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**Topic 7: The Prevalence of Allergens in Foods  
and Threshold Levels for Sensitization**

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## **The prevalence of food allergy.**

The prevalence of food allergy in Australia, based on the Melbourne Atopy Cohort Study (MACS) (1) is shown in Table 1.

**Table 1. The prevalence of common food proteins in Australian Children**

|             |       |
|-------------|-------|
| Egg         | 3.2%  |
| Cow's milk  | 2.0%  |
| Peanut      | 1.9%  |
| Sesame seed | 0.42% |
| Cashew nut  | 0.33% |
| Hazelnut    | 0.18% |
| Walnut      | 0.16% |
| Brazil nut  | 0.07% |
| Almond      | 0.02% |
| Wheat       | 0.15% |
| Soy         | 0.10% |
| Fish        | 0.07% |

The prevalence of cow milk allergy in Australia is similar to that in Denmark (2) and that of peanut allergy, 1.9%, (1) is similar to the 1% prevalence in young children on the Isle of Wight (UK)(3).

In Table 2 the relative frequencies of different food allergies in South East Asia is shown. Investigators from the different countries ranked the frequency of allergy to 8 common foods based on skin tests, measurement of IgE antibodies to foods and/or food challenge procedures (1). Milk and egg allergy were highest in Australia, China, Taiwan and Japan whereas hypersensitivity to crustacea and fish were commonest in the Philippines, Indonesia, Thailand and Singapore where these foods form part of the staple diet in early infancy. Rice allergy was rare.

**Table 2. Ranking Food Allergy in South-East Asia**

|             | Egg | Milk | Peanut | Soy | Wheat | Rice | Fish | Shellfish |
|-------------|-----|------|--------|-----|-------|------|------|-----------|
| Australia   | 1   | 2    | 3      | 5   | 4     | 7    | 6    | 8         |
| China       | 1   | 2    | 3      | 4   | 5     | -    | -    | -         |
| Taiwan      | 1   | 2    | 3      | 4   | 6     | -    | 5    | -         |
| Japan       | 1   | 2    | 7      | 5   | 3     | 6    | 4    | -         |
| Malaysia    | 1   | 3    | -      | 2   | -     | 5    | 4    | -         |
| Singapore   | 3   | 2    | 5      | 6   | 4     | -    | 1    | -         |
| Thailand    | 5   | 6    | 2      | 3   | -     | 4    | -    | 1         |
| Indonesia   | 4   | 5    | 1      | -   | -     | 6    | 3    | 2         |
| Philippines | 4   | 5    | 6      | 7   | 3     | 8    | 1    | 2         |

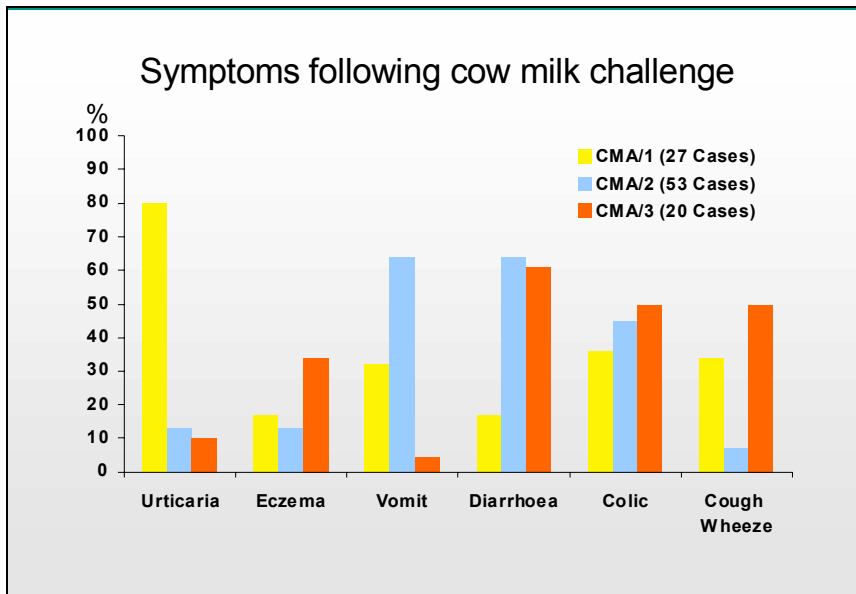
The ranking of food allergy from 1-8 in children from Australia and South-East Asia.

## The Spectrum of Food Allergy in Childhood

The Melbourne Food Allergy Study (MFAS) initially focussed on milk allergy in infancy and childhood as a model of food allergy. The Melbourne Milk Allergy Study (MMAS) described a diverse group of clinical symptoms and syndromes reproduced by cow milk challenge (4). A clustering algorithm identified three clinical groupings that had immunological correlates. The first, an immediate reacting group, were IgE sensitised; the second, a slower reacting group, had predominantly gastrointestinal symptoms; the third (late reacting) group developed symptoms of eczema, bronchitis and/or gastrointestinal disturbance. This late reacting group showed varying degrees of IgE sensitisation and as a group demonstrated greater T-cell sensitisation to milk proteins than the other groups (5).

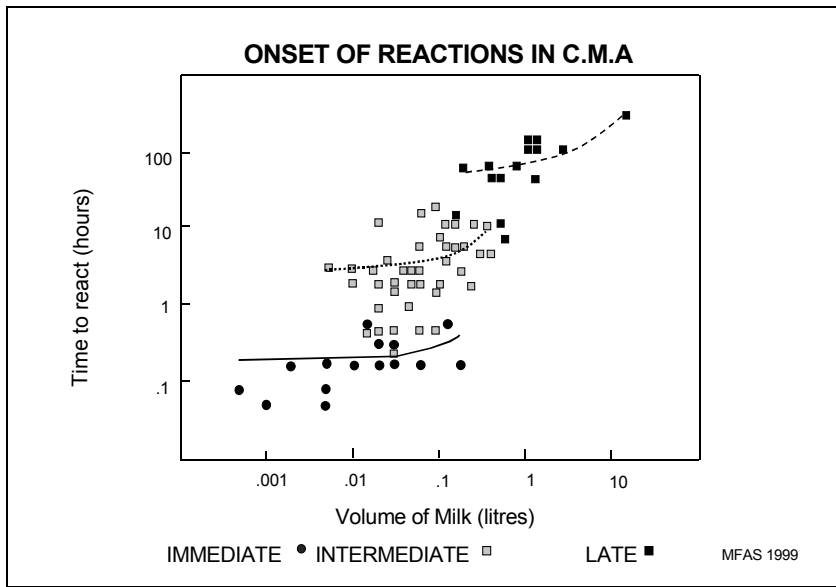
In figure 1 the frequency of common symptoms in each of the three groups is shown, as a percentage of the group affected.

**Figure 1**



In figure 2 the relationship between the time of onset of adverse symptoms and the volume of cow milk administered up to the time of onset of those symptoms is shown for each of the 100 infants. The second degree polynomial, the line of best fit for each of the groups described above, emphasises the distinction between the groups.

**Figure 2.**



In subsequent studies we identified these three patterns of reactions in children with allergy to egg, wheat, soy and extensively hydrolysed formulas (6). Conversely, reactions to peanuts, nuts and seafood were predominantly an immediate anaphylactic type response.

Relevant to the FAO consultancy it should be noted that most studies which have identified food protein hypersensitivity threshold levels have been restricted to immediate anaphylactic responses; milligram amounts of food protein have caused these reactions. The MFAS studies highlight the number of infants and young children who suffer from subtle forms of hypersensitivity to foods which manifest as colic, gastro-oesophageal reflux and atopic dermatitis. These symptoms can be precipitated by microgram or nonogram amounts of dietary protein contained in extensively hydrolysed formula (6,7) or excreted in breast milk (8,9,10).

### **Threshold provoking doses of food allergens elicited by challenge.**

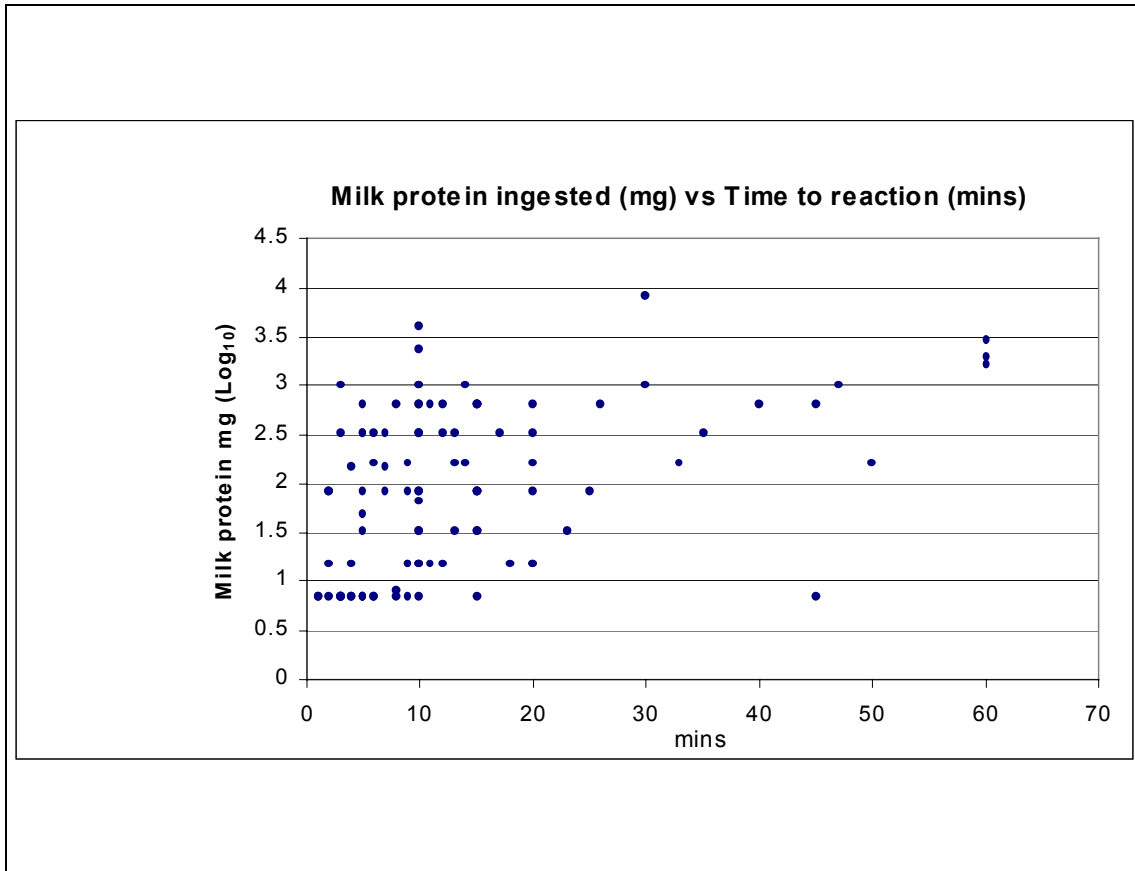
In the Melbourne Food Allergy study the results of groups of one hundred children with hypersensitivity to cow milk, egg or peanut on open food challenge were examined to identify the lowest amount of food protein which caused hypersensitivity reactions to these foods.

#### **a) Cow Milk.**

There were 100 children with cow milk allergy on challenge. They were 3.4 years (range 7 months to 13 years) and two thirds of them were boys. The challenge protocol followed was 0.2ml, 0.5ml, 1.0ml, 2.5ml, 5ml, 10ml, and 30ml given at half hourly intervals on day 1. On day 2, 20ml, 30ml, 60ml and 90ml were administered at half hour intervals. On day 3 normal volumes of cow milk were administered.

In Figure 3 the amount of cow milk protein eliciting an adverse response and the time after initial exposure when the reaction was seen is documented. Log101 is approximately 10mg of cow milk protein, i.e. equivalent to 0.5ml of cow milk.

**Figure 3.**



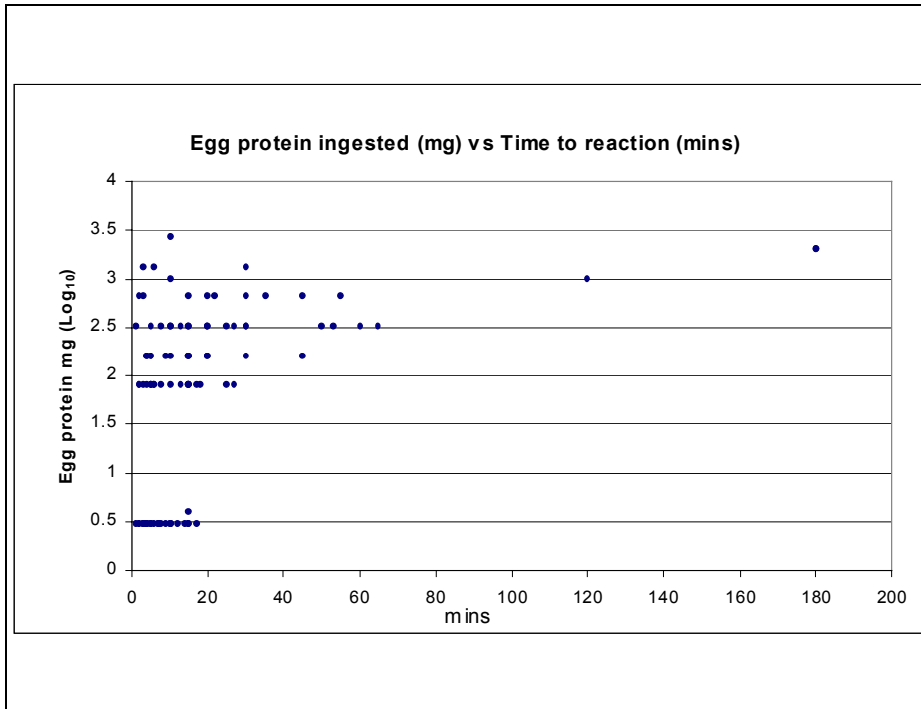
**b) Egg allergy**

The median age of the 100 patients was 4 years (range 7 months to 12 years). Two thirds of the patients were male.

On day 1, 0.02, 0.3, 0.6, 1.25, 2.5 and 5.0ml of raw egg white was administered at thirty minute intervals. On day 2, 10, 20 and 30ml of raw egg was given at 30 minute intervals.

In Figure 4 the amount of egg protein required to elicit adverse symptoms shown in relation to time after commencing exposure when the adverse reaction was seen. The amount of egg protein eliciting adverse symptoms is shown. Log<sub>10</sub>1 is approximately 3mg of egg protein, i.e. the amount contained in one drop.

**Figure 4.**



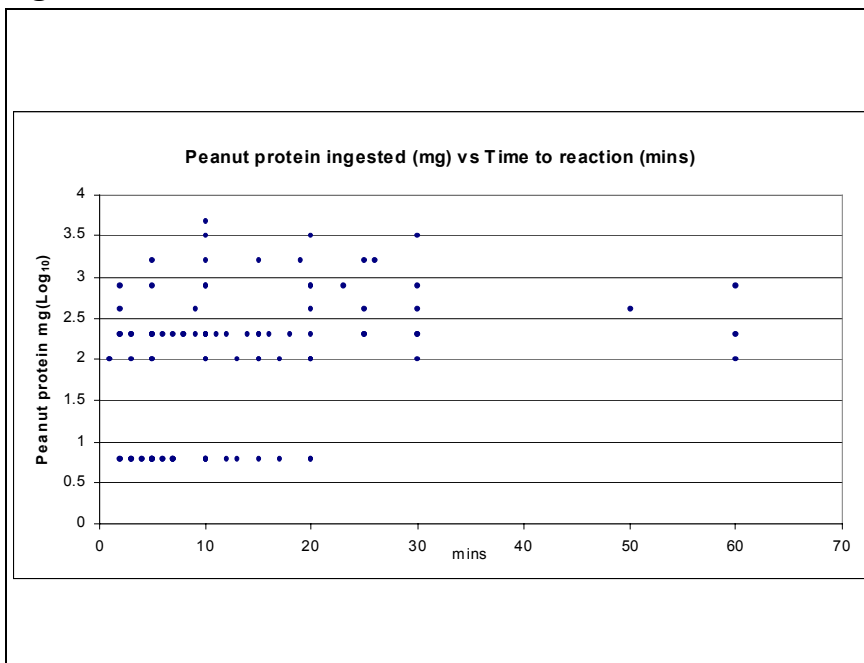
**c) Peanut allergy**

One hundred children aged from 1-20 years were studied. On day 1, 6mg, 100mg, 200mg, 400 mg and 800mg of peanut butter were administered at 30 minute intervals. On day 2, 1600mg of peanut butter was given.

In figure 5 the amount of peanut protein which elicited adverse symptoms and the time of onset of that adverse symptom after the challenge procedure was commenced is shown.

A third of our patients reacted to the first dose of peanut protein, i.e. 6mg.

**Figure 5.**



## Comparison of threshold provoking doses of food allergens in the Melbourne Food Allergy Study with other investigations.

### a) **Beta lacto-globulin.**

We have identified a group of young children with multiple food protein intolerance syndrome (6). They showed hypersensitivity to extensively hydrolysed casein and whey formula. It has been estimated that these formulae contain approximately 0.14 microgram/gm of beta lacto-globulin per gram of formula powder (7). Based on our DBPC studies we estimate that children reacting to beta lacto-globulin in these preparations are ingesting approximately 0.5 micrograms (500 nonograms) of beta lacto-globulin in 240mls of the formula; these amounts elicited adverse reactions (6).

### b) **peanut**

Hourihane et al (11) in a randomised double-blind placebo-controlled food challenge of 14 subjects with allergy to peanut, administered peanut concealed in flour. Hourihane noted that in a few patients, objective adverse symptoms could be demonstrated with 2mg of peanut protein but in retrospect one patient noted transient symptoms when challenged with 100mcg of peanut protein and another to 250 mcg of peanut.

## Exposure to dietary proteins in breast milk.

Recent studies have suggested that colic (12), gastro-oesophageal reflux (13) and atopic dermatitis (9) in breast-fed infants in the first twelve months of life occurs as a result of exposure to “physiological” doses of dietary antigens in breast milk. In table 3 the concentrations of different dietary proteins from cow milk, egg and peanut is shown in relation to the loading dose of these foods administered to the mother.

**Table 3. Dietary Proteins in Breast Milk**

|                     | Positive EBM samples | Conc <sup>n</sup> in EBM (ng/ml) | EBM sample time (hr) | Maternal loading dose |                      |
|---------------------|----------------------|----------------------------------|----------------------|-----------------------|----------------------|
| OA <sup>1</sup>     | 12/22                | 1.26-6.2                         | 2 – 6                | 1 egg                 | Kilshaw (1984)       |
| OM <sup>1</sup>     | 7/9                  | 0.44-2.9                         | 2 – 6                | 1 egg                 | Kilshaw (1984)       |
| BLG <sup>1</sup>    | 10/19                | 0.11-6.4                         | 2 – 6                | 300ml CM              | Kilshaw (1984)       |
| GLIA <sup>2</sup>   | 37/53                | 5.0-95                           | 2 – 4                | 20g Gliadin           | Troncone (1987)      |
| BLG <sup>2</sup>    | 10/17                | 0.01-11.5                        | 2 - 30               | 300-2300 CM           | Jarvinen (1999)      |
| BLG <sup>2</sup>    | 24/47                | 0.12                             | 1 - 2                | 400ml CM              | Sorva (1994)         |
| Peanut <sup>2</sup> | 1/3                  | 0-2000                           | 2                    | 30g peanut            | Hefle, Taylor (2001) |

<sup>1</sup>RIA <sup>2</sup>ELISA

In table 4 the amount of dietary antigen contained in the breast milk required to elicit symptoms in infants being breast-fed is shown. It is noted that some infants developed urticaria attributed to of beta lacto-globulin in breast milk in amounts which could not be detected by the assay, i.e. less than 0.002ng/ml.

**Table 4. Reactions to food in breast milk**

|     | Maternal Intake | Reaction Time (hr) | Subjects | Estimated Provoking Dose ng/ml          | Adverse effect |                 |
|-----|-----------------|--------------------|----------|---|----------------|-----------------|
| BLG | 400ml           | 2                  | 8        | <0.002-3.0<br>Est Vol. 150ml            | U,E,V,D        | Sorva (1994)    |
| BLG | 400ml           | 2                  | 3        | <0.002-0.14<br>Est Vol 0.2ml            | U (contact)    | Sorva (1994)    |
| BLG | 300-2,300ml     | 2-80               | 15       | <0.01-11.54<br>Est Vol. 150-3600ml      | E,D            | Jarvinen (1999) |
| Egg | 6.6gm           | 24                 | 1        | 1.26-6.2 <sup>+</sup><br>Est Vol. 150ml | E,V,D          | Warner (1980)   |

<sup>+</sup> from Kilshaw (1984)

U = urticaria, E = eczema, V = vomit, D = Diarrhea

### **Estimation of minimal levels of dietary antigen exposure required for sensitisation.**

It has been estimated that bottle-fed infants receiving small amounts of cow milk formula, i.e. 40ml of cow milk formula, ingest  $3.6 \times 10^8$  nonograms of beta lacto-globulin; this is equivalent to the amount of beta lacto-globulin an infant receiving 1 litre of human breast milk per day for 7 to 21 years (13). Thus, breast-fed infants with food hypersensitivity disorders are exposed to very small amounts of dietary antigen excreted in breast-milk. Two disorders of breast-fed infants which have been associated with food hypersensitivity are

- a) colic – associated with cow milk protein intolerance (12), and
- b) atopic dermatitis (9) associated with hypersensitivity to cow milk protein, egg and peanut protein.

The following graphs, Figure 6 and 7, estimate the amount of beta lacto-globulin and ovalbumen infants would ingest during the process of sensitisation if they were being entirely breast fed. Our studies (14) suggest that most infants with colic develop symptoms at about two weeks of age, and infants with severe atopic dermatitis develop symptoms from about 4 weeks of age (15, personal observation).

In constructing these figures the following assumptions have been made:

A three month old baby weighing 5.6kgs will ingest 150ml/kg of breast milk per day and will have 5 feeds per day.

#### ***Estimation of exposure to egg protein.***

If mother ingests one egg, most protein is going to be excreted within 4-6 hours of ingestion (10).

One egg → gives 1.2 – 6.2ng/ml ovalbumen in breast milk within 2-6 hours (10).

Thus, a 3 month old 5.6kg infant ingests 150ml breast milk/kg in 5 feeds per day.

Therefore the volume in one feed = 30ml x 5.6kg = 168ml.

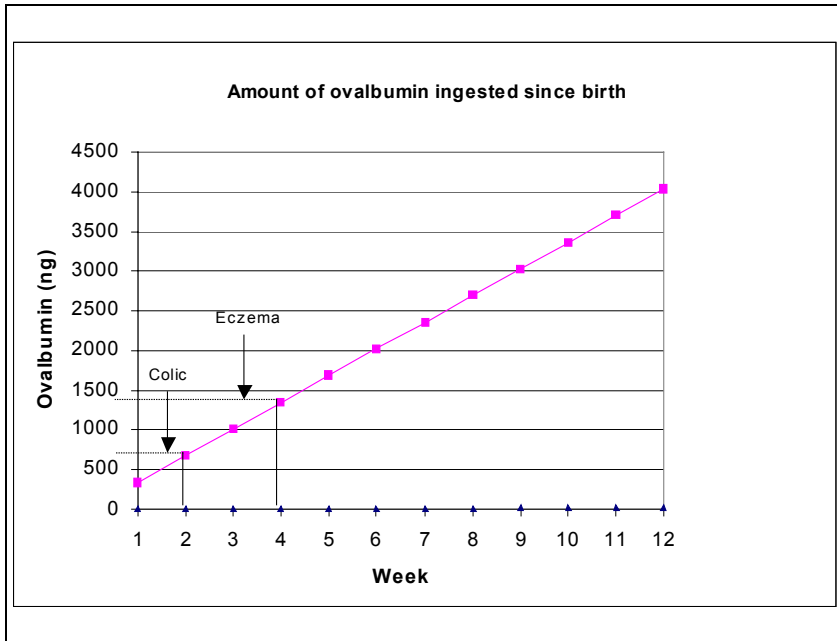
Therefore the amount of ovalbumen ingested is 168ng in one feed.

Our study suggests two eggs per week are ingested, i.e. the infant ingests a total of 336ng of ovalbumen per week via breast milk.



In figure 6, the graph based on these assumptions has been constructed showing the amount of ovalbumin ingested from week 1 – 12. The amounts of egg protein likely to be ingested prior to the onset of colic and prior to the onset of atopic dermatitis is also shown.

**Figure 6.**



**Cow milk**

A three month infant weighing 5.6kg will ingest 150ml/kg of breast milk per day over 5 feeds. Therefore the volume of milk in one feed is  $150\text{ml}/5\text{ml} \times 5.6\text{kg} = 168\text{ml}$ .

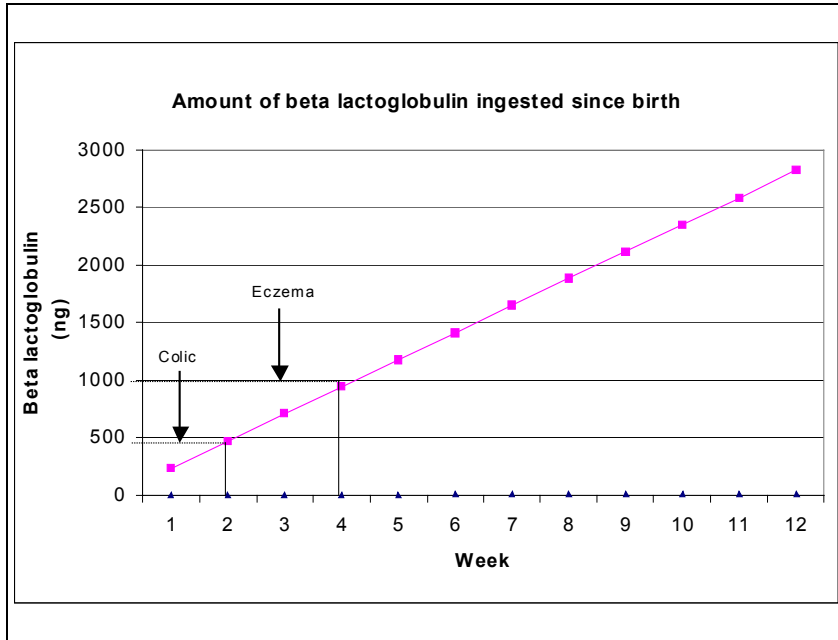
We assume that mother ingests 600ml of cow milk per day and this will be ingested prior to two feeds and will be excreted in two breast milk feeds i.e.  $n 168 \times 2 = 336\text{ml}$  of breast milk. Sorva (8) has reported that the maternal ingestion of 400ml of cow milk → gives EBM levels of 0.1ng/ml of beta lacto-globulin.

Therefore, the amount of beta lacto-globulin ingested daily by the infant =  $336 \times 0.1 = 33.6\text{ng}$  of beta lacto-globulin per day.

For each week the amount =  $33.6 \times 7 = 235.2\text{ng}$  of beta lacto-globulin.

In Figure 7 the amount of beta lacto-globulin ingested by a breast-feeding infant in week 1 to 12 is shown.

**Figure 7.**



Based on the above assumptions we have calculated that a two week old infant developing colic due to cow milk protein intolerance will be approximately 500ng and eczema approximately 1000ng.

### **Conclusion.**

For the purpose of the consultancy we have attempted to deduce the smallest amounts of food protein in cow milk, egg and peanut which may cause sensitisation and precipitate symptoms in food hypersensitivity disorders. Assumptions on which this data has been derived has been described.

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