Impact of the degree of prematurity and time postpartum on milk protease activity in the infant stomach

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“Preterm” is defined as infant birth with less than 37 wks gestation.

GA at birth ↓, risk for health problems ↑ (underdeveloped system)

Gastrointestinal issues:
Necrotizing enterocolitis

Breast milk is the gold standard of food for term infants – may not for preterm.
Digestion of human milk in preterm infant

Immature digestive system in preterm infants:

- produces **less gastric acid**
- has lower gastric **pepsin** and **intestinal protease** activity than in term infants

<table>
<thead>
<tr>
<th></th>
<th>Preterm</th>
<th>Term</th>
<th>Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pepsin activity(^1) (U/mL)</td>
<td>12</td>
<td>125 (10X)</td>
<td>600 (50X)</td>
</tr>
<tr>
<td>Gastric pH (^2)</td>
<td>4.1 – 5.8</td>
<td>3.2 – 5.0</td>
<td>1.8 – 2.0</td>
</tr>
<tr>
<td>Elastase level(^3) (µg/g)</td>
<td>113 – 127</td>
<td>129 – 160</td>
<td>&gt; 200</td>
</tr>
</tbody>
</table>

Adapted from Henderson et al. (2001)\(^1\), Armand et al. (1995, 1996)\(^2\), Mason (1962)\(^2\), Kori et al. (2016)\(^3\).

**Lack of digestive capacity:** critical

Digestion of milk proteins = peptides with antimicrobial and immunological activities
Milk proteases begin to degrade proteins within the mammary gland and continue to act within the infant’s stomach.

Milk has not only intact proteins - some proteins are partially digested.

Milk proteases = important component of the infant’s digestive capacity.

Dallas et al. (2015)
Human milk has many different enzymes that help preterm infant to digest.

System of proteases (active and zymogen forms), activators and protease inhibitors = control protein digestion.
Bioinformatic analysis of enzyme cleavage patterns

- Milk proteases are more active in preterm milk than term milk

- Milk enzymes are still active in the term infant stomach

Dallas et al. (2015)
• Milk protease activity in the preterm stomach remain unexplored

• Study Aims: determine the impact of the degree of **prematurity** and **postpartum time** on the activity of milk proteases in the **preterm infant stomach**

Early versus Late preterm milks

Early versus Late gestational age (GA)
preterm infant stomach

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Study aims

Milk protease activity in the preterm stomach remain unexplored

Study Aims: determine the impact of the degree of **prematurity** and **postpartum time** on the activity of milk proteases in the **preterm infant stomach**

Early versus Late preterm milks

Early versus Late gestational age (GA)
preterm infant stomach

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Early versus Late postpartum time

Days of life (DOL)
Human milk and infant gastric samples were collected from mother-infant pairs (18 preterm infants)

<table>
<thead>
<tr>
<th>Preterm Infant</th>
<th>Gestational age (GA) (weeks)</th>
<th>Days of life (DOL) (days)</th>
<th>Birth weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>24 1/7</td>
<td>2</td>
<td>720</td>
</tr>
<tr>
<td>2</td>
<td>25 1/7</td>
<td>9</td>
<td>620</td>
</tr>
<tr>
<td>3</td>
<td>25 1/7</td>
<td>23</td>
<td>620</td>
</tr>
<tr>
<td>4</td>
<td>25</td>
<td>26</td>
<td>770</td>
</tr>
<tr>
<td>5</td>
<td>25</td>
<td>26</td>
<td>665</td>
</tr>
<tr>
<td>6</td>
<td>24 5/7</td>
<td>31</td>
<td>700</td>
</tr>
<tr>
<td>7</td>
<td>25</td>
<td>42</td>
<td>675</td>
</tr>
<tr>
<td>8</td>
<td>24 5/7</td>
<td>45</td>
<td>760</td>
</tr>
<tr>
<td>9</td>
<td>25 1/7</td>
<td>47</td>
<td>620</td>
</tr>
<tr>
<td>10</td>
<td>27</td>
<td>11</td>
<td>900</td>
</tr>
<tr>
<td>11</td>
<td>29 2/7</td>
<td>5</td>
<td>1170</td>
</tr>
<tr>
<td>12</td>
<td>30</td>
<td>8</td>
<td>1190</td>
</tr>
<tr>
<td>13</td>
<td>30</td>
<td>19</td>
<td>1190</td>
</tr>
<tr>
<td>14</td>
<td>32 2/7</td>
<td>11</td>
<td>1610</td>
</tr>
<tr>
<td>15</td>
<td>27 3/7</td>
<td>31</td>
<td>1010</td>
</tr>
<tr>
<td>16</td>
<td>29 5/7</td>
<td>31</td>
<td>1040</td>
</tr>
<tr>
<td>17</td>
<td>32</td>
<td>30</td>
<td>2210</td>
</tr>
<tr>
<td>18</td>
<td>27</td>
<td>44</td>
<td>890</td>
</tr>
</tbody>
</table>

- Gastric samples were collected via indwelling gastric tubes present for other medical reasons

![Diagram of the digestive system]
Protease activity by fluorometric or spectrometric assays

A) Add supernatant samples to tube
Add standards and blanks in other tubes

B) Add buffer and synthetic substrate* and incubate at 37°C for 60 min

C) Transfer in a microplate

D) Read with a microplate reader

Activity was determined for:
- Total protease
- Plasmin
- Elastase
- Kallikrein
- Thrombin
- Cathepsin D
- Carboxypeptidase

human milk or preterm gastric samples (2X)

Centrifuge at 3,000 rpm, 10 min at 4°C
### Concentration of proteases, antiproteases and protein by ELISA

**Concentration** was determined for:

<table>
<thead>
<tr>
<th>Protease</th>
<th>Antiprotease</th>
<th>Bioactive protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasmin</td>
<td>Antitrypsin</td>
<td>Lactoferrin</td>
</tr>
<tr>
<td>Elastase</td>
<td>Antichymotrypsin</td>
<td>IgA</td>
</tr>
<tr>
<td>Kallikrein</td>
<td>Antiplasmin</td>
<td></td>
</tr>
<tr>
<td>Thrombin</td>
<td>Antithrombin III</td>
<td></td>
</tr>
<tr>
<td>Cathepsin D</td>
<td>SERPINAS5</td>
<td></td>
</tr>
<tr>
<td>Carboxypeptidase B2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytosol aminopeptidase</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Other measurements: pH and total protein
Results: Protease in milk and in the preterm stomach

Changes in proteases between milk and gastric samples in premature infants (24–32 wk GA)

Values are LS means ± SE (n=18 for each group) (**: P ≤ 0.01, ***: P ≤ 0.001 )

At least 83-88% of gastric digestion derives from milk proteases rather than pepsin
Antiproteases and bioactive proteins in milk and in the preterm stomach

Changes in antiproteases and bioactive proteins between milk and gastric samples in premature infants (24–32 wk GA)
Values are LS means ± SE (n=18 for each group) (**: P ≤ 0.01; ***: P ≤ 0.001)

Partial digestion of antiproteases in the stomach

Degradation of bioactive proteins in the stomach
Changes in protease activity in early (EGA, 24–26 wk GA) and late (LGA, 27–32 wk GA) GA preterm infant gastric samples. Values are LS means ± SE (n=9 for each group) (*: P ≤ 0.05; ***: P ≤ 0.001)
Activity of proteases in gastric samples from early GA (EGA, 24–26 wk) and late GA (LGA, 27–31 wk) infants across early (EDOL, 2–30 d) and late (LDOL, 31–50 d) time postpartum. Values are LS means ± SE (n=5). Slope (r) and p-value (p), linear regression.
Antiproteases across postpartum in the preterm infant stomach

Changes of antiproteases and proteins in gastric samples from early GA (EGA, 24–26 wk) and late GA (LGA, 27–31 wk) infants across early (EDOL, 2–30 d) and late (LDOL, 31–50 d) time postpartum. Values are LS means ± SE (n=5).

EDOL: Early days of life
LDOL: Late days of life
EGA: Early gestational age
LGA: Late gestational age
Conclusion

- At least 83-88% of gastric digestion derives from milk proteases rather than pepsin
- Gastric digestion increases across time postpartum in late GA infants, but not early GA infants
- Milk proteases are more active and abundant than pepsin in the preterm infant stomach
- This study confirms our predicted data on the activity of proteases in human milk
- Need more investigation of these changes with increased samples numbers for preterm and term infants
Acknowledgments

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- Research team at OSU

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Explanation for concentration of elastase (ELISA)

Milk < Stomach

Conformation of procathepsin D differ from cathepsin D
= Low binding with antibody

Conformation of proelastase similar to elastase
= High binding with antibody

Cross reactivity with proenzyme differ between antibody

Anti-cathepsin D antibody

Milk = Stomach

Anti-elastase antibody