An INFOGEST international consensus static in vitro digestion model adapted to the general older adult population and its application to dairy products

Didier Dupont, Anaïs Lavoisier, Olivia Ménard, Stefano Nebbia, Martine Morzel

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

International Research Network

Dr. Didier DUPONT, Senior Scientist, INRAE, France

INFOGEST

Scientific objectives

- Compare the existing digestion models, harmonize the methodologies and propose guidelines for performing experiments

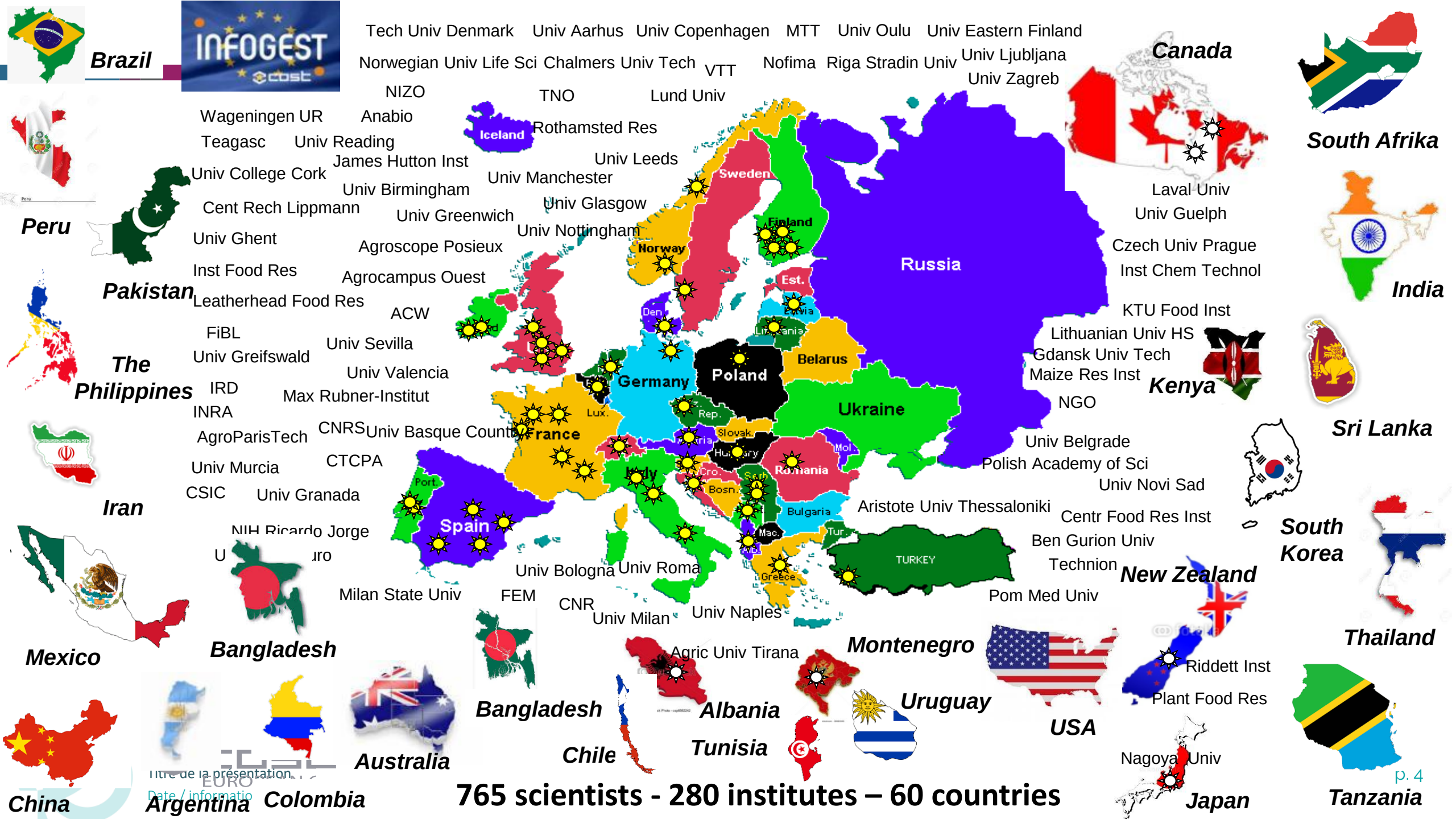
- Develop *in vitro*, *in vivo* and *in silico* models of digestion.
  Validate *in vitro* models towards *in vivo* data (animal and/or human).

- Identify the beneficial/deleterious components that are released in the gut during food digestion

- Determine the effect of the matrix structure on the bioavailability of food nutrients and bioactive molecules

But these goals can only be reached by…

- Gathering scientists from different disciplines (food science, nutrition, gastroenterology, immunology…) to share and improve our knowledge on food digestion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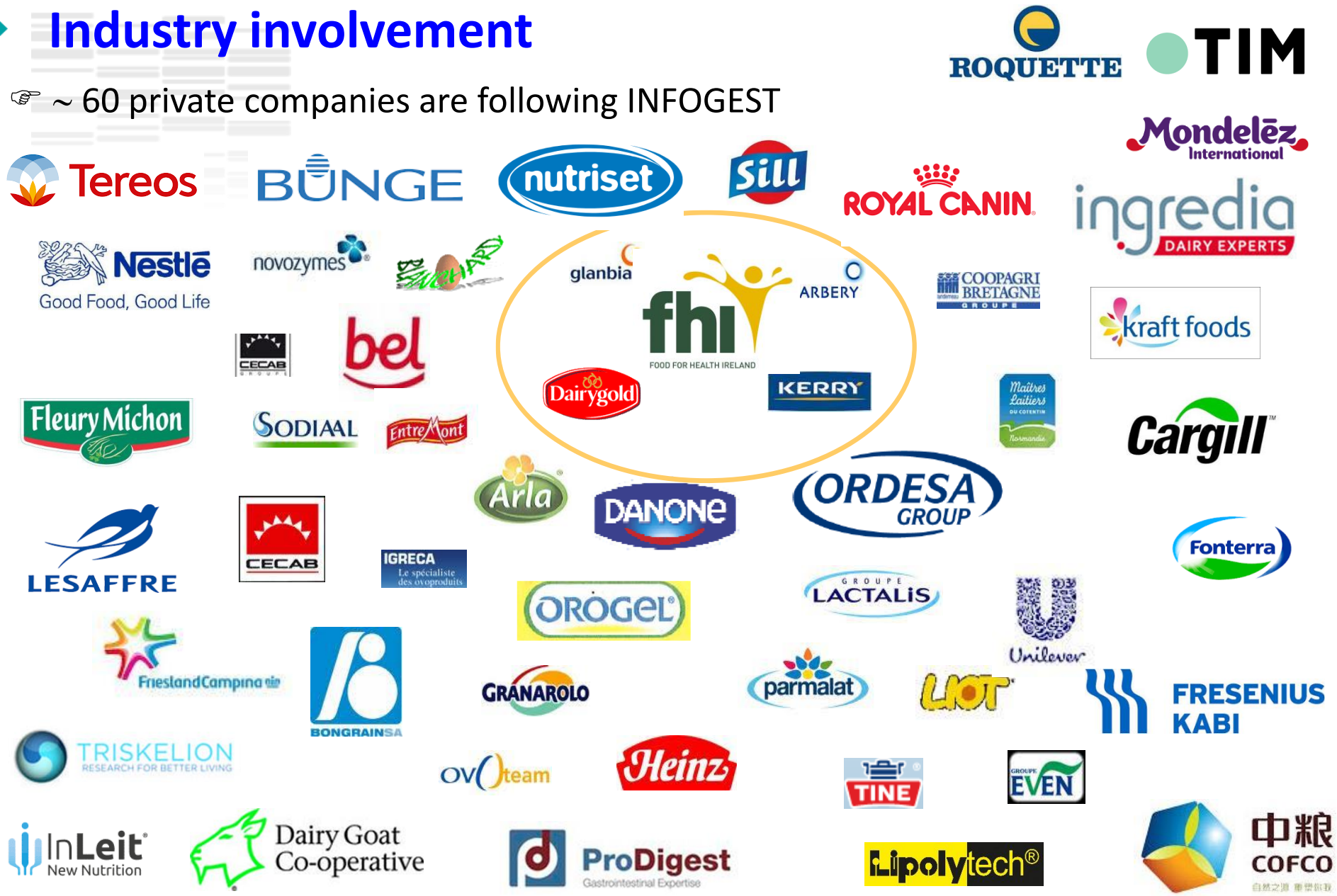
Industry involvement

☞ ~ 60 private companies are following INFOGEST



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Introduction - Digestive functions decline with age

* 10% of the world population is over 65, but this number reaches 16% in the US, 21% in Europe and even 30% in Japan

* In 2050, this number should increase from 10% to 16%

* Gastrointestinal motor function, food transit, chemical food digestion, and functionality of the intestinal wall have been previously shown to be affected by ageing (see Rémond *et al*. 2015)


Oncotarget

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Understanding the gastrointestinal tract of the elderly to develop dietary solutions that prevent malnutrition

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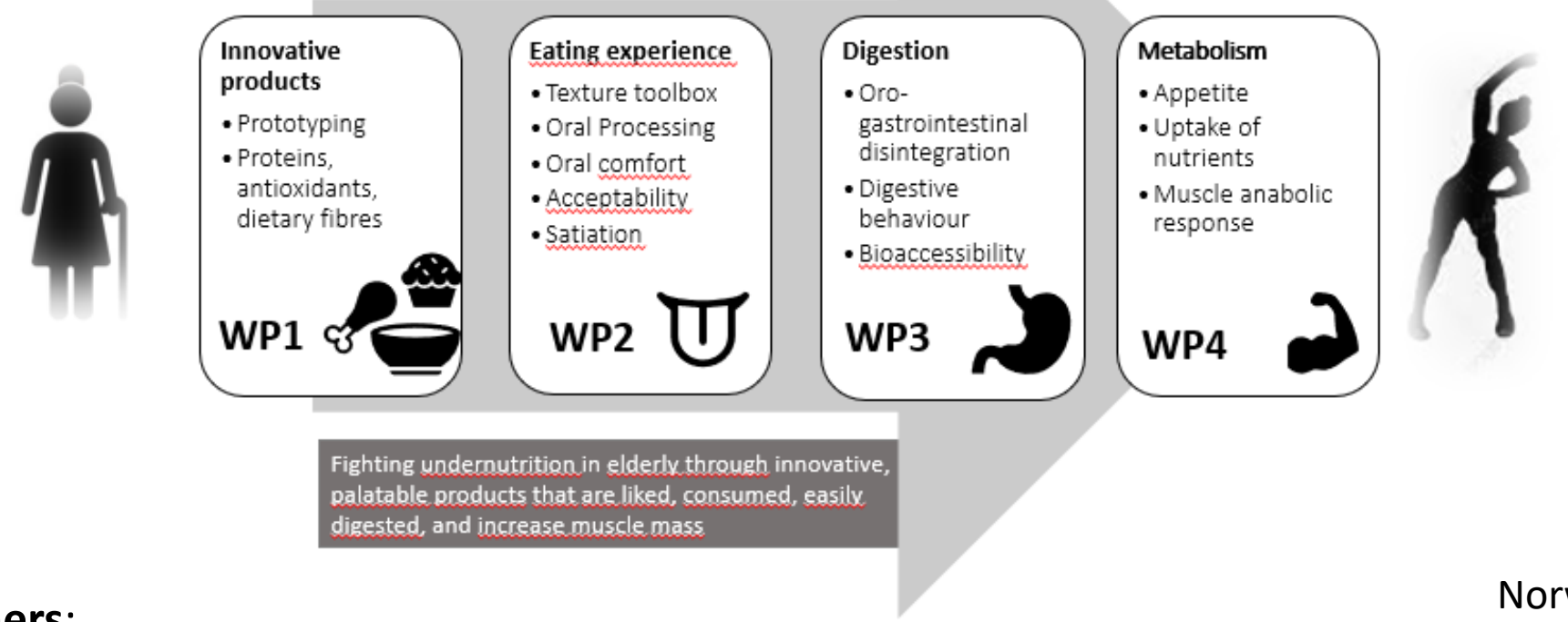
* *In vitro* digestion models must take these changes into account to remain physiologically relevant

* Several *in vitro* digestion models of the elderly have been proposed in the literature by different groups: Levi & Lesmes, 2014; Hernandez-Olivas *et al*. 2020; Plante *et al*. 2020; Aalaei *et al*. 2021; Lee *et al*. 2022

* But all these models are different and harmonization is needed in order to allow comparison between studies

EAT4AGE - Palatable, nutritious and digestible foods for prevention of undernutrition in active aging

Coordinated by NOFIMA
(Dr Paula Varela Tomasco)



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Methodology

Objective: gathering data from the literature in order to build static, semi-dynamic and dynamic *in vitro* digestion models simulating the gastrointestinal tract of the elderly

1st of April 2021	1 List of the physiological parameters to find in the literature
	2 **Exhaustive review of the literature to find data**
6th of October 2021	3 First meeting within the EAT4AGE consortium to identify the gaps of knowledge
	4 New literature search targeted on parameters
18th of January 2022	5 Second plenary meeting to discuss the missing information
	6 Creation of 3 subgroups (oral, gastric, intestinal) and more literature search…
	5 New subgroup and plenary meetings to discuss the model
11th of April 2022	7 All the data available put together into a presentation – Sharing with international experts
2nd and 3rd of May 2022	8 **Workshop in Cork for reaching an international consensus**
22nd of May 2023	9 Publication of the model
November 2023	10 Application of the consensus *in vitro* digestion model for elderly and comparison with the adult model for 3 categories of food studied (cereal, dairy, meat)

Parameters to set up

Oral Phase

* SSF composition
* Dilution with SSF
* pH
* Duration
* Mastication to achieve in order to reach a desired mean particle size
* Salivary amylase

Gastric Phase

* SGF composition
* Dilution with SGF
* pH
* Duration
* Pepsin
* Gastric lipase

Intestinal Phase

* SIF composition
* Dilution with SIF
* pH
* Duration
* Pancreatic lipase
* Pancreatic amylase
* Trypsin
* Chymotrypsin
* Bile

- First question to answer: what is an « older adult »? >60? >65?
- The literature analysis showed that, ideally we would need different models for different ages (the digestive conditions of a 65 y old are different than that of a 95 y old adult) but the literature is poor for some parameters after 70 y.
- So it was decided to build one model of adults older than 65

3-5 days Preparations

Prep-work

- Assay enzyme activity and bile salts concentration
- Prepare SSF, SGF and SIF, and $CaCl_2$ stock solution
- Perform pH adjustment pre-experiment

Parameters differing from the young adult model are in bold

1 Day digestion analysis

Elderly (>65y)

Oral

Dry food : SSF ratio (V/V)	1:1
Salivary amylase (U/mL)	75
Duration (min)	2
pH	7.0

Stomach

Oral bolus : SGF ratio (V/V)	1:1
Pepsin (U/mL)	**1200**
Gastric lipase (U/mL)	**36**
Duration (hour)	**3**
pH	**3.7**

Intestine

Gastric effluent : SIF ratio (V/V)	1:1
$CaCl_2$ [mM]	**1**

Pancreatin & Bile

Pancreatin (U/mL)-	
By trypsin	**80**
By lipase	**1600**
Bile salt [mM]	**6.7**

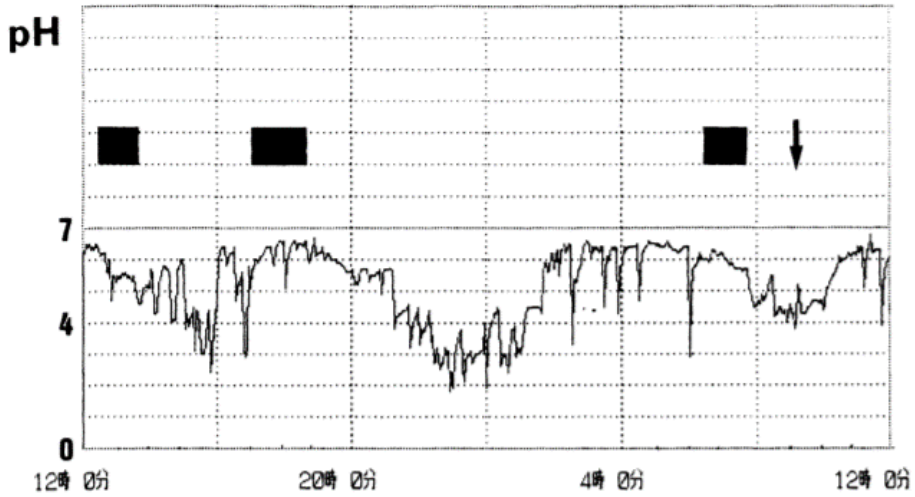
OR Individual components

Trypsin (U/mL)	**80**
α-chymotrypsin(U/mL)	**20**
Pancreatic α-amylase(U/mL)	**160**
Pancreatic lipase (U/mL)	**1600**
Sodium glycodeoxycholate [mM]	**3.35**
Taurocholic acid sodium salt hydrate[mM]	**3.35**

Duration (hour)	2
pH	7.0

Sampling, inactivate enzymes and analyses

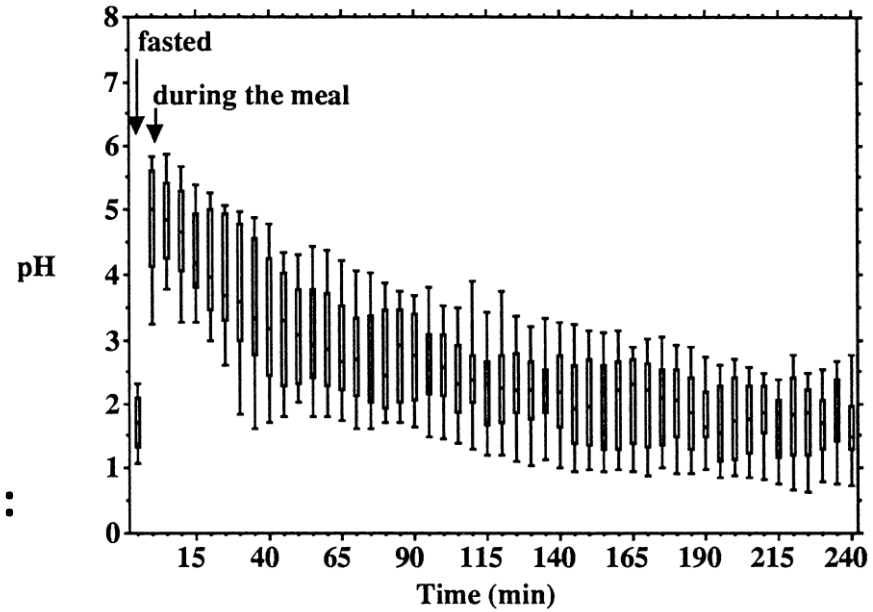
Gastric pH What is the pH at $T_{1/2}$?



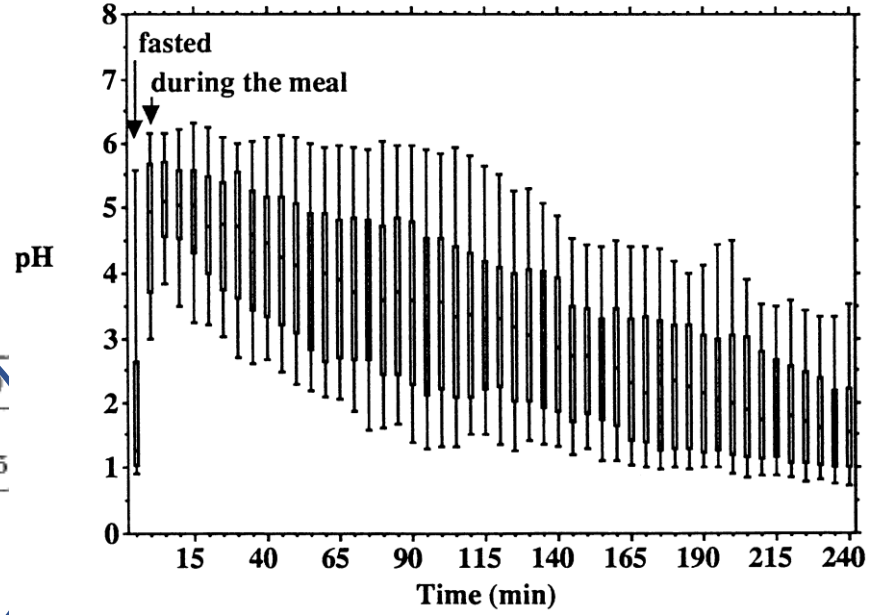
Morihara et al. 2001

Extrapolation from these data, pH at $T_{1/2}$ ~ 3.9

INFOGEST proposal: pH at $T_{1/2}$ = 3.7



Russel et al. 1993



Extrapolation from these data, pH at $T_{1/2}$ = 3.7

TABLE 2

Secretin Test Data: Patients without Pancreatic Disease, by Age Decade

Age range (yrs)	F:M/Total No.	Duodenal (mean ± SD) Age (yrs)	Volume (ml/kg)	HCO_3 (mEq/l)	Amylase (u/kg)	Blood amylase (IU)	Gastric (mean ± SD) ph	% pts pH < 3.5
50–59	469:300/769	54 ± 3	3.5 ± 1.2	109 ± 12	17.0 ± 7.9	76 ± 33	3.5 ± 2.3	64
60–69	352:291/643	64 ± 3	3.5 ± 1.1	109 ± 13	17.0 ± 7.6	68 ± 30	3.7 ± 2.4	60
70–79	108:80/188	73 ± 3	3.5 ± 1.2	109 ± 14	22.8 ± 12.9	77 ± 37	4.4 ± 2.6	50
>80	4:11/15	83 ± 3	3.4 ± 1.0	105 ± 19	19.4 ± 10.7	69 ± 39	4.4 ± 2.1	47

Dreiling et al. 1985

3.7 <gastric $_{pH}$< 4.4

Duration – Gastric phase

Brogna et al. 1999

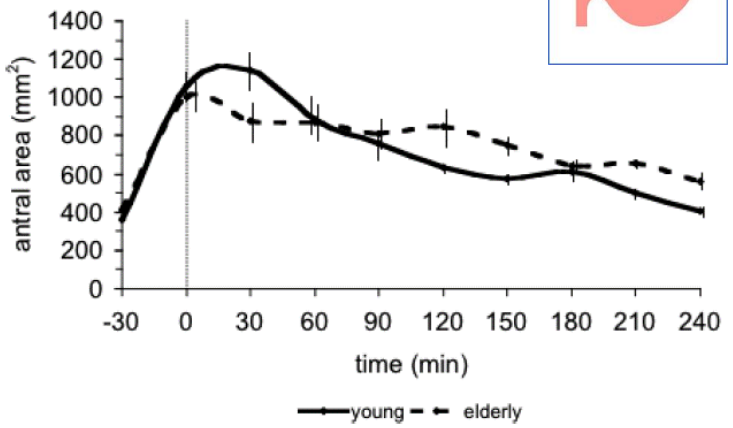
$T_{1/2}$ = 335 min **elderly**
$T_{1/2}$ = 245 min **young**

+37%
Gastric
Emptying Time

Table 1. Gastric Motility Parameters

Parameter	Elderly ($N = 10$)	Young ($N = 9$)	p
Fasting antral area, cm^2	416 ± 182	364 ± 77	.84
Maximal postprandial area, cm^2	878 ± 315	1143 ± 363	.06
Emptying time, min	448 ± 104	306 ± 57	.00

The meal consisted of 60 grams of "macaroni alla bolognese" with 70 grams of meat sauce, 50 grams of ham, 50 grams of soft fatty cheese, one roll, and 250 ml of water. Total energy was 800 kcal (15% proteins, 45% fat, 40% carbohydrates).



Antral area assessed by ultrasonography

448 min in **elderly** *vs* 306 min in **adult**: **+46%**

Di Francesco et al. 2005

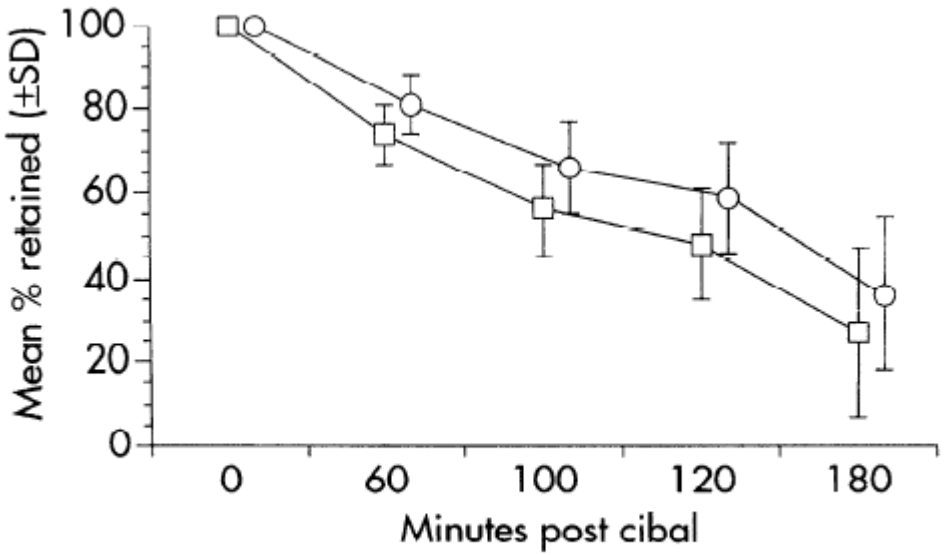


Fig. 2. Gastric emptying of solid component of meal in young (□) and elderly (○) subjects for total stomach. Data are mean values ± SD.

Table 2. *Gastrointestinal transit in young and healthy elderly subjects*

	Young	Elderly	P
Gastric emptying			
Solid $T_{½}$, min	127 ± 13	182 ± 26	<0.05
Liquid $T_{½}$, min	35 ± 3	47 ± 4	<0.05

Clarkston et al. 1997

Elderly/young
+43% solid

INFOGEST proposal: Gastric phase = 3h

Pepsin

	Young (18–34 years old) (n = 85)	Middle-aged (35–64 years old) (n = 99)	Elderly (65 years or older) (n = 22)
Men (%)	51 (60.0)	56 (56.6)	11 (50.0)
Black/white/other	57/23/5	62/35/2	2/20/0[a,b]
Weight (*lb*)	180 ± 46 (170)	177 ± 35 (170)	161 ± 31 (160)
Height (*in*)	68 ± 4 (68)	67 ± 4 (68)	66 ± 4 (66)[a]
Smoking now (%)	42 (49.4)	56 (56.6)	1 (4.5)[a,b]
Smoking now or in the past (%)	52 (61.2)	68 (68.7)	11 (50.0)
H. pylori seropositive (%)	38/84 (45.2)	57/98 (58.2)	18/22 (81.8)[a,b]
H. pylori titer	49 ± 100 (0)	52 ± 80 (29)	125 ± 131 (76)[a,b]
Inflammation in biopsy sample (%)	32 (37.7)	44 (44.4)	14/20 (70)[a,b]
CASG (%)	29 (34.1)	40 (40.4)	9 (45)
CSG (%)	3 (3.5)	2 (2)	0 (0)
CAG (%)[c]	0 (0)	2 (2)	5 (25)[a,b]
H. pylori in biopsy sample (%)	32 (37.7)	49 (49.5)	10/20 (50.0)
BAO (*mmol/h*)	3.5 ± 3.8 (2.0)	3.7 ± 4.1 (2.8)	3.1 ± 5.1 (1.8)
PAO (*mmol/h*)	29.3 ± 12.7 (29.0)	29.9 ± 14.1 (29.6)	19.0 ± 13.2 (21.2)[a,b]
BPO ($IU \times 10^3$/*15 min*)	2.0 ± 1.4 (1.7)	1.9 ± 1.4 (1.5)	1.3 ± 1.3 (1.0)[a,b]
PPO ($IU \times 10^3$/*15 min*)	4.6 ± 2.1 (4.5)	4.6 ± 2.7 (4.2)	2.4 ± 1.9 (2.4)[a,b]
Gastrin level (*pg/mL*)	59 ± 38 (49)	60 ± 35 (46)	69 ± 62 (48)

Feldman, et al. 1996

Basal Pepsin Output:
1.9 vs 1.3 ↘ 31.%

Pentagastrin Pepsin Output:
4.6 vs 2.4 ↘ 47%

Considering 1) a decrease of **40%** (average) in pepsin output
2) adult pepsin level 2000 U/ml (Brodkorb et al. 2019)

EAT4AGE proposal: 1200 U of pepsin/ml of gastric content

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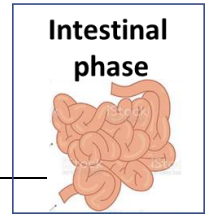
Summary of the data for pancreatic enzyme activities



Reference	Population	Control group?	Lipase	Trypsin	Chymotrypsin	Amylase
Fikry, 1968	**60-72 y.** (n =23)	No, compared with data for healthy adults from previous studies	= (activity)	↘ 30 % (activity)	/	↘ 30 % (activity)
Bartos & Groh, 1969	**61 – 76 y.** (n = 10)	Yes, adults 17 – 33 y. (n = 10)	/	/	/	= (output)
Gullo et al., 1983	2 groups: **61-68 y.** (n = 15) and **71-78 y.** (n = 10)	Yes, adults 18-54 y. (n = 30)	= (			
Dreiling et al., 1985	4 groups: **50 - 59 y.**, 54 ± 3 (n = 769) **59 - 69 y.**, 64 ± 3 (n = 643) **69 - 79 y.**, 73 ± 3 (n = 188) **> 80 y.**, 83 ± 3 (n = 15)	Yes, Adults 20 – 40 y. (n = 300)				
Vellas et al., 1988	**72 ± 3.2 y.** (n = 28)	Yes, adults 36 ± 7.8 y. (n = 27)	↘ (conc ↘ 43.6			
Ishibashi et al., 1991	**65 – 78 y.** (n =18)	Yes, 2 groups: adults < 40 y. and 40 – 65 y.				
Laugier et al., 1991	**16 – 83 y.** (n = 180) **>65 y. n = 20**	/	↘ (conc U			

INFOGEST: decrease of

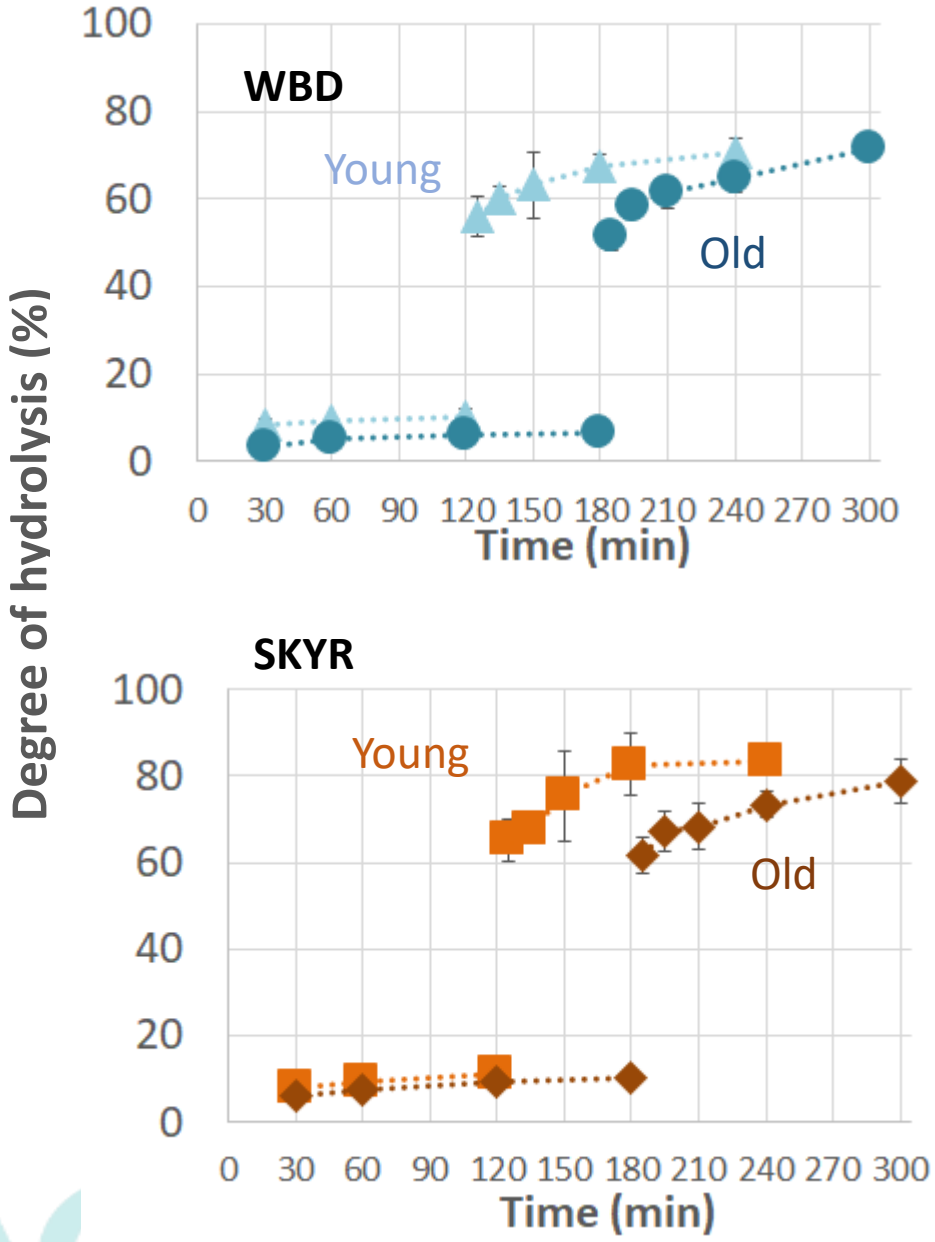
Application of the older adult model to dairy products

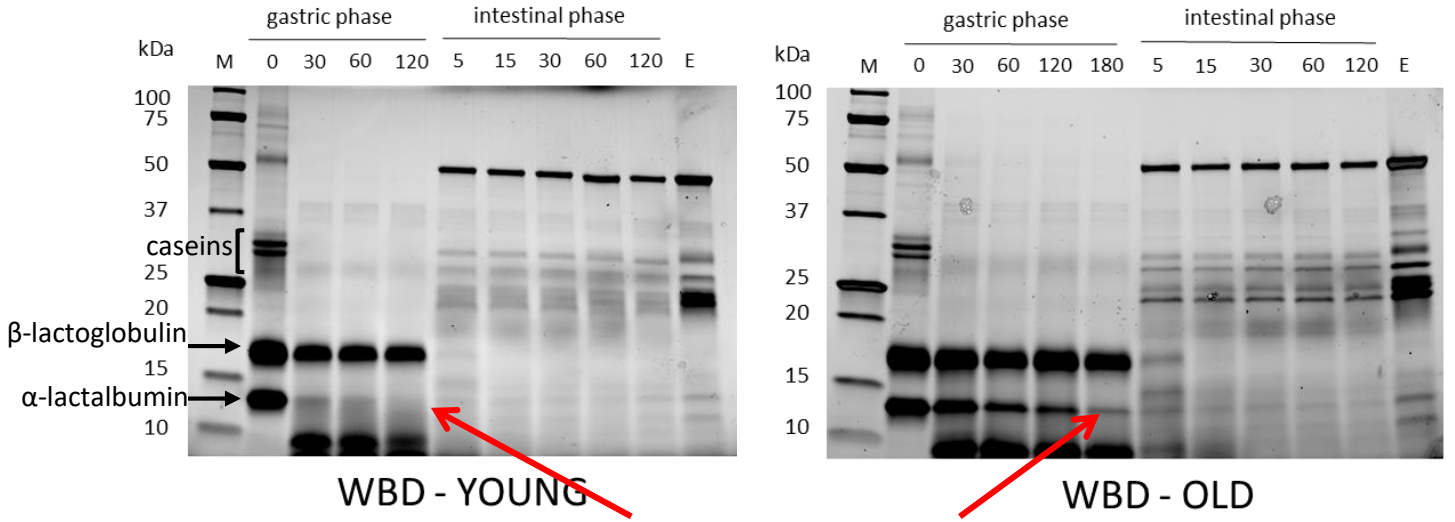
- **Whey-based dessert (WBD)**
- Ratio whey proteins/caseins = 80/20
- 10 % proteins, 2 % lipids, lactic ferments
- Heat treatment at 72°C for 2 min, acidified to pH 4.5 and stirred

VS

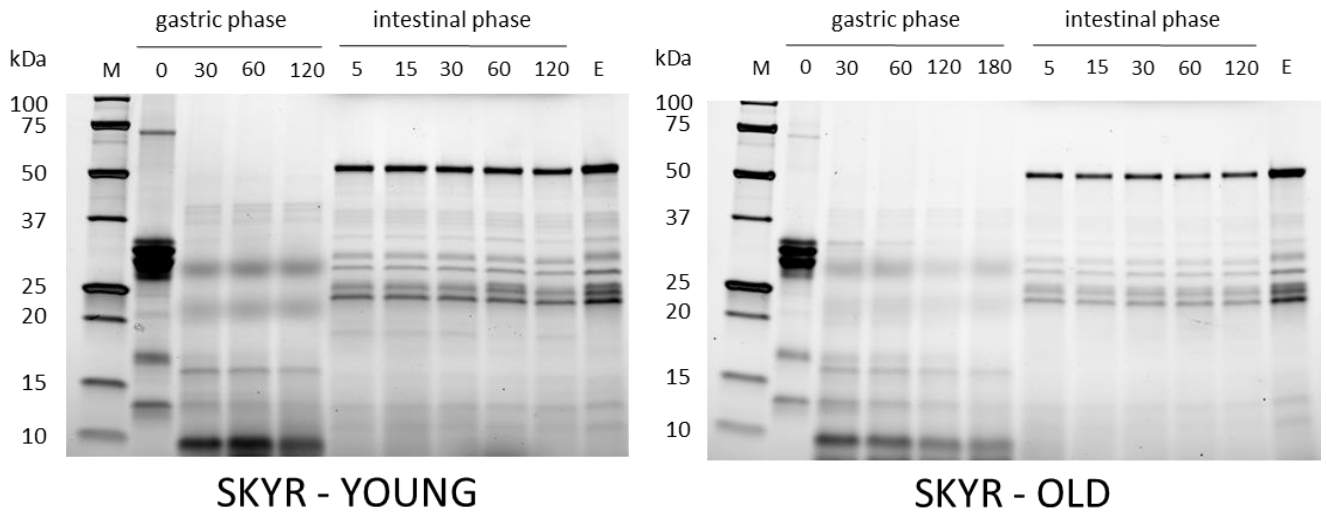
- **Control = commercial Skyr**
- Ratio whey proteins/caseins = 20/80
- 10 % proteins, 2 % lipids, lactic ferments

Protein hydrolysis



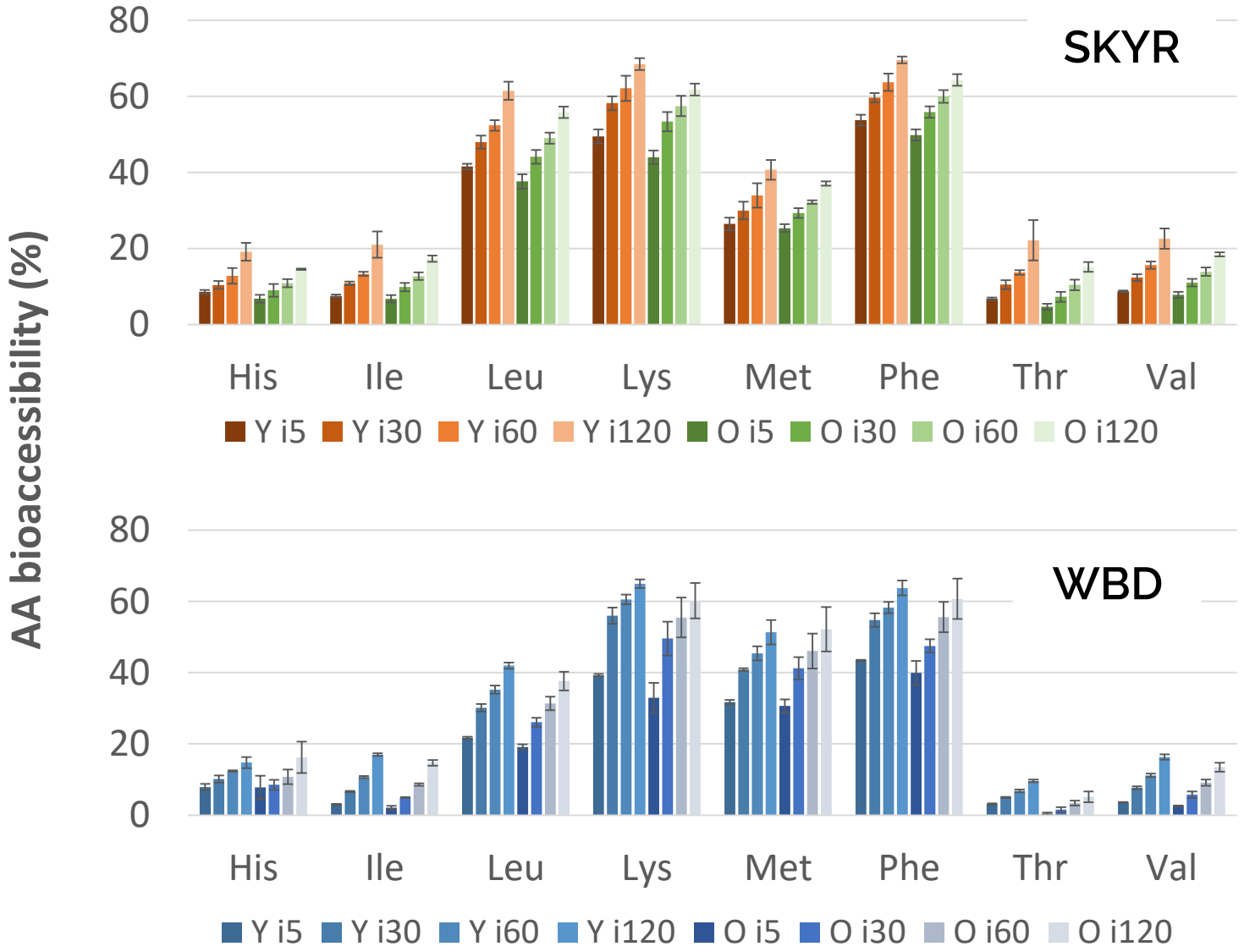


α-la more resistant to pepsin in elderly



Lower values for elderly in the gastric phase – Digestion of milk protein will be completed but after a longer time

Amino acid bioaccessibility



A 10% decrease in leucine bioaccessibility is observed for older adults but overall the designed dairy products exhibit a high digestibility

Conclusion

- A consensus static *in vitro* digestion model has been proposed by the INFOGEST network to simulate the gastrointestinal conditions of adults over 65

- Compares to the adult model, it exhibits :
    - An increase in the duration of the gastric phase (from 2h to 3h)
    - An increase in gastric pH (from 3.0 to 3.7)
    - A decrease in pepsin activity (from 2000 UI/mL to 1200 UI/mL)
    - A 20% decrease in pancreatic enzymes
    - A 40% decrease in bile salts concentration

- The model was applied to dairy products specifically designed for elderly people and showed a good protein digestibility and amino acid bioaccessibility
- It is currently being applied to the digestion of cereal-based and meat-based products within the EAT4AGE European project
- Parameters obtained from the literature are currently being used for building a semi-dynamic and a dynamic *in vitro* digestion model

INFOGEST
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