The origin and effect of leptin on breastfeeding behaviour

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Significance of the breastmilk leptin

- According to World Health Organization (2013) obesity and overweight are one of the major cause of deaths (2.8 million adults/year)
- More than 170 million children are overweight
- Breastfeeding decreases obesity risk
- Breastmilk leptin helps to program infants:
  - Develop areas of hypothalamus that control energy balance and appetite
  - Develop chief cells in stomach that produce leptin
- It is critical to investigate breastmilk leptin: mechanisms of appetite control in infants and their long-term effects to prevent obesity

What is leptin?

- Leptin was identified in 1994 in the human obese gene on chromosome 7
- Responsible for food intake regulation, energy balance, suppressing appetite
- Produced by the adipose tissue and other cells such as epithelial cells
- Found in stomach, skeletal muscles, placenta, cord blood and breastmilk
- Origins of leptin in breastmilk not clear
- Leptin affects gastrointestinal tract in adults – no information about infants

Feedback loop of leptin

- Leptin enters the blood and travels to the brain
- Leptin receptors in the hypothalamus are triggered
- Brain receives information about fat stores in the body
- Body receives the signal to decrease food intake and increase energy expenditure

Pico et al. 2003, Klok, Jakobsdottir and Drent 2006
Leptin effect on infants

- A positive effect of leptin on satiety and energy regulation in breastfed infants
- Adults that were formula-fed as infants have an increased chance of becoming obese compared to those that were breastfed
- Breastfed infants develop better appetite control than formula-fed infants
- Leptin programs important appetite control mechanisms

Aims of the project

1. Examine which cells synthesize leptin in human lactating breast

2. Measure leptin concentration in breastmilk over 24h and determine variability within and between mothers and effect on breastfeeding patterns

3. Examine effects of leptin on infant gastric emptying
Which cells synthesize leptin in human lactating breast

- Stain human resting (n=3) and lactating (n=3) breast tissues with anti-leptin antibody in combination with mammary-specific markers to determine whether breast tissue produces leptin.

- Stain breastmilk cells with anti-leptin antibody and other mammary-specific markers to determine which cells within a breast tissue produce leptin (n=4).
Leptin concentration in breastmilk over 24 hours

- Retrospective pre- and post-feed breastmilk samples, over 24h in exclusively breastfeeding dyads 1-6 months postpartum (n=19)

- Measure:
  - Leptin – sandwich ELISA
  - Protein – Bradford protein assay
  - Lactose – enzymatic lactose assay
  - Fat – creamatocrit method

- Variability of leptin concentration over 24h within and between mothers

- Relationships between feed volume, concentration of macronutrients and time between the feeds

Mitoulas et al. 2002, Kuhn and Lowenstein 1967, Fleet and Linzell 1964
Effects of leptin on infant gastric emptying

- Prospective exclusively breastfeeding dyads (n=20) 1-7 months postpartum
- Take scans of infant’s stomach pre- and post-feed, and at 5-15 min-intervals until the next feed
- Pre- and post-feed breastmilk samples to measure leptin concentration
- Leptin dose calculation:
  \[(\text{pre- concentration} + \text{post- concentration})/2 \times \text{feed volume}\]
Which cells synthesize leptin in human lactating breast

- Leptin stained positive in epithelium layer of the lactating tissue breast
- Alveoli more positive for leptin than ducts
- Variability among different lobules in terms of leptin staining
- Leptin was found in the lumen of ducts and alveoli

Scale bars: 50 µm. Red – leptin antibody Blue – DAPI (nuclei)
Which cells synthesize leptin in human lactating breast

Flow cytometry dot plots showing breastmilk cell analysis for leptin and lactocytes, and stem cells

90% of lactocytes and 5% of breastmilk stem cells (at different stage of maturity) contain leptin (n=4)
Leptin concentration in breastmilk over 24 hours

- Variability of leptin within and between mothers

- Relationship between leptin and:
  - Feed volume
  - Concentration of macronutrients: lactose, protein and fat
  - Time between the feeds

- 24-hour pattern of leptin
Leptin concentration in breastmilk over 24 hours

<table>
<thead>
<tr>
<th>Breastmilk and feeding characteristics</th>
<th>Mean ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total milk production both breasts (mL)</td>
<td>818 ± 166</td>
<td>496 – 1232</td>
</tr>
<tr>
<td>Number of feeds</td>
<td>9 ± 2</td>
<td>7 – 13</td>
</tr>
<tr>
<td>Volume of feeds (mL)</td>
<td>108 ± 46</td>
<td>18 – 220</td>
</tr>
<tr>
<td>Feed interval (min)</td>
<td>172 ± 102</td>
<td>23 – 590</td>
</tr>
<tr>
<td>Skim milk leptin dose per feed (ng)</td>
<td>45.94 ± 21.25</td>
<td>9.62 – 104.84</td>
</tr>
</tbody>
</table>
Leptin concentration in breastmilk over 24 hours

- **Average leptin concentration** was 0.43 ± 0.10 ng/mL (0.23 – 0.80 ng/mL)

- Variation within mothers was 0.002 ng/mL (CV:12.6%) and between mothers was 0.008 ng/mL (CV:21.4%)

- **No difference between breasts** within individuals (p=0.999)

- **No difference between pre- and post-feed** leptin concentration (p=0.623)

- Difference in **pre- and post-feed** leptin concentration was **related to feed volumes** (p=0.014)
Feeds larger than 105 g associated with higher difference in pre- and post-feed leptin levels (p=0.0009)
Leptin concentration in breastmilk over 24 hours

- No relationship between difference in pre- and post-feed leptin concentration and difference in pre- and post-feed lactose (p=0.587) and protein concentrations (p=0.313)

- A marginal relationship between difference in pre- and post-feed leptin concentration and difference in pre- and post-feed fat concentration (p=0.056)

- A significant positive relationship between leptin dose and feed volume (p=0.0001)
Individual relationships between leptin dose and time interval between feeds

No relationship between leptin dose and time until the next feed (p=0.236)
Changes in leptin concentrations over 24 hour period separated for pre- and post-feed measurements
Leptin concentration in breastmilk over 24 hours

¬ Leptin concentration *increases* with maternal BMI (p=0.008)

¬ No relationship between leptin concentration and infant *birth weight* (p=0.715) and *current weight* (p=0.769)

¬ No relationship between leptin concentration and lactation stage (p=0.638)

¬ No relationship between leptin concentration and infant gender (p=0.22)
Examine effects of leptin on infant gastric emptying

- A strong positive association between **leptin dose** and **feed volume** \( (p=0.001) \)

- **No relationship** between **leptin dose** and proportion of **previous feed** remaining \( (p=0.207) \)

- **No relationship** between **leptin dose** and **time** between feeds \( (p=0.907) \)
Conclusions

- Both lactating and resting breasts produce leptin

- One sample can be indicative of mother’s breastmilk leptin levels unless investigating effect of volume of the feed

- Leptin in skim milk appears to have 24-hour pattern

- Skim milk leptin appears not to have a dominant effect on satiety and gastric emptying in infants
Acknowledgements

Hartmann Human Lactation Research Group
Which cells synthesize leptin in human lactating breast

Flow cytometry dot plots showing breastmilk cell analysis for leptin and lactocytes

90% of lactocytes contain leptin (n=4)
Which cells synthesize leptin in human lactating breast

Flow cytometry dot plots showing breastmilk cell analysis for leptin and stem cells

5% of breastmilk stem cells (at different stage of maturity) contain leptin (n=4)
Discussion

- Leptin is produced by the lactating and resting breast, mainly the lactocytes
- Effect on sampling protocol
- Changes in fat, cells, feed volume and leptin
- Circadian pattern of leptin
- ‘Lab effect’ on gastric emptying measurements
- Gastric emptying more complex
1. Methods: Aim 3. Examine effects of leptin on infant gastric emptying rate

- Investigate relationship between breastmilk leptin in term infants and the gastric emptying rate (GER) using ultrasound imaging
- Ultrasound imaging is a non-invasive and validated method to measure GER in term and preterm infants
- Volume of the milk influences infant’s GER: the larger the feed volume the slower the GER
Measure leptin concentration in breastmilk over 24h and determine variability within and between mothers and effect on breastfeeding patterns

- Protein – Bradford protein assay
- Lactose – enzymatic lactose assay
- Fat – creamatocrit method
- Leptin – sandwich ELISA
Why should we look at leptin in breastmilk?

- Leptin is an appetite control hormone present in breastmilk
- Influences self-regulation of appetite
- Important to understand its contribution to appetite control
- Attributed with decrease in risk of obesity in breastfed infants