Unravelling the mechanisms of food digestion to improve our knowledge of the effect of food on human health

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INRA, Rennes, France
By increasing our knowledge on food digestion, we will increase our knowledge on the effect of food on human health.
Our goals

To understand the mechanisms of breakdown of food matrices and their constituents in the gut and identify the beneficial/deleterious food components released during digestion.

To determine the impact of the structure of food matrices on nutrient bioavailability.

To model these phenomena in order to develop a reverse engineering approach.

Bioactivities
- Bioactive peptides
- Amino acids
- Fatty acids
- Minerals…
The digestive process

- **Storage, grinding and mixing in the stomach**
- **Pepsin**
- **Gastric lipase**
- **HCl**
- **Fasted pH 1.3-2.5**
- **Esophagus**
- **Stomach**
- **Pylorus**
- **Duodenum**
- **Jejunum**
- **Ileum**
- **Blood**
- **Small intestine**
- **Large intestine**
- **Mouth**
- **Chewing and deglutition**
- **Trypsin, Chymotrypsin, Pancreatic lipase**
- **pH 6.5-6.8**
- **Intestinal transit**
- **Nutrient absorption**
- **Gastric emptying**

- From Roger Lentle, Massey Univ. NZ

Gastric phase = a very complex but crucial step for the whole digestion process

Kong and Singh, 2008
Models available at INRA for simulating digestion

In vitro static models (infant, adult, elderly)

In vitro dynamic models (infant, adult, elderly)

In silico models

\[ \Phi_{12} = k_{12\text{whey}} \times \left( V_1 - m_{\text{waste}} \times \alpha \right) + k_{12\text{aggr}} \times m_{\text{aggr}} \times \alpha \]

Human models

Animal models

De Oliveira et al. 2016, Am J Clin Nutr
De Oliveira et al. 2017, Clin Nutr

Dupont et al. 2010ab, Mol Nutr Food Res
Minekus et al. 2014, Food Funct

Le Huerou-Luron et al. 2016, Eur J Nutr

Menard et al. 2014, Food Chem
Sanchez et al. 2015, Food Res Int

Le Feunteun et al. 2014, Food Bioprocess Tech

Dupont et al. 2010ab, Mol Nutr Food Res
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Sanchez et al. 2015, Food Res Int

Minekus et al. 2014, Food Funct

Le Feunteun et al. 2014, Food Bioprocess Tech
Food structure as modified by processing affects the kinetics of food digestion

Dupont D.

INRA, Rennes, France
Comparison of 6 dairy products of identical composition but different structure

Fat-free matrices:
40 g/L caseins, 10 g/L whey proteins, 95 g/L lactose and minerals
+ marker of the meal transit (Cr$^{2+}$-EDTA) → Gastric emptying half-time
The multi-canulated mini-pigs

6 minipigs (20 ± 1kg)

1 catheter: abdominal aorta

6 minipigs × 6 matrices × 8 sampling times after ingestion = 288 plasma samples collected

2 cannulas: end of stomach and mid-jejunum

6 minipigs × 6 matrices × 8 sampling times after ingestion × 2 sampling sites = 576 effluent samples collected
Ultra Low Heat powder

unheated milk ("raw" milk)

heated milk

96 min

? min

96 min

352 min

148 min

124 min

Ultra Low Heat powder

unheated milk ("raw" milk)

heated milk

96 min

? min

96 min

352 min

148 min

124 min

Gastric emptying half time
Bioactive peptides released during digestion differ from one matrix to another

More than 16000 peptides identified by LC-MS-MS in the jejunum

- More bioactive peptides identified during digestion of acid gel than rennet gel
- Nature of peptides is identical (clearly defined by the digestive enzyme specificity)
- Kinetics of release are different
The liquid-gel transition

Effect on absorption

- Milk gelation:
  - Delayed proteins transit → delayed AA absorption
  - Maximal AA concentration in the plasma

Potential effect on satiety

Ghrelin (gastrointestinal hormone → appetite stimulation)

- Heated milk vs. acid gel

Barbé et al. Food Chem 2013
Highly cited paper
Differential behaviour of acid/rennet gels in gastric conditions

- Acid/Rennet gel: identical composition, similar rheological properties and pore size
- ≠ Time of residence in the stomach (Acid 148 min / Rennet 352 min)
- How can we explain this difference? Dynamic in vitro digestion of the 2 gels

Ménard et al. Food Chem 2014

StoRM® software

DIDGI®

Stomach
- Pepsine
- Gastric lipase
- Simulated gastric fluid
- HCl

Small intestine
- Pancreatin
- Bile
- Simulated intestinal fluid
- NaHCO₃

Emptying: Elashoff’s model
Behaviour of acid and rennet gels in the stomach during *in vitro* dynamic digestion

Formation of a strong coagulum with rennet gel → slow down the gastric emptying of caseins

The structure that a food adopts in the stomach is essential to understand its digestion
Soleil is a particle (electron) accelerator that produces the synchrotron radiation, an extremely powerful source of light that permits exploration of inert or living matter.

DISCO is a VUV to visible beamline dedicated to biochemistry, chemistry and cell biology. The spectral region is optimized between 60 and 700 nm with conservation of the natural polarization of the light. It allows the imaging of protein intrinsic fluorescence with a UV microscope.
Kinetics of gel particles disintegration

Rennet Gel

Acid Gel
Diffusion of FITC-pepsin in Rennet gel of increasing casein concentration (37°C)

Pepsin diffusion of enzyme through the protein network is possible

Food proteins can effectively be hydrolyzed by pepsin from the interior of the gel pores.

Thevenot et al. Food Chem 2017

Gastric acidic environment → Syneresis and gel contraction → protein concentration → sieving effect of the polymer network → pepsin diffusion → digestion rate
What happens when food have the same macrostructure but different microstructures?
The case of egg white gels

Dupont D.
INRA, Rennes, France
The microstructure of egg-white gels made from different types of aggregates affects the kinetics of proteolysis.

<table>
<thead>
<tr>
<th>Aggregates</th>
<th>Rate of in vitro digestion</th>
</tr>
</thead>
<tbody>
<tr>
<td>linear</td>
<td>+++</td>
</tr>
<tr>
<td>branched</td>
<td>++</td>
</tr>
<tr>
<td>spherical</td>
<td>+</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>pH 9, IS 1M</th>
<th>pH 7, IS 1M</th>
<th>pH 5, IS 1M</th>
</tr>
</thead>
<tbody>
<tr>
<td>80°C/6h</td>
<td>80°C/6h</td>
<td>80°C/6h</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gels</th>
<th>Rate of in vitro digestion</th>
</tr>
</thead>
<tbody>
<tr>
<td>90°C/2.5 h</td>
<td>+</td>
</tr>
</tbody>
</table>

Nyemb et al., 2016. Food Hydrocolloid, 54, 315-27
Nyemb et al., 2016. Food Res. Int., 88, 302-9

SEM
CRYO-TEM
Spatio-temporal evolution of pH during an *in vivo* digestion.

**Gel pH 5**
- Digestion times: 20 min, 60 min, 120 min, 240 min, 360 min
- n=33

**Gel pH 7**
- Digestion times: 20 min, 60 min, 120 min, 240 min, 360 min
- n=33

**Gel pH 9**
- Digestion times: 20 min, 60 min, 120 min, 240 min, 360 min
- n=33

*Esophagus*, *Proximal region*, *Distal region*, *Pylorus*.
Kinetics of proteolysis

Proteolytic activity even at pH 7 and 9: probably driven by local pH
Understanding human milk digestion to design new infant formulas that will have the same behaviour in the GI tract

Deglaire A., Menard O., De Oliveira S., Bourlieu C. & Dupont D.

INRA, Rennes, France
Human milk

Bovine milk

Infant Formula

Casein micelle

Whey Proteins

(Turck et al, 2010)

\[ \Phi = 64 \text{ nm} \]
\((\beta, \kappa\text{-casein})\)

\[ \phi = 182 \text{ nm} \]
\((\alpha_{s1}, \beta\text{-casein})\)
**Human/ bovine milk / Infant Formula**

**Lipid globule structure**

Human milk

Bovine milk

**Native milk fat globule**

(4 - 5 µm)

Glycerophospholipids: P, PC (outer side), PE, PS, PI (inner side)

Adrophilin

Sphingolipids: Sphingomyelin (SM; )

Milk fat globule membrane (MFGM)

Triacylglycerols

Interface

Primary membrane from the endoplasmic reticulum

Bilayer from the plasma membrane

Glycosphingolipids (ceramides, gangliosides)

Xanthin dehydrogenase / oxidoreductase

Bulophilin

Glycosylated proteins (MUC1, MUC15, CD36, PA567 …)

Cholesterol ( )

Raft: sphingolipid and cholesterol-rich domain

(Lopez, 2010)

Infant Formula

**Lipid droplets**

(0,2 - 1 µm)

Triacylglycerols

Casein micelles, sub-micelles or caseins (α, β, κ)

Whey proteins

Thickness: 50-300 nm

(Lopez and Briard-Bion, 2007)
**In vivo study in the preterm infant**

ClinicalTrials.gov Identifier: NCT02112331
ARCHILACT

Preterm hospitalized infants
Feeding nasogastric tube
Fed every 3 hours

**Aim:** to compare meals with similar composition but different structure

Human milk from their *own mother*

GROUP A

- Raw human milk
- Pasteurized human milk

24h before feeding (4°C)
Stored at -20°C (bank milk)

Bank milk

Human milk from *anonymous donor*

GROUP B

- Pasteurized human milk
- Pasteurized-homogenized human milk

Stored at -20°C (bank milk)

Homogenization by sonication

30 min, 62.5°C
Pasteurization affected the initial structure and the emulsion disintegration of HM
(n = 6 infants)

Initial structure

Raw HM

Past HM

→ Protein heat-induced aggregation

Gastric disintegration

35 min

60 min

90 min

Size (µm) (n = 6 infants)

Volume (%)

Volume (%)
Pasteurization selectively impacted gastric proteolysis

Lactoferrin

Serum albumin

α-lactalbumin

β-casein

→ Faster decrease of lactoferrin in Past HM
→ Slower decrease of α-lactalbumin in Past HM (only at 90 min)
→ α-lactalbumin was the most resistant to gastric digestion

Impact on protective qualities of the native lactoferrin?

De Oliveira et al., Am J Clin Nutr 2017
Homogenization accelerates gastric lipolysis

Instantaneous lipolysis level

- Same milk composition, initial pre-lipolysis degree and inactivation of BSSL
- Different structure
- Increase of specific surface of droplets facilitating HGL adsorption

De Oliveira et al., Clin Nutr 2017
Infant formulas: can we create lipid structures biomimetic on the native fat globule?

Formula T1: Interface 100% Proteins 100% vegetable oil

Formula T2: Interface 100% phospholipids 100% vegetable oil

Formula T3: Interface 100% phospholipides 40% vegetable oil + 60% milk fat

Lopez, (2007)
Can the composition of infant formula modulate the physiological response of the neonate?

Automatic meal delivery (10 meals/day)

Effluents:
- SDS-PAGE
- Elisa

Mesenteric Lymph Nodes (MLN)

Collect of effluents and tissues

Tissues:
- Morphometry
- Enzyme Activities
- Intestinal Permeability
- Local immune response
- Microbiota

Veg
- Veg + PL
- Dairy Fat + PL

Rehydration at 20%

Slaughtering after
- 7 days
- 28 days
(90 min postprandial)

+ Mother-fed piglets (MF = + control)
Secretory activity of MLN

**Interferon-g (Th1 pro-inflammatory)**

**Interleukine-10 (Th2 anti-inflammatory)**

Milk lipids ➔ maturation of the piglet’s immune system more similar than with sow’s milk

Le Huerou et al. Eur J Nutr 2017
The composition/structure of the infant formula « orientates » the microbiota

More Proteobacteria with milk fat /
More Firmicutes with plant oil

May bioactive peptides released during digestion have an effect *in vivo*?
In vivo digestion of milk proteins

- 16 subjects were fed with 30g of either:
  - Caseins
  - Whey proteins

- Intestinal contents were collected through a double lumen nasogastric tube placed in their jejunum during 6 h after ingestion (location of the sampling site was controlled by radiography)

- Effluents were then freeze-dried until further use. Before analysis, effluents were rehydrated in 50 mM Tris-HCl buffer pH 8.0 and 2M urea, centrifuged for 10 min at 2000 g

- Samples were characterized by ESI-MS-MS. Only peptides larger than 5 amino acids are considered
Kinetics of peptides release

4704 dietary peptides unambiguously identified

Release of casein peptides throughout digestion

Peptides generated from whey proteins digestion are larger

Generation of a unique database of dietary peptides bioaccessible
A great variability in patterns but some peptides present in all subjects

31 peptides present throughout digestion
14 peptides found in all the subjects

1-6        RELEEL
57-66      SLVYPFPGPI
58-66      LVYPFPGPI
58-68      LVYPFPGPIPN
59-66      VYPFPGPI
59-67      VYPFPGPIPN
59-68      VYPFPGPIPN
60-66      YPFPGPI
60-66      YPFPGPI
61-66      PFPGPI
73-79      NIPPLTQ
73-80      NIPPLTQT
73-82      NIPPLTQTPV
80-91      TPVVPPFLQPE
81-87      PVVVPFPF
81-91      PVVVPFPFLQPE
81-92      PVVVPFPFLQPEV
83-91      VPPFLQPE
84-91      VPPFLQPE
85-901     PFPFLQPE
107-113    KEMPFPK
108-113    EMPFPK
114-119    YPVEPF
134-139    HLPLPL
135-139    LPLPL
156-160    MFPPQ
168-173    VAPFPQ
170-175    VLPVPQ
171-175    LPVPQ
194-201    QEPVLGPV
195-201    EPVLGPV
196-201    PVGLGPV

57-66            SLVYPFPGPI
57-66            SLVYPFPGPI
58-66            LVYPFPGPI
58-66            LVYPFPGPIPN
59-66            VYPFPGPI
59-67            VYPFPGPIPN
59-68            VYPFPGPIPN
60-66            YPFPGPI
60-66            YPFPGPI
61-66            PFPGPI
73-79            NIPPLTQ
73-80            NIPPLTQT
73-82            NIPPLTQTPV
80-91            TPVVPPFLQPE
81-87            PVVVPFPF
81-91            PVVVPFPFLQPE
81-92            PVVVPFPFLQPEV
83-91            VPPFLQPE
84-91            VPPFLQPE
85-901           PFPFLQPE
107-113          KEMPFPK
108-113          EMPFPK
114-119          YPVEPF
134-139          HLPLPL
135-139          LPLPL
156-160          MFPPQ
168-173          VAPFPQ
170-175          VLPVPQ
171-175          LPVPQ
194-201          QEPVLGPV
195-201          EPVLGPV
196-201          PVGLGPV

Among those 5 bioactive peptides

59-66            VYPFPGPI
59-68            VYPFPGPIPN
60-66            YPFPGPI
108-113          EMPFPK
114-119          YPVEPF

β-CN

Anti-hypertensive
Opioïd

Boutrou et al. 2013
Am J Clin Nutr
Bioactive peptides released during digestion can reinforce the defense of the intestinal epithelium.

β-CN(94-123) stimulates the mucus production in vitro (HT-29 MTX) and in vivo (rat).

Sections of duodenum after 10 d of administration of peptide β-CN (94-123)

Plaisancie et al. 2013, J Nutr Biochem
Plaisancie et al. 2015, J Dairy Res
Conclusion

The structure/composition of food regulate the kinetics of protein digestion and the release of amino acids in the bloodstream.

Gastric emptying rate will highly depend on the structure that the product will adopt in the stomach cavity.

Understanding the mechanisms of food particle breakdown in the stomach is critical to control the structure a food will adopt in gastric conditions.

Being able to design food structures for controlling the kinetics of hydrolysis of macronutrients will allow to obtain food particularly adapted to specific population.

Overweight/diabetic

Elderly/Athletes

Release Rate
The Bioactivity & Nutrition group at INRA in Rennes

Head
Didier DUPONT - Senior Scientist

Scientists
Rachel BOUTROU – Junior Scientist
Amélie DEGLAIRE – Lecturer
Juliane FLOURY – Lecturer
Catherine GUERIN - Lecturer
Joëlle LEONIL – Senior Scientist
Steven LE FEUNten – Junior Scientist
Françoise NAU – Professor
Frédérique PEDRONO – Lecturer
Guilherme FURTADO – Post-doc
Xaoxi YU – Post-Doc

PhD students
Linda LEROUX (2016-2019)
Manon HIOLLE (2016-2019)
Yohan REYNAUD (2016-2019)
Amira HALABI (2017-2020)
Léa SALELLES (2018-2021)
Jun WANG (2018-2021)

Technicians
Gwenaëlle HENRY
Yann LE GOUAR
Nathalie MONTHEAN

Engineers
Julien JARDIN
Olivia MENARD
Jordane OSSEMOND

2-3 Masters students

Engineers
Julien JARDIN
Olivia MENARD
Jordane OSSEMOND
Improving health properties of food by sharing our knowledge on the digestive process

International Network

Dr. Didier DUPONT, Senior Scientist, INRA, France
Industry involvement

~ 50 private companies are following INFOGEST
INFOGEST

Chair
Didier Dupont - France
didier.dupont@inra.fr

Vice-chair
Alan Mackie - UK

www.cost-infogest.eu

In vitro models of digestion
WG1
Didier Dupont
Pasquale Ferranti

Food interaction – meal digestion
WG2
Linda Giblin
Milena Corredig

Absorption models
WG3
Myriam Grundy

Digestive lipases and lipid digestion
WG4
Brigitte Graf
Frederic Carriere

Digestive amylases and starch digestion
WG5
Nadja Siegert
Caroline Orfila

In silico models of digestion
WG6
Choi-Hong Lai
Steven Le Feunteun

Absorption models
WG3

In vitro models of digestion
WG1

Food interaction – meal digestion
WG2

Digestive lipases and lipid digestion
WG4

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WG3

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WG4

Digestive amylases and starch digestion
WG5

In silico models of digestion
WG6
Some outputs

*In vitro* gastrointestinal digestion
Consensus INFOGEST protocol

**Oral phase**
Mix 1:1 with Simulated Salivary Fluid (SSF)
salivary amylase (75 U/mL)
2 min, pH 7

**Gastric Phase**
Mix 1:1 with Simulated Gastric Fluid (SGF)
Pepsin (2000 U/mL)
2h, pH 3

**Intestinal Phase**
Mix 1:1 with Simulated Intestinal Fluid (SIF)
Enzymes
- Pancreatin (based on trypsin 100 U/mL) or
- Pure enzymes
Bile (10mM)
2h, pH 7

Minekus et al. 2014
Food & Function, 5,
1113-1124
700 citations
An updated version of the protocol published last week!

INFOGEST static in vitro simulation of gastrointestinal food digestion


5-year Impact Factor=15,269
The consensus model can be learned with videos on YouTube.
Release of a 341-page book on all the in vitro and ex vivo models to study the impact of food bioactives on health

Some outputs

- Release of a 341-page book
- More than 620,000 chapter downloads
- Top 25% most downloaded eBooks in 2017

OPEN ACCESS

* More than 620,000 chapter downloads
* Top 25% most downloaded eBooks in 2017
The International Conference on Food Digestion

The Conference has been created by INFOGEST and is now an event regularly followed by 200 scientists.
We are pleased to announce the next
7th International Conference on Food Digestion

in Cork, Ireland, 2021