In vitro gut barrier models for food absorption

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Supports Agriculture & Food Industry in Ireland via research, advisory and training

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01 **Gut barrier**

02 **In vitro models**

03 **Food Absorption**

04 **Ex vivo models**

Upper GUT:

Mouth (mastication, mixing)

Stomach (enzymes, acid, peristaltic waves)

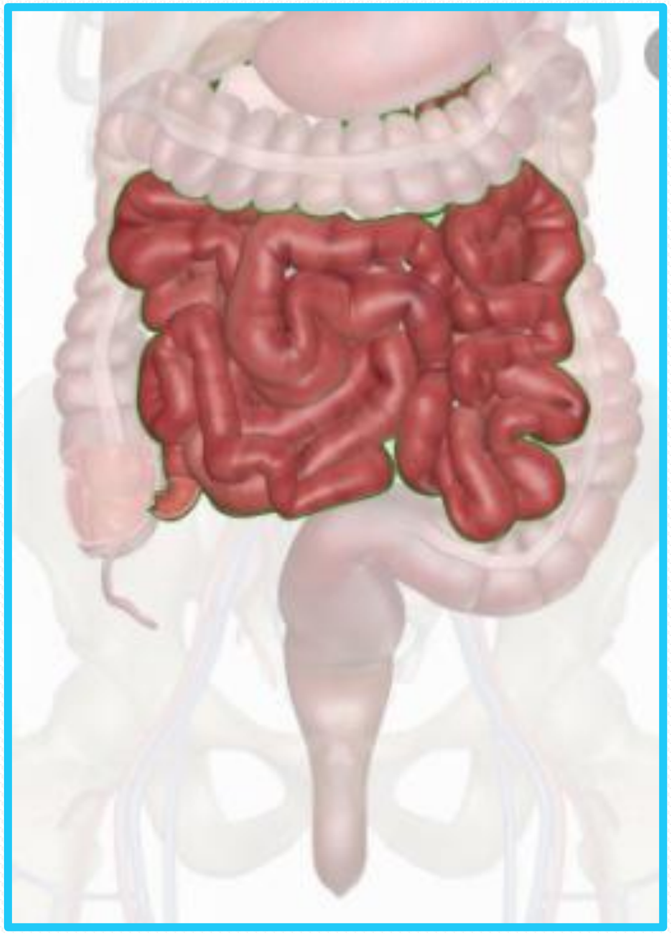
Small intestine (bile acids, pancreatic enzymes, neutral pH, brush border enzymes) main site for food absorption

Lower GUT:

Caecum

Colon

(gut microbiota, uptake of bacterial metabolites, SCFA, vitamins, water retention)

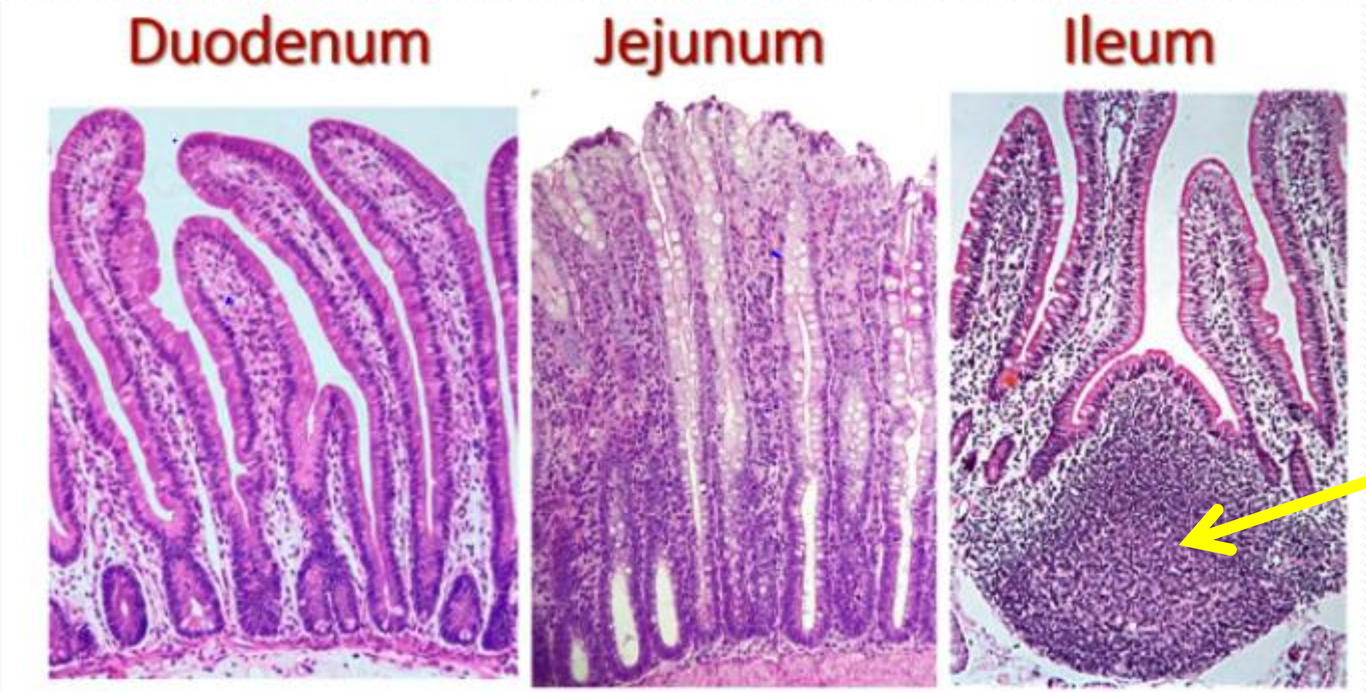


Small Intestine

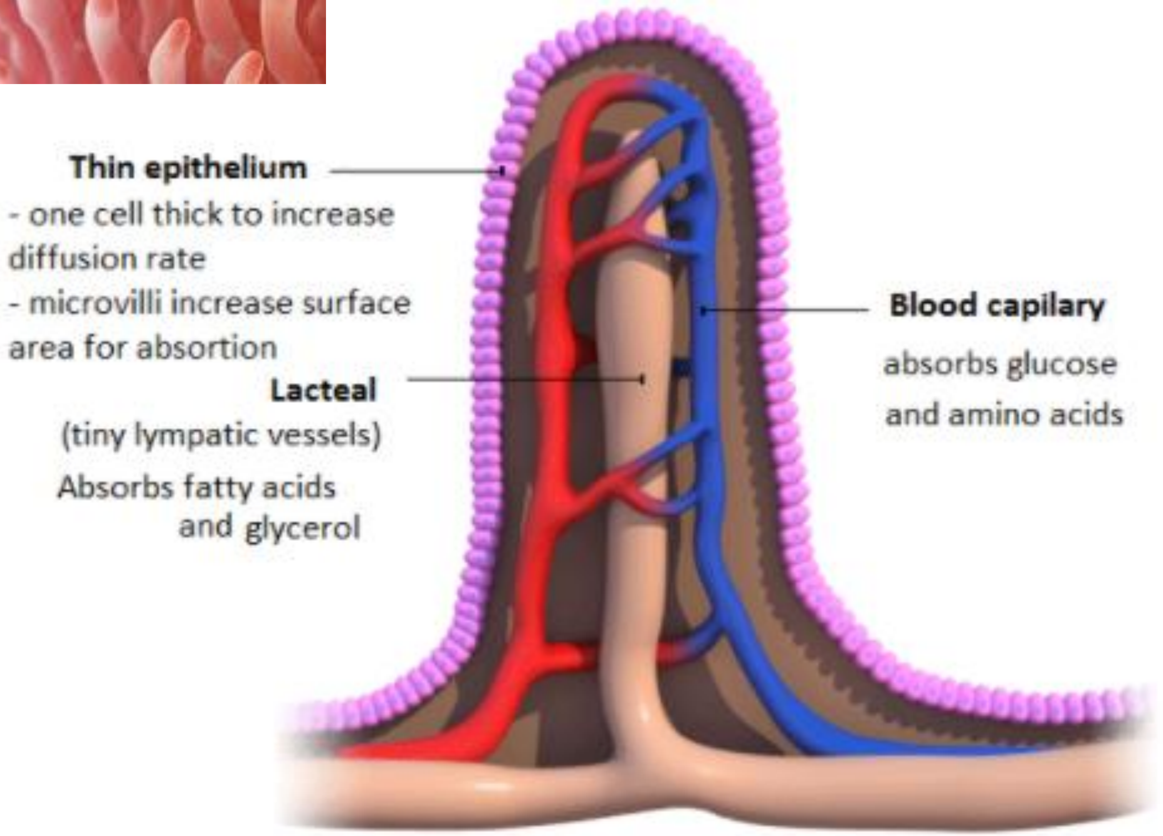
3 sections:
Duodenum
Jejunum
Ileum

6m length
Surface area upto 250m_2 Villi

Villi differ length & width from duodenum to ileum (shorter in ileum)
Villa: have hairlike projections (microvilla, brush border membrane)



Peyers patches
ileum
Immune function



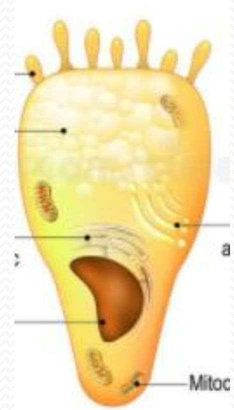
Gut barrier

Single layer of cells

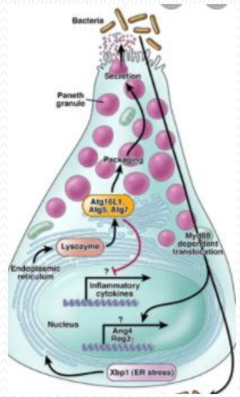
tight junctions with each other (diameter between cells 1-1.3nm)

4 main cell types in barrier

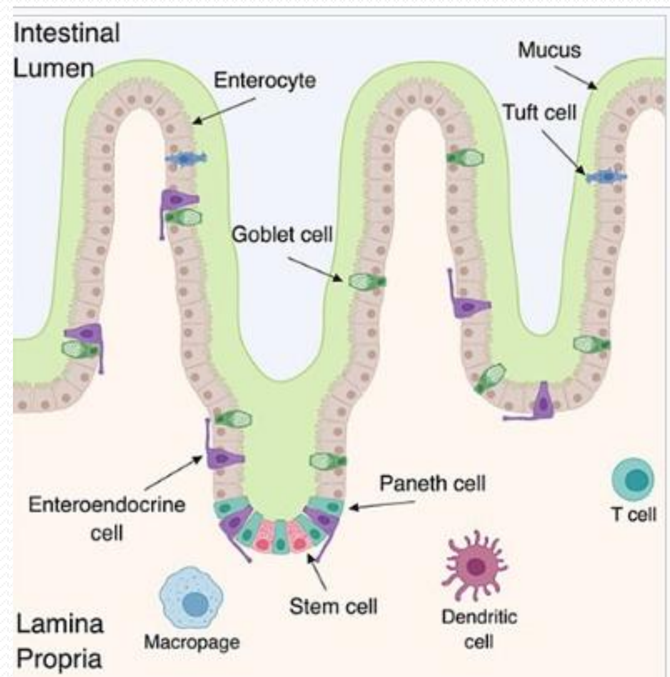
Goblet (4-12%): mucus

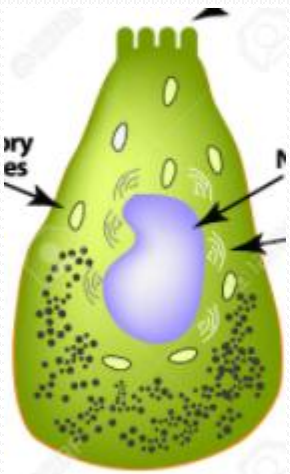


Enterocyte : absorptive most abundant, microvilli



Paneth cells 2%: digestive enzymes, antimicrobial peptides cytokines

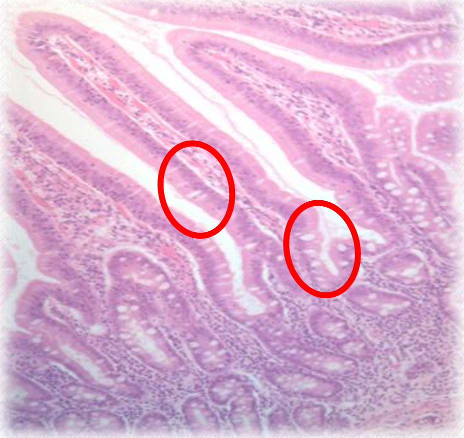




Enteroendocrine 2%: hormones satiety

Microfold (M) cells, Tuft cells

- Barrier Renewed every 5-7 days
- Enterocyte, goblet, endocrine cells migrate up tip as age: ‘exfoliate’ from tip
- Paneth cells in crypt ‘control centre’

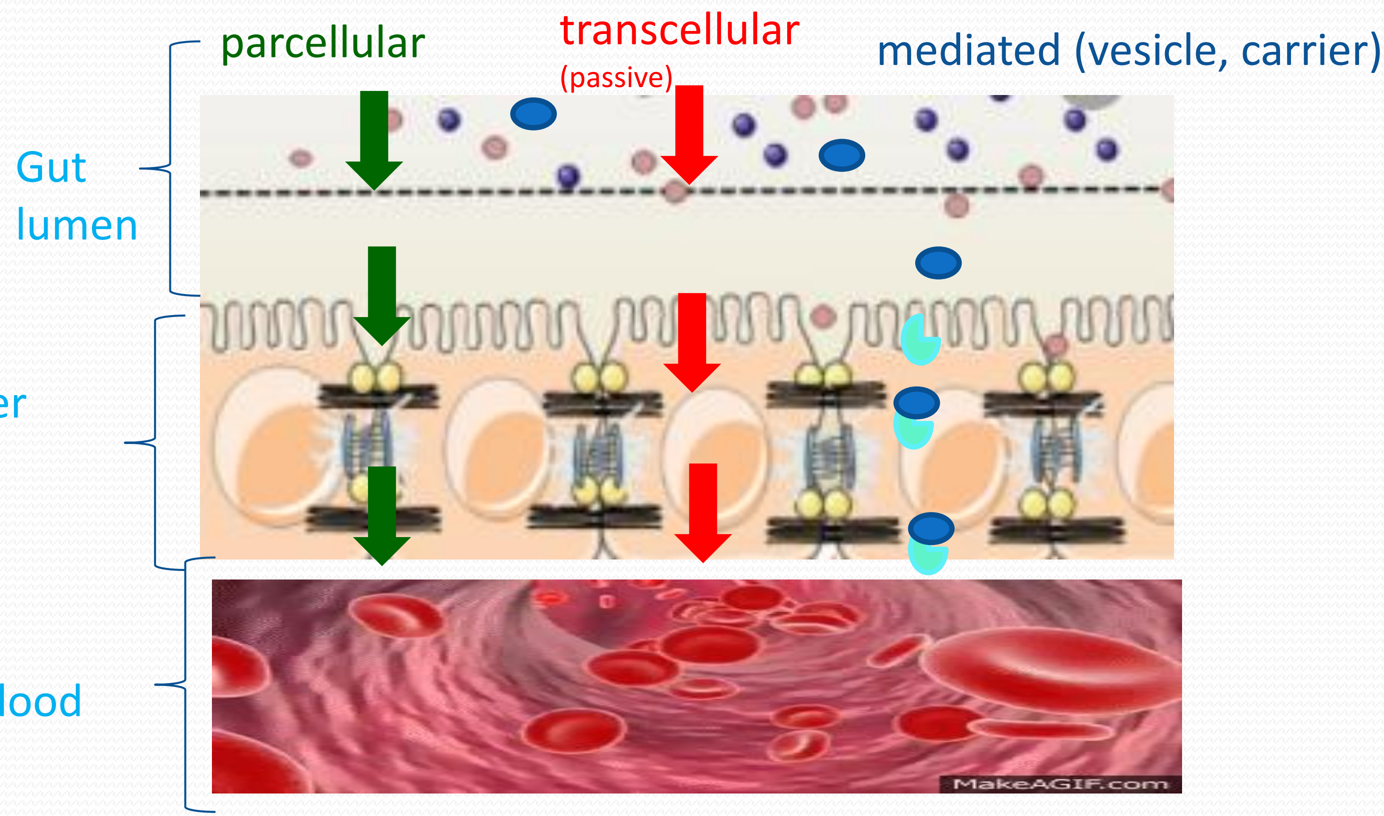


Crypt depth
villus height
Goblets cells

Gut barrier health

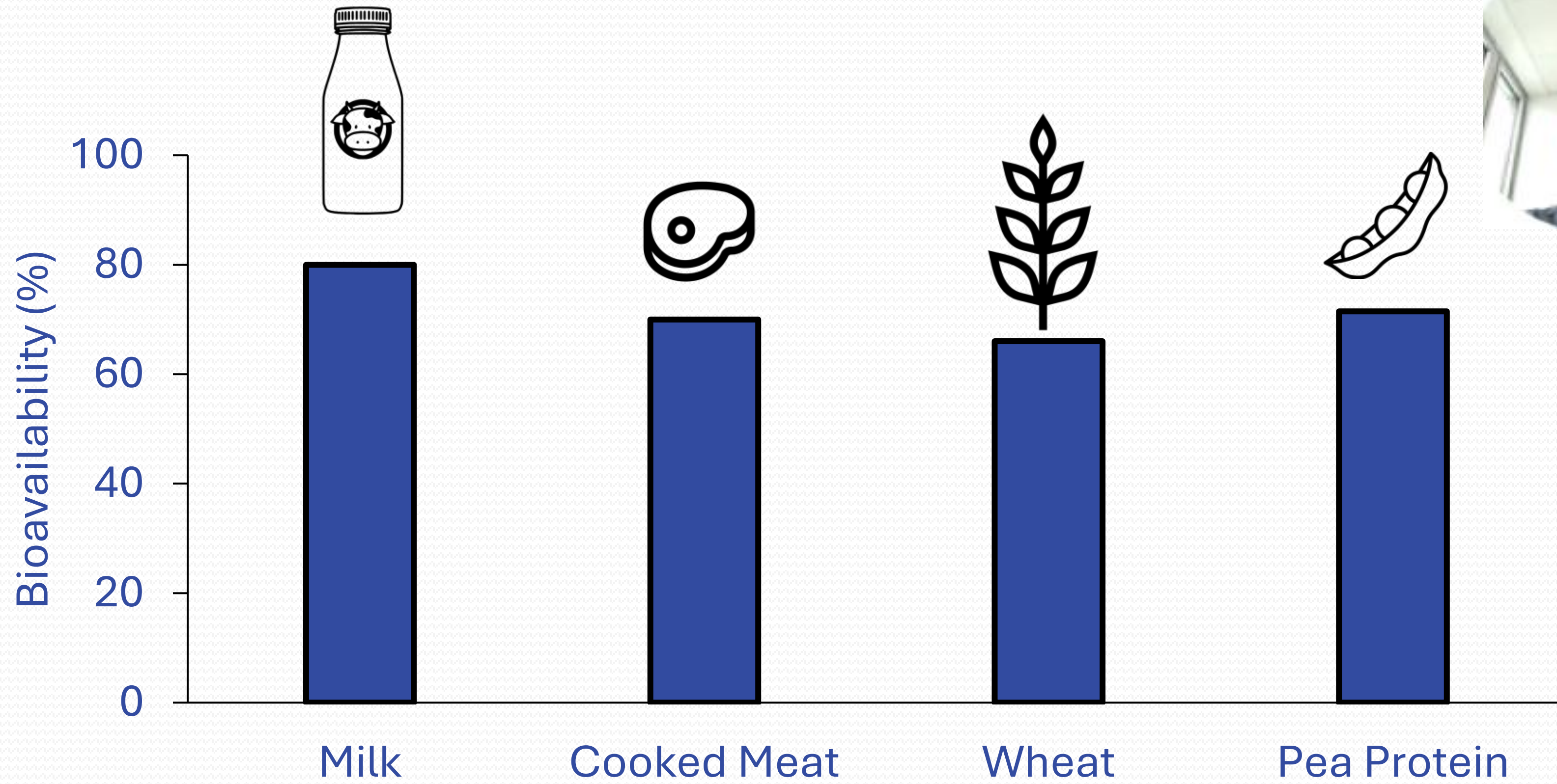
Nutrient Transport across gut barrier

Digestion
- Proteins-amino acids, small (di/tri) peptides
- Fats-fatty acids
- Carbohydrates- mono/disaccharides



Why study protein absorption?

Fact: Not all proteins are digested equally, not all proteins are equal in bioavailability



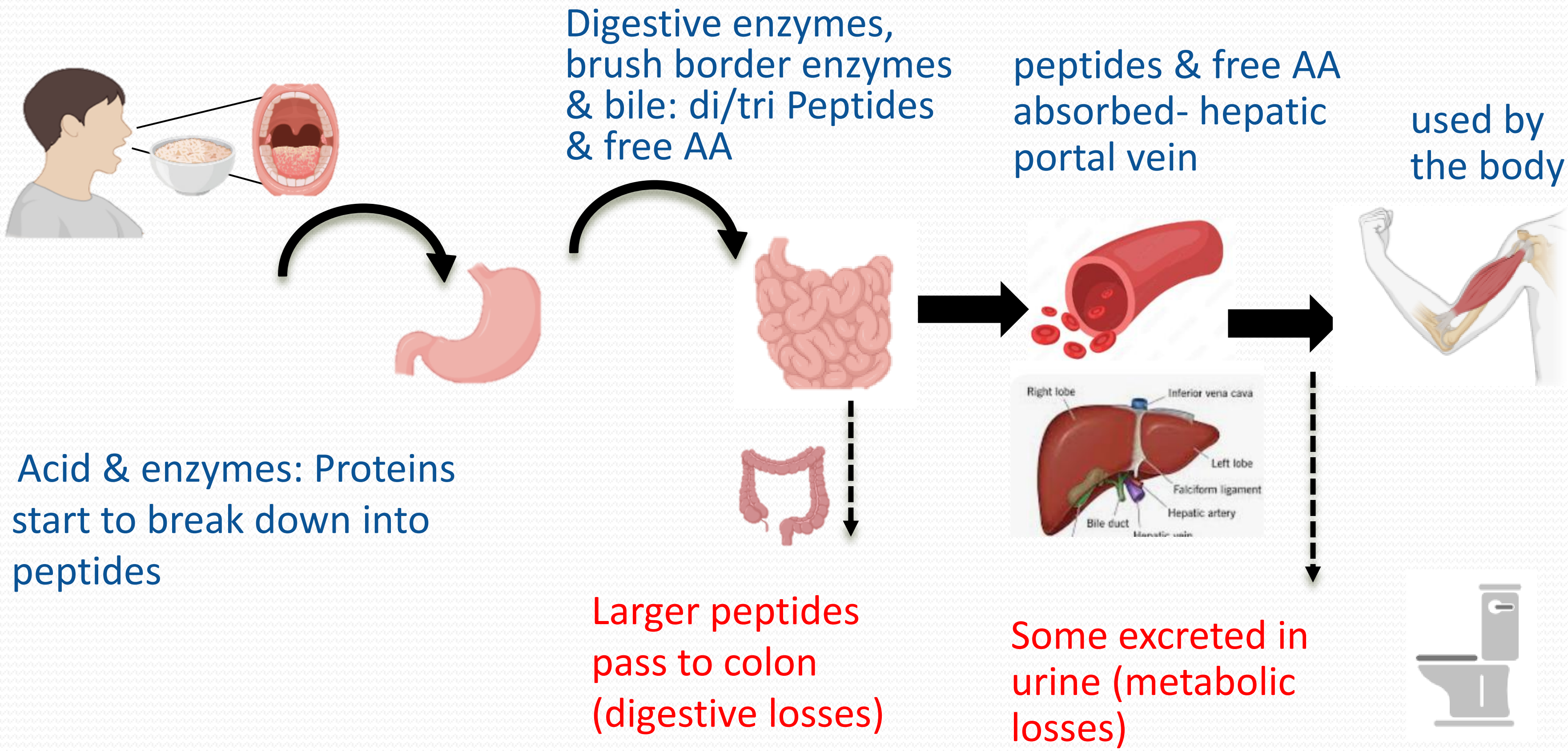
Milk: https://doi.org/10.1093/jn/129.4.890

Cooked meat: https://doi.org/10.3945/jn.115.216838

Pea protein: https://doi.org/10.1093/jn/131.6.1706

Wheat: https://doi.org/10.1093/ajcn/81.1.87

Absorption of proteins



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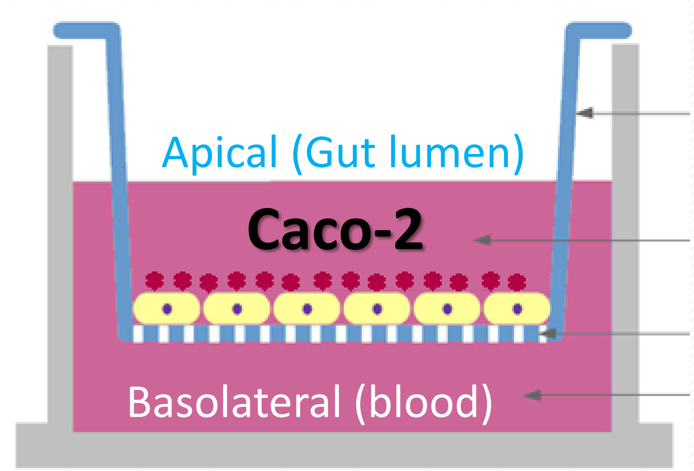
In vitro gut barriers: Caco-2 monolayers

Caco-2 cell line

Orginated from Human
colorectal carcinoma
72 yr caucasian male 1977

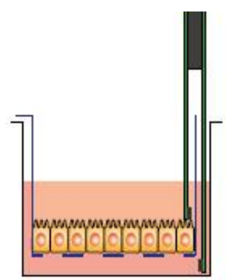


21 days culturing in transwell plates:

- Polarised enterocyte monolayers
- Tight junctions
- Secretory vesicles on apical
- microvilli
- Apical brush border
- 84 known transporters

measure Caco-2 tight junction integrity

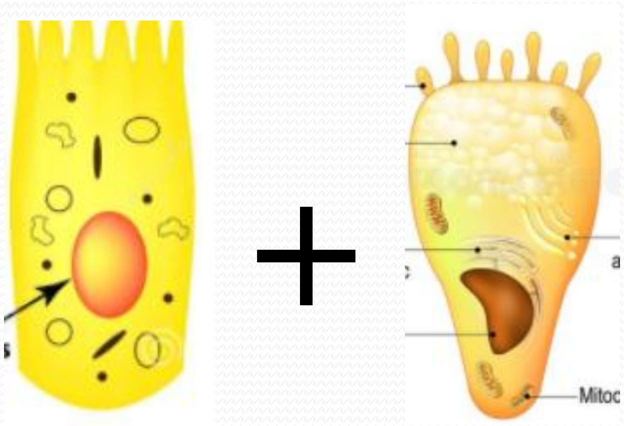
a Transepithelial electrical resistance (TEER)



>500 $\Omega \times cm^2$

Addition of Goblet cells

Add goblets cells w/o disturbing monolayers



HT29 Human colon adenocarcinoma

Female 44 isolated 1964

- Treat Methotrexate MTX
- subclones mature goblet cells producing mucus
- Polarised monolayers with tight junctions

Caco2:HT29MTX Ratios: 90:10 - 70:30

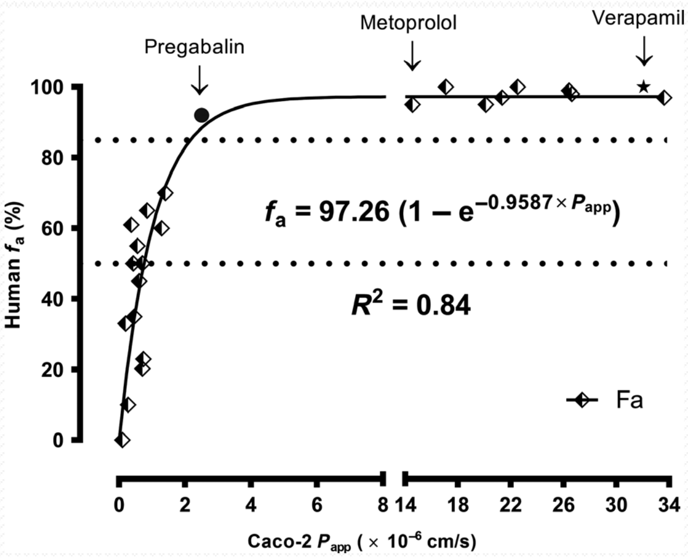
Compounds to measure transport

Compound	Caco-2 Permeability	Determination	Type of transport	Human permeability
Propranolol	17×10^{-6} cm/s	Absorbance. Flouresence	Transcellular passive	100% fraction absorbed
Ketoprofen	20×10^{-6} cm/s	Absorbance	Transcellular passive	95%
Lucifer yellow	0.2×10^{-6} cm/s	Fluorescence	Paracellular passive	
Phenol Red	0.42×10^{-6} cm/s	Colorimetric	Paracellular passive	
Fluorescein isothiocyanate dextran 4,400	0.02×10^{-6} cm/s	Fluorescence	Paracellular passive	
Alfatoxin B1	94×10^{-6} cm/s cm/s	HPLC/FLD	Transcellular passive	
Riboflavin (vitamin B2)	1.52×10^{-5} cm/s	HPLC	Transcellular Active	

in vivo comparisons & Inter-lab

Limitations: *In vitro* barriers based on Caco-2

Permeability Correlation with human gut
R^2= 0.84 (21 drug compounds different solubilities)



Jarc et al., *J. Pharm. Pharmacol.* 2019

- Narrow tight junction 0.4nM (1-1.3nm)

TEER >500 Ωcm² human TEER = 50-100 Ω cm²)

- Solubility, lipophilicity, polar surface will alter permeability

- Substantial inter-lab variability (different Caco2 clones, different culturing protocols)

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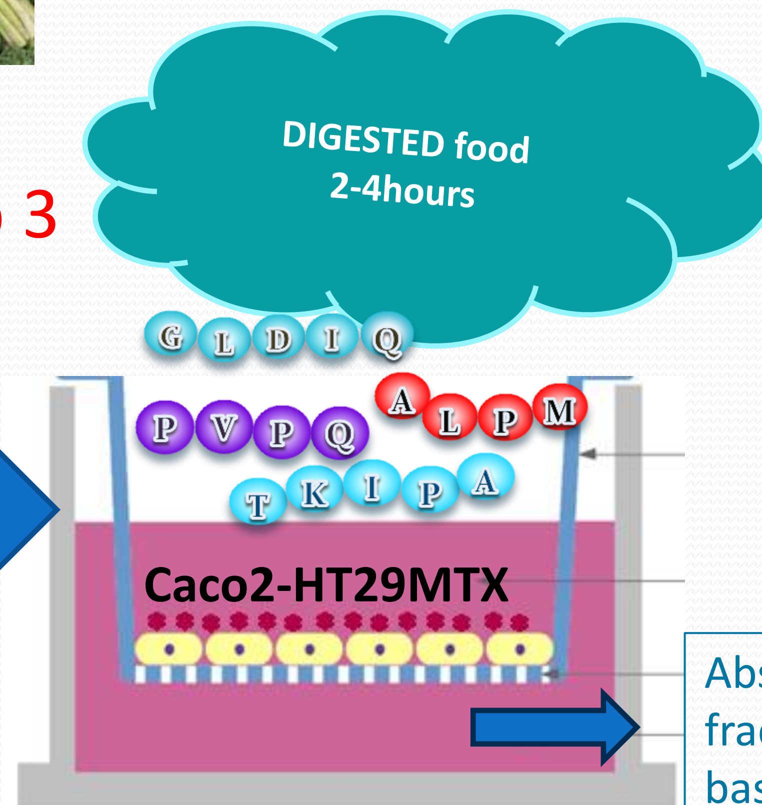
Caco-2/HT29MTX monolayers for food absorption studies

Step 1

Step 2

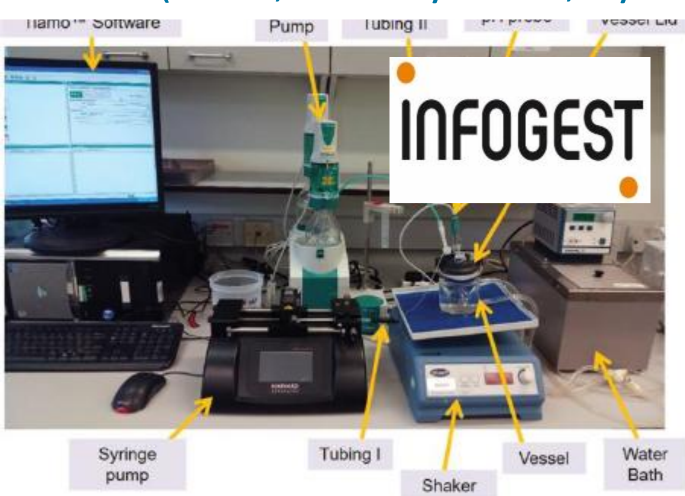
Step 3



digestion

Jejunal aspirates

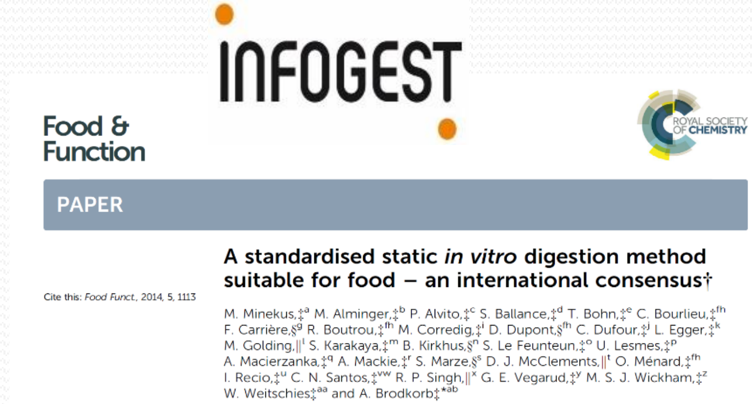
In vitro (static, semi dynamic, dynamic)

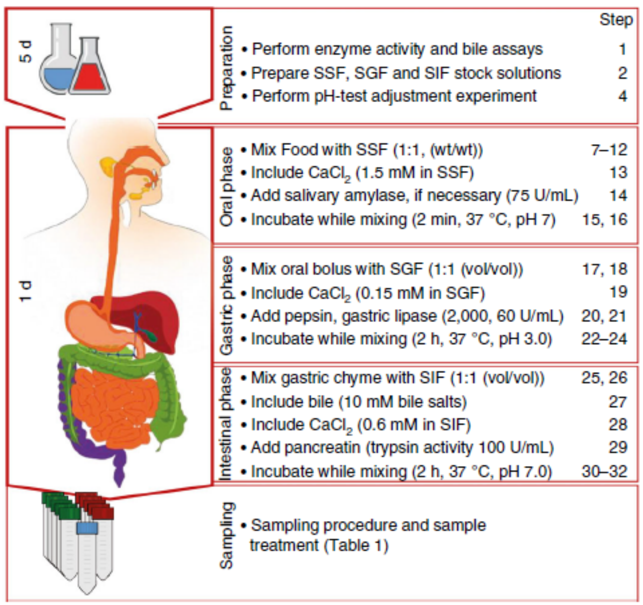


Absorbed fraction basolateral

INFOGEST Protocol

Standardized In vitro Gastrointestinal Digestion protocol - Cost Action 2011-2015





"one pot model"

Oral

Gastric

Intestinal

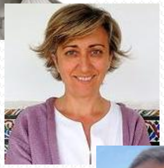
- **Based on available physiological data**
- **Complete step by step protocol**
- **All materials listed; *Critical Steps***

→ **Widely accepted by academia and industry**

→ **High impact >10,000 citation**

INFOGEST

In vitro DIAAS method for the assessment of protein quality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ISO ISO/TC 34/SC 5 **N 1840**

ISO/TC 34/SC 5 "Milk and milk products"
Secretariat: **NEN**
Committee manager: **de Vreeze Marcel Mr**

Form 6 - Result of voting on ISO/NP 24167|IDF 261 (In vitro digestion protocol for the analysis of protein digestibility and in vitro DIAAS)

Document type	Related content	Document date	Expected action
Project / Form	Project: ISO/NP 24167 Ballot: ISO/NP 24167 (restricted	2023-12-15	INFO

World Health Organization
Food and Agriculture Organization of the United Nations
efsa European Food Safety Authority

Draft: ISO/WD 24167:2024(E)
IDF 261:2024(E)

In vitro DIAAS protein digestibility method

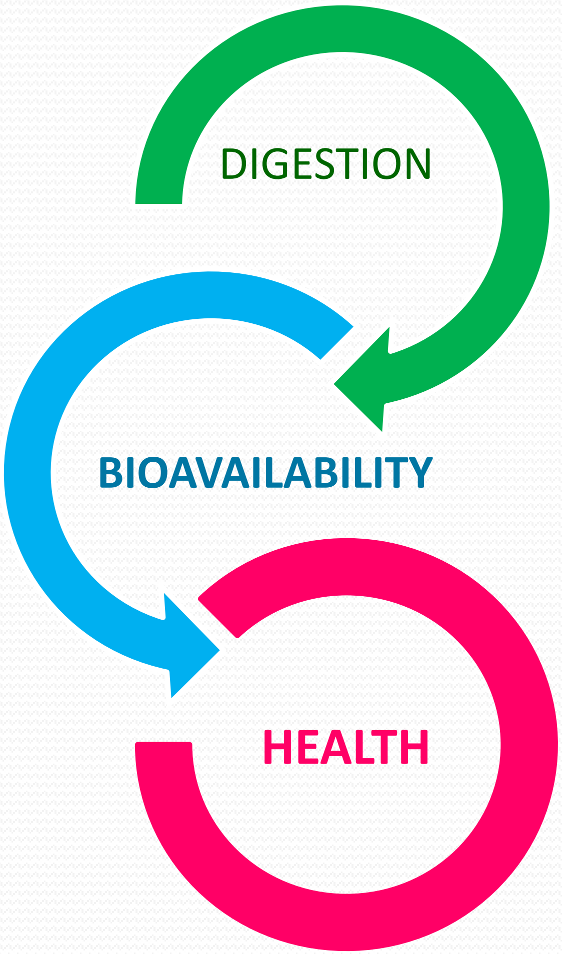
nature protocols PROTOCOL
https://doi.org/10.1038/s41596-018-0119-1

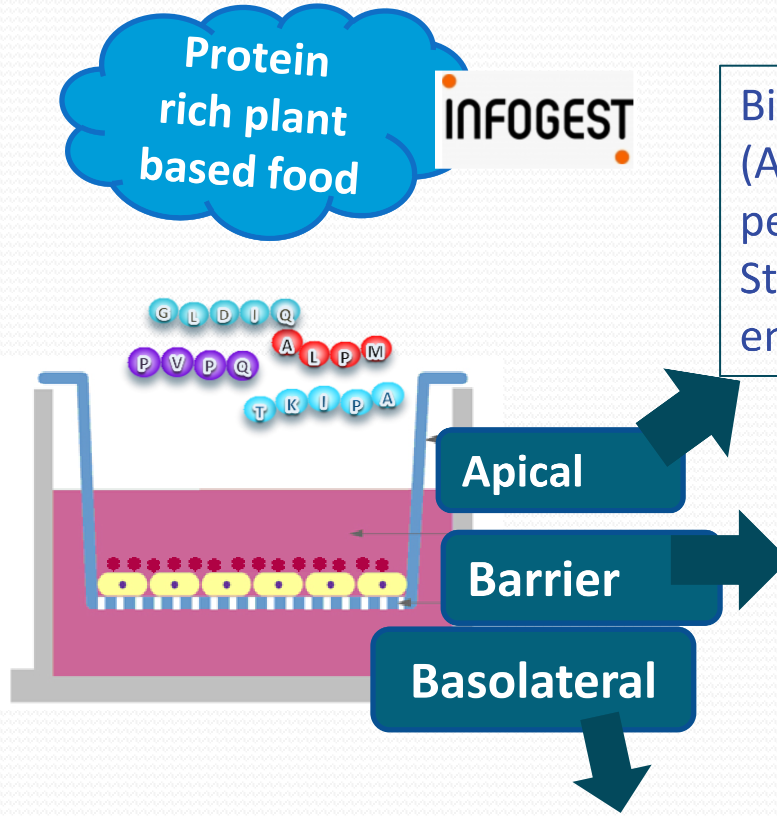
INFOGEST static in vitro simulation of gastrointestinal food digestion

André Brodkorb[1*], Lotti Egger[2], Marie Alminger[3], Paula Alvito[4], Ricardo Assunção[4], Simon Ballance[5], Torsten Bohn[6], Claire Bourlieu-Lacanal[7], Rachel Boutrou[8], Frédéric Carrière[9], Alfonso Clemente[10], Milena Corredig[11], Didier Dupont[8], Claire Dufour[12], Cathrina Edwards[13], Matt Golding[14], Sibel Karakaya[15], Bente Kirkhus[5], Steven Le Feunteun[8], Uri Lesmes, Adam Macierzanka[17], Alan R. Mackie[18], Carla Martins[4], Sébastien Marze[19], David Julian McClements[20], Olivia Ménard[8], Mans Minekus[21], Reto Portmann[2], Cláudia N. Santos[22,23], Isabelle Souchon[24], R. Paul Singh[25], Gerd E. Vegarud[26], Martin S. J. Wickham[27], Werner Weitschies[28] and Isidra Recio[29]

FLAVOURsome

WORK FLOW





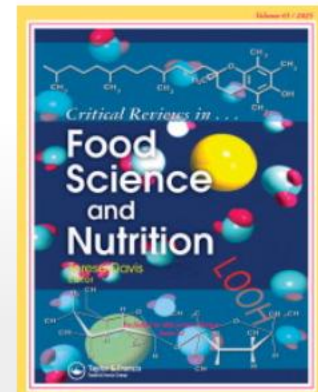
Bioaccessible fraction (Amino acid, peptides), Satiety Studies on enteroendocrine cells

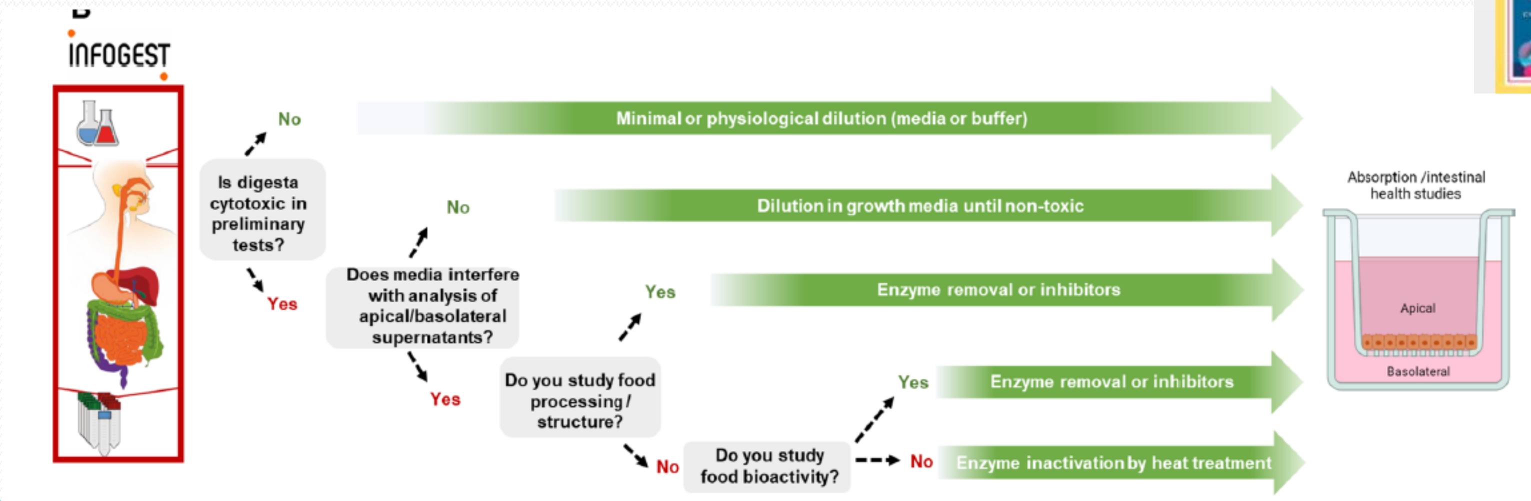
Barrier health check permeability, architecture, inflammation (allergy)

Absorbed fraction (Amino acid, peptides)
Bioactivity by advanced pre-clinical assays
Eg Muscle protein synthesis

Are digested samples biocompatible with Caco-2-HT29MTX monolayers?

- Challenge: Digestive enzymes (trypsin), Bile salts are toxic
- Solutions: Remove or inactivate enzymes, dilute salts
- Warning: eg Enzyme inhibitors can inhibit Caco-2 brush border enzymes, remove enzymes can also remove food components, dilution= below limit of detection

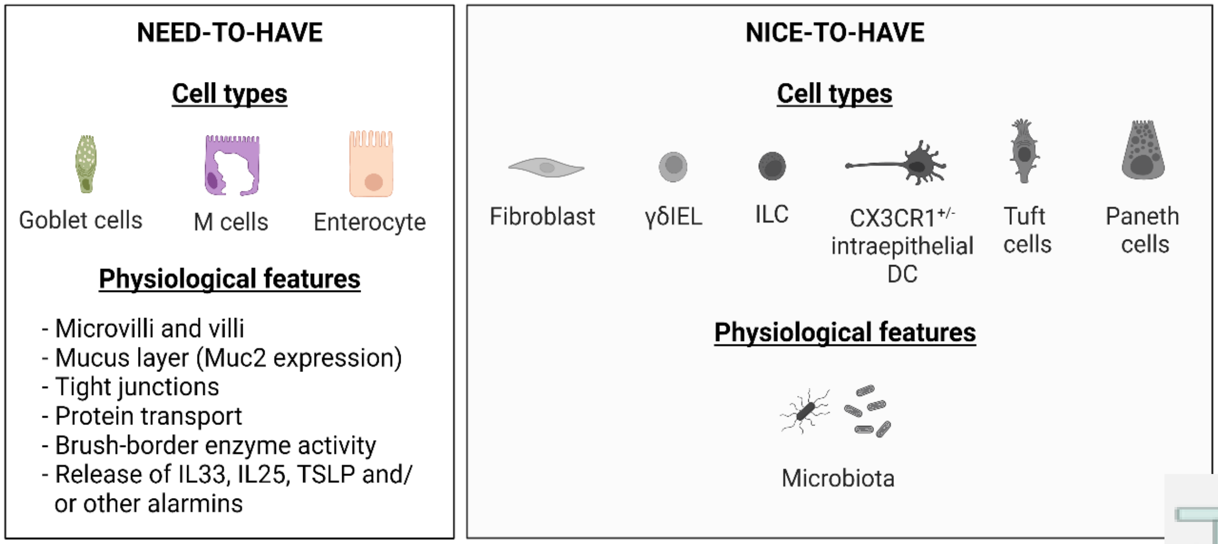




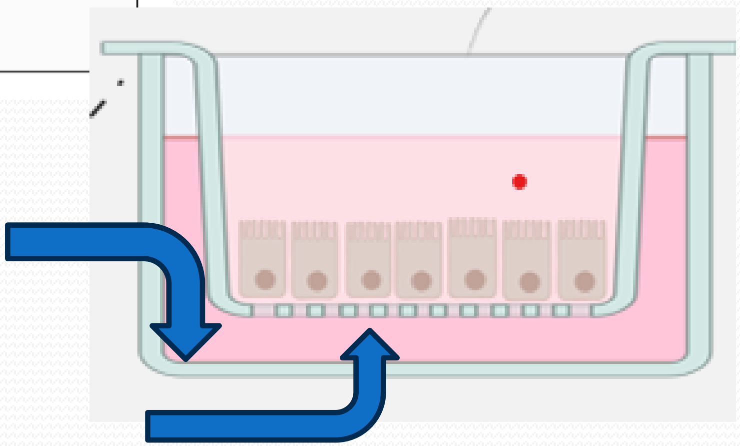
Kondrashina et al. DOI: 10.1080/10408398.2023.2214628

Use of Caco-2/HT29MTX monolayers for Food allergy?

epithelial activation requires addition M cells to barrier model



Dijk et al., DOI: 10.1111/1541-4337.13097



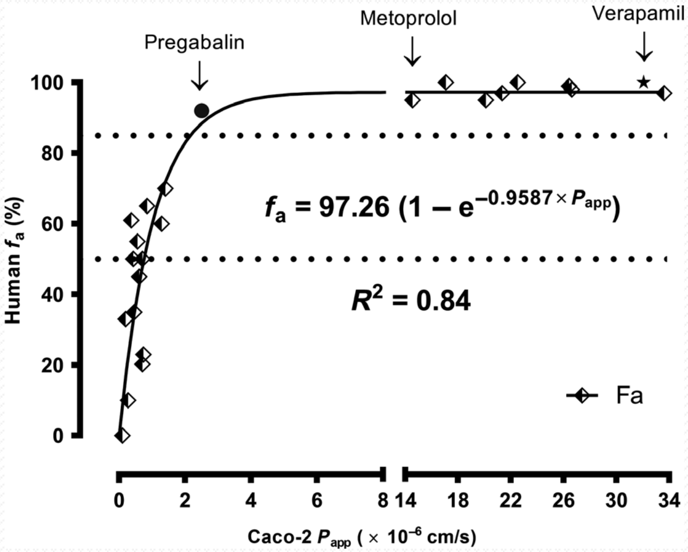
- Raji-B lymphoma cells (M cells) will disturb Caco2/HT29MTX monolayer
- Options: cultured in basolateral chamber, underside transwell membrane

Caco-2 based monolayers

- Tried & tested (lots of data in the literature)
- Workhorse of oral drug industry
- Relatively easy to culture
- Commercially available
- Suitable for Food digesta (clean-up required)
- Suitable for food allergy (M cell addition)

Limitations for food absorption studies

Permeability Correlation with human gut
$R^2 = 0.84$



Jarc et al., *J. Pharm. Pharmacol.* 2019

- Narrow tight junction 0.4nM (1-1.3nm) TEER >500 Ωcm^2 human TEER = 50-100 Ω cm^2)

- Caco-2 may underpredict neutral amino acid transport
- Food matrix, food processing will alter nutrient bioaccessibility in turn will alter bioavailability
- Interlab variation: different clones, different protocols, different monolayer thresholds

International Ring Trial to reduce inter-lab variation

- Consensus protocols for Caco-2/HT29MTX culturing, monolayers differentiation, permeability studies

8 European countries, 17 laboratories, 15 Institutes, 1 private technology centre, 1 company

Teagasc Agriculture and Food Development Authority
IPLA Instituto de Productos Lácteos de Asturias
U.PORTO
CIAL Instituto de Investigación en Ciencias de la Alimentación
Wageningen University & Research
INRAE
Università degli Studi di Napoli Federico II
BIA Biopolymères Interactions Assemblages
STLO
université PARIS-SACLAY
H&H Group inspiring wellness
INL International Iberian Nanotechnology Laboratory
Instituto Nacional de Saúde Doutor Ricardo Jorge
ainia
Aarhus University
University of Surrey
EEZ Estación Experimental del Zaidín
UAM Universidad Autónoma de Madrid

Alternative cell lines which will form barrier

name	animal	location	Derived cancer
IPEC-J2			
IPEC-1			
IP1-21			
T84, SW620			
HUTU-80			
IEC-6, IEC-18			

- All cell line barriers have limitations V real life
- Consider primary research question

Compare & contrast within labs: Caco2

Immune response: IPEC-J2

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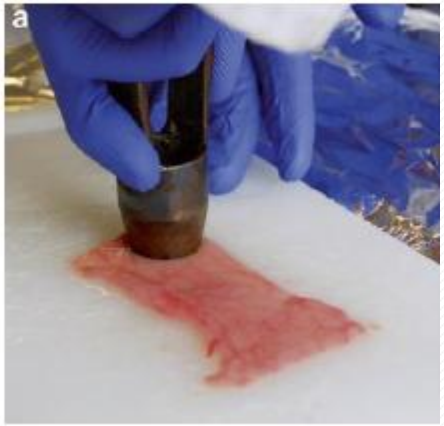
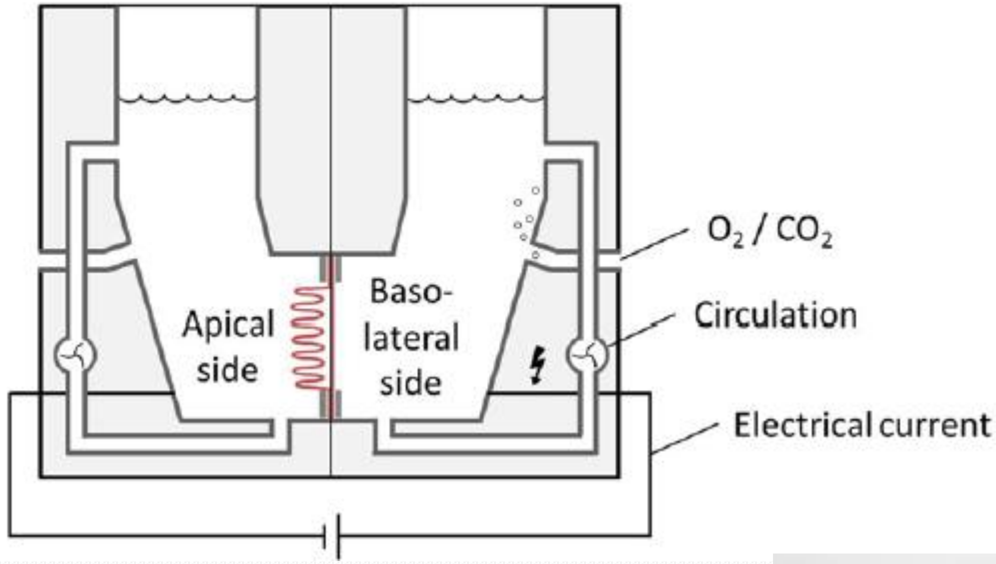
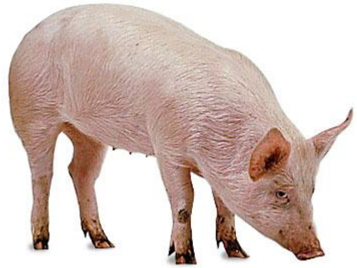
Ex-vivo models Ussing chambers

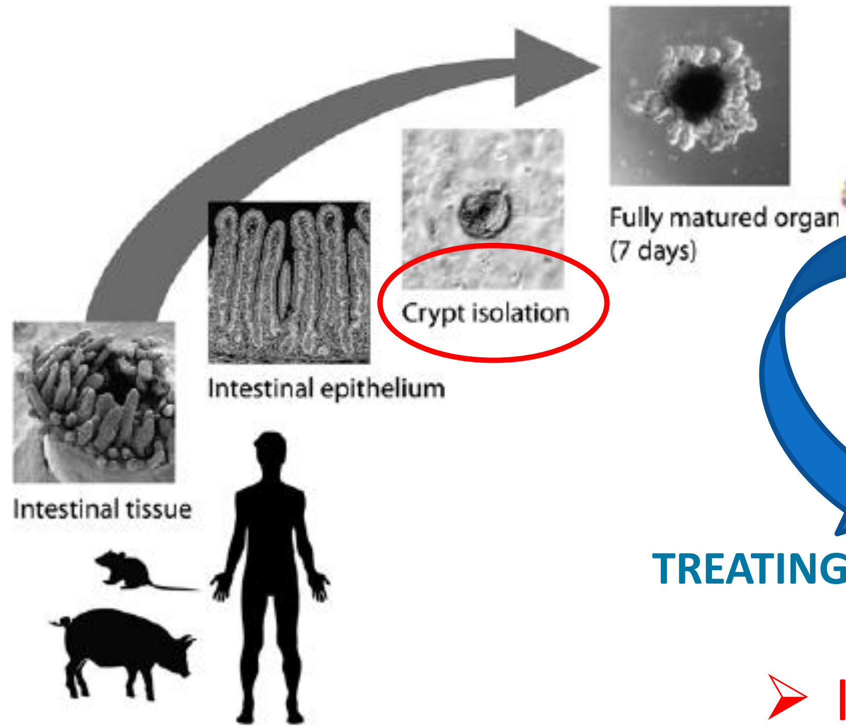


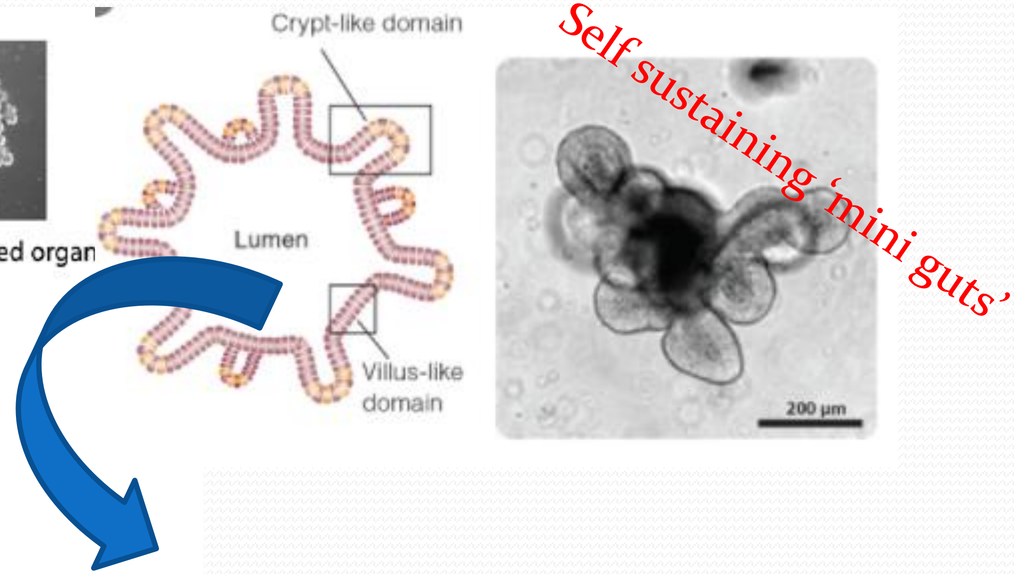


Limitation:
Low throughput
Availability/ethical
Human, Pigs, rats



Intestinal ORGANOIDS





TREATING ORGANOIDS WITH FOOD DIGESTA

- Inward orientation
- Apical side inaccessible
- 2D organoids apical exposed
- Biocompatability with INFOGEST?

Need for dietary protein diversification

Horizon 2020 project

GIANT LEAPS

Wheat, rice and maize ~ 60% of plant-based calorie intake in most diets.

Protein Digestibility (DIAAS) & Bioavailability (absorption)

Upcoming Conferences

INFOGEST

9th International
INFOGEST
Gdansk, Poland
19-21 May 2026

ICF Gdańsk 2026

WG3 leader:
Absorption Models

ISBP
INTERNATIONAL SYMPOSIUM
ON BIOACTIVE PEPTIDES

Limerick, Ireland
14-16 June 2027

Co-host


Thank
You!