The fate of milk proteins and bioactive peptides in the gastrointestinal tract during digestion

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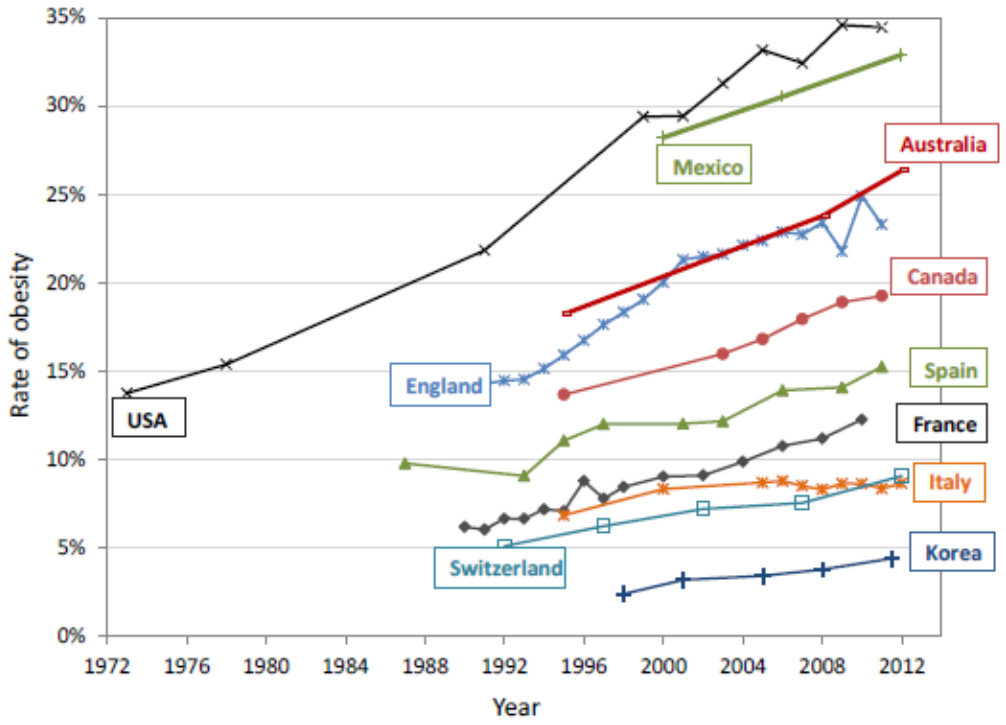
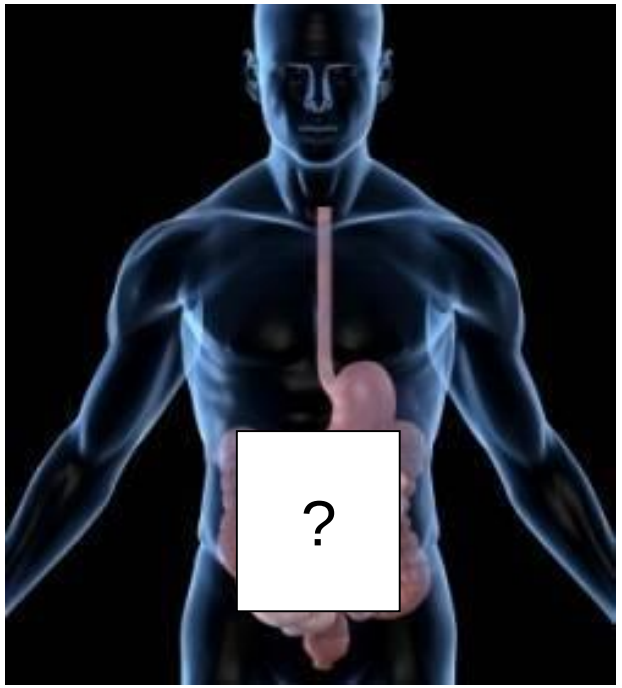
Food and human health: the key role of the digestive process



Diet-related diseases ↑

↳ Prevent these pathologies rather than cure them



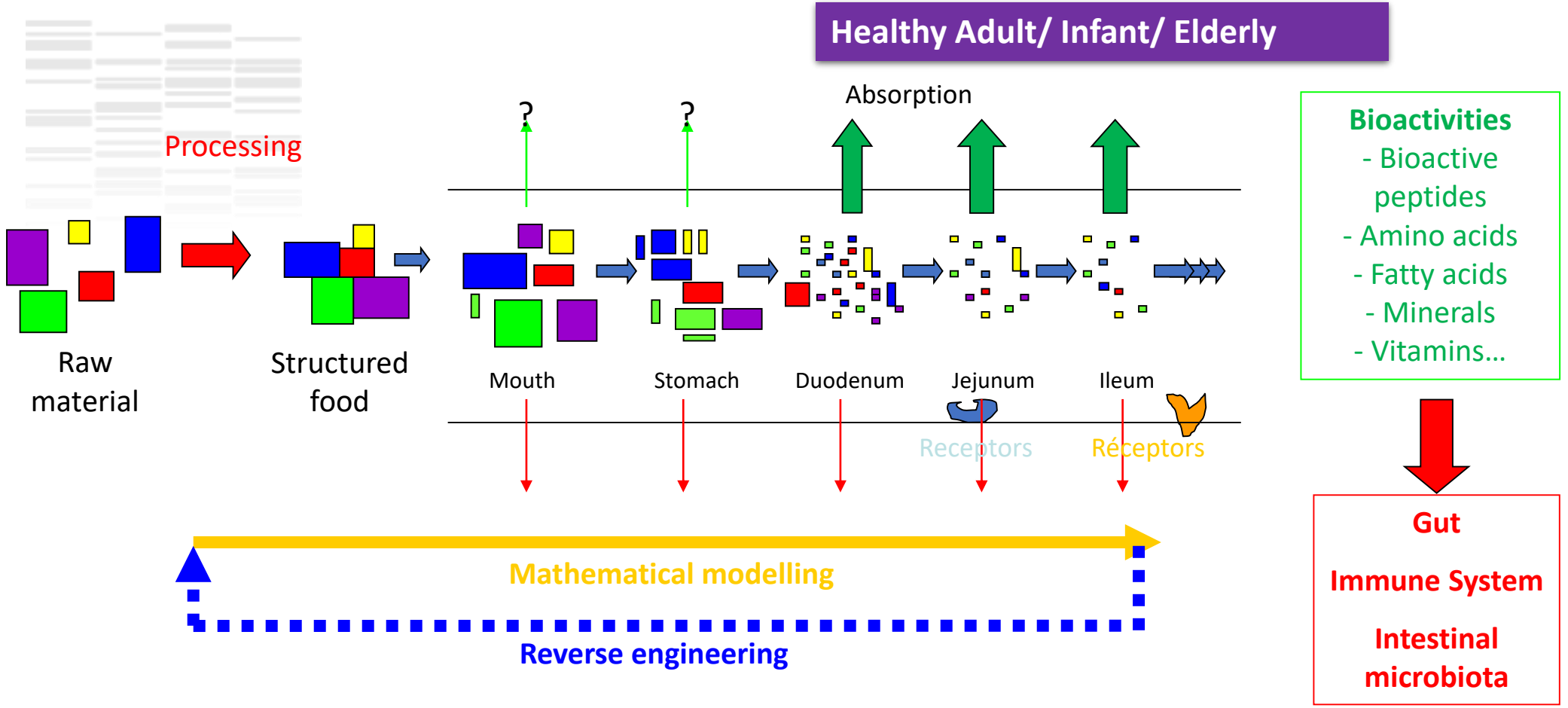
Gut = interface between food and human body

Digestion provides nutrients to the body and releases food components that can have a beneficial or a deleterious effect on human health

… but the mechanisms of food disintegration in the gastrointestinal tract remain unclear and the digestive process has been considered as a black box so far

Increasing our knowledge on food digestion to increase our knowledge on the effect of food on human health

Our goals

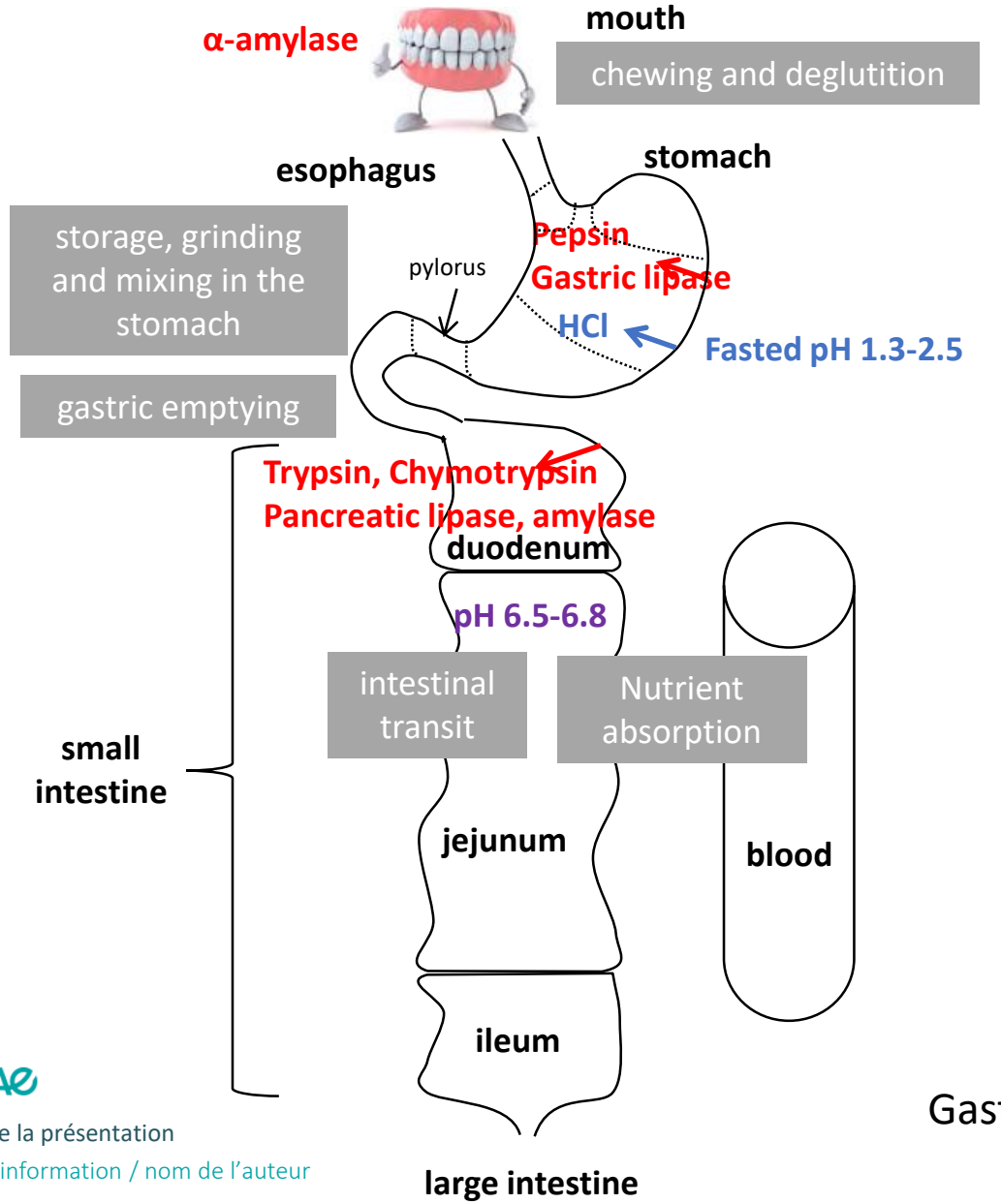


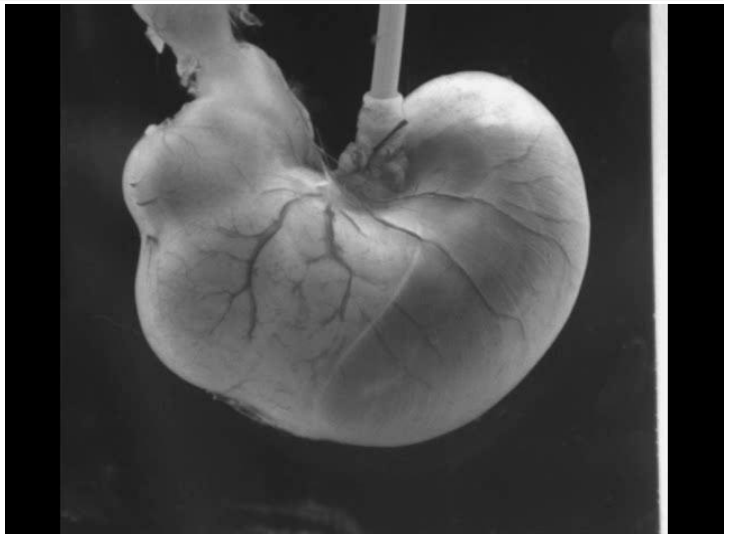
☞To understand the mechanisms of breakdown of food matrices and their constituents in the gut and identify the beneficial/deleterious food components released during digestion

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

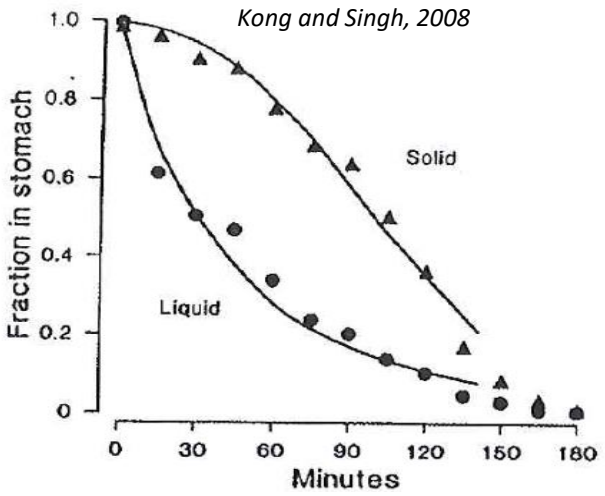
☞To model these phenomena in order to develop a reverse engineering approach

The digestive process





From Roger Lentle, Massey Univ. NZ



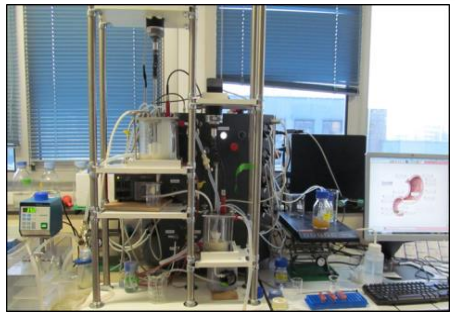
Gastric phase = a very complex but crucial step for the whole digestion process

Models available at INRAE for simulating digestion

Brodkorb et al. 2019, Nat Protocols
Menard et al. 2018, Food Chem

In vitro static models (infant, adult, elderly)

Menard et al. 2014, Food Chem
Sanchez et al. 2015 Food Res Int
Leroux et al. 2020, Foods
Reynaud et al. 2021 Food Chem

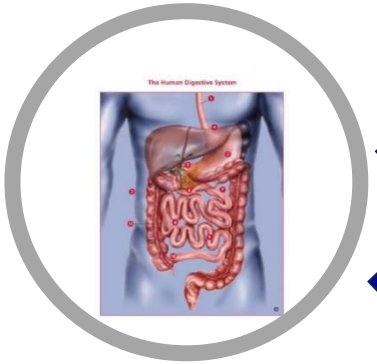


In vitro dynamic models (infant, adult, elderly)

Le Feunteun et al. 2014
Food Bioprocess Tech

In silico models

$$\Phi_{12} = k_{12whey} \times \left(V_1 - m_{caswpd1} \times \alpha\right) + k_{12aggr} \times m_{caswpd1} \times \alpha$$



Human models

Animal models

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

Barbé et al. 2013, 2014 Food Chem
Le Huerou-Luron et al.2016 Eur J Nutr
Lorieau et al 2019 Food Chem
Nau et al. 2019, 2022 Food Chem

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Does the food structure affect the release of bioactive peptides during digestion?

The food matrices

Ultra Low Heat powder

rehydration in water 14.5%

unheated milk ("raw" milk)

heat treatment 90°C-10 min

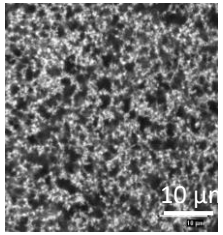
microstructure

heated milk

macrostructure

rennet gel

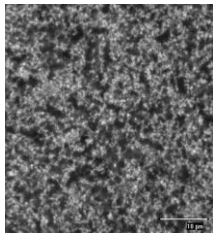
24h-20°C, rennet 0.003 % v/w



pH 6.6

rennet gel

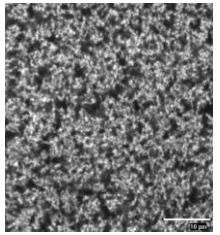
24h-20°C, rennet 0.3 % v/w



pH 6.6

24h-20°C, GDL 3 % w/w

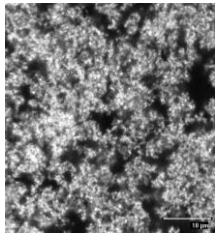
acid gel



pH 4

24h-20°C, GDL 3 % w/w + mixer 2 min

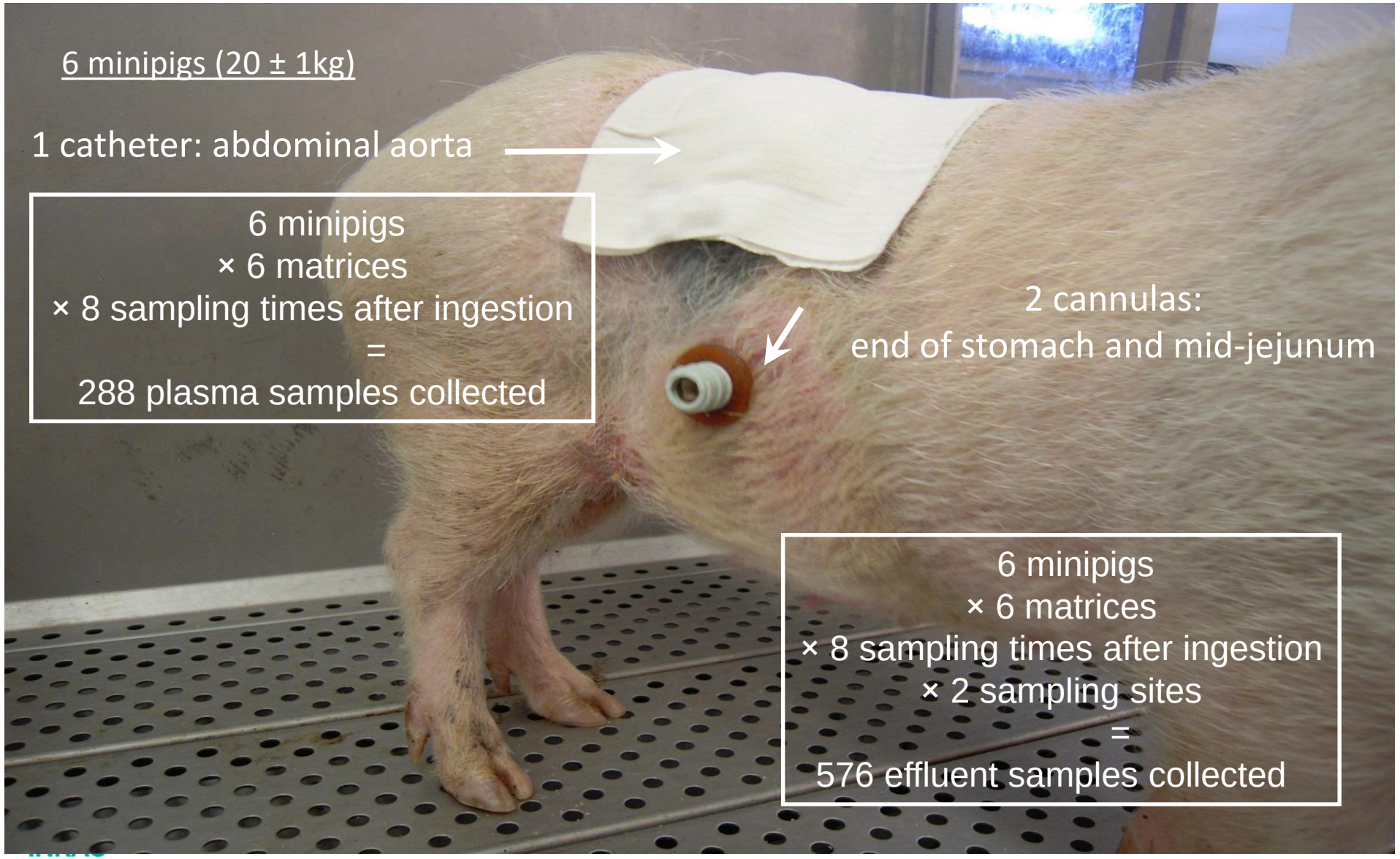
stirred acid gel



pH 4

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



Impact of food structure on gastric emptying half-time

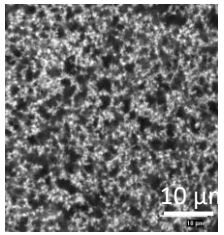
Ultra Low Heat powder

96 min

unheated milk ("raw" milk)

? min

rennet gel



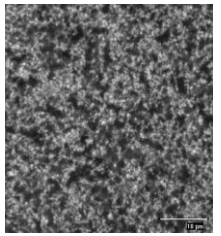
pH 6.6

heated milk

96 min

352 min

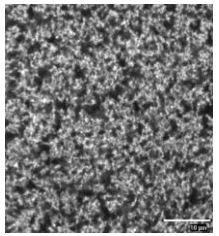
rennet gel



pH 6.6

148 min

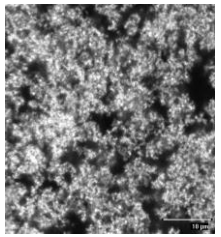
acid gel



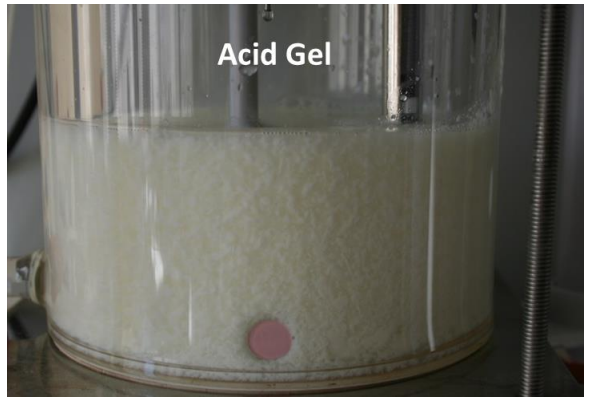
pH 4

124 min

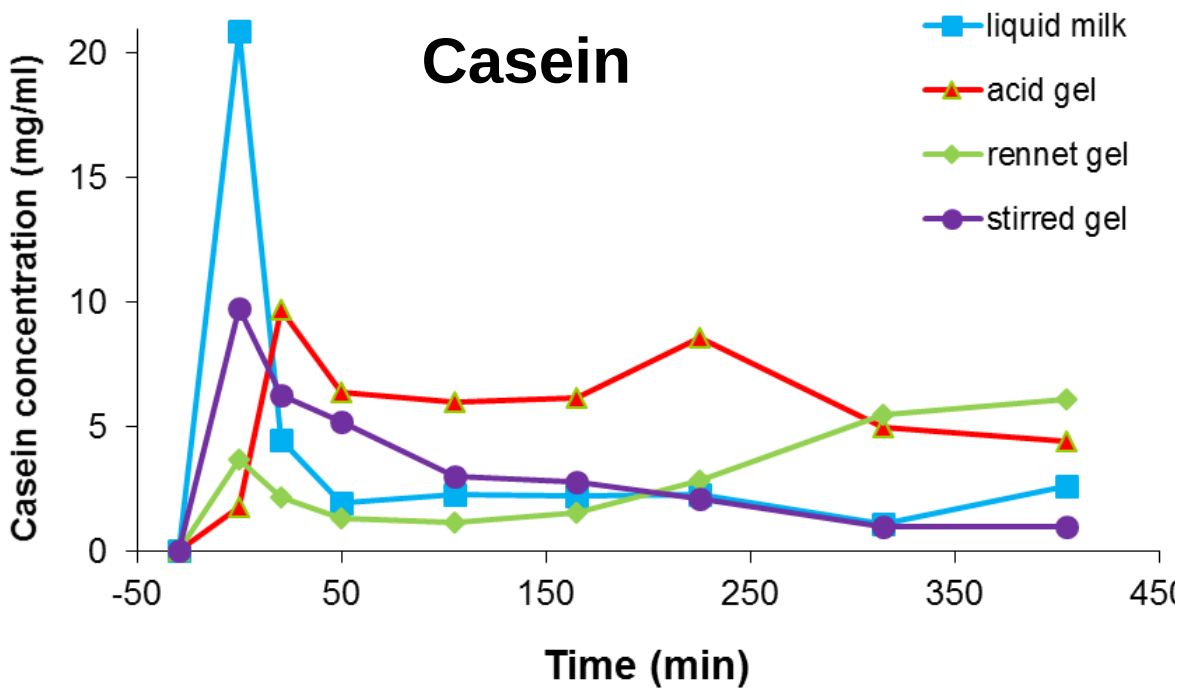
stirred acid gel



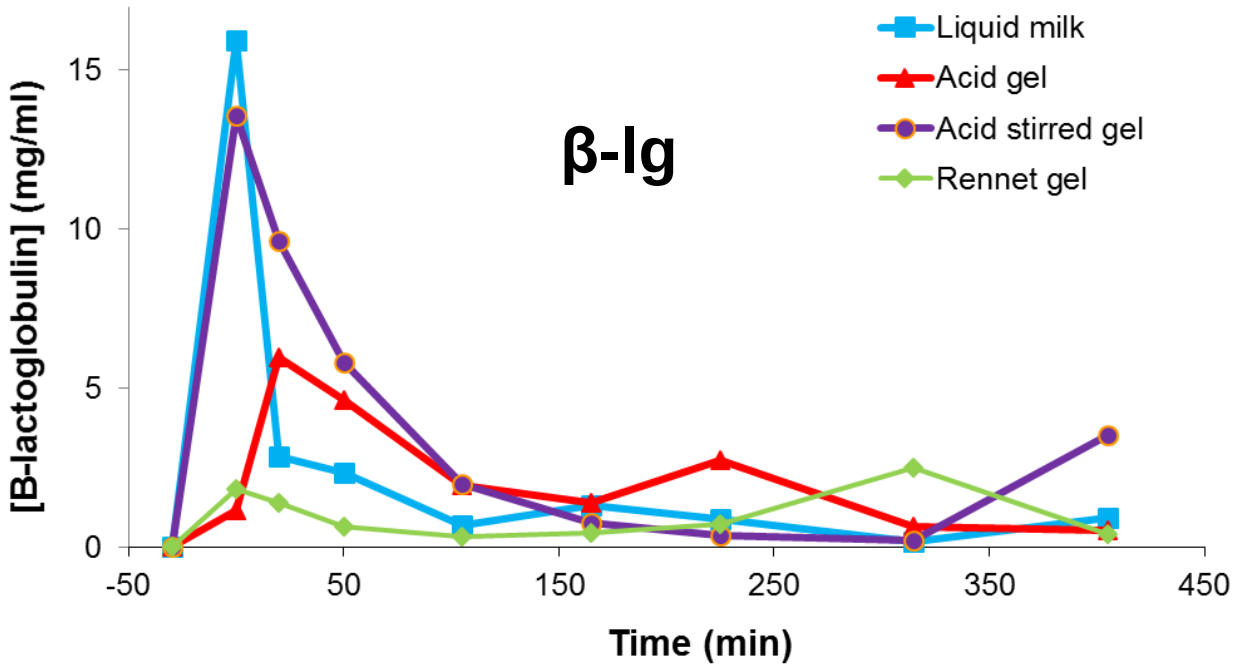
pH 4



Concentration of Milk proteins in the duodenum (ELISA)



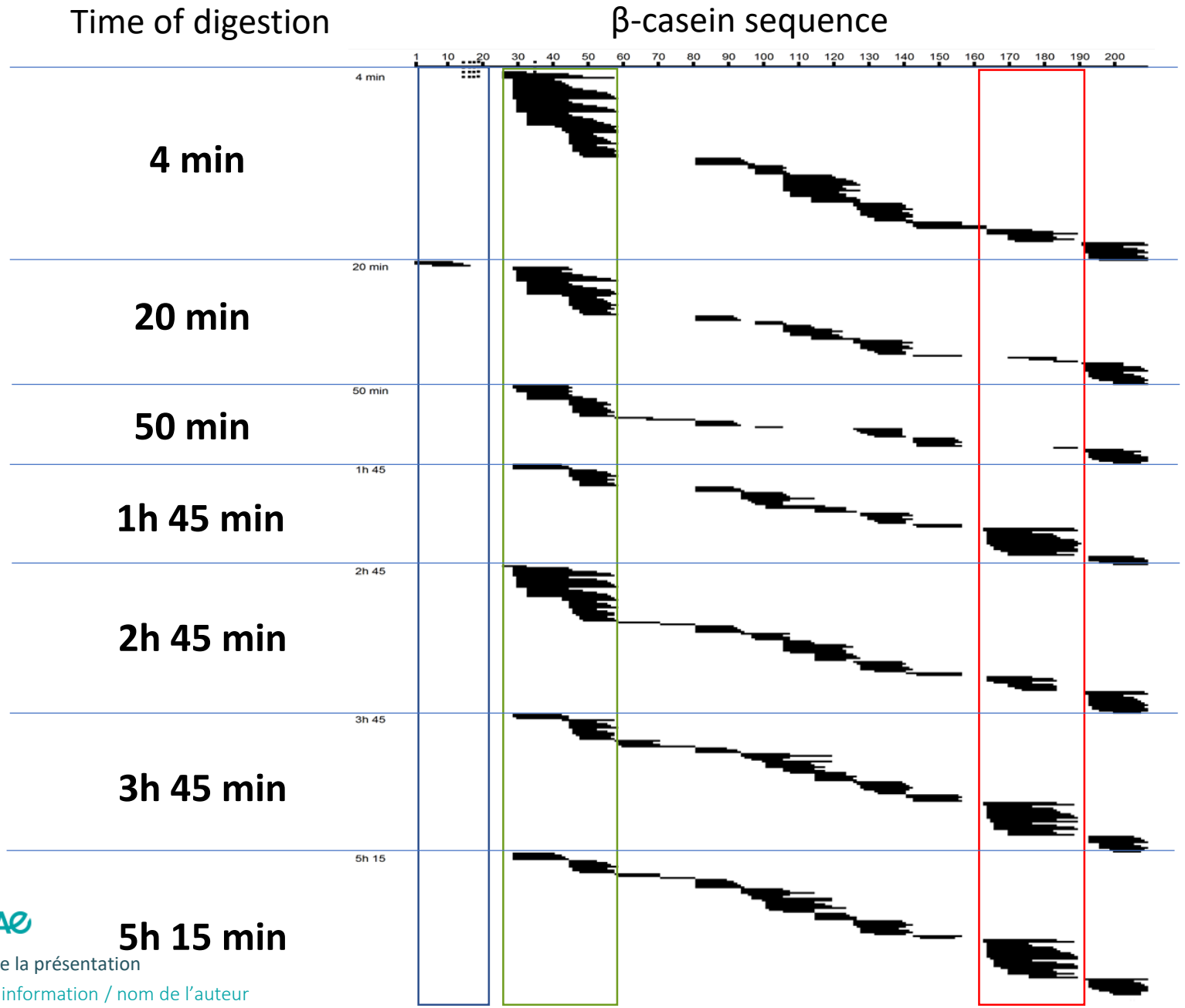
- Intense and early peak with milk/ lower and delayed with gels
- Intermediate behaviour with stirred gel
- Low concentrations with rennet gel but casein release tends to increase over time



- Only traces of milk proteins found in the jejunum
- Dairy products remain highly digestible

Barbé et al. 2013, 2014 Food Chem

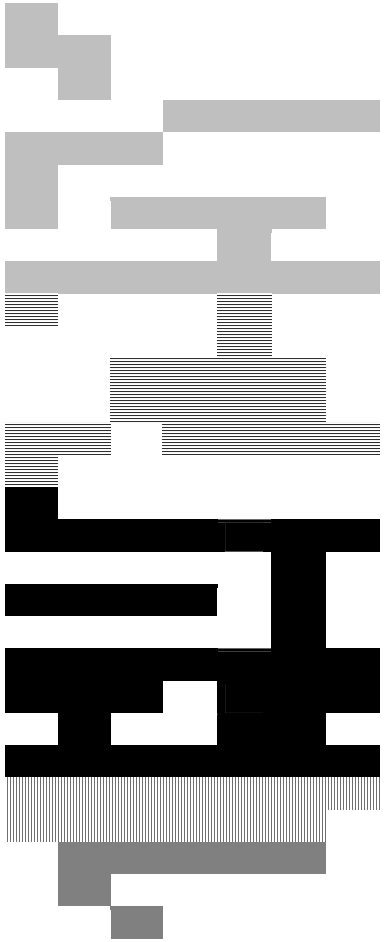
β-casein peptides released in the duodenum during digestion of acid gels



More than 16,000 milk peptides identified in the gastrointestinal tract of pigs

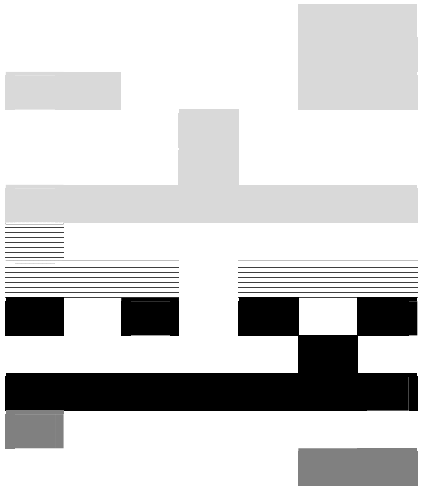
Bioactive peptides released during digestion differ from one matrix to another

Protein	Sequence	Activity	Reference
αs1	1-23	EMUL	Shimizu et al. (1984)
αs1	23-34	HYP	Maruyama & Suzuki (1982)
αs1	30-45	MB	Meisel et al. (1991)
αs1	40-52	MB	Adamson & Reynolds (1996)
αs1	43-58	MB	Meisel et al. (1991)
αs1	91-100	STRE	Miclo et al. (2001)
αs1	99-109	MIC	McCann et al. (2006)
αs1	167-180	MIC	Hayes et al. (2006)
αs1	180-193	MIC	Hayes et al. (2006)
αs2	1-24	MB	Miquel et al. (2005)
αs2	124-146	MB	Miquel et al. (2005)
αs2	183-206	TRAN	Kizawa et al. (1996)
αs2	183-207	MIC	Recio & Visser (1999)
αs2	189-197	HYP	Maeno et al. (1996)
αs2	190-197	HYP	Maeno et al. (1996)
β	1-24	MB	Bouhallab et al. (1999)
β	33-52	MB	Miquel et al. (2005)
β	60-80	OPI	Jinsmaa & Yoshikawa (1999)
β	98-105	OXI	Rival et al. (2001)
β	114-119	OPI	Jinsmaa & Yoshikawa (1999)
β	132-140	HYP	Robert et al. (2004)
β	192-209	IMM	Coste et al. (1992)
β	193-202	IMM	Kayser & Meisel (1996)
β	193-209	IMM	Coste et al. (1992)
κ	18-24	HYP	Lopez-Exposito et al. (2007)
κ	106-116	THR	Jolles et al. (1986)
β–lg	32-40	HYP	Pihlanto-Leppala et al. (2000)
β–lg	92-100	MIC	Pellegrini et al. (2001)
β–lg	142-148	HYP	Mullally et al. (1997)



Acid Gel

Protein	Sequence	Activity	Reference
αs1	40-52	MB	Adamson & Reynolds (1996)
αs1	43-58	MB	Meisel et al. (1991)
αs1	99-109	MIC	McCann et al. (2006)
αs1	167-180	MIC	Hayes et al. (2006)
αs1	180-193	MIC	Hayes et al. (2006)
αs2	1-24	MB	Miquel et al. (2005)
αs2	189-197	HYP	Maeno et al. (1996)
β	33-52	MB	Miquel et al. (2005)
β	166-175	HYP	Hayes et al. (2007)
β	193-202	IMM	Kayser & Meisel (1996)
β–lg	92-100	MIC	(*8*))
β–lg	142-148	HYP	(*9*))



Rennet Gel

- 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

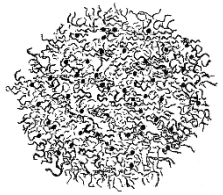
Barbé et al. 2014 Food Res Int

The intestinal peptidome highly depends on food structure

Characterization of bioactive peptides released in the small intestine of human volunteers

In vivo digestion of milk proteins

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



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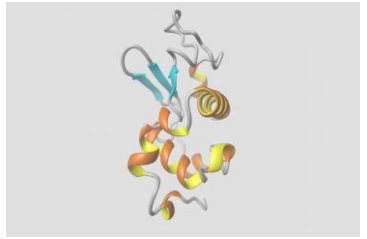
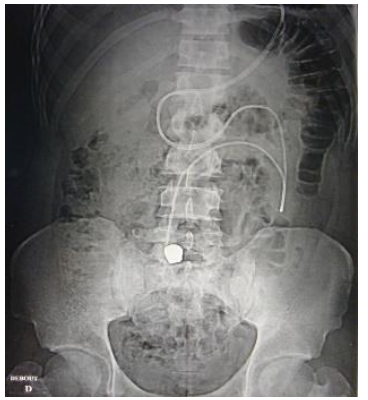
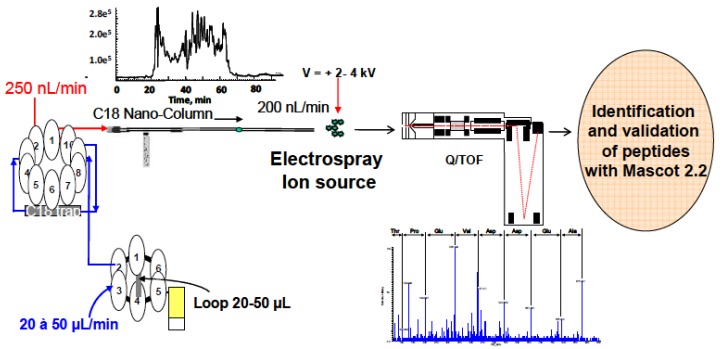


- Intestinal contents were collected through a double lumen nasogastric tube placed in their jejunum during 6 h after ingestion (location of the sampling site was controlled by radiography)



- 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

Example of data collected

prot hit num	prot desc	prot cover	pep exp mr	start	peptide	end	score	res before	pep seq	res after
1	18KD Betalg D bovine	26,5	724,3362	94	94-99	99	25,15	L	VLDTDY	K
1	18KD Betalg D bovine	26,5	771,3746	50	50-56	56	39,24	T	PEGDLEI	L
1	18KD Betalg D bovine	26,5	837,42	93	93-99	99	26,65	V	LVLDTDY	K
1	18KD Betalg D bovine	26,5	1116,4984	125	125-134	134	73,04	R	TPEVDDEALE	K
1	18KD Betalg D bovine	26,5	1203,6312	42	42-51	51	42,99	V	**YVEQLKPTPE**	G
1	18KD Betalg D bovine	26,5	1212,6165	43	43-53	53	55,16	Y	**VEQLKPTPEGD**	L
1	18KD Betalg D bovine	26,5	1226,6485	45	45-55	55	45,06	E	**QLKPTPEGDLE**	I
1	18KD Betalg D bovine	26,5	1244,6017	125	125-135	135	82,11	R	TPEVDDEALEK	F
1	18KD Betalg D bovine	26,5	1272,6174	124	124-134	134	55,85	V	RTPEVDDEALE	K
1	18KD Betalg D bovine	26,5	1325,706	43	43-54	54	60	Y	**VEQLKPTPEGDL**	E
1	18KD Betalg D bovine	26,5	1355,6724	44	44-55	55	62,52	V	**EQLKPTPEGDLE**	I
1	18KD Betalg D bovine	26,5	1375,6718	42	42-53	53	46,74	V	**YVEQLKPTPEGD**	L
1	18KD Betalg D bovine	26,5	1454,7508	43	43-55	55	56,53	Y	**VEQLKPTPEGDLE**	I
1	18KD Betalg D bovine	26,5	1567,8385	43	43-56	56	64,32	Y	**VEQLKPTPEGDLEI**	L
1	18KD Betalg D bovine	26,5	1617,8032	42	42-55	55	36,18	V	**YVEQLKPTPEGDLE**	I
2	24KD cas BetaB bovine	19,6	587,2972	115	115-119	119	28,04	Y	PVEPF	T
2	24KD cas BetaB bovine	19,6	753,4528	81	81-87	87	43,75	T	PVVVPPF	L
2	24KD cas BetaB bovine	19,6	882,4862	73	73-80	80	39,25	Q	NIPPLTQT	P
2	24KD cas BetaB bovine	19,6	888,4869	59	59-66	66	49,43	L	VYPFPGPI	H
2	24KD cas BetaB bovine	19,6	1220,7164	81	81-91	91	48,36	T	PVVVPPFLQPE	V

4704 dietary peptides unambiguously identified

A large proportion of the sequence covered

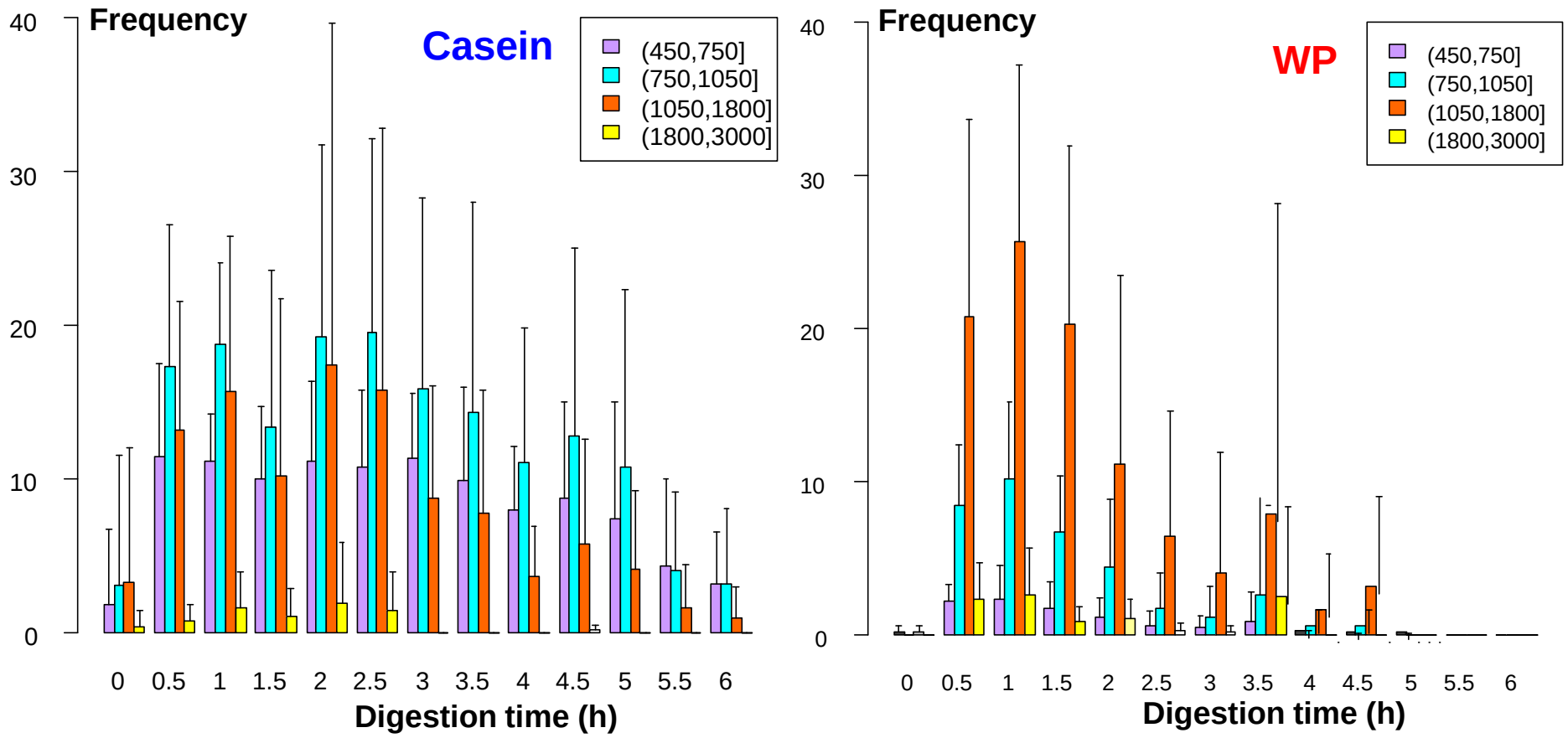
β-lactoglobulin : 70 % of the sequence

```
  1 LIVTQTMKGL DIQKVAGTWY SLAMAASDIS LLDAQSAPLR VYVEELKPTP
 51 EGDLEILLQK WENGECAQKK IIAEKTKIPA VFKIDALNEN KVLVLDTDYK
101 KYLLFCMENS AEPEQSLACQ CLVRTPEVDD EALEKFDKAL KALPMHIRLS
151 FNPTQLEEQC HI
```

β-casein : 67 % of the sequence

```
  1 RELEELNVPG EIVESLSSSE ESITRINKKI EKFQSEKQQQ TEDELQDKIH
 51 PFAQTQSLVY PFPGPIHNSL PQNIPPLTQT PVVVPPFLQP EVMGVSKVKE
101 AMAPKHKEMP FPKYPVEPFT ESQSLTLTDV ENLHLPLPLL QSWMHQPHQP
151 LPPTVMFPPQ SVLSLSQSKV LPVPQKAVPY PQRDMPIQAF LLYQEPVLGP
201 VRGPFPIIV
```

Kinetics of peptides release



☞ Release of casein peptides throughout digestion

☞ Peptides generated from whey proteins digestion are larger

↳ **Generation of a unique database of dietary peptides bioaccessible**

High variability in patterns but some peptides present in all subjects

β-CN

31 peptides present throughout digestion

14 peptides found in all the subjects

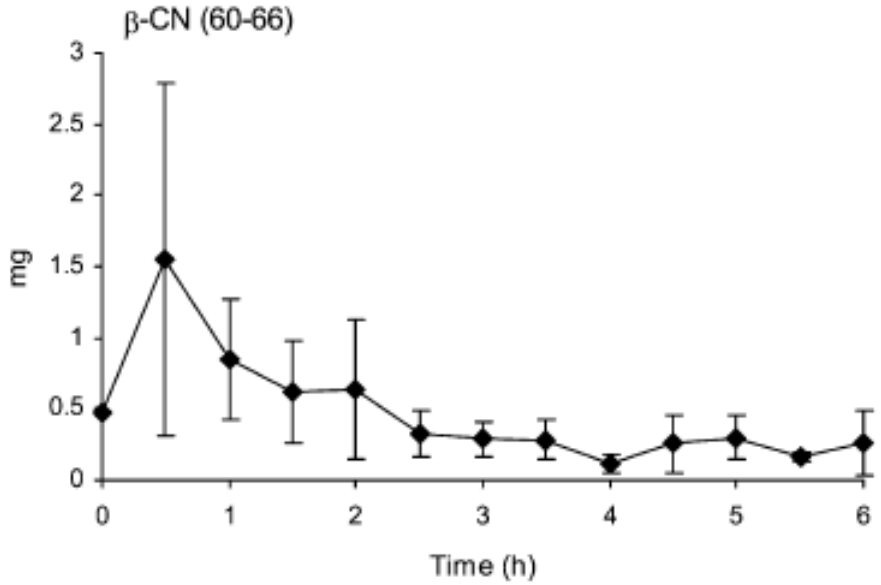
1-6	RELEEL		
57-66	SLVYPFPGPI	57-66	SLVYPFPGPI
58-66	LVYPFPGPI		
58-68	LVYPFPGPIPN		
59-66	VYPFPGPI	59-66	VYPFPGPI
59-67	VYPFPGPIP		
59-68	VYPFPGPIPN	59-68	VYPFPGPIPN
60-66	YPFPGPI	60-66	YPFPGPI
61-66	PFPGPI	60-67	YPFPGPIP
73-79	NIPPLTQ		
73-80	NIPPLTQT		
73-82	NIPPLTQTPV		
80-91	TPVVVPPFLQPE		
81-87	PVVVPPF		
81-91	PVVVPPFLQPE	81-91	PVVVPPFLQPE
81-92	PVVVPPFLQPEV	81-92	PVVVPPFLQPEV
83-91	VVPPFLQPE		
84-91	VPPFLQPE		
85-091	PPFLQPE		
107-113	KEMPFPK		
108-113	EMPFPK	108-113	EMPFPK
114-119	YPVEPF	114-119	YPVEPF
134-139	HLPLPL		
135-139	LPLPL		
156-160	MFPPQ		
168-173	VAPFPQ	168-173	VAPFPQ
170-175	VLPVPQ	170-175	VLPVPQ
171-175	LPVPQ		
194-201	QEPVLGPV	177-182	AVPYPQ
195-201	EPVLGPV	194-201	QEPVLGPV
196-201	PVLGPV	195-201	EPVLGPV

Among those 5 bioactive peptides

Boutrou et al. 2013
Am J Clin Nutr

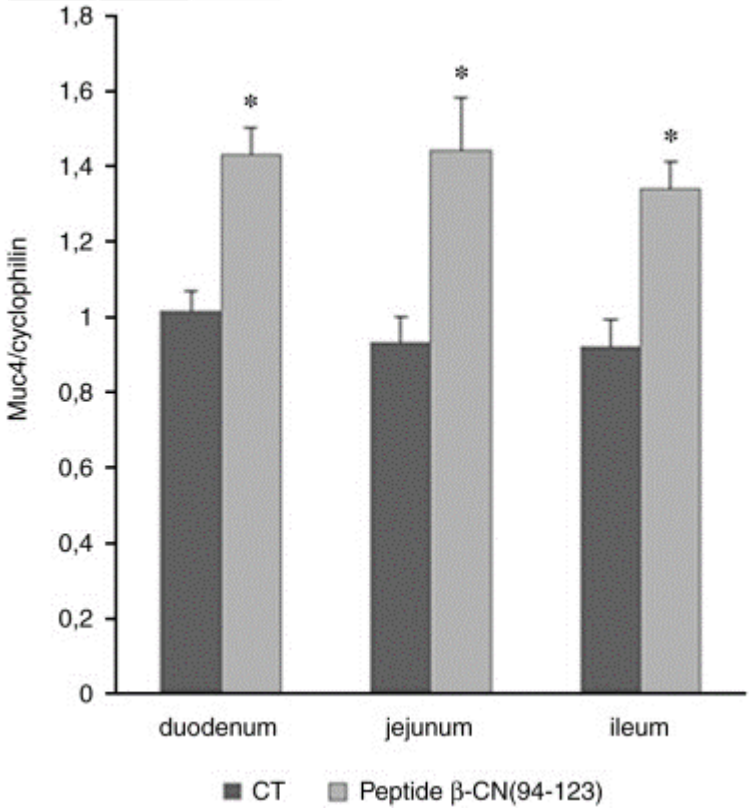
59-66	VYPFPGPI
59-68	VYPFPGPIPN
60-66	YPFPGPI
108-113	EMPFPK
114-119	YPVEPF

Anti-hypertensive Opioïd

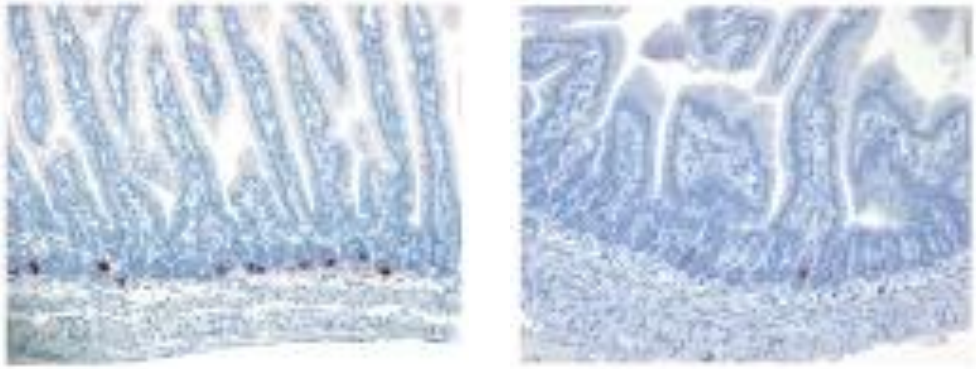


Bioactive peptides are released in the gut lumen at concentrations compatible with a biological action

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)



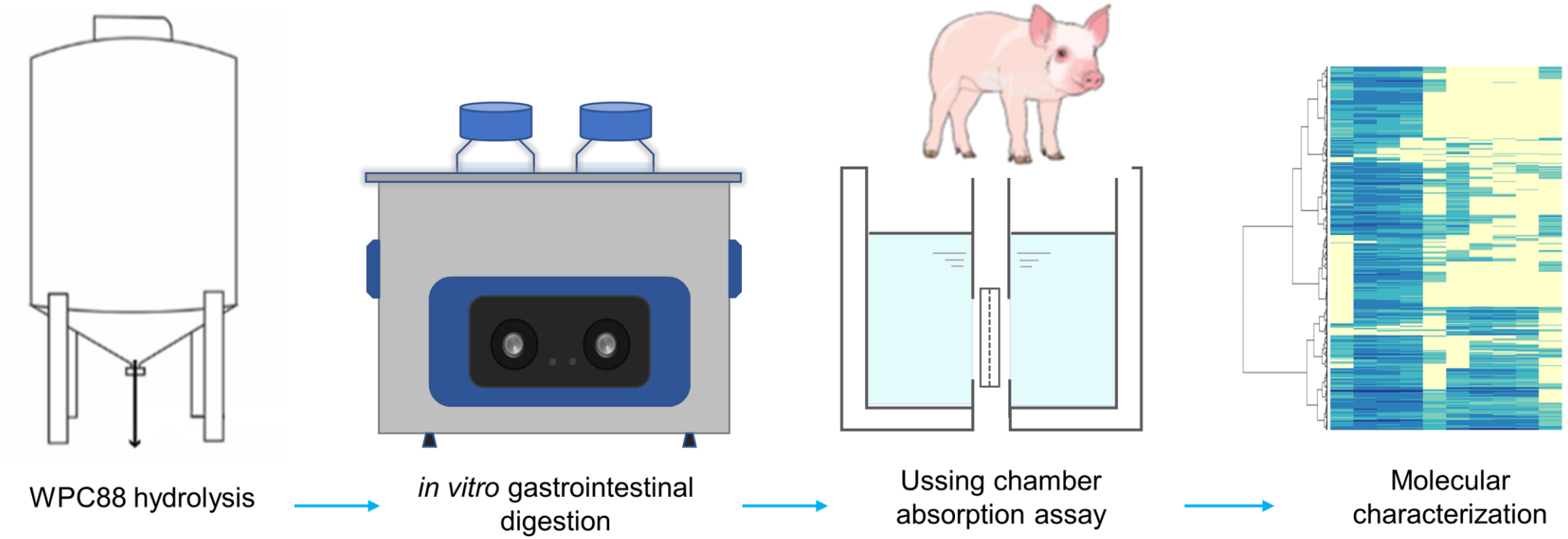
Before After

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
Bruno et al. 2017, J Dairy Sci

Are these bioactive peptides absorbed?

Ex vivo absorption of milk peptides



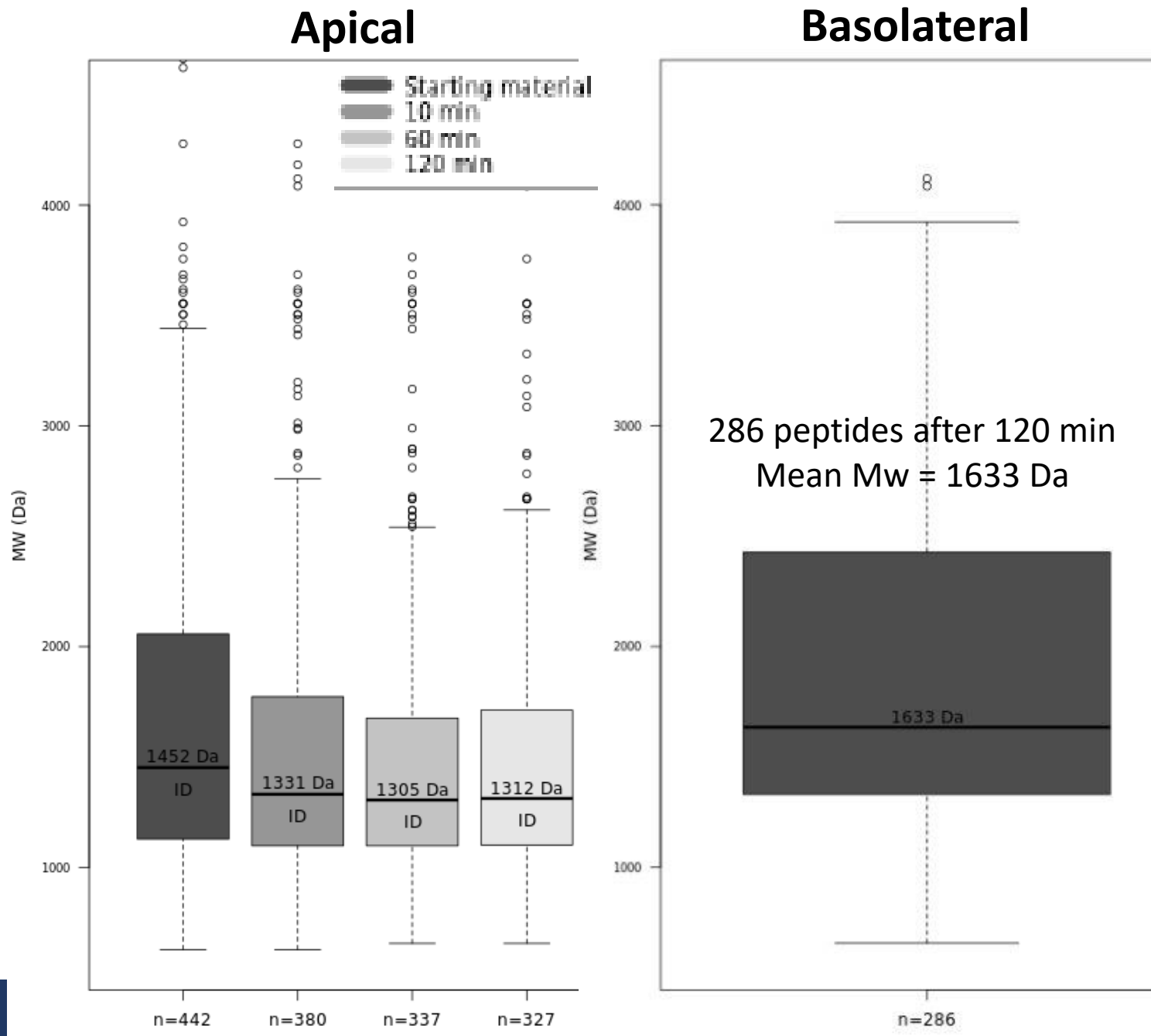
A whey protein hydrolyzate was submitted to *in vitro* digestion
Peptide absorption was investigated by Ussing Chambers
Absorbed peptides were identified by mass spectrometry

Ozorio et al. 2021 Foods

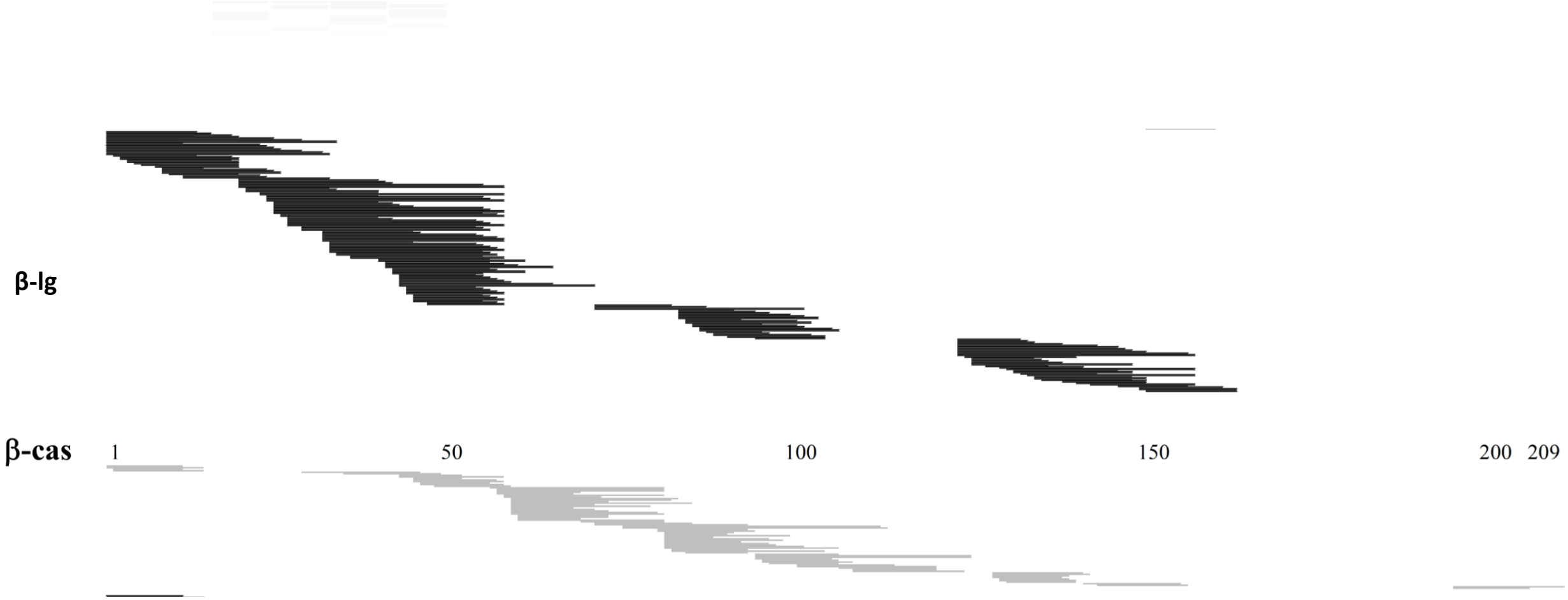
Mw of the peptides identified in the apical and basolateral chambers

Even large peptides (Mw> 4kDa) can go through the epithelial barrier

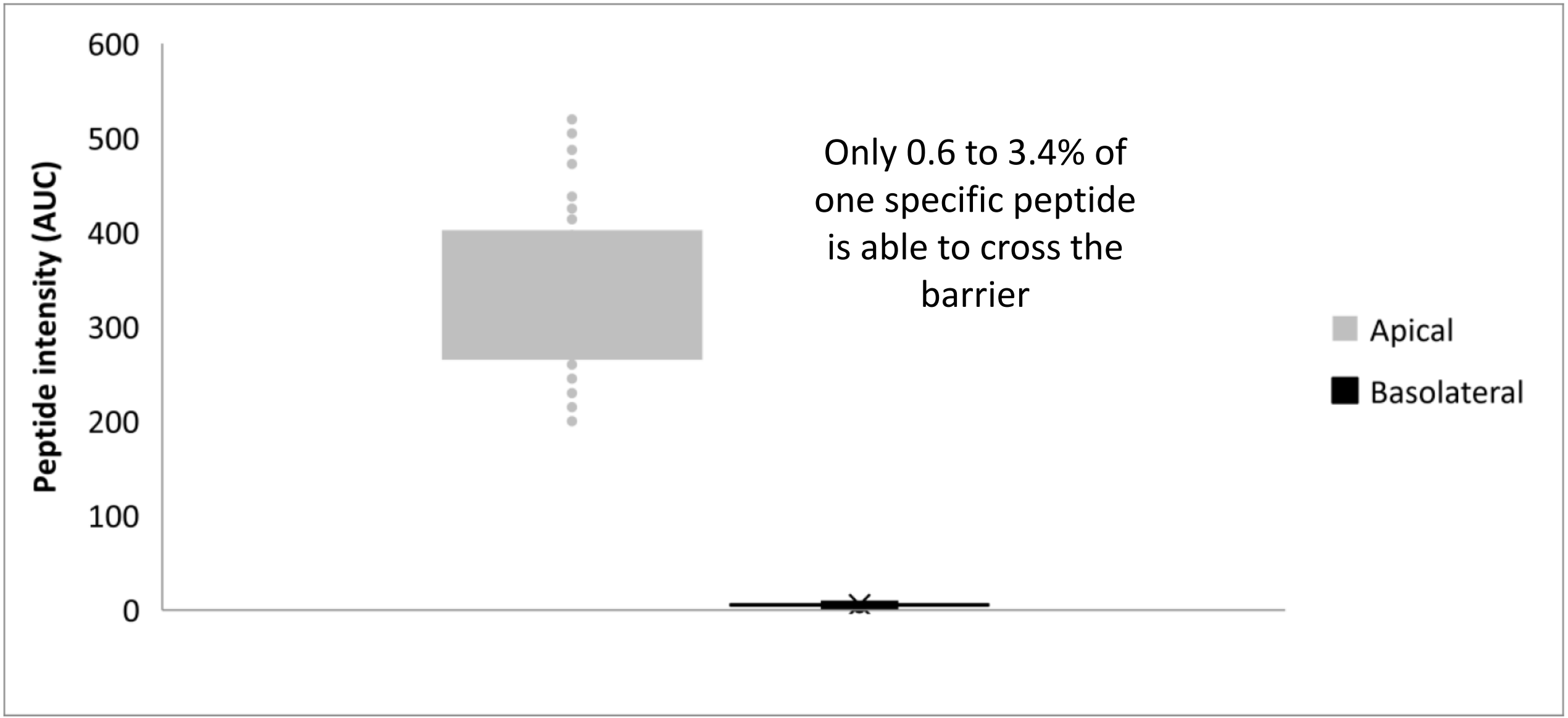
This confirms our preliminary work on the absorption of a 17 AA peptide (Regazzo et al. 2010)



β-lactoglobulin (β-lg) and β-casein (β-cas) peptides identified by NanoLC-MS/MS in the basolateral compartment of the Ussing chambers



Median intensity (AUC) of the 147 peptides identified in both the apical and basolateral compartments of the Ussing chambers



Some of the peptides able to cross the epithelial barrier are bioactive

Protein	Peptide	WPH	ID	Bioactivity	Reference
β-lg	KGLDIQKVAGTW	X	X	ACE-Inhibitory	[42]
	GLDIQK	X	X		[43]
	VLDTDYK		X		
	IVTQTMKG		X		[44]
	VLDTDYKK		X		
	KTKIPAV	X	X		
	VEELKPTPEGDLE	X			
	LEKFDK	X			
	LDIQKVAGTW	X			[45]
	RELKDLKGYGG	X	X	Anti-diabetic	[46]
	LIVTQTMKGLD		X		
	IVTQTMKGLD	X			
	IVTQTMKGLDIQ		X		
	LKPTPEGDL	X	X	DPP-IV inhibitory	[45]
	TPEVDDEALEK	X	X		
	VLVLDTDYK	X	X		
	LVLDTDYK		X	Antimicrobial	[47]
	ALKALPMHI	X			[48]
	GLDIQKVAGT	X	X		[45]
	TPEVDDEALEK	X	X		
	VLVLDTDYK	X	X		
	IDALNENK	X	X		
	SLAMAASDISLL	X	X		
	KPTPEGDLEI	X		Memory function	[49]
	KTKIPAVF	X		Antioxidant	[50]
	NENKVLVLDTDYKKY	X			[51]
α-la	GGVSLPEW	X		ACE-Inhibitory	[52]
	DKVGINYW	X			[45]
	LKGYGGVSLPEW		X		
β-cas	LVYPFPGPIPN	X	X		[9]
	VYPFPGPIPN	X	X		[45]
	YPFPGPIPN	X	X		
	YPFPGPIHNSLPQ	X			
	VENLHLPLPL	X		Anticancer	[53]
	VYPFPGPIPN	X	X	Antioxidant	[45]
	YPFPGPIPN	X	X	DPP-IV inhibitory	
	VYPFPGPIP	X	X	Prolyl endopeptidase-inhibitory	
	PVVVPPFLQPE	X	X	Antimicrobial	
κ-cas	VQVTSTAV	X		Antimicrobial	
α-cas$_{S1}$	SDIPNPIGSENSEK	X	X		

Take home messages

Dietary proteins are hydrolyzed in the gastrointestinal tract into a myriad of bioactive peptides

Concentrations of these bioactive peptides are sufficient to exert a biological activity in the gut lumen (antimicrobial, increase in mucus and defensins production)

Peptides up to 4 kDa are able to cross the epithelial barrier

But only 0.6-3.4% of a peptide will pass through the epithelium

Since the lifetime of a peptide in the bloodstream is a question of minutes, one can wonder whether bioactive peptides targeting a peripheric organ can really exert a biological activity?

The Bioactivity & Nutrition team at INRAE Rennes

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Rozenn LE FOLL *(2020-2023)*
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Masters students

Improving health properties of food by sharing our knowledge on the digestive process

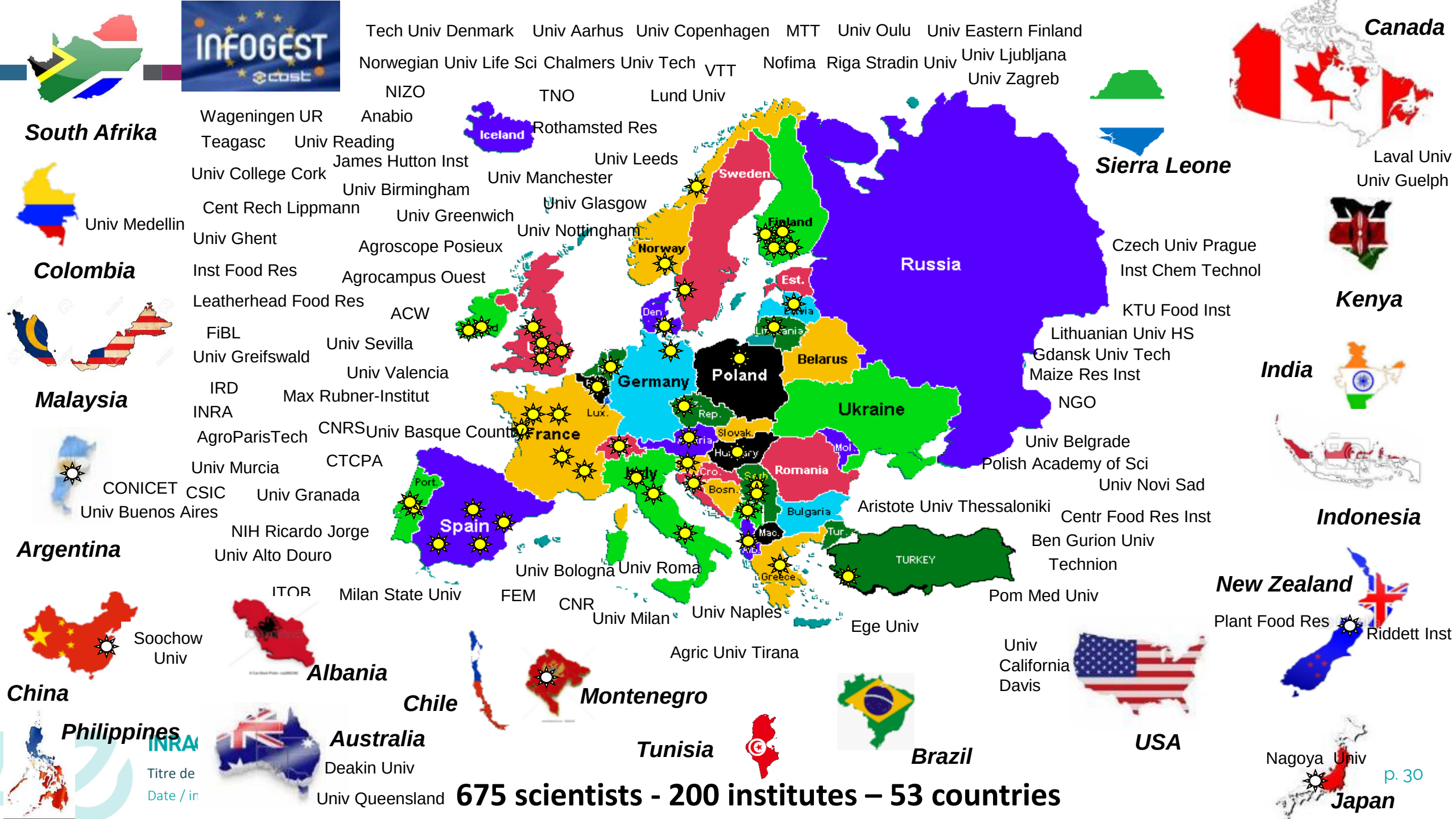
International Network

Dr. Didier DUPONT, Senior Scientist, INRAE, France

INFOGEST

Main objective: understanding the mechanisms of food digestion

- Develop new *in vitro*, *in vivo* and *in silico* digestion models including some for specific populations (infant, elderly)
- Harmonize the methodologies and propose guidelines for performing experiments
- Validate *in vitro* models towards *in vivo* data (animal and/or human)
- Identify the beneficial/deleterious components that are released in the gut during food digestion
- Determine the effect of the food matrix on the bioavailability of food nutrients and bioactive molecules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Univ California Davis
USA
Nagoya Univ
Japan
675 scientists - 200 institutes – 53 countries

Industry involvement

☞ More than 60 companies are following INFOGEST

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Vice-chair
Alan Mackie - UK

In vitro models of digestion **WG1**	Food interaction – meal digestion **WG2**	Absorption models **WG3**	Digestive lipases and lipid digestion **WG4**	Digestive amylases and starch digestion **WG5**	In silico models of digestion **WG6**

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International Conference
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2024

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