In vitro and in vivo approaches for investigating gastrointestinal disintegration of infant formula by the neonate

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General objectives

- To understand the mechanisms of disintegration of infant formula and their proteins in the digestive tract
- To determine the impact of the bioactives released during digestion on the infant health
- To model these phenomena in order to develop a reverse engineering approach

Bioactivities:
- Bioactive peptides
- Proteins
- Amino acids

Peripheric targets:
- Gut
- Muscle

Mathematical modelling
Reverse engineering

Ingredients + Processing
Milk
Infant Formula
Mouth
Stomach
Duodenum
Jejunum
Ileum
Receptors
Absorption

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Does heat treatment of milk affect caseins digestibility?

 mostra CNs = major allergens in infant whereas highly sensitive to digestion

Diagram:
- **Step 1**: Dissolution in milliQ water
- **Step 2**: Heat treatment
  - 80°C/20 s
  - 85°C/3 min
  - 105°C/60 s
- **Step 3**: Spray drying

**Digestion**
Infant gut Model (Dupont et al. Mol Nutr Food Res 2010, 54, 767-780)

Rehydrated milk powders → Gastric phase

- 60 min
- pH 3.0
- + pepsin
- + PC

Aliquots taken after 0, 1, 2, 5, 10, 20, 40 and 60 min digestion

→ Biochemical characterisation (SDS-PAGE, Immunoassays, LC-MS-MS)

Duodenal phase

- 30 min
- pH 6.5
- + trypsin
- + chymotrypsin
- + bile salts

Aliquots taken after 0, 1, 2, 5, 10, 15 and 30 min digestion
Identification of epitopes resistant to digestion by ELISA using specific Mabs

κ-casein

β-casein

αs₂-casein

αs₁-casein

For more information, see poster P26
Residual immunoreactivity of casein fragments

αs$_1$-CN(f129-151)  \( \kappa $-CN(f112-130) \g\)  αs$_2$-CN(f36-75) \p$

\beta$-CN(f1-25) \p$
\beta$-CN(f76-93)
\beta$-CN(f133-150)

Temperature \( p<0.0001 \)
Dry matter \( p<0.01 \)
Antibody array technology

Fluorescent anti-IgG conjugate

αs1-, αs2-, β- or κ-casein specific monoclonal antibodies

Digested sample

Rabbit polyclonal capturing antibody

Functionalized spots

Glass Microarray

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Kinetics of β-casein digestion depend on the area studied.
Pattern of bioactive peptides resistant to digestion depends on the process

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Bioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-CN(f108-113)</td>
<td>Anti-hypertensive</td>
</tr>
<tr>
<td>β-CN(f114-119)</td>
<td>Opioïd agonist</td>
</tr>
<tr>
<td>β-CN(f193-202)</td>
<td>Anti-hypertensive</td>
</tr>
<tr>
<td>β-CN(f193-209)</td>
<td>Immunomodulatory</td>
</tr>
<tr>
<td>αs1-CN(f1-23)</td>
<td>Antimicrobial</td>
</tr>
<tr>
<td>αs1-CN(f23-34)</td>
<td>Anti-hypertensive</td>
</tr>
<tr>
<td>αs1-CN(f91-100)</td>
<td>Anti-stress</td>
</tr>
<tr>
<td>αs1-CN(f144-149)</td>
<td>Antioxidant</td>
</tr>
<tr>
<td>β-lg(f9-14)</td>
<td>Anti-hypertensive</td>
</tr>
</tbody>
</table>
Conclusion 1

- Heat treatment increases caseins resistance to digestion (Dupont et al. 2010, Mol Nutr Food Res 54; Dupont et al. 2010, Food Dig, in Press)

- Increased resistance is associated to the formation of heat-induced aggregates between casein micelles and whey proteins

- Limits of the study:
  - Model matrix with no lipids
  - Static in vitro digestion model that does not consider the flow of the food and pH variations
  - Need to develop dynamic in vitro and in vivo approaches
Predicting matrix viscosity in gastric conditions

Simulation of pH decrease in the stomach using gluconodeltalactone (GDL) in a rheometer

by oscillation:

- determination of the viscoelastic properties of gels formed by acidification of rehydrated milk powders with GDL

- determination of the coagulation time and the firmness of the gel.
Development of dynamic digestion models

Cooperation with INRA UMR GMPA (D. Picque et al.)
In vivo digestion using the piglet as model

Cooperation with INRA UMR SENAH (I. Luron et al.)

Proteins
Lipids
Sugars
Minerals
Eau

Heat treatment + concentration + homogenization
+ Spray-drying

Formula

Effluent Characterization by:
*SDS-PAGE
*Immunoassays
*LC-MS-MS

Stomach
Proximal Jejunum 2m
Median Jejunum 1m 50
Ileum

Automatic milk feeder

Reconstitution 20%

28 days
Kinetics of Infant Formula proteins digestion

In g/100g of ingested protein

Stomach

Proximal jejunum

Ileum

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Peptides identified *in vivo*

For more information, see poster P22

Jejunum at 30 min

β-CN

α_{31}-CN

β-lg

α-la

Jejunum at 90 min

β-CN

α_{32}-CN

κ-CN

β-lg

Jejunum at 210 min

β-CN

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Conclusion 2

• Caseins are rapidly digested in the stomach compares to whey proteins but caseins fragments are still present in the ileum

• Are these findings compatible with the slow/fast proteins concept of milk???

• One of the key factor might be the ability of the caseins to coagulate in the stomach (depends on their concentration, buffering properties of the matrix…)

• Combination of *in vitro* and *in vivo* approaches are necessary to better understand the mechanisms of milk proteins digestion

• Diversity of analytical techniques allows a sharp characterization of the hydrolysis of proteins in different complexe media (food matrices, effluents…)
Improving infant formula for improving human life

Thanks for your kind attention !!!