Breastfeeding & Food Allergies:

WHAT’S THE LINK???

Childhood Allergy & Immunology Research (CAIR)

Jessica Metcalfe | PhD Candidate
Focus on early life prevention of allergic disease

- Study the developing immune system over the first year of life

- Conduct clinical trials using dietary interventions to modulate infant immune responses in the prevention of allergies
Outline:

ALLERGY PREVENTION

NOT ALLERGY TREATMENT

1. Food allergy trends/background
2. Controlled allergen exposure in prevention of allergies
3. Food proteins in breast milk
4. Studies investigating breast feeding and food allergy outcomes
5. Our current research: QuEST Study
The allergy epidemic began over 50 years ago in the 1980’s with a rapid rise in respiratory diseases like asthma.

Food allergy: a new wave in the allergy epidemic

RESPIRATORY ALLERGY EPIDEMIC

20+ year lag

FOOD ALLERGY EPIDEMIC

In Australia, UK, USA (& now accelerating in Asia)
Why are children becoming more sensitised?

• Largely debated – combination of multiple factors as a result of our modern lifestyle
  – Balance of omega-3 to omega-6 polyunsaturated fatty acids in the diet (Calder et al, 2010)
  – Environmental changes: microbial exposure, cigarette smoke, pollutants (Prescott et al, 2011)
  – ‘less exclusive breastfeeding’ (Oddy et al, 2004)
Risk factors for food allergy

1. Family History of Allergies (Genetics)
   - asthma, eczema, hay fever, food allergies
   - One family member, or two family members with allergies greatly increases the risk for a child to develop *any form of allergy*
   - *these children have a 50-80% risk compared to a 20% risk if no family history*

2. Eczema in early life
   - If a child develops eczema early in life, they are at an increased risk of developing a food allergy (36% infants with eczema had a diagnosed egg allergy at 12 months of age, Palmer et al, 2013)
Breakdown of tolerance mechanism: (Tcells)

- Body is continually assessing whether things we encounter in the environment are harmful
- Allergy = *immune reaction* to otherwise harmless protein
- IgE mediated food allergy – results in the production of IgE antibodies after initial exposure to the problematic protein.
Sensitisation:

- Presence of IgE antibodies to a food

Food Allergy:

Symptoms occur after exposure to that food

Can have sensitisation to a food and not have an allergic reaction to that food
Factors affecting the development of sensitisation and allergy

- Environmental factors
- Genetic predisposition
- Immune status
- Allergen exposure

Result:
- Sensitisation
- Allergy
Factors affecting the development of sensitisation and allergy

- Environment
- Genetic predisposition
- Immune status
- Allergen exposure

SENSITISATION

ALLERGY
Factors affecting the development of sensitisation and allergy

- Genetic predisposition
- Immune status
- Allergen exposure

ENVIRONMENT

SENSITISATION

ALLERGY

TIMING
- Early childhood
- Pregnancy
- Lactation
- ???

Dose (Quantity)
- Frequency of exposure
- Form of allergen
  - (raw/cooked/ in breast milk)
Food Allergy: Treatment

• **Complete avoidance of the allergen**
  
  – Risk of accidental exposure and severe reaction

• **Partial exposure may be possible for some food allergies**
  
  – Ie. Egg allergy & Milk allergy
    
    • some kids can tolerate well cooked or baked egg & milk but react to raw
“Controlled allergen exposure”

• Oral immunotherapy
  – Gradual introduction of increasing doses of the food
  – Must be given regularly
  – Still being investigated in RCT’s for efficacy and safety concerns.

*Has shown to be effective for some individuals
Pregnancy    Birth        4 m       5 m       6 m          8 m     10 m       12 m

Food allergy prevention ‘Intentional allergen exposure’

- STAR 33% of infants reacted with first egg exposure (Palmer et al, 2013)
- Indicates allergen exposure before solid foods

Other Allergens
LEAP, PEAAD, EAT,
“The role of breastfeeding in allergy prevention is controversial, but any protective effect is small. Breastfeeding should be recommended because of other beneficial effects.” (Australia: Prescott SL, et al. 2007)
Food proteins in human milk

Food proteins from the mothers diet found in breast milk

*New study shows presence of multiple allergens using micro-array technologies (C. Pastor-Vargas et al, 2015)

✧ Milk (betalactoglobulin)

✧ Egg (ovalbumin)

✧ Peanut

✧ Wheat (gliadin)

✧ Fish (parvalbumin)
Egg proteins in human milk

Effect of cooked and raw egg consumption on OVA content of human milk: a randomised, double-blind, cross-over trial.

- Ovalbumin (OVA) protein is found in breast milk after maternal consumption of cooked egg in most women (68%).
- There is a direct dose response relationship with the amount of cooked egg consumed and the amount of OVA found in breast milk.
- The response is greater to cooked egg when compared with raw egg
- 24% of the women had no detectable OVA in their breast milk 8 hours after any form of egg ingestion.
Breakfast: 1 cooked egg (n=41)

Food proteins in human milk: FRIEND OR FOE?

TOLERANCE

SENSITISATION
Review

Does maternal diet during pregnancy and lactation affect outcomes in offspring? A systematic review of food-based approaches

Merryn J. Netting B.Sc., B.N.D. a,b,
Philippa F. Middleton M.P.H., Grad.Dip.Lib.St., B.Sc.(Hons.) b,c,
Maria Makrides Ph.D., B.Sc., B.N.D. a,b,d,*
### Maternal diet during lactation for *allergy prevention*: Prospective observational studies

<table>
<thead>
<tr>
<th>Author</th>
<th>Allergen exposure studied (n)</th>
<th>Outcome measure</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vance, 2004</td>
<td>Egg (n=229)</td>
<td>Atopy 6,12,18mo</td>
<td>No relationship</td>
</tr>
<tr>
<td>Sausenthaler 2007, Germany</td>
<td>Cow’s milk, eggs, fats, oils, seeds (n=2641)</td>
<td>Sensitisation 1y, 2y</td>
<td>No relationship</td>
</tr>
<tr>
<td>Venter, 2009 UK</td>
<td>Cow’s milk, wheat, fish, peanut (n=969)</td>
<td>Food hypersensitivity 1y</td>
<td>No relationship</td>
</tr>
<tr>
<td>Nwaru, 2011, Finland</td>
<td>General diet, dairy, eggs, fish, cereales (n=652)</td>
<td>Sensitisation 5y</td>
<td>No relationship</td>
</tr>
</tbody>
</table>

*overall no conclusive evidence that allergen exposure while breast feeding is associated with food sensitisation*
### Study or Subgroup

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Restricted Events</th>
<th>Restricted Total</th>
<th>Standard Events</th>
<th>Standard Total</th>
<th>Weight (%)</th>
<th>Risk Ratio M-H, Fixed (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.6.1 cow's milk and egg avoidance: during first 5 y</td>
<td>35</td>
<td>84</td>
<td>37</td>
<td>114</td>
<td>100%</td>
<td>1.28/0.89–1.85</td>
</tr>
<tr>
<td>Falth-Magnusson 1987 RCT</td>
<td>35</td>
<td>84</td>
<td>37</td>
<td>114</td>
<td>100%</td>
<td>1.28/0.89–1.85</td>
</tr>
<tr>
<td>Subtotal (95% CI)</td>
<td>84</td>
<td>114</td>
<td>100%</td>
<td>1.28/0.89–1.85</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total events</td>
<td>35</td>
<td>37</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Heterogeneity: Not applicable
Test for overall effect: $Z = 1.34$ ($P = 0.18$)

### 1.6.2 multiple restrictions+acaricide: at 2 y

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Restricted Events</th>
<th>Restricted Total</th>
<th>Standard Events</th>
<th>Standard Total</th>
<th>Weight (%)</th>
<th>Risk Ratio M-H, Fixed (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arshad 1992 RCT</td>
<td>3</td>
<td>58</td>
<td>16</td>
<td>62</td>
<td>100%</td>
<td>0.20/0.06–0.65</td>
</tr>
<tr>
<td>Subtotal (95% CI)</td>
<td>58</td>
<td>62</td>
<td>100%</td>
<td>0.20/0.06–0.65</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total events</td>
<td>3</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Heterogeneity: Not applicable
Test for overall effect: $Z = 2.67$ ($P = 0.008$)

### 1.6.5 multiple restrictions: definite/probable allergy at 2 y

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Restricted Events</th>
<th>Restricted Total</th>
<th>Standard Events</th>
<th>Standard Total</th>
<th>Weight (%)</th>
<th>Risk Ratio M-H, Fixed (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zeiger 1995 RCT</td>
<td>7</td>
<td>99</td>
<td>34</td>
<td>177</td>
<td>100%</td>
<td>0.37/0.17–0.80</td>
</tr>
<tr>
<td>Subtotal (95% CI)</td>
<td>99</td>
<td>177</td>
<td>100%</td>
<td>0.37/0.17–0.80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total events</td>
<td>7</td>
<td>34</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Heterogeneity: Not applicable
Test for overall effect: $Z = 2.53$ ($P = 0.01$)

Test for subgroup differences: $\chi^2 = 24.11$, df = 4 ($P < 0.0001$), $I^2 = 83.4\%$
Summary

RCT’s maternal diet & atopy outcomes

- were judged to be of moderate to high risk for bias
- very low numbers
- No conclusive evidence to suggest lower rates of sensitisation in infants fed by mothers on exclusion diets

Prospective cohort Studies

- looked at more investigations into relationships between foods and atopic outcomes.
- Most studies showed **no association** between maternal food intake and allergy outcomes in their offspring
“It could be suggested that milk is the communication vehicle between the maternal immune system and the infant, a system actively directing and educating the immune, metabolic and microflora systems within the infant, while conferring multiple means of protection from pathogens.” Catherine Field, 2005, Canada.
Animal models for oral tolerance induction

- **Yamamoto et al. 2012**
  - Induced tolerance to OVA in mice (Egg Protein)
  - OVA sensitised mice who were breast fed by OVA exposed mothers were much less likely to develop a food allergy to OVA

- **Verhasselt et al, 2008**
  - Induced tolerance and asthma in mice who were breast fed by OVA exposed mothers (also mice!)

- **Bernard et al, 2014**
  - Tolerance induction for mice given **HUMAN** milk containing peanut
Can proteins found in breast milk lead to sensitisation?

Isolated peanut proteins from human milk

Bernard et al, Allergy, 2014
Animal models for tolerance induction

Peanut IgE

Peanut Allergy

TOLERANCE

Bernard et al, Allergy, 2014
Allergen exposure via breast milk

- Infant sees ‘allergen’ (egg) in combination with immune modulators found in mother’s breast milk.
- May promote tolerance to food protein.
- Increase in TGF-beta = increased IgA = lower risk of allergy (Kaliliomaki M et al, 1999)
- Every mother’s milk is different
The QuEST Study

QUESTIONING THE ROLE OF EGG IN LACTATION FOR THE INDUCTION OF SPECIFIC TOLERANCE

Childhood Allergy & Immunology Research (CAIR)

Jessica Metcalfe | PhD Candidate
Why Eggs?

• Hen’s egg allergy is the most common food allergy in Australian children
  • 8.9% have an egg allergy by 12 months of age (Koplin et al, 2012)
  • 3 times more common than peanut allergy
  • Only 1 in 5 children outgrow their egg allergy by 5 years of age
  • Becoming more severe – more cases of anaphylaxis
Food allergy prevention

‘Intentional allergen exposure’

- STAR: 33% of infants reacted with first egg exposure (Palmer et al, 2013)
- Indicates allergen exposure before solid foods

Other Allergens
LEAP, PEAAD, EAT,
Hypothesis:

Regular exposure to egg proteins in breast milk will induce neonatal tolerance to egg and prevent sensitisation.

Primary Outcome:

To compare the effects of varying maternal dietary egg intake during lactation on egg protein levels in breast milk

✨ Quantified by the measurement of OVA protein in breast milk
**Study Design:**  
Randomised Controlled Trial

**Inclusion Criteria**

Eligible women included those who:

1. Were planning to breastfeed

2. Had a maternal history of allergic disease (diagnosed asthma, eczema, hay fever, or food allergy)

3. Did not have an egg allergy

**Recruitment strategy:**

120 women were recruited in pregnancy from antenatal clinics and classes in across various Perth hospitals.
In a **three-group** design, women were randomly allocated a dietary group from **birth to 6 weeks** of lactation:

1. **‘high egg’** 4-6 eggs per week (n=37)
2. **‘low egg’** 1-3 eggs per week (n=44)
3. **‘egg free’** avoiding all egg and egg containing foods (n=39)

◆ **Women in egg groups (1&2)** included **all forms of egg** and egg containing foods (quiche, muffins, pancakes)

◆ **Diary cards** were used to capture the amount and types of egg consumed throughout the intervention
Study Timeline

**Intervention (Maternal)**
High egg (n=40), Low egg (n=40), Egg free (n=40)

**36-40wks**
- Antenatal Appointment
- Maternal Blood Collection

**Birth**
- Cord Blood Collection
- Breast milk Telephone calls

**2 wks**
- Appointment
- Breast milk
- Infant Blood
- TEWL
- Eczema assess

**4 wks**
- Appointment
- Breast milk
- Infant Blood
- Maternal Blood
- TEWL
- Eczema assess

**6 wks**
- Appointment
- Breast milk
- Infant Blood
- Maternal Blood
- TEWL
- Eczema assess

**16 wks**
- Appointment
- Breast milk
- Infant Blood
- TEWL
- Eczema assess
Progress Update
& Study Timeline

Intervention (Maternal)
High egg (n=37), Low egg (n=44), Egg free (n=39)

- Antenatal appointment for randomisation: n=120
  - Maternal blood: n=112

Birth
- 6 wks
- 4 wks
- 2 wks
- 36-40 wks

Babies born
- n=117
- Cord Blood: n=83

Lost to follow up during intervention: n=4

Appointment: n=108
  - Breast milk: n=88
  - Infant blood: n=87
  - Withdrew: n=0
  - Lost to follow up: n=2

Breast milk
- n=109
- phone calls: n=115

Breast milk
- n=106
- phone calls: n=111

Breast milk
- n=102
- Infant blood: n=84
  - Withdrew: n=1

Infant blood
- n=102
  - Breast milk: n=84
  - Maternal blood: n=109

Maternal blood
- n=109

Withdrawn after diet allocation: n=3 (Egg Free)
QuEST Planned Future Analysis:

- Measurement of breast milk OVA protein using ELISA methodology
- Breast permeability – measured via breast milk
- Breast milk macro nutrients (total protein, fat, lactoferrin and lysozyme) and micro nutrients (selenium, zinc, choline)
- Maternal and infant serum egg protein levels
- Variations in maternal and infant skin integrity (transepidermal water loss ‘TEWL’) AquaFLUX 200
- Infant sensitisation to egg (specific IgE) at 4 months of age prior to the introduction of egg containing solid foods into the diet of the infant
Translational Application

 מיוחדת, ברוש, מסדר, סדר

 ◇ Results from this study will translate to better evidence-based advice about dietary egg intake for breast-feeding mothers in regards to allergic sensitisation for their baby.
Breastfeeding & Food Allergies:

WHAT’S THE LINK???

Summary

1. There is no evidence to suggest maternal ingestion of allergenic foods while breast feeding leads to sensitisation – might induce tolerance!

2. Avoidance diets do not result in lower rates of sensitisation

3. More research needed into human breast milk and allergen exposure.
Acknowledgements

The CAIR Team

Supervisors: W/Prof Susan Prescott, A/Prof Debbie Palmer, & Dr. Nina D’Vaz

QuEST Study Participants

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