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Determinants of Severe and Fatal Anaphylaxis

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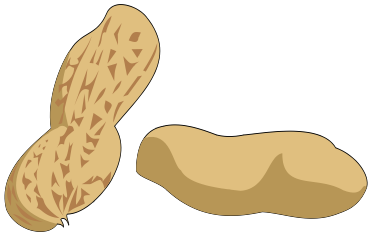


The Problem

Peanut … the most common cause of fatal
and near-fatal allergic reactions in the U.S. and U.K.

Estimated prevalence of peanut allergy
as high as 1 – 2% of 4-year old children

Peanut allergy starts early

Peanut is a very potent allergen

Peanut allergy only rarely remits

The Problem

Most peanut allergic individuals have only
mild to moderate symptoms upon ingestion.

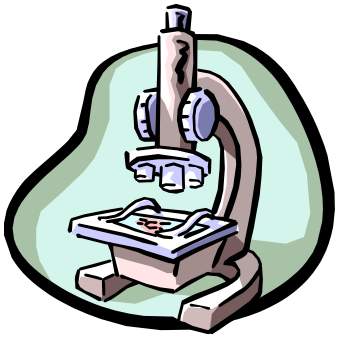
However … there are no reliable means of stratifying peanut-sensitive individuals to reliably identify those at risk for potentially life-threatening reactions.

All peanut-allergic individuals are required to exercise tremendous vigilance in avoiding exposure.

This is difficult, stressful and sometimes unnecessary.

The Problem

- diagnosis of peanut allergy may be very disruptive in the home / school environment
- extraordinary precautions are often required at home, in restaurants, schools or daycare environments
- most peanut-allergic individuals at risk for only minor reactions and only a small subset at risk for life-threatening reactions



Background

- ✓ the cells largely responsible for anaphylaxis are mast cells
- ✓ chemicals released from mast cells include histamine, tryptase, leukotrienes, prostaglandin D2 and platelet activating factor (PAF)
- ✓ release of these chemical mediators is responsible for manifestations of anaphylaxis

Background: PAF

Platelet Activating Factor

- the most potent lipid mediator identified
- active at concentrations as low as 10^{-12} M
- biologic effects of PAF include
  - vascular permeability
  - decreased cardiac output
  - smooth muscle contraction (gut, uterus and airways)
  - circulatory collapse

Platelet Activating Factor

PAF: 1-0-alkyl-2-acetyl-sn-glycero-3-phosphocholine.
Biologically active phospholipid, active at concentrations as low as 10^{-12} M.

Actions

- signalling and activation of proinflammatory cells
- increased vascular permeability
- bronchospasm
- pulmonary hypertension
- death

Sources

- mast cells
- platelets
- leukocytes
- endothelial cells

Background: PAF

PAF Receptor Antagonists

Drugs that block PAF

- WEB 2170 prevented death due to anaphylaxis in ovalbumin-sensitized mice
- WEB 2086 prevented anaphylactic death in rabbits

PAF is produced by mast cells during anaphylaxis and is capable of reproducing manifestations of anaphylaxis. PAF receptor antagonists protect against death in animal models of anaphylaxis.

Effects of PAF Antagonists on Systemic Anaphylaxis

- WEB 2170 pretreatment prevented death due to anaphylaxis in ovalbumin-sensitized mice.
- WEB 2086 pretreatment of rabbits inhibited anaphylactic alterations in right ventricular pressure, total pulmonary resistance, and decrease in dynamic compliance
- WEB 2086 ablated the anaphylactic increase in pulmonary resistance in rabbits.

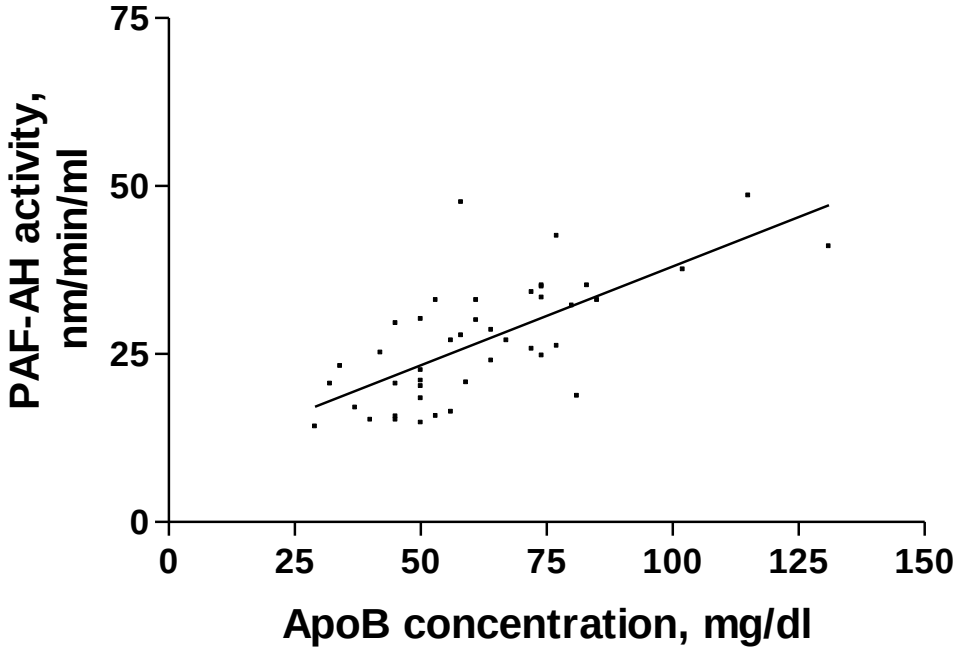
PAF Receptor Knock-Out

- Mice developed lacking PAF receptor
- PAF receptor knock-out mice resistant to the effects of exogenous authentic PAF
- PAF receptor knock-out mice resistant to anaphylaxis

Platelet Activating Factor Acetylhydrolase (PAF-AH)

- PAF is degraded to a biologically inactive form by enzyme PAF-AH
- PAF-AH circulates as a complex with cholesterol particle LDL
- PAF-AH activity in plasma is strongly correlated with the plasma concentration of LDL

Scatterplot of plasma PAF-AH activity and ApoB concentration



n=43

Effect of Human PAF-AH in Two Anaphylactic Shock Models

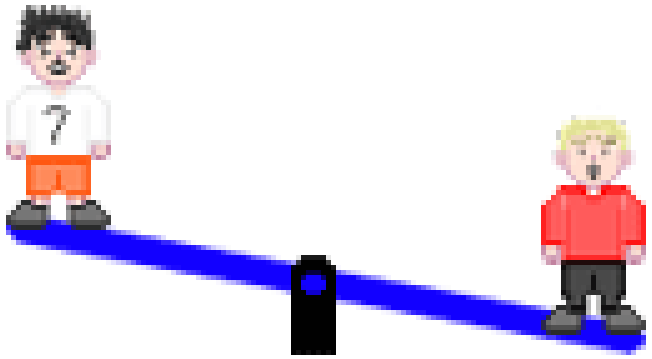
Fukuda Y et al *Eur J Pharmacol* 2000;390(1-2):203-207

Examined effects of recombinant human PAF-AH in 2 mouse models of anaphylaxis

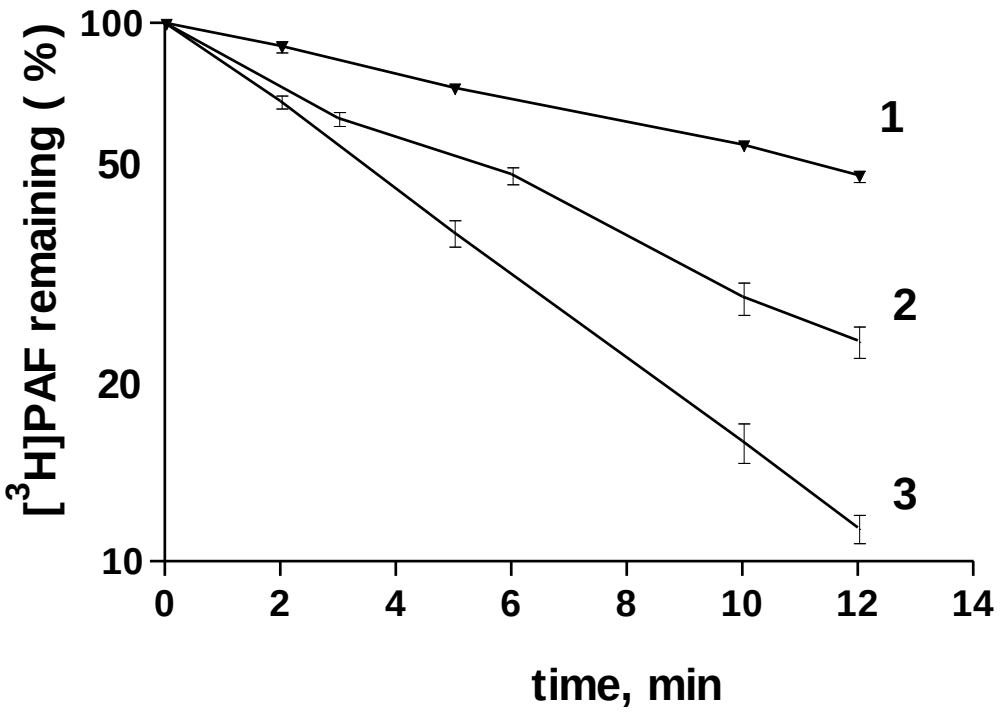
- in a mouse model of PAF-induced death, infusion of PAF-AH reduced mortality in a dose-dependent manner, with complete prevention of mortality at 1.0 mg/kg
- in a mouse model of anaphylactic shock to bovine serum albumin, treatment with PAF-AH reduced mortality in a dose-dependent manner with significant protection at 1.0 mg/kg

Hypothesis

- Patients with lower levels of PAF-AH will breakdown PAF more slowly during acute anaphylactic reactions
- As PAF is produced during acute anaphylaxis, higher levels (as a result of slower inactivation) will lead to more severe physiologic effects

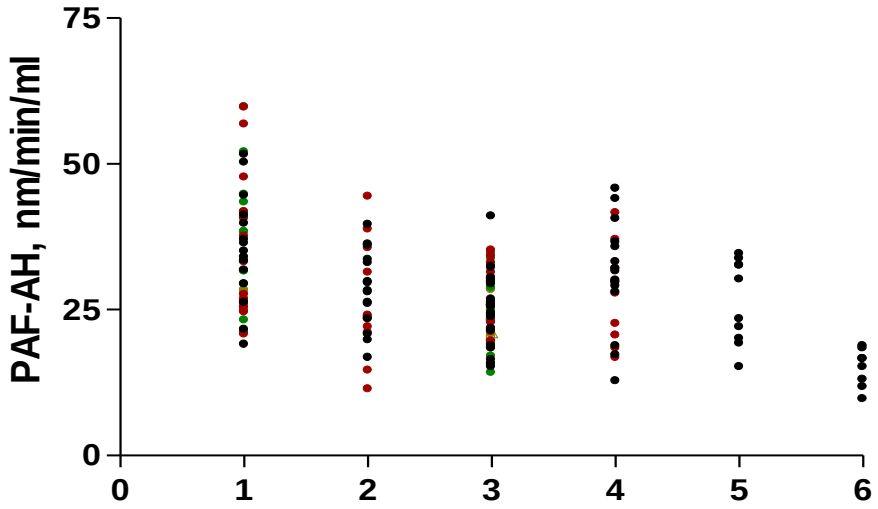


Metabolism of PAF in human serum



1 - PAF-AH = 7.8 nmol/min/ml
2 - PAF-AH = 24.8 nmol/min/ml
3 - PAF-AH = 48.6 nmol/min/ml

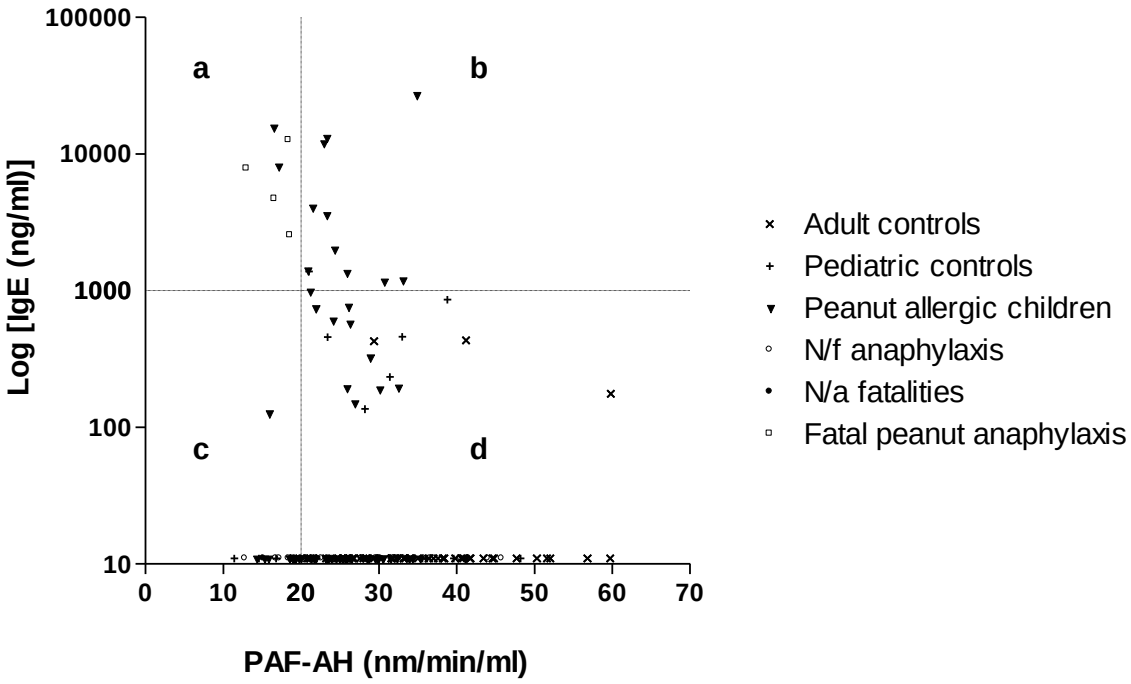
Relationship of PAF-AH to Fatal Peanut Anaphylaxis



1 - Adult controls (49)
2 - Pediatric controls (26)
3 - Peanut allergic children (63)
4 - Non-fatal peanut anaphylaxis (24)
5 - Non-anaphylactic fatalities (10)
6 - Fatal peanut anaphylaxis (7)

- Those individuals with normal levels of PAF-AH had low risk of anaphylaxis

- Those individuals who were deficient in PAF-AH were at high risk of fatal anaphylaxis



a : PAF-AH < 20 nm/min/ml; IgE >1000 ng/ml

b : PAF-AH > 20 nm/min/ml; IgE >1000 ng/ml

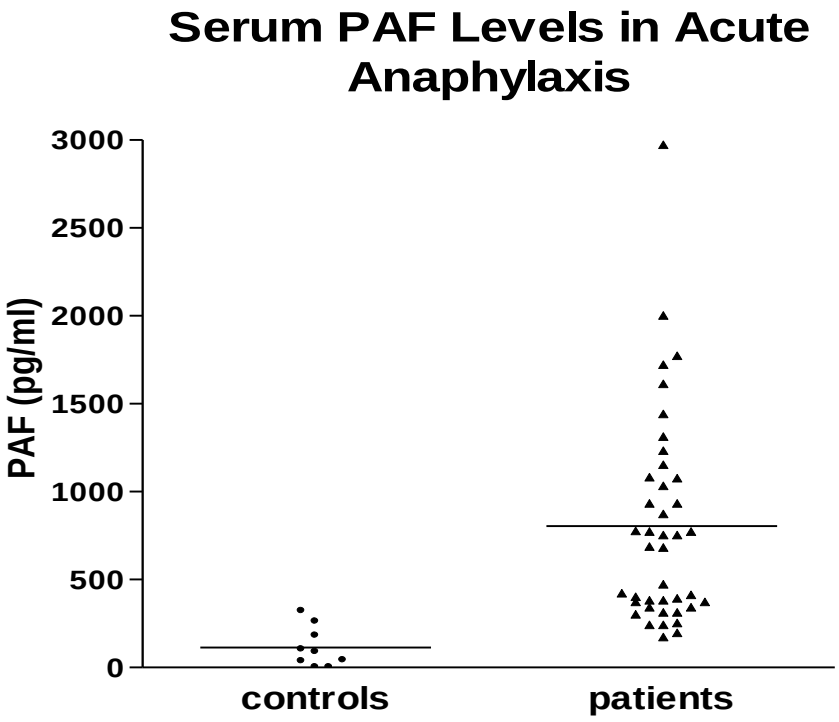
c : PAF-AH < 20 nm/min/ml; IgE <1000 ng/ml

d : PAF-AH > 20 nm/min/ml; IgE <1000 ng/ml

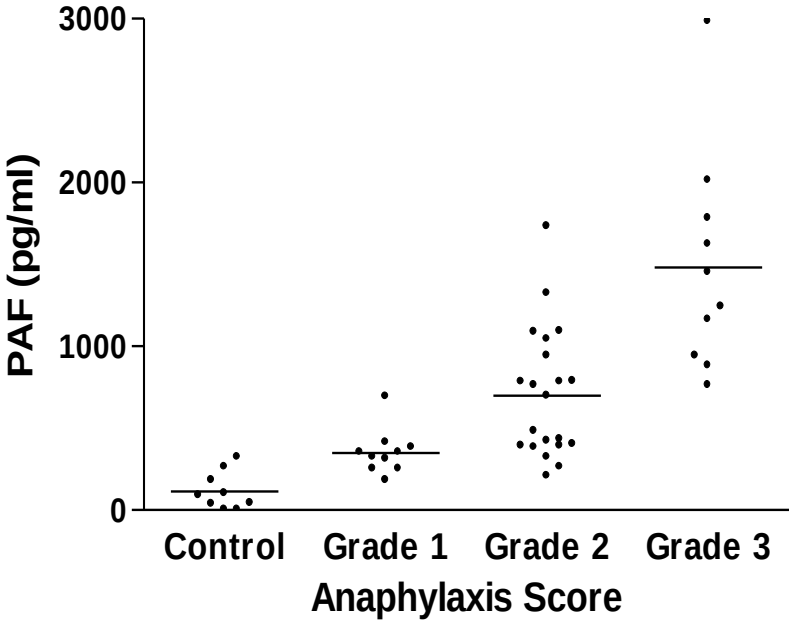
Summary

- Mean serum PAF-AH activity in patients with fatal peanut anaphylaxis was significantly lower than in
  - patients with non-fatal peanut reactions
  - patients who died of causes unrelated to allergy / anaphylaxis
  - healthy pediatric and adult control groups

- Low PAF-AH activity was a necessary but not sufficient factor predisposing to fatal peanut anaphylaxis. High levels of peanut-specific IgE were a requisite co-factor.


Serum PAF Levels in Acute Anaphylaxis
PAF (pg/ml)
3000
2500
2000
1500
1000
500
0
controls
patients

Relationship between Anaphylaxis Score and PAF Levels



According to S.G.A. Brown, J Allergy Clin Immunol 2004;114:371-6

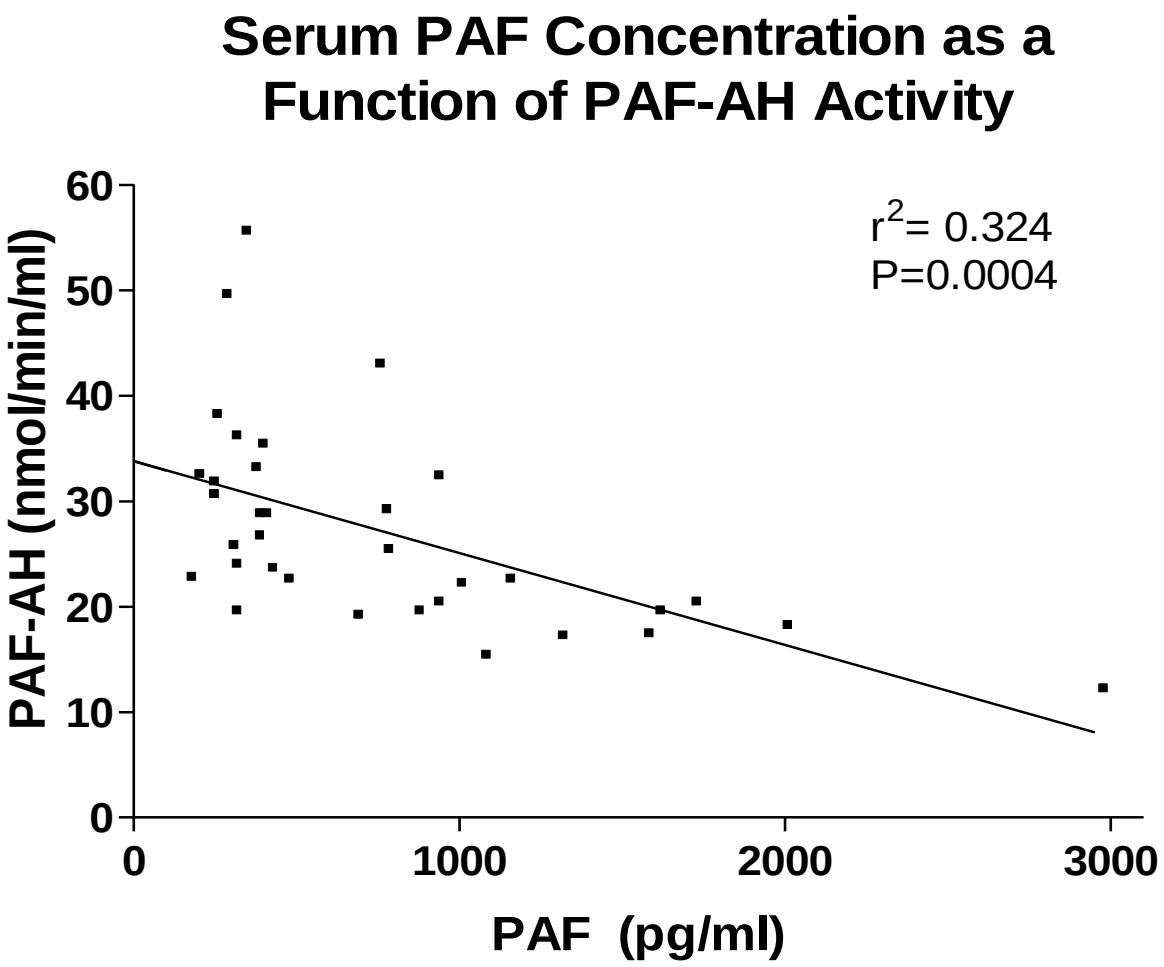
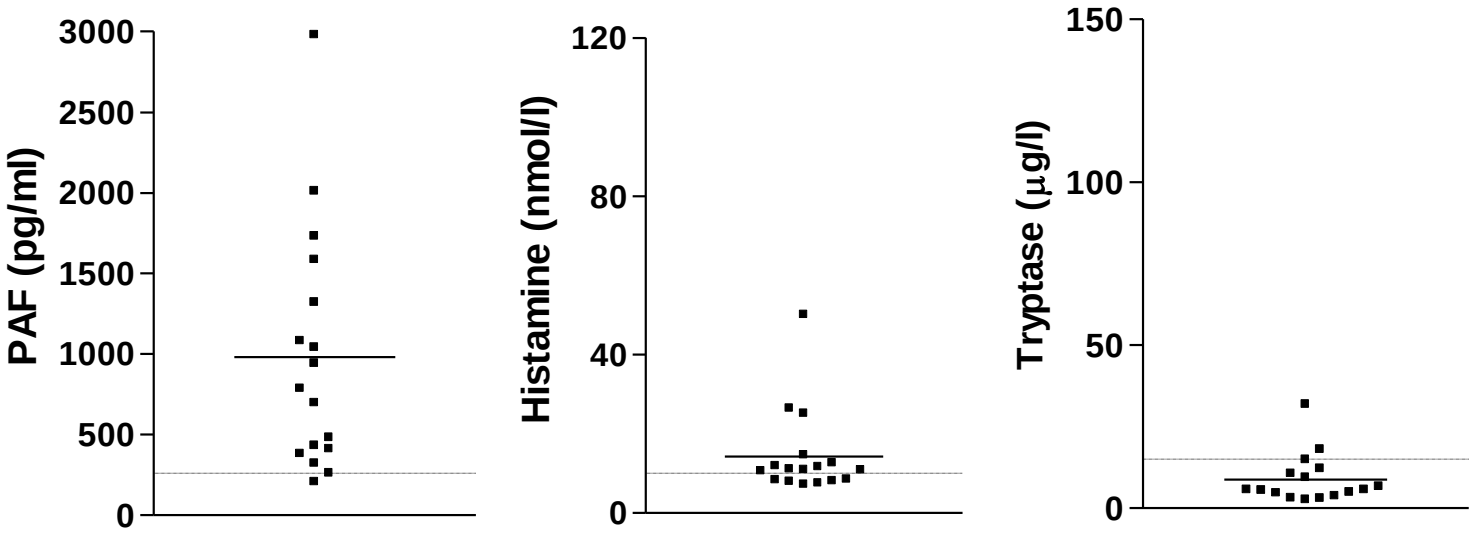

Serum PAF Concentration as a Function of PAF-AH Activity
r2= 0.324
P=0.0004
PAF-AH (nmol/min/ml)
60
50
40
30
20
10
0
0
1000
2000
3000
PAF (pg/ml)

Table I. Rate of elevated PAF, histamine and tryptase according to anaphylaxis grade.

	Grade I n = 10 (%)	Grade II n = 21 (%)	Grade III n = 10 (%)
PAF > 400 pg/ml	2 (20)	15 (71.5)	10 (100)
Histamine > 10 nmol/L	4 (40)	12 (57.1)	7 (70)
Tryptase > 15 µg/L	0	1 (4.7)	6 (60)

PAF, histamine and tryptase levels in anaphylaxis caused by food



Summary

- PAF reproduces acute physiologic changes of anaphylaxis in experimental animals
- PAF is produced acutely during anaphylaxis in both experimental animals and in humans
- PAF levels correlate with the severity of anaphylaxis in animals and in humans

Summary

- PAF is a more reliable indicator of the relative severity of anaphylaxis than two conventional mediators, histamine and tryptase
- Medications that block the action of PAF prevent anaphylaxis in experimental animals

Conclusions

- PAF is produced during anaphylaxis and reproduces the manifestations of anaphylaxis.
- Individuals with decreased circulating levels of PAF-AH are less able to inactivate PAF than those with normal levels of PAF-AH.
- PAF-AH deficiency (in conjunction with high levels of allergen-specific IgE) is associated with fatal anaphylaxis.

Significance

Data described herein provide a means of stratifying risk in food-allergic subjects.

Individuals with PAF-AH deficiency will benefit from more intensive education to reinforce preventative and treatment strategies.

Over the long term, drug treatments may provide a way of reversing PAF-AH deficiency to minimize the severity of allergic reactions.

Table 1

Variable	Peanut Fatals (8) Mean □ SD (Range)	Other Peanut (63) Mean □ SD (Range)	p value	R-Square
PAF-AH	16.65 ± 2.59 (13 – 18.6)	25.35 ± 5.57 (14.2 – 41)	0.003	0.13 (13%)
log (PAF-AH)	1.22 ± 0.07 (1.1 – 1.3)	1.4 ± 0.10 (1.2 – 1.6)	0.0008	0.16 (16%)
log IgE	3.77 ± 0.30	1.15 ± 1.57	0.0016	0.15 (15%)

There were highly significant differences between mean PAF (whether untransformed or log transformed) and log IgE titres between the fatal peanut cases and the mild reactions to peanut.

Of note, mean PAF-AH activity in mild peanut reactors (25-35) was almost identical to that seen in non-anaphylactic deaths.

Table 2

Variable	Peanut Fatals (8) Mean □ SD (Range)	Other Deaths (10) Mean □ SD (Range)	p value	R-Square
PAF-AH	16.65 ± 2.59 (13 – 18.6)	26.36 ± 7.15 (15.2 – 34.6)	0.0234	0.36 (36%)
log (PAF-AH)	1.22 ± 0.07 (1.1 – 1.3)	1.4 ± 0.13 (1.2 – 1.5)	0.017	0.39 (39%)

Mean serum PAF-AH activity in peanut-induced fatal anaphylaxis was 16.65 nmol/min/ml *vs* 26.36 nmol/min/ml in non-anaphylactic fatalities ($p = 0.0234$, $r^2 = 0.36$)

Table 3

PAF-AH Activity	Fatal Anaphylaxis	Other Deaths	Totals
< 20	8	3	11
> 20	0	7	7
Totals	8	10	18

PAF-AH activity as a categorical variable cut-off
(cut-off at less than or equal to 20 nmol/min/ml)