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

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Premature birth report by National Center for Health Statistics 2014

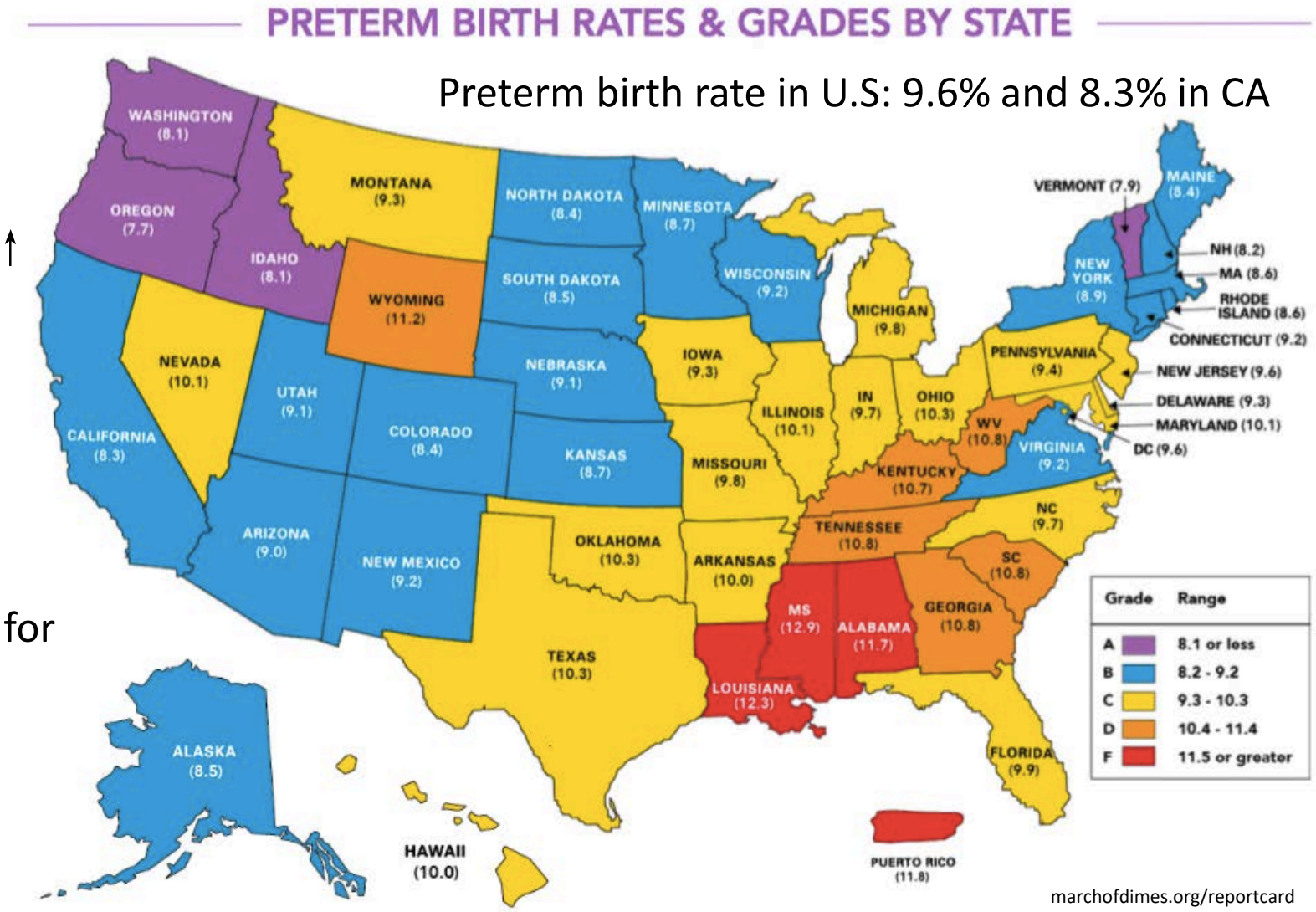
"**Preterm**" is defined as infant birth with less than 37 wks gestation

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

Preterm birth rate in U.S: 9.6% and 8.3% in CA



marchofdimes.org/reportcard

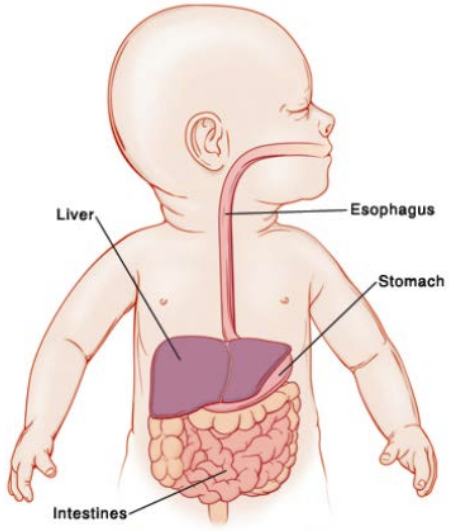
Digestion of human milk in preterm infant

Immature digestive system in preterm infants:

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

	Preterm	Term	Adult
Pepsin activity[1] (U/mL)	12	125 (10X)	600 (50X)
Gastric pH [2]	4.1 – 5.8	3.2 – 5.0	1.8 – 2.0
Elastase level[3] (μg/g)	113 – 127	129 – 160	> 200

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



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Lack of digestive capacity: critical

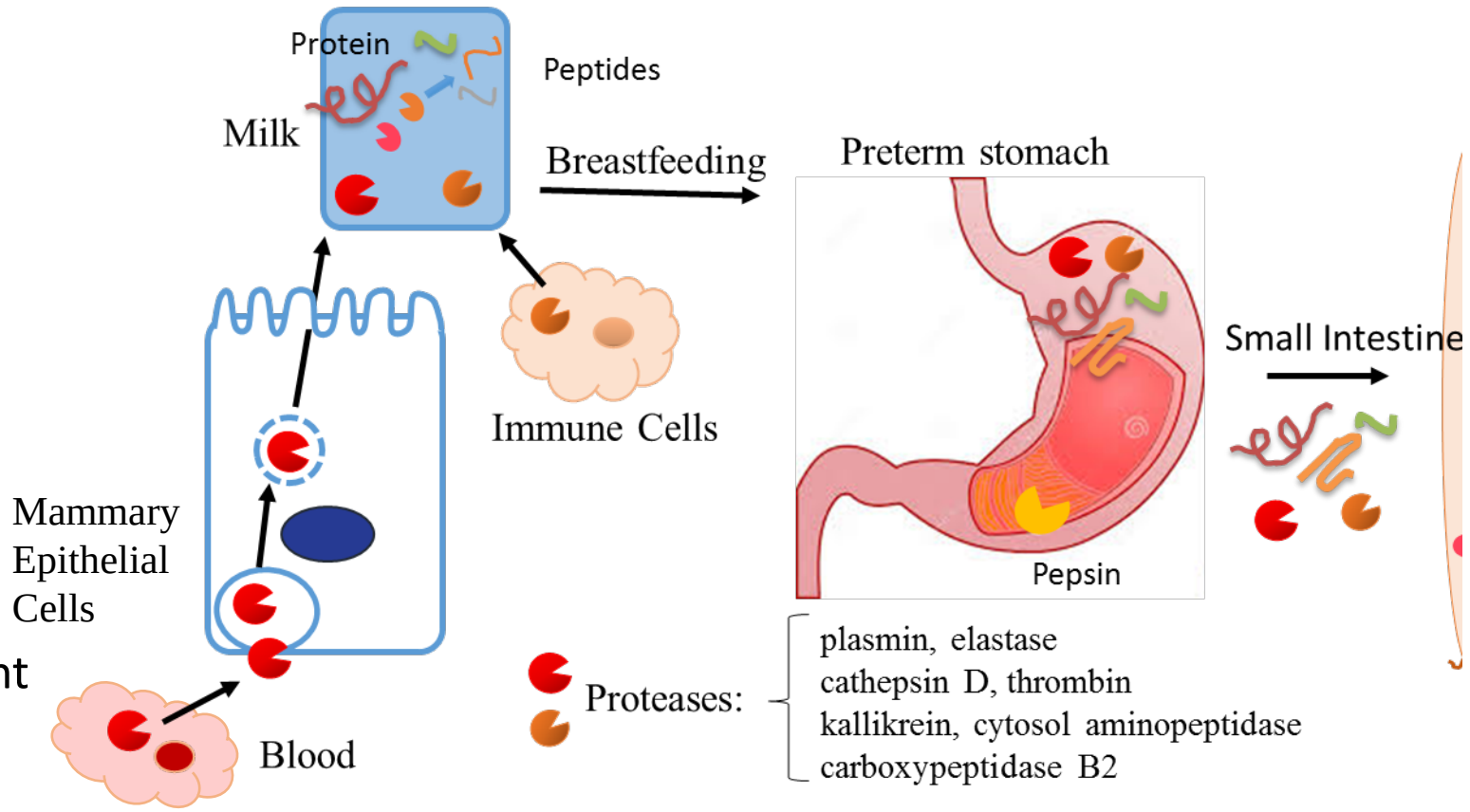
Digestion of milk proteins = peptides with antimicrobial and immunological activities

Our previous findings and hypothesis

- **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

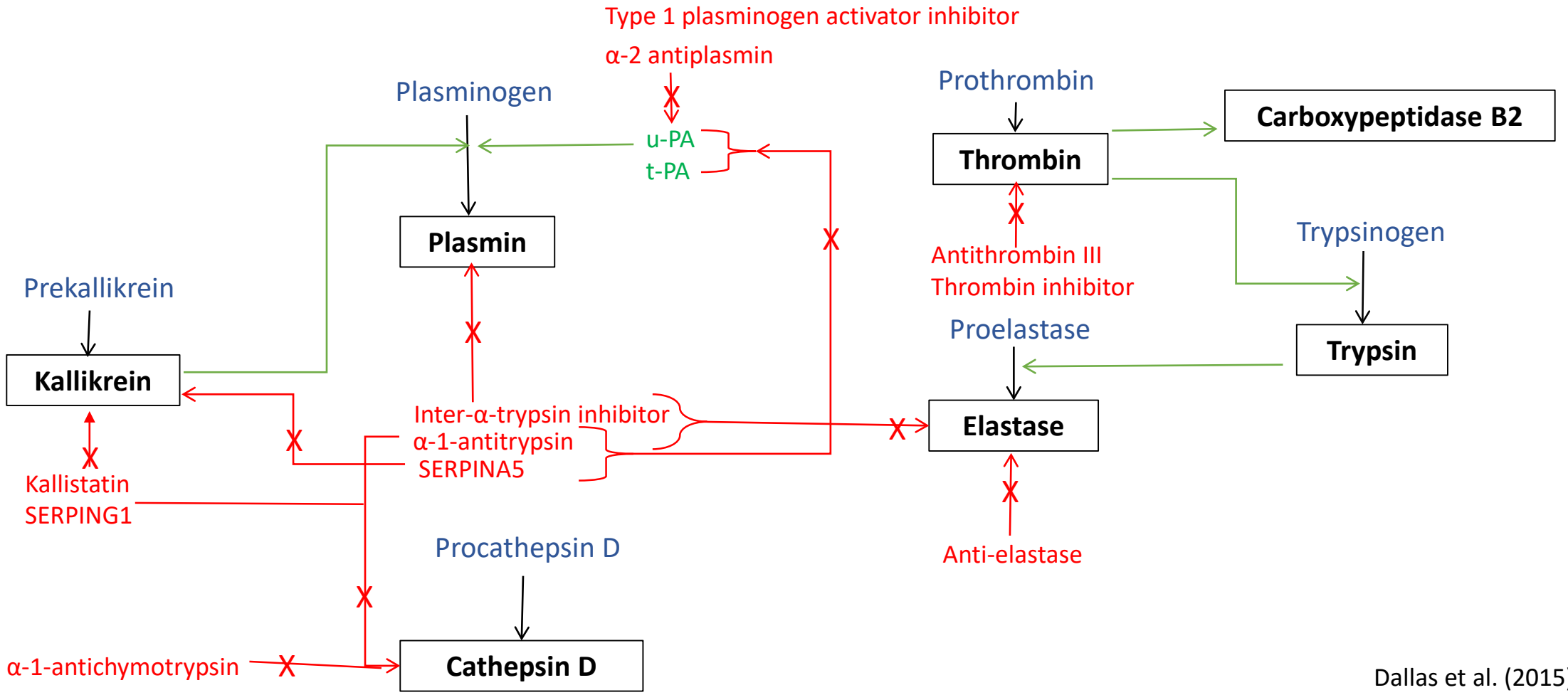
- Milk proteases = important component of the **infant's digestive capacity**



Dallas et al. (2015)

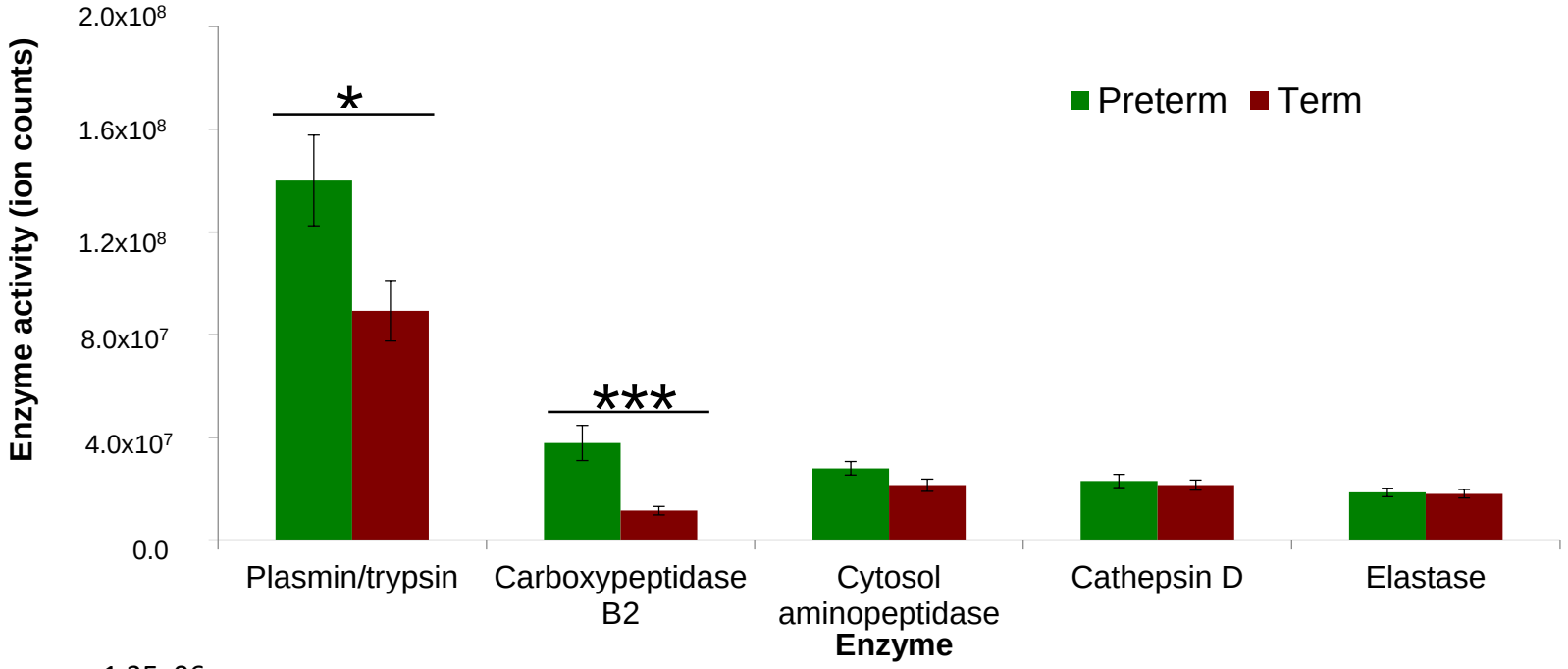
System of proteases and antiproteases

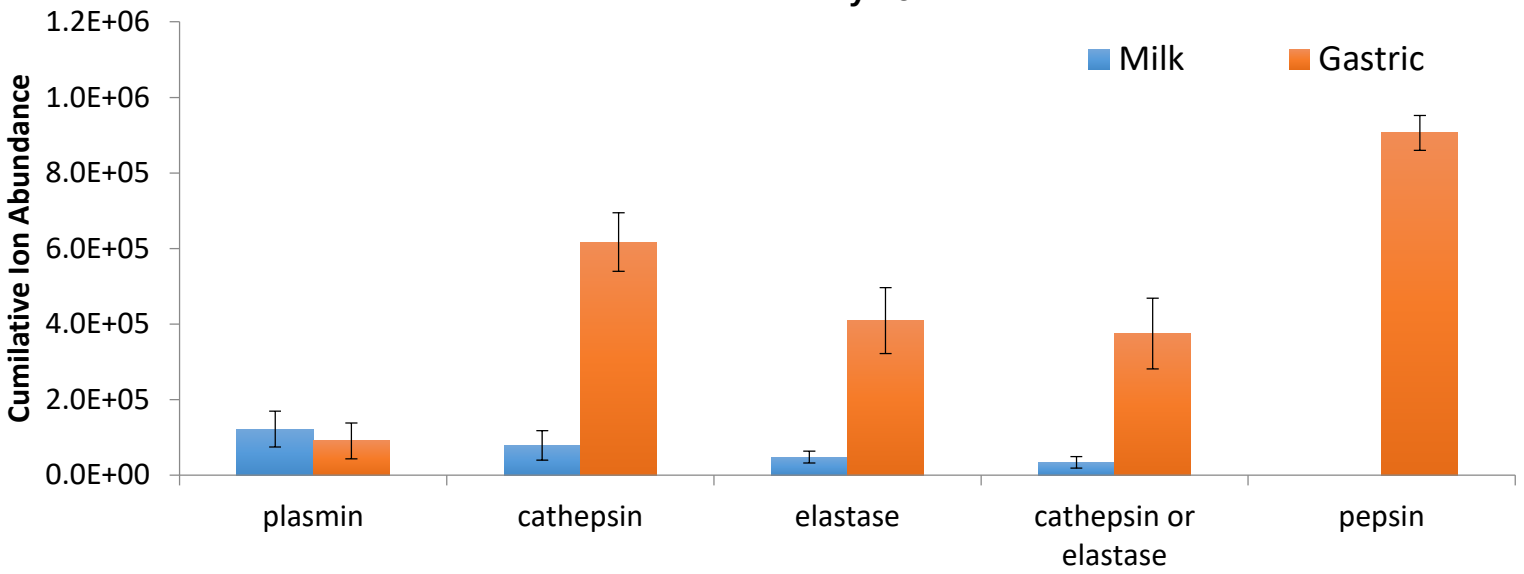
- Human milk has many different enzymes that help preterm infant to digest
- System of proteases (**active** and zymogen forms), activators and protease inhibitors = control protein digestion



Dallas et al. (2015)

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)

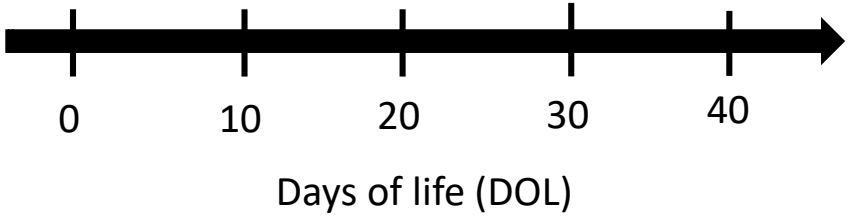
Study aims

- 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 postpartum time



Early versus Late gestational age (GA) preterm infant stomach

Material and Methods: collection of milk and gastric samples

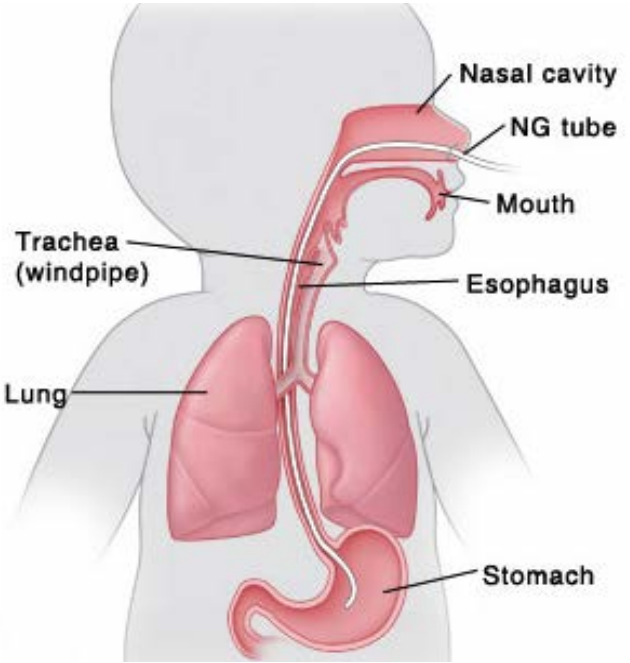
Human milk and infant gastric samples were collected from mother-infant pairs (18 preterm infants)

9 early GA

9 late GA

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

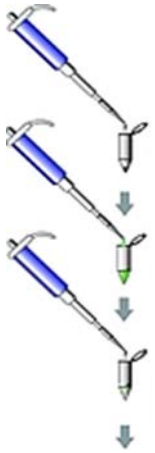
- Gastric samples were collected via indwelling gastric tubes present for other medical reasons



Protease activity by fluorometric or spectrometric assays

human milk or preterm gastric samples (2X)

Centrifuge at 3,000 rpm, 10 min at 4°C

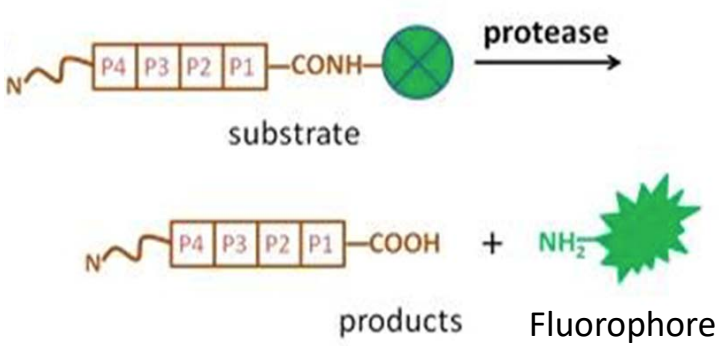


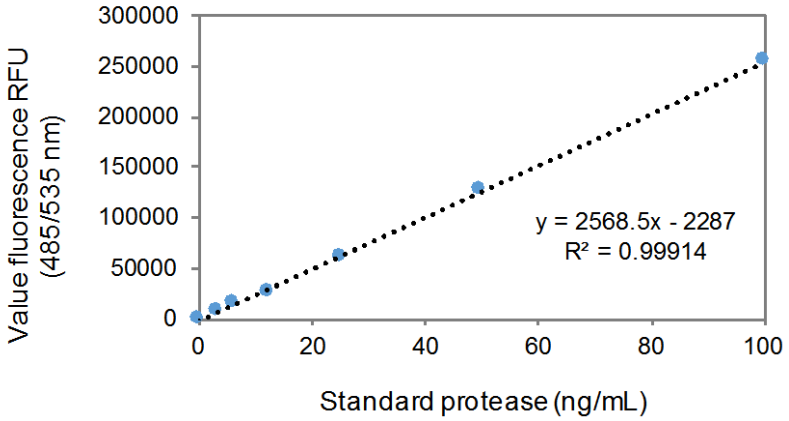
A) Add supernatant samples to tube
Add standards and blanks in other tubes

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

Concentration of proteases, antiproteases and protein by ELISA

Concentration was determined for:

Protease

- Plasmin
- Elastase
- Kallikrein
- Thrombin
- Cathepsin D
- Carboxypeptidase B2
- Cytosol aminopeptidase

Antiprotease

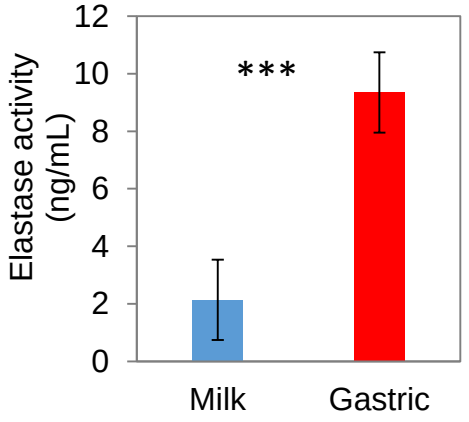
- Antitrypsin
- Antichymotrypsin
- Antiplasmin
- Antithrombin III
- SERPINA5

Bioactive protein

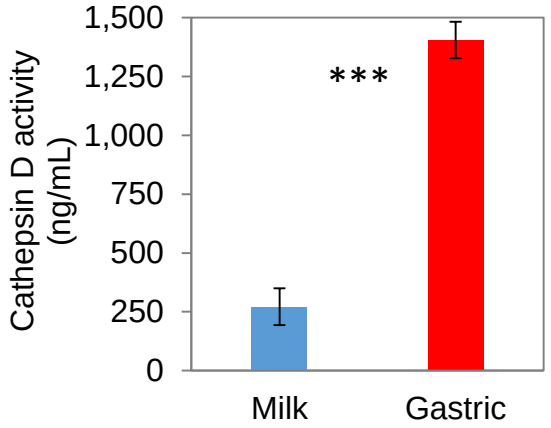
- Lactoferrin
- IgA

Other measurements : pH and total protein

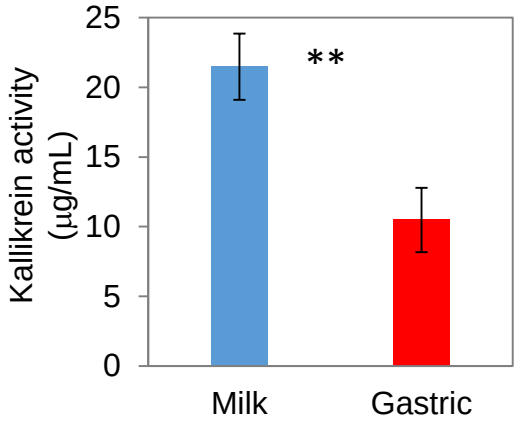
Results: Protease in milk and in the preterm stomach



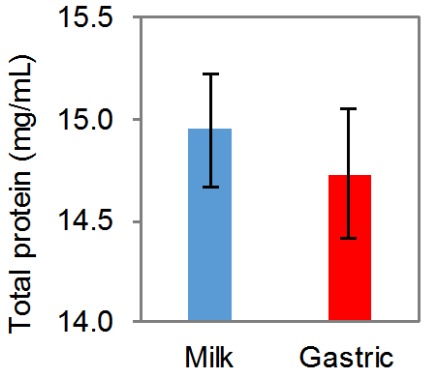
pH optimal of elastase : 8.0

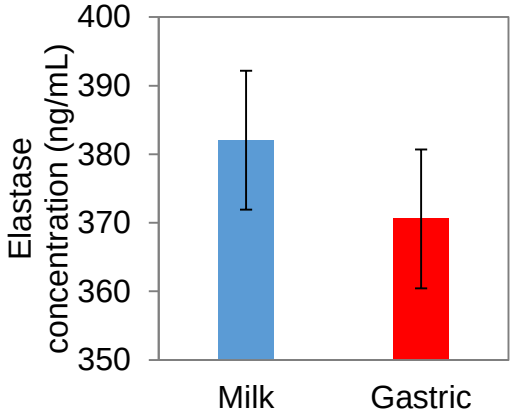


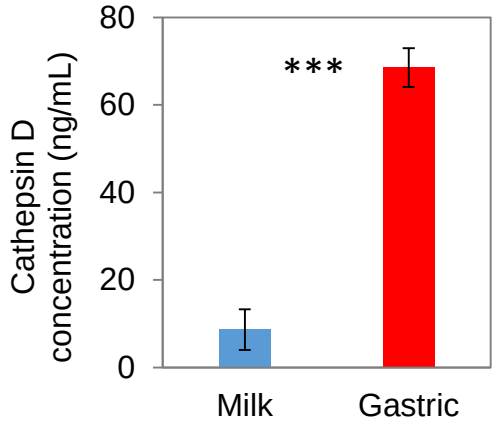
pH optimal of cathepsin D : 4.0

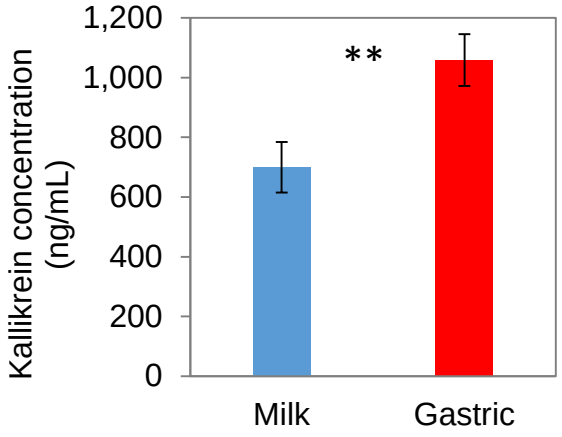


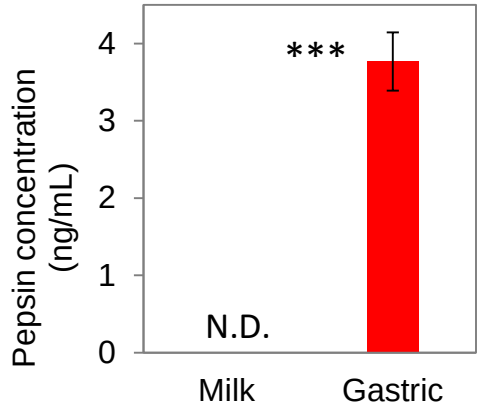
pH optimal of kallikrein : 6.5









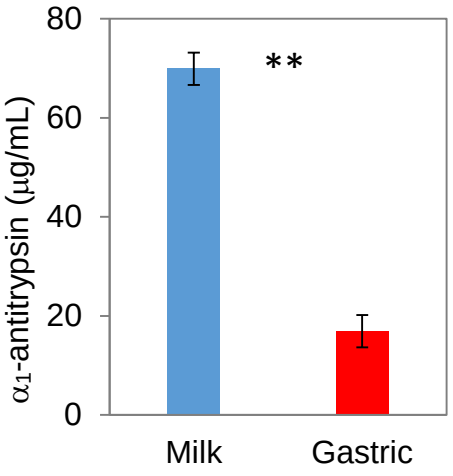


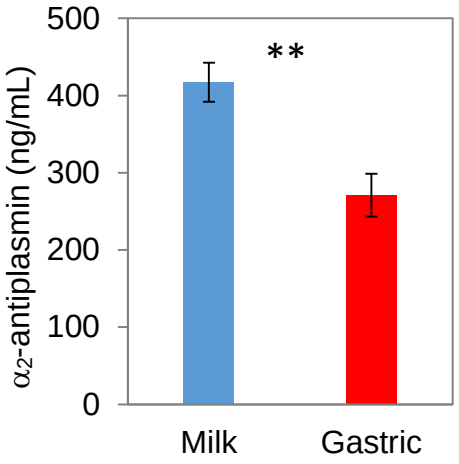
Changes in proteases between milk and gastric samples in premature infants (24–32 wk GA)
Values are LS means ± SE (n=18 for each group) (**: P ≤ 0.01, ***: P ≤ 0.001)

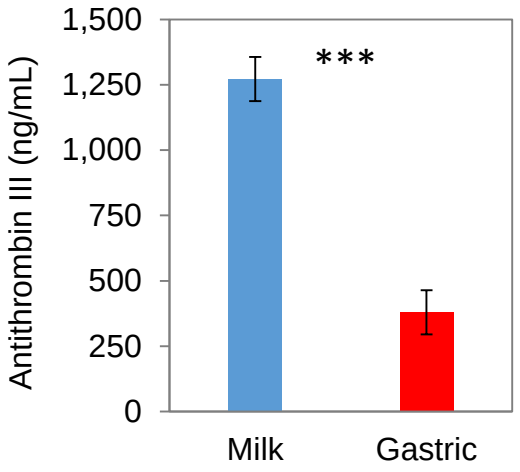
Milk proteases was more active and abundant than **pepsin**

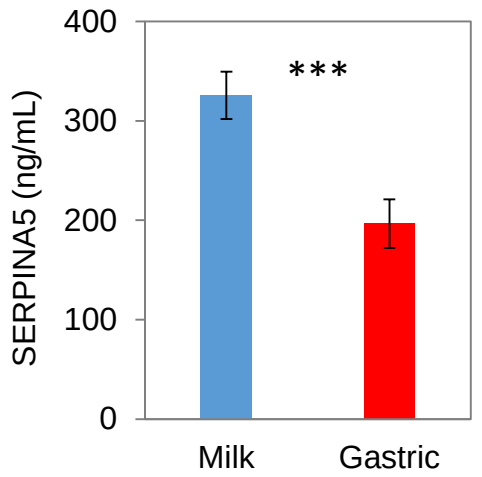
At least 83-88% of gastric digestion derives from **milk proteases** rather than **pepsin**

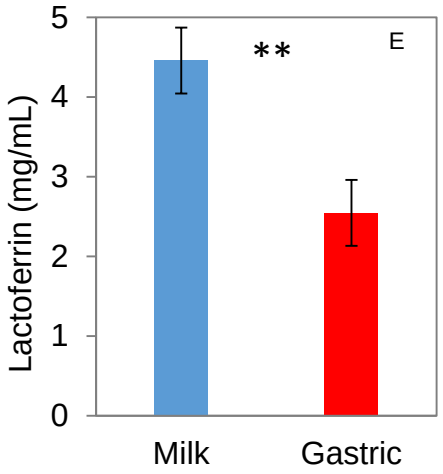
Antiproteases and bioactive proteins in milk and in the preterm stomach

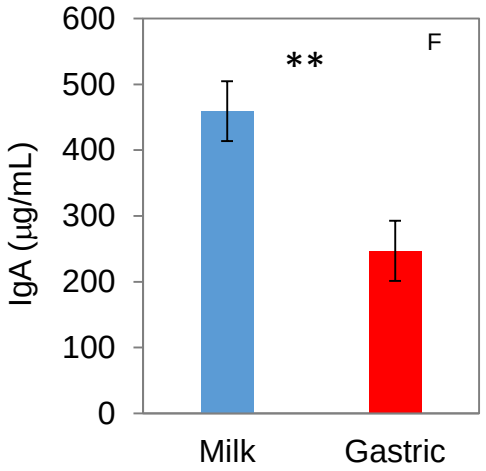


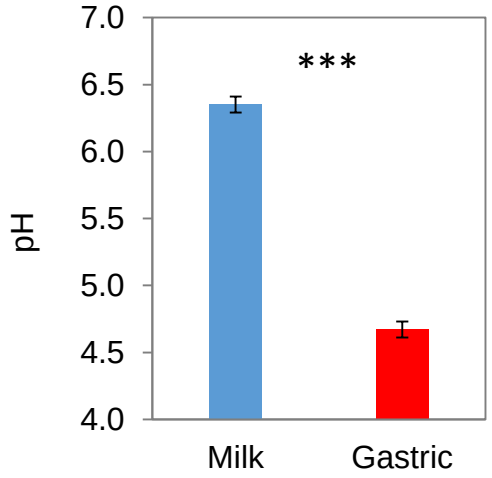












Partial digestion of **antiproteases** in the stomach

Degradation of **bioactive proteins** in the stomach

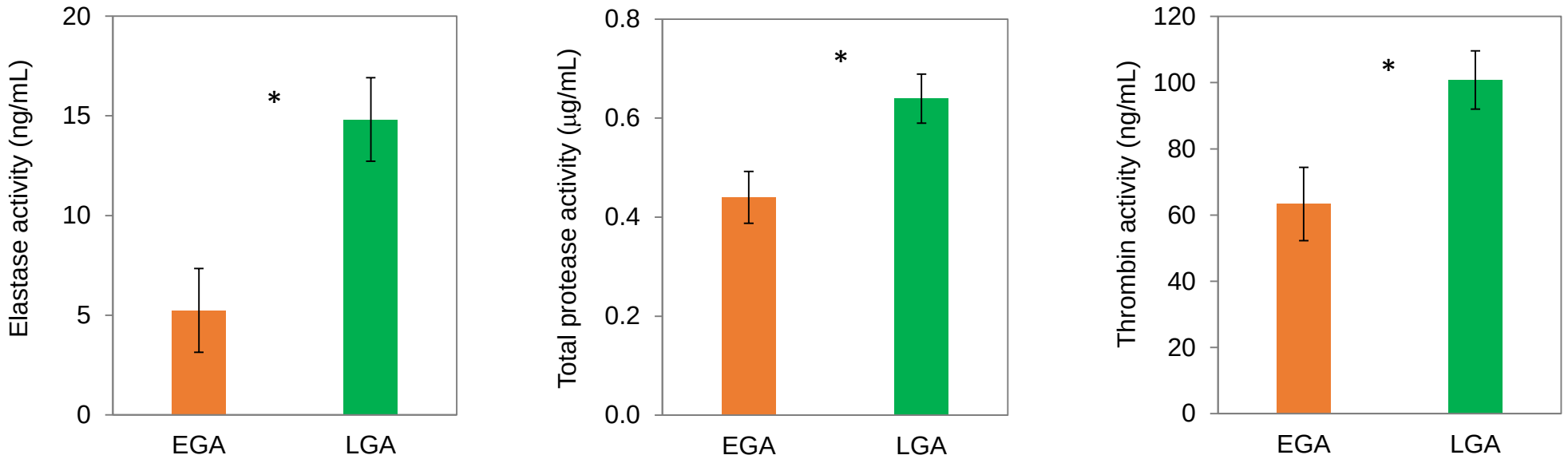
Changes in antiproteases and bioactive proteins between milk and gastric samples in premature infants (24–32 wk GA)
Values are LS means ± SE (n=18 for each group) (**: P ≤ 0.01; ***: P ≤ 0.001)

Protease activity across gestational age in the preterm stomach

Preterm infant stomach

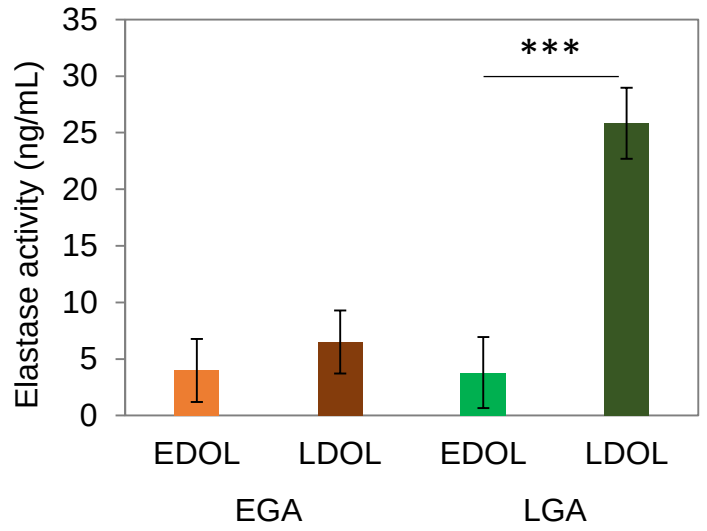
EGA: Early gestational age **LGA**: Late gestational age

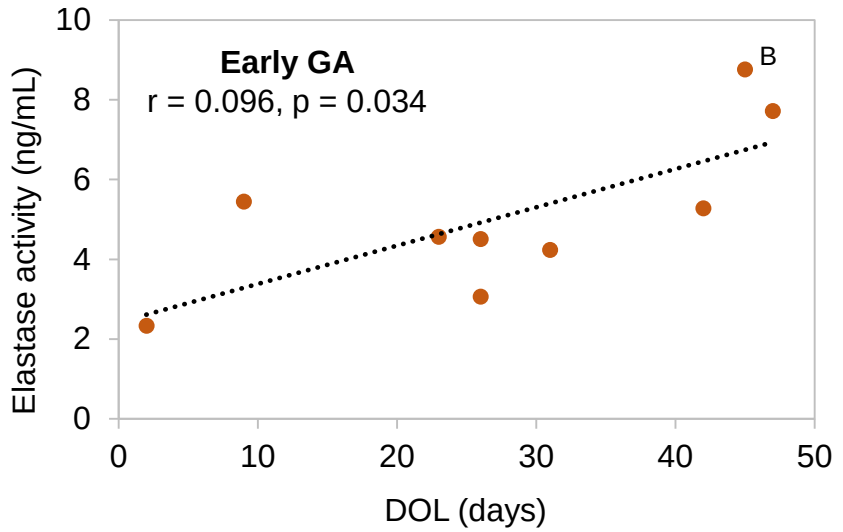
- **Early premature** infants may have an **immature digestive system**

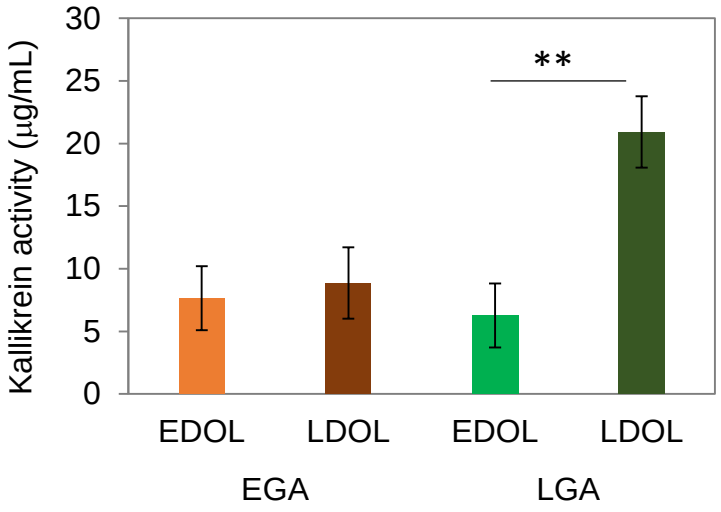


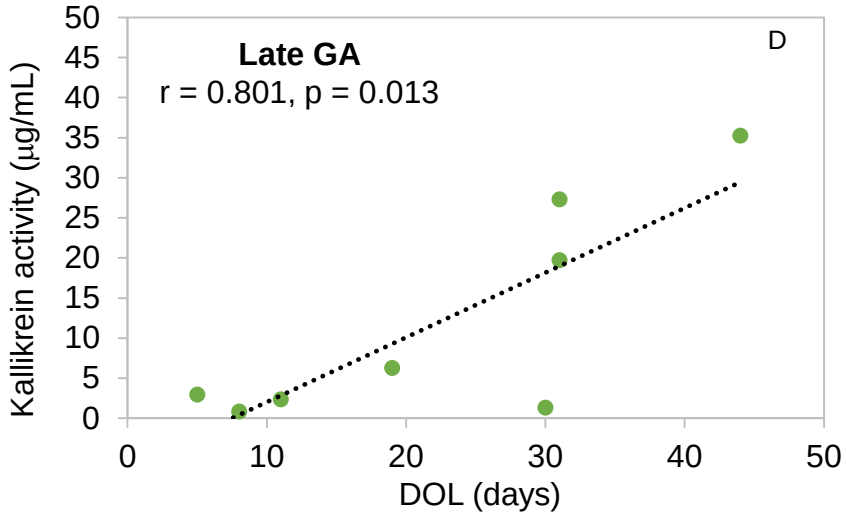
Changes in protease activity in early (EGA, 24–26 wk GA) and late (LGA, 27–32 wk GA) GA preterm infant gastric samples. Values are LS means ± SE (n=9 for each group) (*: P ≤ 0.05; ***: P ≤ 0.001)

Protease activity across time postpartum in the preterm infant stomach







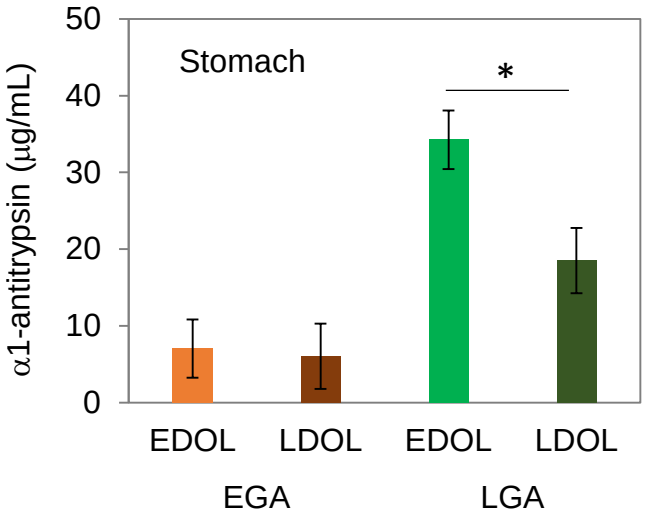


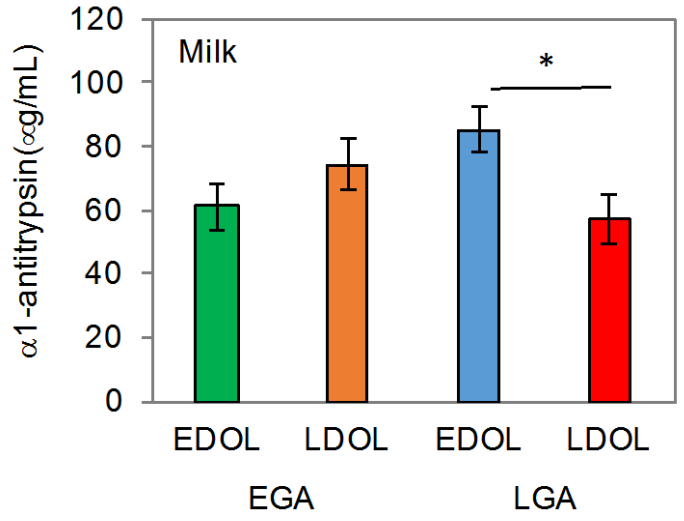
EDOL: Early days of life
LDOL: Late days of life

EGA: Early gestational age
LGA: Late gestational age

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.

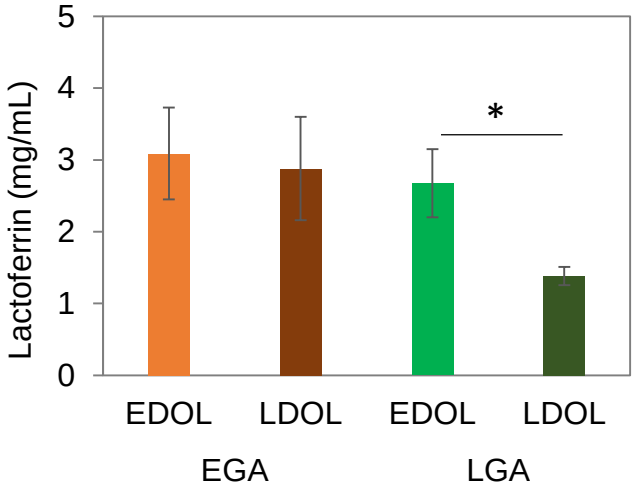
Antiproteases across postpartum in the preterm infant stomach

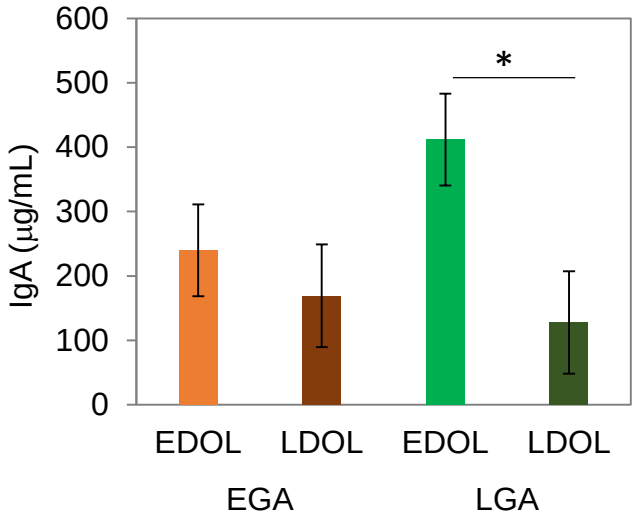


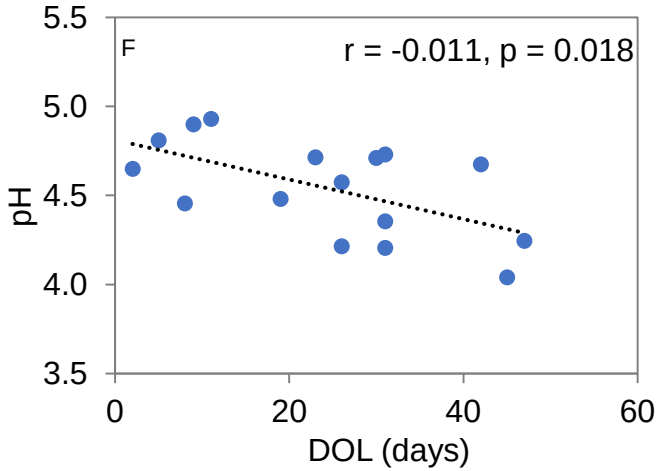


EDOL: Early days of life
LDOL: Late days of life

EGA: Early gestational age
LGA: Late gestational age







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).

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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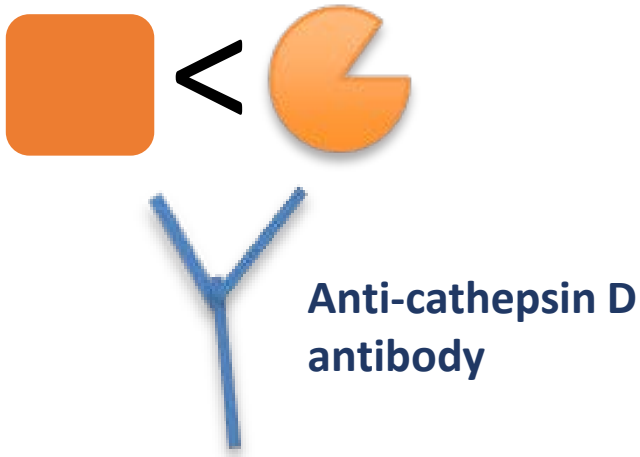
Explanation for concentration of elastase (ELISA)

Milk < Stomach

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



Cross reactivity with proenzyme differ between antibody

Milk = Stomach

Conformation of proelastase similar to elastase
= High binding with antibody