mean and 95% CI.

4.3.3.6.2 ST segment in lead V2:

Change in ST segment on lead V2 is shown in Figure 74. A significant decrease in ST segment was seen at J point on active day at time of OCR (mean decrease -0.05mm, 95% CI [-0.08, -0.01], Figure 74A) but no differences were seen at 40 or 60ms. No changes were seen on active day compared to placebo day at any of the 3-time points.

Figure 74 Change in ST segment on lead V2:

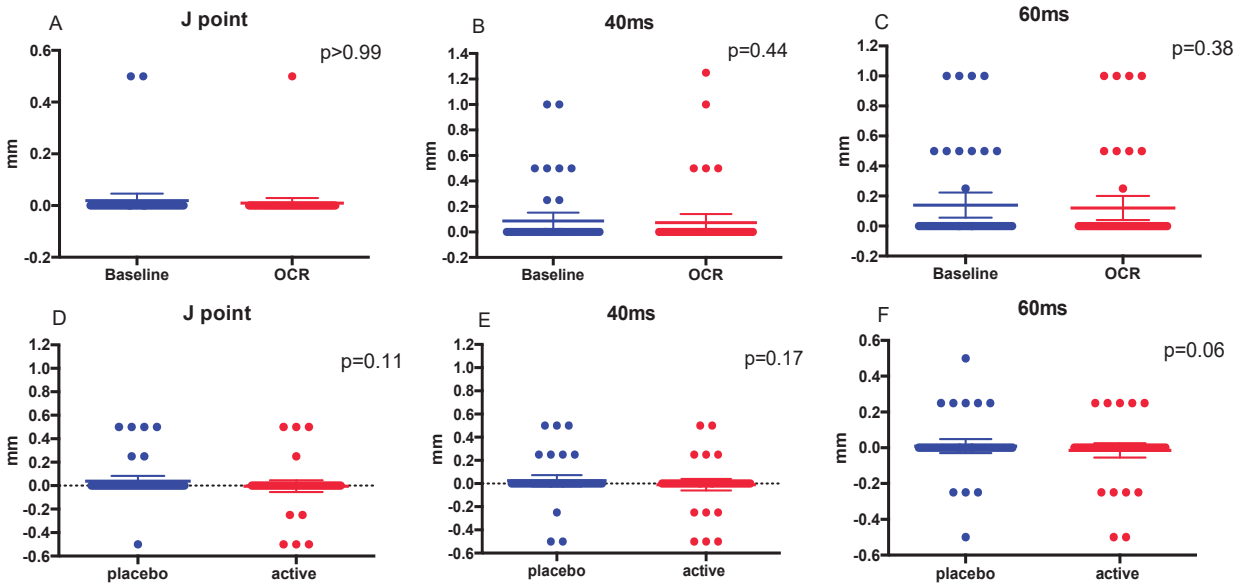


Change on active day at time of OCR at (A) J point, (B) 40ms, (C) 60ms and on active day compared to placebo day at (D) J point, (E) 40ms and (F) 60ms. Shown is mean and 95% CI.

4.3.3.6.3 ST segment in lead V6:

Change in ST segment on lead V6 is shown in Figure 75. No significant differences were seen at any of the 3 time points on active at time of OCR and on active day compared to placebo day.

Figure 75 Change in ST segment on lead V6:



Change on active day at time of OCR at (A) J point, (B) 40ms, (C) 60ms and on active day compared to placebo day at (D) J point, (E) 40ms and (F) 60ms. Shown is mean and 95% CI.

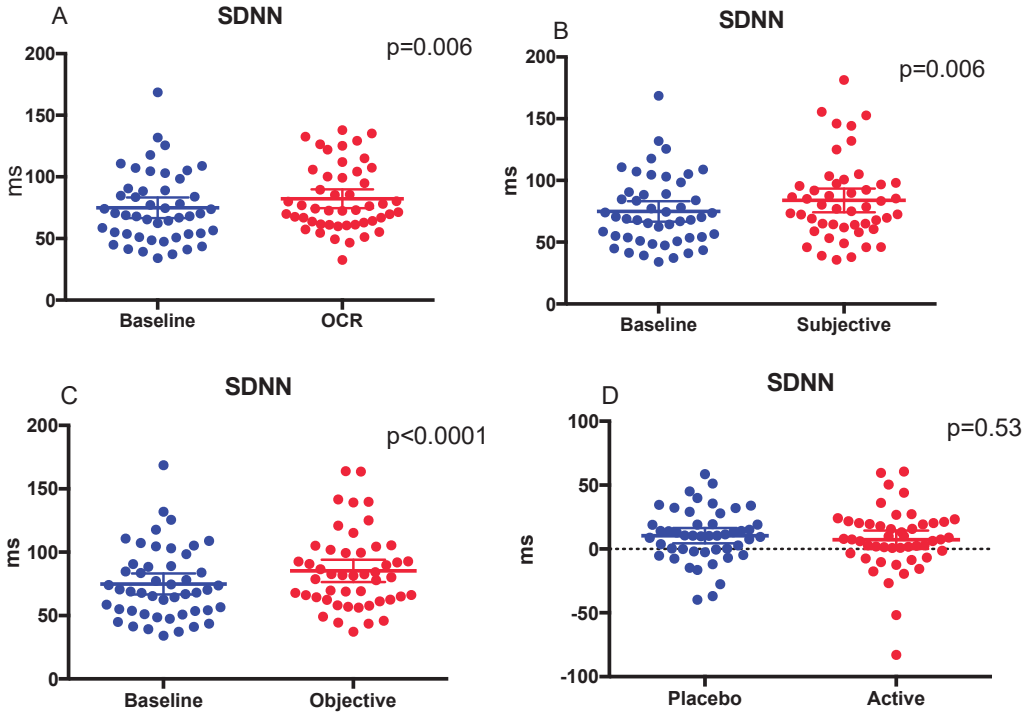
4.3.4 Change in Heart Rate Variability (HRV) during peanut allergic reaction:

Data for 51 participants was analysed, reasons for missing data were ECG data not available (n=5) or too much artefact and therefore data not suitable for analysis (n=1) in one or both challenge days. For time course, data was available for 48 participants; reason for missing data was too much artefact (n=3) on active day.

4.3.4.1 Time domain:

Change in time domain SDNN show a significant increase on active day, at onset of subjective and objective symptoms and at time of OCR (mean increase 7.3ms, 95% CI [0.15, 14.5], Figure 76C) but this change was not seen on active day compared to placebo day, shown in Figure 76.

Figure 76 Change in SDNN on baseline challenge:

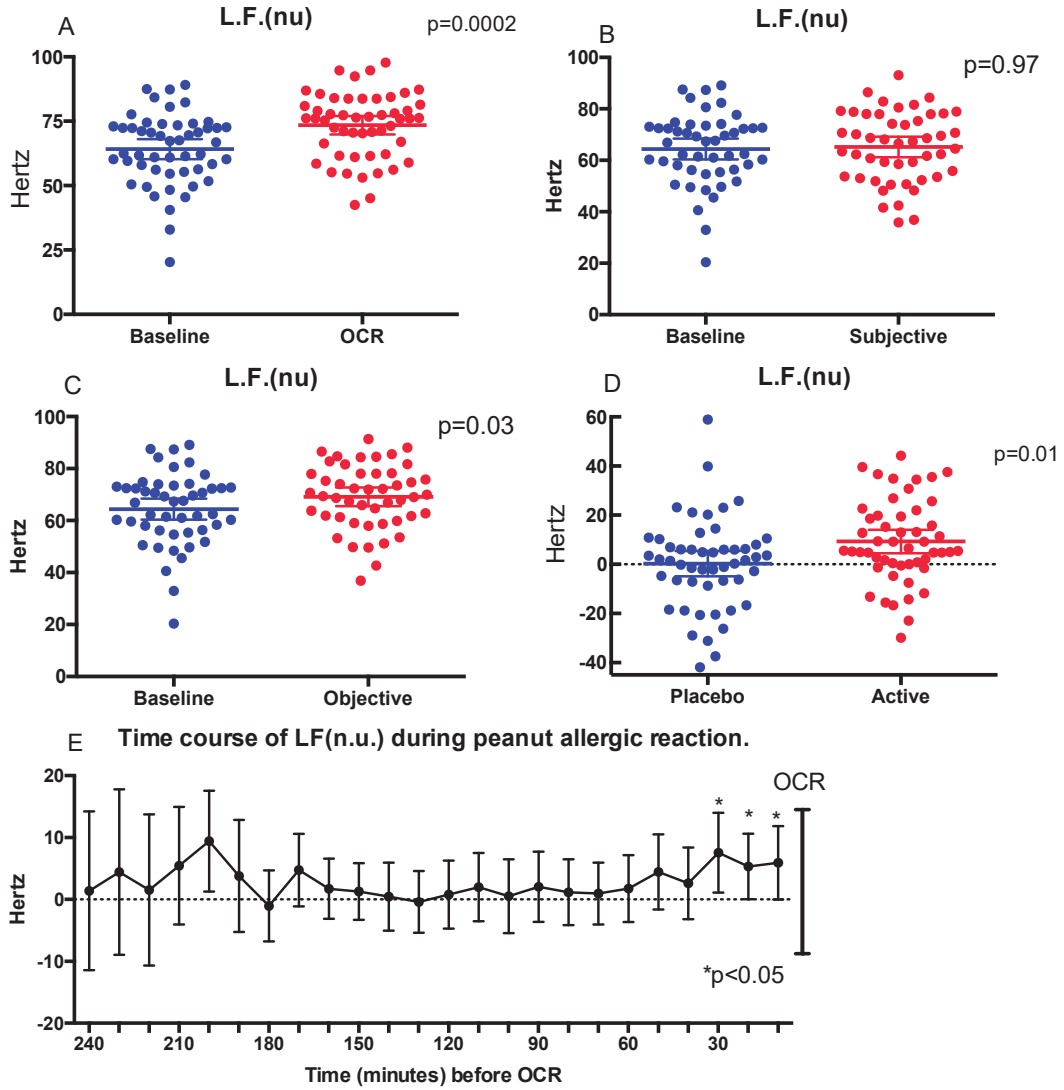


Change in SDNN on active day at time of OCR (A), change in HR at onset of subjective (B) or objective symptoms (C) prior to peanut allergic reaction, and (D) change in HR on placebo and active days. Shown is mean and 95% CI.

4.3.4.2 Frequency domain:

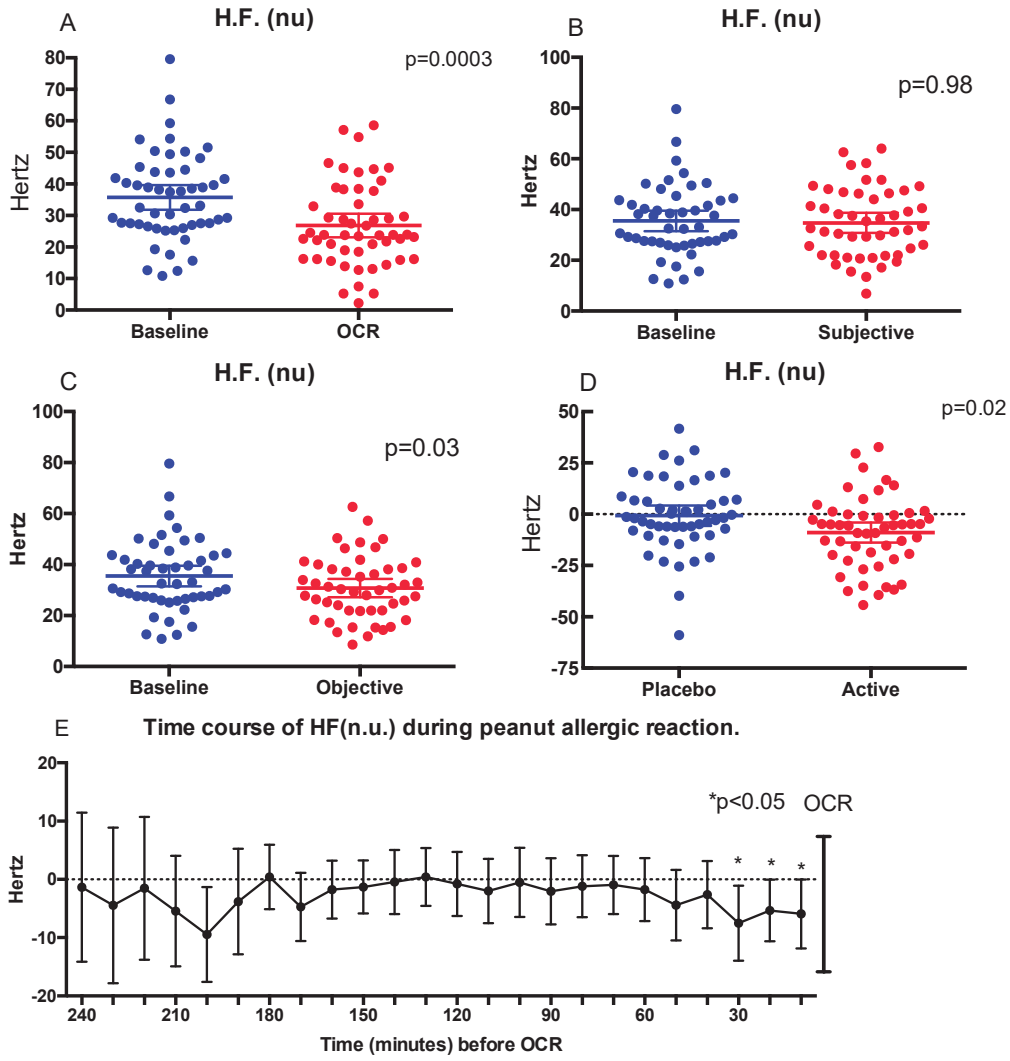
Change in frequency domain is shown in Figure 77 and 78. Significant increase was found for low frequency normalise unit (L.F.(nu)) and a significant decrease was seen for high frequency normalise unit (H.F.(nu)) on active day at onset of objective symptoms (mean increase 9.3Hertz, 95% CI [4.5, 14] (Figure 77C) and mean decrease -8.9ms, 95% CI [-13.8, -4] (Figure 78C)), at time of OCR (Figure 77A and 78A) and on active day compared to placebo day (Figure 77D and 78D). These changes are statistically significant from 30 minutes prior to meeting criteria for OCR.

Figure 77 Change in L.F. (nu) on baseline challenge:



Change on active day at time of OCR (A), change in HR at onset of subjective (B) or objective (C) symptoms prior to peanut allergic reaction, change in HR on placebo and active days (d), and (E) the time course of change in HR during peanut allergic reaction. Shown is mean and 95% CI.

Figure 78 Change in H.F. (nu) on baseline challenge:

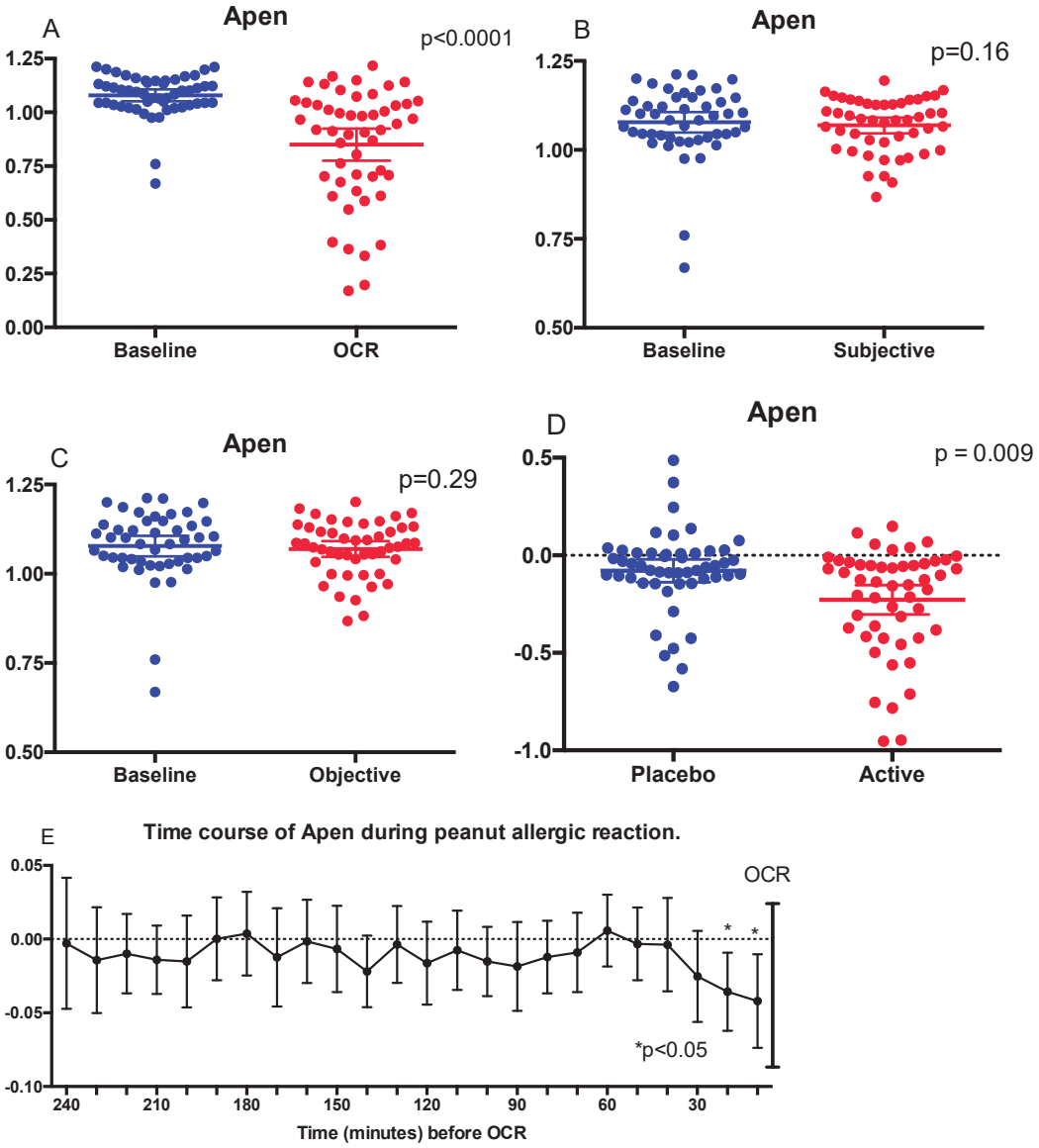


Change in H.F. (n.u.) during peanut allergic reaction (A), change in HR at onset of subjective (B) or objective (C) symptoms prior to peanut allergic reaction, change in HR on placebo and active days (D), and (E) the time course of change in HR during peanut allergic reaction. Shown is mean and 95% CI.

4.3.4.3 Non-linear domains:

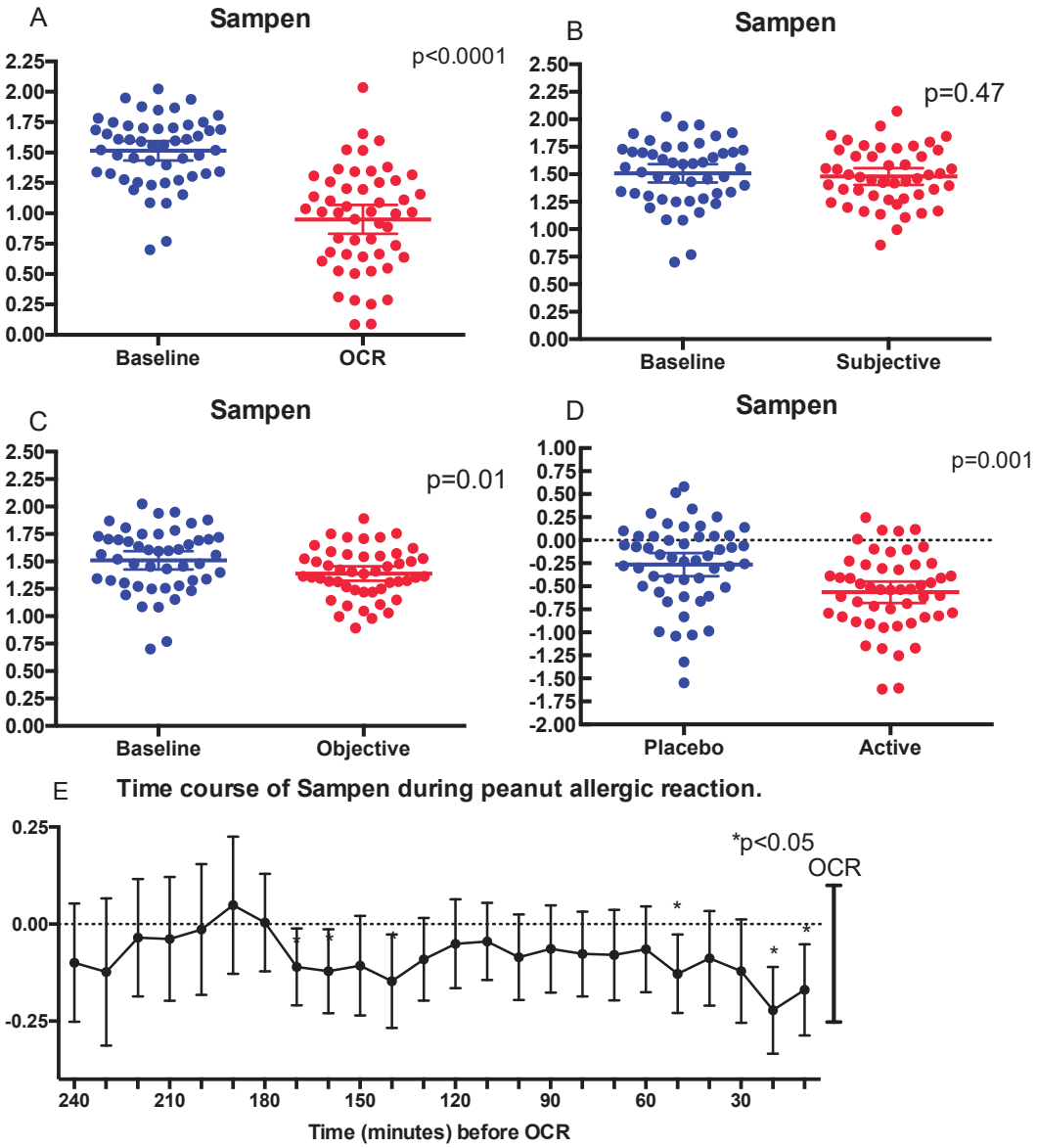
The changes in non-linear domains are shown in figures 79 to 80. Significant decrease was found in both Apen and Sampen on active day at time of OCR (Figure 79A and 80A) and on active day compared to placebo day (Figure 79D and 80D). No significant differences were seen for DFA-1 on active day at time of OCR and on active day compared to placebo day (Figure 81). These changes are statistically significant from 20 minutes prior to meeting criteria for OCR.

Figure 79 Change in Apen on baseline challenge:



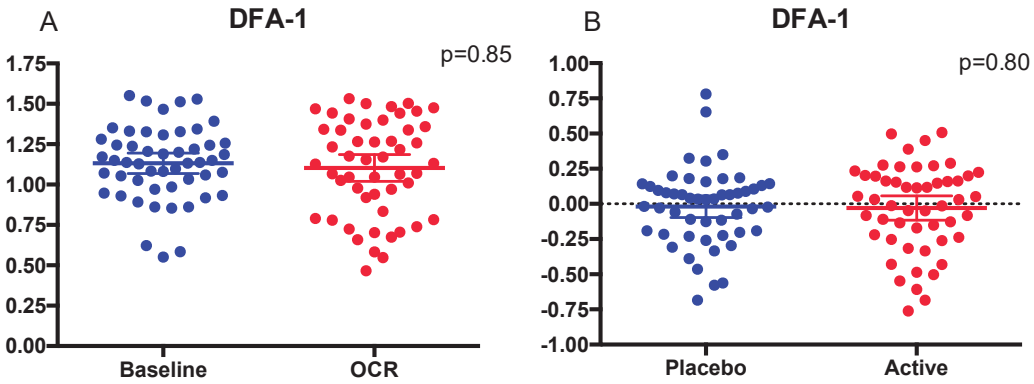
Change in Apen on active at time of OCR (A), change in HR at onset of subjective (B) or objective (C) symptoms prior to peanut allergic reaction, (D) change in HR on placebo and active days, and (E) the time course of change in HR during peanut allergic reaction. Shown is mean and 95% CI.

Figure 80 Change in Sampen on baseline challenge:



Change on active day at time of OCR (A), change in HR at onset of subjective (B) or objective (C) symptoms prior to peanut allergic reaction, change in HR on placebo and active days (D), and (E) the time course of change in HR during peanut allergic reaction. Shown is mean and 95% CI.

Figure 81 Change in DFA-1 on baseline challenge:

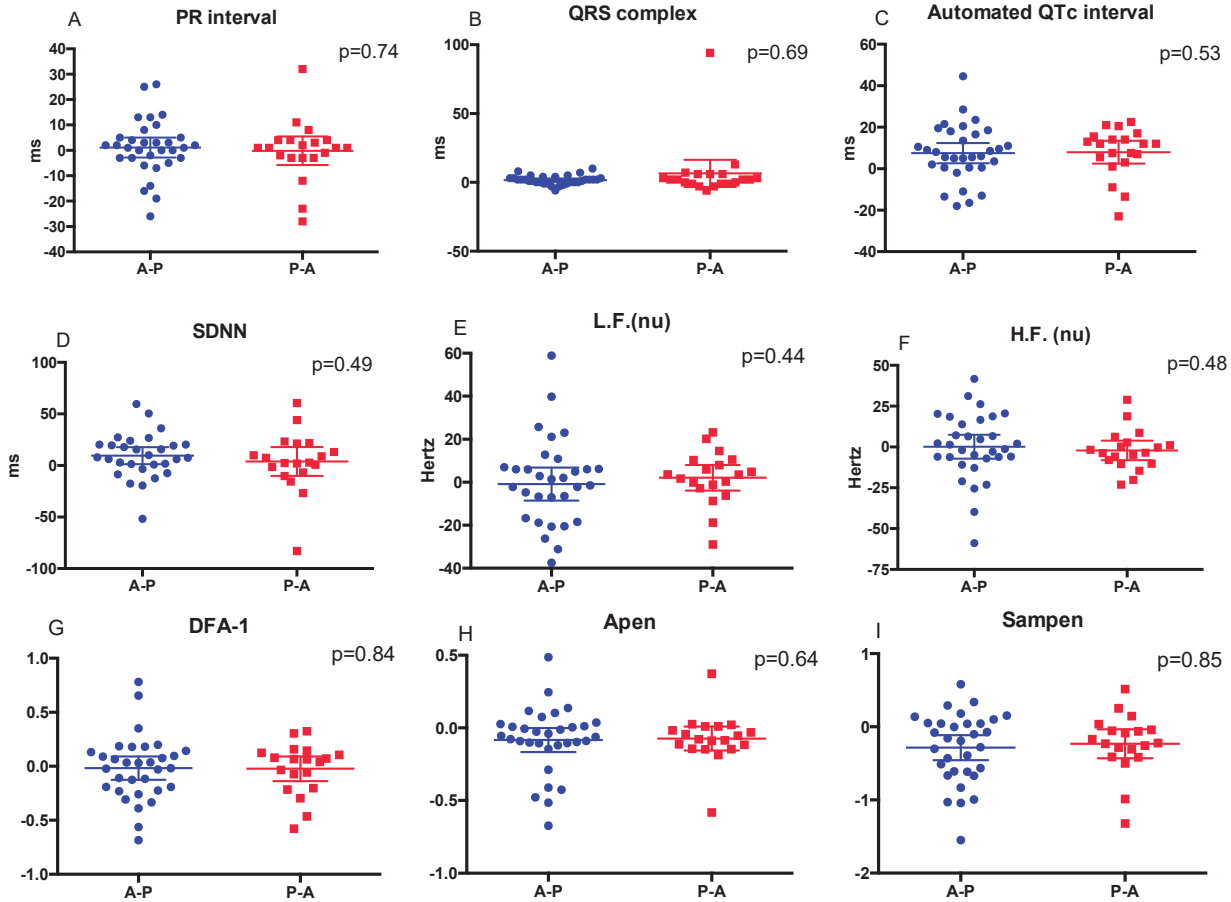


Change on active day at time of OCR (A) and on placebo and active days (B). Shown is mean and 95% CI.

4.3.5 Impact of challenge order:

Results in Figure 82 show no significant difference in any of the ECG and HRV parameters analysed by challenge order of the DBPCFC on the placebo challenge day, between participants who had a placebo challenge before compared with after their peanut allergic reaction.

Figure 82 Difference according to challenge order:



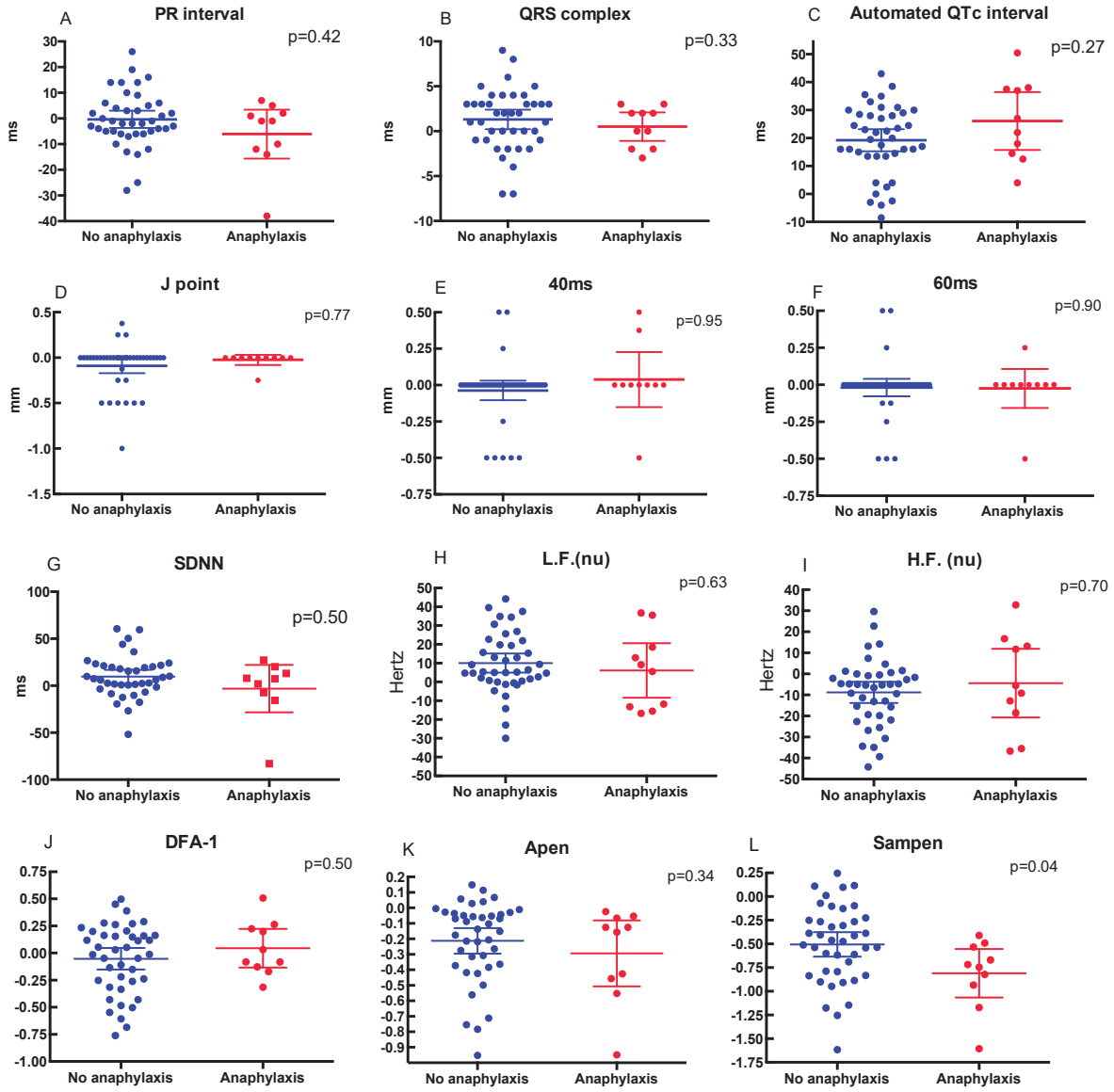
Change in (A) PR interval, (B) QRS complex, (C) QTc interval, (D) SDNN, (E) LF (n.u.), (F) HF (n.u.), (G) DFA-1, (H) Apen and (I) Sampen on placebo day challenges according to challenge order during DBPCFC. A-P corresponds to those participants having placebo second and P-A corresponds to those participants having placebo first as their order for DBPCFC. Shown is mean and 95% CI.

4.3.6 Association between cardiac rhythm changes and severity of reaction:

4.3.6.1 Comparison of cardiac rhythm changes and different severity scores:

Figure 83 shows a significance decrease in HRV non-linear parameter Sampen between those participants who required IM adrenaline as rescue medication and those who did not (Figure 83L). No significant changes were seen for any of the other parameters analysed for cardiac rhythm.

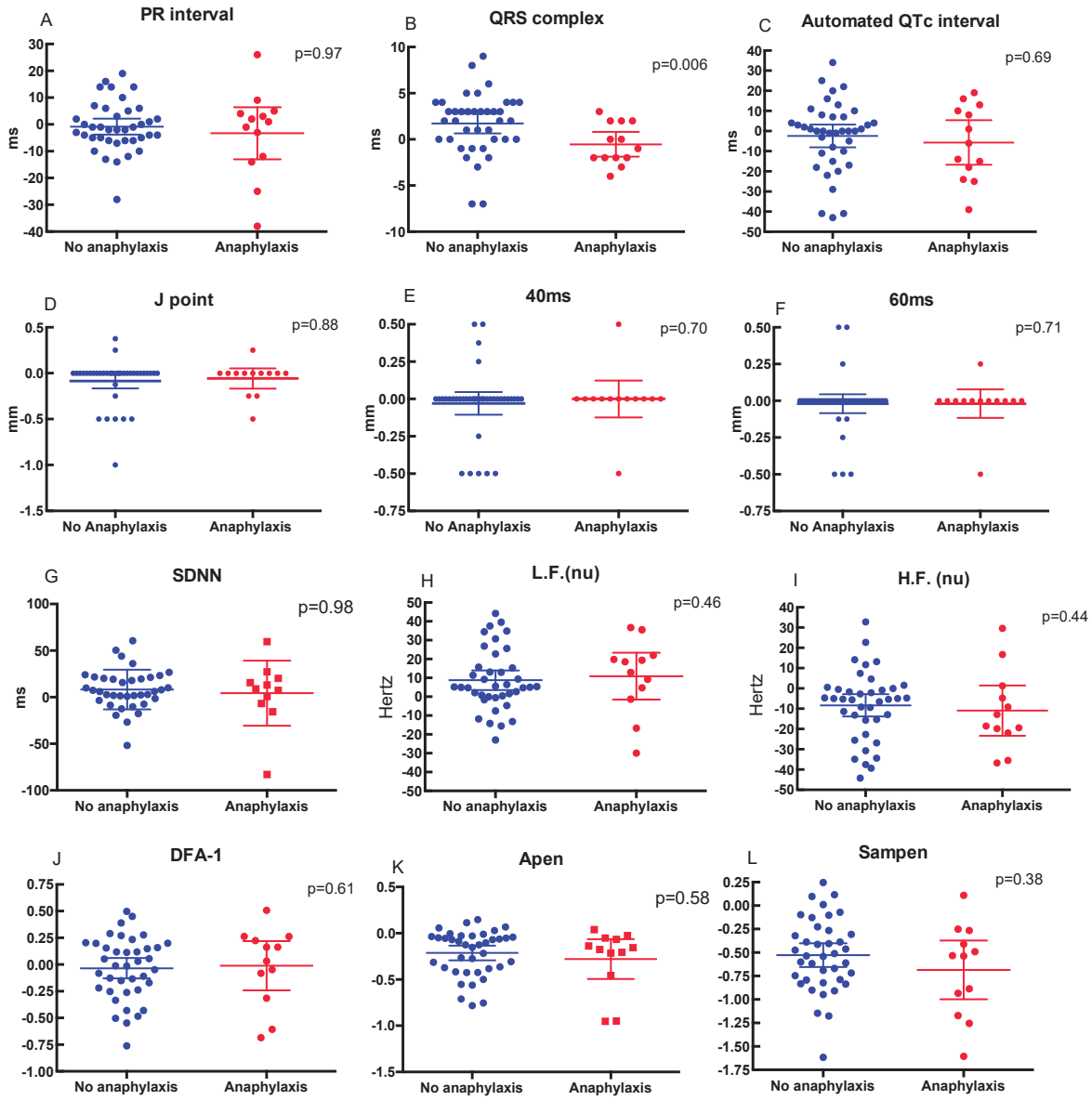
Figure 83 Differences in cardiac conductance changes according to the use of IM adrenaline:



Difference in (A) PR interval, (B) QRS complex, (C) automated QTc interval, ST segment at (D) J point, (E) 40ms, (F) 60 ms, (G) SDNN, (H) L.F.(nu), (I) H.F.(un), (J) DFA-1, (K) Apen and (L) Sampen between those participants who requiered IM adrenaline as rescue medication and those who did not. For ST segment results shown correspond to lead II. Shown is mean and 95% CI.

Results in Figure 84 shows a significant decrease in QRS complex (median decrease -1ms, IQR [-2, 2]) between those participants who had anaphylaxis according to NIAID classification and those who did not. No significant changes were found for any of the other ECG or HRV parameters.

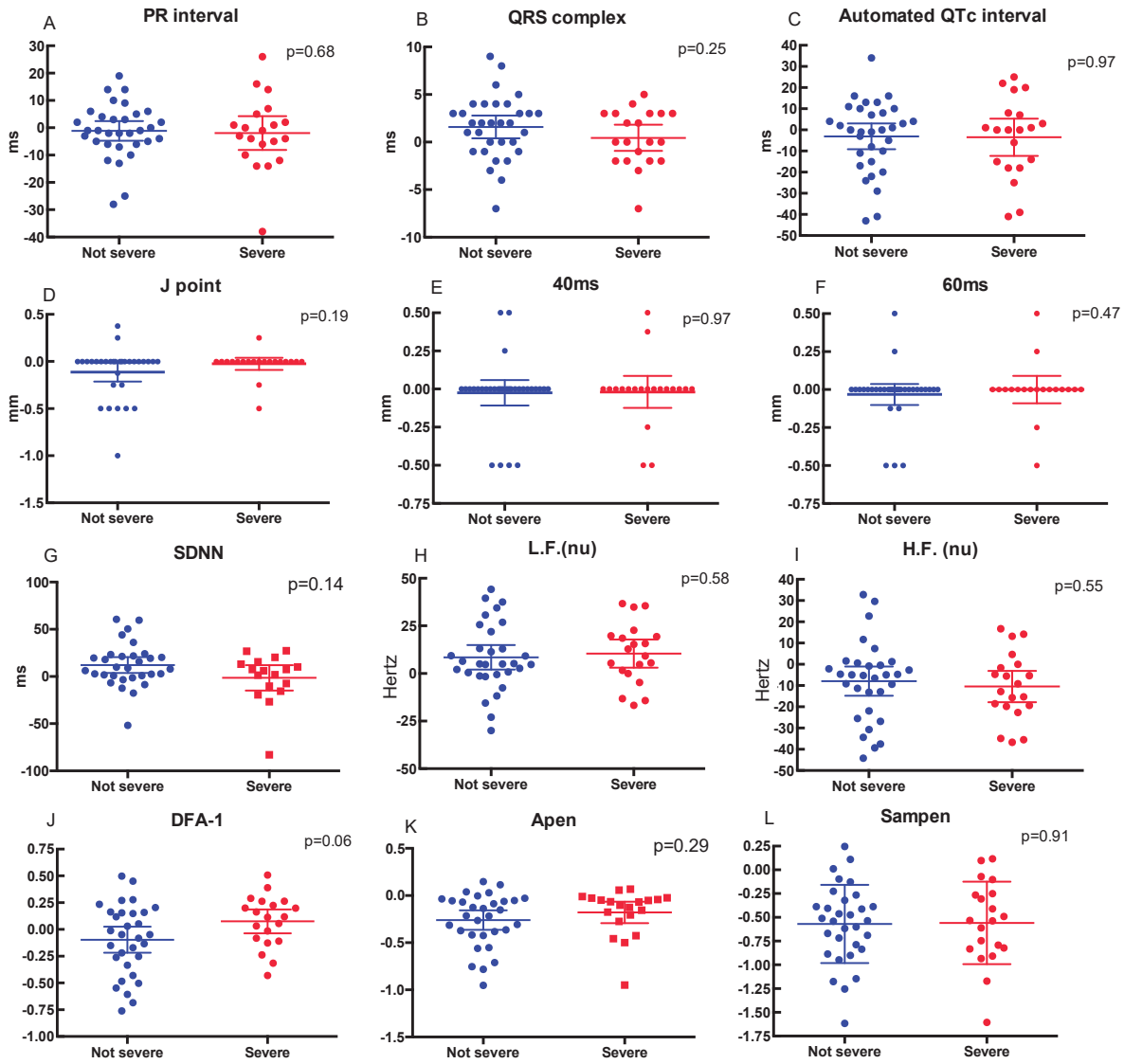
Figure 84 Differences in cardiac conductance changes according to the NIAID classification:



Difference in (A) PR interval, (B) QRS complex, (C) automated QTc interval, ST segment at (D) J point, (E) 40ms, (F) 60 ms, (G) SDNN, (H)L.F.(nu), (I) H.F.(un), (J) DFA-1, (K) Apen and (L) Sampen between those participants classified as anaphylaxis and non-anaphylaxis according to the NIAID classification. For ST segment results shown correspond to lead II. Shown is mean and 95% CI.

Results in Figure 85 show no significant differences in any of the ECG and HRV parameters analysed between those participants classified as having a mild reaction compared to those classified as having a moderate/severe reaction according to Ewan and Clark classification.

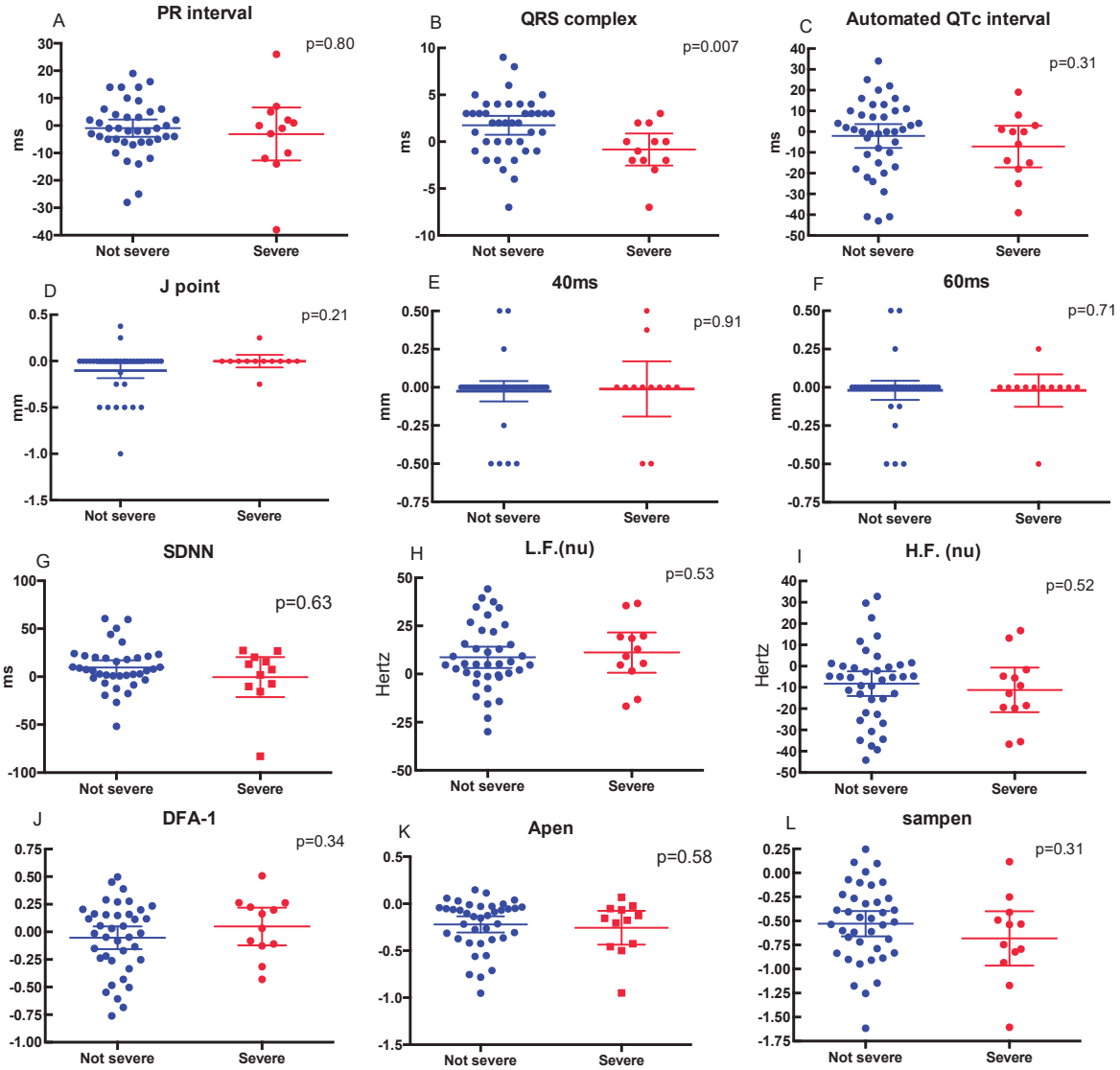
Figure 85 Differences in cardiac conductance changes according to Ewan&clark classification:



Difference in (A) PR interval, (B) QRS complex, (C) automated QTc interval, ST segment at (D) J point, (E) 40ms, (F) 60 ms, (G) SDNN, (H)L.F.(nu), (I) H.F.(un), (J) DFA-1, (K) Apen and (L) Sampen between those participants classified as mild (1-3) and those classified as moderate/severe (4-5) reactions by Ewan and Clark severity scoring. For ST segment results shown correspond to lead II. Shown is mean and 95% CI.

Results in Figure 86 show a significant decrease in QRS complex (median decrease -0.5ms, IQR [-2, 1.5]) between those participants classified as having a mild reaction compared to those classified as having a moderate/severe reaction according to WAO classification of severity. No significant differences were seen for any other ECG or HRV parameters.

Figure 86 Differences in cardiac conductance changes according to WAO classification:



Difference in (A) PR interval, (B) QRS complex, (C) automated QTc interval, ST segment at (D) J point, (E) 40ms, (F) 60 ms, (G) SDNN, (H)L.F.(nu), (I) H.F.(un), (J) DFA-1, (K) Apen and (L) Sampen between those participants classified as mild (1-3) and those classified as moderate/severe (4-5) reaction according to the WAO classification for subcutaneous immunotherapy systemic reaction grading system. For ST segment results shown correspond to lead II. Shown is mean and 95% CI.

ECG and HRV parameters were correlated with the VAS score recorded by both the participants and clinician on the day of the allergic reaction, for different organ systems and the overall assessment of global severity. Table 16 shows, overall, a poor correlation between the change in ECG and HRV parameters and participant´s VAS severity score.

A moderate correlation was found between DFA-1 and participant´s VAS score for gastrointestinal symptoms. Similar results were found for the correlation between the ECG and HRV parameters and the clinician´s VAS score (Appendix 20).

Table 16 Relationship between cardiac conductance and symptoms:

	VAS skin score	**VAS GI score**	**VAS upper resp score**	**VAS lower resp score**	**VAS anxiety score**	**VAS overall score**
PR interval	r= -0.13 (p=0.36)	r= -0.06 (p=0.70)	r= -0.18 (p=0.20)	r= -0.09 (p=0.54)		r= -0.03 (p=0.82)
QRS complex	r=0.07 (p=0.60)	r= -0.12 (p=0.40)	r=0.02 (p=0.91)	r= -0.24 (p=0.09)		r= -0.17 (p=0.23)
Automated QTc interval	r= -0.11 (p=0.44)	r= -0.19 (p=0.17)	r= -0.16 (p=0.24)	r= -0.07 (p=0.61)		r= -0.12 (p=0.41)
SDNN	r= -0.17 (p=0.24)	r= -0.09 (p=0.57)	r= -0.11 (p=0.44)	r= -0.09 (p=0.54)	r=0.27 (p=0.07)	r= -0.13 (p=0.36)
LF (n.u.)	r= 0.07 (p=0.62)	r=0.24 (p=0.09)	r= -0.12 (p=0.41)	r= -0.08 (p=0.60)	r= -0.11 (p=0.44)	r= -0.08 (p=0.59)
HF (n.u.)	r= -0.07 (p=0.63)	r=0.25 (p=0.08)	r=0.11 (p=0.45)	r=0.004 (p=0.99)	r=0.08 (p=0.56)	r=0.07 (p=0.64)
Apen	r=0.12 (p=0.41)	r=0.09 (p=0.54)	r=0.05 (p=0.70)	r=0.01 (p=0.54)	r= -0.03 (p=0.81)	r=0.07 (p=0.61)
Sampen	r=0.08 (p=0.60)	r=0.02 (p=0.86)	r= -0.07 (p=0.61)	r= -0.18 (p=0.22)	r=0.01 (p=0.94)	r= -0.02 (p=0.88)
DFA-1	r=0.02 (p=0.88)	**r=0.38 (p=0.02)**	r=0.10 (p=0.47)	r=0.25 (p=0.08)	r= -0.07 (p=0.63)	r=0.06 (p=0.69)

Spearman correlation.

4.3.7 Correlation between HRV parameters and laboratory measurements:

No correlation was found between HRV parameters and laboratory measurements (MCT and plasma adrenaline) of an allergic reaction, shown in Table 17.

Table 17 Correlation between HRV and laboratory parameters:

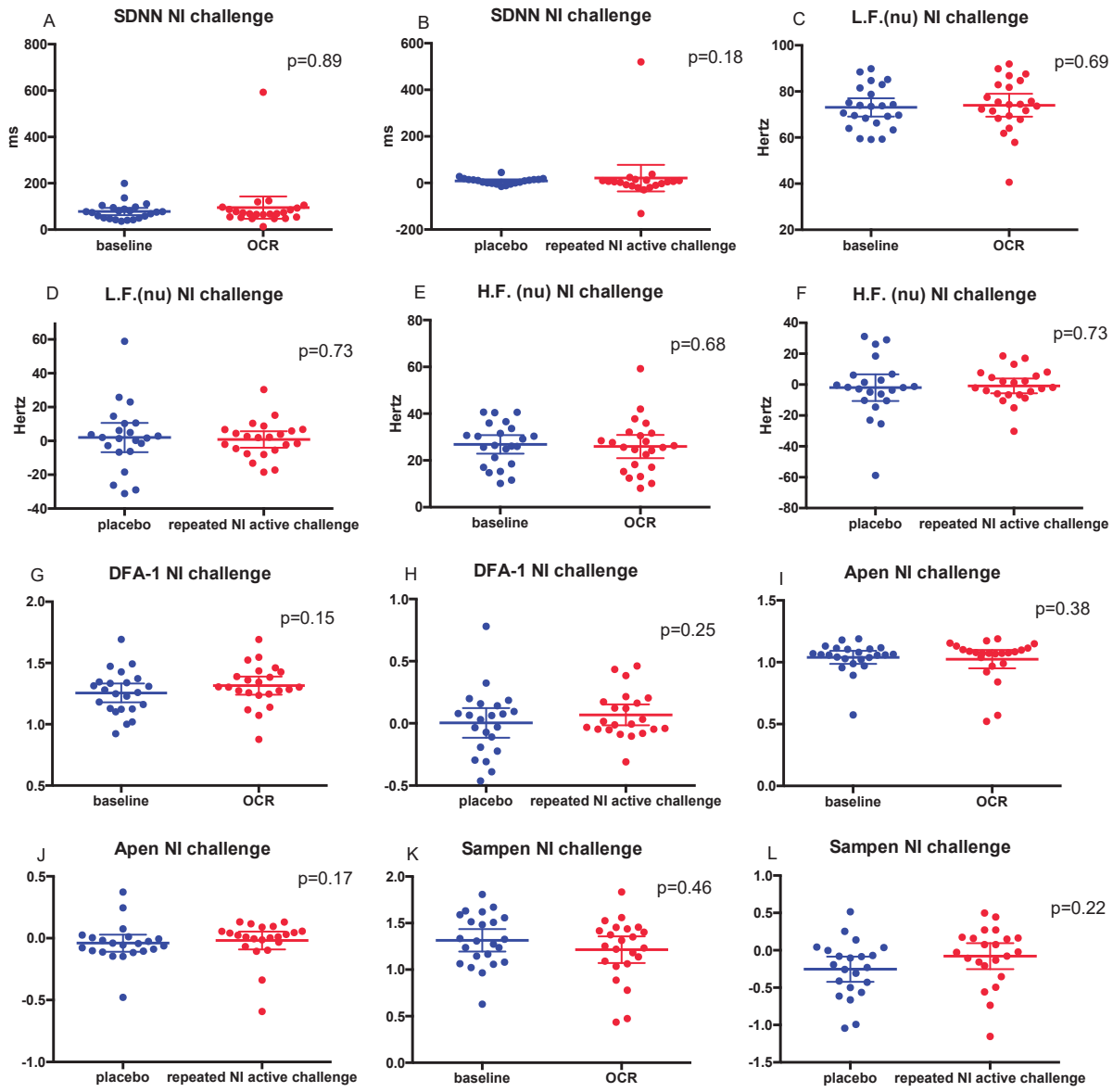
HRV parameters	**Peak % MCT**	**% MCT at OCR**	**Plasma adrenaline**
SDNN	r=-0.13 (p=0.38)	r=-0.17 (p=0.24)	r=0.19 (p=0.21)
L.F. (n.u.)	r=0.22 (p=0.13)	r=0.06 (p=0.64)	r=0.25 (p=0.10)
H.F. (n.u.)	r=-0.22 (p=0.13)	r=-0.07 (p=0.61)	r=-0.25 (p=0.09)
DFA-1	r=0.06 (p=0.70)	r=0.13 (p=0.36)	r=-0.02 (p=0.87)
Apen	r=-0.15 (p=0.30)	r=-0.19 (p=0.18)	r=-0.23 (p=0.12)
Sampen	r=-0.27 (p=0.06)	r=-0.19 (p=0.19)	r=-0.23 (p=0.12)

Spearman correlation. Peak % MCT= peak % change in MCT from OCR to 2hr after.

4.3.8 Changes in repeated NI challenges:

No clinically significant changes were seen for ECG parameters of PR interval, QT and QTc interval, QRS complex and ST segment on baseline DBPCFC therefore we only repeated the analyses of HRV parameters on repeated NI challenges shown in Figure 87.

Figure 87 Change in HRV:



Change on active day at time of OCR for (A) SDNN, (C) L.F. (n.u.), (E) H.F. (n.u.), (G) DFA-1, (I) Apen and (K) Sampen and on active day compared to placebo day for (B) SDNN, (D) L.F. (n.u.), (F) H.F. (n.u.), (H) DFA-1, (J) Apen and (L) Sampen during repeated NI challenge. Shown is mean and 95% CI.

4.3.8.2 Association between HRV changes and severity of reaction:

The reactions of those participants who underwent a repeated NI challenge were classified according to the need of IM adrenaline as rescue medication, NIAID, Ewan & Clark and WAO severity scoring in the same way as for baseline challenges. Results show no significant difference for HRV parameters between those participants who had anaphylaxis and those who did not and no significant difference between those participants who were classified as having a severe reaction with those who did not. Results for this are shown in Appendix 21.

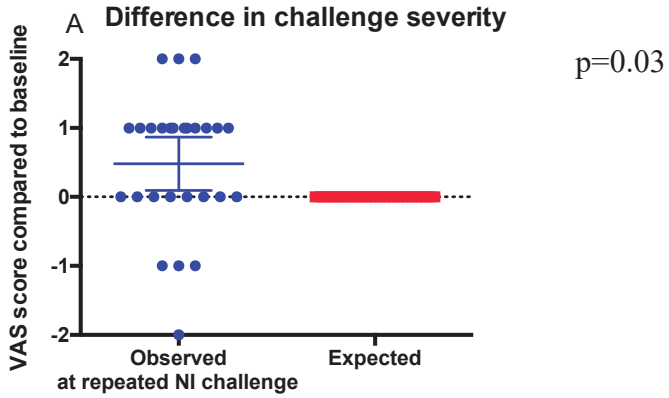
No significant differences were seen in a comparison of participants´ organ VAS score between baseline and NI challenges, when participants were asked to 'score' their reaction in isolation, as shown in Table 18. However, when participants were asked how their reaction at NI compared to their reaction at baseline challenge, they scored their NI reaction as significantly less severe (p=0.03), as shown in Figure 88.

Table 18 Differences in participant´s VAS score, for each reaction (baseline and NI challenge) scored independently:

Organ	VAS baseline challenge	VAS NI challenge	p value
Skin	3 (1, 6)	4 (2, 6)	p=0.88
GI	7 (5, 8)	7 (5, 7)	p=0.31
Upper respiratory	4,5 (3, 5)	4, 5 (3, 6)	p=0.73
Lower respiratory	2 (0, 4)	2 (0, 5)	p=0.91
Anxiety	4 (1, 6)	3 (1, 5)	p=0.45
Overall	6 (5, 7)	6 (4, 7)	p=0.59

Results show median and IQR.

Figure 88 Difference in challenge severity:

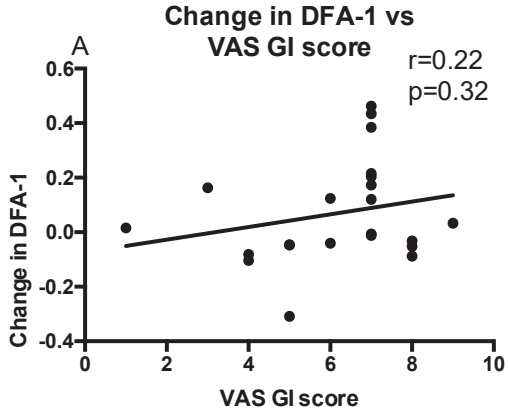


Difference in participant´s VAS score between baseline and NI challenge, where the null hypothesis is that there is no difference between the severity of the reactions. Shown is mean and 95% CI.

Legend: 0=no difference, -1=worse, -2=much worse, 1=better, 2=much better.

We were unable to reproduce the moderate association between DFA-1 and GI symptom score on these repeated NI challenges shown in Figure 89. Correlation between HRV parameters and both participant´s and clinician´s VAS score is shown in Appendix 22.

Figure 89 Relationship between DFA-1 and GI symptoms:



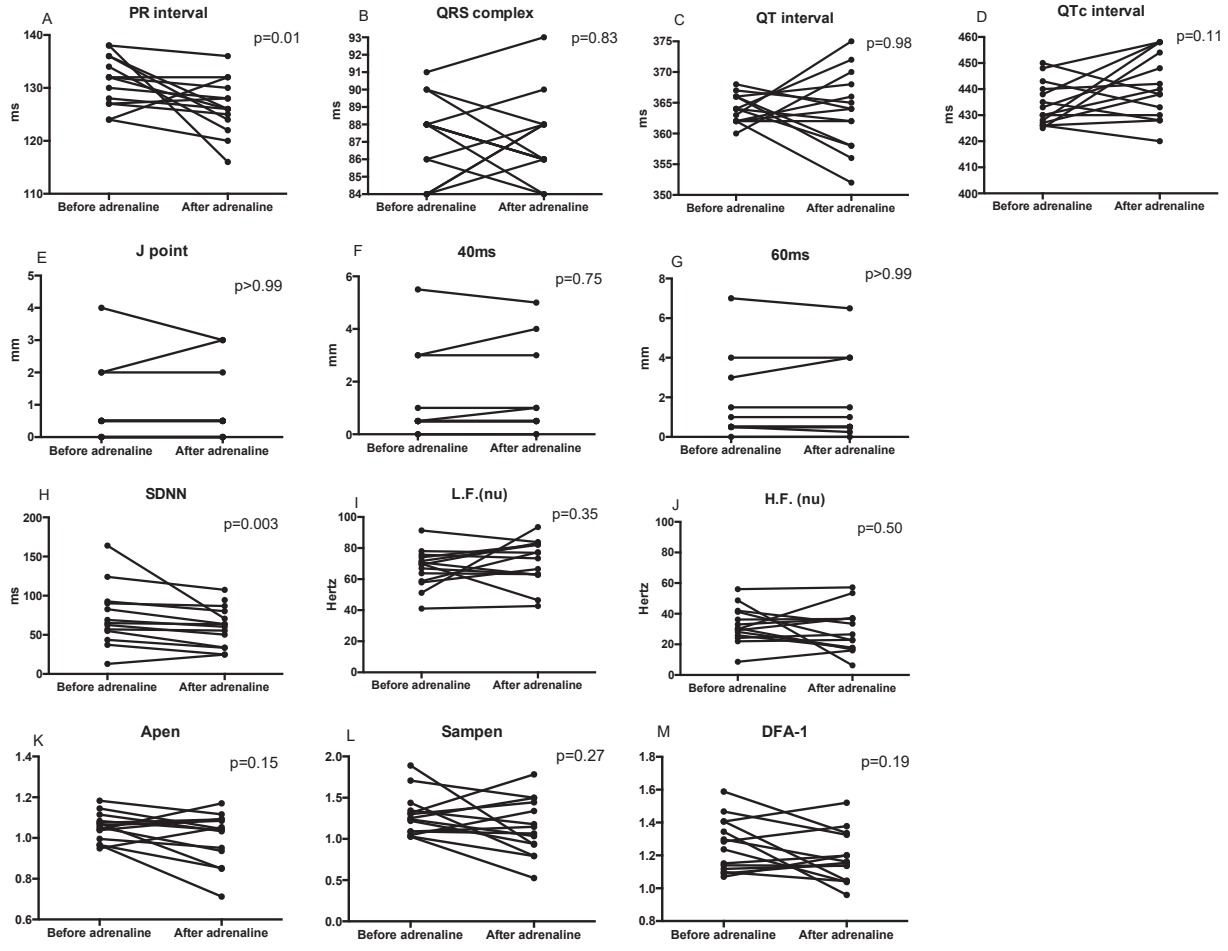
Spearman correlation between DFA-1 and participant´s GI symptom score on repeated NI challenges.

4.3.9 Effects of IM adrenaline in ECG and HRV parameters:

Seventeen participants, with a total of 21 challenges, required IM adrenaline as a rescue medication; data for 12 participants, in 14 challenges, for electrical conductance changes were studied. Reasons for missing data were: monitor not available (n=1), technical malfunction of the monitor (n=1), data not recorded (n=1) or data from exercise challenge (n=4), which were not included in this thesis. The data for this section of results includes baseline, sleep deprivation and non-intervention challenges as part of the TRACE study described in Methods section 2.1 No data is shown for the analysis of the comparison for those participants who required IM adrenaline on one challenge but not on a repeated challenge as only good quality data for 2 participants was available.

Figure 90 shows a significant shortening of the PR interval (median shortening -2ms, IQR [-1.5, -22]) and decrease in SDNN (median decrease -11.2ms, IQR [-1.6, -93.1]) during the 10 minutes after administration of IM adrenaline. No other significant differences were seen in any of the cardiovascular parameters after IM adrenaline was administered.

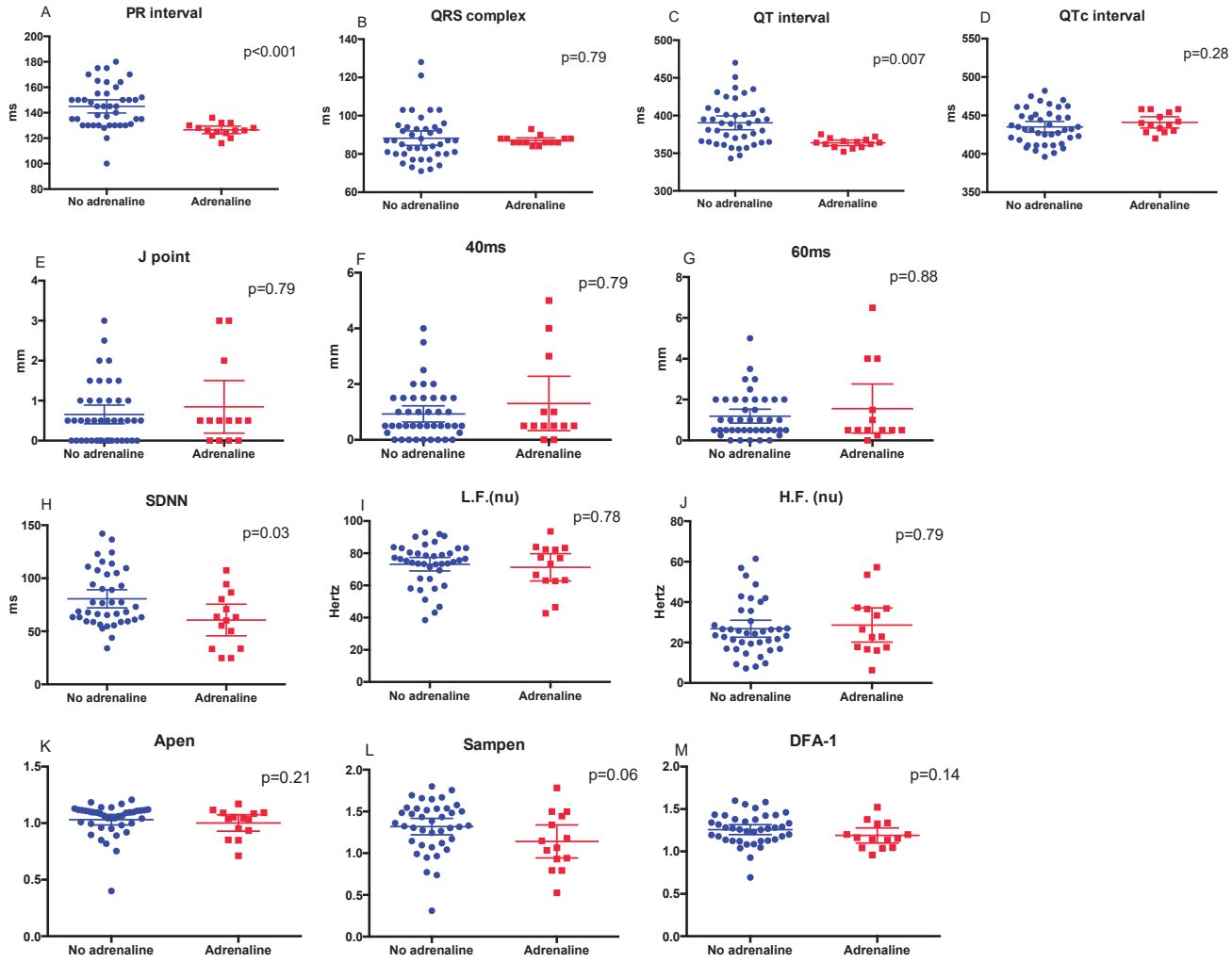
Figure 90 Difference in cardiac conductance changes with the use of IM adrenaline:



Change in (A) PR interval, (B) QRS complex, (C) QT interval, (D) QTc interval, (E) J point, (F) 40ms, (G) 60ms, (H) SDNN, (I) L.F.(n.u.), (J) H.F.(n.u.), (K) Apen, (L) Sampen and (M) DFA-1 before and after 0.5mg of IM adrenaline were administered.

Figure 91 shows a shortening of PR interval, QT interval and a decrease in SDNN between those participants who required IM adrenaline as rescue medication as those who did not during peanut allergic reaction.

Figure 91 Difference in cardiac conductance changes according to the use of IM adrenaline:



Change in (A) PR interval, (B) QRS complex, (C) QT interval, (D) QTc interval, (E) J point, (F) 40ms, (G) 60ms, (H) SDNN, (I) L.F.(n.u.), (J) H.F.(n.u.), (K) Apen, (L) Sampen and (M) DFA-1 between the 10 minutes after administration of IM adrenaline and the 10 minutes after other non-IM adrenaline treatment during the baseline allergic reaction. Shown is mean and 95% CI.

4.4 Discussion:

In this observational study of ECG changes during allergic reactions to peanut, in young adults with primary IgE-mediated peanut allergy, we found no consistent evidence for electrocardiographic changes either during anaphylactic or non-anaphylactic reactions. We did identify two individual cases of type 2 AV block, one of which was associated with clinically significant symptoms, however the first case was not reproducible on subsequent challenges. The second case may have been triggered by a pain/vasovagal stimulus rather than an allergic reaction. We found no consistent evidence for more widespread ECG changes across this population of 52 peanut allergic adults.

Results from initial 'baseline' peanut challenges suggested that during peanut-induced allergic rections, there was a reduction in HRV in adults similar to that previously described in a paediatric population [197], but no significant changes were observed in other ECG parameters, namely PR interval, QRS complex, QT/QTc interval and ST segment changes. The HRV changes are consistent with sympathetic activation, as evidenced by the decrease in High frequency (H.F. (n.u.)) activity. Results for plasma cathecolamines (chapter 3) found that only adrenaline was significantly increased during peanut allergic reactions, suggesting that the adrenergic pathway may be selectively activated by the sympathetic nervous system, although we were unable to find a correlation between HRV parameters and the change in plasma cathecolamine. HRV has been related to emotional arousal and the H.F. domain has been found to decrease under conditions of anxiety[337]. As discussed in chapter 3, the study design incorporated a modified version of the PRACTALL consensus[288] in order to determine the stopping criteria for the challenge in as objective manner as possible, however subjects undoubtedly experienced some anxiety, which might have influenced these changes. The possibility that anxiety caused these HRV changes is supported by the finding that these changes were not reproducible on repeat 'non-intervention' peanut challenge.

There were clear differences between our findings at baseline challenge and NI challenge, where we found no evidence for a change in HRV parameters at the repeat open NI challenge. This could, in part, be related to a lower level of anxiety as participants had become familiar with what to expect from an active challenge. Indeed, althougho no correlation was found between HRV parameters and participant anxiety score, participants did score the severity of reaction at NI challenge significantly less severe when compared to their baseline challenge ($p=0.03$). They also reported lower median anxiety scores at NI challenge than at baseline, although this difference was not statistically significant. All this suggests that perhaps some of the changes seen at baseline challenge may be related to

anxiety over their first experience of a controlled peanut challenge. Unfortunately, data related to catecholamine levels were not available for the repeat open NI challenges, which might have been able to confirm the lower adrenergic stimulus compared with baseline challenge.

We did find some changes in ECG parameters during peanut allergic reactions, in ST segment at the J point, and in QT/QTc interval. However these changes were not consistent across different methods of measurement. The ST changes which we found were inconsistent. There was a decrease in J point in lead V2 (-0.02mm) on the active challenge day at time of OCR, and on lead II (-0.08mm) on active day compared to placebo day. Deviations of the ST segment due to non-ischemic aetiologies are often seen, and ST elevation due to non-ischemic aetiologies has been reported in up to 15% in the general population [338], more frequently in young and healthy males [339] and in the precordial leads, specifically V2 [340]. However, the magnitude of the change in ST segment found in this study is not thought to be clinically relevant, as ST elevation has been described to be pathological from 1mm or 0.1mV[341] and ST depression has been described as pathological when this is 0.5 mm or more at the J-point in at least 2 contiguous leads[342]. Besides, none of our participants complained of chest pain, which is the most common symptom reported in Kounis syndrome [327]. Finally the change was not consistent when ST segment elevation was assessed in different ways i.e. at 40ms and 60ms after the J point. We chose to measure changes in ST segment at three points (J point, 40 and 60ms after the J point) as there is a lack of consensus amongst cardiologists as to the best location to assess. A recent study has shown a specificity and sensitivity of 77% and 96% respectively at J point, 75% and 96% respectively at 40 ms, and 67% and 98% respectively at 60ms for diagnosis of STEMI, best results were found at 10ms after J point [343].

The QT interval is a measure of the duration of ventricular depolarization and repolarization. QT prolongation is associated with an increased risk for cardiac arrhythmias and sudden death[344-347]. We found no significant changes in QT interval at time of OCR on active challenges but a significant increase in QT interval was seen on placebo days, such that the difference between active and placebo days was significant. QT interval also increased prior to OCR on active days, i.e. at onset of subjective and first objective symptoms on active day (Figure 68B and 68C). The increase in QT interval on placebo days may be explained by the fact that the QT interval is subject to a diurnal variation that has been previously described in healthy participants with an increase in QT interval that peaks shortly after awakening and increase in QT interval variability in the morning hours[348]. Thus, the increase in QT

observed during placebo days, and on active days at onset of subjective and objective symptoms, probably reflects changes in diurnal sympathetic activity. The relative decrease in QT interval on active days, compared with placebo days, is likely to be an artefact of increased HR, as shown in Figure 69E, which shortens the QT interval.

Changes in QT interval are largely dependant on HR, calculating a corrected QT (QTc) interval reduces this somewhat, but the effect of correction when calculating QTc interval varies depending on the correction method used. We manually measured QTc interval using Fridericia´s and Framingham´s formula, in addition to the automated results given by the MARS programme using Bazett´s formula. Bazett´s[349] and Fridericia´s formulae tend to overcorrect (i.e. give a longer QTc interval) for higher HR values (when HR is over 60bpm), while Framingham´s formula results in an overcorrection at lower HR values. The active challenge is associated with changes in cardiovascular outcomes (as outlined in Chapter 3) including a signficant increase in heart rate, therefore there is no optimal formula to use under dynamic and acute conditions such as a food challenge. Correction using Bazett's and Fridericia's formulae both revealed apparent QTc interval prolongation at OCR and on active day compared to placebo day, but this can be explained by the tendency to over-correct QTc interval with increasing HR. Likewise, correction with Famingham's formula revealed a shortening of QTc interval on active day compared to placebo day, but this can be explained by an over-correction of QTc interval on placebo days when participants tended to be more relaxed, together with our participants being mostly young, healthy and athletic people with low resting HR seen on placebo challenge days. We therefore conclude that apparent changes in QT and QTc interval are probably due to diurnal patterns, increased HR and artefact introduced by attempts to correct QT for HR, rather than an actual change in QT/QTc interval due to reaction.

No significant changes were seen with regard to the PR interval, in contrast to what has been previously described in healthy volunteers in response to intravenous infusion of histamine, in which AV block was the most common arrythmia and the incidence and duration of the AV block was proportional to the quantity of histamine[325]. This discrepancy may be due to a relatively lower level of histamine being produced during the peanut-induced allergic reactions monitored in this thesis, consistent with the relative absence of severe reactions due to the nature of the challenge protocol, which limits reaction severity.

AV block type 2 was found in two participants ECG strip during active peanut allergic reactions. On the first case (participant 02008) this AV block was limited to 2 heartbeats and only mild symptoms of an allergic reaction (persistent stomach pain and nausea) were experienced, which could be triggered by local mast cell degranulation and therefore lower

levels of plasma histamine, in comparison to those changes seen when histamine is infused in healthy volunteers in which probably higher levels are infused and therefore the AV block is sustained for longer periods of time. This participant was given 2 more doses of peanut after the time at which the AV block was observed with no further arrhythmias being observed, this together with no other cardiovascular signs or symptoms observed we believe this AV block was not relevant to the allergic reaction.

Vagal mediated AV block has been described and defined as paroxysmal AV block, includes all types of second degree and complete AV blocks and it's a benign condition[350]. We believe this is what happened to our second participant given that she had had a very low dose of peanut protein (300ug) to which less than 5% of peanut allergic patients react[60] and there was manipulation of the cannula with symptoms of pallor and light-headedness typical of vasovagal conditions.

A significant shortening of the PR and QT interval was observed in those participants who were given IM adrenaline as rescue medication. The reason for this could be, as previously described for PR interval[351] and QT interval, due to a significant increase in HR, which in our cohort was seen after administration of IM adrenaline (chapter 3 section 3.3.12.1). A relationship was also described between HR and SDNN HRV time domain parameter[352], which could be the reason for the significant difference that was found for SDNN at 10 minutes post-reaction between those participants who required IM adrenaline and those who did not.

As with all research findings, there are some limitations to the data presented in this chapter. As previously discussed in chapter 3, there is no consensus on how to classify severity of food allergic reaction. Changes in ECG parameters were not related to apparent severity of reaction although the study was limited by the relative mild anaphylaxis occurring during food challenges, due to the importance of safety. Given that individual cases of type 2 AV block were recorded, we cannot exclude a role for cardiac conduction disturbances in more severe cases such as fatal or near-fatal food anaphylaxis. However, the absence of reproducible evidence for widespread ECG changes during allergic reactions should provide some reassurance, that the precursors of serious arrhythmias are not commonly present. This contrasts with our findings for cardiovascular changes during allergic reactions to peanut (chapter 3), where a fall in stroke volume appears to be common even in those undergoing non-anaphylactic reactions and reproducible in repeated peanut allergic reaction. This work has highlighted the potential impact of anxiety on cardiac changes recorded during peanut allergic reactions. Caution is needed when interpreting these findings, since some of the

changes seen during allergic reactions, such as increased heart rate and blood pressure, may be exaggerated by a sympathetic response in study participants.

In conclusion, we did not identify consistent ECG changes triggered by allergic reactions to peanut, in a population of young adults without pre-existing cardiovascular disease. We did identify individual cases of arrhythmias. This work does not suggest that routine continuous ECG monitoring is necessary during provoked allergic reactions to peanut, but the possibility of arrhythmia should be considered in patients with relevant symptoms.

5. RELATIONSHIP BETWEEN LOCAL VASCULAR RESPONSE TO SKIN PRICK TEST AND PHENOTYPE OF ALLERGIC REACTION TO PEANUT:

ABSTRACT:

Oral food challenge remains as the "gold standard" diagnostic test for food allergic reactions. To date the other diagnostic tests available in everyday clinics have good sensitivity and specificity but cannot risk stratify our patients and therefore they are all treated as high-risk patients, which has an impact on their quality of life and in health costs.

Some published data for egg allergy suggest that modified skin prick test may be a valid diagnostic test to identify those patients with more severe reactions.

We performed modified SPT in 57 participants undergoing DBPCFC by means of titrated SPT and time to resolution of SPT. Duplicate titrated peanut SPT (1:1, 1:10, 1:100, 1:1000, $1:10^4$, $1:10^5$) were performed on the left forearm and by the same physician on both active and placebo days. This was also repeated on the non-intervention peanut challenge day. The wheal size was initially measured after 20 minutes (PMAX). The wheal to undiluted peanut extract was subsequently measured every 30 minutes thereafter, until the wheal had disappeared or the patient was discharged (PT3). Additional data on specific IgE, peanut components, demographic characteristics, BHR and VAS was obtained and used for bivariate and multivariate analyses.

Results showed that participants with larger SPT wheal size (PMAX) and lower threshold of SPT reactivity (PC3) tend to have lower threshold of clinical reactivity and a significant difference was found between those participants who required IM adrenaline and those who did not for the PT3, this latter was also seen for those participants who had more severe reactions following several scoring systems. The overall VAS score was significantly correlated with PT3. However these results were not reproduced on repeated peanut challenges on a smaller sample size of the same population.

Preliminary results from DBPCFC to peanut suggest that time to resolution of SPT is related to severity of reaction on the day of the challenge whilst titrated SPT is related to threshold of reactivity, never the less further studies on bigger cohorts and different food triggers are needed to confirm these findings.

5.1.Introduction:

5.1.1 Food allergy (FA):

The European Academy of Allergy and Clinical Immunology (EAACI) defines FA as an adverse reaction to food triggered by an immunological mechanism, involving specific IgE (IgE-mediated), cell-mediated mechanisms (non-IgE-mediated) or both IgE-and cell-mediated mechanisms (mixed IgE-and non-IgE-mediated)[353]. For this chapter I will study IgE-mediated FA. Food allergic reactions can vary from mild self-limited to cardiorespiratory arrest. Severe reactions are termed anaphylaxis, defined by the World Allergy Organization (WAO) as severe, life-threatening generalized or systemic hypersensitivity reaction, which is characterized by being rapid in onset with life-threatening airway, breathing or circulatory problems, usually associated with skin and mucosal changes[13].

Primary IgE-mediated FA involves sensitization to the primary allergen(s), depending on geographical location there can be secondary IgE-mediated FA as part of cross-sensitization to inhalant allergens. In northern Europe secondary peanut allergy is part of sensitisation to birch pollen, which cross-reacts with homologous epitopes in peanut. For this study we used subjects with primary sensitisation to peanut rather than as part of pollen food allergy syndrome.

IgE-mediated FA affects 4-7% of primary school children[36], 6-8% of children of all ages and 3% of adults[16, 34, 202, 354] when diagnosed with oral food challenge (OFC). Different studies have shown an increased prevalence in the diagnosis of food allergy[37, 39, 40] and food anaphylaxis[42] in recent decades. The most common offending foods in primary IgE-mediated FA and anaphylaxis are milk, egg, peanut, tree nuts, fish, shellfish, wheat and soy for young children and peanut, tree nut and fish, shellfish for adults[42, 43]. In Northern Europe, fruits and vegetables are common causes of IgE-mediated FA in adults, usually as part of the pollen food syndrome (secondary food allergy) when eaten raw, but these foods only rarely cause anaphylaxis[24].

5.1.1.1 Peanut allergy:

Peanut (*Arachis hypogaea*) is a groundnut, considered a legume crop. A systematic review performed by the EAACI showed a point prevalence of peanut allergy of 1.7% for SPT positive, 8.6% for positive IgE, 0.2% for positive OFC and 1.6% for positive OFC or history of peanut allergy[355]. Prevalence varies depending on geographical region and it

is estimated to affect between 0.2-3% of the population[16, 355]. Prevalence of peanut allergy may have increased in children between 3-4 years old in the last 20 years, in some populations [40] and seems to be persistent through to adolescence [356] with only around 20% of cases resolving spontaneously[111]. In the UK and USA, peanut allergy is the most common cause of fatal food anaphylaxis [42, 51].

5.1.1.2 Diagnostic tests in Food Allergy (FA):

The "gold standard" diagnostic test for food allergic reactions is a double-blind placebo control food challenge (DBPCFC), and the European Academy of Allergy and Clinical Immunology (EAACI), following recommendations after PRACTALL study, have released recommendations that provides criteria, which should be met so that DBPCFC is used appropriately and consistently[288]. However, the DBPCFC is limited in practice by the potential to induce anaphylaxis, time needed to perform a food challenge (typically one day), need for multiple resources, possible reactions to placebo – all of which can invalidate the results[357-359]. Furthermore, little is understood about the reproducibility of a challenge, and how the information gleaned on one day might be relevant to another occasion.

Given these limitations, the most commonly used diagnostic test in many Allergy Clinics is the skin prick test (SPT), as it is minimally invasive, inexpensive, results are available within 15-20 minutes and when carried out by trained health professionals the results can be reproducible. Sensitivity and specificity for food allergens range from 30-90% and 20-60% respectively[91], which is the main limitation of the technique. SPT can also be utilized to test less common allergens, such as fresh fruits and vegetables using the prick-prick technique where no specific IgE antibody measurements are available, or relevant epitopes are easily degraded. Overall, the larger a SPT wheal, the greater the likelihood of allergy[360].

Currently, it is difficult to diagnose more detailed information about the disease, for patients with IgE-mediated food allergy. For example, it is not possible to reliably predict which patients with primary food allergy are at greatest risk for anaphylaxis[280], and it is not possible to reliably predict threshold of reactivity without undergoing oral food challenge. Therefore, healthcare professionals often treat all such patients as at risk of reaction from low dose exposures, and at risk of fatal food anaphylaxis. This means that strict avoidance and provision of an adrenaline auto injector device for emergency use are usual care for patients with primary IgE mediated food allergy. This blanket approach may lead to excessive dietary restrictions and anxiety in some participants, and does not allow for tailored advice to those participants at highest risk of severe reactions, or

reactions to trace exposures. Adrenaline autoinjector device prescription has increased significantly in the last 20 years [361]. Current diagnostic testing cannot help risk-stratify patients reliably, and anecdotally there is variability both within and between individuals in terms of threshold and reaction severity[60].

It would be clinically useful if SPT could predict the threshold or severity of an allergic reaction resulting from exposure, but attempts at identifying features of SPT, which might predict threshold or severity of clinical reaction have reported inconclusive results to date [15, 297]. One way of modifying SPT to predict severity may be to use the end-point titration (EPT), which involves using serial dilutions of SPT extract, was found to predict the reaction to hen's egg at food challenge in children[92]. EPT represents threshold of reactivity in the skin, to SPT; so may be able to predict threshold of reactivity to oral ingestion[291].

Severity of clinical reaction to orally ingested allergen may be partly determined by the efficiency of mechanisms, which terminate a reaction. Mast cells and basophils have well-characterised self-inhibitory mechanisms, which may be relevant to the duration and/or severity of a clinical reaction[27, 362, 363]. In this chapter, we elected to study the time course of natural resolution of SPT wheal to peanut, as a possible measure of a patient's mast cell and basophil self-inhibitory mechanisms. Our hypothesis was that duration of wheal in response to peanut SPT may provide a diagnostic marker for severity of reaction to orally ingested peanut, in individuals with primary IgE-mediated peanut allergy.

The aims of this chapter are to:

1. Investigate the relationship between threshold of SPT reaction, using endpoint titration, and threshold of clinical reactivity (assessed at DBPCFC) in young adults with IgE-mediated peanut allergy.
2. Investigate the relationship between time to resolution of SPT wheal and severity of clinical reaction at DBPCFC in young adults with IgE-mediated peanut allergy.
3. Try to develop a model, which can predict threshold or severity of reaction, in young adults with IgE-mediated peanut allergy.

5.2. Methods:

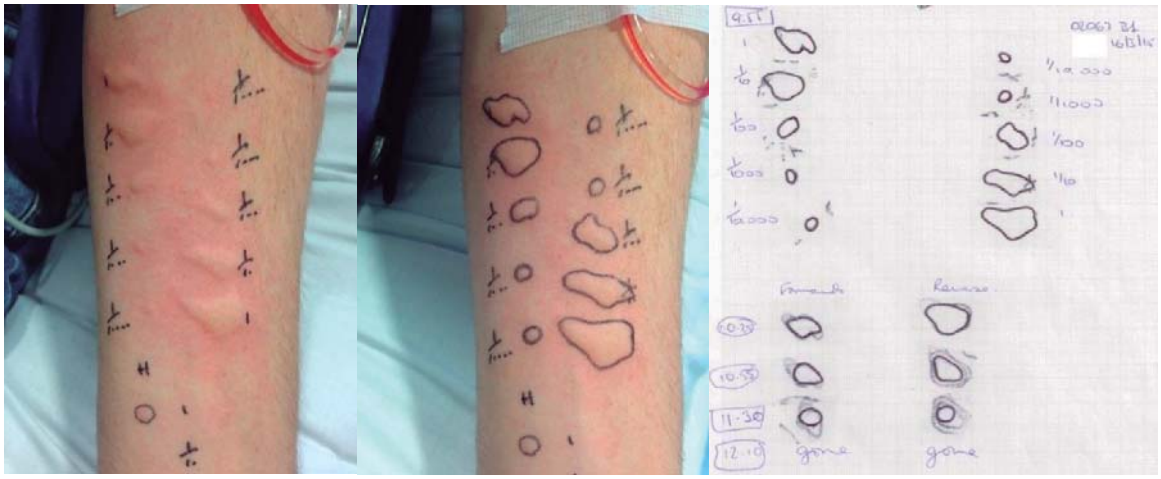
5.2.1 Study protocol:

The protocol for the TRACE peanut allergy study has been previously outlined in the methods chapter section 2.1. This chapter describes evaluation of SPT responses at baseline DBPCFC, and again at subsequent open 'non-intervention' challenge.

5.2.2 Study procedures:

Screening visits to assess eligibility for the study were performed in all participants. A full medical history and SPT to commercial peanut extract and a range of other commercial food extracts and aeroallergens (Stallergenes, Paris, France) were completed for each subject (the different extracts used are explained in Methods section). Subjects were excluded at this point if they did not have a positive SPT (≥3mm) to commercial peanut extract. Blood concentrations of specific IgE to peanut and Ara h1, h2, h3, h8 and h9 were measured and bronchial hyper-responsiveness (BHR) was assessed with inhaled histamine using the dosimeter method (SOP Appendix 8). All patients were given questionnaires on asthma control (ACQ), eczema (POEM) and rhinitis (Total Nasal Symptom Score (TNSS)) severity. Subjects did not continue with the study if they had a presentation consistent with pollen food allergy syndrome, mono-sensitisation to Ara h 9, or poorly controlled asthma (BTS treatment step 3 or higher[364]). Before participants underwent the DBPCFC, titrated SPT to peanut was performed in duplicate according to European guidelines[91] on both active and placebo days. This was also repeated on the non-intervention peanut challenge day. Dilutions of 1:1, 1:10, 1:100, 1:1000, $1:10^4$, $1:10^5$ commercial peanut extract (Stallergenes, Paris, France) were applied to the volar side of the left forearm of the patient using ALK lancets (ALK-Abelló, Hørsholm, Denmark). Singlicate titrated SPT to 1% histamine and 9% codeine phosphate at 1:1, 1:10, 1:100, 1:1000 was also performed. To reduce variability, SPT was performed and read by the same clinician. The wheal size was initially measured after 10 minutes for histamine and codeine, and after 20 minutes for peanut extract. The wheal to undiluted peanut extract was subsequently measured every 30 minutes thereafter, until the wheal had disappeared or the patient was discharged. SPT results were recorded by drawing around the circumference of the wheal and transferring to paper with cellophane tape shown in Figure 92 and explained in the SOP Appendix 12.

Figure 92 Skin prick test technique:



A. B. C.

Technique of SPT (A) 20 minutes after it was performed, (B) after drawing around the wheals with permanent marker at 20 minutes and (C) scan of wheal sizes transferred to paper, above, initial duplicated measurements and below, sizes of wheals to undiluted extract over time.

5.2.3 Data analyses:

Mean wheal diameter (the average of the largest diameter and then the perpendicular diameter) was measured at each concentration and time point. The mean of the wheal diameters to undiluted extract was called PMAX. Cut off points of 3mm and 6mm were interpolated by finding the highest concentration to induce a 3mm and 6mm wheal respectively and labelled as PC3 and PC6 respectively, shown in Figure 93. Results in section 5.3.3 of this chapter show a linear relationship between concentration of peanut extract and SPT wheal, which justifies the formula used to calculate PC3 and PC6.

Figure 93 Formula to calculate concentration to induce a 3 or 6mm wheal:

$$PC = C1 + (R - W1) \times \frac{C2 - C1}{W2 - W1}$$

R= defined wheal diameter we are investigating (3 or 6).

C1= concentration that induced a wheal diameter closest to and less than R.

W1= size of wheal at C1.

C2= concentration that induced a wheal diameter closest to and greater than R.

W2= size of wheal at C2.

A similar formula was used to determine the time taken for PMAX to diminish to a 6mm and 3mm wheal labelled as PT6, and PT3 respectively, shown in Figure 94. Results in section 5.3.4 of this chapter show a linear relationship between time to resolution and SPT wheal, which justifies the formula used to calculate PT3 and PT6.

Figure 94 Formula to calculate the time needed to reduce the SPT wheal size to 3 or 6mm wheal:

$$PT = T1 + (R - W1) \times \frac{T2 - T1}{W2 - W1}$$

R= defined wheal diameter we are investigating (3 or 6).

T1= time when wheal diameter had shrunk to just below R.

W1= size of wheal at T1.

T2= time when wheal diameter had shrunk to just above R.

W2= size of wheal at T2.

5.2.4 Statistical analyses:

The general approach to statistical analyses has been explained in in the Methods section 2.9.3 and is summarised below.

Initial statistical analysis was done following Mann-Whitney test and Spearman correlation. When we look at multiple variables with a relatively small n number the continuous variables didn´t follow normal distribution so we consulted external statistical who advice to do bootstrapping.

Initially bivariate analyses examining associations between the dependent and independent variables calculating Spearman correlation for continuous variables, chi- square test for categorical variables and independent t-tests for continuous measures using SPSS version 23 (New York, United States) were performed. Where continuous data distributions were clearly non-normal, bootstrapping (bias-corrected and accelerated; based on 2000 bootstrap samples) was employed to calculate 95% confidence intervals of mean difference and associated p values.

To analyse the combined contribution of the most important dependent and independent variables, multivariable logistic and binary regression models were constructed for each outcome measure. Age and cumulative dose where always included in the models, furthermore to evaluate the robustness of associations when controlled for potential confounding variables, and to develop a model to predict threshold dose or severity of reaction any variable from bivariate analyses indicating at least marginal significance ($p<0.10$) was included. The analysis was done using ENTER method in SPSS.

For threshold dose model the dependent variable was cumulative dose of peanut ingested in mg of peanut protein, although we also explored whether relationships changed if final dose ingested was used in place of cumulative dose (no differences were found, data not shown). For severity of reaction model, we used several separate measures, since there is no consensus on how best to measure reaction severity in food allergy[280]. Measures of severity used were Ewan&Clark classification of severity[287], NIAID classification of anaphylaxis[286], WAO grading system for systemic reactions[316] and use of IM adrenaline during DBPCFC, all of which were made binary as 'anaphylaxis' versus 'no anaphylaxis' or "severe" versus "not severe". We also used as measure of severity participant´s and investigator´s VAS overall reaction severity, scored from 1 to 10 with 10 being the most severe reaction, plasma adrenaline level and MCT peak % change from baseline as objective physiological markers of reaction severity. The independent explanatory variables, which we analysed, were: PMAX, PT3, age, sex, BHR, PC3 (ln-transformed) and Ara h 2 (ln-transformed). Cumulative dose was also used as an explanatory variable, in relation to severity outcomes.

5.3 Results:

5.3.1 Study population:

Data from baseline challenges (Table 19) were available for 57 participants for SPT wheal at 20 minutes (PMAX) and PC3, but only 56 participants for PC6 since 1 participant had a PMAX less than 6mm. Results for time to resolution of SPT (PT3 and PT6) were available in 47 participants, because PT3 and PT6 were only recorded after the first 10 participants had completed their baseline challenge. Titrated SPT was performed as duplicate in 49 participants and as singlicate in the first 8 participants. Twenty-eight participants underwent a further open non-intervention (NI) challenge at which similar data were acquired, data for 20 participants were available for PMAX, PC3 and PC6 and data for 18 participants were available for time to resolution of SPT (PT3 and PT6). SPT was not repeated in the remaining participants.

Table 19 Characteristics of the study population:

	Baseline challenge	**Repeated challenge (NI)**
Age at enrolment	24 (20,29) years	24 (22, 29)
Age at time of diagnosis	2 (1, 6) years	2 (1, 4) years
Sex (female)	33 (58%)	12 (60%)
Asthma	32 (56%)	10 (50%)
Rhinitis	44 (77%)	17 (85%)
Eczema	31 (54%)	11 (55%)
Total IgE	251 KU/L (114, 690)	208 KU/L (74.5, 427)
Specific IgE to peanuts	11 KUA/L (3.6, 26)	17KUA/L (3.4, 31.9)
Specific IgE to Ara h 2	5 KUA/L (2, 18)	10 KUA/L (2, 13.7)
Wheal size SPT to peanut extract Stallergenes®	11mm (9, 15)	11mm (9, 13)
Sensitised to other nuts	31 (54%)	11 (55%)
Sensitised to other non-nut foods	30 (53%)	10 (50%)
Cumulative dose of peanut protein ingested	133mg (33, 433)	133mg (58, 433)
BHR	16mg/ml (3.6, 16)	16mg/ml (3.7, 16)
PC3	0.0007mg/ml (0.0002, 0.006)	0.001microg/ml (0.0002, 0.004)
PT3	156mins (117, 196)	151mins (106, 170)
IM adrenaline during peanut challenge	11 (19%)	4 (20%)
NIAID (anaphylaxis)	14 (25%)	6 (30%)
Ewan&Clark (moderate/severe)	19 (33%)	8 (40%)
WAO (moderate/severe)	9 (16%)	4 (20%)
Mast Cell Tryptase (% peak change)	24% (7, 46)	N/A
Adrenaline (change from baseline)	0.29nmol/L (-0.08, 0.83)	N/A
Noradrenaline (change from baseline)	0.01nmol/L (-0.09, 0.11)	N/A

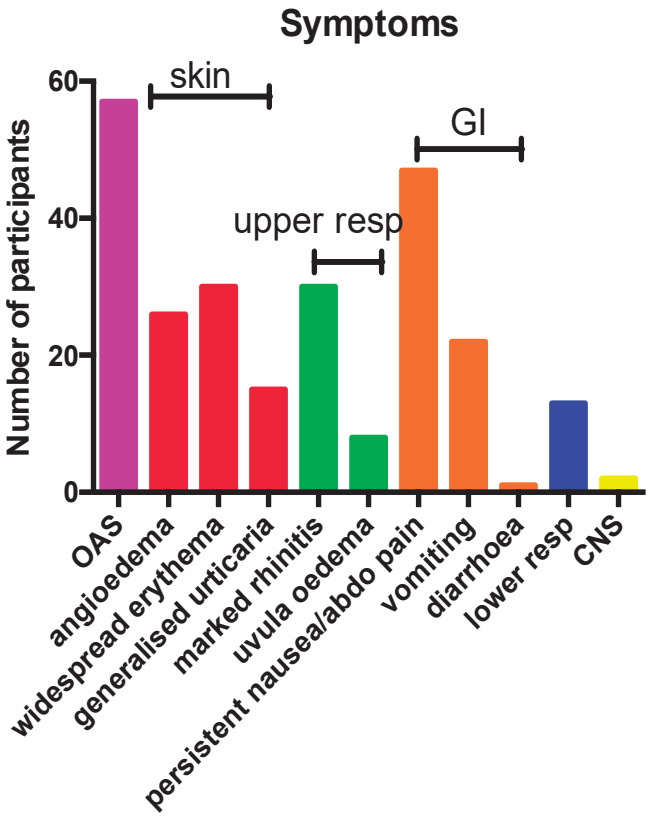
All data are shown as median and IQR unless specified otherwise. N/A: Data not available.

5.3.2 Summary of symptoms during peanut allergic reaction:

During baseline challenge, all participants had oropharyngeal symptoms ('OAS'), most participant´s had gastrointestinal symptoms too, typically persistent nausea and stomach pain (summarised in Figure 95). Almost 50% of participants experienced upper respiratory symptoms, the most common being marked rhinitis. Ten participants had lower respiratory symptoms and required IM adrenaline as rescue medication and 2 participants experienced altered level of consciousness lasting for less than 2 minutes with no recurrence or progression to other organs. Participant´s VAS scoring rated throat and abdominal symptoms as most severe, although severe symptoms were reported in every domain by at least one participant (Figure 96).

Severity of reaction was classified using three different published scoring systems, which have been previously described in Methods chapter section 2.8 and section 5.2.4 of this chapter. The distribution of our participants according to these classifications is shown in Table 20 where we can appreciate that there is some discordance between the 3 scoring systems when trying to classify the same reaction. Ewan and Clark scoring system classifying more reactions as "severe" than the other two classifications.

Figure 95 Symptoms during baseline peanut allergic reactions for the 57 participants analysed in this chapter:



Summary of the most common symptoms participants experienced during an allergic reaction separated by organ. GI: gastrointestinal; OAS: oral allergy syndrome; CNS: central nervous system.

Figure 96 Symptom severity reported by participants at baseline challenge for the 57 participants analysed in this chapter:

	Skin	Upper resp	Lower resp	GI	Anxiety	Overall
02003	8	6	0	0	4	4
02004	0	7	2	7	2	6
02005	9	10	1	10	3	9
02008	1	2	0	1	1	5
02010	6	6	4	9	8	6
02012	7	9	2	4	8	7
02014	0	0	0	3	10	9
02016	7	6	5	7	3	5
02018	4	4	1	8	3	5
02019	8	6	6	8	7	7
02023	1	8	3	8	1	6
02024	0	6	1	5	5	5
02025	1	4	3	7	4	4
02034	3	8	7	4	4	7
02037	5	6	8	7	0	7
02040	1	6	1	7	6	7
02045	6	8	8	7	8	8
02047	0	6	6	8	6	8
02048	8	5	3	8	5	5
02049	6	7	0	3	5	4
02051	4	6	2	7	2	4
02053	2	1	0	4	5	4
02055	6	3	0	6	1	4
02056	7	5	2	8	5	6
02063	5	4	0	0	1	4
02064	3	8	1	3	0	4
02065	0	8	0	4	1	7
02066	7	6	2	8	6	7
02067	7	8	0	6	7	7
02073	1	3	0	6	3	3
02074	7	7	0	8	3	7
02075	0	5	0	8	9	3
02078	5	4	5	6	3	5
02079	0	8	2	10	7	7
02080	3	6	3	8	2	6
02081	4	6	4	9	2	6
02087	4	2	7	4	7	7
02090	2	3	0	3	3	3
02091	3	6	4	1	5	3
02092	7	5	4	0	4	6
02093	3	5	3	5	0	5
02094	2	6	0	7	4	5
02097	3	4	0	7	1	4
02098	0	3	0	6	10	10
02100	5	8	7	0	0	6
02101	3	7	1	6	5	4
02103	2	3	3	6	2	4
02104	1	7	6	7	1	8
02106	8	6	6	6	9	8
02108	2	8	4	4	4	5
02109	0	6	0	8	2	7
02111	3	2	3	0	1	1
02112	3	5	2	6	6	5
02117	0	4	2	4	2	6
02120	6	5	0	0	7	6
02121	3	1	0	0	2	2
02124	5	7	1	4	3	6

Heat map for the VAS symptom score by participants for each organ and overall reaction score. Symptoms were reported on a 10-point VAS, where 10 is the most severe, and 0 means no symptoms.

Table 20 Concordance of anaphylaxis or severe reaction classification across 3 different published classification systems.

	E&C only	NIAID only	WAO only	E&C and NIAID	E&C and WAO	NIAID & WAO	All scores
Baseline	23/57 (40%)	13/57 (23%)	13/57 (23%)	8/57 (14%)	13/57 (23%)	8/57 (14%)	8/57 (14%)
NI Challenge	10/28 (36%)	6/28 (21%)	5/28 (18%)	4/28 (14%)	5/28 (18%)	4/28 (14%)	4/28 (14%)

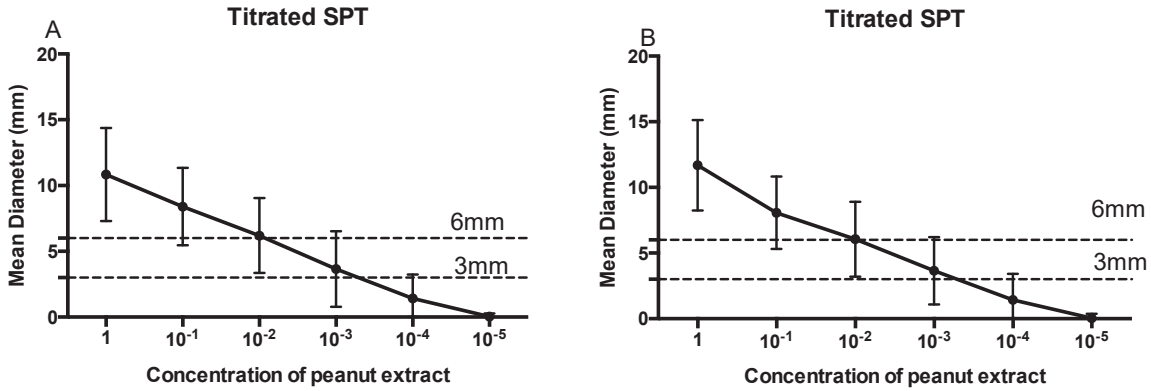
Data represent the proportion (%) of participants categorised as having anaphylaxis or severe reaction using 3 different published classification systems. E&C= Ewan and Clark.

5.3.3 Investigation of titrated SPT with peanut extract during peanut allergic reaction:

Results in Figure 97 show titrated SPT on both active and placebo days of the baseline challenge. Results are for skin prick test wheal at 20 minutes (PMAX median 10.5mm, IQR [8.5, 13.1]), PC3 (10^{-4}IQR [10^{-4}, 10^{-3}]) and PC6 (10^{-2}, IQR [10^{-3}, 10^{-2}]).

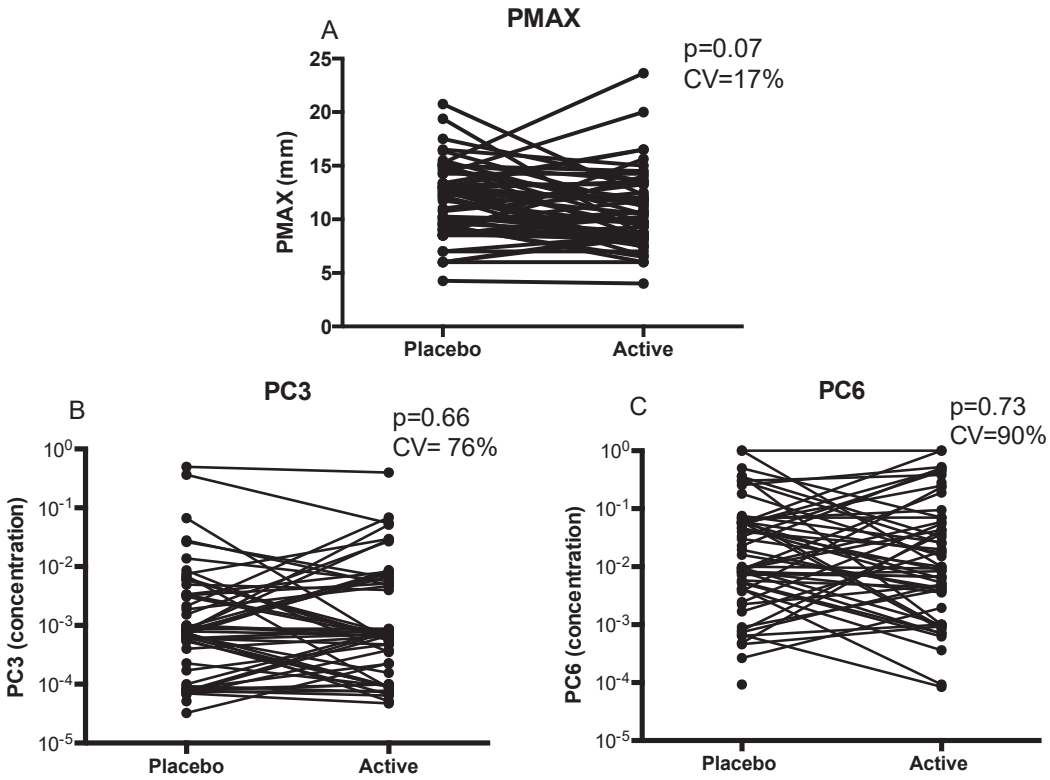
Similar results were found on both days and no consistent difference was seen for PMAX, PC3 and PC6 between active and placebo days. But there was significant variability for PC3 and PC6 between placebo and active challenge days, shown in Figure 98. For correlations with severity and threshold of reaction, we elected to use PC3 only, due to the lower CV of PC3, and the high correlation between PC3 and PC6 ($r=0.75$, $p<0.0001$).

Figure 97 Titrated SPT during baseline challenge:



Difference on titrated SPT on (A) active day and (B) placebo day. Shown are mean and SD.

Figure 98 Measures of titrated SPT on baseline challenge:

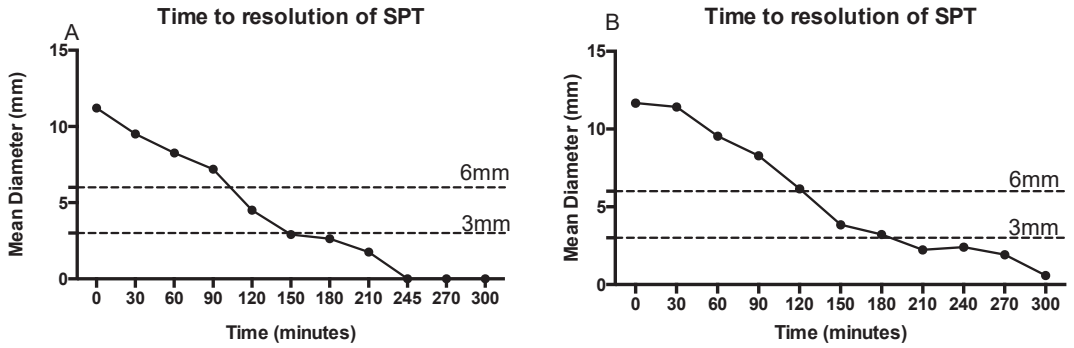


Change in (A) PMAX, (B) PC3 and (C) PC6 between active and placebo days. P value shows the difference between placebo and active day.

5.3.4 Investigation of time to resolution of SPT during peanut allergic reaction:

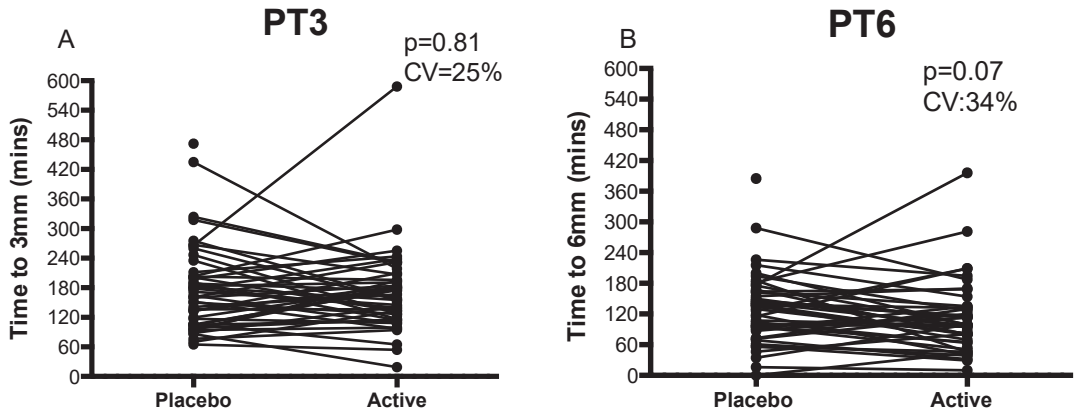
Results in Figure 99 show results for time to resolution of SPT for both baseline challenge days, placebo and active. Similar results were found on active and placebo days. Results for PT3 (median 156mins, IQR [117, 196]) and PT6 (98mins, IQR [65, 133]) are shown in Figure 100, no significant differences were seen for any of these parameters between active and placebo days, and the CVs were acceptable. For correlations with severity and threshold of reaction, we elected to use PT3 only due to lower CV than PT6, and due to a high correlation between PT3 and PT6 ($r=0.81$, $p<0.0001$).

Figure 99 SPT time to resolution during baseline challenge:



Difference in time to resolution of undiluted wheal in (A) active and (B) placebo challenge day. Shown are mean.

Figure 100 Measure SPT time to resolution during baseline challenge:

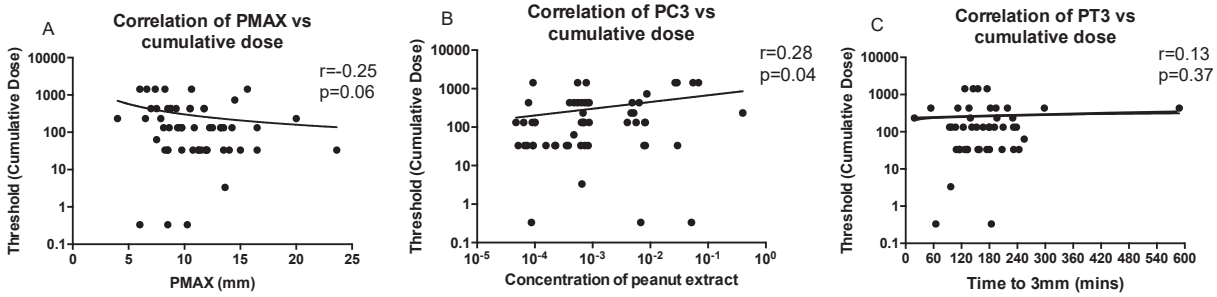


Change in (A) PT3 and (B) PT6 between active and placebo days.

5.3.5 Relationship between measures of SPT response to peanut and threshold of clinical reactivity in young adults with IgE-mediated peanut allergy:

Unadjusted analyses suggest that participants with larger SPT wheal size and lower threshold of SPT reactivity tend to have lower threshold of clinical reactivity. There was no relationship between PT3 and threshold dose. Correlation coefficients did not suggest a strong relationship for PMAX and PC3, and the statistical significance of these relationships was only modest (Figure 101).

Figure 101 Relationship between titrated SPT and threshold:

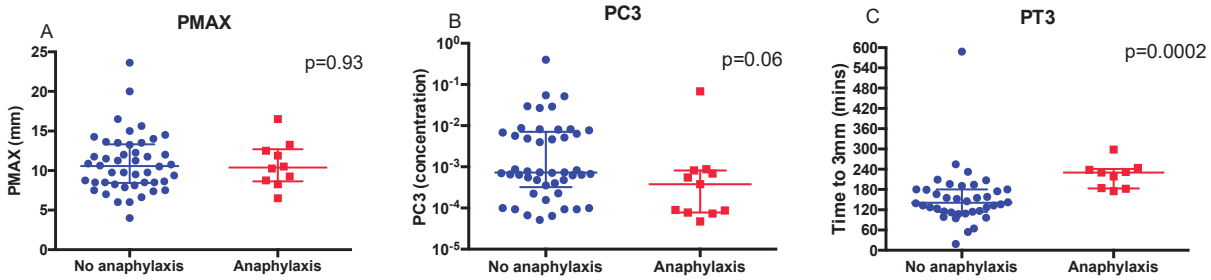


Spearman correlation between cumulative threshold dose and (A) PMAX, (B) PC3 and (C) PT3.

5.3.6 Relationship between measures of SPT response to peanut, and measures of severity of reaction, in young adults with IgE-mediated peanut allergy:

A significant difference was found between those participants who required IM adrenaline and those who did not for the time to resolution of SPT to 3mm, shown in Figure 102 (also for 6mm, data not shown p=0.04). Similarly, PT3 was also longer in participants with more severe reactions when assessed using other scoring systems, as described below. No significant differences were found for PMAX and PC3. Given that treatment with IM adrenaline and the clinical reaction itself could affect the time to resolution of SPT, we explored PT3 in 2 other different ways; only in those participants who SPT reached a 3mm wheal before OCR and on placebo challenge days. Results in Appendix 24, Figure 13 show that when excluding those participants with PT3 longer than time to OCR, results are similar to those for the complete dataset. When we evaluated PT3 on placebo day and measures of severity of reaction on the active day, prolonged PT3 in those with NIAID classification of anaphylaxis was found, but no relationship between PT3 and severity using other measures, as shown in Appendix 24, Figure 14.

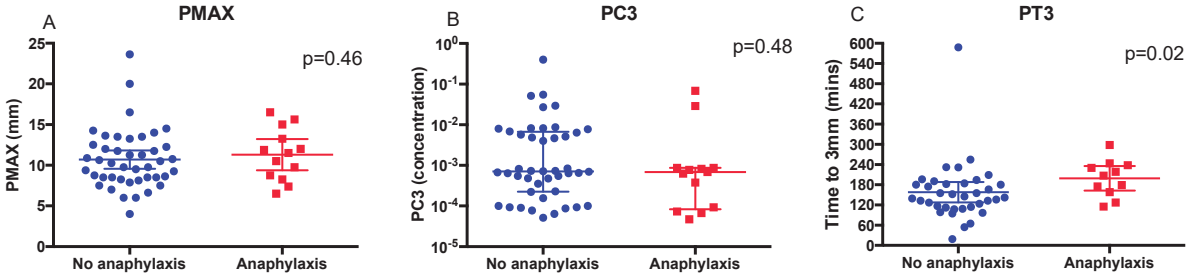
Figure 102 Differences in SPT measurements in those participants who required IM adrenaline:



Change in (A) PMAX, (B) PC3 and (C) PT3 between those participants who required IM adrenaline and those who did not.

A significant difference was seen for PT3 between those participants classified as having anaphylaxis compared to those who did not by the NIAID classification, shown in Figure 103.

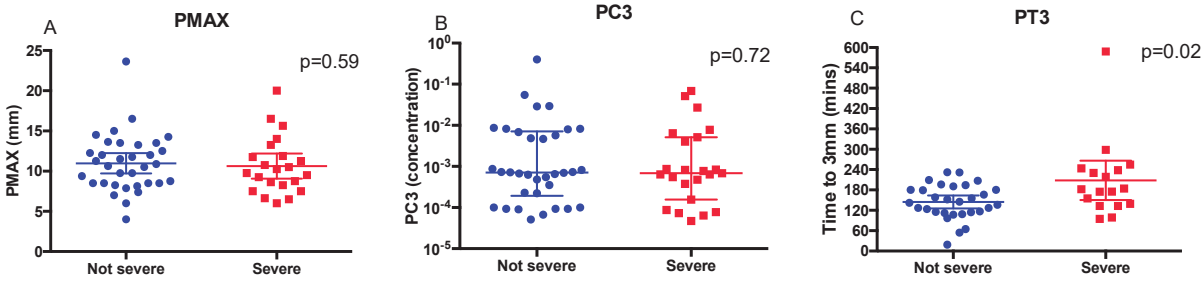
Figure 103 Differences in SPT measurements when reaction severity is classified by NIAID:



Change in (A) PMAX, (B) PC3 and (C) PT3 according to NIAID classification of anaphylaxis.

A significant difference was seen for PT3 between those participants classified as having a severe reaction compared to those who did not according to Ewan&Clark classification of food allergic reaction, shown in Figure 104.

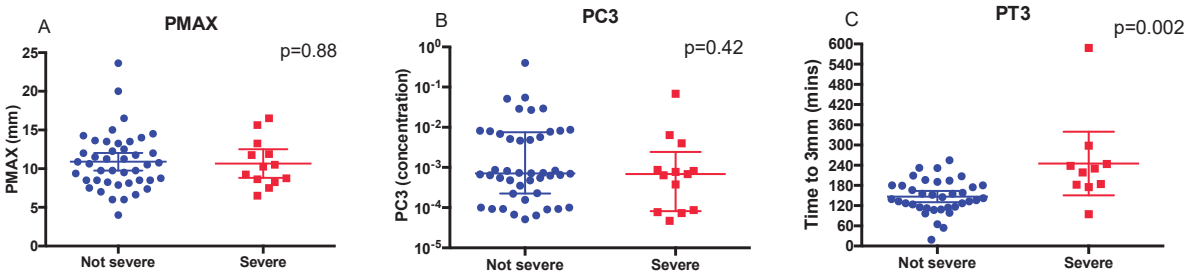
Figure 104 Differences in SPT measurements when reaction severity is classified by Ewan&Clark:



Change in (A) PMAX, (B) PC3 and (C) PT3.

A significant difference was seen for PT3 between those participants classified as having a severe reaction compared to those who did not according to WAO classification, shown in Figure 105.

Figure 105 Difference in SPT measurements when reaction severity is classified by WAO:



Change in (A) PMAX, (B) PC3 and (C) PT3.

Participant-rated severity of reaction using a VAS score. The overall VAS score was significantly correlated with PT3, but not with PC3 or PMAX, as shown in Table 21. Similar results were seen for relationship between investigator VAS overall score and measures of SPT shown in Appendix 25.

Table 21 Relationship between SPT measurements and participant´s VAS score on baseline challenge:

	VAS skin	Vas GI	VAS upper respiratory	VAS lower respiratory	VAS overall
PMAX	r= -0.07 (p=0.62)	r=0.23 (p=0.09)	r=0.05 (p=0.68)	r= -0.15 (p=0.26)	r= -0.14 (p=0.30)
PC3	r= -0.15 (p=0.28)	r= -0.12 (p=0.29)	r= -0.09 (p=0.51)	r= -0.09 (p=0.53)	r= -0.08 (p=0.58)
PT3	r=0.21 (p=0.16)	r=0.07 (p=0.63)	r= -0.08 (p=0.58)	r=0.19 (p=0.21)	**r=0.40 (p=0.006)**

Spearman correlation. Highlighted p<0.05.

5.3.7 Association between CVS, HRV parameters and SPT:

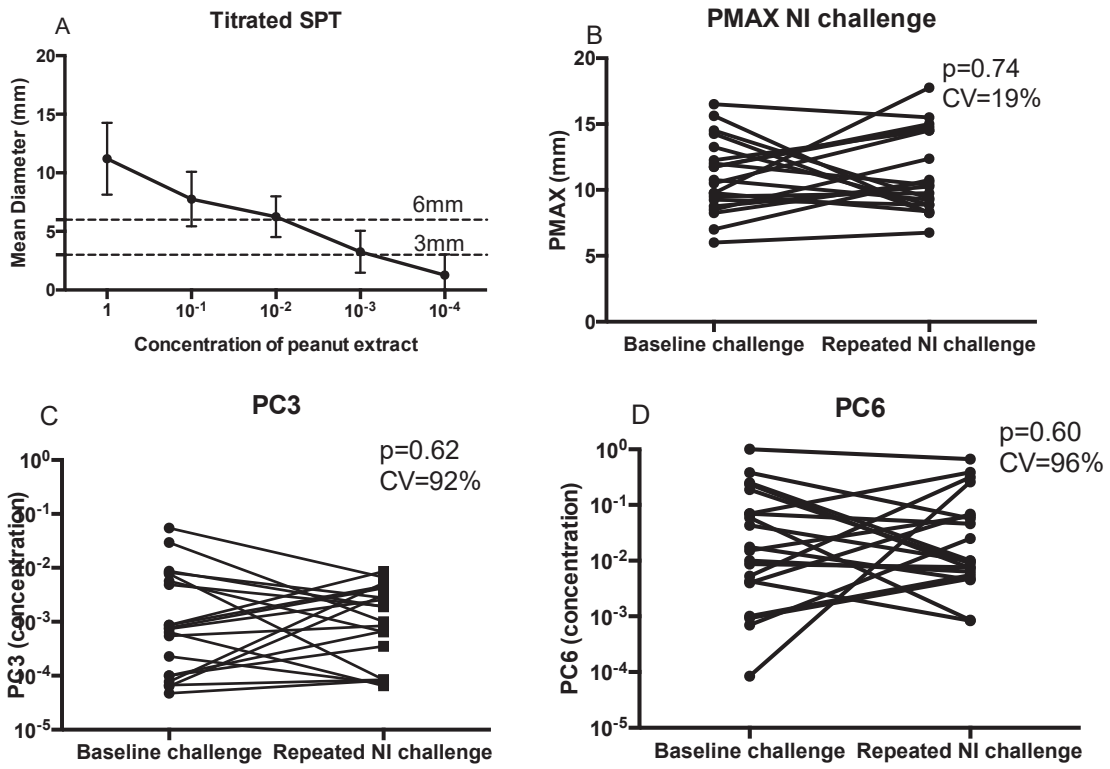
No correlation was found between any of the CVS or HRV parameters and the SPT parameters explored. This is shown in Appendix 26.

5.3.8 Changes in repeated NI challenges:

5.3.8.1 Investigation of titrated SPT during repeated NI peanut allergic reaction:

No significant differences were seen for PMAX (median 10.3mm, IQR [8.9, 14.5]) but again a great variability was seen for PC3 (10^{-3}, IQR [10^{-4}, 10^{-3}]) and PC6 (10^{-2}, IQR [10^{-3}, 10^{-2}] between baseline and NI active challenge day, shown in Figure 106.

Figure 106 Measures of titrated SPT on repeated NI challenge:



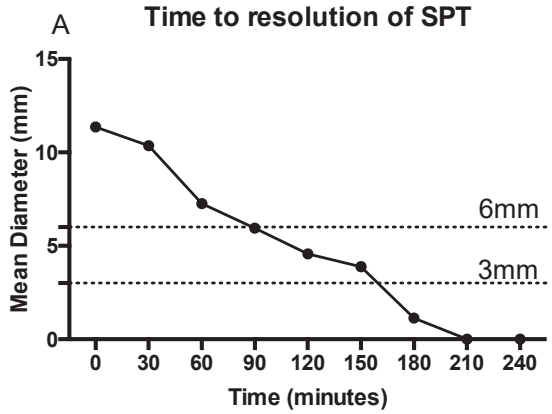
Mean titrated SPT (A), changes in (B) PMAX, (C) PC3 and (D) PC6 between baseline and repeated NI challenge.

5.3.8.2 Investigation of time to resolution SPT during peanut allergic reaction:

Figure 107 shows the time to resolution of SPT during repeated NI challenges.

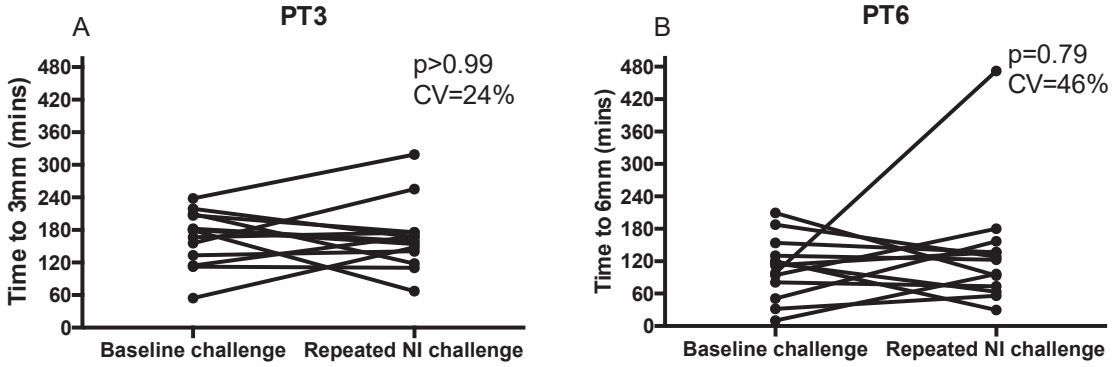
No consistent direction of difference, and fair consistency, were seen for PT3 (median 151mins, IQR [106, 170]) and PT6 (95mins, IQR [59, 137]) between baseline and NI active challenge day, shown in Figure 108.

Figure 107 SPT time to resolution on repeated NI challenge:



Mean time to resolution of undiluted wheal.

Figure 108 Measures of SPT time to resolution on repeated NI challenge:

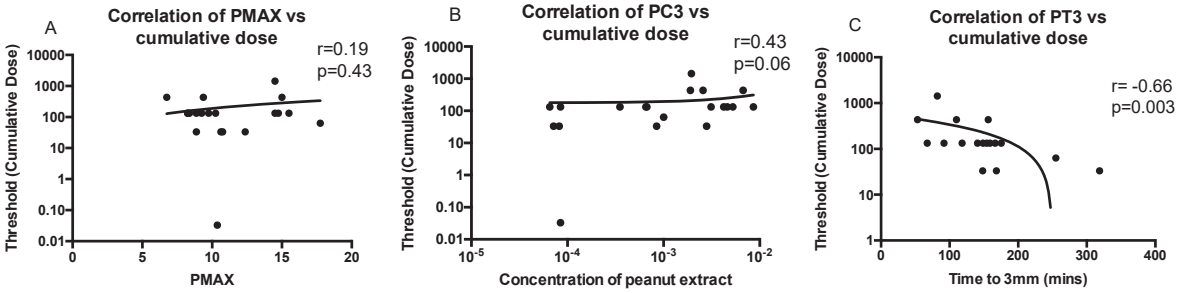


Change in (A) PT3 and (B) PT6 between active and repeated NI challenge.

5.3.8.3 Relationship between measures of SPT response to peanut and threshold of clinical reactivity during repeated NI challenge:

Unadjusted analyses suggest that participants with longer SPT wheals tend to have lower threshold of clinical reactivity with a strong correlation between them (Figure 109). There was no relationship between size of SPT of threshold of SPT reactivity and threshold dose as seen during baseline challenge.

Figure 109 Relationship between titrated SPT and threshold on repeated NI challenge:



Spearman correlation between cumulative threshold dose and (A) PMAX, (B) PC3 and (C) PT3.

5.3.8.4 Association between SPT and severity of reaction during repeated NI challenge:

We were unable to reproduce the results found for PT3 seen during baseline challenge, however the number of anaphylaxis cases was generally too small for meaningful analysis. We did find in this dataset, surprisingly, that there was significant smaller PMAX in those participants classified as having anaphylaxis by the use of IM adrenaline as rescue medication and NIAID classification of anaphylaxis and between those classified as having a severe reaction according to WAO classification. This is shown in Appendix 27.

5.4 Predictive models:

5.4.1 Bivariate analyses on baseline challenge data:

Table 22 shows bivariate regression between the dependent and independent variables, using bootstrapping for all analyses with a non-binary dependent variable. Both PC3 and Ara h 2 were statistically associated with cumulative dose i.e. threshold of skin prick test reactivity was associated with threshold of clinical reactivity to an oral dose, in peanut allergy, and specific IgE to Ara h 2 was inversely correlated with cumulative threshold. Similar associations were seen in the NI challenges undertaken in a subset of these participants (Appendix 28, Table 11).

For measures of severity of reaction, the most consistent association was with PT3; participants who required IM adrenaline, were classified as having anaphylaxis or severe reaction, or whose reaction was rated as more severe by participant´s or investigator´s VAS score, had more persistent SPT wheal reactions and therefore the time for the SPT to reduce below 3mm was increased. This association was not seen for the objective markers of reaction severity or response to reaction (plasma adrenaline and MCT), nor in the NI challenges (Appendix 28, Table 11). Bivariate analyses using only the subset of participants who reached PT3 before OCR is shown on Appendix 27, Table 12, these results show significant correlation with use of IM adrenaline and participant´s VAS overall score. No significant correlation was seen for bivariate analyses using measurements for PT3 on placebo day (Appendix 28, Table 13).

Participants with more severe reactions tended to have received higher cumulative dose, and to have lower PC3, than participants with less severe reactions. However this was only statistically significant for cumulative dose and MCT, and for PC3 and investigator VAS score, again these associations were not seen in the NI challenges (Appendix 28, Table 11). BHR to a lower concentration of histamine was associated with anaphylaxis by Ewan and Clark classification, again not seen in the NI challenges (Appendix 28, Table 11). Specific IgE level to Arah2 was only associated with plasma adrenaline, but not to clinical markers of reaction severity, nor to MCT, either in baseline or NI challenges (Appendix 28, Table 11).

Sex did not appear to be related to any measure of reaction severity in baseline or NI challenges, but younger age was associated with increased severity on some measures in both baseline and NI challenges. Cumulative dose was higher in males than females in both baseline and NI challenges, but not significantly (Appendix 28, Table 11). SPT wheal size (PMAX) showed no association with severity in the baseline challenges, and a counter-

intuitive inverse association with some measures of severity in the NI challenges (Appendix 28, Table 11). None of these analyses were corrected for multiple separate comparisons, so statistical significance levels need to be interpreted with appropriate caution.

Table 22 Bivariate analysis of potential predictors of threshold and severity of reaction, and measures of threshold or severity of reaction, in baseline peanut challenge:

	Cum. Dose	NIAID	E&C (binary)	WAO (binary)	Use of IM adrenaline	Participant´s VAS overall score	Investigator´s VAS overall score	Plasma adrenaline (nmol/L)	Peak % MCT (%)
Cum. Dose		Anaph 433 (133, 800) No Anaph 190 (109, 295) p=0.23	Severe 346 (154, 596) Not Severe 188 (100, 314) p=0.25	Severe 396 (167, 733) Not Severe 208 (105, 335) p=0.24	Adr 363 (128, 674) No Adr 218 (115, 353) p=0.42	r=0.10 (p=0.45)	r= -0.05 (p=0.74)	r= -0.13 (p=0.37)	**r=0.33 (p=0.02)**
PMAX (mm)	r= -0.25 (p=0.06)	Anaph 10 (8.7, 12.4) No Anaph 11 (10, 12.8) p=0.52	Severe 11 (9, 13) Not Severe 11 (10, 13) p=0.87	Severe 11 (9, 13) Not Severe 11 (10, 13) p=0.62	Adr 11 (9, 13) No Adr 11 (10, 13) p=0.69	r= -0.14 (p=0.30)	r=0.03 (p=0.81)	r=0.25 (p=0.08)	r=0.14 (p=0.34)
PT3 (mins)	r=0.13 (p=0.37)	Anaph 203 (170, 238) No Anaph 163 (135, 201) p=0.12	**Severe 215 (170, 276) Not Severe 146 (124, 166) p=0.051**	**Severe 262 (201, 362) Not Severe 148 (130, 165) p=0.01**	**Adr 222 (198, 248) No Adr 159 (132,195) p=0.01**	**r=0.40 (p=0.006)**	**r=0.35 (p=0.02)**	r=0.19 (p=0.30)	r=0.16 (p=0.30)
Sex (female)	M 417 (235, 633) F 258 (143, 400) p=0.20	Anaph 9/13 (69%) No Anaph 24/44 (55%) p=0.35	Severe 14/23 (61%) Not Severe 19/34 (60%) p=0.71	Severe 8/13 (62%) Not Severe 25/44 (57%) p=0.76	Adr (males) 6/11 (55%) No Adr 28/46 (61%) p=0.35	M 5.5 (4.7, 6.2) F 5.7 (5.1, 6.3) p=0.63	M 5.9 (5.4, 6.4) F 5.9 (5.5, 6.2) p=0.31	M 0.40 (0.07, 0.75) F 0.57 (0.23, 0.95) p=0.51	M 35 (20, 54) F 33 (20, 51) p=0.64
Age (years)	r= -0.02 (p=0.88)	Anaph 28 (23, 33) No Anaph 26 (23, 28) p=0.53	Severe 24 (21, 27) Not Severe 28 (25, 31) p=0.08	Severe 24 (20, 28) Not Severe 27 (24,29) p=0.22	**Adr 22 (20, 25) No Adr 27 (25, 30) p=0.01**	r= -0.22 (p=0.11)	r= -0.07 (p=0.62)	r= -0.003 (p=0.98)	r= -0.19 (p=0.19)
BHR (mg/ml)	r=0.14 (p=0.31)	Anaph 12 (8.5, 15.4) No Anaph 11 (8.8, 13.3) p=060	**Severe 8.5 (5.5, 11.7) Not Severe 13.2 (10.9, 15) p=0.02**	Severe 11.8 (7.7, 15.3) Not Severe 11.2 (9, 13.3) p=0.80	Adr 11.8 (8.1, 15.3) No Adr 11.2 (9.2, 13.5) p=0.81	r= -0.16 (p=0.22)	r= -0.06 (p=0.67)	r=-0.02 (p=0.92)	r=0.20 (p=0.19)
Ln PC3 (mg/ml)	**r=0.29 (p=0.03)**	Anaph -7.1 (-8.5, -5.5) No Anaph -6.9 (-7.7, -6.1) p=0.83	Severe -7.1 (-8.1, -6.1) Not Severe -6.9 (-7.7, -5.9) p=0.69	Severe -7.7 (-9.1, -6.1) Not Severe -6.7 (-7.5, -6.0) p=0.23	Adr -7.9 (-9.2, -6.3) No Adr -6.7 (-7.4, -5.9) p=0.20	r= -0.08 (p=0.54)	**r= -0.27 (p=0.04)**	r= -0.16 (p=0.26)	r= -0.06 (p=0.69)
Ln Ara h 2 (KU/L)	**r= -0.35 (p=0.009)**	Anaph 1.6 (0.59, 2.48) No Anaph 1.6 (0.86, 2.3) p=0.94	Severe 1.4 (0.3, 2.3) No Severe 1.7 (1, 2.4) p=0.58	Severe 1.4 (0.6, 2.3) Not Severe 1.6 (0.9, 2.3) p=0.68	Adr 1.7 (1, 2.4) No Adr 1.6 (0.8, 2.2) p=0.78	r=0.04 (p=0.78)	r=0.07 (p=0.61)	**r=0.34 (p=0.02)**	r=0.19 (p=0.21)

Spearman correlation, chi-square and bootstrap t-test between dependent variables in columns and independent variables in rows. Highlighted $p<0.05$.
PC3 and Ara h 2 were log transformed but other continuous variable did not approximate to a normal distribution after log transformation, so raw data were used.

5.4.2 Multivariate analyses:

Table 23 shows multivariate regression analyses between the dependent and independent variables, using bootstrapping for all analyses with a non-binary dependent variable. Similar to the univariate analyses, PC3 was moderately associated with cumulative dose i.e. threshold of skin prick test reactivity was associated with threshold of clinical reactivity to an oral dose, in peanut allergy, the same positive association was seen in the multivariate analysis of the NI dataset, but was not statistically significant (Appendix 29, Table 14).

For measures of severity of reaction, there was an association between PT3 and severity or reaction when measured using Ewan and Clark, WAO and requirement for IM adrenaline, but not using other measures of severity. This association was not seen in the NI dataset (Appendix 29, Table 14), although numbers there may have been insufficient for reliable multivariate analysis. Multivariate analyses using PT3 measurements only for those participants who reached a 3mm wheal before OCR showed no association with measurements of severity seen in the bivariate analyses (Appendix 29, Table 15). An association was seen between PT3 measurements on placebo day with severity of reaction when this was measured using NIAID classification of anaphylaxis (Appendix 29, Table 16)

Younger age was associated with increased severity of reaction as judged by Ewan and Clark and by requirement for IM adrenaline, but not by other measures. This was also seen in the NI dataset, but only for severe reactions classified by Ewan and Clark scoring system (Appendix 29, Table 14).

The strongest association seen was between specific IgE to Ara h 2 and plasma adrenaline in the baseline challenge; plasma adrenaline samples were not taken in the NI challenge.

Overall, associations between the proposed explanatory variables and measures of severity of reaction were not consistent between datasets, but inconsistent signals were seen for PT3 and for younger age.

5.4.2.1 Quality of predictive models:

When determining the quality of a model using multivariate analyses there are different measurements that can be used. For binary logistic regression the Hosmer–Lemeshow test is a statistical test for goodness of fit and assess whether observed events match the expected events, a $p>0.05$ indicates a good fit for the model. The omnibus test is a likelihood test, the significance value of $p<0.05$ indicates that the current model outperforms the null model. This is similar to the analyses of variance (ANOVA) for linear regression models. Table 22 also shows the results for quality of each model. The threshold model is significant shown by an ANOVA $p=0.006$.

Models of severity using different classifications as the dependent variables all showed a good fit and a significance omnibus test except when using NIAID as the dependent variable. Severity models were also significant when using participants´ VAS scores and plasma adrenaline as dependent variables.

Table 23 Multivariate analysis of potential predictors of threshold and severity of reaction, and measures of threshold or severity of reaction, in baseline peanut challenge:

	Cum. Dose	**NIAID**	**E&C (binary)**	**WAO (binary)**	**Use of IM adrenaline**	**Participant´s VAS overall score**	**Investigator´s VAS overall score**	**Plasma adrenaline (nmol/L)**	**Peak % MCT (%)**
Cum. Dose		1.001 (0.999, 1.002) p=0.30	1.003 (1.000, 1.006) p=0.08	1.001 (0.999, 1.004) p=0.15	1.002 (0.999, 1.004) p=0.13	0.002 (0.000, 0.004) p=0.08	0.001 (0.000, 0.002) p=0.14	0.000 (0.000, 0.001) p=0.53	0.022 (-0.001, 0.049) p=0.11
PMAX (mm)	-1.3 (-32.4, 37.6) p=0.94	-	-	-	-	-	-	**0.1 (0.023, 0.200) p=0.028**	-
PT3 (mins)	-	-	**1.011 (1.000, 1.023) p=0.050**	**1.025 (1.004, 1.047) p=0.019**	**1.011 (1.001, 1.021) p=0.027**	0.003 (-0.001, 0.018) p=0.47	0.002 (0.000, 0.010) p=0.23	-	-
Sex (female)	-	-	-	-	-	-	-	-	-
Age (years)	8.2 (-4.9, 24.4) p=0.27	1.036 (0.948, 1.132) p=0.44	0.833 (0.690, 1.005) p=0.056	0.940 (0.805, 1.098) p=0.43	**0.800 (0.647, 0.990) p=0.04**	-0.059 (-0.174, 0.053) p=0.31	-0.008 (-0.056, 0.059) p=0.78	0.40 (0.001, 0.081) p=0.056	-1.385 (-3.341, 0.496) p=0.16
BHR (mg/ml)	-	-	0.902 (0.798, 1.019) p=0.10	-	-	-	-	-	-
logPC3 (mg/ml)	70.0 (13.0, 142.4) p=0.058	-	-	-	-	-	-0.152 (-0.317, -0.005) p=0.057	-	-
logArah2 (KU/L)	-36.3 (-102.3, 28.9) p=0.26	-	-	-	-	-	-	**0.247 (0.077, 0.391) p=0.006**	-
ANOVA	p= 0.006					p=0.047	p=0.077	**p=0.002**	p=0.157
Omnibus test		p=0.41	p= 0.002	p=0.002	p=0.01				
Hosmer and Lemeshow test		p=0.352	p=0.556	p=0.267	p=0.811				

Beta coefficients for continuous dependent variables, and Exp(B) for binary dependent variables, both with 95% confidence intervals. Dependent variables are shown as columns, and independent (explanatory) variables as rows. Highlighted p<0.05.

5.5 Discussion:

In this observational study of repeated peanut challenges in young adults with IgE-mediated peanut allergy, we found a consistent relationship between threshold of SPT reactivity and threshold of clinical reactivity, suggesting that EPT may be used to estimate threshold of reactivity for patients and their carers. We also found weaker and less consistent evidence that time to resolution of SPT wheal may be related to severity of reaction. Overall these data support the possibility that precise measurements of carefully conducted SPT have the potential to provide clinically relevant information regarding threshold or severity of reaction, in young adults with peanut allergy. Further work in separate cohorts is needed to validate these findings, and to establish whether they can be generalised to other IgE-mediated FA and/or other populations. These findings also provide insight into potential mechanisms underlying the wide variation in threshold and severity of reaction, which is seen in food allergy, in contrast to inhalant allergy.

A relationship was found between younger age and severity of reaction on DBPCFC, which we were able to reproduce on repeated NI challenge for Ewan&Clark classification only. This is consistent with previous publications where younger age is associated with life-threatening anaphylaxis and death [42, 176]. An association was found between Ara h 2 and cumulative dose suggesting that those with higher levels of Ara h 2 had a significant lower clinical threshold of reactivity to peanut. Similar results have been previously found both for children and adults [241, 285].

In our dataset we found no relationship between severity of reaction and cumulative dose. Previous work has suggested the possibility, intuitively attractive, that those who ingest higher doses of allergen will have a more severe reaction, but we could not confirm such a relationship in our cohort. Severe reactions have been reported with all levels of allergen intake previously[15, 365]. The relationship between dose of exposure and the symptoms experienced is unclear and the severe reactions can happen at all levels of threshold. OFC symptoms, under controlled conditions do, not always reproduce real life reactions and we know that patients who experienced anaphylaxis previously only had mild reactions in most cases during this study.

We found no systematic differences for PMAX, PC3 and PT3 between the active and placebo day. However, we observed a high level of intra-participant variability, particularly for PC3, PC6 and PT6 despite having controlled for sources of variability by performing and reading the SPT by the same trained professional, always on the same site and with the same device. High intra- and inter- participant variability has previously been described when comparing techniques and sites for SPT [366, 367].

A significant positive correlation was found between PC3 and cumulative dose, suggesting that those participants with a lower threshold of skin reactivity also have a lower threshold of clinical reactivity. This is consistent with a previous report in which SPT was performed to 9 different foods including peanuts prior to DBPCFC[95]. Given the limitations of the conventional SPT technique, titrated SPT has been suggested as an alternative way to determine reaction severity. This has been previously described for severity of hen´s egg allergic reactions[92] but we suggest that it probably is a better predictor of threshold, at least for peanut allergic reactions as seen in our results for both univariate and multivariate analyses. This is also backed up by results from oral immunotherapy studies reporting a decrease in EPT in those participants undergoing active treatment[291-294], again suggesting that skin reactivity maybe more related to clinical reactivity rather than reaction severity.

The time to resolution to 3mm wheal of SPT (PT3) was found to be significantly longer in those participants classified as having severe reactions and/or anaphylaxis as per Ewan & Clark classification, WAO classification of severity of reaction after immunotherapy and use of IM adrenaline as rescue medication. This suggests a possible difference in the "switch-off" mechanisms in these participants. However, these results were not reproducible for PT3 on repeated NI challenges perhaps in part because the NI reactions were overall milder than DBPCFC as evidenced by the VAS data. The main limitation for NI challenge results is the small number of our population with relevant and complete data. Time to resolution of SPT performed during active challenge could be influenced by the mediators released during a positive oral food challenge and by the treatment given, mainly antihistamine and IM adrenaline. Given this limitation in this SPT measurement, we also analysed time to resolution of SPT just with those participants who reached the 3mm wheal before OCR and also on placebo day. This extended analysis gave inconclusive results for PT3 although the direction of the change was similar throughout the different approaches. To our knowledge this is the first time such results have been described in food allergic reactions and for SPT.

No relationship was seen between SPT measurements and the changes found in CVS and cardiac conductance changes.

Results for multivariate analyses suggest that PC3 is the most significant independent variable to predict threshold of reaction, which was already described in the univariate analyses, whilst both PT3 and age seem to be the most significant independent variables to predict severity of reaction.

On the basis of these results, we believe modified SPT both with EPT and time to resolution of SPT may add greater diagnostic information for adult patients with peanut allergy when

measured as independent variables but also when analysed in a model with other variables. Some of our results were not reproducible in a repeated challenge probably given the reduced power, as it was a smaller cohort. Further studies in different populations and using different food allergens are required to validate our findings.

6.DISCUSSION:

In this thesis, I have characterised cardiovascular changes during IgE-mediated peanut allergic reactions in adults. The experimental protocol allowed for partial replication of observations in a sub-cohort of patients, which contributed significantly to our confidence in the reproducibility and consistency of the findings. We found that reduced cardiac stroke volume, likely secondary to vasodilation and fluid redistribution, is common during allergic reactions to peanut, irrespective of reaction severity. Electrocardiographic measurements indicated sympathetic activation during allergic reactions to peanut, which was not present at repeat reaction, and we found evidence that anxiety related to the initial allergic reaction may underlie these changes. Evaluation of local vascular responses to skin prick test, as the time to resolution of the wheal to 3mm (PT3), identified a prolongation of the time to resolution for the wheal as a predictor of reaction severity: thus, a failure to compensate for fluid extravasation (resulting in increased time to resolution) may be a risk factor for a more severe reaction. These data support the early use of intravenous fluids in the management of allergic reactions to food, for example, to treat reactions that are resistant to initial parenteral treatment with adrenaline, and suggest that failure to limit fluid extravasation, perhaps due to ongoing release of inflammatory mediators, may be an important determinant of reaction severity.

6.1 Cardiovascular changes during IgE-mediated FA:

It is clear from this thesis that IgE-mediated allergic reactions to peanut are accompanied by significant cardiovascular changes: a decrease in stroke volume with an increase in peripheral blood flow and compensatory response resulting in increased heart rate and blood pressure to maintain cardiac output. Some of these compensatory changes were greater on the first reaction than at repeat reaction, and this may reflect an influence of anxiety/stress as shown by a maintained CO despite a significant decrease in SV but a smaller increase in HR than at baseline challenge. However, the reduction in stroke volume was consistently seen in both baseline and repeat reactions, and cannot be explained by increased sympathetic activation due to anxiety: this would be expected to cause an increase in stroke volume, rather than a decrease which is what was observed. Indeed, the fall in stroke volume was at least as great as at baseline challenge. Our assessment of stroke volume measurements relied on non-invasive, indirect assessment using an FDA-validated monitor; this method correlated well with echocardiographic measurement of stroke volume where these could be undertaken. The primary events leading to these cardiovascular changes are likely to be peripheral vasodilation and sub-clinical fluid redistribution due to capillary leak, triggered

by vasoactive mediators released during acute allergic reactions to peanut. This is the first detailed description of such changes in IgE-mediated food-induced allergic reactions in humans.

We believe these findings are of importance as reduced cardiac preload and stroke volume can potentially lead to cardiac arrest due to what has been colloquially termed "empty heart" syndrome; this may explain the association between posture change and fatal outcome which has been described in observational post mortem studies of anaphylaxis due to food and other causes[89, 164]. These findings have important clinical implications in the management of food allergic reactions, by providing indirect evidence to support earlier use of fluids and more aggressive use of fluid therapy in those severe reactions refractory to initial treatment with intramuscular adrenaline. Although cardiovascular collapse is most frequent in anaphylactic reactions to anaesthetic[368] these findings show that cardiovascular impairment is also common in IgE-mediated FA. Notably, our findings were seen with reactions of any severity, although results in baseline challenges suggest a trend to greater decrease in SV in more severe reactions. We did not see a relationship between SV decrease and severity of reaction in the repeated challenges, most likely due to the small number of anaphylaxis reactions seen at repeat challenge.

Guidelines for the management of anaphylaxis[74, 369] include fluid therapy as a 3rd line treatment and our findings suggest that this should possibly be elevated to a 2nd line treatment together with second administration of IM adrenaline. Data from case series of anaesthetic reactions are consistent with a loss of fluid equivalent in one third of the circulating blood volume within minutes of onset of anaphylaxis[189]. We have demonstrated that a similar – albeit less severe – process may occur during peanut-induced allergic reactions. A poor response to adrenaline should therefore prompt more aggressive fluid resuscitation.

Our experience from the food challenges conducted during this research is that abdominal symptoms were frequent during controlled food challenges to peanut. In contrast, however, patients did not describe this (at initial screening) as a frequent symptom during accidental reactions occurring in the community. We did not find a consistent relationship between symptoms experienced by the participants and the changes seen in the CVS. The fall in stroke volume implies fluid redistribution and/or fluid leak, and given the absence of significant clinical peripheral oedema, we hypothesise that the majority of fluid shifts are occurring in the gut. This would be consistent with a single case report of a patient experiencing anaphylaxis who underwent CT scan during reaction due to persistent GI symptoms, which demonstrated significant bowel wall oedema consistent with "shock

bowel" [165]. With this in mind, we considered whether the occurrence of gastrointestinal symptoms might be linked to the degree of change in stroke volume. Although there was a relationship between SV, HR and severity of gastrointestinal symptoms during the initial DBPCFC, this was not reproduced during the repeat food challenges.

In a recent study of 412 allergic reactions of any trigger presenting to an emergency department, of which 76% were anaphylaxis, altered level of consciousness was seen in 23% of all reactions [168]. It is unclear how many of the reactions with altered level of consciousness were food anaphylaxis. Our own clinical observations in this thesis suggest that some individuals experience subtle changes in cognition during allergic reactions, which could be explained by the effect of local vasoactive mediators released.

A significant drop in lung function was observed at time of objective clinical reaction and although a drop in SV has been described in patients suffering from asthma due to increased intrathoracic pressure[324], we did not find any correlation between lung function and changes in SV, and we note that the magnitude of the drop in FEV1/PEFR is smaller than that typically observed during acute asthma exacerbations.

6.2 Cardiac conductance changes during IgE-mediated FA:

Results from this thesis showed no consistent changes in cardiac conductance during food allergic reactions of any severity. Initially, changes in HRV parameters consistent with sympathetic activation were seen during DBPCFC up to 30 minutes before OCR, as previously reported by others[197], however these changes were not reproduced during the repeated food challenges. There was some evidence that this difference in HRV between the 2 challenges (DBPCFC and repeat open challenge) might be explained by reduced anxiety at repeat reaction, since participants reported less anxiety and subjective reaction severity at repeat reaction compared with initial DBPCFC reaction. HRV has been related to emotional arousal and the H.F. domain has been found to decrease under conditions of anxiety[337].

No changes were seen for ECG conductance changes at any challenge day, which is difference to that observed in the literature from drug and venom anaphylaxis describing different types of conductance changes being SVT[189] and ST elevation[196] amongst the most common findings suggesting a possible different shock organ depending on the trigger but probably also severity of the reaction and that, in general, food reactions happen in the community and away from a medical facility were cardiac monitoring can be performed and to the relative cardiovascular fitness of young adults with peanut allergy compared with older individuals with drug or venom allergy. Our observations are also in contrast to those seen following infusion of histamine in healthy volunteers e.g. AV block [325]. This may be due the fact that a variety of mediators are released at varying biological concentrations

during an allergic reaction rather than a single potent pharmacological stimulus; furthermore, histamine metabolism is tightly regulated so that the levels of endogenous histamine released during most allergic reactions are probably less than that used during the histamine infusion studies reported in the literature.

To our knowledge this is the first report of continuous cardiac conductance monitoring during the entirety of supervised oral food challenges in a cohort of adults. Given the inconsistency of the results, we do not recommend cardiac conductance monitoring, unless the patient reports characteristic symptoms which accompany ECG changes during allergic reactions or experiences a significant anaphylaxis in which case there is a (albeit low) risk of adrenaline-induced arrhythmias. We did not identify any evidence of coronary artery vasospasm in this series of allergic reactions, although no cases of anaphylactic shock were recorded; coronary artery vasospasm may be a relatively late event associated with severe anaphylaxis.

6.3 Assessment of the relationship between skin prick test (SPT) reactivity and phenotype of peanut allergic reaction:

When evaluating the local vascular response to peanut SPT, we found evidence for a relationship between such responses and threshold and severity of reaction to peanut. There was a significant and consistent relationship between threshold of SPT reactivity by means of end-point titration as the concentration required to reach a 3mm wheal (PC3) and threshold dose of clinical reactivity, which is consistent with some previous literature[291]. These results suggest that although further studies and more accurate EPT is required, prediction of threshold may be possible in the future, at least for peanut allergy. There was also a relationship between time to resolution of SPT as the time taken to reach a 3mm wheal (PT3) and severity of reaction. To our knowledge this is the first time such an association has been described.

The relationship between Ara h 2 IgE level and threshold for clinical reaction suggests that density of Ara h 2 IgE expression on tissue resident mast cells might explain variation in threshold between subjects. However, variations in oromucosal permeability may modify this relationship, and thereby explain the wider variation in threshold of clinical reactivity in oral food challenge, compared with nasal grass pollen challenge[370].

The relationship between PT3 and reaction severity raises the intriguing possibility that severity of reaction in peanut allergy may be in part determined by an individual's ability to limit vascular leak, or to limit the production or activity of vasoactive mediators. PT3 is likely to relate to time to recovery of vascular endothelial integrity following a vasoactive inflammatory stimulus; or to time to cessation of short-lived vasoactive mediator production

by inflammatory cells such as mast cells. Of note, we did not find a relationship between PT3 and a single measure of serum mast cell tryptase. The vascular effects of histamine depend on the receptors sensitivity to histamine, in duration of the effect, and in the mechanism of their production[371-373]. The vasodilator effects of histamine are mediated by nitric oxide (NO), which diffuses to smooth muscle increasing the levels of cyclic guanine monophosphate (cGMP) leading to vascular smooth muscle relaxation and vasodilation[374, 375]. Failure to compensate these effects, represented in our results as SPT prolongation, could therefore be related to more severe reactions.

From these results we suggest that further work in separate cohorts is justified, to determine the value of using modified SPT by means of end-point titration for prediction of threshold rather than previously suggested as predictor of severity[92] and time to resolution of SPT to 3 mm wheal as a predictor of severity as those with SPT taking longer to disappear have more severe reactions possibly given their failure to compensate the reaction experienced by the mediators released translated in a wheal which takes longer to reduce in size.

With these findings we have opened a line of investigation for the future where similar challenges are performed with bigger cohorts and other food triggers. Time to resolution of SPT could be of great help before ordinary food challenges where SPT and IgE levels have not disappeared completely and a risk of a positive reaction exists. Furthermore with bigger cohorts and multiple food triggers ROC curves could be perform and cut-off points obtained for threshold of reactivity.

6.4 Effects of IM adrenaline on cardiac measurements:

No significant cardiovascular changes were found following IM adrenaline during anaphylaxis, aside from a statistically borderline increase in HR, whilst IM adrenaline was associated with increased airway calibre as measured by lung function. This suggests that IM adrenaline may not have a major effect on the cardiovascular system in the context of peanut anaphylaxis. No changes were found in cardiac conductance after administration of IM adrenaline including ST elevation which has previously been described as part of Kounis syndrome[376]. These results support the safety of using IM adrenaline to treat anaphylaxis, but raise questions about treatment efficacy, although it should be noted that the design of the study has some limitations for evaluating IM adrenaline effects as this was not the primary focus of the study.

6.5 Strengths and limitations of this thesis:

TRACE study had a complex design involving repeated challenges in peanut allergic participants. The participants involved in this thesis were recruited as part of TRACE study

and therefore one of the strengths of this thesis is the possibility of studying a well characterized adult population with primary IgE-mediated FA, representing the population age at higher risk of near fatal and fatal anaphylaxis and who underwent repeated supervised allergic reactions to peanut. This means that initial findings could be checked for reproducibility, which was valuable in our interpretation of findings. For the reduction in stroke volume seen during reactions, reproducibility adds confidence to the finding; for changes in ECG during reactions, the lack of reproducibility effectively dismissed the findings; and for the relationship between PC3, PT3 and phenotype of clinical reaction, partial reproducibility added strength to the findings. For the duration of the study, all challenges performed were supervised by the same clinician, increasing the consistency of the application of stopping criteria and reducing the variability of this when multiple clinicians supervise food challenges or when different stopping criteria are applied as might be the case in retrospective studies or meta-analyses of food allergy pathophysiology. This same strength can be applied to data collection and analysis, which was performed by the same investigator throughout (i.e. myself), with appropriate senior support.

Another strength of this thesis is that the stopping criterion applied is a modified PRACTALL consensus. PRACTALL applies colours to symptoms, which are then used to determine the termination of the food challenge. This modified version, explained in section 2.1.3.3 of the Methods chapter, in which some of the very mild objective symptoms initially coded as yellow (moderate) are degraded to green (mild) increasing the threshold for challenge termination and therefore allowing that probably further mechanisms affecting the reaction could take place.

Limitations to the study design include a lack of placebo at the repeat reaction, the limitations inherent in evaluating severity of allergic reactions, where no universal standard is available; and a lack of blinding for evaluation of the relationship between PC3, PT3 and phenotype of clinical reaction. PT3 may also have been affected by clinical reactions, however findings were similar when using PT3 measures from placebo days, and when excluding subjects where PT3 occurred after clinical reaction. Lack of blinding is unlikely to have influenced evaluation of the relationship between PC3, PT3 and phenotype of reaction, with the supervising clinical team focussed on the main TRACE clinical study outputs rather than these mechanistic evaluations during the study and the fact that tracings were made of the SPT wheal in real-time, and measured later when wheal sizes could be more objectively determined. However, the possibility that reactions occurring in subjects with long PT3 or a low PC3 were recorded as more severe due to investigator awareness of the results cannot be completely discounted.

At the same time the stopping criteria is a limitation to the study as safety was the main priority for TRACE study and therefore for this thesis. Most patients experienced mild, self-limiting reactions and only the minority had typically mild anaphylaxis: this limits our ability to assess changes in more severe reactions. In addition, the protocol rightly mandated treatment to be given promptly to participants, therefore not allowing the study of the natural evolution of IgE-mediated FA, which could have been of importance for the pathophysiology and the effects of treatment on IgE-mediated FA.

One of the main difficulties of TRACE study, and therefore of this thesis, was recruitment of participants: this in turn limited the number of patients recruited for mechanistic assessments and further repeat challenges, with some participants withdrawing prior to the repeat challenge visits which were used to assess reproducibility of our initial findings.

For pragmatic and ethical reasons the cardiovascular monitoring of the heart was performed by non-invasive mechanisms and although results from CHEETAH NICOM^{TM} monitor have met FDA standards for validation against pulmonary artery catheter methods, there was variability in the measurements, mostly due to movement of participants, correct placement of pads and patients' physiognomy. However, we did find good correlation for SV results between the non-invasive CHEETAH NICOM^{TM} monitor and cardiac echocardiography where this could be performed at the appropriate times by a highly trained physician.

We assessed peanut-induced allergic reactions, as this is the most common cause of primary IgE-mediated food allergy in UK adults, and the most frequent cause of fatal and near-fatal food anaphylaxis in adults[42, 51]. It is likely that our findings are valid for reactions due to most other triggers, but may not necessarily be generalised to other age groups. The data are unlikely to be directly relevant to secondary food allergy or non-IgE mediated food allergy.

Unfortunately, there is no consensus on how to classify severity of IgE-mediated FA reactions, which limited our ability to relate our findings with severity. As shown in chapter 5, section 5.3.2 Table 20 there is discrepancy between the most common used classifications of allergic reactions specially when trying to discriminate between non-severe and severe reactions. We have found that the main difference in severity scoring is in Ewan and Clark classification, which classifies throat tightness (subjective) as a severe symptom.

Overall the main strengths of this thesis are the CVS analyses and the extended SPT analyses, and their possible clinical implications.

6.6 Clinical and research implications of the findings:

The validation and reproducibility of the CVS data has important clinical implications: a significant drop in SV was seen irrespective of reaction severity. This highlights the importance of measures to increase cardiac preload in reactions which require treatment beyond an initial dose of IM adrenaline, particularly in food-induced reactions were clinical cardiovascular compromise is not typically noted. Such measures might include positioning and more aggressive fluid resuscitation than that currently specified in guidelines. At the same time the inconsistency of cardiac conductance measurements has shown that, unless characteristic symptoms are present, ECG cardiac monitoring is not recommended during IgE-mediated FA.

Anxiety has also shown to be a factor affecting cardiac monitoring, especially HRV, and that the changes initially found are most probably driven solely by sympathetic activation and not by IgE-mediated FA mediators. Therefore, HRV parameters are currently not well characterized in IgE-mediated FA for them to be used as determinants for positive food challenges.

Risk stratification of patients with IgE-mediated FA remains a challenge for clinicians, from the results of this thesis we do suggest that modified SPT in the form of end-point titration and time to resolution of SPT might potentially add greater diagnostic information for adults with IgE-mediated peanut allergy, however this may be limited to SPT on the day of exposure, and needs further prospective assessment in other cohorts.

One method to extend the data and findings from this thesis would be to undertake food challenges at the same time as additional imaging techniques, such as MRI (with cardiac imaging). This would better help delineate the cardiovascular changes and shed light on where fluid redistribution may be occurring. Sedation of participants could be considered, to reduce the effects of anxiety on these measures although this might likewise impact on the ability of participants to communicate symptoms and therefore challenge safety. The study of paediatric populations, larger cohorts and inclusion of those with previous severe anaphylaxis may all add to these findings significantly, although not without ethical challenges. Further work is required to develop a consistent model that can predict clinical phenotype of food allergy.

In the introduction to this thesis (section 1.7.1) we suggested a model for severity of food allergic reactions (Figure 4), where increased airway mast cell density (for example, due to persistent aeroallergen exposure in sensitised subjects) might be a major determinant of severity of food-induced allergic reactions. However, our data do not support this hypothesis: there was no relationship between change in mast cell tryptase and CVS

changes, nor between the change in lung function and CVS changes which might have been predicted. However, our findings are limited by the lack of severe reactions at challenge in this study: airway mast cell density may still be a risk factor (although probably not the only factor) for truly life-threatening reactions in the wider food-allergic population. Further data to support this could come from assessment of MC density in the lungs (either from routine biopsies or post mortem studies of fatal anaphylaxis where airway sections are commonly taken but not stained specifically for mast cells). Alternatively, future studies could consider whether MC density in nasal biopsies /curette samples correlate with MC density in the respiratory mucosa.

6.7 Conclusions:

During IgE-mediated allergic reaction to peanut, we found significant cardiovascular changes, specifically a fall in stroke volume with evidence of compensation, irrespective of reaction severity with no clear relation to specific organ symptoms. In contrast to our initial hypotheses, we did not find any significant and reproducible ECG changes which could be attributed to reaction rather than anxiety. We cannot therefore recommend HRV changes as an objective measure of reaction during food challenges in adults.

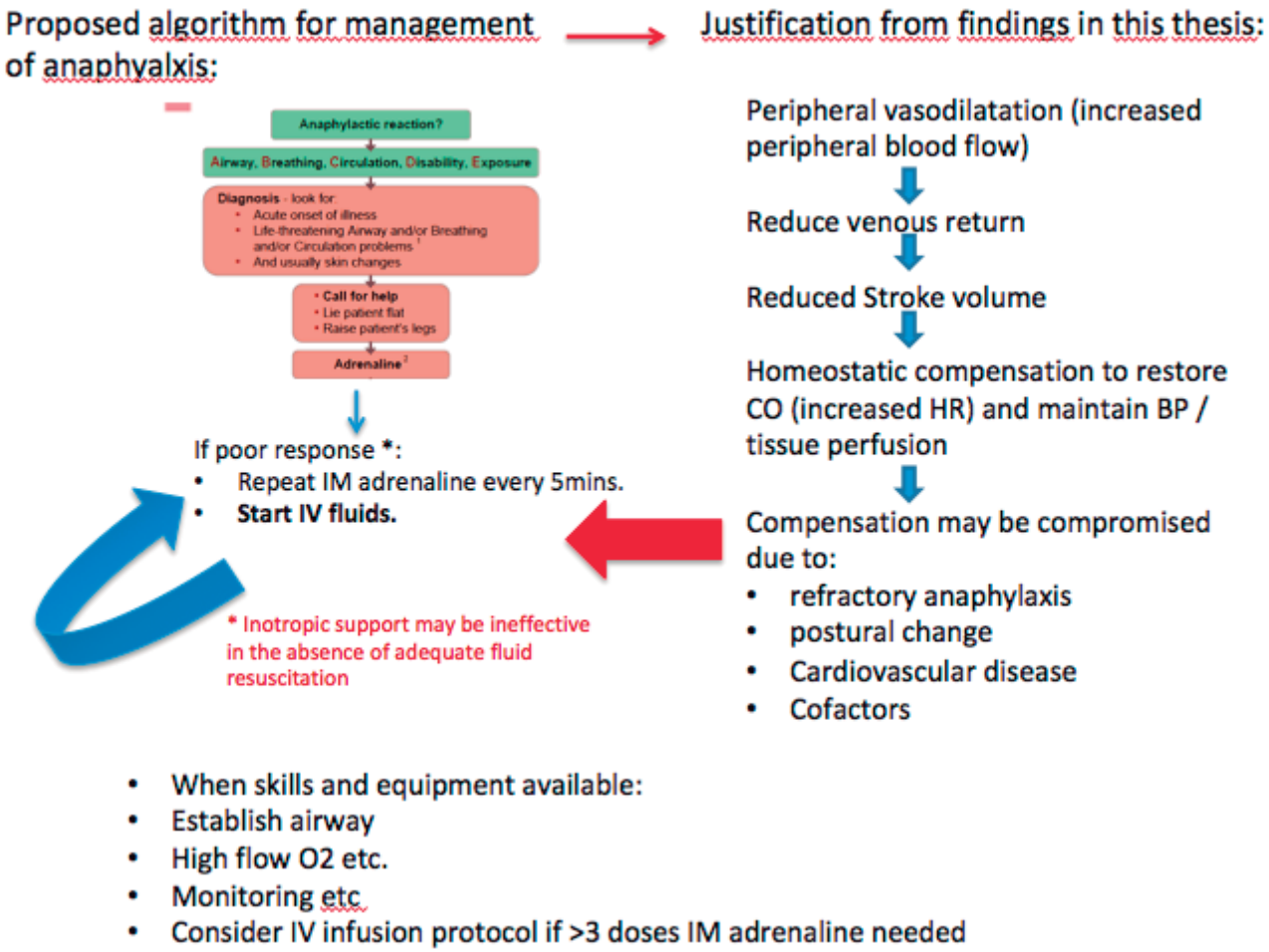
The local vascular response (as assessed using titrated SPT and time to resolution) might help predict clinical phenotype more consistently in terms of clinical threshold and reaction severity, at least at challenge, although further studies in different cohorts, age groups and using different food triggers are required to establish whether these can contribute to clinically-relevant diagnostics.

We propose that the cardiovascular systemic changes identified have important implications in the management and possible outcome of allergic reactions and we propose modifications to the current algorithms for the management of anaphylaxis as shown in Figure 110, with more emphasis on fluid therapy for those reactions refractory to initial treatment with IM adrenaline.

We have previously mentioned that fluid therapy in these patients needs to be more aggressive than usual, especially as they are in general healthy people and will tolerate this well. Resuscitation algorithms for management of anaphylaxis need to be revised and fluid therapy pushed upwards and placed together with the first line of treatment, which is still IM adrenaline. Allergists but also other specialists and paramedics come into contact with patients suffering from food anaphylaxis who need to be aware of these findings and how it changes the management of the disease with meetings in hospitals and GP practice.

We are aware that the changes seen for SV will not have any physiological consequences on healthy individuals who are not undergoing a food challenge, however, and given the post mortem results, we believe these findings are of importance. Having said this further studies probably including cardiac echocardiography or cardiac MRI will find more accurate results including data on cardiac contractility.

Figure 110 Modification to algorithm for management of anaphylaxis:



REFERENCES:

1. Adams, F., *TheGenuineWorksofHippocrates.* Williams, 1939.
2. Panzani, R., *Cypress and food allergy: was it suspected in antiquity?* J Asthma, 1985. **22**(4): p. 223-6.
3. Hare, F., *TheFoodFactorinDisease* , ed. Longmans. Vol. I, II. 1905.
4. Von Pirquet, C., *Allergie.* Munch Med Wochenschr, 1906. **52**: p. 1457.
5. Schofield, A.T., *ACaseofEggPoisoning.* Lancet, 1908: p. 716.
6. M., S.O., *ACaseofAllergytoCommonFoods.* Am J Dis Child, 1912. **3**: p. 341.
7. OM., S., *A case of food allergy. Idiosincrasy to eggs, almonds and oats, due to anaphylaxis..* Arch Paediat, 1912. **29**: p. 219.
8. Rowe, A.H., *Food Allergy: Its Manifestation, Diagnosis, and Treatment.* 1931: Lea&Febiger.
9. Vaughan, W.T., *PractiveofAllergy* . 1948.
10. Vaughan, W.T., *StrangeMalady* . 1941.
11. Coca, A.F., *ThePulseTest.* 1956.
12. Rajan, T.V., *The Gell-Coombs classification of hypersensitivity reactions: a re-interpretation.* Trends Immunol, 2003. **24**(7): p. 376-9.
13. Johansson, S.G., et al., *Revisednomenclatureforallerg yforglobaluse:Reportof theNomenclatureReviewCommitteeofthe WorldAllergyOrganization,October 2003.* J Allergy Clin Immunol, 2004. **113**(5): p. 832-6.
14. Muraro, A., et al., *EAACIfoodallergyandanaphyla xisguidelines:diagnosisand managementoffoodallergy.* Allergy, 2014. **69**(8): p. 1008-25.
15. Blumchen, K., et al., *Modified oral food challenge used with sensitization biomarkersprovidesmorereal-lifeclin icalthresholdsforpeanutallergy.* J Allergy Clin Immunol, 2014. **134**(2): p. 390-8.
16. Osborne, N.J., et al., *Prevalence of challenge-proven IgE-mediated food allergy usingpopulation-basedsamplingandpredeterminedchallengecriteriaininfants.* J Allergy Clin Immunol, 2011. **127**(3): p. 668-76 e1-2.
17. Lack, G., *Epidemiologic risks for food allergy.* J Allergy Clin Immunol, 2008. **121**(6): p. 1331-6.
18. Kelleher, M.M., et al., *Skinbarrierimpairmentatbirth predictsfoodallergyat2 yearsofage.* J Allergy Clin Immunol, 2016. **137**(4): p. 1111-1116 e8.
19. Brown, S.J., et al., *Loss-of-functionvariantsinthefilaggringeneareasignificant riskfactorforpeanutallergy.* J Allergy Clin Immunol, 2011. **127**(3): p. 661-7.
20. Irvine, A.D., W.H. McLean, and D.Y. Leung, *Filaggrin mutations associated with skinandallergicdiseases.* N Engl J Med, 2011. **365**(14): p. 1315-27.
21. Ramirez, D.A., Jr. and S.L. Bahna, *Food hypersensitivity by inhalation.* Clin Mol Allergy, 2009. **7**: p. 4.
22. Sampson, H.A., *Foodallergy.Part1:immunopath ogenesisandclinicaldisorders.* J Allergy Clin Immunol, 1999. **103**(5 Pt 1): p. 717-28.
23. Moriyama, T., *DiversityofFoodAllergy.* J Nutr Sci Vitaminol (Tokyo), 2015. **61 Suppl**: p. S106-8.
24. Katelaris, C.H., *Should patients with pollen fr uit syndrome be prescribed an automaticepinephrineinjector?* Curr Opin Allergy Clin Immunol, 2016. **16**(4): p. 370-4.
25. Flinterman, A.E., et al., *Lipidtransferprotein-linkedhazelnutallergyinchildren from a non-Mediterranean birch-endemic area.* J Allergy Clin Immunol, 2008. **121**(2): p. 423-428 e2.

26. Schocker, F., et al., *IgEbindingtouniquehazelnutallergens:identificationofnon pollen-related and heat-stable hazelnut allergens eliciting severe allergic reactions.* Eur J Nutr, 2000. **39**(4): p. 172-80.
27. Galli, S.J. and M. Tsai, *IgEandmastcellsinallergicdisease.* Nat Med, 2012. **18**(5): p. 693-704.
28. Geha, R.S., H.H. Jabara, and S.R. Brodeur, *The regulation of immunoglobulin E class-switchrecombination.* Nat Rev Immunol, 2003. **3**(9): p. 721-32.
29. Larche, M., C.A. Akdis, and R. Valenta, *Immunological mechanisms of allergen-specificimmunotherapy.* Nat Rev Immunol, 2006. **6**(10): p. 761-71.
30. Takhar, P., et al., *Class switch recombination to Ig E in the bronchial mucosa of atopic and nonatopic patients with asthma.* J Allergy Clin Immunol, 2007. **119**(1): p. 213-8.
31. Coeffier, M., et al., *Epsilongerm-lineandIL-4trans criptsareexpressedinhuman intestinal mucosa and enhanced in patients with food allergy.* Allergy, 2005. **60**(6): p. 822-7.
32. Stone, K.D., C. Prussin, and D.D. Metcalfe, *IgE, mast cells, basophils, and eosinophils.* J Allergy Clin Immunol, 2010. **125**(2 Suppl 2): p. S73-80.
33. Jr, J.C., *Effectormechanismsinallergicreactions.* The immune system in health and disease. 5th Edition. , 2001.
34. Rona, R.J., et al., *The prevalence of food al lergy: a meta-analysis.* J Allergy Clin Immunol, 2007. **120**(3): p. 638-46.
35. Woods, R.K., et al., *Reportedadversefoodreactions overestimatetruefoodallergy inthecommunity.* Eur J Clin Nutr, 2002. **56**(1): p. 31-6.
36. Nwaru, B.I., et al., *Theepidemiologyoffoodallergyin Europe:asystematicreview andmeta-analysis.* Allergy, 2014. **69**(1): p. 62-75.
37. Gupta, R., et al., *Timetrendsinallergicdisordersin theUK.* Thorax, 2007. **62**(1): p. 91-6.
38. Gupta, R., et al., *Increasing hospital admissions for systemic allergic disorders in England:analysisofnationaladmissionsdata.* BMJ, 2003. **327**(7424): p. 1142-3.
39. Kotz, D., C.R. Simpson, and A. Sheikh, *Incidence,prevalence,andtrendsofgeneral practitioner-recorded diagnosis of pea nut allergy in England, 2001 to 2005.* J Allergy Clin Immunol, 2011. **127**(3): p. 623-30 e1.
40. Venter, C., et al., *Timetrendsintheprevalenceofpe anutallergy:threecohortsof childrenfromthesamegeographicallocationintheUK.* Allergy, 2010. **65**(1): p. 103-8.
41. Burney, P.G., et al., *The prevalence and distribution of food sensitization in Europeanadults.* Allergy, 2014. **69**(3): p. 365-71.
42. Turner, P.J., et al., *Increaseinanaphylaxis-relatedho spitalizationsbutnoincrease infatalities:ananalysisofUnitedKingdomnationalanaphylaxisdata,1992-2012.* J Allergy Clin Immunol, 2015. **135**(4): p. 956-63 e1.
43. Vetander, M., et al., *Anaphylaxisandreactionstofoodsinchildren--apopulation-basedcasestudyofemergencydepartmentvisits.* Clin Exp Allergy, 2012. **42**(4): p. 568-77.
44. Vereda, A., et al., *Peanut allergy: Clinical and immunologic differences among patients from 3 differ ent geographic regions.* J Allergy Clin Immunol, 2011. **127**(3): p. 603-7.
45. Liu, A.H., et al., *National prevalence and risk fa ctors for food allergy and relationship to asthma: results fr om the National Health and Nutrition Examination Survey 2005-2006.* J Allergy Clin Immunol, 2010. **126**(4): p. 798-806 e13.

46. Brough, H.A., et al., *Atopic dermatitis increases the effect of exposure to peanut antigen in dust on peanut sensitiz ation and likely peanut allergy.* J Allergy Clin Immunol, 2015. **135**(1): p. 164-70.
47. Brough, H.A., et al., *Peanut allergy: effect of environmental peanut exposure in children with filaggrin loss-of-function mutations.* J Allergy Clin Immunol, 2014. **134**(4): p. 867-875 e1.
48. Baek, J.H., et al., *The link between serum vitamin D level, sensitization to food allergens, and the severity of atopic dermatitis in infancy.* J Pediatr, 2014. **165**(4): p. 849-54 e1.
49. Allen, K.J. and J.J. Koplin, *Prospects for Prevention of Food Allergy.* J Allergy Clin Immunol Pract, 2016. **4**(2): p. 215-20.
50. Grundy, J., et al., *Rising prevalence of allergy to peanut in children: Data from 2 sequential cohorts.* J Allergy Clin Immunol, 2002. **110**(5): p. 784-9.
51. Bock, S.A., A. Munoz-Furlong, and H.A. Sampson, *Fatalities due to anaphylactic reactions to foods.* J Allergy Clin Immunol, 2001. **107**(1): p. 191-3.
52. Burks, A.W., et al., *Identification of a major peanut allergen, Ara h I, in patients with atopic dermatitis and positive peanut challenges.* J Allergy Clin Immunol, 1991. **88**(2): p. 172-9.
53. Rabjohn, P., et al., *Molecular cloning and epitope anal ysis of the peanut allergen Arah3.* J Clin Invest, 1999. **103**(4): p. 535-42.
54. Marsh, J., et al., *Purification and characterisation of a panel of peanut allergens suitable for use in allergy diagnosis.* Mol Nutr Food Res, 2008. **52 Suppl 2**: p. S272-85.
55. Burks, A.W., et al., *Identification and characterization of a second major peanut allergen, Ara h II, with use of the sera of patients with atopic dermatitis and positive peanut challenge.* J Allergy Clin Immunol, 1992. **90**(6 Pt 1): p. 962-9.
56. Koppelman, S.J., et al., *Purification and immunoglobulin E-binding properties of peanut allergen Ara h 6: evidence for cross-reactivity with Ara h 2.* Clin Exp Allergy, 2005. **35**(4): p. 490-7.
57. A, P., *identification of Arah7 in peanut extract.* Allergy, 2009. **64**: p. 109.
58. Krause, S., et al., *Lipid transfer protein (Ara h 9) as a new peanut allergen relevant for a Mediterranean allergic population.* J Allergy Clin Immunol, 2009. **124**(4): p. 771-8 e5.
59. Kleber-Janke, T., et al., *Selective cloning of peanut allergens, including profilin and 2S albumins, by phage display technology.* Int Arch Allergy Immunol, 1999. **119**(4): p. 265-74.
60. Taylor, S.L., et al., *Threshold dose for peanut: Risk characterization based upon diagnostic oral challenge of a series of 286 peanut -allergic individuals.* Food Chem Toxicol, 2010. **48**(3): p. 814-9.
61. Scadding, G.W., et al., *Effect of grass pollen immuno therapy on clinical and local immune response to nasal allergen challenge.* Allergy, 2015. **70**(6): p. 689-96.
62. Taylor, S.L., et al., *Threshold dose for peanut: risk characterization based upon published results from challenges of peanut-allergic individuals.* Food Chem Toxicol, 2009. **47**(6): p. 1198-204.
63. Gupta, R., et al., *The economic impact of childhood food allergy in the United States.* JAMA Pediatr, 2013. **167**(11): p. 1026-31.
64. Bilaver, L.A., et al., *Socioeconomic Disparities in the Economic Impact of Childhood Food Allergy.* Pediatrics, 2016. **137**(5).
65. Teufel, M., et al., *Psychological burden of food allergy.* World J Gastroenterol, 2007. **13**(25): p. 3456-65.
66. Knibb, R.C., et al., *Psychological characteristics of people with perceived food intolerance in a community sample.* J Psychosom Res, 1999. **47**(6): p. 545-54.

67. MacKenzie, H., et al., *Teenagers'experiencesofliving withfoodhypersensitivity:a qualitativestudy.* Pediatr Allergy Immunol, 2010. **21**(4 Pt 1): p. 595-602.
68. Mikkelsen, A., et al., *Thefoodhypersensitivityfamily impact(FLIP)questionnaire -developmentandfirstresults.* Pediatr Allergy Immunol, 2013. **24**(6): p. 574-81.
69. McBride, C., K. McBride-Henry, and K. van Wissen, *Parenting a child with medicallydiagnosedseverefoodallergiesinNewZealand:theexperienceofbeing unsupported in keeping their children healthy and safe.* Contemp Nurse, 2010. **35**(1): p. 77-87.
70. Soller, L., J. Hourihane, and A. DunnGalvin, *Theimpactoforalfoodchallengetests onfoodallergyhealth-relatedqualityoflife.* Allergy, 2014. **69**(9): p. 1255-7.
71. Knibb, R.C., et al., *The psychological impact of diagnostic food challenges to confirm the resolution of peanut or tree nut allergy.* Clin Exp Allergy, 2012. **42**(3): p. 451-9.
72. Warren, C.M., et al., *Quality of Life Among Food Al lergic Patients and Their Caregivers.* Curr Allergy Asthma Rep, 2016. **16**(5): p. 38.
73. Choo, K.J., E. Simons, and A. Sheikh, *Glucocorticoids for the treatment of anaphylaxis:Cochranesystematicreview.* Allergy, 2010. **65**(10): p. 1205-11.
74. Muraro, A., et al., *Anaphylaxis:guidelinesfromtheEuropeanAcademyofAllergy andClinicalImmunology.* Allergy, 2014. **69**(8): p. 1026-45.
75. Simons, F.E., et al., *Internationalconsensuson(ICON)anaphylaxis.* World Allergy Organ J, 2014. **7**(1): p. 9.
76. Patterson, R., et al., *Idiopathicanaphylaxis.Anatte mpttoestimatetheincidence intheUnitedStates.* Arch Intern Med, 1995. **155**(8): p. 869-71.
77. Greenberger, P.A. and P. Lieberman, *Idiopathic anaphylaxis.* J Allergy Clin Immunol Pract, 2014. **2**(3): p. 243-50; quiz 251.
78. Simons, F.E., et al., *WorldAllergyOrganizationan aphylaxisguidelines:summary.* J Allergy Clin Immunol, 2011. **127**(3): p. 587-93 e1-22.
79. Beyer, K., et al., *Anaphylaxis in an emergency se tting - elicitors, therapy and incidenceofsevereallergicreactions.* Allergy, 2012. **67**(11): p. 1451-6.
80. Pumphrey, R., *Anaphylaxis: can we tell who is at risk of a fatal reaction?* Curr Opin Allergy Clin Immunol, 2004. **4**(4): p. 285-90.
81. Umasunthar, T., et al., *Incidenceoffoodanaphylaxisin peoplewithfoodallergy:a systematicreviewandmeta-analysis.* Clin Exp Allergy, 2014.
82. Poulos, L.M., et al., *Trends in hospitalizations for anaphylaxis, angioedema, and urticaria in Australia, 1993-1994 to 2004 -2005.* J Allergy Clin Immunol, 2007. **120**(4): p. 878-84.
83. Canani, R.B., et al., *Hospital admissions for food-induced anaphylaxis in Italian children.* Clin Exp Allergy, 2012. **42**(12): p. 1813-4.
84. Vyas, D., *Increase in Intensive Care Unit Admi ssion for Anaphylaxis in the United Kingdom2008-2012.* Journal of Allergy and Clinical Immunology. **137**(2).
85. Gibbison, B., et al., *AnaphylaxisadmissionstoUKcr iticalcareunitsbetween2005 and2009.* Anaesthesia, 2012. **67**(8): p. 833-9.
86. Noimark, L., et al., *Theuseofadrenalineautoinjectorsbychildrenandteenagers.* Clin Exp Allergy, 2012. **42**(2): p. 284-92.
87. Gold, M.S. and R. Sainsbury, *First aid anaphylaxis management in children who were prescribed an epinephrin e autoinjector device (EpiPen).* J Allergy Clin Immunol, 2000. **106**(1 Pt 1): p. 171-6.
88. Umasunthar, T., et al., *Incidence of fatal food anaphy laxis in people with food allergy:asystematicreviewandmeta-analysis.* Clin Exp Allergy, 2013. **43**(12): p. 1333-41.
89. Mullins, R.J., et al., *Increases in anaphylaxis fatalities in Australia from 1997 to 2013.* Clin Exp Allergy, 2016. **46**(8): p. 1099-110.

90. Ma, L., T.M. Danoff, and L. Borish, *Case fatality and population mortality associated with anaphylaxis in the United States.* J Allergy Clin Immunol, 2014. **133**(4): p. 1075-83.
91. Heinzerling, L., et al., *The skin prick test - European standards.* Clin Transl Allergy, 2013. **3**(1): p. 3.
92. Tripodi, S., et al., *Predicting the outcome of oral food challenges with hen's egg throughskintestend-pointtitration.* Clin Exp Allergy, 2009. **39**(8): p. 1225-33.
93. Fleischer, D.M., et al., *Sublingual immunotherapy for peanut allergy: a randomized, double-blind, plac ebo-controlled multicenter trial.* J Allergy Clin Immunol, 2013. **131**(1): p. 119-27 e1-7.
94. Burks, A.W., et al., *Sublingual immunotherapy for peanut allergy: Long-term follow-up of a randomized multicenter trial.* J Allergy Clin Immunol, 2015. **135**(5): p. 1240-8 e1-3.
95. Ta, V., et al., *UseofSpecificIgEandSkinPrick TesttoDetermineClinicalReaction Severity.* Br J Med Med Res, 2011. **1**(4): p. 410-429.
96. Sampson, H.A. and D.G. Ho, *Relationshipbetweenfood-s pecificIgEconcentrations andtheriskofpositivefoodchal lengesinchildrenandadolescents.* J Allergy Clin Immunol, 1997. **100**(4): p. 444-51.
97. Dang, T.D., et al., *Increasingtheaccuracyofpea nutallergydiagnosisbyusingAra h2.* J Allergy Clin Immunol, 2012. **129**(4): p. 1056-63.
98. Nicolaou, N., et al., *Quantification of specific Ig E to whole peanut extract and peanutcomponentsinpredictionofpeanutallergy.* J Allergy Clin Immunol, 2011. **127**(3): p. 684-5.
99. Vazquez-Ortiz, M., et al., *BaselinespecificIgElevelsar eusefultopredictsafetyof oral immunotherapy in egg-allergic children.* Clin Exp Allergy, 2014. **44**(1): p. 130-41.
100. Vazquez-Ortiz, M., et al., *Ovalbumin-specific IgE/IgG4 ratio might improve the prediction of cooked and uncooked egg tolerance development in egg-allergic children.* Clin Exp Allergy, 2014. **44**(4): p. 579-88.
101. Hourihane, J.O., et al., *Doesseverityoflow-dose, double-blind,placebo-controlled foodchallengesreflectseverityofallerg icreactionstopeanutinthecommunity?* Clin Exp Allergy, 2005. **35**(9): p. 1227-33.
102. Johnson, M.J., et al., *Practices in the prescription of adrenaline autoinjectors.* Pediatr Allergy Immunol, 2012. **23**(2): p. 124-7.
103. Martorell, A., et al., *The predictive value of specific immunoglobulin E levels in serum for the outcome of the development of tolerance in cow's milk allergy.* Allergol Immunopathol (Madr), 2008. **36**(6): p. 325-30.
104. Saarinen, K.M., et al., *Clinical course and prognosis of cow's milk allergy are dependentonmilk-specificIgEstatus.* J Allergy Clin Immunol, 2005. **116**(4): p. 869-75.
105. Host, A. and S. Halken, *Aprospectivestudyofcowmilk allergyinDanishinfants during the first 3 years of life. Clinical course in relation to clinical and immunologicaltypeofhypersensitivityreaction.* Allergy, 1990. **45**(8): p. 587-96.
106. Dieguez, M.C., et al., *Utility of diagnostic tests in the follow-up of egg-allergic children.* Clin Exp Allergy, 2009. **39**(10): p. 1575-84.
107. Venter, C., et al., *Prevalenceandcumulativeincidenceoffoodhypersensitivityin thefirst3yearsoflife.* Allergy, 2008. **63**(3): p. 354-9.
108. Savage, J.H., et al., *The natural history of egg allergy.* J Allergy Clin Immunol, 2007. **120**(6): p. 1413-7.
109. Skripak, J.M., et al., *The natural history of IgE-mediated cow's milk allergy.* J Allergy Clin Immunol, 2007. **120**(5): p. 1172-7.

110. Santos, A., A. Dias, and J.A. Pinheiro, *Predictivefactorsforthepersistenceofcow's milkallergy.* Pediatr Allergy Immunol, 2010. **21**(8): p. 1127-34.
111. Skolnick, H.S., et al., *Thenaturalhistoryof peanutallergy.* J Allergy Clin Immunol, 2001. **107**(2): p. 367-74.
112. Ho, M.H., et al., *Earlyclinicalpredictorsofremiss ionofpeanutallergyinchildren.* J Allergy Clin Immunol, 2008. **121**(3): p. 731-6.
113. Fleischer, D.M., et al., *The natural progression of pea nut allergy: Resolution and thepossibilityofrecurrence.* J Allergy Clin Immunol, 2003. **112**(1): p. 183-9.
114. Perry, T.T., et al., *The relationship of allergen-spe cific IgE levels and oral food challengeoutcome.* J Allergy Clin Immunol, 2004. **114**(1): p. 144-9.
115. Peters, R.L., et al., *ThenaturalhistoryofIgE-mediate dfoodallergy:canskinprick tests and serum-specific IgE predict the resolution of food allergy?* Int J Environ Res Public Health, 2013. **10**(10): p. 5039-61.
116. Leung, D.Y., et al., *Effect of anti-IgE therapy in patients with peanut allergy.* N Engl J Med, 2003. **348**(11): p. 986-93.
117. Vickery, B.P., et al., *Sustained unresponsiveness to peanut in subjects who have completedpeanutoralimmunotherapy.* J Allergy Clin Immunol, 2014. **133**(2): p. 468-75.
118. Anagnostou, K., et al., *Assessing the efficacy of oral immunotherapy for the desensitisation of peanut allergy in children (STOP II): a phase 2 randomised controlledtrial.* Lancet, 2014. **383**(9925): p. 1297-304.
119. Hofmann, A.M., et al., *Safetyofapeanutoralimmunotherapyprotocolinchildren withpeanutallergy.* J Allergy Clin Immunol, 2009. **124**(2): p. 286-91, 291 e1-6.
120. Tang, M.L., et al., *Administrationofaprobioticwi thpeanutoralimmunotherapy: Arandomizedtrial.* J Allergy Clin Immunol, 2015. **135**(3): p. 737-44 e8.
121. Jones, S.M., et al., *Epicutaneous immunotherapy for the treatment of peanut allergy in children and young adults.* J Allergy Clin Immunol, 2017. **139**(4): p. 1242-1252 e9.
122. Begin, P., et al., *Phase 1 results of safety and tolerability in a rush oral immunotherapy protocol to multiple foods using Omalizumab.* Allergy Asthma Clin Immunol, 2014. **10**(1): p. 7.
123. Beyer, K., et al., *Effectsofcookingmethod sonpeanutallergenicity.* J Allergy Clin Immunol, 2001. **107**(6): p. 1077-81.
124. Thyagarajan, A., et al., *Peanutoralimmunotherapyis notreadyforclinicaluse.* J Allergy Clin Immunol, 2010. **126**(1): p. 31-2.
125. Varshney, P., et al., *Adverse reactions during peanut oral immunotherapy home dosing.* J Allergy Clin Immunol, 2009. **124**(6): p. 1351-2.
126. Pumphrey, R.S., *Lessons for management of anap hylaxis from a study of fatal reactions.* Clin Exp Allergy, 2000. **30**(8): p. 1144-50.
127. Rudders, S.A., et al., *Age-related differences in the clinical presentation of food-inducedanaphylaxis.* J Pediatr, 2011. **158**(2): p. 326-8.
128. Sampson, H.A., et al., *Second symposium on the definition and management of anaphylaxis:summaryreport--SecondNationa lInstituteofAllergyandInfectious Disease/Food Allergy and Anaphylaxis Network symposium.* J Allergy Clin Immunol, 2006. **117**(2): p. 391-7.
129. Pumphrey, R.S. and I.S. Roberts, *Postmortem findings after fatal anaphylactic reactions.* J Clin Pathol, 2000. **53**(4): p. 273-6.
130. Simons, F.E., *Anaphylaxis:Recentadvancesinassessmentandtreatment.* J Allergy Clin Immunol, 2009. **124**(4): p. 625-36; quiz 637-8.
131. Worm, M., et al., *Symptom profile and risk factor s of anaphylaxis in Central Europe.* Allergy, 2012. **67**(5): p. 691-8.

132. Finkelman, F.D., et al., *Molecularmechanismsofanaph ylaxis:lessonsfromstudies withmurinemodels.* J Allergy Clin Immunol, 2005. **115**(3): p. 449-57; quiz 458.
133. Finkelman, F.D., M.V. Khodoun, and R. Strait, *HumanIgE-independentsystemic anaphylaxis.* J Allergy Clin Immunol, 2016. **137**(6): p. 1674-1680.
134. Smit, J.J., et al., *Contribution of classic and alternative effector pathways in peanut-inducedanaphylacticresponses.* PLoS One, 2011. **6**(12): p. e28917.
135. Allen, K.J., et al., *Allergenreferencedosesforprec autionarylabeling(VITAL2.0): clinicalimplications.* J Allergy Clin Immunol, 2014. **133**(1): p. 156-64.
136. Smedegard, G., et al., *Anaphylactic shock in monkeys passively sensitized with humanreaginicserum.I.Hemodynamicsandcardiacperformance.* Acta Physiol Scand, 1981. **111**(3): p. 239-47.
137. Martin, T.R., et al., *Roleofmastcellsinanaphylax is.Evidencefortheimportance ofmastcellsinthecardiopulmonaryalterationsanddeathinducedbyanti-IgEin mice.* J Clin Invest, 1989. **83**(4): p. 1375-83.
138. Takeishi, T., et al., *Differences in the expression of the cardiopulmonary alterationsassociatedwithanti-immunogl obulinE-inducedoractiveanaphylaxis in mast cell-deficient and normal mice. Mast cells are not required for the cardiopulmonarychangesassociatedwithcertainfatalanaphylacticresponses.* J Clin Invest, 1991. **88**(2): p. 598-608.
139. Karczewski, W. and J.G. Widdicombe, *The role of the vagus nerves in the respiratory and circulatory reactions to anaphylaxis in rabbits.* J Physiol, 1969. **201**(2): p. 293-304.
140. Faye, N., et al., *Macromolecular capillary leakage is involved in the onset of anaphylactichypotension.* Anesthesiology, 2012. **117**(5): p. 1072-9.
141. Dewachter, P., et al., *Anaphylactic shock: a form of distributive shock without inhibitionofoxygenconsumption.* Anesthesiology, 2005. **103**(1): p. 40-9.
142. Levine, S. and A. Saltzman, *Distributionofsmallintestinallesionsinanaphylaxis ofrats.* Int Arch Allergy Immunol, 1998. **115**(4): p. 312-5.
143. Zhang, W., et al., *Rathepaticandsplanchnicvascularresponsestoanaphylactic shock,comparedwithhemorrhagic orvasodilator-inducedshock.* In Vivo, 2013. **27**(4): p. 485-93.
144. Takano, H., et al., *Hepaticmicrovascularpressureduringanaphylacticshockin anesthetizedrats.* Microvasc Res, 2009. **78**(2): p. 169-73.
145. Martin, T.R., et al., *Mast cells contribute to the changes in heart rate, but not hypotension or death, associated with active anaphylaxis in mice.* J Immunol, 1993. **151**(1): p. 367-76.
146. Cui, S., et al., *Venousresistanceincreasesduringratanaphylacticshock.* Shock, 2008. **29**(6): p. 733-9.
147. Davidson, J., et al., *Anaphylacticshockdecreasescerebralbloodflowmorethan whatwouldbeexpectedfrom severearterialhypotension.* Shock, 2012. **38**(4): p. 429-35.
148. Shinomiya, S., et al., *Nitricoxideandbeta(2)-adrenoceptoractivationattenuate pulmonary vasoconstriction during anap hylactic hypotension in anesthetized BALB/cmice.* Exp Lung Res, 2013. **39**(3): p. 119-29.
149. Arias, K., et al., *Concurrentblockadeofplatelet-activatingfactorandhistamine prevents life-threatening peanut -induced anaphylactic reactions.* J Allergy Clin Immunol, 2009. **124**(2): p. 307-14, 314 e1-2.
150. Osterfeld, H., et al., *Differential roles for the IL-9/IL-9 receptor alpha-chain pathway in systemic and oral antigen-induced anaphylaxis.* J Allergy Clin Immunol, 2010. **125**(2): p. 469-476 e2.
151. Forbes, E.E., et al., *IL-9- and mast cell-mediated intestinal permeability predisposestooralantigenhypersensitivity.* J Exp Med, 2008. **205**(4): p. 897-913.

152. Ahrens, R., et al., *Intestinal mast cell levels control severity of oral antigen-inducedanaphylaxisinmice.* Am J Pathol, 2012. **180**(4): p. 1535-46.
153. Jiang, W., et al., *5-HT(3) and histamine H(1) receptors mediate afferent nerve sensitivity to intestinal anaphylaxis in rats.* Gastroenterology, 2000. **119**(5): p. 1267-75.
154. MacNaughton, W.K., A.G. Catto-Smith, and D.G. Gall, *A role for phospholipid-derivedinflammatorymediatorsinintestinalanaphylaxisintherat.* J Pharmacol Exp Ther, 1992. **260**(2): p. 773-9.
155. Oliver, M.R., D.T. Tan, and R.B. Scott, *Intestinal anaphylaxis: mediation of the responseofcoloniclongitudinalmuscleinrat.* Am J Physiol, 1995. **268**(5 Pt 1): p. G764-71.
156. Bartnikas, L.M., et al., *Epicutaneous sensitization results in IgE-dependent intestinal mast cell expansion and food-induced anaphylaxis.* J Allergy Clin Immunol, 2013. **131**(2): p. 451-60 e1-6.
157. Strait, R.T., et al., *Ingested allergens must be absorbed systemically to induce systemicanaphylaxis.* J Allergy Clin Immunol, 2011. **127**(4): p. 982-9 e1.
158. Krishnamurthy, D., et al., *Monitoring neutrophils and platelets during casein-induced anaphylaxis in an experimental BALB/c mouse model.* Clin Exp Allergy, 2012. **42**(7): p. 1119-28.
159. Perrier, C., et al., *Allergen-specific antibody and cy tokine responses, mast cell reactivity and intestinal permeability up on oral challenge of sensitized and tolerizedmice.* Clin Exp Allergy, 2010. **40**(1): p. 153-62.
160. Vinuesa, M., et al., *Immunopathological modifications in the rectal mucosa from ananimalmodeloffoodallergy.* Rev Esp Enferm Dig, 2005. **97**(9): p. 629-36.
161. Sun, J., et al., *Impact of CD40 ligand, B cells, an d mast cells in peanut-induced anaphylacticresponses.* J Immunol, 2007. **179**(10): p. 6696-703.
162. Shibamoto, T., et al., *PAF, rather than histamin e, participates in mouse anaphylactichypotension.* Pharmacology, 2008. **82**(2): p. 114-20.
163. Khodoun, M., et al., *Peanuts can contribute to anaphylactic shock by activating complement.* J Allergy Clin Immunol, 2009. **123**(2): p. 342-51.
164. Pumphrey, R.S., *Fatal posture in anaphylactic shock.* J Allergy Clin Immunol, 2003. **112**(2): p. 451-2.
165. Jacobsen, R.C. and M.C. Gratton, *Acaseofunrecognizedprehospitalanaphylactic shock.* Prehosp Emerg Care, 2011. **15**(1): p. 61-6.
166. Schwartz, L.B., et al., *Time course of appearance and disappearance of human mastcelltryptaseinthecirculationafteranaphylaxis.* J Clin Invest, 1989. **83**(5): p. 1551-5.
167. Sampson, H.A. and P.L. Jolie, *Increased plasma histamine concentrations after foodchallengesinchildrenwithatopicdermatitis.* N Engl J Med, 1984. **311**(6): p. 372-6.
168. Brown, S.G., et al., *Anaphylaxis:clinicalpatterns, mediatorrelease,andseverity.* J Allergy Clin Immunol, 2013. **132**(5): p. 1141-1149 e5.
169. Sala-Cunill, A., et al., *Usefulness and limitations of sequential serum tryptase for the diagnosis of anaphylaxis in 102 patients.* Int Arch Allergy Immunol, 2013. **160**(2): p. 192-9.
170. Vadas, P., et al., *Platelet-activating factor, PA F acetylhydrolase, and severe anaphylaxis.* N Engl J Med, 2008. **358**(1): p. 28-35.
171. Vadas, P., B. Perelman, and G. Liss, *Platelet-activating factor, histamine, and tryptase levels in human anaphylaxis.* J Allergy Clin Immunol, 2013. **131**(1): p. 144-9.
172. Brown, S.G., *Clinical features and severi ty grading of anaphylaxis.* J Allergy Clin Immunol, 2004. **114**(2): p. 371-6.

173. van Odijk, J., et al., *Measurements of eosinophil act ivation before and after food challenges in adults with food hypersensitivity.* Int Arch Allergy Immunol, 2006. **140**(4): p. 334-41.
174. Lewis, S.A., et al., *The promiscuity of immunoglob ulin E binding to peanut allergens, as determined by Western blotting, correlates with the severity of clinical symptoms.* Clin Exp Allergy, 2005. **35**(6): p. 767-73.
175. Caffarelli, C., et al., *Blood pressure monitoring in children undergoing food challenge: association with anaphylaxis.* Ann Allergy Asthma Immunol, 2012. **108**(4): p. 285-6.
176. Sampson, H.A., L. Mendelson, and J.P. Rosen, *Fatal and near-fatal anaphylactic reactions to food in children and adolescents.* N Engl J Med, 1992. **327**(6): p. 380-4.
177. Santos, A.F., et al., *Basophil activation test discriminates between allergy and tolerance in peanut-sensitized children.* J Allergy Clin Immunol, 2014. **134**(3): p. 645-52.
178. Glaumann, S., et al., *Basophil allergen threshold sensitivity, CD-sens, IgE-sensitization and DBPCFC in peanut-sensitized children.* Allergy, 2012. **67**(2): p. 242-7.
179. Javaloyes, G., et al., *Performance of different in vitro techniques in the molecular diagnosis of peanut allergy.* J Investig Allergol Clin Immunol, 2012. **22**(7): p. 508-13.
180. Ocmant, A., et al., *Basophil activation tests for the diagnosis of food allergy in children.* Clin Exp Allergy, 2009. **39**(8): p. 1234-45.
181. Sperr, W.R., et al., *The human cardiac mast cell: localization, isolation, phenotype, and functional characterization.* Blood, 1994. **84**(11): p. 3876-84.
182. Patella, V., et al., *Human heart mast cells. Isolation, purification, ultrastructure, and immunologic characterization.* J Immunol, 1995. **154**(6): p. 2855-65.
183. Ginsburg, R., et al., *Histamine receptors in the human heart.* Life Sci, 1980. **26**(26): p. 2245-9.
184. Ginsburg, R., et al., *Histamine provocation of clinic al coronary artery spasm: implications concerning pathogenes is of variant angina pectoris.* Am Heart J, 1981. **102**(5): p. 819-22.
185. Detopoulou, P., et al., *Platelet activating factor in heart failure: potential role in disease progression and novel target for therapy.* Curr Heart Fail Rep, 2013. **10**(2): p. 122-9.
186. Wasserman, S.I., *The heart in anaphylaxis.* J Allergy Clin Immunol, 1986. **77**(5): p. 663-6.
187. Vigorito, C., et al., *Effects of histamine on coronary hemodynamics in humans: role of H1 and H2 receptors.* J Am Coll Cardiol, 1987. **10**(6): p. 1207-13.
188. Braquet, P., et al., *Perspectives in platelet-activating factor research.* Pharmacol Rev, 1987. **39**(2): p. 97-145.
189. Fisher, M.M., *Clinical observations on the pa thophysiology and treatment of anaphylactic cardiovascular collapse.* Anaesth Intensive Care, 1986. **14**(1): p. 17-21.
190. Heytman, M. and A. Rainbird, *Use of alpha-agonists for management of anaphylaxis occurring under anaest hesia: case studies and review.* Anaesthesia, 2004. **59**(12): p. 1210-5.
191. Kounis, N.G., *Coronary hypersensitivity disorder: the Kounis syndrome.* Clin Ther, 2013. **35**(5): p. 563-71.
192. Nachmias, B. and D. Leibowitz, *Kounis syndrome second ary to food allergy.* Br J Hosp Med (Lond), 2014. **75**(11): p. 654-5.

193. Zavras, G.M., et al., *Kounis syndrome secondary to allergic reaction following shellfishingestion.* Int J Clin Pract, 2003. **57**(7): p. 622-4.
194. Helbling, A., et al., *Incidence of anaphylaxis with circulatory symptoms: a study over a 3-year period comprising 940,000 inhabitants of the Swiss Canton Bern.* Clin Exp Allergy, 2004. **34**(2): p. 285-90.
195. Yocum, M.W., et al., *Epidemiology of anaphylaxis in Olmsted County: A population-basedstudy.* J Allergy Clin Immunol, 1999. **104**(2 Pt 1): p. 452-6.
196. Abdelghany, M., et al., *In reply: Kounis syndrome: A review article on epidemiology, diagnostic findings, mana gement and complications of allergic acutecoronarysyndrome:Mastocytosisandpost-mortemdiagnosis.* Int J Cardiol, 2017. **242**: p. 39.
197. Twomey, N., et al., *Automated detection of perturbe d cardiac physiology during oral food allergen challenge in children.* IEEE J Biomed Health Inform, 2014. **18**(3): p. 1051-7.
198. Summers, C.W., et al., *Factorspredictinganaphylaxistopeanutsandtreenutsin patientsreferredtoaspecialistcenter.* J Allergy Clin Immunol, 2008. **121**(3): p. 632-638 e2.
199. Xu, Y.S., et al., *Anaphylaxis-related deaths in O ntario: a retrospective review of casesfrom1986to2011.* Allergy Asthma Clin Immunol, 2014. **10**(1): p. 38.
200. Bock, S.A., A. Munoz-Furlong, and H.A. Sampson, *Further fatalities caused by anaphylacticreactionstofood,2001-2006.* J Allergy Clin Immunol, 2007. **119**(4): p. 1016-8.
201. Pumphrey, R.S. and M.H. Gowland, *Furtherfatalallergicreactionstofoodinthe UnitedKingdom,1999-2006.* J Allergy Clin Immunol, 2007. **119**(4): p. 1018-9.
202. Branum, A.M. and S.L. Lukacs, *Foodallergyamongchildreninthe UnitedStates.* Pediatrics, 2009. **124**(6): p. 1549-55.
203. Clark, A.T. and P.W. Ewan, *Goodprognosis,clinicalfeatures,andcircumstancesof peanut and tree nut reactions in children treated by a specialist allergy center.* J Allergy Clin Immunol, 2008. **122**(2): p. 286-9.
204. Hompes, S., et al., *Provoking allergens and treatment of anaphylaxis in children and adolescents--data from the anaphylaxis registry of German-speaking countries.* Pediatr Allergy Immunol, 2011. **22**(6): p. 568-74.
205. Simons, F.E., *Anaphylaxis.* J Allergy Clin Immunol, 2010. **125**(2 Suppl 2): p. S161-81.
206. Iribarren, C., et al., *Asthma and the prospective risk of anaphylactic shock and other allergy diagnoses in a large inte grated health care delivery system.* Ann Allergy Asthma Immunol, 2010. **104**(5): p. 371-7.
207. Wickman, M., et al., *Childhood-to-adolescence evolution of IgE antibodies to pollens and plant foods in the BAMSE cohort.* J Allergy Clin Immunol, 2014. **133**(2): p. 580-2.
208. Court, C.S., D.G. Cook, and D.P. Strachan, *The descriptive epidemiology of house dust mite-specific and total immunoglobin E in England using a nationally representativesample.* Clin Exp Allergy, 2002. **32**(7): p. 1033-41.
209. Skripak, J.M., et al., *A randomized, double-blind, placebo-controlled study of milk oralimmunotherapyforcow'smilkallergy.* J Allergy Clin Immunol, 2008. **122**(6): p. 1154-60.
210. Staden, U., et al., *Specific oral tolerance inductio n in food allergy in children: efficacyandclinicalpatternsofreaction.* Allergy, 2007. **62**(11): p. 1261-9.
211. Vazquez-Ortiz, M., et al., *Safety and predictors of adverse events during oral immunotherapyformilkallergy:severityof reactionatoralchallenge,specificIgE andpricktest.* Clin Exp Allergy, 2013. **43**(1): p. 92-102.

212. Rajakulasingam, K., et al., *Enhanced expression of high-affinity IgE receptor (Fc epsilon RI) alpha chain in human allergen-induced rhinitis with co-localization to mast cells, macrophages, eosinophils, and dendritic cells.* J Allergy Clin Immunol, 1997. **100**(1): p. 78-86.
213. Rajakulasingam, K., et al., *Increased expression of high affinity IgE (FcepsilonRI) receptor-alpha chain mRNA and protein-bearing eosinophils in human allergen-induced atopic asthma.* Am J Respir Crit Care Med, 1998. **158**(1): p. 233-40.
214. Wilson, D.R., et al., *Grass pollen immunotherapy inhibits seasonal increases in basophils and eosinophils in the nasal epithelium.* Clin Exp Allergy, 2001. **31**(11): p. 1705-13.
215. Dirks, C.G., et al., *Does absorption across the buccal mucosa explain early onset of food-induced allergic systemic reactions?* J Allergy Clin Immunol, 2005. **115**(6): p. 1321-3.
216. Poulsen, L.K., et al., *Immunochemical and biological quantification of peanut extract.* Arb Paul Ehrlich Inst Bundesamt Sera Impfstoffe Frankf A M, 2003(94): p. 97-105; discussion 106.
217. Akdis, C.A., K. Blaser, and M. Akdis, *Apoptosis in tissue inf l ammation and allergic disease.* Curr Opin Immunol, 2004. **16**(6): p. 717-23.
218. Ryan, J.J., et al., *Mast cell homeostasis: a fundame ntal aspect of allergic disease.* Crit Rev Immunol, 2007. **27**(1): p. 15-32.
219. Medoff, B.D., S.Y. Thomas, and A.D. Luster, *T cell trafficking in allergic asthma: the ins and outs.* Annu Rev Immunol, 2008. **26**: p. 205-32.
220. Grimbaldeston, M.A., et al., *Mast cell-derived interleukin 10 limits skin pathology in contact dermatitis and chroni c irradiation with ultraviolet B.* Nat Immunol, 2007. **8**(10): p. 1095-104.
221. Serhan, C.N., S. Yacoubian, and R. Yang, *Anti-inflammatory and proresolving lipid mediators.* Annu Rev Pathol, 2008. **3**: p. 279-312.
222. Opal, S.M. and V.A. DePalo, *Anti-inflammatory cytokines.* Chest, 2000. **117**(4): p. 1162-72.
223. Letterio, J.J. and A.B. Roberts, *Regulation of immune responses by TGF-beta.* Annu Rev Immunol, 1998. **16**: p. 137-61.
224. Li, M.O. and R.A. Flavell, *Contextual regulation of inflammation: a duet by transforming growth factor-beta and interleukin-10.* Immunity, 2008. **28**(4): p. 468-76.
225. Burgel, P.R., et al., *Human eosinophils induce mucin production in airway epithelial cells via epidermal growth factor receptor activation.* J Immunol, 2001. **167**(10): p. 5948-54.
226. Vignali, D.A., L.W. Collison, and C.J. Workman, *How regulatory T cells work.* Nat Rev Immunol, 2008. **8**(7): p. 523-32.
227. Rivera, J. and A.M. Gilfillan, *Molecular regulation of mast cell activation.* J Allergy Clin Immunol, 2006. **117**(6): p. 1214-25; quiz 1226.
228. Kraft, S. and J.P. Kinet, *New developments in FcepsilonRI regulation, function and inhibition.* Nat Rev Immunol, 2007. **7**(5): p. 365-78.
229. Kobayasi, T. and G. Asboe-Hansen, *Degranulation and regranulation of human mast cells. An electron microscopic study of the wheal ing reaction in urticaria pigmentosa.* Acta Derm Venereol, 1969. **49**(4): p. 369-81.
230. Dvorak, A.M., R.P. Schleimer, and L.M. Lichtenstein, *Morphologic mast cell cycles.* Cell Immunol, 1987. **105**(1): p. 199-204.
231. Dvorak, A.M., et al., *Human mast cells use conservation and condensation mechanisms during recovery from degranul ation. In vitro studies with mast cells purified from human lungs.* Lab Invest, 1986. **54**(6): p. 663-78.

232. Burwen, S.J., *Recycling of mast cells follow ing degranulation in vitro: an ultrastructuralstudy.* Tissue Cell, 1982. **14**(1): p. 125-34.
233. Xiang, Z., et al., *IgE-mediatedmastcelldegranulationandrecoverymonitoredby time-lapsephotography.* J Allergy Clin Immunol, 2001. **108**(1): p. 116-21.
234. Mills, E.N., et al., *Impact of food processing on the structural and allergenic propertiesoffoodallergens.* Mol Nutr Food Res, 2009. **53**(8): p. 963-9.
235. Grimshaw, K.E., et al., *Presentation of allergen in different food preparations affectsthenatureoftheallerg icreaction--acaseseries.* Clin Exp Allergy, 2003. **33**(11): p. 1581-5.
236. van Odijk, J., et al., *Double-blindplacebo-controlledchallengesforpeanutallergy the efficiency of blinding procedures and the allergenic activity of peanut availabilityintherecipes.* Allergy, 2005. **60**(5): p. 602-5.
237. Nowak-Wegrzyn, A. and A. Fiocchi, *Rare, medium, or well done? The effect of heating and food matrix on food protein allergenicity.* Curr Opin Allergy Clin Immunol, 2009. **9**(3): p. 234-7.
238. Wang, J., et al., *CorrelationofIgE/IgG4milkepitop esandaffinityofmilk-specific IgE antibodies with different phen otypes of clinical milk allergy.* J Allergy Clin Immunol, 2010. **125**(3): p. 695-702, 702 e1-702 e6.
239. Kukkonen, A.K., et al., *Arah2andAra6arethebest predictorsofseverepeanut allergy:adouble-blind placebo-controlledstudy.* Allergy, 2015. **70**(10): p. 1239-45.
240. Asarnoj, A., *IgE-levelstopeanutallergencomp onentArah2:relationtopeanut symptomsin8-year-olds.* Clin Transl Allergy. , 2013. **3**: p. 92.
241. Eller, E. and C. Bindslev-Jensen, *Clinicalvalueofcomponent-resolveddiagnostics inpeanut-allergicpatients.* Allergy, 2013. **68**(2): p. 190-4.
242. Ciprandi, G., et al., *Walnutanaphylaxis:theusefulnessofmolecular-basedallergy diagnostics.* Immunol Lett, 2014. **161**(1): p. 138-9.
243. Fukutomi, Y., et al., *Clinical relevance of IgE to recombinant Gly m 4 in the diagnosisofadultsoybeanallergy.* J Allergy Clin Immunol, 2012. **129**(3): p. 860-863 e3.
244. De Swert, L.F., et al., *Secondarysoyallergyinchildrenwithbirchpollenallergy may cause both chronic and acute symptoms.* Pediatr Allergy Immunol, 2012. **23**(2): p. 117-23.
245. van Zuuren, E.J., et al., *Anaphylaxisafterconsumingsoyproductsinpatientswith birchpollinosis.* Allergy, 2010. **65**(10): p. 1348-9.
246. Flinterman, A.E., et al., *Peanut epitopes for IgE and IgG4 in peanut-sensitized children in relation to severity of peanut allergy.* J Allergy Clin Immunol, 2008. **121**(3): p. 737-743 e10.
247. Santos, A.F., et al., *Distinctparametersofthebasoph ilactivationtestreflectthe severity and threshold of al lergic reactions to peanut.* J Allergy Clin Immunol, 2015. **135**(1): p. 179-86.
248. Wainstein, B.K., et al., *Prediction of anaphylaxis duri ng peanut food challenge: usefulness of the peanut skin prick test (SPT) and specific IgE level.* Pediatr Allergy Immunol, 2010. **21**(4 Pt 1): p. 603-11.
249. Turner, P.J. and B.K. Wainstein, *Crossing the threshold: can outcome data from foodchallengesbeusedtopredictriskofanaphylaxisinthecommunity?* Allergy, 2017. **72**(1): p. 9-12.
250. Wongkaewpothong, P., et al., *The utility of serum trypt ase in the diagnosis of food-inducedanaphylaxis.* Allergy Asthma Immunol Res, 2014. **6**(4): p. 304-9.
251. Lin, R.Y., et al., *Histamine and tryptase levels in patients with acute allergic reactions:Anemergencydepartment-basedstudy.* J Allergy Clin Immunol, 2000. **106**(1 Pt 1): p. 65-71.

252. Arias, K., et al., *Distinct immune effector pathways contribute to the full expression of peanut-induced anaphylactic reactions in mice.* J Allergy Clin Immunol, 2011. **127**(6): p. 1552-61 e1.
253. Hitomi, Y., et al., *Associations of functional NLRP3 polymorphisms with susceptibility to food-induced anaphylaxis and aspirin-induced asthma.* J Allergy Clin Immunol, 2009. **124**(4): p. 779-85 e6.
254. Karasawa, K., et al., *Plasma platelet activating factor-acetylhydrolase (PAF-AH).* Prog Lipid Res, 2003. **42**(2): p. 93-114.
255. Serghini-Idrissi, N., et al., *[Food allergy in the chronic alcoholic and alcohol in foodallergy:aproposof38cases].* Allerg Immunol (Paris), 2001. **33**(10): p. 378-82.
256. Vidal, C., et al., *Serum IgE levels in liver cirrhosis. Contrasting results in alcoholic and non-alcoholic patients.* Clin Exp Allergy, 1994. **24**(6): p. 540-8.
257. Gonzalez-Quintela, A., et al., *Increased serum IgE in alcohol abusers.* Clin Exp Allergy, 1995. **25**(8): p. 756-64.
258. Gonzalez-Quintela, A., et al., *Serum cytokines and increased total serum IgE in alcoholics.* Ann Allergy Asthma Immunol, 1999. **83**(1): p. 61-7.
259. Bjerke, T., et al., *Several genetic and environmental factors influence cord blood IgE concentration.* Pediatr Allergy Immunol, 1994. **5**(2): p. 88-94.
260. McCarthy, D.A. and M.M. Dale, *The leucocytosis of exercise. A review and model.* Sports Med, 1988. **6**(6): p. 333-63.
261. Fry, R.W., et al., *Cell numbers and in vitro responses of leucocytes and lymphocyte subpopulations following maximal exerci se and interval training sessions of different intensities.* Eur J Appl Physiol Occup Physiol, 1992. **64**(3): p. 218-27.
262. Shadick, N.A., et al., *The natural history of exercise -induced anaphylaxis: survey results from a 10-year follow-up study.* J Allergy Clin Immunol, 1999. **104**(1): p. 123-7.
263. Matsuo, H., et al., *Exercise and aspirin increase levels of circulating gliadin peptides in patients with wheat-de pendent exercise-induced anaphylaxis.* Clin Exp Allergy, 2005. **35**(4): p. 461-6.
264. Gisolfi, C.V., et al., *Effects of cycle exercise on intestinal absorption in humans.* J Appl Physiol (1985), 1991. **71**(6): p. 2518-27.
265. Chen, J.Y., J. Quirt, and K.J. Lee, *Proposed new mechanism for food and exercise induced anaphylaxis based on case studies.* Allergy Asthma Clin Immunol, 2013. **9**(1): p. 11.
266. Uguz, A., et al., *Allergic reactions in the community: a questionnaire survey of members of the anaphylaxis campaign.* Clin Exp Allergy, 2005. **35**(6): p. 746-50.
267. Treudler, R., Y. Kozovska, and J.C. Simon, *Severe immediate type hypersensitivity reactions in 105 German adults: when to diagnose anaphylaxis.* J Investig Allergol Clin Immunol, 2008. **18**(1): p. 52-8.
268. Fukata, M., A.S. Vamadevan, and M.T. Abreu, *Toll-like receptors (TLRs) and Nod-like receptors (NLRs) in inflammatory disorders.* Semin Immunol, 2009. **21**(4): p. 242-53.
269. Metz, M. and M. Maurer, *Mast cells--key effector cells in immune responses.* Trends Immunol, 2007. **28**(5): p. 234-41.
270. Santos, J., et al., *Release of mast cell mediators into the jejunum by cold pain stress in humans.* Gastroenterology, 1998. **114**(4): p. 640-8.
271. Fujii, H., et al., *Food-dependent exercise-induced anaphylaxis induced by low dose aspirin therapy.* Allergol Int, 2008. **57**(1): p. 97-8.
272. Cardona, V., et al., *Co-factor-enhanced food allergy.* Allergy, 2012. **67**(10): p. 1316-8.

273. Hovmark, A. and E. Asbrink, *Effects of a beta-receptor blocking agent (propranolol) on synthesis of IgE in vit ro by peripheral blood lymphocytes from atopicpatients.* Allergy, 1981. **36**(6): p. 391-6.
274. Inomata, N., *Recent advances in drug-induced angioedema.* Allergol Int, 2012. **61**(4): p. 545-57.
275. Nassiri, M., et al., *Ramipril and metoprolol intake aggravate human and murine anaphylaxis: evidence for direct mast cell priming.* J Allergy Clin Immunol, 2015. **135**(2): p. 491-9.
276. Untersmayr, E., et al., *Antacid medication inhibits digestion of dietary proteins and causes food allergy: a fish allergy model in BALB/c mice.* J Allergy Clin Immunol, 2003. **112**(3): p. 616-23.
277. Michael, J.G., *The role of digestive enzymes in orally induced immune tolerance.* Immunol Invest, 1989. **18**(9-10): p. 1049-54.
278. Untersmayr, E., et al., *Anti-ulcer drugs promote IgE formation toward dietary antigens in adult patients.* FASEB J, 2005. **19**(6): p. 656-8.
279. Untersmayr, E., et al., *Incomplete digestion of codfish represents a risk factor for anaphylaxis in patients with allergy.* J Allergy Clin Immunol, 2007. **119**(3): p. 711-7.
280. Turner, P.J., et al., *Can we identify patients at risk of life-threatening allergic reactions to food?* Allergy, 2016. **71**(9): p. 1241-55.
281. Zurzolo, G.A., et al., *Peanut Allergen Threshold S tudy (PATS): validation of eliciting doses using a novel si ngle-dose challenge protocol.* Allergy Asthma Clin Immunol, 2013. **9**(1): p. 35.
282. Squier, C.A., *The permeability of oral mucosa.* Crit Rev Oral Biol Med, 1991. **2**(1): p. 13-32.
283. Cockcroft, D.W., et al., *Determinants of allergen-induced asthma: dose of allergen, circulating IgE antibody concentration, and bronchial responsiveness to inhaled histamine.* Am Rev Respir Dis, 1979. **120**(5): p. 1053-8.
284. Cockcroft, D.W., et al., *Prediction of airway responsive ness to allergen from skin sensitivity to allergen and airway responsiveness to histamine.* Am Rev Respir Dis, 1987. **135**(1): p. 264-7.
285. Klemans, R.J., et al., *Objective eliciting doses of peanut-allergic adults and children can be combined for risk assessment purposes.* Clin Exp Allergy, 2015. **45**(7): p. 1237-44.
286. Sampson, H.A., et al., *Second symposium on the definition and management of anaphylaxis: summary report--second National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network symposium.* Ann Emerg Med, 2006. **47**(4): p. 373-80.
287. Ewan, P.W. and A.T. Clark, *Efficacy of a management plan based on severity assessment in longitudinal and case-controlled studies of 747 children with nut allergy: proposal for good practice.* Clin Exp Allergy, 2005. **35**(6): p. 751-6.
288. Sampson, H.A., et al., *Standardizing double-blind, pl acebo-controlled oral food challenges: American Academy of Allergy, Asthma & Immunology-European Academy of Allergy and Clinical Immunology PRACTALL consensus report.* J Allergy Clin Immunol, 2012. **130**(6): p. 1260-74.
289. Benhamou, A.H., S.A. Zamora, and P.A. Eigenmann, *Correlation between specific immunoglobulin E levels and the severity of reactions in egg allergic patients.* Pediatr Allergy Immunol, 2008. **19**(2): p. 173-9.
290. Vazquez-Ortiz, M., et al., *Ovalbumin-specific IgE/total IgE ratio improves the prediction of tolerance development in eg g-allergic children aged >/=5 years.* Pediatr Allergy Immunol, 2015. **26**(6): p. 580-3.

291. Varshney, P., et al., *Arandomizedcontrolledstudyofpeanutoralimmunotherapy: clinical desensitization and modulation of the allergic response.* J Allergy Clin Immunol, 2011. **127**(3): p. 654-60.
292. Begin, P., R.S. Chinthrajah, and K.C. Nadeau, *Oral immunotherapy for the treatmentoffoodallergy.* Hum Vaccin Immunother, 2014. **10**(8): p. 2295-302.
293. Narisety, S.D. and C.A. Keet, *Sublingualvsoralimmunotherapyforfoodallergy: identifyingtherightapproach.* Drugs, 2012. **72**(15): p. 1977-89.
294. Fernandez-Rivas, M., et al., *Randomizeddouble-blind,placebo-controlledtrialof sublingualimmunotherapywithaPrup3quantifiedpeachextract.* Allergy, 2009. **64**(6): p. 876-83.
295. Sheikh, A., et al., *H1-antihistaminesforthetreatm ent of anaphylaxis: Cochrane systematicreview.* Allergy, 2007. **62**(8): p. 830-7.
296. Sheikh, A., et al., *Adrenalineforthetreatmentofan aphylaxis:cochranesystematic review.* Allergy, 2009. **64**(2): p. 204-12.
297. Sicherer, S.H., E.H. Morrow, and H.A. Sampson, *Dose-response in double-blind, placebo-controlled oral food challenges in children with atopic dermatitis.* J Allergy Clin Immunol, 2000. **105**(3): p. 582-6.
298. Cochrane, S.A., et al., *Development of a standardize d low-dose double-blind placebo-controlled challenge vehi cle for the EuroPrevall project.* Allergy, 2012. **67**(1): p. 107-13.
299. Miller, M.R., et al., *Standardisation of spirometry.* Eur Respir J, 2005. **26**(2): p. 319-38.
300. Mills, E.N., et al., *The prevalence, cost and basis of food allergy across Europe.* Allergy, 2007. **62**(7): p. 717-22.
301. Payne, V. and P.C. Kam, *Mastcelltryptase:areviewofitsphysiologyandclinical significance.* Anaesthesia, 2004. **59**(7): p. 695-703.
302. Squara, P., et al., *Noninvasive cardiac output monitoring (NICOM): a clinical validation.* Intensive Care Med, 2007. **33**(7): p. 1191-4.
303. Raval, N.Y., et al., *Multicenter evaluation of noninvasive cardiac output measurement by bioreactance technique.* J Clin Monit Comput, 2008. **22**(2): p. 113-9.
304. Waldron, N.H., et al., *A prospective comparison of a noninvasive cardiac output monitor versus esophageal Doppler monit or for goal-directed fluid therapy in colorectalsurgerypatients.* Anesth Analg, 2014. **118**(5): p. 966-75.
305. Marque, S., et al., *ComparisonbetweenFlotrac-VigileoandBioreactance,atotally noninvasivemethodforcardiacoutputmonitoring.* Crit Care, 2009. **13**(3): p. R73.
306. Squara, P., et al., *ComparisonofmonitoringperformanceofBioreactancevs.pulse contourduringlungrecruitmentmaneuvers.* Crit Care, 2009. **13**(4): p. R125.
307. Costello, B.T., et al., *Evaluation of a brachial cuff and suprasystolic waveform algorithmmethodtononinvasiv elyderivecentralbloodpressure.* Am J Hypertens, 2015. **28**(4): p. 480-6.
308. Park, C.M., et al., *Arterial pressure: agreement between a brachial cuff-based deviceandradialtonometry.* J Hypertens, 2014. **32**(4): p. 865-72.
309. Bochmann, R.P., et al., *Brachialanddigitalarterypulsepressuresinhypertensive andnormotensivesubjects.* J Hum Hypertens, 1996. **10**(8): p. 539-46.
310. Tanaka, H. and O. Thulesius, *Effect of temperature on finger artery pressure evaluatedbyvolumeclamptechnique.* Clin Physiol, 1993. **13**(5): p. 535-45.
311. *Heart rate variability: standards of me asurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology.* Circulation, 1996. **93**(5): p. 1043-65.

312. McCraty, R. and F. Shaffer, *Heart Rate Variability: New Perspectives on Physiological Mechanisms, Assessment of Self-regulatory Capacity, and Health risk.* Glob Adv Health Med, 2015. **4**(1): p. 46-61.
313. Carley, S.D., et al., *What'sthepointofSTelevation?* Emerg Med J, 2002. **19**(2): p. 126-8.
314. Tandberg, D., K.D. Kastendieck, and S. Meskin, *Observervariationinmeasured ST-segmentelevation.* Ann Emerg Med, 1999. **34**(4 Pt 1): p. 448-52.
315. Lin, R.Y., E.R. Erlich, and P.C. Don, *Skinpricktestresponsestocodeine,histamine, andragweedutilizingtheMultitestdevice.* Ann Allergy, 1990. **65**(3): p. 222-6.
316. Cox, L., et al., *Speaking the same language: The World Allergy Organization Subcutaneous Immunotherapy Systemic Reaction Grading System.* J Allergy Clin Immunol, 2010. **125**(3): p. 569-74, 574 e1-574 e7.
317. Worm, M., et al., *FirstEuropeandatafromthenetwor kofsevereallergicreactions (NORA).* Allergy, 2014. **69**(10): p. 1397-404.
318. Solmazgul, E., et al., *Anaphylactic reactions prese nting with hypertension.* Springerplus, 2016. **5**(1): p. 1223.
319. Marone, G., et al., *The human heart as a shock organ in anaphylaxis.* Novartis Found Symp, 2004. **257**: p. 133-49; discussion 149-60, 276-85.
320. Brown, S.G., *Cardiovascular aspects of anaphy laxis: implications for treatment anddiagnosis.* Curr Opin Allergy Clin Immunol, 2005. **5**(4): p. 359-64.
321. Smith, P.L., et al., *Physiologicmanifestationsofhumananaphylaxis.* J Clin Invest, 1980. **66**(5): p. 1072-80.
322. Joint Task Force on Practice, P., et al., *The diagnosis and management of anaphylaxis: an updated practice parameter.* J Allergy Clin Immunol, 2005. **115**(3 Suppl 2): p. S483-523.
323. Sheikh, A., et al., *Adrenaline(epinephrine)forth etreatmentofanaphylaxiswith andwithoutshock.* Cochrane Database Syst Rev, 2008(4): p. CD006312.
324. Hedlin, G. and U. Freyschuss, *Cardiac output and blood pressure in asthmatic childrenbeforeandduringinducedasthma.* Acta Paediatr Scand, 1984. **73**(4): p. 441-7.
325. Wolff, A.A. and R. Levi, *Histamineandcardiacarrhythmias.* Circulation Research, 1986. **58**(1): p. 1-16.
326. Kounis, N.G., *Kounis syndrome: an update on epidemiology, pathogenesis, diagnosis and therapeutic management.* Clin Chem Lab Med, 2016. **54**(10): p. 1545-59.
327. Abdelghany, M., et al., *Kounis syndrome: A review article on epidemiology, diagnostic findings, management and comp lications of allergic acute coronary syndrome.* Int J Cardiol, 2017. **232**: p. 1-4.
328. Franca da Silva, A.K., et al., *ApplicationofHeartRateVa riabilityinDiagnosisand Prognosis of Individuals with Diab etes Mellitus: Systematic Review.* Ann Noninvasive Electrocardiol, 2016. **21**(3): p. 223-35.
329. Mazzeo, A.T., et al., *Heart rate variability: a diagnostic and prognostic tool in anesthesiaandintensivecare.* Acta Anaesthesiol Scand, 2011. **55**(7): p. 797-811.
330. Quintana, M., et al., *Heartratevariabilityasameansofassessingprognosisafter acutemyocardialinfarction.A3-yearfollow-upstudy.* Eur Heart J, 1997. **18**(5): p. 789-97.
331. Fridericia, L.S., *Thedurationofsystoleinanel ectrocardiograminnormalhumans andinpatientswithheartdisease.1920.* Ann Noninvasive Electrocardiol, 2003. **8**(4): p. 343-51.
332. Sagie, A., et al., *An improved method for adjusting the QT interval for heart rate (theFraminghamHeartStudy).* Am J Cardiol, 1992. **70**(7): p. 797-801.

333. Electrophysiology, T.F.o.t.E.S.o.C.t.N.A.S., *Heart Rate Variability : Standards of Measurement, Physiological Interpretation, and Clinical Use.* Circulation, 1996. **93**(5): p. 1043-1065.
334. Burr, R.L., *Interpretation of normalized spectral heart rate variability indices in sleepresearch:acriticalreview.* Sleep, 2007. **30**(7): p. 913-9.
335. HC, B., *Ananalysisofthetime - relationsoftheelectrocardiograms.* Heart, 1920. **7**: p. 353-370.
336. LS, F., *Diesystolendauerimelektrokardiogr ammbeinormalenmenschenundbei herzkranken.* Acta Med Scand., 1920. **53**: p. 469-486.
337. Jonsson, P., *Respiratorysinusarrhythmiaasafunctionofstateanxietyinhealthy individuals.* Int J Psychophysiol, 2007. **63**(1): p. 48-54.
338. Deshpande, A. and Y. Birnbaum, *ST-segment elevation: Distinguishing ST elevation myocardial infarction from ST elevation secondary to nonischemic etiologies.* World J Cardiol, 2014. **6**(10): p. 1067-79.
339. Surawicz, B. and S.R. Parikh, *Prevalence of male and fe male patterns of early ventricularrepolarizationinthenormal ECGofmalesandfemalesfromchildhood tooldage.* J Am Coll Cardiol, 2002. **40**(10): p. 1870-6.
340. Hiss, R.G., L.E. Lamb, and M.F. Allen, *Electrocardiographic findings in 67,375 asymptomaticsubjects.X.Normalvalues.* Am J Cardiol, 1960. **6**: p. 200-31.
341. Thygesen, K., et al., *Third universal definition of myocardial infarction.* Circulation, 2012. **126**(16): p. 2020-35.
342. Thygesen, K., et al., *Universal definition of myocardial infarction.* Eur Heart J, 2007. **28**(20): p. 2525-38.
343. Man, S., et al., *Position of ST-deviation measure ments relative to the J-point: Impactforischemiadetection.* J Electrocardiol, 2017. **50**(1): p. 82-89.
344. van Noord, C., M. Eijgelsheim, and B.H. Stricker, *Drug-andnon-drug-associated QTintervalprolongation.* Br J Clin Pharmacol, 2010. **70**(1): p. 16-23.
345. Noseworthy, P.A., et al., *QT interval and long-term mortality risk in the FraminghamHeartStudy.* Ann Noninvasive Electrocardiol, 2012. **17**(4): p. 340-8.
346. Schouten, E.G., et al., *QTintervalprolongationpredictscardiovascularmortality inanapparentlyhealthypopulation.* Circulation, 1991. **84**(4): p. 1516-23.
347. Nielsen, J.B., et al., *Risk prediction of cardiovascular death based on the QTc interval: evaluating age and gender differences in a large primary care population.* Eur Heart J, 2014. **35**(20): p. 1335-44.
348. Bonnemeier, H., et al., *CircadianprofileofQTintervalandQTintervalvariability in172healthyvolunteers.* Pacing Clin Electrophysiol, 2003. **26**(1 Pt 2): p. 377-82.
349. Malik, M., *Problems of heart rate correction in assessment of drug-induced QT intervalprolongation.* J Cardiovasc Electrophysiol, 2001. **12**(4): p. 411-20.
350. Alboni, P., A. Holz, and M. Brignole, *Vagally mediated atrioventricular block: pathophysiologyanddiagnosis.* Heart, 2013. **99**(13): p. 904-8.
351. Carruthers, S.G., et al., *Relationships between heart ra te and PR interval during physiological and pharmacological interventions.* Br J Clin Pharmacol, 1987. **23**(3): p. 259-65.
352. Kazmi, S.Z., et al., *InverseCorrelationbetweenHear t Rate Variability and Heart RateDemonstratedbyLinearandNonlinearAnalysis.* PLoS One, 2016. **11**(6): p. e0157557.
353. Muraro, A., et al., *EAACI food allergy and anaphylaxis guidelines: managing patientswithfoodallergyinthecommunity.* Allergy, 2014. **69**(8): p. 1046-57.
354. Bock, S.A., *Prospective appraisal of complaints of adverse reactions to foods in childrenduringthefirst3yearsoflife.* Pediatrics, 1987. **79**(5): p. 683-8.

355. Nwaru, B.I., et al., *Prevalence of common food allergies in Europe: a systematic reviewandmeta-analysis.* Allergy, 2014. **69**(8): p. 992-1007.
356. Venter, C., et al., *Theprevalence,naturalhistoryandtimetrendsofpeanutallergy overthefirst10yearsoflifeintwocoho rtsborninthesamegeographicallocation 12yearsapart.* Pediatr Allergy Immunol, 2016. **27**(8): p. 804-811.
357. Asero, R., et al., *Double-blind, placebo-controlled food challenge in adults in everyday clinical practice: a reappraisal of their limitations and real indications.* Curr Opin Allergy Clin Immunol, 2009. **9**(4): p. 379-85.
358. Vlieg-Boerstra, B.J., et al., *Placebo reactions in doub le-blind, placebo-controlled foodchallengesinchildren.* Allergy, 2007. **62**(8): p. 905-12.
359. Sampson, H.A., *Immunologically mediated food al lergy: the importance of food challengeprocedures.* Ann Allergy, 1988. **60**(3): p. 262-9.
360. Sporik, R., D.J. Hill, and C.S. Hosking, *Specificity of allergen skin testing in predictingpositiveopenfoodchallenges tomilk,eggandpeanutinchildren.* Clin Exp Allergy, 2000. **30**(11): p. 1540-6.
361. Diwakar, L., et al., *Prescriptionratesofadrenaline auto-injectorsforchildrenin UKgeneralpractice:aretrospectivecohortstudy.* Br J Gen Pract, 2017. **67**(657): p. e300-e305.
362. Gilfillan, A.M. and J. Rivera, *The tyrosine kinase netw ork regulating mast cell activation.* Immunol Rev, 2009. **228**(1): p. 149-69.
363. Li, X., et al., *NecroX-5 suppresses IgE/Ag-stimulated anaphylaxis and mast cell activationbyregulatingtheSHP-1-Syksignalingmodule.* Allergy, 2016. **71**(2): p. 198-209.
364. British Thoracic, S. and N. Scottish Intercollegiate Guidelines, *Britishguideline onthemanagementofasthma.* Thorax, 2014. **69 Suppl 1**: p. 1-192.
365. Rolinck-Werninghaus, C., et al., *Outcome of oral food challenges in children in relation to symptom-eliciting allerg en dose and allergen-specific IgE.* Allergy, 2012. **67**(7): p. 951-7.
366. Werther, R.L., et al., *Variability in skin prick test results performed by multiple operatorsdependsonthedeviceused.* World Allergy Organ J, 2012. **5**(12): p. 200-4.
367. Radoslaw Spiewak, M.D., *Inter-individual and intra-individual variability of skin reactivitytohistamineatpricktesting* Dermatology Online Journal, 1995. **1**.
368. Fisher, M.M. and B.A. Baldo, *The incidence and clinical features of anaphylactic reactionsduringanesthesiainAustralia.* Ann Fr Anesth Reanim, 1993. **12**(2): p. 97-104.
369. Burton, C. and A. Worth, *UK Resuscitation Council guidelines on emergency treatment of anaphylactic reactions: a primary care perspective.* Prim Care Respir J, 2008. **17**(2): p. 60-1.
370. Durham, S.R., et al., *Long-termclinicalefficacyof grass-pollenimmunotherapy.* N Engl J Med, 1999. **341**(7): p. 468-75.
371. Hudgins, P.M. and G.B. Weiss, *Differential effects of calcium removal upon vascular smooth muscle contraction induced by norepinephrine, histamine and potassium.* J Pharmacol Exp Ther, 1968. **159**(1): p. 91-7.
372. Ebeigbe, A.B., M. Cabanie, and T. Godfraind, *Effects of cicletanine on histamine-induced contractions of isolated rabbit mesenteric arteries.* Fundam Clin Pharmacol, 1989. **3**(3): p. 223-35.
373. Leurs, R., M.J. Smit, and H. Timmerman, *Molecular pharmacological aspects of histaminereceptors.* Pharmacol Ther, 1995. **66**(3): p. 413-63.
374. Furchgott, R.F. and J.V. Zawadzki, *The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine.* Nature, 1980. **288**(5789): p. 373-6.

375. Moncada, S., R.M. Palmer, and E.A. Higgs, *Biosynthesis of nitric oxide from L-arginine. A pathway for the regulation of cell function and communication.* Biochem Pharmacol, 1989. **38**(11): p. 1709-15.
376. Kounis, N.G., et al., *Kounissyndrome:Areviewarticleonepidemiology,diagnostic findings, management and complications of allergic acute coronary syndrome: Mastocytosisandpost-mortemdiagnosis.* Int J Cardiol, 2017. **242**: p. 38.

Appendix 1: Invitation letter to participants.

TRACE
PEANUT STUDY

Dear ….

I am writing to you as one of our patients to tell you about a clinical study that we are carrying out at Addenbrooke's / the Royal Brompton hospital that you may be interested in taking part in.
We are investigating thresholds of reaction in peanut allergy and how these thresholds are affected by factors such as exercise and sleep deprivation. The purpose of the research is to improve peanut advisory labelling on foods (i.e. 'this may contain traces…').

By taking part in this ground-breaking study you will learn more about your allergy and be compensated up to £800 for your time.

For more information and to register your interest please visit www.tracestudy.com

Yours truly

Dr Andrew Clark
TRACE Study
Allergy Clinic
Addenbrooke's Hospital
Cambridge
CB2 0QQ

Appendix 2: Participant information sheet.

Addenbrooke's Hospital NHS
Cambridge University Hospitals NHS Foundation Trust

Royal Brompton & Harefield NHS
NHS Foundation Trust

TRACE
PEANUT STUDY

Information sheet for adults

For further information please telephone:
01223 762 603 and ask to speak to
Yvonne King
Version 6 (10.10.2014)

Study website
http://www.tracestudy.com

Study Co-ordinator

Yvonne King,
Department of Allergy,
Clinic2a,
Addenbrookes NHS Trust,
Cambridge, CB2 2QQ

Part 1- Basic Information

Invitation

We would like to invite you to take part in this study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take the time to read the following information carefully and ask us if there is anything that is not clear or if you would like more information.
Thank you for reading this.

What is the purpose of the study?

As somebody with peanut allergy, you will be aware of food products which carry a 'may contain peanuts' or similar warning on the packaging. In the UK, food manufacturers generally use these warnings because they can't be sure whether the products may, by accident, contain peanut. This may be because an ingredient they buy in from a supplier may be made in a factory where peanuts are used, or because the manufacturer uses peanuts in their own factory. In most cases, even though manufacturers try to eliminate peanut, (e.g. by segregating different products or by cleaning), they don't know whether the controls they have put in place are good enough, and use 'may contain' or 'traces' warnings to discourage peanut allergic people from eating them. These warnings can be unhelpful and are often ignored by people with peanut allergy. The UK government (Food Standards Agency) has commissioned this study to help food manufacturers improve their practice and reduce the need for these warnings. The underlying problem is that it is not known with certainty how much peanut can be tolerated in everyday foods. We want to find out exactly how much peanut will cause an allergic reaction, in people with peanut allergy. Also, we know that this amount, (the lowest safe level known as a 'threshold') can change from one day to the next in the same person and may be affected by a range of other things ('extrinsic factors') such as exercise or tiredness.

We are performing carefully controlled peanut food tests or 'challenges' on about 100 people with peanut allergy. Each participant undergoes four peanut challenges spread out over a 12-month period. This will tell us the average amount or 'threshold' of peanut consumption that causes reactions. Two 'extrinsic' factors also known to influence these thresholds will also be studied during challenges (exercise and tiredness).

The final outcome of the study will be used by the Food Standards Agency to improve the clarity of labelling for peanut allergic consumers in the UK.

Why have I been invited to take part?

You have been asked to join because you are an adult with peanut allergy

Do I have to take part?

It is your decision whether or not to take part in the study. If you do wish to be involved, please keep this information sheet. You will be free to withdraw from the study at any time without giving a reason. This will not affect the medical care you receive.

What do I have to do?

Please read this alongside the flowchart. We will ask you to attend the research ward in either London (Royal Brompton Hospital) or Cambridge (Addenbrooke's Hospital), whichever is most convenient to you, for an initial screening visit. We will ask you for your informed consent to undertake the study and then ask about your allergy history,

take some blood, perform an allergy skin test, do an electrocardiogram (a painless electrical recording of the heart) and ask you to do a 20 minute exercise test on a static bike and some breathing test. These include spirometry and bronchial hyperreactivity. Bronchial hyperreactivity testing is a common clinical test where we will ask you to inhale tiny doses of histamine (a

natural chemical that causes the airway to narrow) to assess how responsive your lungs are. Should you develop any symptoms, such as wheeziness, we will give you salbutamol (ventolin) to relieve them. We will ask you to fill in a short questionnaire about your quality of life related to peanut allergy.

At this visit we will decide whether you are eligible to continue into the rest of the study. If so, we will invite you to return for the first peanut challenge, which takes place over two days. Again, this will be undertaken at the nearest centre to you.

On each day you will be given eight small portions of chocolate to eat at half hour intervals. On one day all the chocolate will contain peanut (active day), on the other none will contain peanut (placebo day); both will look and taste identical. If the chocolate contains peanut, then the peanut dose increases with each portion, starting with a minimal amount. Before starting on each day we will insert a small plastic catheter into one of the veins on your arm (intravenous cannula) so that we can administer medicines if required, and take blood samples. We will repeat the breathing test (spirometry and collect some exhaled air for analysis). We will also perform a test which monitors blood flow in the skin, including in response to histamine (a natural substance which causes itch during allergic reactions). You will also be asked to provide urine and spit (saliva) samples. During the challenge, we will monitor your heart and breathing using non-invasive monitors: these will be attached using a finger probe and/or sticky pads (similar to an ECG trace). We will also measure blood flow in the skin using a non-invasive monitor, and later on during the challenge, and ask you to suck a sweet (like a lollypop) to help us monitor any reaction in your mouth. The peanut challenge is stopped once visible signs of a reaction begin to develop, and we will give you any treatment that is necessary. We may ask you to do some simple tests of concentration and memory during the peanut challenge, and we may ask you to return the day after your peanut challenge for a further assessment and blood test. The order of days is randomised and hidden from you and the doctors and nurses until after the challenge. The two days will be at least 7 days apart. This initial challenge has no extrinsic factors applied and we call it a ‘baseline challenge’. Over the next 12 months you will be invited back to have three more challenges (each will occur on a single day), at 3-4 month intervals. One is similar to the first (baseline), the second is an exercise challenge and the third is a sleep challenge. The order in which they are performed will be random.

For the exercise challenge we will ask you to come to the ward and undergo a baseline challenge with the addition of some moderate exercise on a treadmill, in-between doses.

For the sleep challenge we will ask you to come to the research ward on the evening before the challenge day and spend the night in the research ward, which is comfortably equipped to accommodate you. We will ask that you sleep in bed for only two hours during the night, so that we can understand the effect of sleepiness on peanut allergy. The nursing staff will assist you in staying awake, and DVDs and computer games will be available for your use. Refreshments will be available. The following day you will undergo a peanut challenge. For the exercise and sleep challenges we will ask you to wear a small heart monitor (actiheart), and a GPS device (like a wristwatch).

How much time is involved?

We ask you to attend for one screening visit (2hrs) and then undergo an initial peanut challenges (2 days), followed by 3 more challenges (1 day each) (one baseline, one exercise and one sleep). This is equivalent to five days, and one night spread out over a year). There will be gaps of at least a week between each challenge and appointments can be negotiated with some flexibility.

What are the possible benefits from taking part?

You will learn a lot about your own peanut allergy, and will be helping other people with peanut allergy. Studies have shown that having a food challenge improves quality of life in

people with food allergy, perhaps due to the reduced uncertainty and increased understanding that people acquire from having a challenge. Peanut allergy resolves over time in about one fifth of patients. You may not have had a reaction or had an allergy test for some years and therefore may no longer know whether you are still allergic. Also, it may not be clear to you how much peanut is required to make you react. It is also possible that the nature of your reactions has changed; you may have become more, or less sensitive. By participating, you will benefit first of all from a thorough allergy assessment, taking all your allergies into account (e.g. asthma, eczema and hayfever). You will then learn whether your peanut allergy is still present, and about your individual reaction threshold (how much peanut is required to initiate a reaction), both at a baseline and also when extrinsic factors are applied (these are likely to be different to the baseline threshold). Therefore you will find out how much peanut you can eat before a reaction begins, what symptoms you are likely to encounter, and what effect exercise and tiredness have upon your reactions. We will give you personalised feedback on these results, and explain what they mean for you in practice. After each completed challenge we will contact you with details of your reaction threshold. We can also show you where your threshold is in relationship to those of the other participants, and what this means about your own peanut allergy.

Taking part will also benefit other people with peanut allergy. The study is intended to identify a 'safe' level of peanut contamination in foods for the population. We will identify the level of contamination at which only a small proportion of the allergic population begin to react (e.g. 5-10%), and use this to define a cut-off for food labelling. This is particularly intended to help improve the accuracy of 'may contain' labelling, and reduce the number of foods labelled unnecessarily as unsuitable for people with nut allergy.

What are the possible disadvantages of taking part?

The blood, skin tests and intravenous cannula will cause some discomfort. The actiheart monitor attaches to the chest wall with a postage stamp sized sticky pad, this can cause some discomfort on removal and it is sometimes necessary to shave a small area before attaching it. The screening exercise challenge will involve running for approximately 20 minutes, and the two exercise challenge days will involve running for 10 minutes with eight repetitions each. The bronchial reactivity test may make you feel wheezy and tight chested, but we will give you some medicine immediately to make you feel better.

We anticipate that the peanut challenges will cause symptoms of an allergic reaction. There are several mechanisms built into this project to ensure your safety, which is our primary concern. The challenge will always begin with a dose, which is so low that no

one is expected to react to it (3 micrograms of peanut protein). The dose will then be increased slowly, always allowing sufficient time for the previous dose to be absorbed before proceeding to the next.

Our intention is for any reaction to be as mild as possible whilst still being clearly identifiable: mouth itching, mild stomach-ache and hay fever symptoms occur commonly. Great care is taken in determining whether to continue a challenge in the light of developing mild symptoms and a clear protocol has been developed to ensure your safety. The researchers will strictly adhere to this protocol. Vomiting, a nettle sting – type rash, or swelling may also occur. These symptoms are transient and resolve rapidly after treatment. We expect wheezing or throat-tightening to be uncommon but if they occur you will be treated immediately to halt the reaction. The research teams are all specialists in allergy who are experienced in performing peanut challenges in other studies with peanut allergic patients, and will be present throughout the challenges. Medicines used for treating allergic reactions, including adrenaline, will be immediately available. As the trial progresses, the

safety of challenges will be regularly reviewed by an independent panel of experts (Data Monitoring Committee).

Part 2-Further information

What will happen if I don't want to carry on with the study?

You are free to withdraw at any time without having to explain why. We will ask for your permission to use any data and samples collected up until that time.

Will I receive any money or compensation for undertaking the research?

We will pay you £160 for each challenge day you attend, up to a maximum of 5 challenge visits. This is intended to compensate for the inconvenience of attending and undergoing challenges, and to cover loss of income and travel expenses. If you attend for the screening visit and are found not to be eligible for the rest of the study, then we will reimburse any reasonable travel expenses that you have incurred (eg second class train fare, bus fare).

What will happen to the blood and saliva samples?

These will be analysed to help us understand allergic reactions to peanut in more detail, and to look for reasons for variability in threshold of reactivity or severity of reaction. DNA and RNA will be extracted from the samples to look for genetic variations which might be important in peanut allergy. Some of the samples may be sent to laboratories overseas, including outside the European Union, for analysis. Some of the samples will be stored and may be used in future, ethically approved research studies. All samples will be anonymised so that any identifiable information such as your name or date of birth will not be available to the laboratories undertaking the analyses.

What if there is a problem?

If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions (Cambridge 01223 762603). You can also contact the Patient Advice and Liaison Services at your nearest site (Addenbrooke's Hospital: 01223 216 756 or Royal Brompton Hospital: 020 7352 8121)

In the event that something does go wrong and you are harmed during the research and this is due to someone's negligence then you may have grounds for a legal action for

compensation, but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you (if appropriate). However, in the event that you are harmed during the research and no-one has been negligent, no specific insurance or indemnity will apply. Accordingly, in these circumstances there would be no legal action or applicable indemnity or insurance cover.

Would my taking part in the study remain confidential?
Any personal information, which you give us during the course of the study, will be kept strictly confidential. Only the researchers at the hospital you attend (Royal Brompton and Addenbrooke's Hospitals) will have access to personal data (e.g. contact details), this is necessary so that they can contact you to arrange appointments. Personal data will be kept on paper records in a secure location, and on a secure electronic database at each site. The local teams will enter your clinical study data onto a secure online database administered by researchers in Manchester University who will be involved in analysing the clinical data. No information which could directly identify you as an individual (e.g. name and address) will be transferred to, or appear on the database; you will be referred to only by a participant number and the month and year of your birth. It will not be possible to directly identify any individuals on the database. A link between your participant number and your personal details will be

kept in a secure location by the local team. This is called 'linked-anonymisation'. This is done to protect your identity, but also to allow (only) the researchers to refer back to your original paper records if it becomes necessary during the study. The study researchers in Manchester, Cambridge and London will have password access to the secure linked-anonymised database for the duration of the study.

Notification of your family doctor
Your family doctor will be informed of your participation

What will happen to the results of the research study?
We will publish the results in peer-reviewed scientific journals and at international scientific meetings. A final report will be prepared for the Food Standards Agency and it is anticipated that this will contribute to FSA policy regarding food labelling. Individuals will not be identifiable in the publications. Your own personal results will be available and we will discuss these with you to provide feedback on what they mean.

Who can I ask for advice?
You can find out more and register your interest on our website (http://www.tracestudy.com/). The independent organisation 'Involve' can tell you more about your rights as a research subject (www.involve.co.uk). You can also contact the Patient Advice and Liaison Services at your nearest site (Addenbrooke's Hospital: 01223 216 756 or Royal Brompton Hospital: 020 7352 8121)

Who is organising and funding this research?
The work was commissioned by the Food Standards Agency who provides all the funding. The study is being performed by the allergy research teams at Addenbrooke's Hospital in Cambridge led by Dr Andrew Clark, by Dr Robert Boyle at St Mary's Hospital in Paddington and by Dr Isabel Skypala at the Royal Brompton Hospital in Chelsea. The study has been approved by the Hertfordshire committee of the National Research Ethics Service, and the Research and Development Committees at each site have approved the study and the facilities in which it is to be performed. The study protocol and the patient information sheets have been designed in cooperation with

members of the Anaphylaxis Campaign, and two members of the Anaphylaxis Campaign sit on the Trial Steering Committee which provides oversight on how the trial is conducted.

Royal Brompton & Harefield NHS
NHS Foundation Trust
TRACE
PEANUT STUDY

Appendix 3: Consent form.

Consent to participate in a clinical trial

Ethics Reference Number: 12/EE/0289 NRES Committee East of England – Hertfordshire

Title of Project: The Study of Extrinsic Factors in Food Allergy (TRACE)
Dr Andrew Clark / Dr Pamela Ewan / Dr Robert Boyle / Professor Stephen Durham

Initial boxes

Hereby give my permission fully and freely to participate in this study

Please initial box

- I confirm that I have read and understand the information sheet dated **Version 6 (10.10.2014)** for the above study and have had the opportunity to ask questions. ☐
- I understand that participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐
- I understand that sections of any of my medical notes may be looked at by responsible individuals from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records. ☐
- I am willing that my general practitioner is notified of their participation in this research. ☐
- I agree to my taking part in the above study. ☐
- I agree for any samples taken in this study, to be used in future ethically approved research ☐
- I agree for any samples taken in this study, to be sent to overseas laboratories for analysis if needed, including laboratories outside the European Union ☐
- I agree for a DNA sample to be taken, and understand that any genetic analysis will be anonymous and I will not receive any results from this. ☐

Name of participant *(Please print)*	Date	Signature
Name of Research Team member *(Please print)*	Date	Signature

3 copies required: top copy for researcher; one copy for patient; one copy to be kept with research subject's notes.

Appendix 4: Asthma control questionnaire.

Asthma UK is the only charity dedicated to the health and well-being of the 5.2 million people in the UK with asthma. By taking control of their asthma, most people's day-to-day lives should be free from disruption such as troubled sleep or not being able to exercise.

Asthma Control Test™

asthma UK

Why take the Asthma Control Test™?

The Asthma Control Test™ will provide you with a snapshot of how well your asthma has been controlled over the last four weeks, giving you a simple score out of 25. Asthma symptoms can vary from month to month, so it is worth keeping the test handy to see if your score changes. You can also share your results with your doctor or asthma nurse to help explain just how your asthma affects you.

Are you in control of your asthma? Or is your asthma in control of you? Here's how to find out

Step 1: Read each question below carefully, circle your score and write it in the box.
Step 2: Add up each of your five scores to get your total Asthma Control Test ™ score.
Step 3: Use the score guide to learn how well you are controlling your asthma.

Q1 During the **past 4 weeks**, how often did your asthma prevent you from getting as much done at work, school or home?

All of the time	Most of the time	Some of the time	A little of the time	None of the time	Score:
1	2	3	4	5	

Q2 During the **past 4 weeks**, how often have you had shortness of breath?

More than once a day	Once a day	3-6 times a week	1-2 times a week	Not at all	Score:
1	2	3	4	5	

Q3 During the **past 4 weeks**, how often did your asthma symptoms (wheezing, coughing, chest tightness, shortness of breath) wake you up at night or earlier than usual in the morning?

4 or more times a week	2-3 nights a week	Once a week	Once or twice	Not at all	Score:
1	2	3	4	5	

Q4 During the **past 4 weeks**, how often have you used your reliever inhaler (usually blue)?

3 or more times a day	1-2 times a day	2-3 times a week	Once a week or less	Not at all	Score:
1	2	3	4	5	

Q5 How would you rate your **asthma control** during the **past 4 weeks?**

Not controlled	Poorly controlled	Somewhat controlled	Well controlled	Completely controlled	Score:
1	2	3	4	5	

Total Score

What does your score mean?

Score: 25 – WELL DONE
- Your asthma appears to have been UNDER CONTROL over the last 4 weeks.
- However, if you are experiencing any problems with your asthma, you should see your doctor or nurse.

Score: 20 to 24 – ON TARGET
- Your asthma appears to have been REASONABLY WELL CONTROLLED during the past 4 weeks.
- However, if you are experiencing symptoms your doctor or nurse may be able to help you.

Score: less than 20 – OFF TARGET
- Your asthma may NOT HAVE BEEN CONTROLLED during the past 4 weeks.
- Your doctor or nurse can recommend an asthma action plan to help improve your asthma control.

What can you do now?

Like many other people in the UK, it is possible that your asthma could have less impact on your everyday life. You can get a free pack full of information about how to take control of your asthma, including an action plan to fill in with your doctor or asthma nurse, from Asthma UK.

US English version modified for use in UK. The production of this leaflet has been supported by GlaxoSmithKline

Registered charity number 802364

Appendix 5: Screening case record form (CRF).

CENTRE: ☐☐

Participant initials: ☐☐☐

subject code (patient's unique identifier): ☐☐ ☐☐☐☐

Date of examination (dd/mm/yyyy):

Date of written consent (dd/mm/yyyy):
The written consent should be kept with the hard copy of the CRF

Patient's demographic data:

Birth date (dd/mm/yyyy): / /

Age at visit to clinic (years): [18-45]

Sex: Male ☐ Female ☐

1. **Age at onset of the first adverse reaction to peanut** : . (0-45)

2. **Number of adverse reactions:** [0-20]

Regarding the most severe reaction induced by peanut:

3. **Type of food: …….**

4. **Minimum intake to trigger the first complaint:**

A bite / a swallow
¼ helping
½ helping
One normal helping (according to patient's age)
Unknown

5. **Interval between the food intake and the onset of symptoms:**

< 5 minutes 5- 15 minutes >15- 30 minutes
>30 – 60 minutes > 1- 2 hours > 2 hours
Unknown

6. **Symptoms associated with the most severe reaction induced by peanut**

A. Complaints of the oral cavity

Oral allergy syndrome only
Oral itching

B. Skin complaints

Urticaria

Angioedema
Erythema / flushing
Itching

C. Digestive complaints
Nausea
Vomiting
Stomach pain
Cramps
Diarrhoea
Dysphagia

D. Airway complaints
Asthma (dyspnea, wheezing, cough, chest tightness)
Rhinitis
Dysphonia
Thightness of the throat

E. Eye complaints
Conjunctivitis

F. Cardiovascular complaints
Cardiac arrhythmia
Myocardial ischaemia (angina, infarction)
Hypotension

G. Neurologic complaints
Disorientation, confusion
Dizziness
Seizures
Incontinence
Loss of conciousness

H. Anaphylaxis ***(tick all the applicable)***
with severe bronchospasm
with severe laryngeal oedema
with hypotension (anaphylactic shock)
EIA (exercise induced anaphylaxis)

7. Medication received to control the reaction:

Yes: antihistamines corticosteroids adrenaline intravenous fluids vasopressors oxygen mechanical ventilation

No/unknown

7. Emergency care assistance and/or hospitalization after the reaction:

Yes
No/unknown

8. Time elapsed since the last (any) reaction to peanut (until today)**:**

Up to 1 month > 1- 6 months >6-12 months
> 12-24 months >2-5 years >5 years
Unknown

9. Does the patient have any other food allergies (including any of the matrix components?)

Yes No

If yes denote which ones below and complete additional food adverse reactions form for each.

Foods involved in immediate (≤ 2 hours) adverse reactions.

	yes	**no**		**yes**	**no**
Cow's milk			Sesame seed		
Hen's egg			Pecan nut		
Brazil nut					
Almond					
Hazelnut					
Walnut					
Cashew					
Pistachio					
Pine nut					

OTHER ASSOCIATED CONDITIONS

ASTHMA

10. Do you have asthma? Yes No Unknown

Asthma Control Test score….. [0-25]

11. Triggers

Dust
Pollen
Animal dander
Fungal spores
NSAIDS
Infection
Exercise
Cold air

11b. If pollen related asthma present denote period of symptoms:

January		May		September	
February		June		October	
March		July		November	
April		August		December	

12. Current treatment:

Short acting B2 agonist
Inhaled corticosteroid

Long acting B2 agonist
Combination device
Systemic corticosteroids
Additional agents

13. Number of courses of oral corticosteroids with the last 2 years [0-10]

14. Number of previous asthma related hospital admissions [0-10]

15. Number of previous ITU/HDU admissions [-0-5]

RHINITIS/RHINOCONJUNCTIVITIS

16. Do you suffer from rhinitis/rhinoconjunctivitis?

Yes No
If yes Total Nasal Symptom Score [0-12]

17. **Triggers**

Dust
Pollen
Animal dander
Fungal spores

18. Seasonal Yes No Perennial Yes No

19. If seasonal denote period of symptoms

January		May		September	
February		June		October	
March		July		November	
April		August		December	

20. **Treatment required**

Antihistamines
Nasal spray/drops
Eye drops
Oral steroids
Leukotriene antagonists

ECZEMA AND SKIN CONDITIONS

21. Associated atopic dermatitis: Yes No Unknown
POEM score [0-28]

22. Associated urticaria/angioedema Yes No

PAST MEDICAL HISTORY:

23. Do you suffer from any major illnesses or conditions including:

	Yes	No
Gastric or duodenal ulcer		
Eosinophilic oesophagitis	Yes	No
Coronary artery disease	Yes	No
A past medical history of clinically significant ECG abnormalities	Yes	No

Other significant illness which may prevent inclusion…….

Are you currently pregnant?_____ Yes No

CURRENT MEDICATION:

24. Any drug allergies Yes No

25. **Are you on any current medication including:**

Systemic corticosteroids	Yes		No	
Immunosuppressants		Yes		No
Beta blockers	Yes		No	
ACE inhibitors		Yes		No
Antacid medication	Yes		No	
Tricyclic antidepressants	Yes		No	
Sedatives	Yes		No	

Other ……[one line free text].

SOCIAL:

26. Alcohol consumption **….. units/week [0-40]**

27. Smoker **…..pack year history [0-60]**

28. Occupation…… **Night shift worker Yes** **No**

FAMILY HISTORY

29. Family background of atopy

Mother: Yes
Father: Yes
Sibling(s): Yes

Investigations
Skin prick tests
[

	Wheal in mm	Flare in mm
Negative control	[0-5]	[0-50]
Histamine	[0-30]	[0-50]

Nut

	Wheal	Flare
Peanut	[0-30]	[0-50]
Brazil	[0-30]	[0-50]
Almond	[0-30]	[0-50]
Hazelnut	[0-30]	[0-50]
Walnut	[0-30]	[0-50]
Cashew	[0-30]	[0-50]
Pistachio	[0-30]	[0-50]
Macadamia	[0-30]	[0-50]
Pecan	[0-30]	[0-50]

Other foods

	Wheal	Flare
Milk	[0-30]	[0-50]
Wheat	[0-30]	[0-50]
Egg (white)	[0-30]	[0-50]
Soya	[0-30]	[0-50]
Sesame	[0-30]	[0-50]
Lupine flour	[0-30]	[0-50]
Cod	[0-30]	[0-50]
Shrimp	[0-30]	[0-50]
Peach	[0-30]	[0-50]

Aeroallergens

	Wheal	Flare
5 grasses	[0-30]	[0-50]
Dermatophagoides farinae	[0-30]	[0-50]
Dermatophagoides pteronyssinus	[0-30]	[0-50]
Alternaria alternata	[0-30]	[0-50]
Aspergillus	[0-30]	[0-50]
Cladosporium (Cladosporoides, herbarum)	[0-30]	[0-50]
Alder	[0-30]	[0-50]
Birch	[0-30]	[0-50]
Hazel	[0-30]	[0-50]
Plane	[0-30]	[0-50]
Cat	[0-30]	[0-50]
Dog	[0-30]	[0-50]

Investigations (continued)

ECG ok for challenge? Yes No

Spirometry

Pre exercise
FEV_1 litres/minute ☐☐☐ [2.0-5.5]

Post VO2 max exercise
FEV_1 litres/minute ☐☐☐ [2.0-5.5]

Fall in FEV1 >15% suggesting possible exercise induced asthma Yes No

Exercise test

VO2 max test ☐☐☐☐ [0-100] mL/kg/min

Maximum heart rate achieved ☐☐☐ 100-250 bpm

Target heart rate for exercise challenge [85% maximal heart rate] ☐☐☐ 100-250 bpm

Blood test results
FBC normal? Y N

Renal function normal? Y N

Test	Result	Range
Baseline tryptase		[2.0-25.0] ng/ml
IgE		[0-10000] KU/L
Peanut specific Ige		[0-5000] KUa/L
Arah1		[0-500] KUa/L
Arah2		[0-500] KUa/L
Arah3		[0-500] KUa/L
Arah8		[0-500] KUa/L
Arah9		[0-500] KUa/L

Appendix 6: Patient- Orientated Eczema Measure.

Patient-Oriented Eczema Measure (POEM)
(Adult version)

Patient details:	Date:
	Total POEM score: (maximum 28)

Please circle one response for each of the seven questions below about your eczema. Please leave blank any questions you feel unable to answer.

1. Over the last week, on how many days has your skin been itchy because of your eczema?

No days 1-2 days 3-4 days 5-6 days Every day

2. Over the last week, on how many nights has your sleep been disturbed because of your eczema?

No days 1-2 days 3-4 days 5-6 days Every day

3. Over the last week, on how many days has your skin been bleeding because of your eczema?

No days 1-2 days 3-4 days 5-6 days Every day

4. Over the last week, on how many days has your skin been weeping or oozing clear fluid because of your eczema?

No days 1-2 days 3-4 days 5-6 days Every day

5. Over the last week, on how many days has your skin been cracked because of your eczema?

No days 1-2 days 3-4 days 5-6 days Every day

6. Over the last week, on how many days has your skin been flaking off because of your eczema?

No days 1-2 days 3-4 days 5-6 days Every day

7. Over the last week, on how many days has your skin felt dry or rough because of your eczema?

No days 1-2 days 3-4 days 5-6 days Every day

Appendix 7: British Thoracic Society treatment levels.

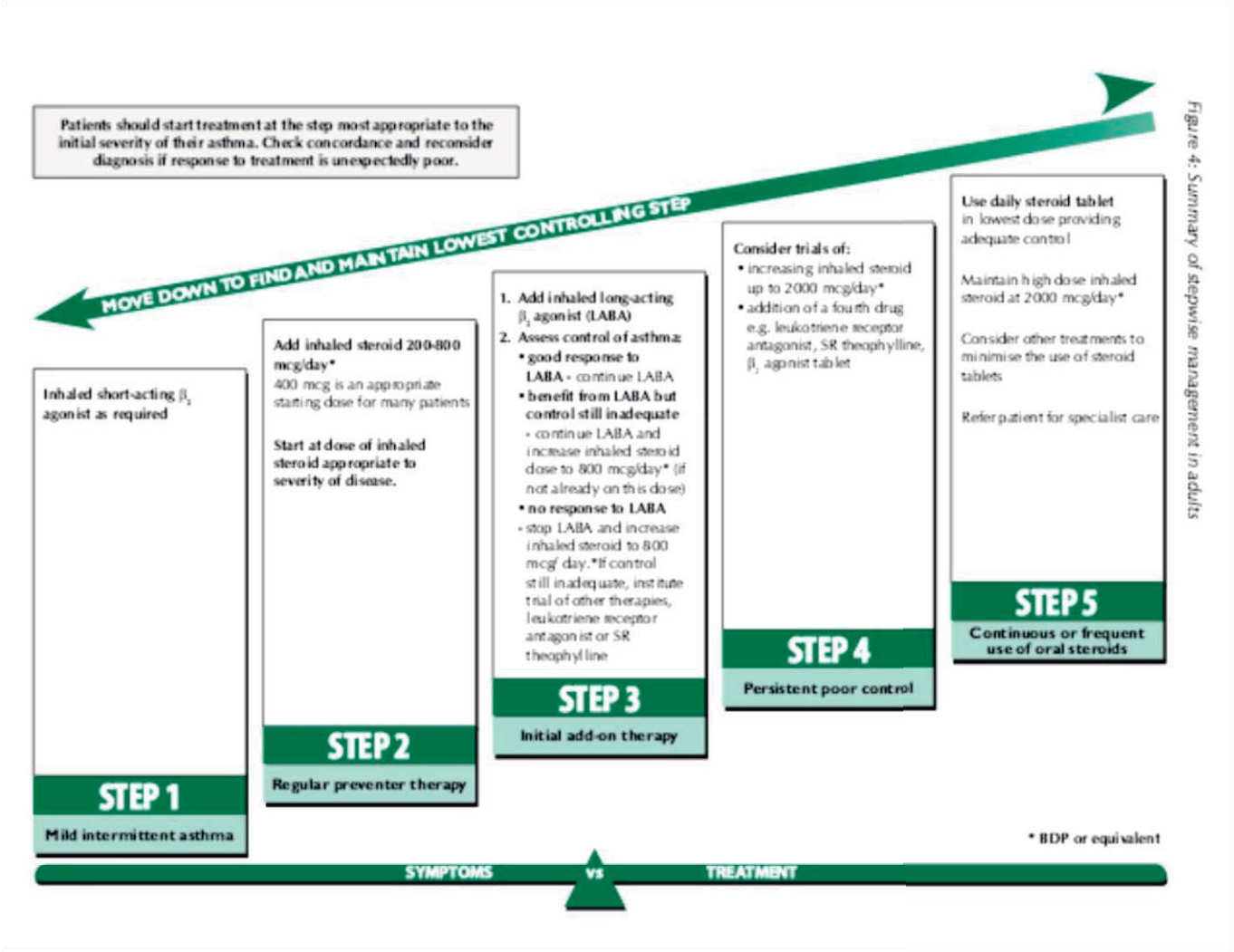


Figure 4: Summary of stepwise management in adults

Appendix 8: Bronchial hyper-responsiveness (BHR) standard operating procedure (SOP).

Before starting the provocation test a baseline spirometry was performed and if the participant´s FEV1 was below 80% of predicted for height, weight, age and sex the test was not performed.
Participant´s were restrained from using their asthma rescue medication short-acting Beta-Agonist (SABA) the day of the challenge.

Ampoule of histamine at a concentration of 32mg/ml was used.
Challenge by dosimeter technique started with saline and concentrations of histamine (mg/ml) were performed diluting the 32mg/ml ampoule by ½ and then each subsequent vial also diluted ½ until reached a 0,125mg/ml. Concentrations of histamine (mg/ml) used: 32, 16, 8, 4, 2, 1, 0,5, 0,25, 0,125 and 0.06.

The concentration of histamine causing a 20% fall in FEV1 from the baseline value was named PC20 and it´s calculated:

$$PC_{20} = C1 + (20 - R1) \times \frac{C2 - C1}{R2 - R1}$$

C1: concentration of histamine that induced a % fall in FEV1 closest to and less than 20%.
C2: concentration of histamine that induced a % fall in FEV1 bigger than or equal to 20%.
R1: % fall in FEV1 closest to and less than 20%.
R2: % fall in FEV1 bigger than or equal to 20%.

Appendix 9: Baseline challenge SOP.

TRACE
PEANUT STUDY

Baseline challenge Standard Operating Procedure

Authors: Dr Andrew Clark, Dr Laura Watson, Dr Robert Boyle, Professor Clare Mills, Dr Pamela Ewan, Dr Isabel Skypala, Dr Chris Palmer, Dr Simon Bond, Dr Paul Turner, Dr Laura Pasea, Dr Monica Ruiz-Garcia, Mrs Emily Wilson, Dr Shelley Dua, Professor Stephen Durham

Chief Investigator: Dr Andrew Clark

Lead Centre: Cambridge Biomedical Campus

Collaborating centres: Imperial College London and the University of Manchester

Clinical Trials.Gov number: NCT01429896

Version number: V3

Release date: 05.12.2014

Baseline Oral Food Challenge Standard Operating Procedure Manual

Background

Guidelines recommend using oral food challenges for diagnosing Food Allergy (FA). While a single-blind or an open-food challenge may be considered diagnostic under certain circumstances the double blind placebo controlled food challenge (DBPCFC) is the gold standard approach [1].
The DBPCFC markedly reduces potential bias of patients and supervising health care professionals that may interfere with the appropriate interpretation of oral food challenges, and corresponds most closely to the natural ingestion of food.
The DBPCFC is time consuming, expensive, and, like any form of oral food challenge, exposes the patient to potential severe allergic reactions. When negative, they may be considered diagnostic in ruling out FA, and when positive (i.e., when ''immediate'' objective allergic symptoms are elicited), they may be considered diagnostic in patients who have a supportive medical history and laboratory data. Investigators agree that verification of clinical reactivity requires well-designed oral food challenge testing [2].
Because of the inherent risk of serious reactions, an oral food challenge must be conducted at a medical facility with medical supervision, appropriate medicines and resuscitation facilities on hand. An adult intensive care unit should be located within close proxmity (ideally on-site).
The challenge test is carried out while the patient is on minimal or no symptomatic medication. The test should be designed and performed under medical supervision to document the dose that provokes the reaction and to administer symptomatic treatment, which may require management of anaphylaxis. The medical personnel should have experience in carrying out such challenges. Ideally the oral food challenge begins with a low dose (intended to be lower than a dose that can induce a reaction [3]. While monitoring for any allergic symptoms, the dose is gradually increased, until a cumulative dose at least equivalent to a standard portion for age is consumed. Because of the risk of a severe reaction, intentional challenge should be avoided in patients who have recently experienced a life-threatening reaction to a particular food, particularly if it occurred more than once.
Patients who will react to the screening DBPCFC with a severe reaction (WAO grade 4= respiratory failure or hypotension with or without loss of consciousness) will not be randomized.
Studies differ in starting doses, typically in the range of the quantity of the food for the most sensitive subjects to produce an objective, but mild reaction. Studies also differ in challenge procedures, the form of the food used, the matrix in which the allergen was presented and the weight accorded to subjective and objective manifestations.
Though there is currently no internationally accepted, standardized protocol for performing and interpreting DBPCFCs, there is a wealth of publications on Oral Food Challenges. Efforts to standardize OFC began in 2004 with the position paper from the European Academy of Allergology and Clinical Immunology [4], and more recently the Adverse Reactions to Food Committee of the American Academy of Allergy, Asthma & Immunology published a review of Oral Food Challenge tests [5]. The present protocol has been developed in alignment with the current guidelines and published literature and complies with the recently published PRACTALL consensus document from the American Academy of Allergy, Asthma & Immunology-European Academy of Allergy and Clinical Immunology [6].

This protocol and the attached SOPs have been developed by the Trial Management Group (TMG: Dr Andrew Clark, Dr Laura Watson, Dr Robert Boyle, Professor Clare Mills, Dr Pamela Ewan, Dr Isabel Skypala, Dr Chris Palmer, Dr Simon Bond, Dr Paul Turner, Dr Laura Pasea, Dr Monica Ruiz-Garcia, Mrs Emily Wilson, Dr Shelley Dua, Professor Stephen Durham) and reviewed by the Trial Steering Group (TSG), external experts (Prof Kirsten Beyer and Prof Jonathan Hourihane) and Food Standards Agency (FSA, funder). The Trial Steering committee members have also contributed: Prof Graham Roberts, Dr Stephen Till, Dr Victoria Cornelius, Dr Phillipa Caudwell, Mrs Moira Austin and Mrs Hazel Gowland (representing the Anaphylaxis

Campaign), Dr Pina Rotiroti, and Prof Ian Kimber, Dr Sarah Hardy and Miss Nathalie Shapiro from the Food Standards Agency.

Site Requirements

The personnel involved in the food challenge procedure must be trained in management of acute allergic reactions and equipment for resuscitation must be present on the research unit and immediately available. The clinical fellows responsible for performing challenges will have undergone a recent suitable Advanced Life Support Course before commencing challenges.

Investigators should ensure they have the emergency contact/bleep details of the on call anaesthetist, intensive care unit and the local principle investigator before commencing each challenge.

Emergency medication/equipment should be placed in the room in which the challenge will take place. This should include:

Adrenaline vials x4 vials, 1mg in 1ml for injection, one vial drawn up for immediate use.

1ml syringe and needle for IM injections

Intravenous chlorphenamine and hydrocortisone

Salbutamol 2.5mg nebules

Adult nebulizer

Supplemental oxygen supply sufficient to provide 5-15 litres/min

Oral cetirizine tablet or syrup (or equivalent)

Intravenous cannulation equipment (the patient should be cannulated before the challenge commences)

Normal saline (0.9% sodium chloride solution) 1 litre for IV administration

Challenge will be performed in settings with access to intensive / high dependency care units.

The first 20 challenges for *each* clinical fellow will be carried out under the direct supervision of the local PI.

As a minimum standard, there will be a rolling stop point review of challenge safety by TMG/IDMC after recruitment of 5, 25, 50, 75 participants, OR after 6 month intervals whichever is sooner. A summary of these reviews will be communicated to the TSC.

Participants

Patients who fulfill the eligibility criteria for the study will have a screening DBPCFC. The exclusion criteria are the same as the ones for this study.

For each DBPCFC, there will be two test days, i.e. one day with peanut and one day without peanut in a random order. Study staff (except for the independent nurse preparing the challenge materials), investigator nor patient will know if the challenge will be with (peanut challenge) or without (placebo challenge) or which one will be tested first.

Placebo challenge and active challenge will be performed on 2 separate days and test day 2 should only be performed when it is confirmed the patient is well, any symptoms have be resolved and required medication wash out is adhered to.

Patient preparation

Patients must be in good health and otherwise at screening comply with the eligibility criteria of the study (see Inclusion and exclusion criteria). Sleep diaries should be collected (participants will have completed these over the 2 weeks before each challenge)

In addition, some considerations need to be taken into account:

Patients must be in good health, and any concomitant allergic diseases — asthma, allergic rhinitis, or atopic dermatitis — should be under optimal control. Patients with a history of pollen-induced asthma should not be challenged during their pollen season when they are symptomatic. If the patient presents

with a concurrent illness, e.g. common cold, the OFC should be postponed until the patient has recovered.
Ensure that consent to continue in the study is recorded
If the participant develops severe anaphylaxis during any challenge or field reaction then they will not undergo any further challenges, but their data will be retained.
Severe anaphylaxis to peanut as defined by hypoxia (SpO2 < 92%) or hypotension (>30% drop in systolic blood pressure), with or without neurological compromise, or a reaction, which in the opinion of the investigator, TMG or IDMC was clearly life-threatening.

The presence of any of the following will prevent the participant from undergoing challenge. If these factors have resolved or been overcome, then the investigator could at their discretion allow the next challenge to proceed:

Poorly controlled asthma manifest by FEV1 < 80% of predicted

Fasting for less than 2 hours before the first challenge dose

Caffeine or cow's milk ingestion in the past 12hrs

Regular treatment with: systemic immunosuppressant, beta blocker, ACE inhibitor, or other anti-hypertensive drugs, sedative or antidepressant drugs

Alcohol or drug misuse

A sleep or psychiatric disorder which in the opinion of the investigator could impair the participants ability to perform the study procedures

Night-shift working within the past month

Musculo-skeletal disease which in the opinion of the investigator could impair the participants ability to perform the exercise challenge, coronary artery disease, eosinophilic oesophagitis, gastric or duodenal ulcer

Any current symptoms of allergic disease specified in table 1 below

Significant illness with systemic features (e.g. fever >37.5 degrees Celcius) within two (2) weeks prior to challenge

A significant clinical reaction to peanut within the previous three months

Pregnancy

A pregnancy test should be performed at the screening visit and at subsequent visit if there is any possibility of pregnancy (record date of last period). The challenge will not proceed if the test is positive.
For 72hrs before the OFC, patients should avoid unaccustomed intense physical activity (sufficient to induce breathlessness) and alcohol. Medications that interfere with the interpretation of the OFC should be avoided for the times stated in table 1-1.
The patient will not eat for at least 2 hours before or during challenge . Starting the challenge at the patient's normal breakfast time will allow the challenge material plus vehicle to serve as a meal as the dosing progresses through the morning. Allow to drink water freely during the challenge.
Intravenous access must be in place before initiation of the first baseline challenge.
Before the OFC, it must be ensured patients have not been taking any medication that may impact the outcome or the interpretation of the outcome of the challenge. See Table 1-1 for medication wash-out requirements.

Table 1-1 Guidelines for discontinuation of medications that might interfere with interpretation of OFC

Medication	Last dose before OFC
Short acting oral antihistamines	48 h
Long acting oral antihistamines	7 d
Antihistamine nose spray	12h
Oral H2 receptor antagonist	12h
Oral/intramuscular/intramuscular/intravenous steroids*.	30 days
Short acting beta-agonist	6h
* Participants on inhaled corticosteroids (1-400 mcg twice daily) or intranasal corticosteroids should continue on their usual prescribed doses.	

Clinical assessment

Prior to each dose, patients will have a brief clinical examination including inspection of the skin and upper airway, auscultation of the chest and abdomen, and determination of the blood pressure (x2 measurements at baseline, x1 after each dose), heart rate, peak expiratory flow rate, respiratory rate, and O_2 saturation measurement. Patients will be questioned about pruritus (mouth mucosa and/or skin), laryngeal symptoms, abdominal symptoms, e.g. nausea, pain, chest tightness, dizziness, etc.

The physical examination will provide objective symptoms. Complaints arising from the patient without observable changes will be classified as subjective and if isolated will not account for a positive challenge. Physical findings will be documented to serve as a reference. Baseline peak flow will be collected in all patients.

Initial challenge dose and escalation scheme

The starting dose will be 3 µg of peanut protein.

The general challenge schedule will consist of increasing doses in semi-logarithmic increases 3µg, 30 µg, 300 µg, 3mg, 30mg, 100mg, 300mg and 1000 mg of peanut protein (or placebo).

Based on a recent publication, [7]], about 10% of peanut patients may react to exposure levels lower than 3mg (ED10) however, these more sensitive subjects do not seem to represent a sub group at a higher risk for more severe reactions. Therefore the increments are logarithmic from 3µg to 30mg, and semi-logarithmic thereafter. Schedules using semi-logarithmic increases are associated with good safety. Smaller increments (e.g. doubling) may further enhance safety but would also significantly increase the time necessary to complete the challenge procedure. Therefore, the proposed scheme is expected to represent a suitable challenge regimen, balancing safety and practicability.

Measure out the correct volume for each dose, one at a time, using weighing scales assuming 1ml=1g of dessert. The dose can be presented on a spoon with a plate underneath. The participant should be asked to eat the dessert without undue delay.

Interval

The aforementioned discrete doses of allergen will be delivered at least 30 minutes intervals with the exception of doses intervals 6-7 and 7-8 which will be 60 minutes. A time interval of 30 minutes between doses is in most cases suitable for investigation of IgE associated reactions [6]. It should be remembered that the volume of matrix administered with higher doses (≥ 300mg protein) may delay absorption of peanut and, at the discretion of the investigator, intervals of up to one hour may be appropriate. After each dose, any reactions swill be carefully reviewed, and options of stopping the challenge, repeating the last dose or waiting longer before giving the next dose should be considered. The symptoms elicited at each dose should be considered in the context of symptoms elicited by previous doses. Apply particular caution to up-

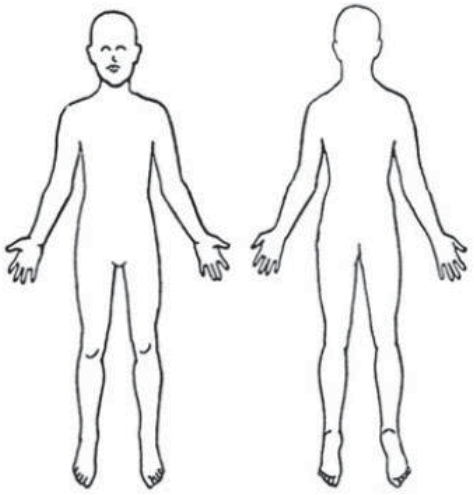
dosing after the development of a 'new' cough during challenge. If at least two concurrent yellow symptoms, or one lower respiratory (including cough) yellow symptom
– Delay next dose for 60m
– Next dose not to be given unless symptom-free for 30m

Maximum dose
The increment will continue until the top dose of 1000mg has been reached (dose 8), or until the patient reacts, whatever happens first.
Scoring and stopping
A unified approach to scoring and stopping the challenges will be essential to both the veracity of data and safety of participants, not least because the study is taking place in several sites and relies on interpretation by different individuals. We have derived our scoring and stopping rules from the recently published Practall guidelines, which has achieved broad consensus from US and European allergists [6]. Our adapted criteria were developed following pilot challenges.
Challenges are typically considered positive, and dosing stopped, when objective symptoms occur. In some situations, however, mild objective symptoms may be considered insufficient to discontinue dosing or to consider a challenge positive (e.g., one or two transient urticarial lesions, perioral hives from contact with the food or one episode of vomiting in a patient with anxiety and a distaste for the challenge substance) [3].
Subjective symptoms may in some circumstances indicate a positive response to a challenge and present a good reason to stop dosing, for example, by having repetitive symptoms or multiple subjective symptoms in several organ systems. However, stopping a challenge for subjective symptoms increases the risk of a false positive test compared to only accepting objective symptoms as an indication of a positive test.
When mild objective or subjective symptoms occur, decisions include stopping the challenge, waiting longer before administering the next dose, or repeating the previous or same dose [3]. Judgments about proceeding must balance safety against the certainty of the challenge outcome.
A scoring system for acute allergic responses based upon the PRACTALL consensus is shown [6] in Table 1-2 to Table 1-5 and will be used for this protocol. The scoring system indicates symptoms and signs that may warrant caution (repeating a dose, delaying a dose, consideration for stopping) or are clear enough to warrant stopping a challenge and declaring the result positive. Investigators will report in detail how symptoms were assessed with regard to stopping dosing and determination of positive, negative or inconclusive challenge results.
The algorithm describes the decision schemes:
Green symptoms:
Not usually an indication to alter dosing and not generally sufficient to consider a challenge positive unless symptoms persist for at least 120 minutes.
Yellow symptoms (scores increasing to yellow):
The presence of three concurrent yellow symptoms/signs is likely to be indicative of a reaction and if clinically indicated, dosing should stop.
After the appearance of single yellow symptoms, proceed with caution, dosing could proceed, be delayed or be repeated rather than escalated.
If clinically indicated, dosing is stopped.
3 or more scoring areas (within the same organ or across different organs) in yellow represent a positive challenge.
Red symptoms:
Positive challenge, stop dosing and administer treatment (See Section 16.9).
NOTE: investigators can override the stopping criteria at any time for safety reasons.

Table 1-2 Scoring system on skin symptoms

SKIN	
Erythematous Rash - % area involved	See body surface area diagram in Figure 2.1
Pruritus	Absent
	Green - Occasional scratching
	Green - Scratching continuously for > 2 minutes at a time
	Yellow - Hard continuous scratching ➔ excoriations
Urticaria/Angioedema	Absent
	Yellow - < 3 hives, or mild lip oedema
	Red - < 10 hives but ≥3, or significant lip or face oedema
	Red – Generalized involvement
Rash	Absent
	Green – Few areas of faint erythema
	Yellow – Areas of erythema,
	Red – Generalized marked erythema (>50%)

Figure 2-1 Body surface area diagram for scoring Erythematous Rash



	Adult
Head	4.5%
Neck	1%
Anterior trunk	18%
Posterior trunk	18%
Leg	18%
Arm	9%

Table 1-3 Scoring system in respiratory symptoms

UPPER RESPIRATORY	
Sneezing/Itching	Absent
	Green - Itching in ear canal
	Green – Rare bursts, occasional sniffing
	Green – Bursts < 10, intermittent rubbing of nose, and/or eyes or frequent sniffing Yellow– Continuous rubbing of nose and/or eyes
	Yellow - periocular swelling and/or long bursts of sneezing,
	Yellow - persistent rhinorrhoea
LOWER RESPIRATORY	
Wheezing	Absent
	Green – chest tightness without any fall in PEFR
	Green - chest tightness with a <10% fall in PEFR
	Yellow - chest tightness with a 10-20% fall in PEFR
	Red – Expiratory or inspiratory wheeze
	Red – Use of accessory muscles and/or audible wheezing (or silent lung)

UPPER RESPIRATORY	
Laryngeal	Absent
	Green – throat tingling / altered sensation in throat
	Yellow –>3 discrete episodes of throat clearing or cough, or persistent throat tightness/pain Red – Hoarseness, frequent dry cough
	Red – Stridor

Table 1-4 Scoring system in gastrointestinal symptoms

GASTROINTESTINAL	
Nausea/pain	Absent
	Green – transient nausea
	Green – transient abdominal pain
	Yellow – persistent nausea
	Yellow –Persistent abdominal pain
Emesis/diarrhoea	Absent
	Yellow – 1 episode of emesis or diarrhoea
	Red – >1 episodes of emesis or diarrhoea or 1 of each

Table 1-5 Scoring system in cardiovascular/neurological symptoms

Cardiovascular	
	Normal heart rate or BP for age/baseline
	Yellow - Subjective response (weak, dizzy), or tachycardia
	Red - Drop in blood pressure and/or >20% from baseline, or significant change in mental status. Red - Cardiovascular collapse, signs of impaired circulation (unconscious)
Neurological	Altered consciousness (record GCS score)

Challenges should not start if there are baseline symptoms meeting descriptions in green.

Completion of the food challenge: treatment of positive reactions and discharge procedures

General considerations

If the subject has hoarseness, or has difficulty swallowing or speaking, IMMEDIATELY DISCONTINUE THE FOOD CHALLENGE, NOTIFY THE SUPERVISING CHALLENGE PHYSICIAN AND ADMINISTER TREATMENT.

All positive reactions that lead to the termination of the DBPCFC as per section 14.5 should be immediately treated in order to prevent the potential progression to more severe manifestations and in order to restore to baseline condition as soon as possible. These guidelines are not intended to replace each investigator's normal management of emergent reactions, and each investigator is expected to use their own clinical judgment and experience when treating reactions. The investigator should be aware of the following reasons why a more cautious approach may be warranted:

Peanut challenges under normal clinical circumstances are performed in people who are likely to have outgrown their allergy, or in whom the diagnosis is uncertain. In contrast, under this protocol we will be challenging patients who are highly likely to react.

Being adults with a clinical diagnosis of peanut allergy, the target population of this study represents on average a more severe patient population than the one investigative sites may deal with in their normal clinical activities. It is assumed that adult peanut allergic patients have a more severe disease phenotype than children and as such require a more aggressive approach to treatment.

The dessert matrix used to conceal peanut flour for the DBPCFC in this trial may also result in different absorption of peanut than the matrix routinely used by each investigative site. This may result in the sudden emergence of severe symptoms due to cumulative absorption of large amounts of allergen.

Treatment of positive reactions

This information is meant to harmonize the management of positive reactions during the DBPCFC by providing guidance relative to treatment options based on the outcomes defined in the scoring system of the procedure's protocol (Tables 1.2 – 1.5).

Treatment of "red symptoms"

The following Table 1-6 summarizes the recommended treatment approach for the "red symptoms" as defined in section 1.6. These recommendations should be interpreted in context of the speed of onset of reactions and the cumulative ingested dose of peanut allergy.

Table 1-6 Recommended Treatment for Red Symptoms

Signs and symptoms	Stopping criteria	Recommended treatment
Skin		
Urticaria/Angioedema	< 10 hives but ≥3, or significant lip or face oedema	In isolation: follow local procedures, consider fast acting anti-histamines (eg. cetirizine) first In combination with any symptom from a different system, consider: 0.5 mL adrenaline (1:1000) IM
	Generalized involvement	0.5 mL adrenaline (1:1000) IM
Rash	Generalized marked erythema (>50%)	0.5 mL adrenaline (1:1000) IM
Lower respiratory		
Wheezing	Expiratory wheezing on auscultation	0.5 mL adrenaline (1:1000) IM +SABA
	Mild audible (inspiratory and) expiratory wheezing	0.5 mL adrenaline (1:1000) IM +SABA
	Use of accessory muscles and/or audible wheezing (or silent lung)	0.5 mL adrenaline (1:1000) IM +SABA
Laryngeal	Hoarseness, frequent dry cough	0.5 mL adrenaline (1:1000) IM, consider nebulised adrenaline (1mg in 5ml saline).
	Stridor	0.5 mL adrenaline (1:1000) IM, consider nebulised adrenaline (1mg in 5ml saline). Notify anaesthetist / ICU.
Gastrointestinal		
Emesis/diarrhoea	2-3 episodes of emesis or diarrhoea or 1 of each	0.5 mL adrenaline (1:1000) IM)+ 1000 mL, consider 1 litre 0.9% saline bolus over 1-3 minutes
	>3 episodes of emesis or diarrhoea or 2 of each	0.5 mL adrenaline (1:1000) IM + 1000 mL 0.9% saline bolus over 1-3 minutes
Cardiovascular/neurologic		
	Drop in blood pressure and/or >20% from baseline, or significant change in mental status. Cardiovascular collapse, signs of impaired circulation (unconscious)	0.5 mL adrenaline (1:1000) IM. Inform ICU/ anaesthetist +1000 mL 0.9% saline bolus over 1-3 minutes (repeat as required) Consider IV adrenaline; diluted to at least 1:10,000, Start infusion at 5-15 µg/min. ECG /P/BP monitoring essential. Contact ICU / anaesthetist.

Treatment of yellow and persistent green symptoms

≥3 yellow symptoms

With the exception of three yellow symptoms confined within the skin (=one organ) any other combination of yellow symptoms implies multi-system involvement and the use of adrenaline should be favoured. This constellation may represent a situation that could rapidly evolve into more severe manifestations and it is important to timely block such potential progression. Short acting beta agonists (SABA) should be added in case of involvement of the lower airways.

<3 yellow symptoms or persistent (≥120min) green symptoms

The choice of the treatment should be aligned with the clinical judgment that led to the decision to stop the procedure. It is recommended to consider adrenaline in case of combination with other organ systems and of SABA in case of involvement of the lower airways.

Discharge procedures

Patients who do not exhibit a positive reaction at the end of the DBPCFC (=negative DBPCFC). As a late reaction cannot be excluded, prior to discharge, all patients will be provided with rescue medication consisting

of an adrenalin auto-injector, antihistamines (non-sedating long acting preferred, e.g. cetirizine, loratidine) and short acting beta-agonists (only for asthma patients). Patients will also receive specific information (counselling) on how to recognize such late reactions and on how to use rescue medication.

Patients using adrenaline due to a suspected reaction after discharge should immediately return to the investigative site or go to the closest emergency department for additional assessment.

Patients who exhibit a positive reaction to the DBPCFC and promptly respond to initial treatment

Patients whose symptoms return to baseline levels (vital signs and PEF are unchanged from baseline in the investigator's assessment and no symptoms of a systemic reaction are exhibited) within three hours of initial treatment will be discharged.

As a late reaction cannot be excluded, prior to discharge, all patients will be provided with rescue medication consisting of an adrenaline auto-injector, antihistamines (non-sedating, long-acting preferred, e.g. cetirizine, loratidine) and short-acting beta-agonists (only for asthma patients). Consider providing oral corticosteroids to patients with a diagnosis of asthma. Patients will also receive specific information (counselling) on how to recognize such late reactions and on how to use rescue medication. Patients will be counselled not to undertake vigorous or unaccustomed physical exercise, take ingest alcohol or take NSAIDs for 4 hours after the last challenge dose,

Patients using adrenaline due to a suspected reaction after discharge should immediately return to the investigative site or go to the closest emergency department for additional assessment.

Patients who exhibit a positive reaction to the DBPCFC and only partially respond to initial treatment

Patients whose symptoms show an improvement to the initial treatment but are NOT returned to baseline within three hours require a prolonged observation and further treatment as appropriate following investigator's practice.

If there is no recurrence of clinically significant symptoms, the subject may be discharged.

Patients who exhibit a life-threatening reaction to the DBPCFC and/or do not respond to repeated treatment

Patients who immediately exhibit a potentially life-threatening reaction to the DBPCFC or require more than 2 intramuscular injections of adrenaline with/without intravenous hydration will require IMMEDIATE REFERRAL TO AN EMERGENCY DEPARTMENT OR A FACILITY WITH AN INTESIVE CARE UNIT for further evaluation and, upon clinical evaluation, may be hospitalized to provide appropriate treatment.

Arrangements for next challenge day – restrictions

Participants will not be permitted to attend for a further challenge day if they have experienced severe anaphylaxis to peanut as defined above under patient preparation. Data up to the point where the participant left the study will be stored and analysed.

1.7 Laboratory and Physiological assessments

1.7.1 Sampling of blood and other biological fluids

1.7.1.1 Sampling prior to challenge

Blood will be collected from intravenous cannula sited prior to challenge.. Saliva will be collected using the Salivette system at the same time. Subjects will be asked to urinate (void to waste) prior to challenge.

1.7.1.2 Sampling post challenge

At onset of objective symptoms, and *after* any essential medical treatment has been initiated, blood and saliva will be collected and processed. Further blood samples will be collected at 30, 60 and 120 minutes after cessation of challenge (or final dose where no reaction occurs). Saliva will also be collected at 60 minutes post cessation of challenge / final dose. Urine will be collected during the challenge (as provided) with a final collection 120 minutes after cessation of challenge / final dose.

1.7.2 Physiological assessments

Subjects will be connected to a non-invasive cardiac monitor (NICOM Cheetah) during both the challenge and recovery period. Endothelial function, forearm skin perfusion and exhaled nitric oxide will be monitored non-invasively prior to challenge and following cessation of challenge, by a trained technician. Finally, absorption in the mouth will be determined by asking subjects to suck a sweet (lollypop) containing whey milk protein, once the challenge has been ceased. Any absorbed whey milk protein will then be measured in the blood samples to be collected at the time intervals described above.

References

Boyce JA, Assa'ad A, Burks AW, Jones SM, Sampson HA, Wood RA, et al. Guidelines for the diagnosis and management of food allergy in the United States: report of the NIAID-sponsored expert panel. J Allergy Clin Immunol 2010; 126(suppl):S1-58.

Sicherer SH, Morrow EH, Sampson HA. Dose-response in double-blind, placebo- controlled oral food challenges in children with atopic dermatitis. J Allergy Clin Immunol 2000;105:582-6.

Niggemann B, Beyer K. Pitfalls in double-blind, placebo-controlled oral food challenges. Allergy 2007;62:729-32.

Bindslev-Jensen C, Ballmer-Weber BK, Bengtsson U, Blanco C, Ebner C, Hourihane J, et al. Standardization of food challenges in patients with immediate reactions to foods—position paper from the European Academy of Allergology and Clinical Immunology. Allergy 2004;59:690-7.

Nowak-Wegrzyn A, Assa'ad AH, Bahna SL, Bock SA, Sicherer SH, Teuber SS. Work Group report: oral food challenge testing. J Allergy Clin Immunol 2009; 123(suppl):S365-83.

Sampson HA, Gerth van Wijk R, Bindslev-Jensen C, Sicherer S, Teuber SS, Burks AW, Dubois AE, Beyer K, Eigenmann PA, Spergel JM, Werfel T, Chinchilli VM. J Allergy Clin Immunol. 2012;130:1260-74. Standardizing double-blind, placebo-controlled oral food challenges: American Academy of Allergy, Asthma & Immunology-European Academy of Allergy and Clinical Immunology PRACTALL consensus report.

Taylor SL, Moneret-Vautrin DA, Crevel RW, Sheffield D, Morisset M, Dumont P, Remington BC, Baumert JL. Threshold dose for peanut: Risk characterization based upon diagnostic oral challenge of a series of 286 peanut-allergic individuals. Food Chem Toxicol

Appendix 10: Challenge CRF.

Baseline Peanut DBPCFC:
Study Participant UID:
Supervising clinician:
Nurse:

Challenge Day 1 Date |__|__| |__|__| |__|__|
Day Month Year

Challenge SOP version used:

1. Has the participant given consent to continue?
Yes ❒ No ❒

2. Type of challenge
Baseline❒ No intervention ❒ Sleep ❒ Exercise ❒

3. Pre-challenge history

	Yes	No
Asthma control:		
Is FEV1 >80% predicted	❒	❒
Asthma control test score above 20?	❒	❒
A significant clinical reaction to peanut within the previous three months	❒	❒
Significant illness with systemic features (e.g. fever >37.5 degrees Celcius) within two (2) weeks prior to challenge	❒	❒
Any current symptoms of allergic disease (urticaria, angiodema, eczema, rhinitis, asthma)	❒	❒
Musculoskeletal disease which could impair the participant's ability to perform the exercise challenge	❒	❒
Any stomach pain, sickness, diarrhoea, bloating?	❒	❒
Has subject fasted for at least 2 hours?	❒	❒
Has intense exercise been avoided for 12 hours?	❒	❒
No caffeine intake in last 12 hours	❒	❒
Has alcohol been avoided for 24 hours?	❒	❒
Alcohol or drug misuse	❒	❒
Night shift working within the last month	❒	❒

Drugs that may alter reactivity and influence the outcome of the DPT if taken concomitantly:

Guidance provided in study SOP:

	Yes	No
Corticosteroids (systemic) in previous 2 weeks	❒	❒
Antihistamine in previous		
3 days (short-acting eg chlorpheniramine)	❒	❒
5 days (long acting eg cetirizine, fexofenadine).	❒	❒
Regular treatment with: systemic immunosuppressants, beta blockers, ACE inhibitor, antacid medication, antidepressant (tricyclic) or sedatives	❒	❒
Contraindication to the administration of adrenaline (e.g., ischaemic heart Disease, poorly controlled hypertension or cardiac arrhythmia)	❒	❒
Any clinically significant disease that can affect patient's safety or can make implementation of the protocol or interpretation of the results difficult, and has arisen subsequent to the screening visit?	❒	❒
Pregnancy (if applicable).....	❒	❒

Date of last period (if applicable)..... |__|__| |__|__| |__|__|
Day Month Year

Pregnancy test (dipstick) result, if applicable Positive ❒ Negative ❒

Does the patient have rhinitis? Y ❒ N ❒

Score each symptom below 1 (mild) 2(moderate) 3 (severe) (Total score 12)

Runny nose ☐ Sneeze ☐ Nasal itch☐ Congestion☐

Does the patient have eczema; Y ❒ N ❒

Patient oriented eczema measure …….. [0-28]

SLEEP DETAILS

Average number of hours sleep per night in 2 weeks prior to challenge?☐☐[0-12]

Has the patient received 3 hours sleep or less the night before the challenge? Y ❒ N ❒

If no, record how many hours of sleep the patient has had❒❒ 0-10

4. Pre-challenge examination

Baseline observations (Pre-Dose1): Time (hour/min) |__|__|:|__|__|

Temperature °C ☐☐.☐[36.0-42.0]

If above 37.5 no challenge

Blood pressure (mmHg) ☐☐☐systolic [60-200] ☐☐☐diastolic [30-120]

Heart rate beats/minute ☐☐☐ [30-150]

Respiratory rate /minute ☐☐ [4-40]
SpO2 ☐☐☐% [90-100]
Peak expiratory flow rate (PEFR) litres/minute ☐☐☐ [300-800]

% of predicted PEFR ☐☐☐% [0-150]
If less than 80% no challenge predicted

FEV_1 litres/minute ☐☐[2.0-5.5]
Percentage predicted ☐☐☐ % [0-150]

Vital signs stable (SO_2, PEFR, BP, Pulse, respiratory rate) Y ❒ N ❒
Examination

	Normal	Abnormal	If abnormal provide details
Oral cavity	☐☐		_____
Skin	☐☐		_____
Nasal passages	☐☐		_____
Respiratory system	☐☐		_____
Cardiovascular system	☐☐		_____
Gastrointestinal system	☐☐		_____

Room temperature °C ☐☐.☐[36.0-42.0]

5. Challenge Scheduling

Yes No
DBPCFC to be rescheduled due to abnormal examination finding ❒
❒

6. Pre-Challenge Set-up

	Yes	No
i.v. access	☐	☐
Emergency medications available in challenge room?	☐	☐

Challenge meal batch number and expiry date ……..
Challenge randomization code:

8. POST CHALLENGE

***Day 1- Post last dose* observations:** Time (hour/min) |__|__| |__|__|

Temperature °C ☐☐.☐ [36.0-42.0]

Blood pressure (mmHg) ☐☐☐systolic [60-200] ☐☐☐diastolic [30-120]

Heart rate beats/minute ☐☐☐ [30-150]

Respiratory rate /minute ☐☐ [4-40]

SpO2 ☐☐☐% [90-100]

Peak expiratory flow rate (PEFR) litres/minute ☐☐☐ [200-800] ☐☐☐% predicted [0-150]

PEFR 20% drop litres/minute ☐☐☐

FEV_1 litres/minute ☐☐☐ [2-5.5] ☐☐☐ % predicted [0-150]

VISUAL ANALOGUE SCORE RESULTS

Particpant:

Skin ☐☐ [0-10] Nose ☐☐[0-10] Throat ☐☐ [0-10] Breathing ☐☐ [0-10]

Abdominal ☐☐[0-10]

Other (free text here)☐☐ [0-10] Anxiety ☐☐[0-10]

Overall reaction severity ☐☐ [0-10]

Total VAS score ☐☐ [0-80]

In relation to baseline challenge ☐☐ [-2 to +2]

Investigator:

Skin ☐☐ [0-10] Nasal☐☐[0-10] Oropharyngeal☐☐ [0-10] Lower respiratory☐☐ [0-10] Gastrointestinal☐☐[0-10]Other (free text) ☐☐ [0-10] Anxiety ☐☐[0-10]

Overall reaction severity ☐☐ [0-10]

Total VAS score☐☐ [0-80]

In relation to baseline challenge ☐☐ [-2 to +2]

9. Treatment given during challenge

Oral antihistamine Dose 1 ☐ Dose 2 ☐ and time of doses
IV antihistamine Dose 1 ☐ and time of dose
IM adrenaline Dose 1 ☐ Dose 2 ☐ Dose 3 ☐ Dose 4 ☐ and time of doses
Nebulised adrenaline Dose 1 ☐ Dose 2 ☐ Dose 3 ☐ Dose 4 ☐ and time of doses
IV Saline bolus 1 Litre Dose 1 ☐ Dose 2 ☐ Dose 3 ☐ Dose 4 ☐ and time of administration
IV adrenaline infusion Dose 1 ☐ time infusion started
Other inotrope infusion Dose 1 ☐ time infusion started
High flow oxygen [yes button]
Other treatment_____

10. Summary of observations during challenge

Record the peak symptom severity during the first 2 hours of the allergic reaction as below:

Lowest blood pressure recorded during reaction ☐☐☐systolic [60-200] / ☐☐☐diastolic [30-120]

Highest heart rate recorded during reaction ☐☐☐/minute [30-150]

Lowest peak expiratory flow rate recorded during reaction ☐☐☐ litres/minute ☐☐☐ % [50-800]

Highest respiratory rate recorded during reaction ☐☐☐ /minute [4-40]

Lowest SaO2 recorded during reaction ☐☐☐% [0-100]

Time to complete resolution of symptoms [hours]☐☐ [0-72]

11. Disposal:

Home
Admitted to hospital
Where
Admitted to intensive care
Where
Treatment plan given? Yes ☐No ☐

12. Post-challenge examination

Yes No
examination of oral cavity, skin, lung performed ☐☐
withdraw i.v. access ☐☐
Blood pressure (mmHg) ☐☐☐systolic [60-200] ☐☐☐diastolic [30-120]
Heart rate beats/minute ☐☐☐ [30-150]
* post-challenge PEF ..... [200-800] (..... % of predicted) [0-150]
* post-challenge FEV_1 ..... [2-5.5] (..... % of predicted) [0-150]

OUTCOME OF DAY 1 CHALLENGE: **Reactive** **Nonreactive** **Inconclusive**

LATE ONSET REACTIONS
(after discharge)

Yes No
Unknown

Did the patient report a late onset reaction after challenge day 1? □□□

Did the patient report a late onset reaction after challenge day 2? □□□

Keep a record of the late reactions together with the hard copy of the DBPCFC form in the CRF

Insert Day 2 (complete repeat of record)

13. Decryption of DBPCFC

[link to randomisation]

	Active	placebo
Challenge of day 1		□□□
Challenge of day 2		□□□

CHALLENGE DOSE :

Any persistent symptoms from previous dose? Yes ☐ No ☐

If yes which?
……
…….

Pre-dose observations [DOSE]:

Temperature °C ☐☐.☐ [36.0-42.0]

Blood pressure (mmHg) ☐☐☐systolic [60-200] ☐☐☐diastolic [30-120]

Heart rate beats/minute ☐☐☐ [30-150]

Respiratory rate /minute ☐☐ [4-40]

(PEFR) litres/minute ☐☐☐ [300-800]

SpO2 ☐☐☐% [90-100]

Dose double checked Yes ☐ No ☐

Time Dose given: ☐☐hr ☐☐min

Whole dose ingested? yes ☐ no ☐ If no: specify ingested amount in g: _ _ _

Water ingestion – specify volume

Whole exercise period undertaken? Yes ☐ No ☐

If no please state how many minutes were undertaken☐☐ [0-10]

Target heart rate maintained during exercise Yes ☐ No ☐

Symptoms	**Time of onset**	**Time of resolution**	**Observations**	**Treatment**

Table 1 Symptom table

Symptom		Percentage area
SKIN		
Pruritus -Occasional scratching [Green]		
Pruritus- scratching continuously for >2 mins at a time [Green]		
Hard continuous scratching>excoriations [Yellow]		
Urticaria-<3 hives or mild lip oedema [Yellow]		
Urticaria- <10 hives ≥ 3or significant lip or face oedema [Red]		
Urticaria-generalised involvement [Red]		
Rash- Few areas of faint erythema [Green]		
Rash- Areas of erythema [Yellow]		
Rash- Generalised marked erythema>50% [Red]		
UPPER RESPIRATORY [Total Nasal Symptom Score] 0-12		
Itching in inner ear canal [green]		
Rare bursts of sneezingoccasional sniffing [green]		
I Bursts < 10, intermittent rubbing of nose, and/or eyes or frequent sniffing [green]		
Continuous rubbing of nose and/or eyes, [Yellow]		
Periocular swelling and/or long bursts of sneezing, [Yellow]		
Persistent rhinorrhoea [Yellow]		
Throat tingling/altered sensation in throat [Green]		
> 3 discrete episodes of throat clearing or cough [Yellow]		
Persistent throat tightness [Yellow]		
Hoarseness or frequent dry cough		

[Red]		
Stridor [Red]		
LOWER RESPIRATORY		
Chest tightness without any fall in PEFR [Green]		
Chest tightness with a <10% fall in PEFR [green]		
Chest tightness with a 10-20% fall in PEFR [yellow]		
Chest tightness with a >20% fall in PEFR [red]		
Expiratory or inspiratory wheeze [Red]		
Use of accessory muscles [Red]		
GASTROINTESTINAL		
Oral itching [Green]		
Transient nausea [green]		
Persistent nausea [yellow]		
Transient abdominal pain [green]		
Persistent abdominal pain [yellow]		
Emesis/diarrhoea (1 episode) [Yellow]		
Emesis/diarrhoea (more than 1 episode) [Red]		
CARDIOVASCULAR		
Weak/dizzy or tachycardia [Yellow]		
Drop in BP and/or >20% from baseline [Red]		
Cardiovascular collapse/signs of impaired circulation [Red]		
NEUROLOGICAL		
Altered level of consciousness [Red]		

Stopping criteria applied?	Yes	No
IF YES, TICK THE CRITERIA USED		
Green symptoms >120 minutes		
Three or more yellow symptoms		
One red symptom		
Participant request		

Stopping Criteria Applied? Yes ☐ ***No*** ☐

Appendix 11: ECG Holter and HRV SOP.

A. ECG HOLTER MONITOR SOP:

1. Have the data in a direct drive i.e c or d but NOT in a folder.

2. Start MARS programme (dongle needed).

3. Acquire data> Shape review> set N to normal QRS, S to supraventricular, V to ventricular and X to noise. *It's very imp to do this correctly as the rest of the analysis will depend on it.*

4. Go to View 12SL and for the five 10 minute epochs of our chosen timepoints select the 10 one minute ECG that corresponds for each. This can then be printed> to print this select FILE for each of them and in report review they will all be included.

5. Values for HR, PR, QRS, QT, QTc will need to be written down manually for each minute in the 10-minute epoch. This is found in the ECG that has been printed out.

B. HRV USING KUBIOS PROGRAMME:

Mars data can be exported and saved for each pt:

1. System> Research Utilities> MIT annotation formats> save in home> trace >HEA and ANN formats (always remember to safe both!)
2. Annotation files can be changed into text files using CYGWIN Terminal on pc desktop.

   ann2rr –r (participants ID) –a ann –i s –p N >(participants ID).txt

3. Txt files are then opened in Kubios and artefact correction applied to remove excessively long/short R-R intervals. "Very low" is selected for artefact correction, or custom, ensuring that variability of the R-R intervals is not compromised.

4. Two five-minute samples are then added for analysis for each ten-minute epoch giving 10 five-minute samples. These should be added in chronological order from the first epoch to the last.

5. The file may then be saved as an ASCII file, which can be imported into Excel as a table, using tab and ";" as the custom separator.

6. Heart rate variability is analysed in three ways and the values we will be looking at are:
   For Time Domain: SDNN.
   For Frequency Domain: LF(nu), HF(nu).
   For Non-linear: DFA1, Apen, Sampen.

Appendix 12: Histamine, codeine and peanut skin prick testing SOP.

MATERIALS

- 1 ml Syringes (12 units)
- Needles (13 units)
- 0,9% Physiological Saline (10,8 ml)
- Gloves
- Histamine, Phosphate codeine and peanut extracts
- Cleaning wipes
- Lancets
- Water resistant pen
- Cello tape
- Graph pad paper
- Ruler

METHODS

1. Preparing the dilutions:

- Wash your hands and put your gloves.
- Put 0, 9 ml of saline in each of the 12 syringes using the same needle. After this procedure, throw this needle.
- Using the water resistant pen, mark each syringe with the correspondent extract and dilution.

2. How to prepare histamine, phosphate codeine and peanut dilutions:

Histamine dilutions

We will prepare **1, 1/10, 1/100 and 1/1000** using one needle for each dilution.

1: Histamine extract "as it".

1/10: Put 0, 1 ml of histamine extract in one of the syringes containing 0, 9 ml of saline.

1/100: Put 0, 1 of the 1/10 histamine dilution in one of the syringes containing 0, 9 ml of saline.

1/1000: Put 0,1 ml of the 1/100 histamine dilution in one of the syringes containing 0, 9 ml of saline.

Phosphate Codeine dilutions

We will prepare **1, 1/10, 1/100 and 1/1000** using one needle for each dilution.

1: Phosphate codeine extract "as it".

1/10: Put 0, 1 ml of codeine extract in one of the syringes containing 0, 9 ml of saline.

1/100: Put 0, 1 of the 1/10 codeine dilution in one of the syringes containing 0, 9 ml of saline.

1/1000: Put 0, 1 ml of the 1/100 codeine dilution in one of the syringes containing 0, 9 ml of saline.

Peanut dilutions

We will prepare 1, 1/10, 1/100, 1/1000, $1/10^{4}$, $1/10^{5}$ using one needle for each dilution.

1: Peanut extract "as it".

1/10: Put 0, 1 ml of peanut extract in one of the syringes containing 0, 9 ml of saline.

1/100: Put 0, 1 ml of the 1/10 peanut dilution in one of the syringes containing 0, 9 ml of saline.

1/1000: Put 0, 1 ml of the 1/100 peanut dilution in one of the syringes containing 0, 9 ml of saline.

1/10^4: Put 0, 1 ml of the 1/1000 peanut dilution in one of the syringes containing 0, 9 ml of saline.
1/10^5: Put 0, 1 ml of the 1/10^4peanut dilution in one of the syringes containing 0, 9 ml of saline.

How to make skin prick testing:

- After preparing the dilutions, clean the patient forearm and start with the skin prick testing in this location using one lancet for each drop.
- Each extract will have a Reading time:
    - **Histamine and Codeine:** 10 minutes
    - **Peanut:** 20 minutes and before each dose or every 30 minutes until it disappears.
- Mark each wheal with the water resistant pen, put cello tape over the wheal and stick it in the graph pad paper.

Appendix 13: NIAID severity scoring.

Figure 1: Clinical criteria for diagnosing anaphylaxis according to NIAID[128]:

Anaphylaxis is highly likely when any <u>one</u> of the following 3 criteria are fulfilled:

1. Acute onset of an illness (minutes to several hours) with involvement of the skin, mucosal tissue, or both (eg, generalized hives, pruritus or flushing, swollen lips-tongue-uvula)

AND AT LEAST ONE OF THE FOLLOWING

a. Respiratory compromise (eg, dyspnea, wheeze-bronchospasm, stridor, reduced PEF, hypoxemia).

b. Reduced BP or associated symptoms of end-organ dysfunction (eg, hypotonia [collapse], syncope, incontinence).

2. Two or more of the following that occur rapidly after exposure *to a <u>likely</u> allergen for that patient* (minutes to several hours):

a. Involvement of the skin-mucosal tissue (eg, generalized hives, itch-flush, swollen lips-tongue-uvula).

b. Respiratory compromise (eg, dyspnea, wheeze-bronchospasm, stridor, reduced PEF, hypoxemia).

c. Reduced BP or associated symptoms (eg, hypotonia [collapse], syncope, incontinence).

d. Persistent gastrointestinal symptoms (eg, crampy abdominal pain, vomiting).

3. Reduced BP after exposure to *<u>known</u> allergen for that patient* (minutes to several hours):

a. Infants and children: low systolic BP (age specific) or greater than 30% decrease in systolic BP.

b. Adults: systolic BP of less than 90 mm Hg or greater than 30% decrease from that person's baseline.

Appendix 14: Ewan and Clark Severity Scoring.

Table 1: Classification of IgE-mediated FA according to Ewan&Clark classification of severity[287]:

Grade	Reaction	Clinical Features
1	Mild	Localised cutaneous erythema/ urticarial/ angioedema/ oral pruritus.
2	Mild	Generalised erythema/ urticarial/ angioedema.
3	Mild	At least 1or 2 plus gastro-intestinal symptoms/ rhinoconjuntivitis.
4	Moderate	Mild laryngeal oedema (voice change/ tightening of throat)/ mild asthma.
5	Severe	Marked dyspnoea/ hypotensive symptoms (light-headedness/ collapse/ loss of consciousness).

Appendix 15: World Allergy Organization Subcutaneous Immunotherapy Systemic Reaction Grading System.

Table 2: WAO severity scoring[316]:

Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Symptom(s)/sign(s) of 1 organ system present* **Cutaneous** Generalized pruritus, urticaria, flushing, or sensation of heat or warmth† **or** Angioedema (not laryngeal, tongue or uvular) **or** **Upper respiratory** Rhinitis - (eg, sneezing, rhinorrhea, nasal pruritus and/ or nasal congestion) **or** Throat-clearing (itchy throat) **or** Cough perceived to originate in the upper airway, not the lung, larynx, or trachea **or** **Conjunctival** Erythema, pruritus or tearing **Other** Nausea, metallic taste, or headache	***Symptom(s)/sign(s) of more than 1 organ system present*** **or** **Lower respiratory** Asthma: cough, wheezing, shortness of breath (eg, less than 40% PEF or FEV_1 drop, responding to an inhaled bronchodilator) **or** **Gastrointestinal** Abdominal cramps, vomiting, or diarrhea **or** **Other** Uterine cramps	**Lower respiratory** Asthma (eg, 40% PEF or FEV_1 drop NOT responding to an inhaled bronchodilator) **or** **Upper respiratory** Laryngeal, uvula, or tongue edema with or without stridor	**Lower or upper respiratory** Respiratory failure with or without loss of consciousness **or** **Cardiovascular** Hypotension with or without loss of consciousness	Death

Patients may also have a feeling of impending doom, especially in grades 2, 3, or 4.
Note: Children with anaphylaxis seldom convey a sense of impending doom and their behavior changes may be a sign of anaphylaxis; eg, becoming very quiet or irritable and cranky.
Scoring includes a suffix that denotes if and when epinephrine is or is not administered in relationship to onset of symptom(s)/sign(s) of the SR:a, ≤ 5 minutes; b, >5 minutes- to ≤10 minutes; c: >10 to ≤20 minutes; d:>20 minutes; z, epinephrine not administered.
The final grade of the reaction will not be determined until the event is over, regardless of the medication administered. The final report should include the first symptom(s)/sign(s) and the time of onset after the subcutaneous allergen immunotherapy injection*** and a suffix reflecting if and when epinephrine was or was not administered, **eg, Grade 2a; rhinitis:10 minutes**.

Appendix 16: Visual analogue scale for rating reaction severity.

For upper respiratory total score we took the nose and throat scoring and divided it by 2.

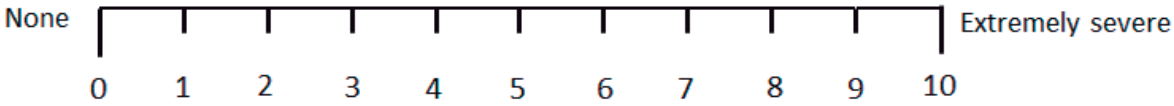
V1 18.10.2013

Assessment of symptoms

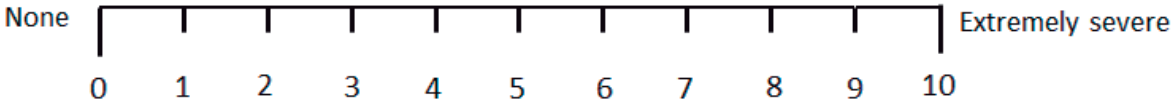
Challenge visit: _____

Please rate your symptoms using the following scales:

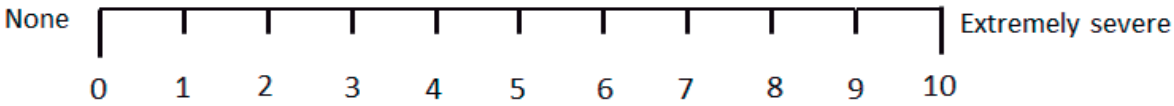
Skin symptoms



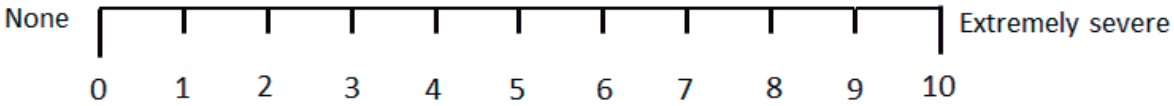
Nose symptoms



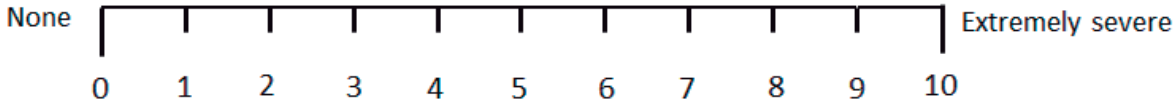
Throat symptoms



Breathing symptoms

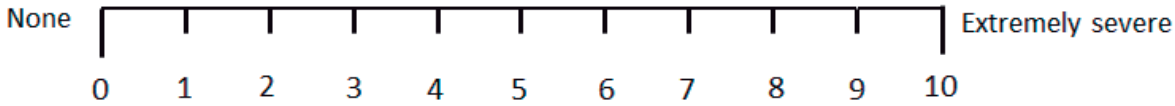


Abdominal symptoms

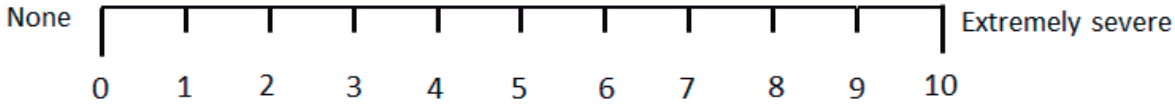


Other symptoms

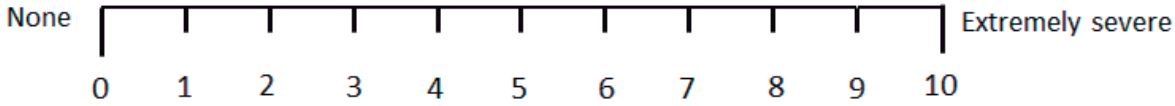
Please define: _____



Anxiety



Overall how would you rate your reaction severity?



Appendix 17: Correlation between investigator´s VAS score and CVS parameters analysed.

Table 3: Relationship between CVS and investigator´s VAS score on baseline challenge:

	VAS skin score	**VAS GI score**	**VAS upper resp score**	**VAS lower resp score**	**VAS overall score**
SV	r= 0.23 (p=0.11)	**r= -0.30 (p=0.04)**	r= 0.10 (p=0.50)	r= -0.17 (p=0.22)	r= -0.13 (p=0.21)
HR	r= -0.10 (p=0.40)	**r= 0.31 (p=0.02)**	r= -0.13 (p=0.38)	r= 0.11 (p=0.46)	**r= 0.29 (p=0.04)**
sBP	r= 0.10 (p=0.48)	r= 0.20 (p=0.18)	r= 0.10 (p=0.49)	r= 0.07 (p=0.65)	r= 0.14 (p=0.33)
dBP	r= 0.02 (p=0.91)	**r= 0.29 (p=0.04)**	r= 0.13 (p=0.26)	r= 0.06 (p=0.67)	r= 0.16 (p=0.28)
Blood flow	r= -0.004 (p=0.98)	r= 0.27 (p=0.11)	r= -0.24 (p=0.15)	r= -0.17 (p=0.32)	r= -0.08 (p=0.67)

Spearman correlation. Highlighted $p<0.05$.

Appendix 18: Difference in CVS parameters between baseline and repeated challenge.

Figure 2 shows no significant difference in CO between OCR and baseline at repeated challenge. Figure 3 shows no significant differences between baseline and repeated challenge for SV, sBP, dBP and a significant difference between baseline and repeated challenge for HR and CO.

Figure 2 Change in CO at repeated NI challenge:

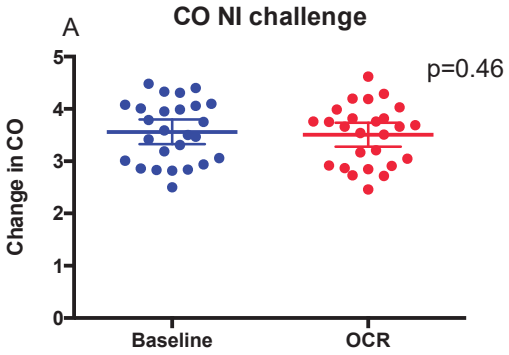
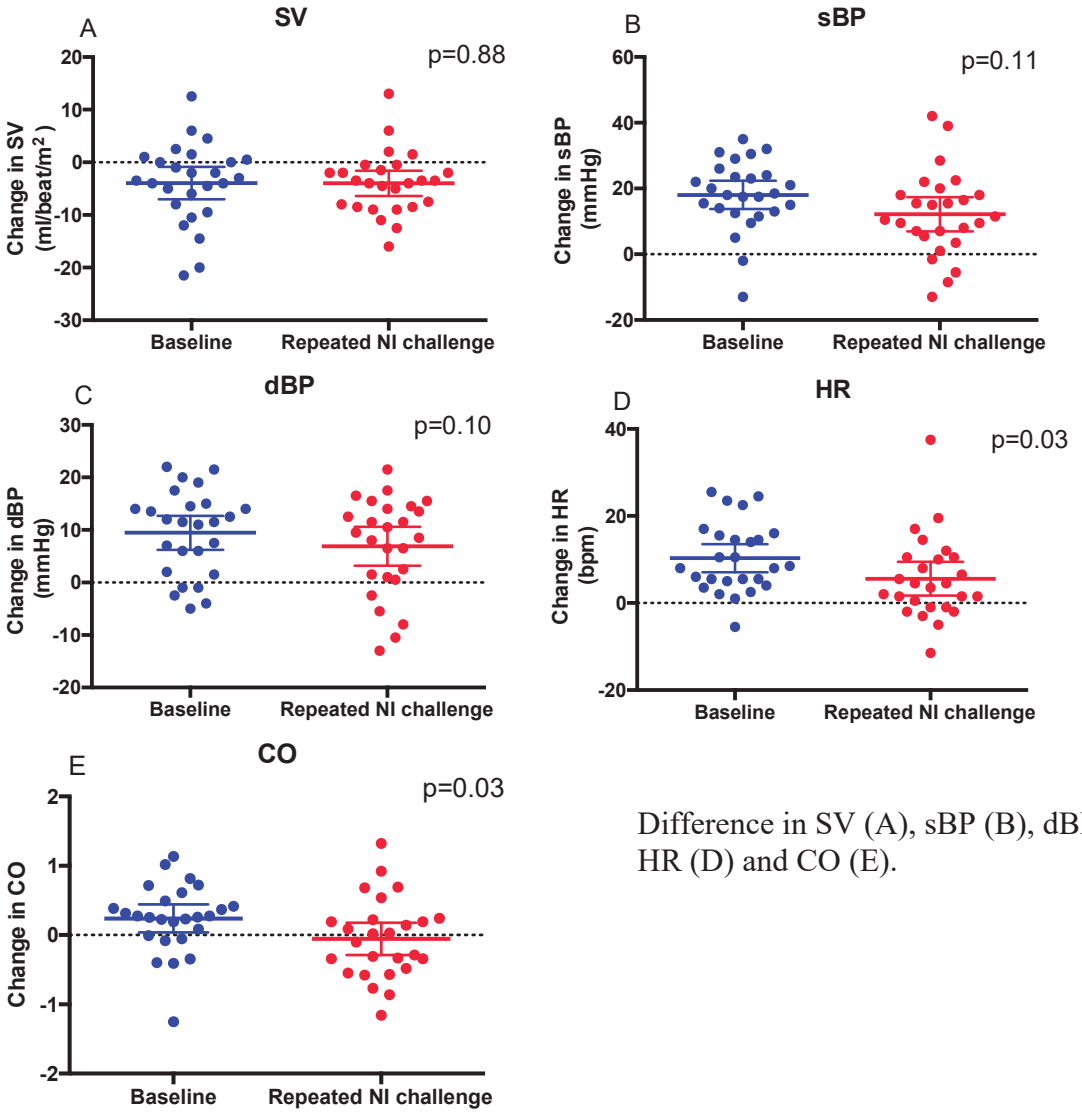


Figure 3 Difference in CVS parameters between baseline and repeated NI challenge:

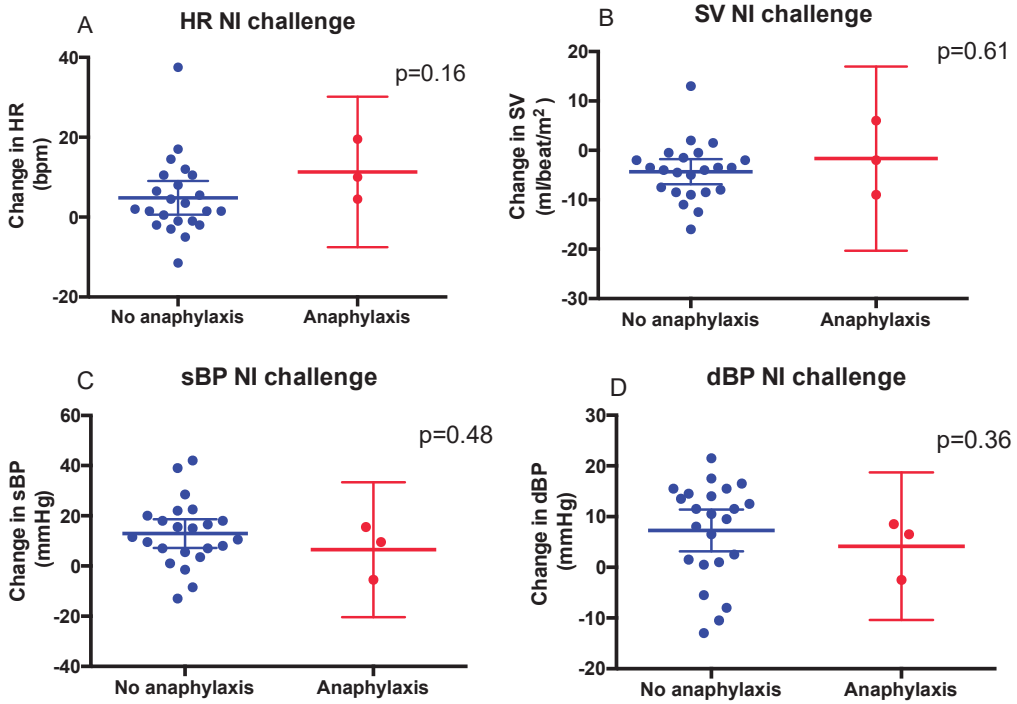


Difference in SV (A), sBP (B), dBP (C), HR (D) and CO (E).

Appendix 19: CVS measurements and severity of reaction on repeated NI challenge.

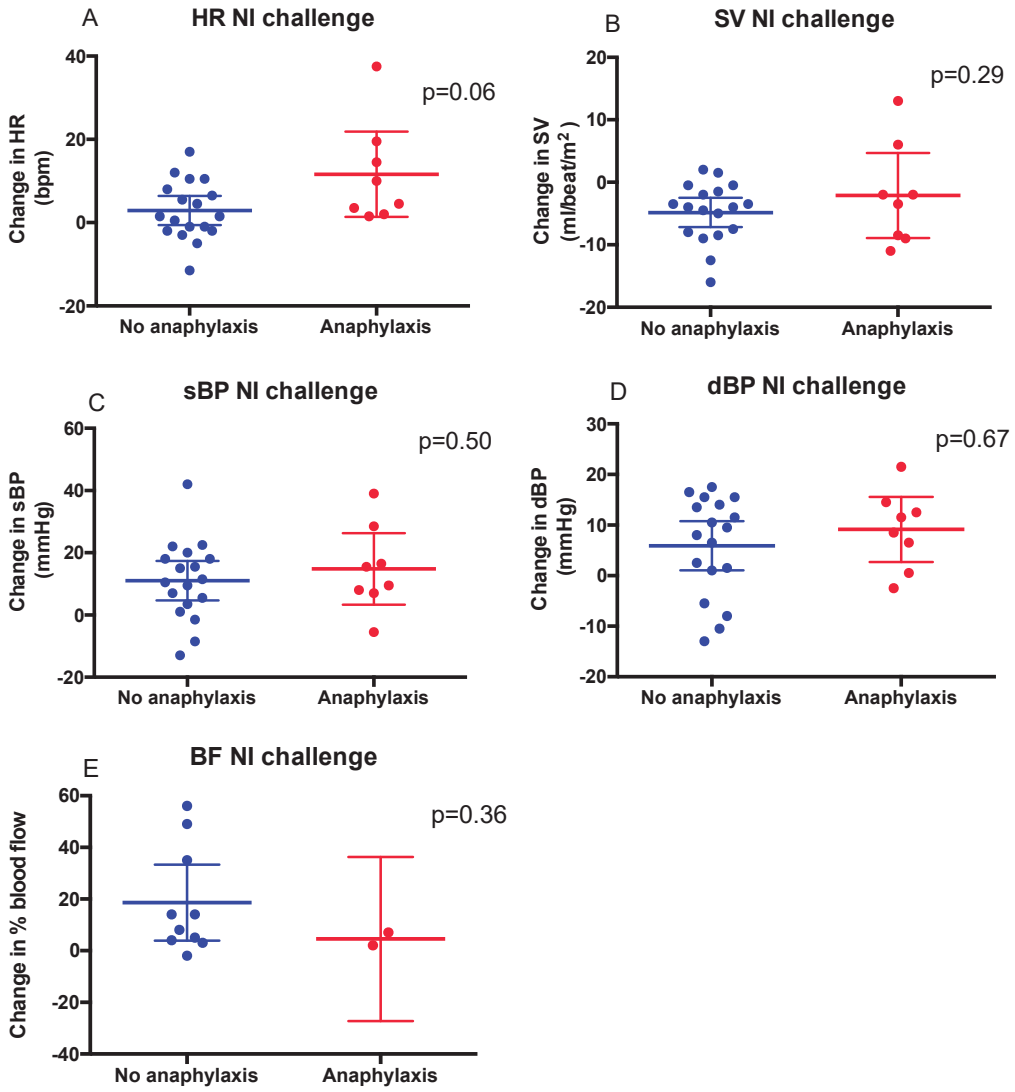
Appendix 19 shows results for classification of severity of reaction for HR, SV, sBP, dBP and blood flow during repeated NI challenges. Analysis for IM adrenaline and WAO classification was not performed for blood flow, as optimal data was only available for one participant for blood flow and only one participant was classified as having severe reaction according to WAO classification on a non-intervention challenge.

Figure 4 Change in CVS parameters according to the use of IM adrenaline:



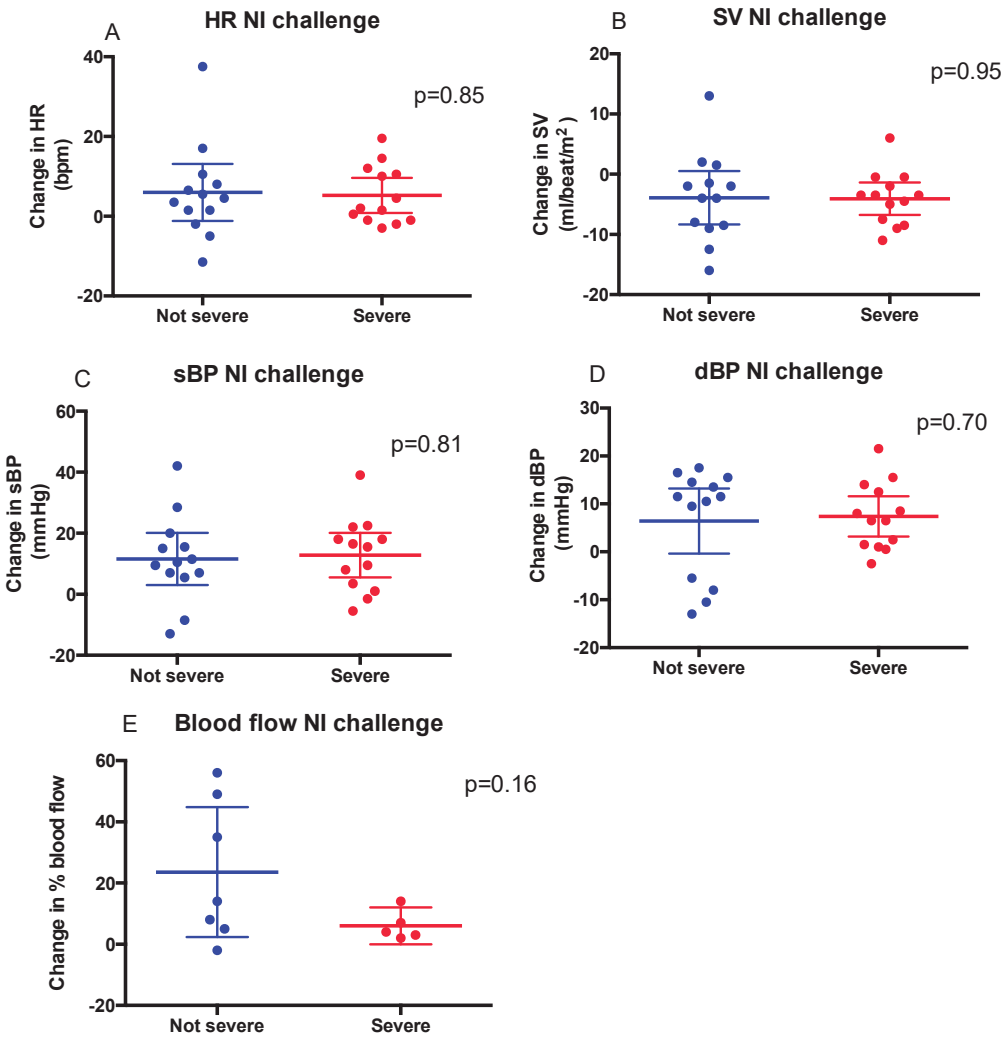
Change in (A) HR, (B) SV, (C) sBP and (D) dBP..

Figure 5 Change in CVS parameters according to NIAID classification of anaphylaxis:



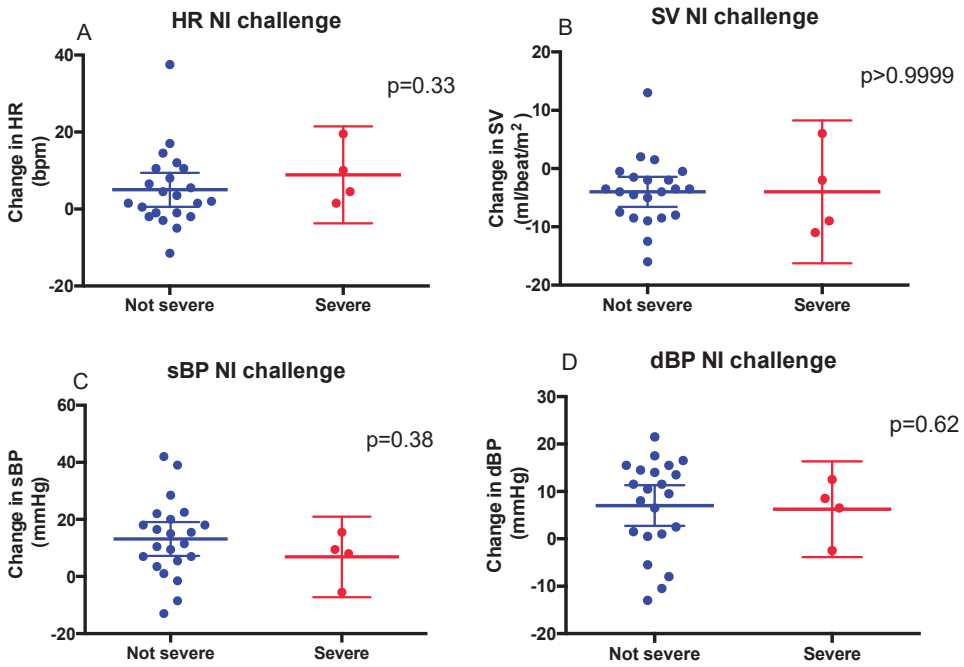
Change in (A) HR, (B) SV, (C) sBP, (D) dBP and (E) blood flow.

Figure 6 Change in CVS parameters according to Ewan&Clark classification:



Change in (A) HR, (B) SV, (C) sBP, (D) dBP and (E) blood flow.

Figure 7 Change in CVS parameters according to WAO classification of severity:



Change in (A) HR, (B) SV, (C) sBP and (D) dBP.

A good correlation, but in opposite direction compared to baseline challenge, was found between participant´s VAS score for GI symptoms, overall score and change in SV, shown in Table 4. Different to what was seen at baseline challenge, no correlation was found between change in HR and participant´s VAS score for GI symptoms (r^2=0.25, p=0.23) and overall severity score (r^2=0.29, p=0.27). Similar results were found for correlation between CVS parameters and investigator´s VAS symptom score for repeated non-intervention challenges, shown in Table 5. This analysis was not repeated for BP or blood flow as no correlation was found between this parameters and VAS scoring for baseline DBPCFC.

Table 4: Relationship between CVS and participant´s VAS score on repeated NI challenge:

	VAS skin score	**VAS GI score**	**VAS upper resp score**	**VAS lower resp score**	**VAS overall score**
SV	r= 0.07 (p=0.75)	**r= 0.40 (p=0.04)**	r= 0.36 (p=0.07)	r= 0.22 (p=0.28)	**r= 0.55 (p=0.005)**
HR	r= 0.12 (p=0.47)	r= 0.28 (p=0.17)	r= 0.14 (p=0.50)	r= 0.12 (p=0.57)	r= 0.30 (p=0.14)

Spearman correlation. Highlighted p<0.05.

Table 5: Relationship between CVS and investigator´s VAS score on repeated NI challenge:

	VAS skin score	**VAS GI score**	**VAS upper resp score**	**VAS lower resp score**	**VAS overall score**
SV	r= -0.08 (p=0.72)	**r= 0.39 (p=0.05)**	r= 0.21 (p=0.43)	r= 0.13 (p=0.52)	r= 0.33 (p=0.10)
HR	r= 0.17 (p=0.53)	r= 0.25 (p=0.23)	r= 0.07 (p=0.72)	r= 0.28 (p=0.18)	r= 0.29 (p=0.17)

Spearman correlation. Highlighted p≤0.05.

Appendix 20: Correlation between the ECG and HRV parameters and investigator´s VAS score.

Table 6: Relationship between cardiac conductance parameters and investigator´s VAS score in baseline challenge:

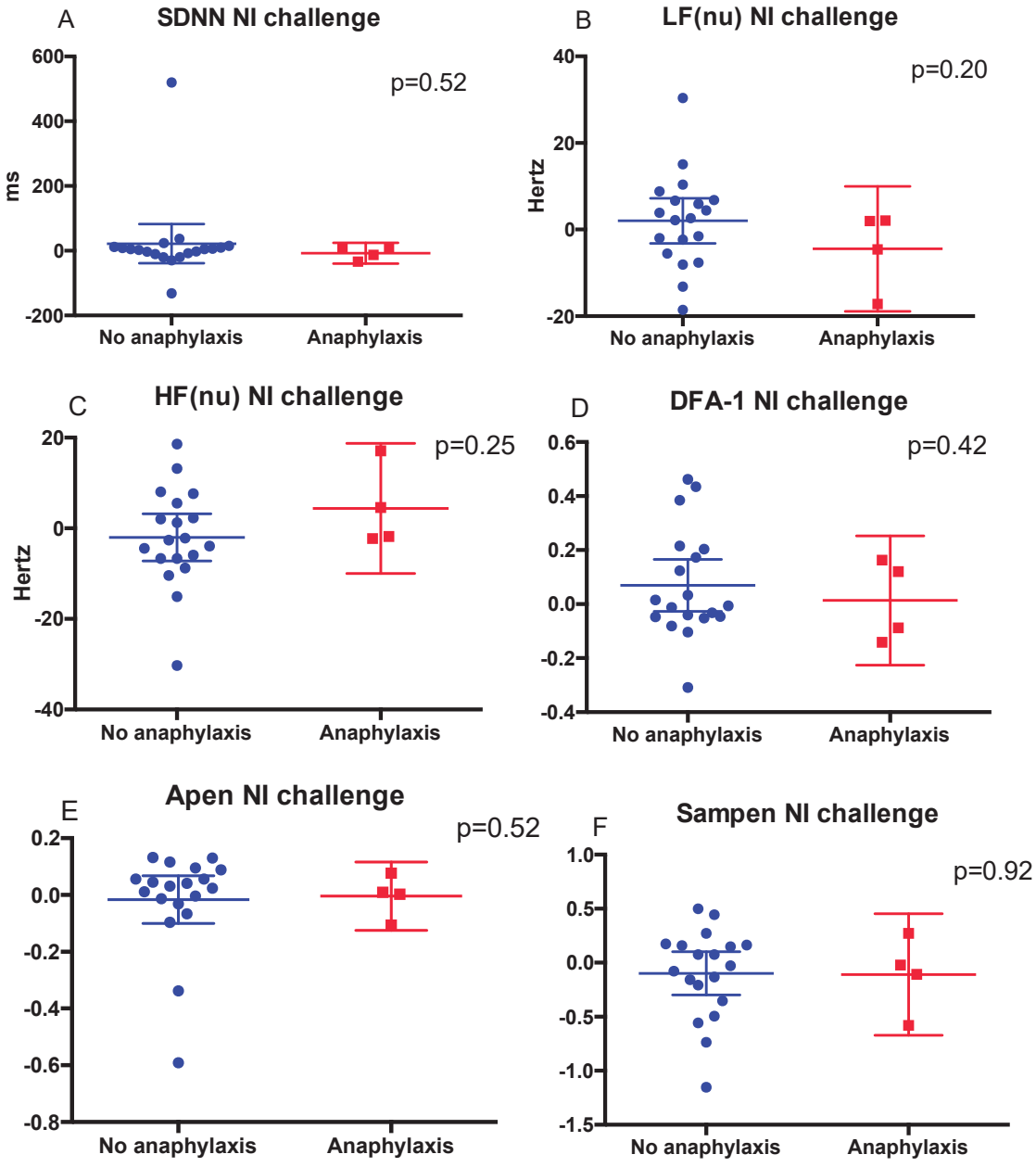
	VAS skin score	**VAS GI score**	**VAS upper resp score**	**VAS lower resp score**	**VAS overall score**
PR interval	r= -0.25 (p=0.07)	r=0.05 (p=0.75)	r= -0.06 (p=0.67)	r= -0.03 (p=0.85)	r= -0.16 (p=0.27)
QRS complex	r=0.06 (p=0.70)	r= -0.15 (p=0.28)	r=0.13 (p=0.38)	r= -0.25 (p=0.08)	**r= -0.37 (p=0.007)**
Automated QTc interval	r= -0.12 (p=0.39)	r= -0.26 (p=0.06)	r=0.04 (p=0.75)	r=0.04 (p=0.79)	r= -0.17 (p=0.24)
SDNN	r=0.15 (p=0.31)	r= -0.02 (p=0.89)	r=0.03 (p=0.83)	r= -0.12 (p=0.43)	r= -0.02 (p=0.82)
LF (n.u.)	r=0.003 (p=0.98)	r=0.21 (p=0.15)	r= -0.09 (p=0.52)	r= -0.06 (p=0.67)	r= -0.06 (p=0.67)
HF (n.u.)	r=0.07 (p=0.60)	r= -0.21 (p=0.13)	r=0.09 (p=0.52)	r=0.07 (p=0.67)	r=0.05 (p=0.70)
Apen	**r=0.29 (p=0.04)**	r=0.13 (p=0.37)	r= -0.22 (p=0.13)	r=0.04 (p=0.78)	r=0.05 (p=0.72)
Sampen	r=0.27 (p=0.06)	r=0.01 (p=0.93)	r= -0.15 (p=0.29)	r= -0.03 (p=0.85)	r= -0.04 (p=0.78)
DFA-1	r= -0.06 (p=0.68)	**r=0.41 (p=0.003)**	r=0.02 (p=0.87)	r=0.19 (p=0.18)	r=0.22 (p=0.13)

Spearman correlation. Highlighted p<0.05.

Appendix 21: HRV parameters and severity of reaction on repeated NI challenge.

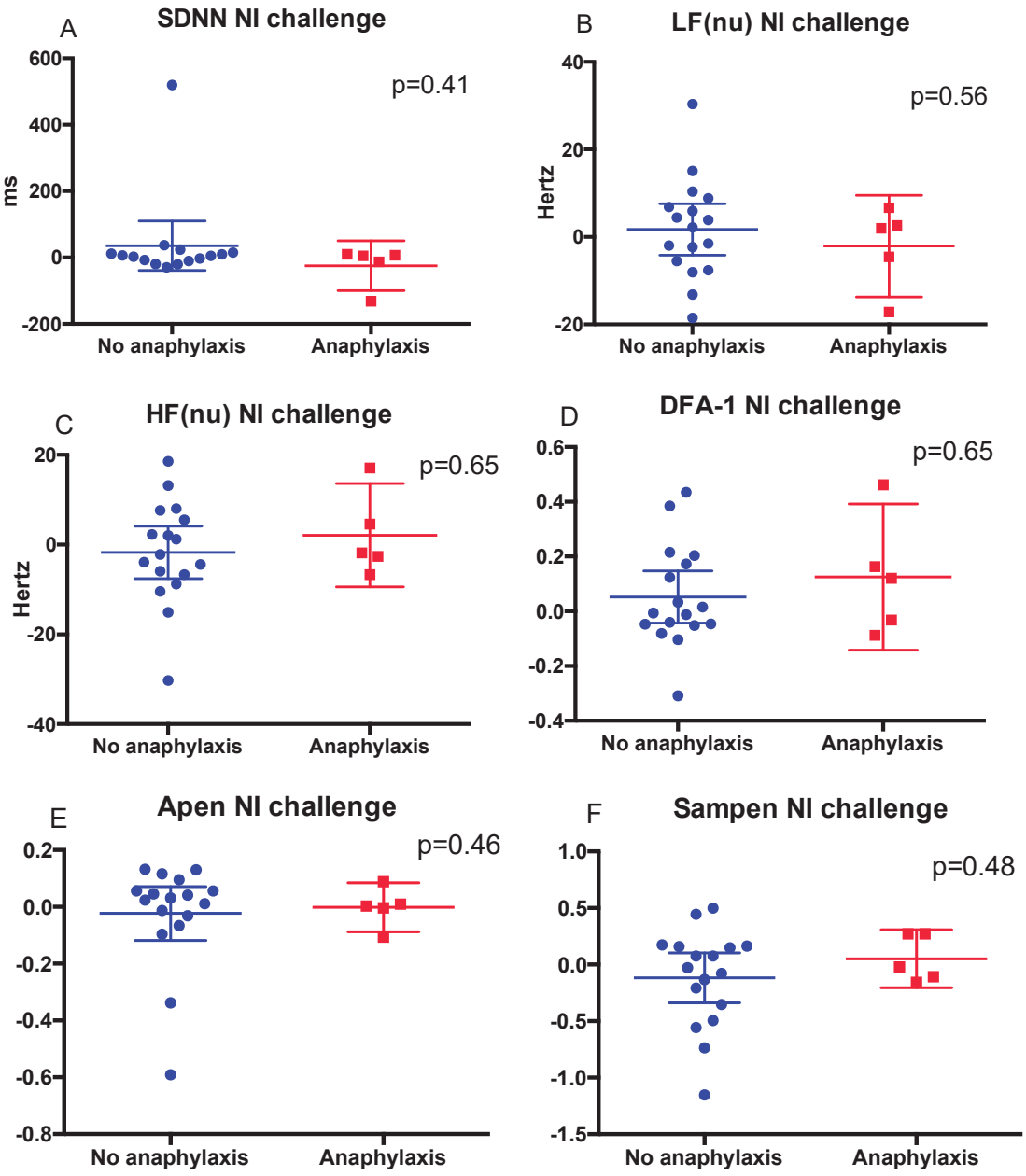
Figures 8-11 show differences between HRV parameters between those participants who required IM adrenaline as rescue medication, those classified as having anaphylaxis and those who did not and those classified as having moderate reactions compared to those classified as having severe reactions.

Figure 8 Change in HRV parameters according to the use of IM adrenaline:



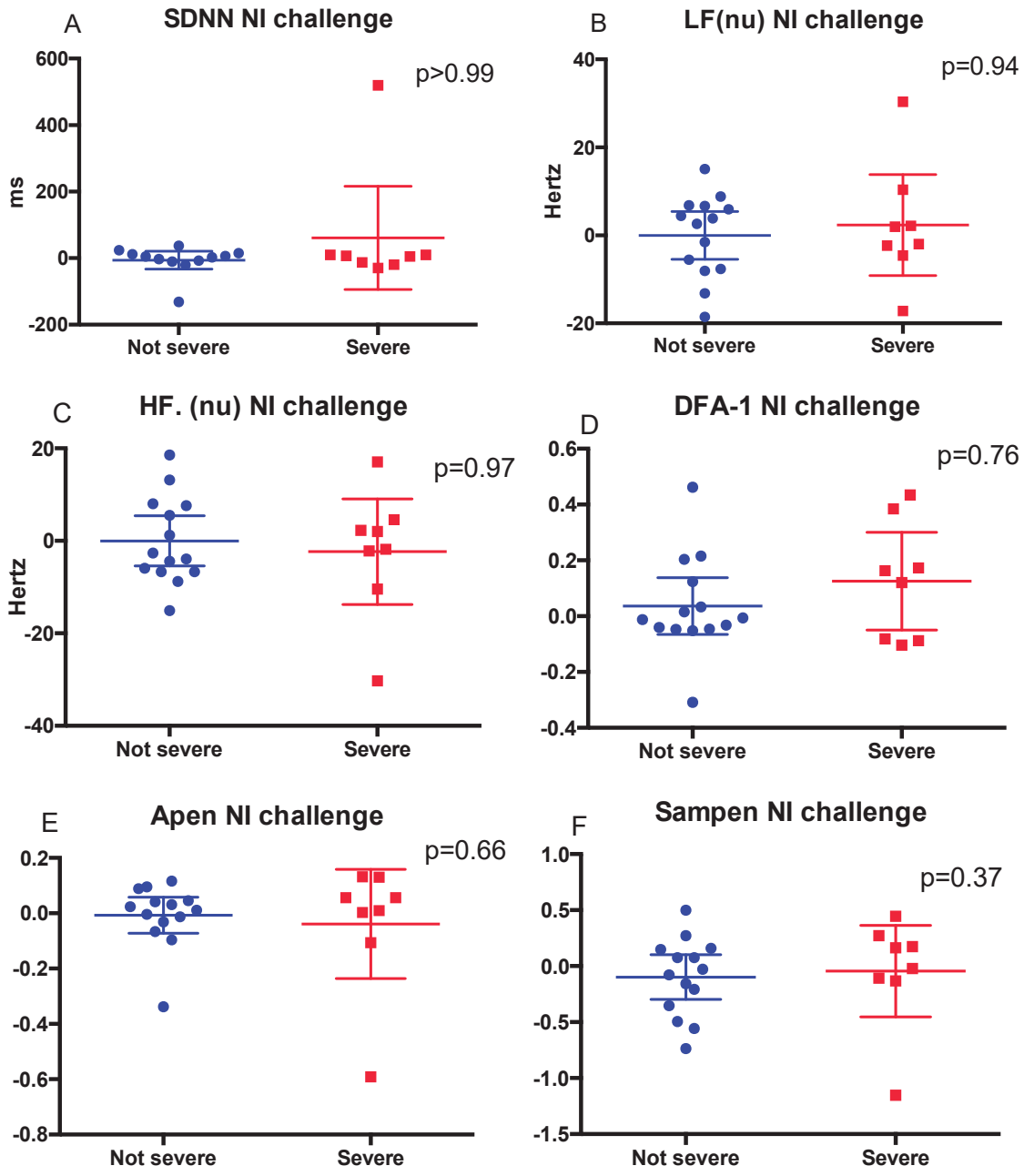
Changes in (A) SDNN, (B) L.F.(nu), (C) H.F.(un), (D) DFA-1, (E) Apen and (F) Sampen.

Figure 9 Change in HRV parameters according to NIAID classification of anaphylaxis:



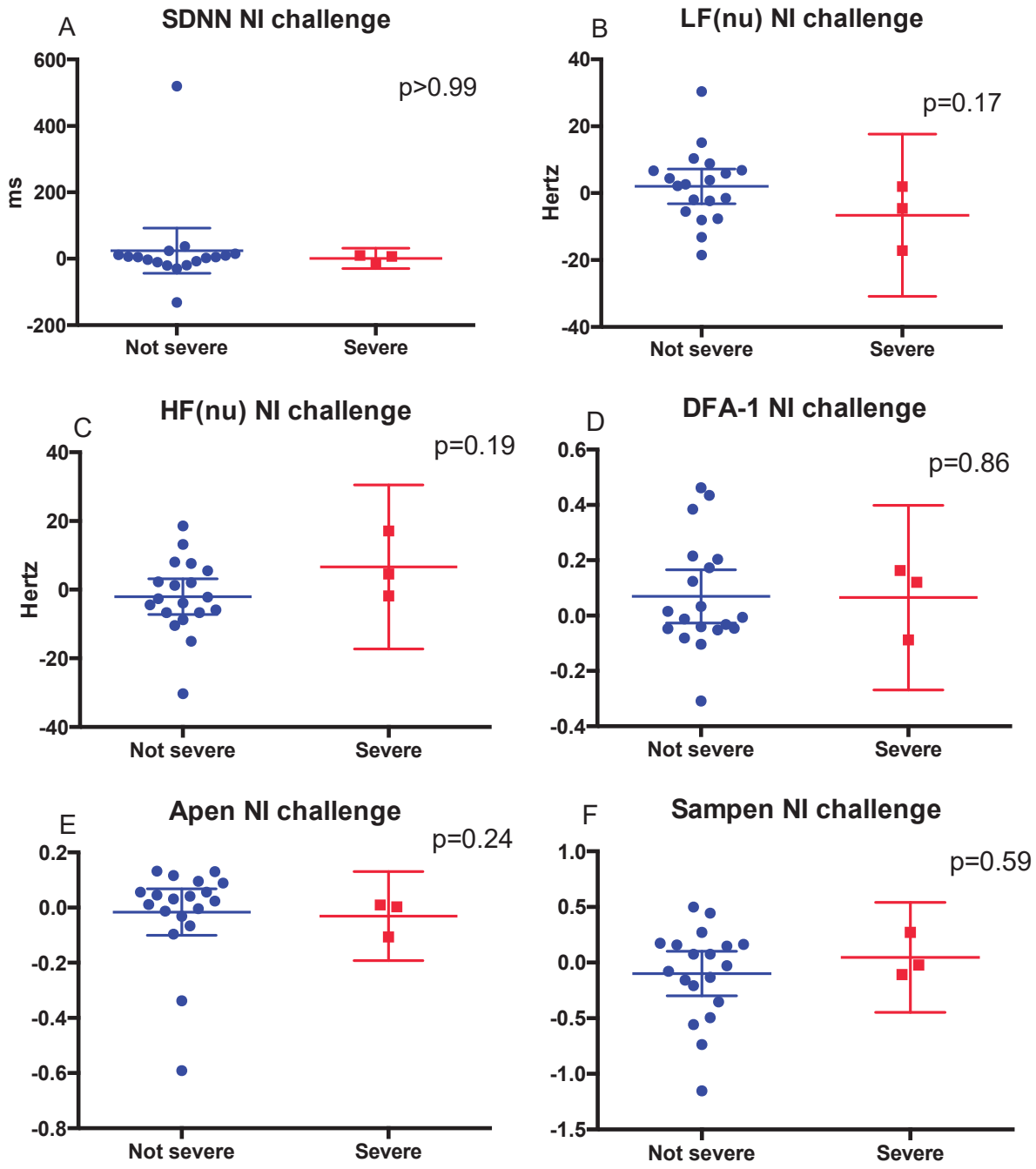
Changes in (A) SDNN, (B) L.F.(nu), (C) H.F.(un), (D) DFA-1, (E) Apen and (F) Sampen.

Figure 10 Change in HRV parameters according to Ewan&Clark classification:



Changes in (A) SDNN, (B) L.F.(nu), (C) H.F.(un), (D) DFA-1, (E) Apen and (F) Sampen.

Figure 11 Change in HRV parameters and WAO classification of severity:



Changes in (A) SDNN, (B) L.F.(nu), (C) H.F.(un), (D) DFA-1, (E) Apen and (F) Sampen.

Appendix 22: Relationship between HRV and VAS score on repeated NI challenge.

Table 7 show correlation between HRV parameters and participant´s VAS score and Table 8 show correlation between HRV and investigator´s VAS score on repeated NI challenges.

Table 7: Relationship between HRV parameters and participant´s VAS score on repeated NI challenge:

	VAS skin score	**VAS GI score**	**VAS upper resp score**	**VAS lower resp score**	**VAS anxiety score**	**VAS overall score**
SDNN	r=0.17 (p=0.56)	r=0.08 (p=0.73)	r=-0.35 (p=0.11)	r=0.03 (p=0.88)	r=0.21 (p=0.35)	r= -0.22 (p=0.33)
LF (n.u.)	r= -0.07 (p=0.77)	r=0.04 (p=0.85)	r= -0.33 (p=0.13)	r= -0.17 (p=0.45)	r= -0.19 (p=0.40)	r= -0.30 (p=0.18)
HF (n.u.)	r=0.06 (p=0.78)	r= -0.04 (p=0.85)	r=0.32 (p=0.14)	r=0.17 (p=0.46)	r=0.20 (p=0.37)	r=0.29 (p=0.18)
Apen	r=0.24 (p=0.29)	r= -0.35 (p=0.11)	r=0.12 (p=0.61)	r= -0.05 (p=0.84)	r= -0.05 (p=0.84)	r= -0.06 (p=0.69)
Sampen	r=0.20 (p=0.36)	**r= -0.47 (p=0.03)**	r=0.27 (p=0.23)	r=0.09 (p=0.68)	r= -0.03 (p=0.90)	r= -0.04 (p=0.78)
DFA-1	r= -0.003 (p=0.99)	r=0.22 (p=0.32)	r=0.01 (p=0.95)	r=0.19 (p=0.18)	r= -0.22 (p=0.32)	r=0.09 (p=0.70)

Spearman correlation. Highlighted p<0.05.

Table 8: Relationship between HRV parameters and investigator´s VAS score on repeated NI challenge:

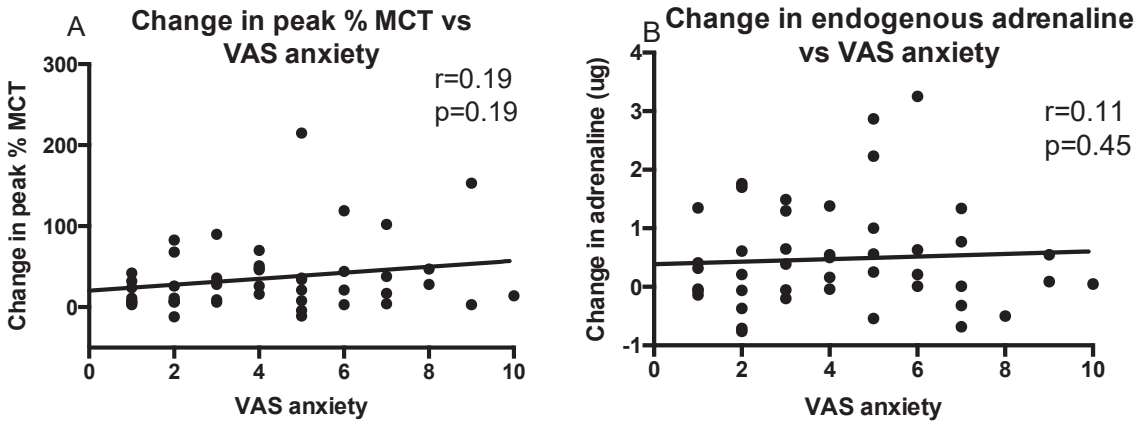
	VAS skin score	**VAS GI score**	**VAS upper resp score**	**VAS lower resp score**	**VAS overall score**
SDNN	r=0.28 (p=0.21)	r=0.09 (p=0.68)	r=-0.09 (p=0.68)	r=0.05 (p=0.83)	r= -0.32 (p=0.15)
LF (n.u.)	r=0.04 (p=0.86)	r=0.40 (p=0.07)	r= -0.13 (p=0.56)	r= -0.15 (p=0.50)	r= -0.16 (p=0.47)
HF (n.u.)	r= -0.04 (p=0.87)	r= -0.40 (p=0.06)	r=0.14 (p=0.54)	r=0.15 (p=0.50)	r=0.16 (p=0.49)
Apen	r= -0.07 (p=0.77)	**r= -0.52 (p=0.01)**	r= -0.03 (p=0.88)	r= -0.08 (p=0.71)	r= -0.007 (p=0.98)
Sampen	r=0.14 (p=0.53)	r= -0.40 (p=0.06)	r=0.37 (p=0.07)	r=0.07 (p=0.75)	r=0.39 (p=0.07)
DFA-1	r=0.09 (p=0.69)	r=0.42 (p=0.06)	r=0.07 (p=0.72)	r=0.04 (p=0.86)	r=0.13 (p=0.57)

Spearman correlation. Highlighted p<0.05.

Appendix 23: Relationship between anxiety and tryptase and adrenaline levels.

Figure 12 shows no correlation between VAS score for anxiety and laboratory measurements.

Figure 12 Relationship between mediators and anxiety:

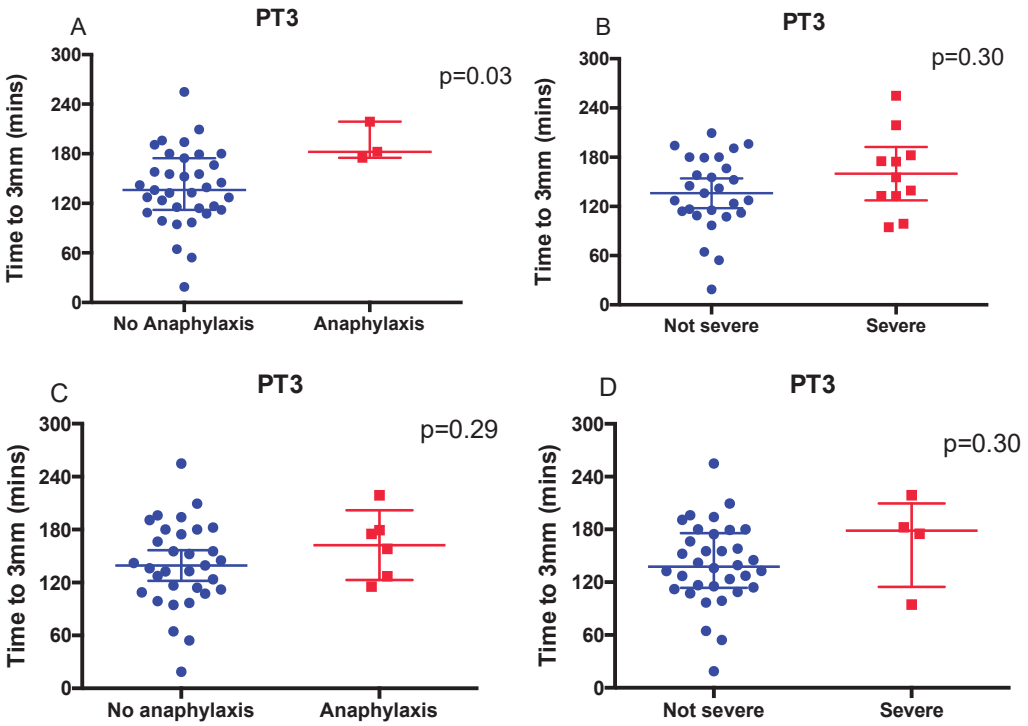


Spearman correlation between participant´s VAS score for anxiety and (A) change in endogenous adrenaline and (B) peak % change in MCT on active baseline challenge.

Appendix 24: Difference in PT3 measurement and severity of reaction on baseline challenge.

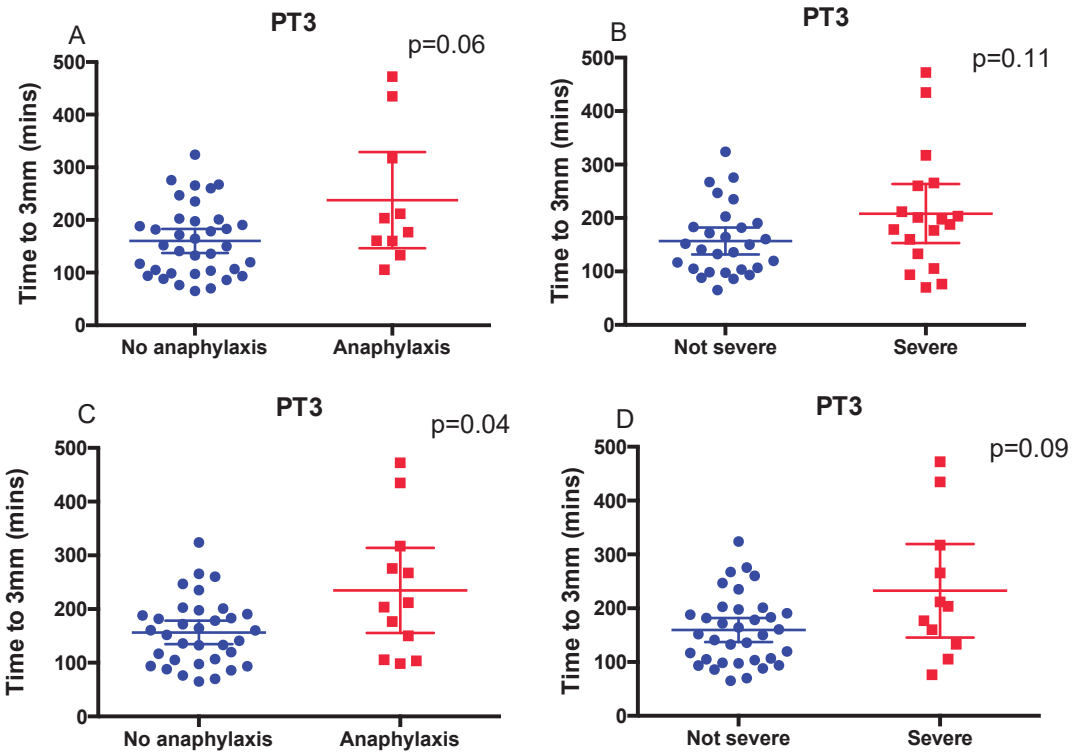
A significant difference was found between those participants who required IM adrenaline and those who didn´t when only measures of PT3 reached before OCR were included in the analysis shown in Figure 13. A significant difference was found for NIAID classification of anaphylaxis on active day for PT3 measurements on the same participants placebo day shown in Figure 14.

Figure 13 Difference in PT3 and measures of reaction severity:



Change in PT3 according to the use of IM adrenaline (A), Ewan&Clark classification of FA reaction (B), NIAID classification of anaphylaxis (C) and WAO classification of severity (D).

Figure 14 Difference in PT3 on placebo days and reaction severity classification on active day:



Change in PT3 according to the use of IM adrenaline (A), Ewan&Clark classification of FA reaction (B), NIAID classification of anaphylaxis (C) and WAO classification of severity (D).

Appendix 25: Relationship between SPT measurements and VAS score on baseline challenge.

A significant relationship was found between PC3 measurement and investigator´s VAS overall score and between PT3 measurement and investigator´s VAS lower respiratory and overall score shown in Table 9.

Table 9: Relationship between measurements of SPT and investigator´s VAS score on baseline challenge:

	VAS skin	**Vas GI**	**VAS upper respiratory**	**VAS lower respiratory**	**VAS overall**
PMAX	r= -0.14 (p=0.31)	r=0.14 (p=0.32)	r=0.13 (p=0.35)	r=0.07 (p=0.63)	r=0.03 (p=0.81)
PC3	r= -0.19 (p=0.16)	r= -0.008 (p=0.95)	r= -0.18 (p=0.19)	r= -0.18 (p=0.19)	**r= -0.27 (p=0.04)**
PT3	r=0.19 (p=0.20)	r= -0.09 (p=0.55)	r= 0.05 (p=0.76)	**r=0.30 (p=0.04)**	**r=0.35 (p=0.02)**

Spearman correlation. Highlighted p<0.05.

Appendix 26: Relationship between SPT measurements and cardiac parameters analysed.

No correlation was found between CVS, HRV parameters and SPT measurements shown in Table 10.

Table 10: Relationship between CVS, HRV and SPT measurements:

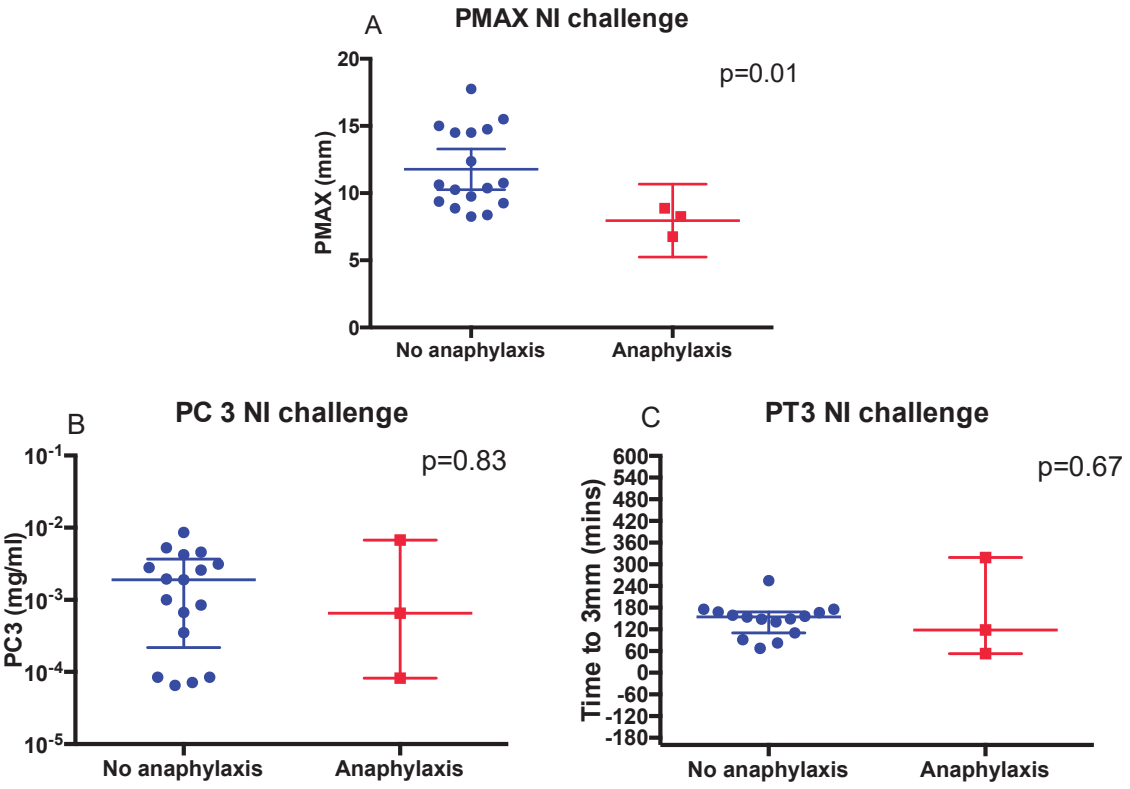
	SV	**HR**	**sBP**	**dBP**	**LF (n.u)**	**HF (n.u.)**	**DFA-1**	**Apen**	**Sampen**
PMAX	r=0.02 (p=0.88)	r=0.10 (p=0.47)	r=0.003 (p=0.99)	r=0.05 (p=0.72)	r=0.04 (p=0.80)	r= -0.03 (p=0.86)	r=0.01 (p=0.94)	r=0.22 (p=0.13)	r=0.08 (p=0.60)
PT3	r= -0.26 (p=0.09)	r=0.14 (p=0.38)	r=0.01 (p=0.92)	r= -0.20 (p=0.19)	r= -0.03 (p=0.85)	r=0.03 (p=0.87)	r= -0.005 (p=0.98	r=0.15 (p=0.35)	r= -0.10 (p=0.55)
PC3	r=0.04 (p=0.77)	r= -0.21 (p=0.14)	r=0.10 (p=0.47)	r=0.18 (p=0.21)	r= -0.10 (p=0.48)	r=0.11 (p=0.46)	r= -0.17 (p=0.24)	r= -0.15 (p=0.29)	r=0.06 (p=0.71)

Spearman correlation.

Appendix 27: Difference in SPT measurements and reaction severity on repeated NI challenge.

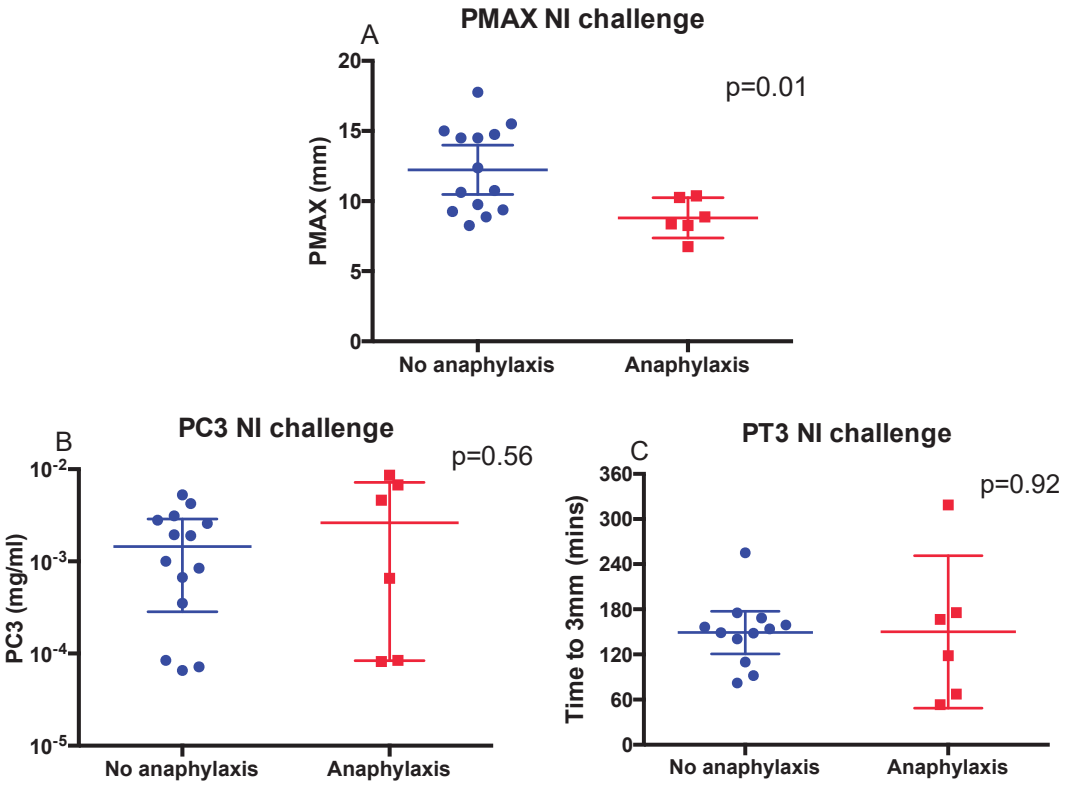
Difference between SPT on repeated NI challenges and severity of reaction are shown on Figure 15-18.

Figure 15 Differences in measures of SPT according to the use of IM adrenaline:



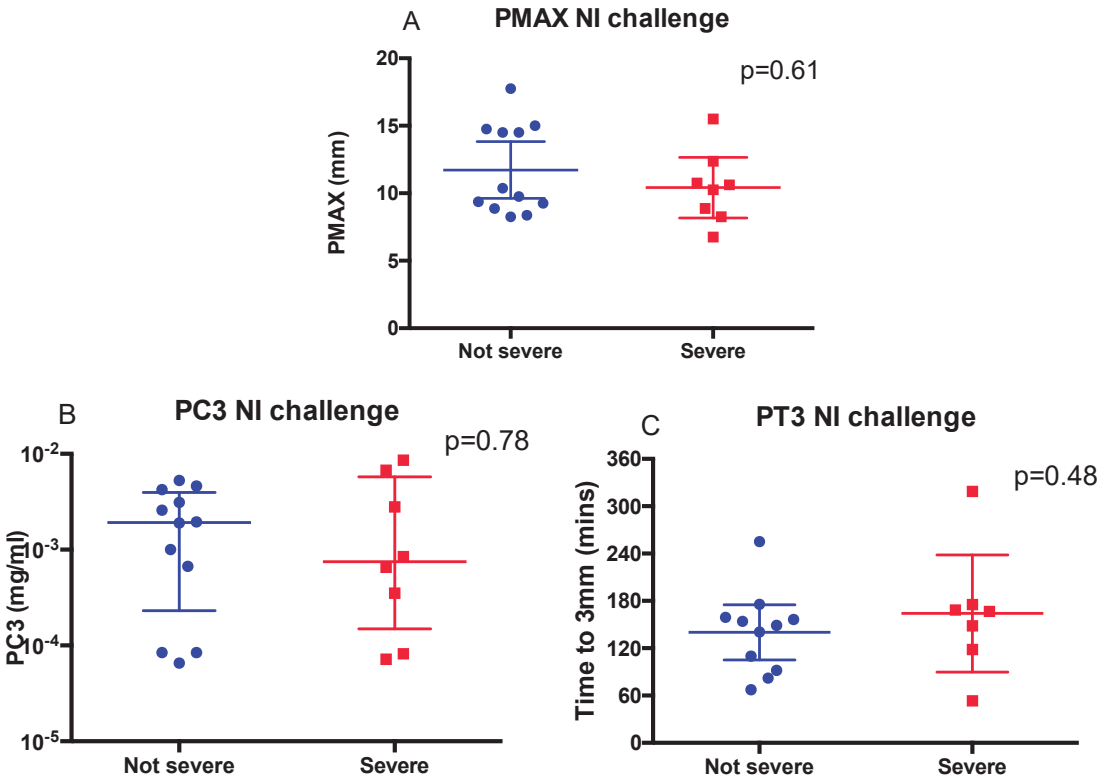
Change in (A) PMAX, (B) PC3 and (C) PT3.

Figure 16 Differences in measures of SPT according to NIAID classification of anaphylaxis:



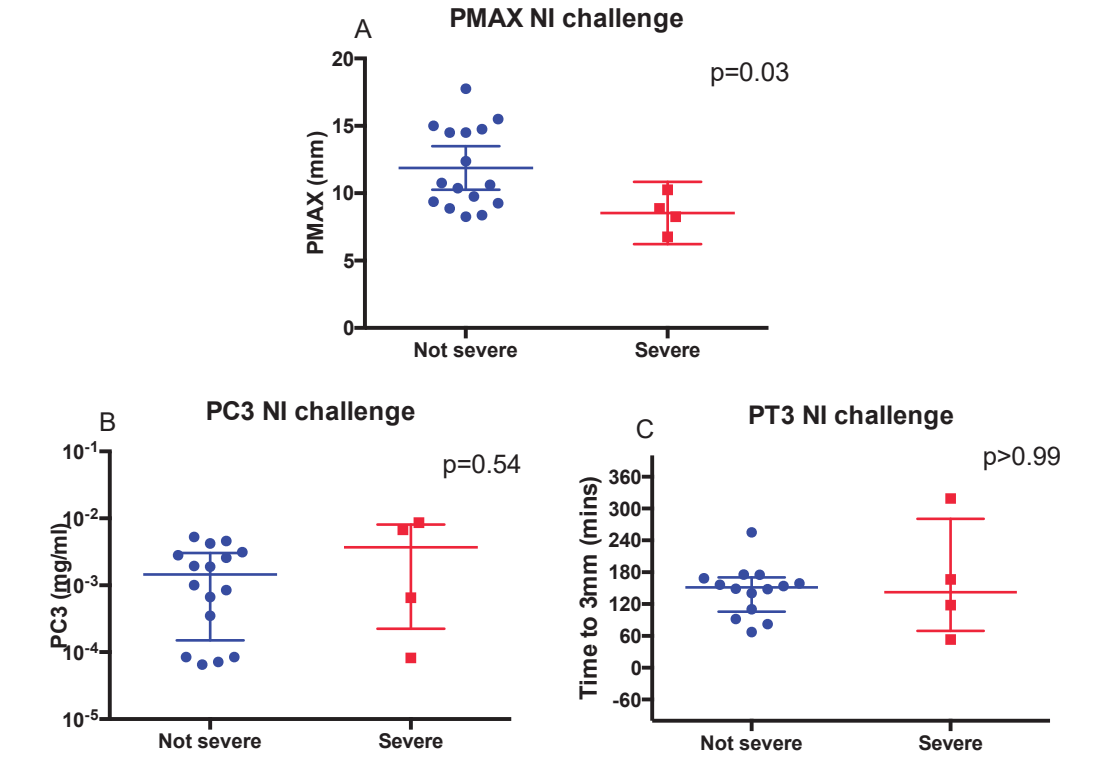
Change in (A) PMAX, (B) PC3 and (C) PT3.

Figure 17 Differences in measures of SPT according to Ewan&Clark classification of food allergic reactions:



Change in (A) PMAX, (B) PC3 and (C) PT3.

Figure 18 Differences in measures of SPT according to WAO classification:



Change in (A) PMAX, (B) PC3 and (C) PT3.

Appendix 28: Bivariate analyses in repeated NI challenges.

Table 11 shows bivariate regression between the dependent and independent variables, using bootstrapping for all analyses with a non-binary dependent variable. For measures of severity of reaction, the most consistent association was with PMAX and age, participants who required IM adrenaline, were classified as having anaphylaxis or severe reaction, or whose reaction was rated as more severe by investigator, had smaller SPT wheal (PMAX) and a younger age, although this association was not found in all measures of severity.

Table 11: Bivariate analysis in repeated NI challenge:

	Cum. Dose	**NIAID**	**E&C (binary)**	**WAO (binary)**	**Use of IM adrenaline**	**Participant´s VAS overall score**	**Investigator´s VAS overall score**
Cum. Dose		Anaph 144 (43, 283) No anaph 269 (102, 512) p=0.35	Severe 133 (53, 258) Not Severe 288 (112, 553) p=0.27	Severe 183 (33, 433) Not Severe 240 (104, 457) p=0.67	Adr 200 (33, 433) No Adr 233 (101, 435) p=0.84	r= -0.13 (p=0.59)	r= -0.07 (p=0.78)
PMAX (mm)	r= -0.10 (p=0.66)	**Anaph 9 (8, 10)** **No Anaph 12 (10, 14)** **p=0.009**	Severe 10 (8, 12) Not Severe 11 (10, 13) p=0.38	**Severe 9 (7, 10)** **Not Severe 12 (10, 13)** **p=0.02**	**Adr 8 (7, 9)** **No Adr 12 (10, 13)** **p=0.005**	r= -0.32 (p=0.30)	**r= -0.47** **(p=0.043)**
PT3 (mins)	**r=-0.53** **(p=0.02)**	Anaph 150 (81, 232) No Anaph 158 (130, 189) p=0.87	Severe 164 (108, 227) Not Severe 149 (115, 185) p=0.68	Severe 164 (53, 319) Not Severe 152 (123, 181) p=0.85	Adr 164 (53, 319) No Adr 153 (127, 182) p=0.91	r= -0.01 (p=0.97)	r= -0.14 (p=0.59)
Sex (female)	M 300 (73, 685) F 156 (87, 240) p=0.43	Anaph 4/6 (67%) No Anaph 8/14 (57%) p=0.69	Severe 5/8 (63%) Not Severe 7/12 (58%) p=0.85	Severe 2/4 (50%) Not Severe 10/16 (63%) p=0.65	Adr 2/3 (67%) No Adr 10/17 (59%) p=0.79	M 5.5 (4.7, 6.2) F 5.7 (5.1, 6.3) p=0.63	M 5.9 (5.4, 6.4) F 5.9 (5.5, 6.2) p=0.31
Age (years)	r= -0.28 (p=0.24)	Anaph 25 (20, 32) No Anaph 26 (23, 30) p=0.76	Severe 23 (20, 26) Not Severe 27 (23, 32) p=0.20	**Severe 21 (18, 24)** **Not Severe 27 (24, 31)** **P=0.01**	**Adr 20 (18, 24)** **No Adr 26 (23, 30)** **p=0.05**	r= -0.07 (p=0.79)	**r= -0.57** **(p=0.01)**
BHR (mg/ml)	r=0.03 (p=0.89)	Anaph 10.9 (6.1, 16) No Anaph 12.4 (8.1, 16) p=0.66	Severe 13.4 (9.8, 16) Not Severe 10.9 (6.4, 14.8) p=0.39	Severe 11.4 (3.6, 16) Not Severe 12 (8.3, 15.1) P=0.87	Adr 11.9 (3.6, 16) No Adr 11.9 (8.5, 14.9) p=0.99	r= -0.36 (p=0.13)	r= -0.24 (p=0.31)
PC3 log (mg/ml)	r=0.43 (p=0.059)	Anaph -6.9 (-8.6, -5.1) No Anaph -6.6 (-7.3, -6.0) p=0.79	Severe -6.8 (-8.0, -5.5) Not Severe -6.7 (-7.6, -5.9) p=0.89	Severe -6.6 (-9.4, -4.8) Not Severe -6.7 (-7.4, -6.1) p=0.93	Adr -7.2 (-9.4, -5.0) No Adr -6.6 (-7.3, -5.9) p=0.67	r= -0.17 (p=0.48)	r=0.11 (p=0.66)
Arah2 log (KU/L)	**r= -0.63** **(p=0.003)**	Anaph 1.8 (-0.09, 2.8) No Anaph 1.4 (0.3, 2.5) p=0.74	Severe 1.6 (-0.2, 3.1) Not Severe 1.5 (0.3, 2.4) p=0.87	Severe 1.4 (-2.7, 3.1) Not Severe 1.6 (0.6, 2.5) p=0.90	Adr 1.0 (-2.7, 3.1) No Adr 1.6 (0.7, 2.4) p=0.78	r=0.19 (p=0.43)	r=0.08 (p=0.74)

Spearman correlation analyses, chi-square and bootstrap t-test between dependent variables in columns and independent variables in rows. Highlighted $p<0.05$.

Bivariate analysis between PT3 including only those participants who reached a 3mm wheal before OCR and all the dependent variables is shown in Table 12. A significant relationship was found between PT3 in this dataset of participants the use of IM adrenaline and participant´s VAS overall score. Bivariate analysis between PT3 on placebo day and all the dependent variables is shown in Table 13. No association was found between this measurement of PT3 and threshold or severity of reaction.

Table 12: Bivariate analysis without those participants who's PT3 went over OCR and dependent variables:

	Cum. Dose	**NIAID**	**E&C (binary)**	**WAO (binary)**	**Use of IM adrenaline**	**Participant´s VAS overall score**	**Investigator´s VAS overall score**	**Plasma adrenaline (nmol/L)**	**Peak % MCT (%)**
PT3	r=0.22	Anaph 162 (129, 193)	Severe 160 (134, 190)	Severe 168 (111, 219)	**Anaph 192 (175, 219)**	**r=0.36**	r=0.12	r= -0.06	=0.03
	(p=0.19)	No Anaph 139 (123, 156)	Not Severe 136 (117, 153)	Not Severe 140 (124, 155)	**No Anaph 139 (123, 154)**	**(p=0.02)**	(p=0.49)	(p=0.74)	(p=0.88)
		(p=0.23)	(p=0.18)	(p=0.38)	**(p=0.03)**				

Spearman correlation and bootstrap t-test between dependent variables in columns and independent variable in row. Highlighted $p<0.05$.

Table 13: Bivariate analysis of PT3 on placebo day and all the dependent variables:

	Cum. Dose	**NIAID**	**E&C (binary)**	**WAO (binary)**	**Use of IM adrenaline**	**Participant´s VAS overall score**	**Investigator´s VAS overall score**	**Plasma adrenaline (nmol/L)**	**Peak % MCT (%)**
PT3	r= -0.14	Anaph 235 (165, 307)	Severe 208 (159, 261)	Severe 233 (162, 311)	Anaph 238 (164, 318)	r=0.24	r=0.22	r=0.12	= -0.0004
placebo	(p=0.36)	No Anaph 157 (137, 179)	Not Severe 157 (134, 182)	Not Severe 160 (139, 182)	No Anaph 160 (138, 183)	(p=0.10)	(p=0.16)	(p=0.44)	(p=0.99)
		(p=0.08)	(p=0.11)	(p=0.11)	(p=0.12)				

Spearman correlation analysis and bootstrap t-test between dependent variables in columns and independent variable in row.

Appendix 29: Multivariate analyses in repeated NI challenge.

Table 14 shows multivariate regression analyses between the dependent and independent variables, using bootstrapping for all analyses with a non-binary dependent variable. No association was found between by any of the independent variables and determinants of threshold or severity.

Table 14: Multivariate analysis in repeated NI challenge:

	Cum. Dose	**NIAID**	**E&C (binary)**	**WAO (binary)**	**Use of IM adrenaline**	**Participant´s VAS overall score**	**Investigator´s VAS overall score**
Cum. Dose		0.998 (0.993, 1.003) p=0.48	0.990 (0.97, 1.007) p=0.23	0.997 (0.991, 1.003) p=0.28	0.998 (0.993, 1.003) p=0.40	-0.001 (-0.011, 0.007) p=0.65	-0.001 (-0.013, 0.001) p=0.39
PMAX (mm)	22.4 (-13.2, 96.4) p=0.56	-	-	-	-	-	-
PT3 (mins)	-	-	0.978 (0.946, 1.011) p=0.185	0.983 (0.950, 1.018) p=0.35	0.987 (0.958, 1.018) p=0.41	0.000 (-0.023, 0.013) p=0.99	-0.004 (-0.017, 0.007) p=0.54
Sex (female)	-	-	-	-	-	-	-
Age (years)	-14.3 (-45.7, 3.8) p=0.46	0.925 (0.781, 1.095) p=0.37	0.555 (0.291, 1.057) p=0.07	0.424 (0.153, 1.177) p=0.09	0.481 (0.196, 1.182) p=0.11	0.019 (-0.380, 0.123) p=0.82	-0.199 (-0.305, 0.012) p=0.055
BHR (mg/ml)	-	-	1.460 (0.977, 2.180) p=0.065	-	-	-	-
Ln PC3	17.1 (-67.6, 72.8) p=0.54	-	-	-	-	-	-0.309 (-0.817, 0.248) p=0.42
Ln Ara h 2 (KU/L)	-88.3 (-196.9, -17.4) p=0.31	-	-	-	-	-	-
ANOVA	p=0.056					p=0.938	p=0.01
Omnibus test		p=0.473	p=0.035	p=0.04	p=0.113		
Hosmer and Lemeshow test		p=0.318	p=0.428	p=0.605	p=0.577		

Beta coefficients for continuous dependent variables, and Exp(B) for binary dependent variables, both with 95% confidence intervals. Dependent variables are shown as columns, and independent (explanatory) variables as rows. Highlighted p<0.05

Multivariate analysis for those dependent variables in which the new dataset and measurements of PT3 was significant or at least marginal significance is shown in Table 15 and 16.

Table 15: Multivariate analysis with measurements only of those participant´s in who´s OCR was after PT3.

	Use of IM adrenaline	**Pt VAS overall score**
Cum. Dose	1.004 (1.000, 1.007) (p=0.06)	0.002 (0.000, 0.004) (0.06)
PMAX (mm)	-	.
PT3 (mins)	1.050 (0.991, 1.111) (p=0.10)	0.009 (-0.004, 0.023) (p=0.24)
Sex (female)	.	.
Age (years)	0.911 (0.700, 1.185) (p=0.49)	-0.038 (-0.187, 0.096) (p=0.64)
BHR (mg/ml)	-	-
Ln PC3	-	-
Ln Ara h 2 (KU/L)	-	-

Beta coefficients for continuous dependent variables, and Exp(B) for binary dependent variables, both with 95% confidence intervals. Dependent variables are shown as columns, and independent (explanatory) variables as rows.

Table 16: Multivariate analysis for NIAID using PT3 measurements on placebo day:

	NIAID
Cum. Dose	1.001 (0.999, 1.003) (p=0.34)
PMAX (mm)	-
PT3 placebo (mins)	**1.011 (1.002, 1.020) (p=0.018)**
Sex (female)	.
Age (years)	1.035 (0.933, 1.147) (p=0.52)
BHR (mg/ml)	-
Ln PC3	-
Ln Ara h 2 (KU/L)	-

Beta coefficients for continuous dependent variables, and Exp(B) for binary dependent variables, both with 95% confidence intervals. Dependent variables are shown as columns, and independent (explanatory) variables as rows. Highlighted $p<0.05$.