Bioactive peptides from milk and fish – can they cross the barrier?

Dr George Grimble
Centre for Gastroenterology and Nutrition
University College London

g.grimble@ucl.ac.uk

Outline

- Why marine species?
- Interesting and useful things which marine species make.
- Hydrolysed fish protein.
- Bioactive peptides from animal protein.
- Strategies for identifying them.
- Critical issues.
- Clinical studies with bioactive peptides
- Fish, themselves. No oil.

Why marine species?

- Fish consumption is good for health.
  e.g. cardiovascular disease, inflammatory disease, T2D
- Oily fish are favoured.
- This effect is related to fish-oil content.
- Is that it?

Why marine species?

Sustainability issues

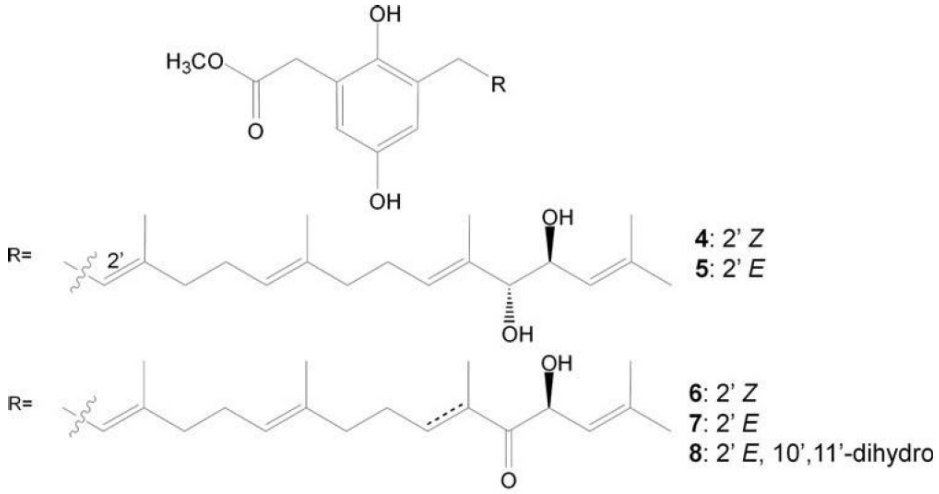
Little impact on health	Waste less Shop, cook and store efficiently
Synergy with health	Eat less food with limited nutritional value
Antagonistic with health	**Eat less fish** Only choose seasonal, locally produced F&V
BIG issue in synergy with health	Eat less meat and dairy products

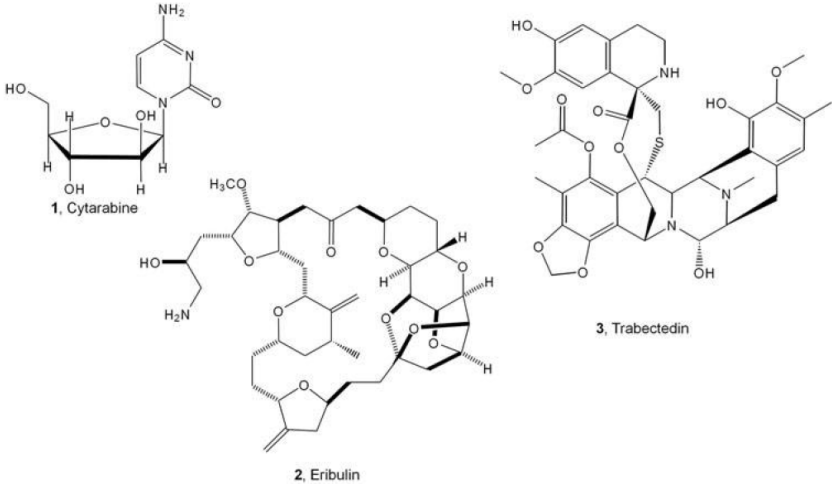
More efficient use of marine harvests

Interesting / useful things made by marine species

Species	Compounds
Crustaceans	Chitosan
Seaweed and Marine algae	Betaine – 1C nutrient Antioxidants - chlorophyll, carotenoids, phycobilipigments, phlorotannins Anticancer agents – Cytarabine, trabectedin, Eribulin

Park and Pezzuto. Antioxidant marine products in cancer chemoprevention. Antioxid Redox Signal 19 (2):115-138, 2013.
Misurcova et al. Health benefits of algal polysaccharides in human nutrition. Adv.Food Nutr.Res. 66:75-145, 2012.





Interesting / useful things in fish

Species	Compounds
Fish	N-3 oils Compounds from other marine species Protein Bioactive peptides

EU rules will mean that fishing should become more efficient

Less of the catch thrown back and unusual species recycled

Skin, viscera, fish heads and carcasses recycled

Bloodwater and “stickwater” recycled

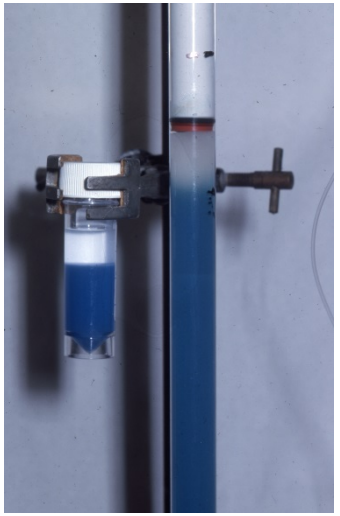
Protein hydrolysates are the great “leveller”

What is a protein hydrolysate?

A partial hydrolysate of food protein

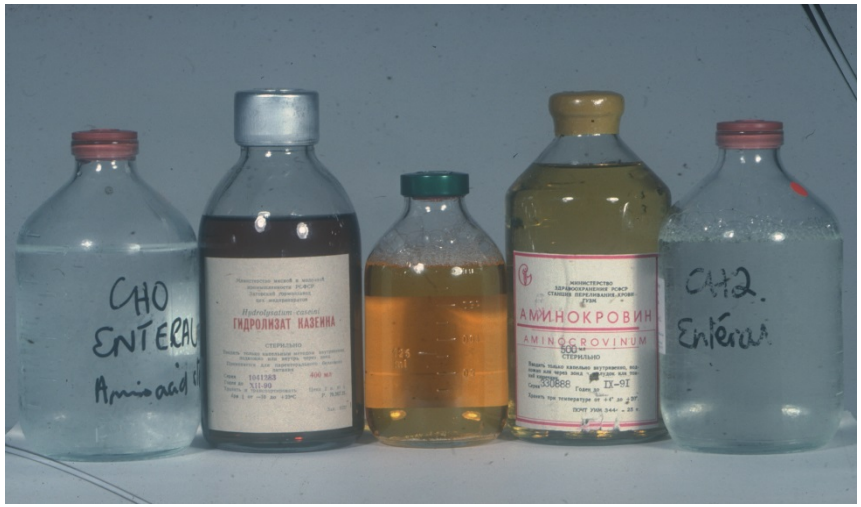
With a huge range of properties

Amino acid composition – compare whey protein with spent grains in brewing
Chain-length profile – mainly long, mainly short, variable amino acids, mixture of all
Sequences – some may be bioactive
Taste – “umami” versus “bitter peptides”!
Solubility – more soluble than the parent protein
Surfactant – shampoos
Antioxidants – great chelators



Medical uses of protein hydrolysates

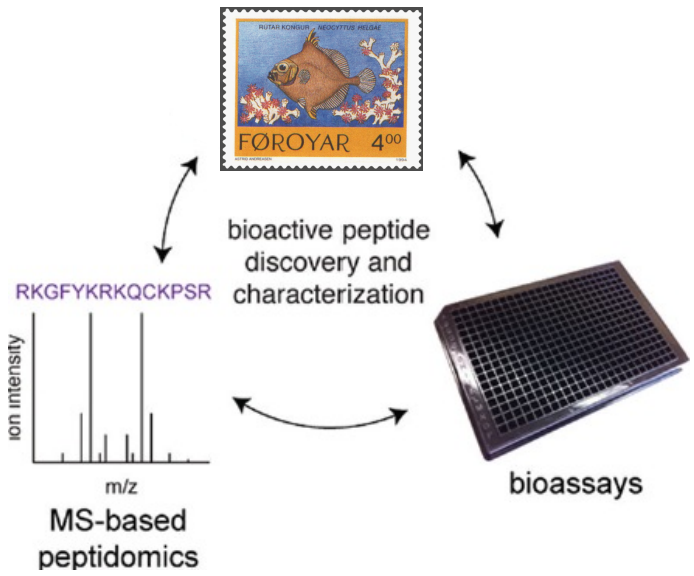
- For oral/tube feeding the patient with impaired intestinal absorption
- For intravenous feeding of patients with intestinal failure, as a substitute for intravenous amino acids
- As a source of bioactive peptides



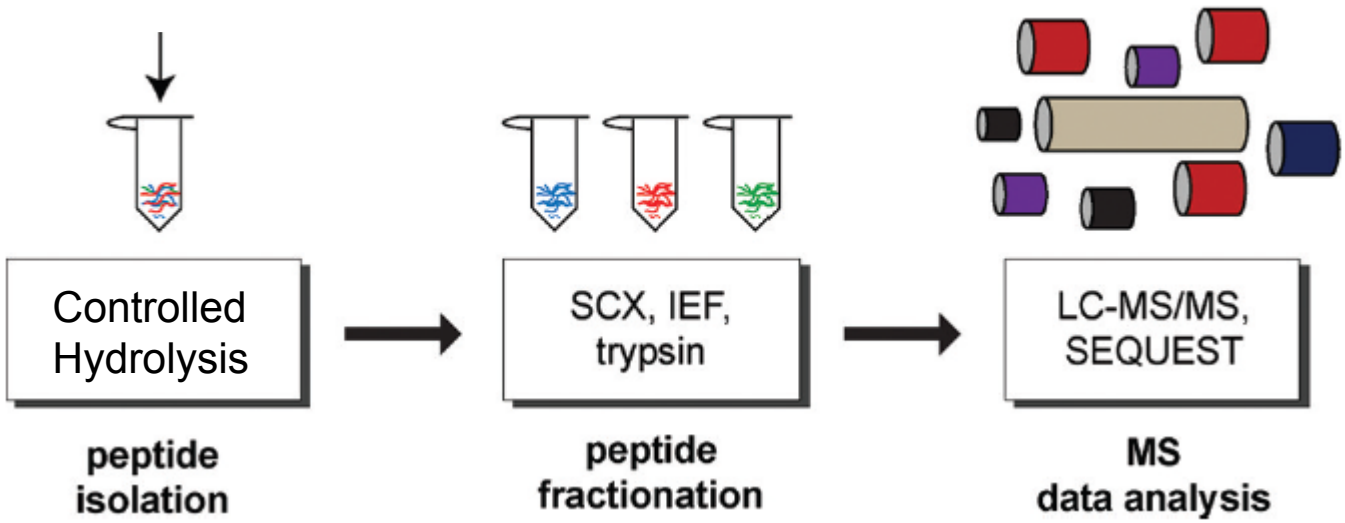
Finding bioactive peptides

Tinoco and Saghatelian. Biochemistry. 50 (35):7447-7461, 2011.

Enzymic protein hydrolysis

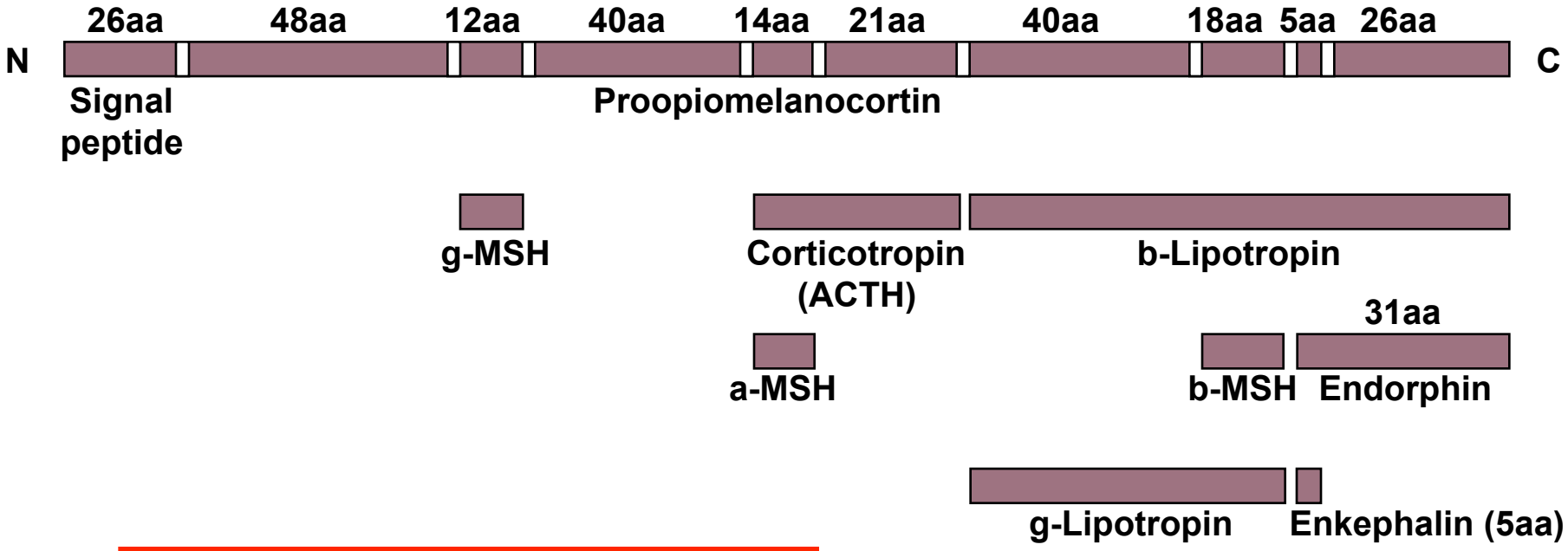


Food protein



Pre-proopiomelanocortin

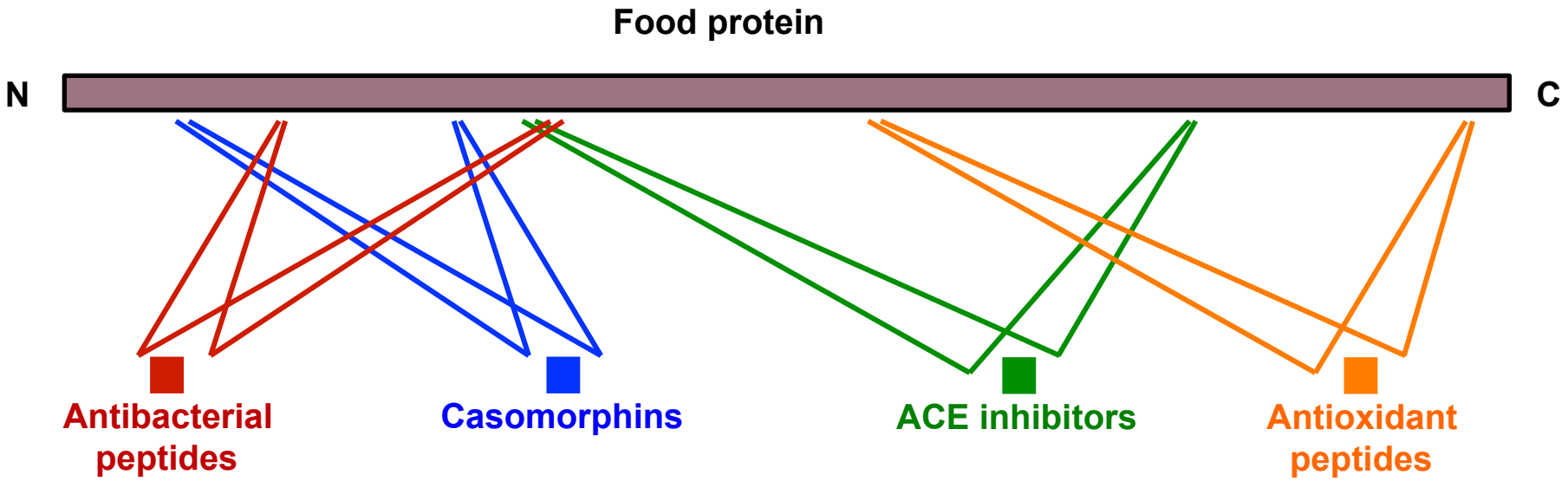
Multiple functional polypeptides from a single precursor
Processed in a cell-specific manner
Purposeful



Important in energy homeostasis

Bioactive peptides from protein hydrolysates

Multiple functional peptides from a single precursor
Released by specific proteases
Purposeful, but opportunistic



The presence of bioactive peptides in food proteins can be

1. Predicted from the sequence – e.g. PeptideLocator programme
Mooney et al. Bioinformatics. 29 (9):1120-1126, 2013.
2. Measured by LC/MS methods

In vitro - Di Bernardini et al. Peptides. 32 (2):388-400, 2011, Harscoat-Schiavo et al. Anal Bioanal.Chem 403 (7):1939-1949, 2012.

In vivo - Boutrou et al. Am.J.Clin.Nutr. 97 (6):1314-1323, 2013.

Current status of bioactive peptides

Vegetables

Garlic, Buckwheat, Mushroom, Mung bean, Rice, Chickpea, Peanut, soybean, Yam, Potato, Maize, Sesame, Flaxseed, Wheat, Alfalfa, Sunflower, Hempseed, Cocoa

Garcia et al. Vegetable foods: a cheap source of proteins and peptides with antihypertensive, antioxidant, and other less occurrence bioactivities. Talanta 106:328-349, 2013.

Milk

Casein, Whey, Yoghurt

Muro Urista et al. Review: Production and functionality of active peptides from milk. Food Sci Technol.Int 17 (4):293-317, 2011.

Marine species

Macroalgae (phycobiliproteins), Microalgae, Fish muscle, Fish albumins, Mollusks, Crustaceans

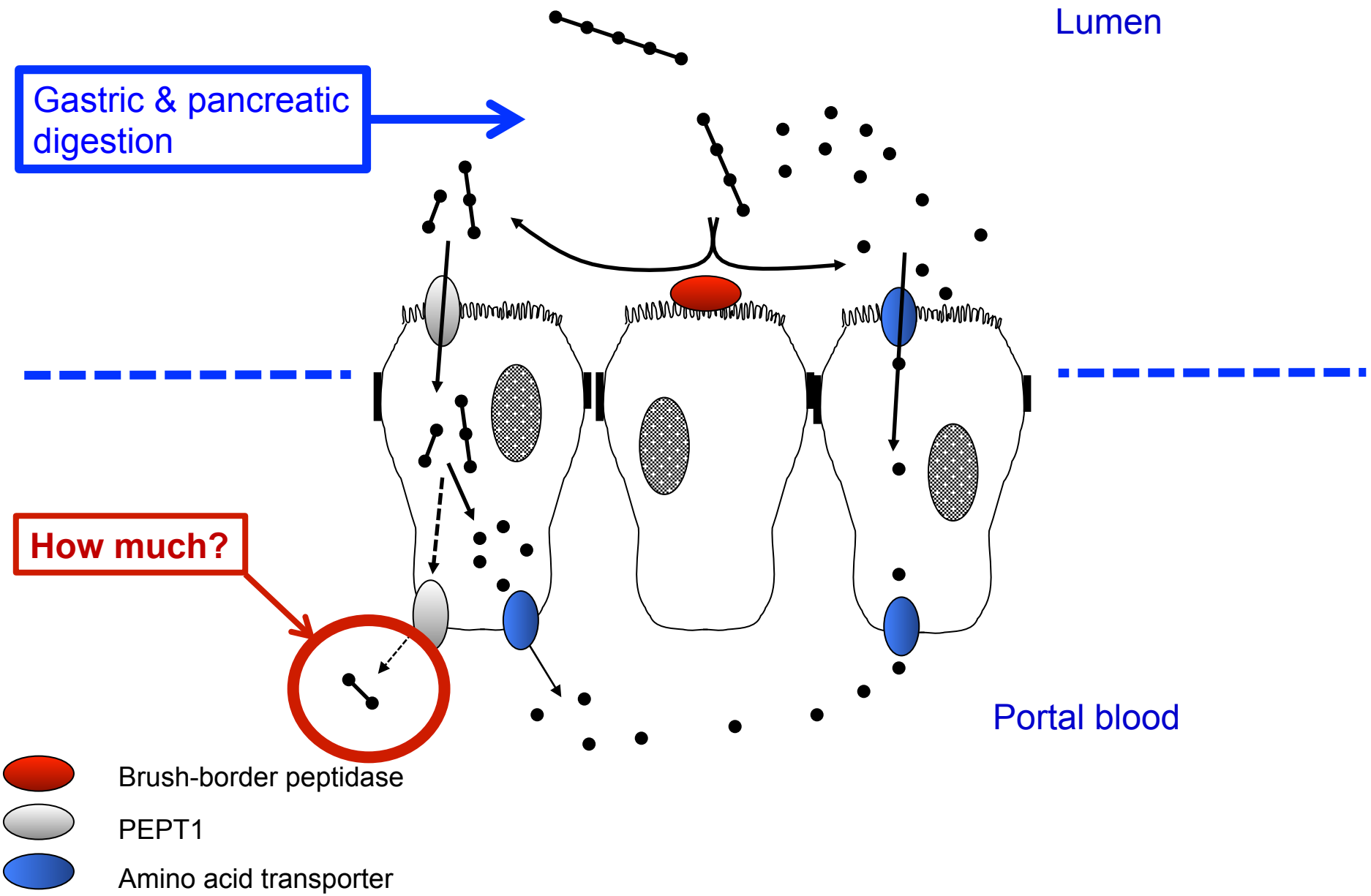
Lordan et al. Marine bioactives as functional food ingredients: potential to reduce the incidence of chronic diseases. Marine Drugs 9 (6):1056-1100, 2011.

Peptide activity	Source
ACE inhibition	Fish frame, algae
Anticoagulative	Fish frame
Antidiabetic	Fish frame
Antimicrobial	Marine invertebrates, fish
Antioxidative	Algae protein waste, fish frame

The elephant in the room

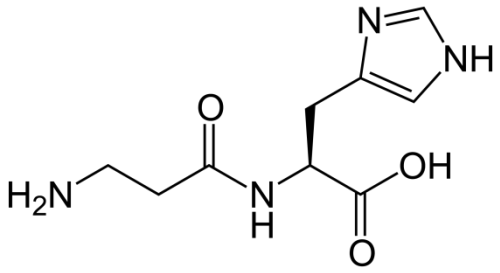
Are these peptides bioactive, in humans eating food?

Protein digestion and assimilation in the gut



Some dipeptides get through

Gardner et al. J.Physiol.(Lond). 439:411-422, 1991.



Dipeptide - β-alanine and histidine

QED. It was absorbed intact

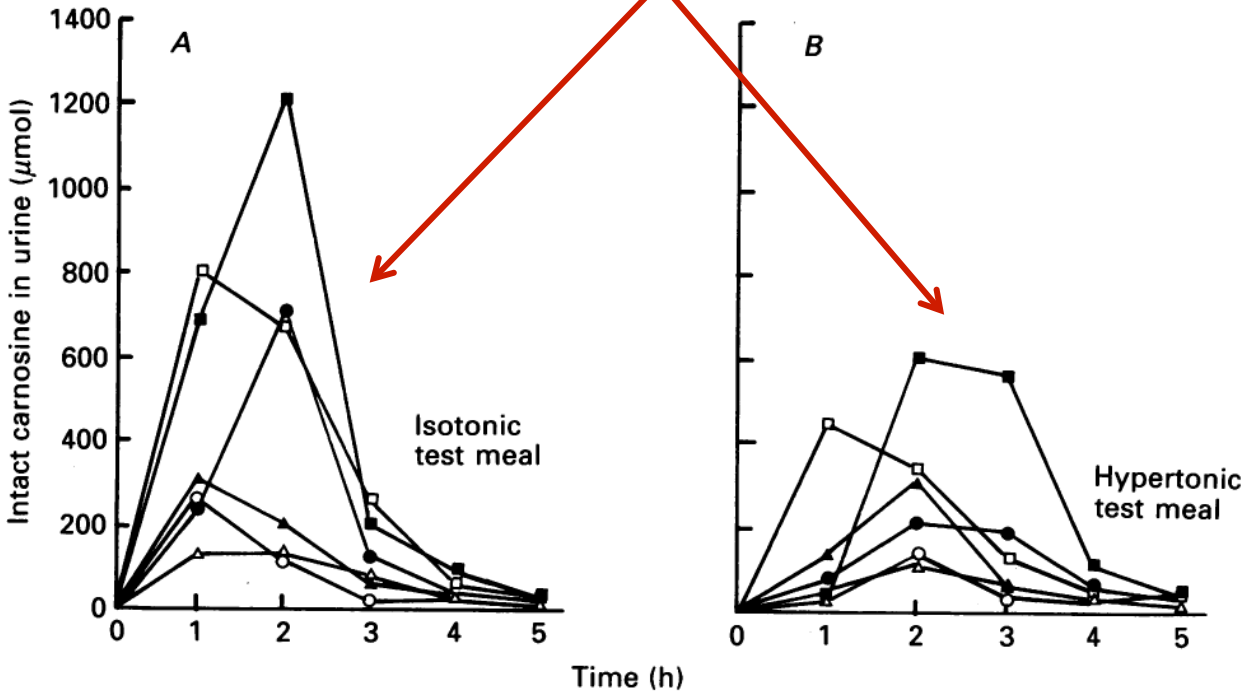
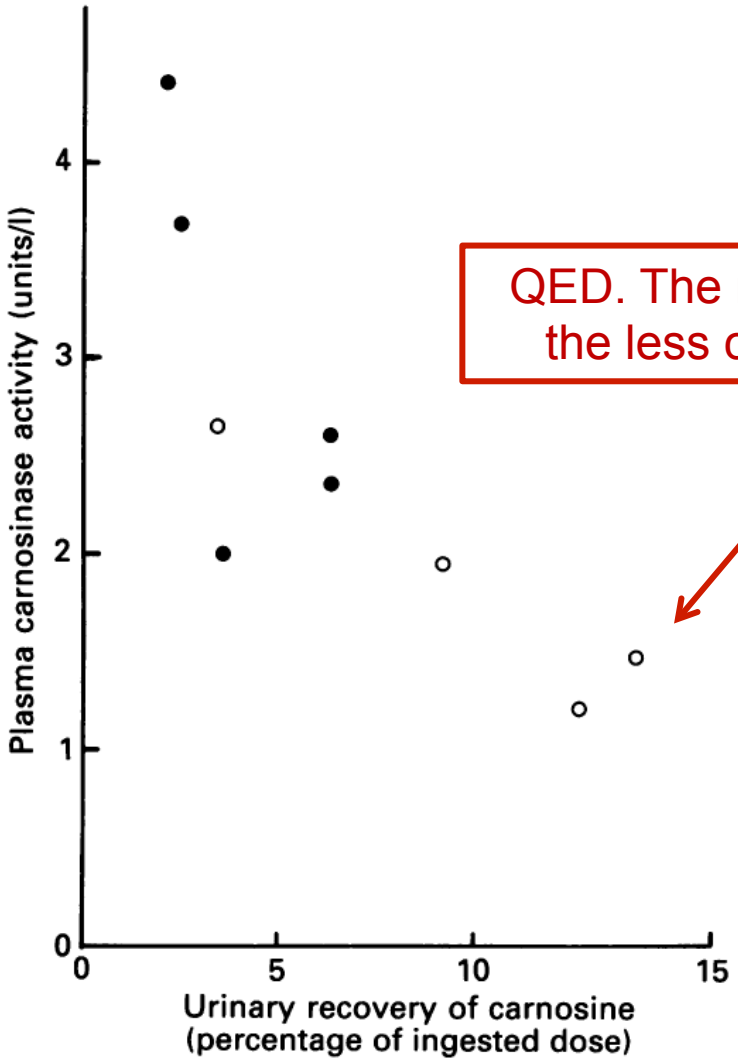


Fig. 1. Hourly urinary output of intact carnosine following ingestion of 4 g carnosine with an isotonic (*A*) or a hypertonic (*B*) test meal. Each symbol represents a different subject.

Ingested carnosine (L-β-alanine-L-histidine) in urine

Gardner et al. J.Physiol.(Lond). 439:411-422, 1991.



QED. The more plasma peptidase; the less carnosine gets through

What about protein breakdown?

There are two pathways of protein breakdown

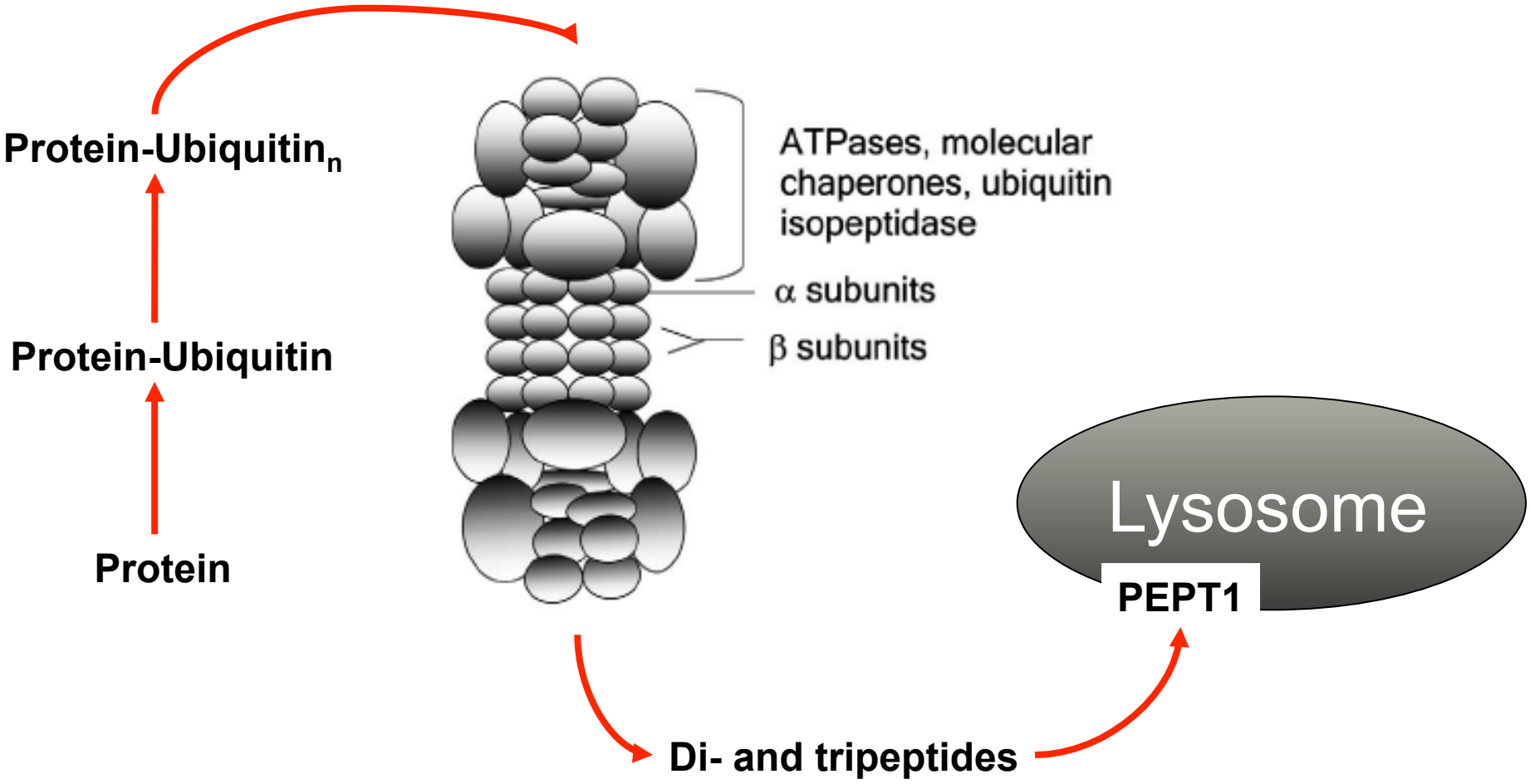
Lysosomal – *"Acidic organelles that contain a battery of degradative enzymes"*

Endocytosis and phagocytosis
Not uncontrolled
Not "suicide bags"

Finn PF, Dice JF. Proteolytic and lipolytic responses to starvation. Nutrition 2006;22:830-44.
Mitch WE, Goldberg AL. Mechanisms of muscle wasting. The role of the ubiquitin-proteasome pathway. N Engl J Med 1996;335:1897-905.

The proteasome

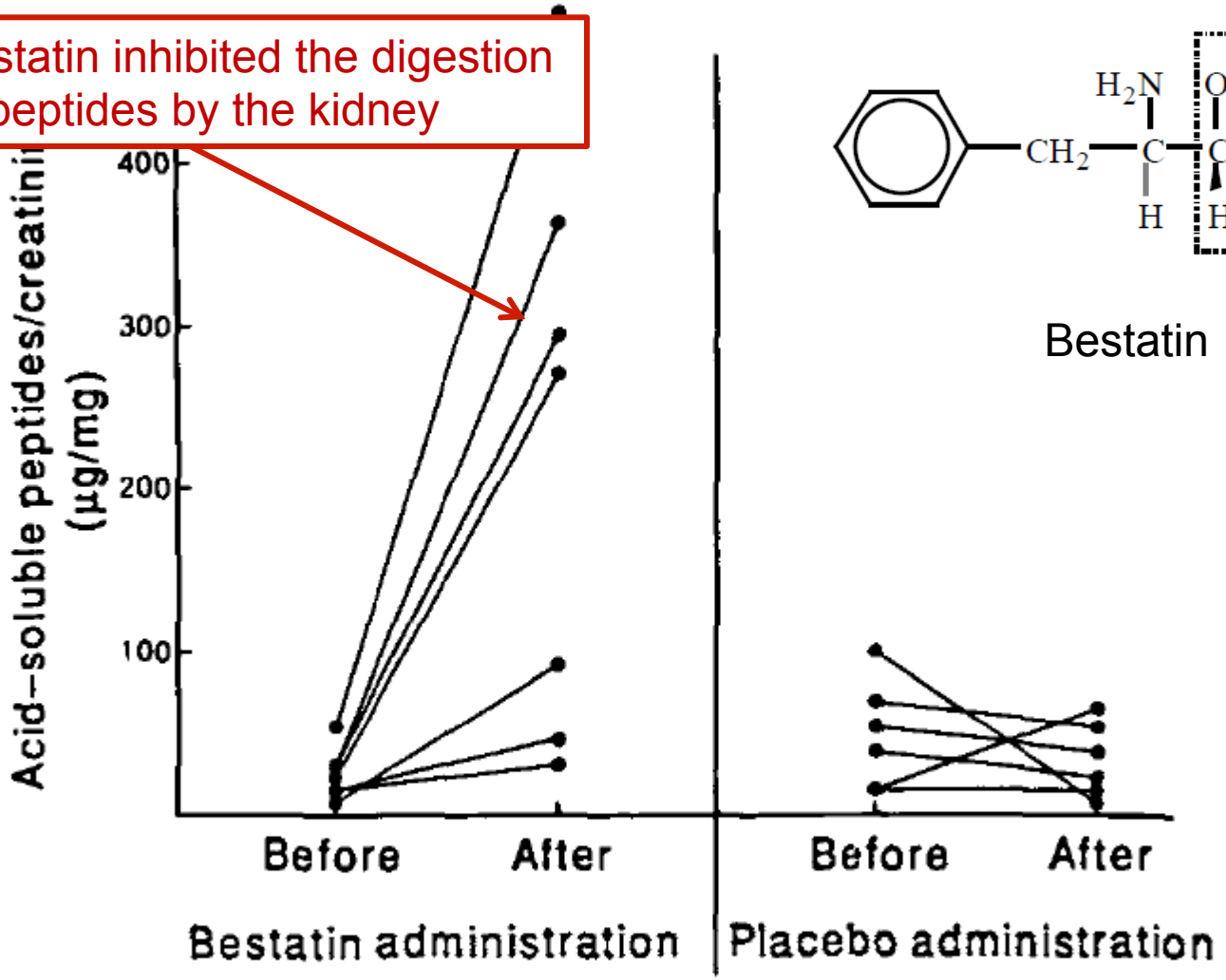
Finn PF, Dice JF. Proteolytic and lipolytic responses to starvation. Nutrition 2006;22:830-44



Inhibiting aminopeptidases ↑urine peptides

Hirano and Sakamoto. Urinary excretion of acid-soluble peptides in children with Duchenne muscular dystrophy. Acta Paediatr.Jpn. 36 (6):627-631, 1994.

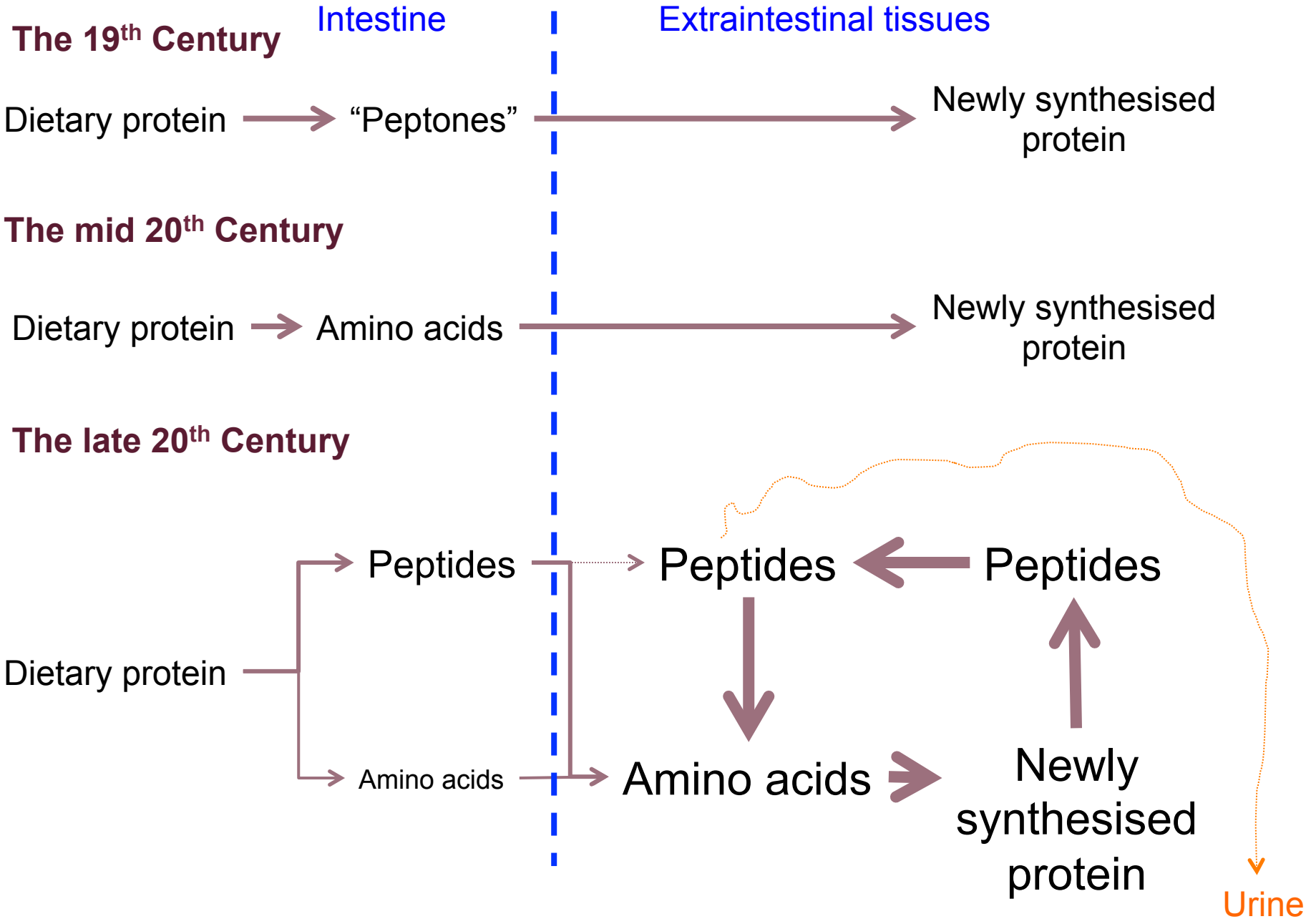
QED. Bestatin inhibited the digestion of blood peptides by the kidney

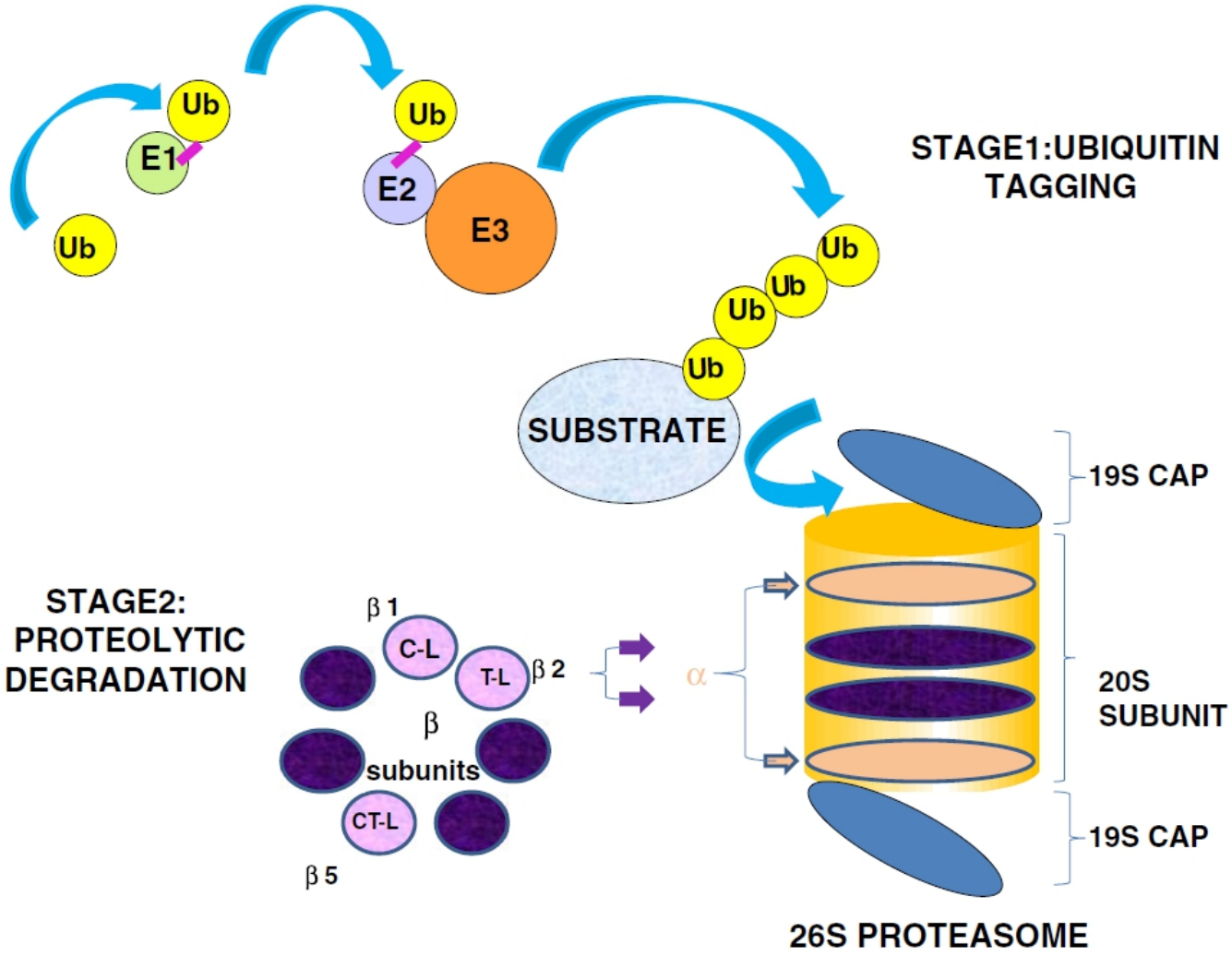


Bestatin

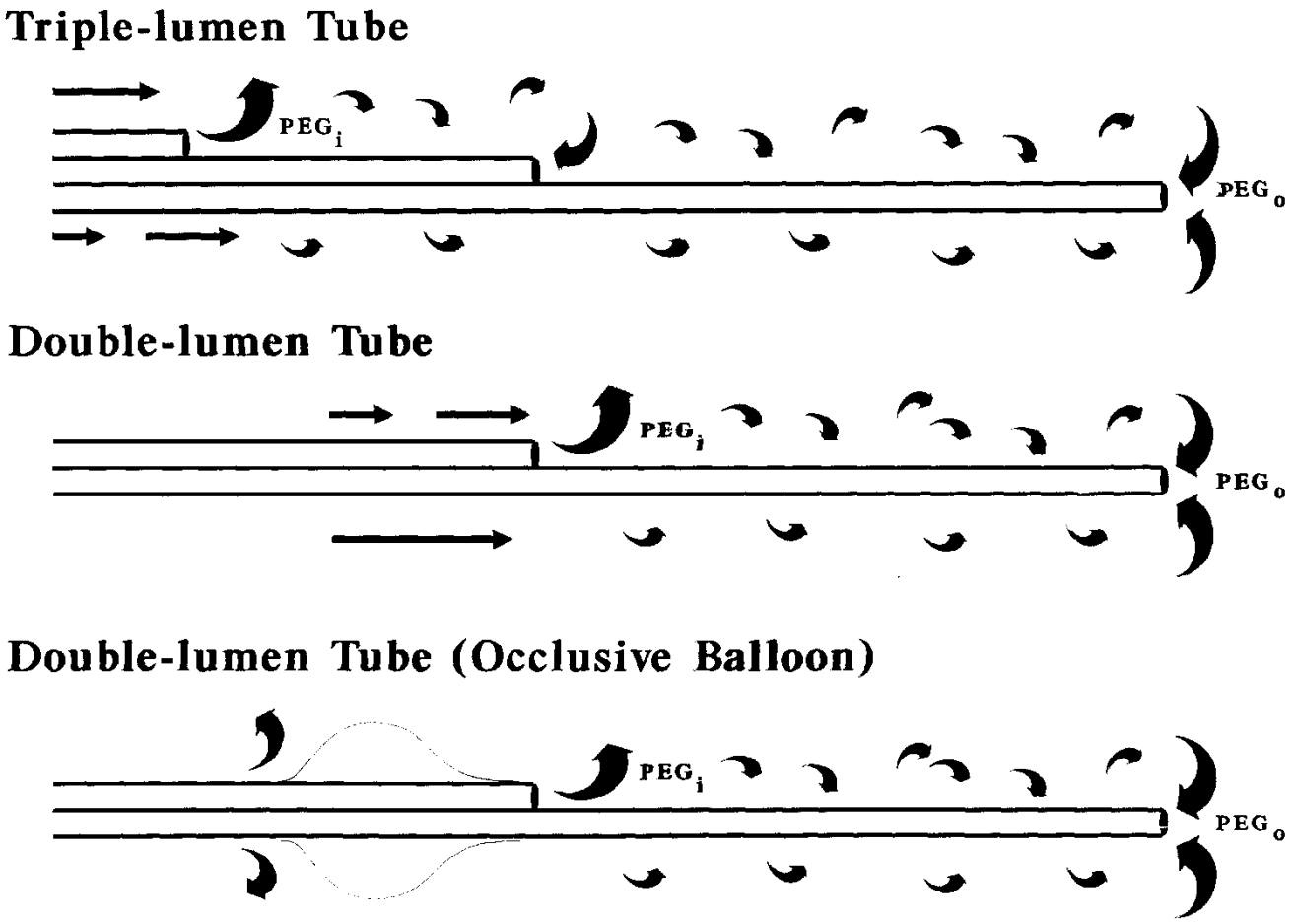
Fig. 4 Change in urinary acid-soluble peptide (ASP) ratio after oral administration of bestatin for 9 months.

Changing thoughts on peptides

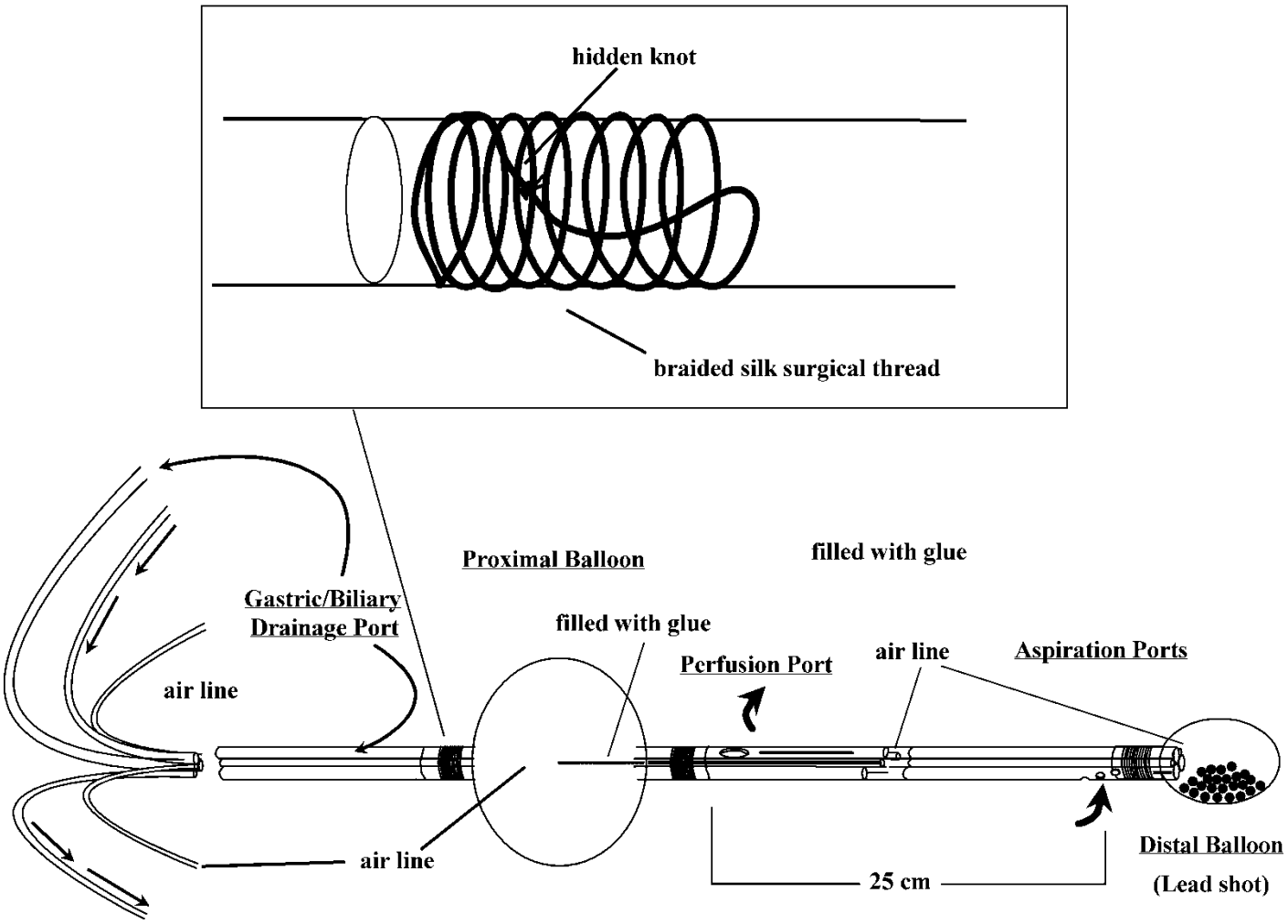



Ub
E1
Ub
Ub
E2
E3
Ub
Ub
Ub
Ub
SUBSTRATE
STAGE1:UBIQUITIN TAGGING
19S CAP
STAGE2: PROTEOLYTIC DEGRADATION
β 1
C-L
T-L
β 2
β
subunits
CT-L
β 5
α
20S SUBUNIT
19S CAP
26S PROTEASOME

Measuring absorption – intestinal perfusion

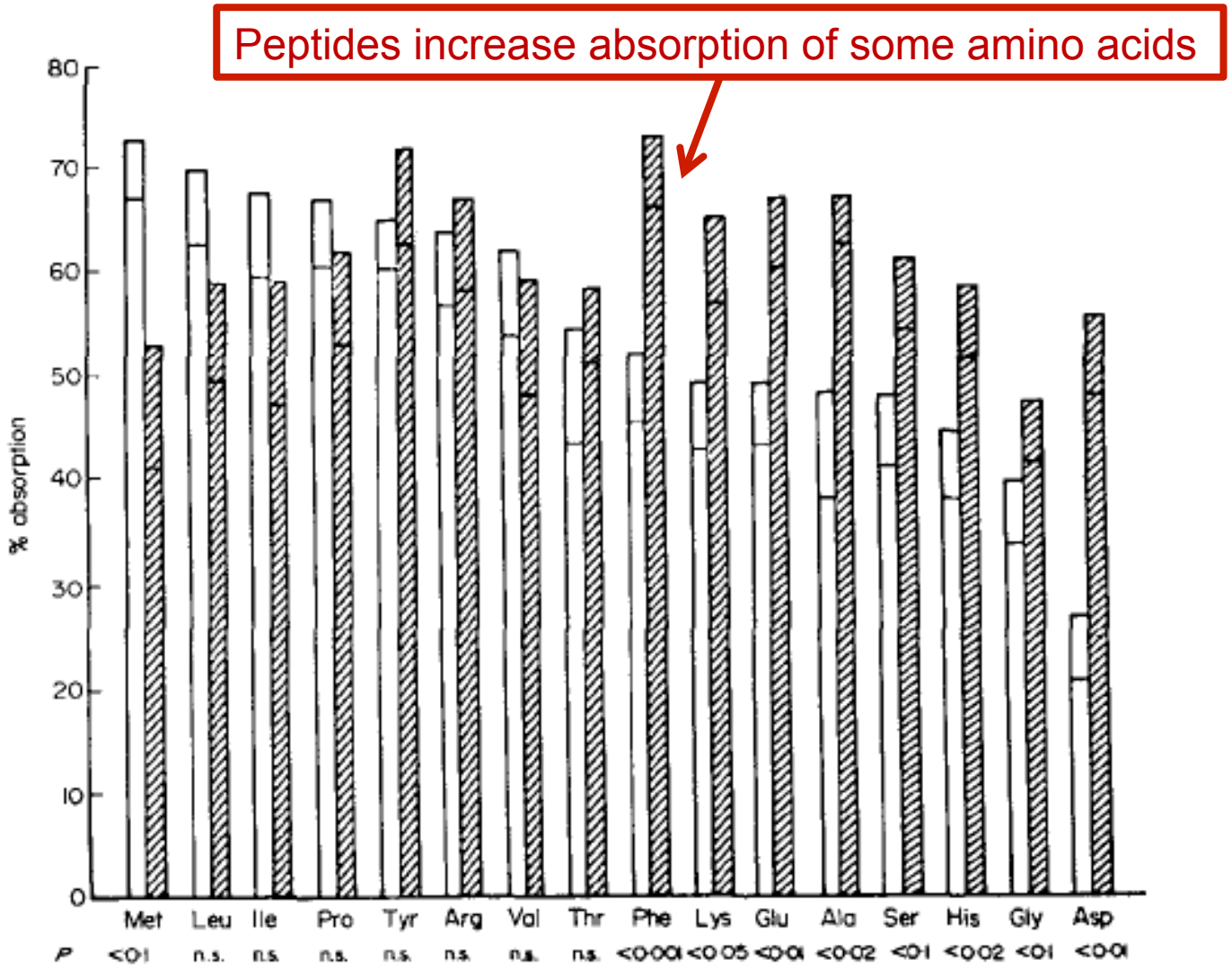


Perfusion tube

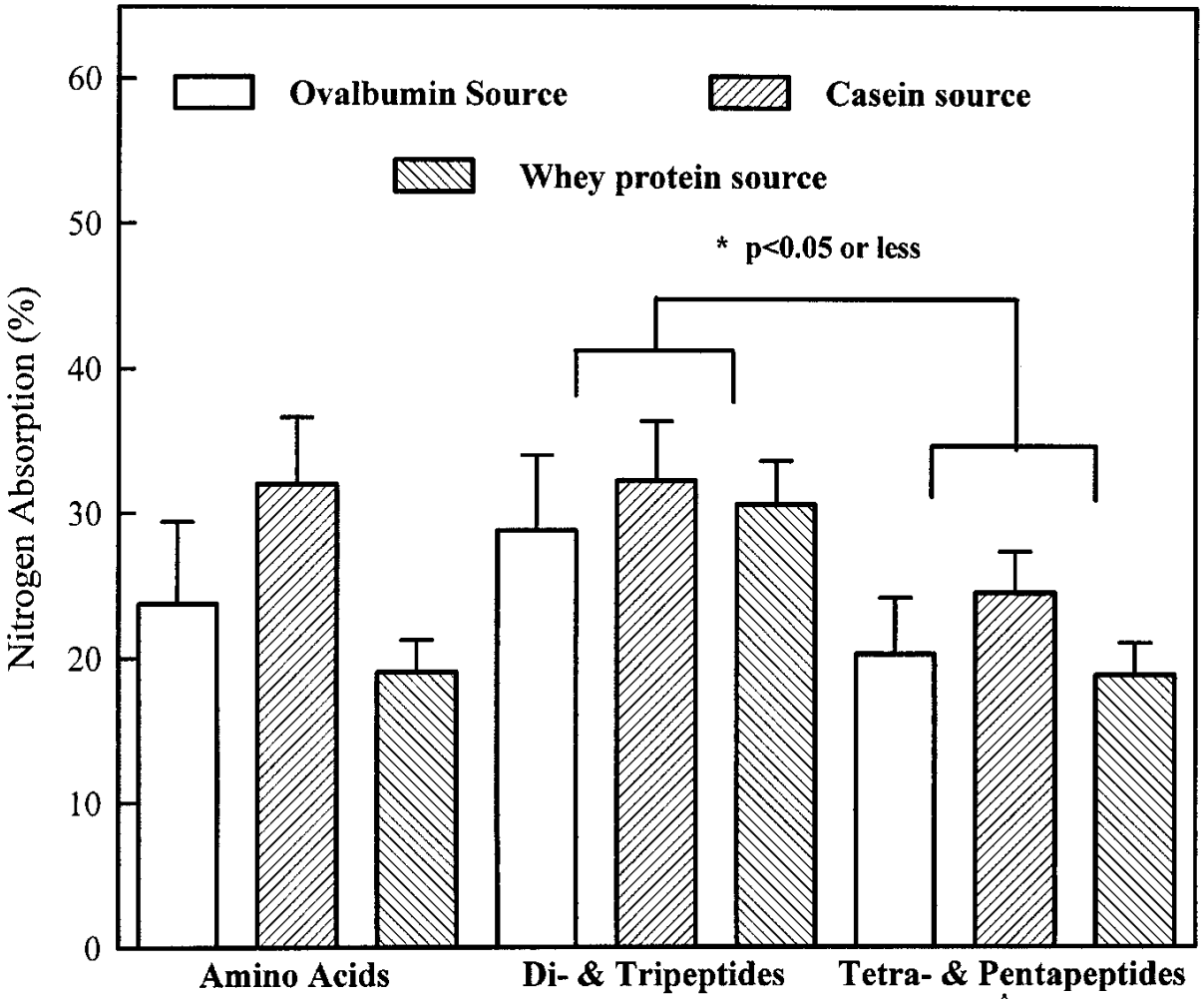


Kinetic advantage of peptides

Silk et al. *J.Parent.Ent.Nutr.* 4 (6):548-553, 1980



Summary of perfusion studies



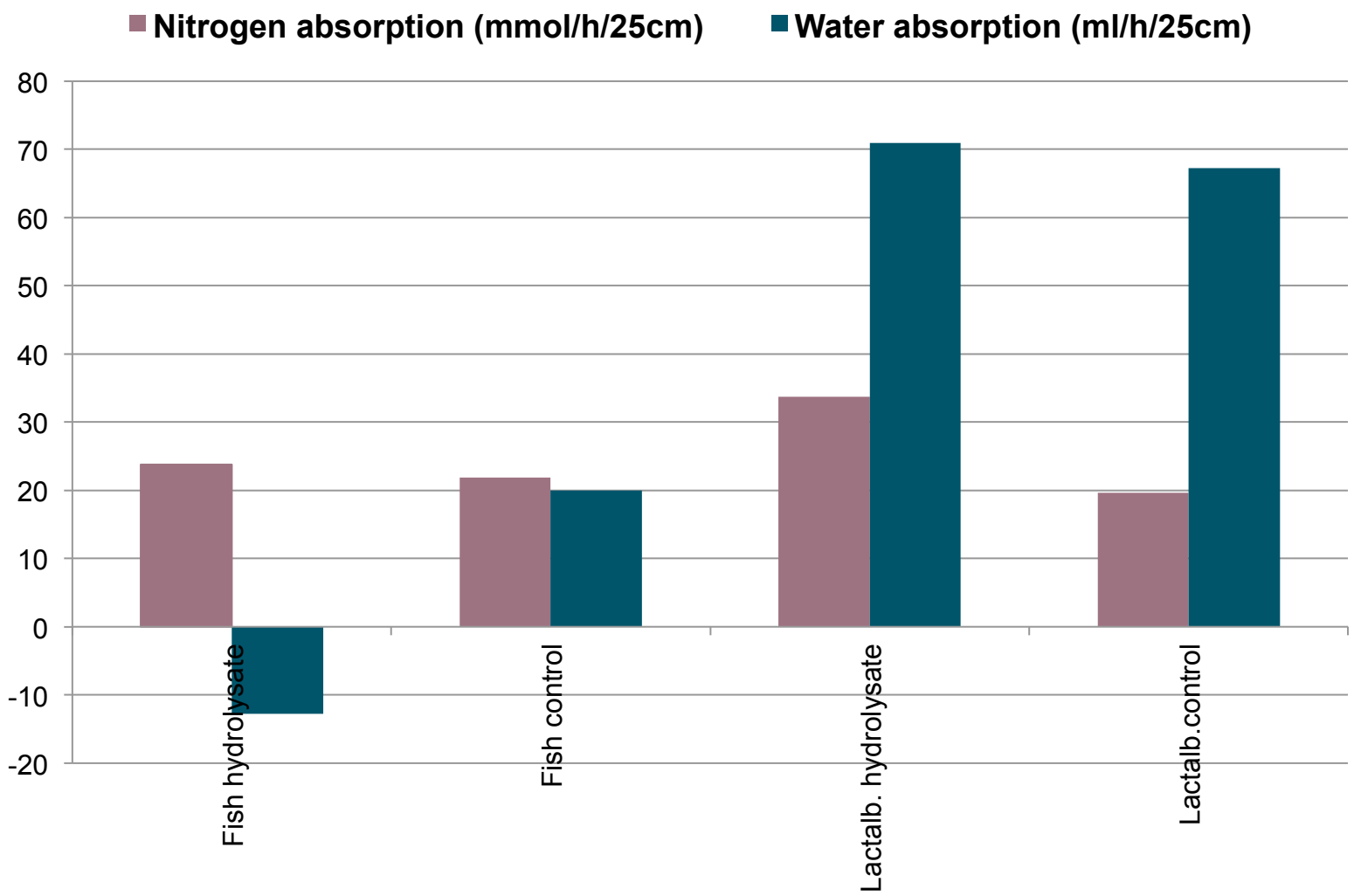
Grimble et al 1987, 1988, 1994

Helix Biotechnology

Helix Biotechnology Ltd
Darwin Building
Gower Street
London WC1E 6BT
Telephone 0171-383 5721
FAX 0171-380 7018/7193

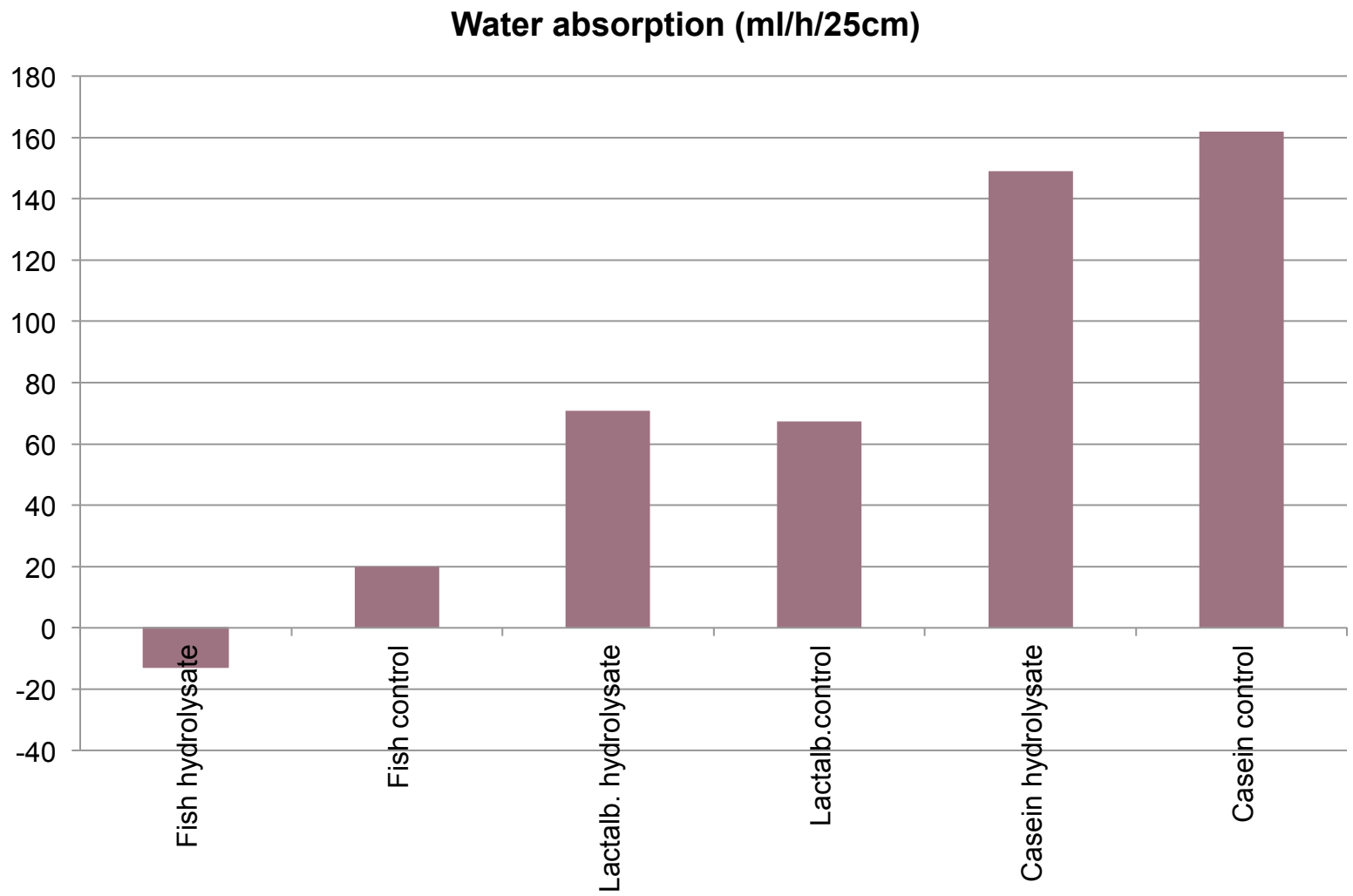
Something “fishy” going on!

Fairclough et al. Comparison of the absorption of two protein hydrolysates and their effects on water and electrolyte movements in the human jejunum. Gut. 21:829-834, 1980.



Something “fishy” going on!

Silk et al. Use of a peptide rather than free amino acid nitrogen source in chemically defined "elemental" diets. J.Parent.Ent.Nutr. 4 (6):548-553, 1980.



Bioactivity in protein hydrolysates

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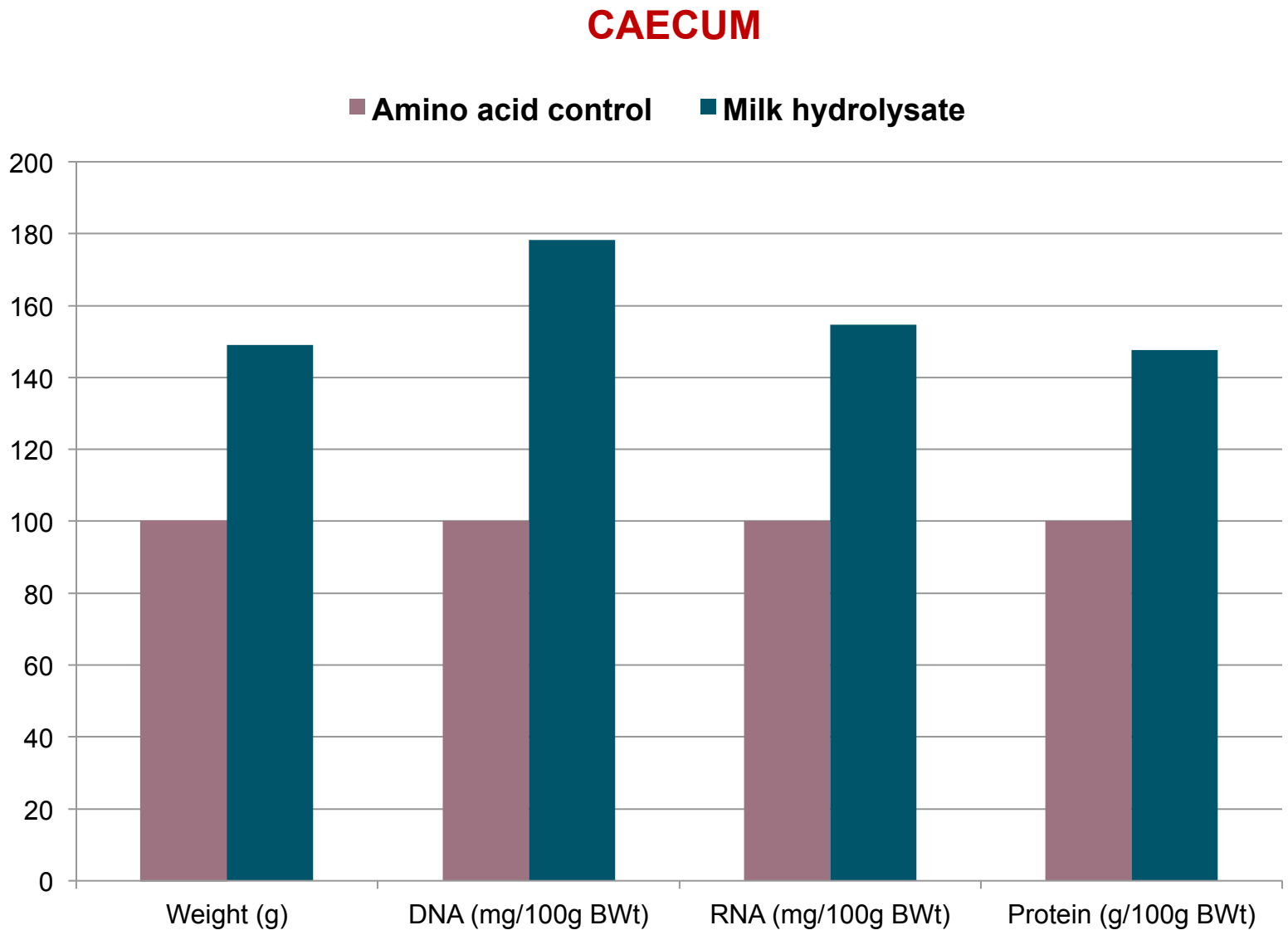

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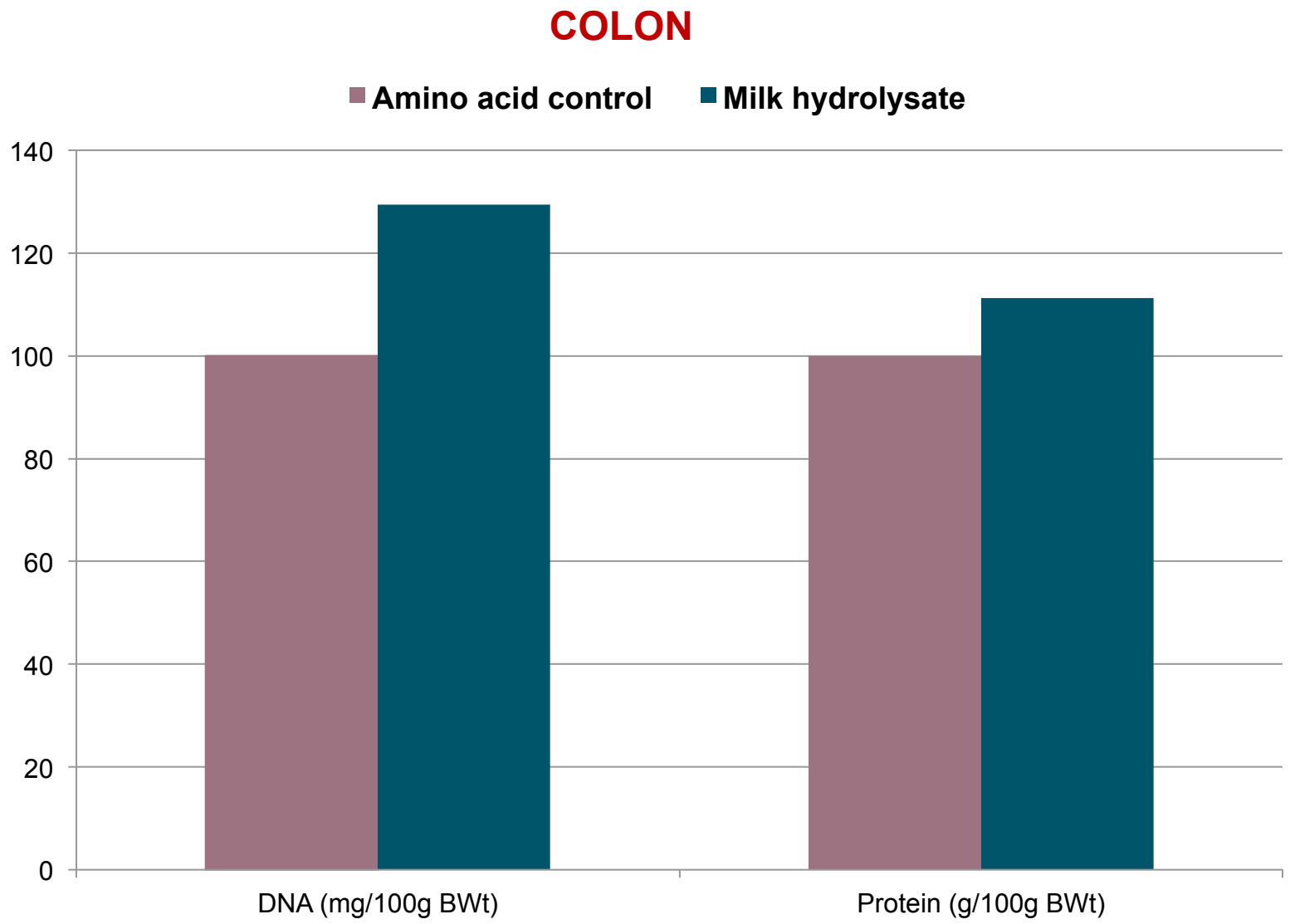
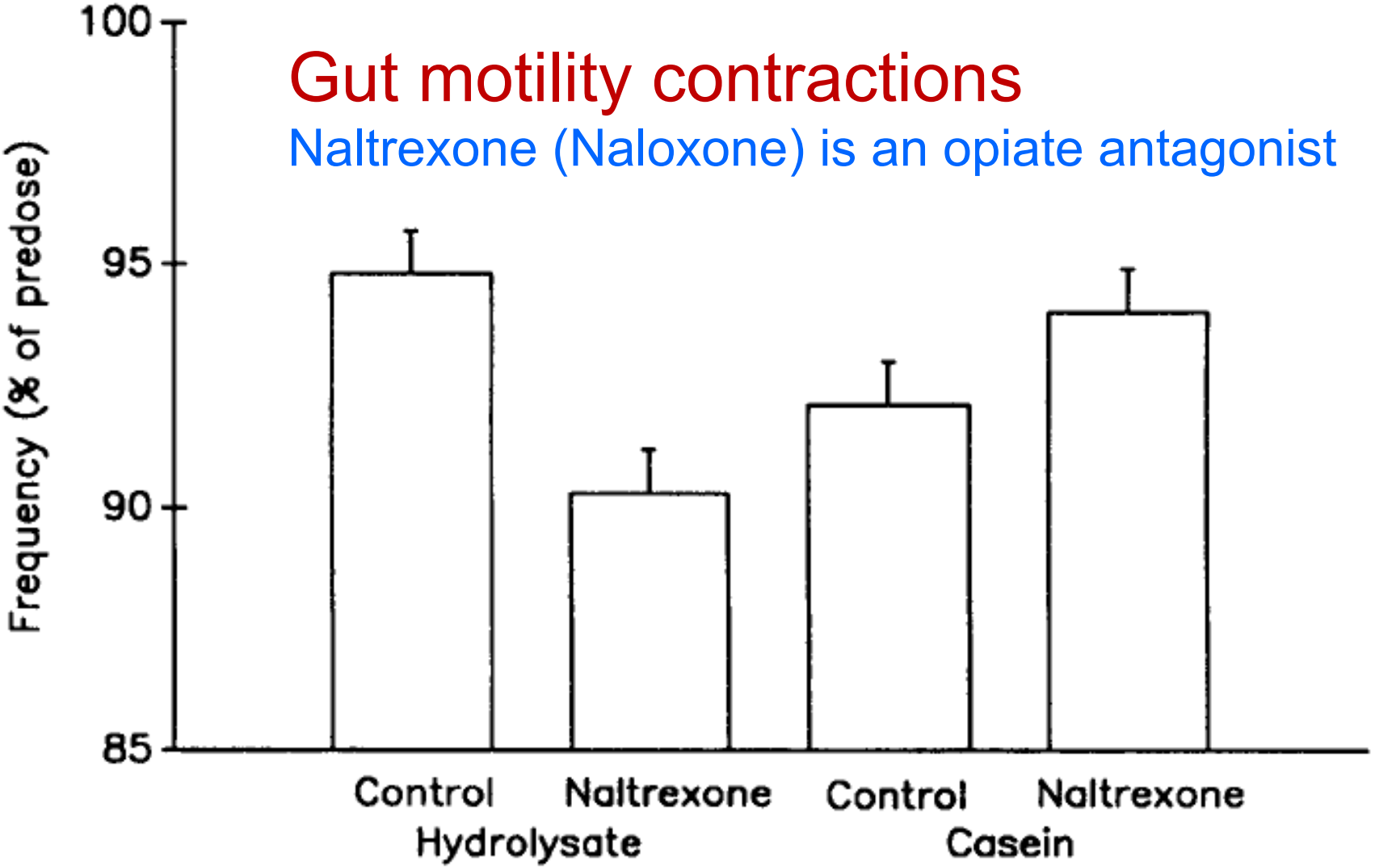


Bioactive peptides from fish

Froetschel. Bioactive peptides in digesta that regulate gastrointestinal function and intake. J.Anim.Sci. 74 (10):2500-2508, 1996.

Gut motility contractions

Naltrexone (Naloxone) is an opiate antagonist



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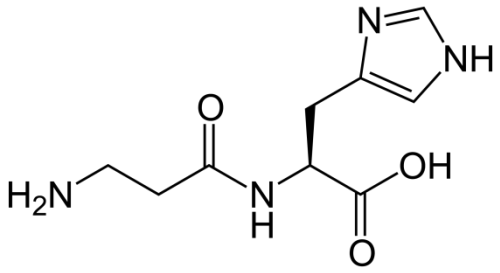

Dipeptide - β-alanine and histidine

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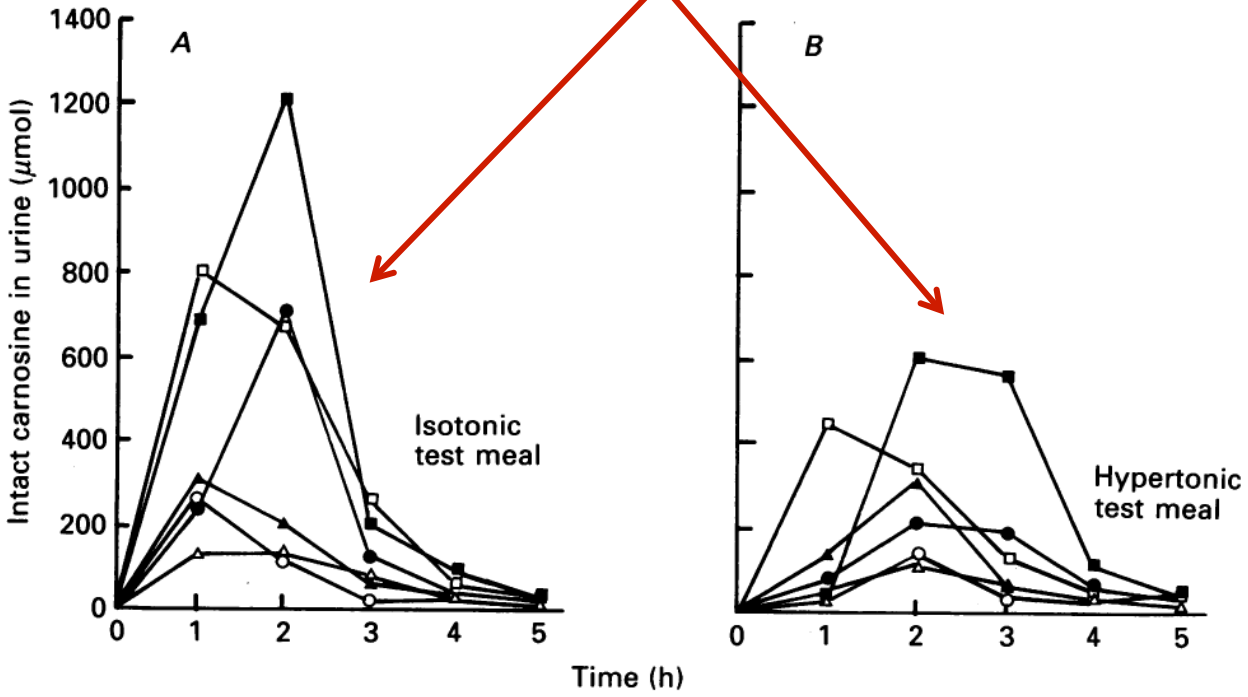
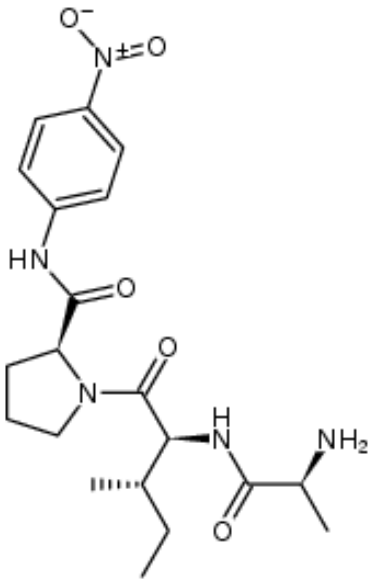


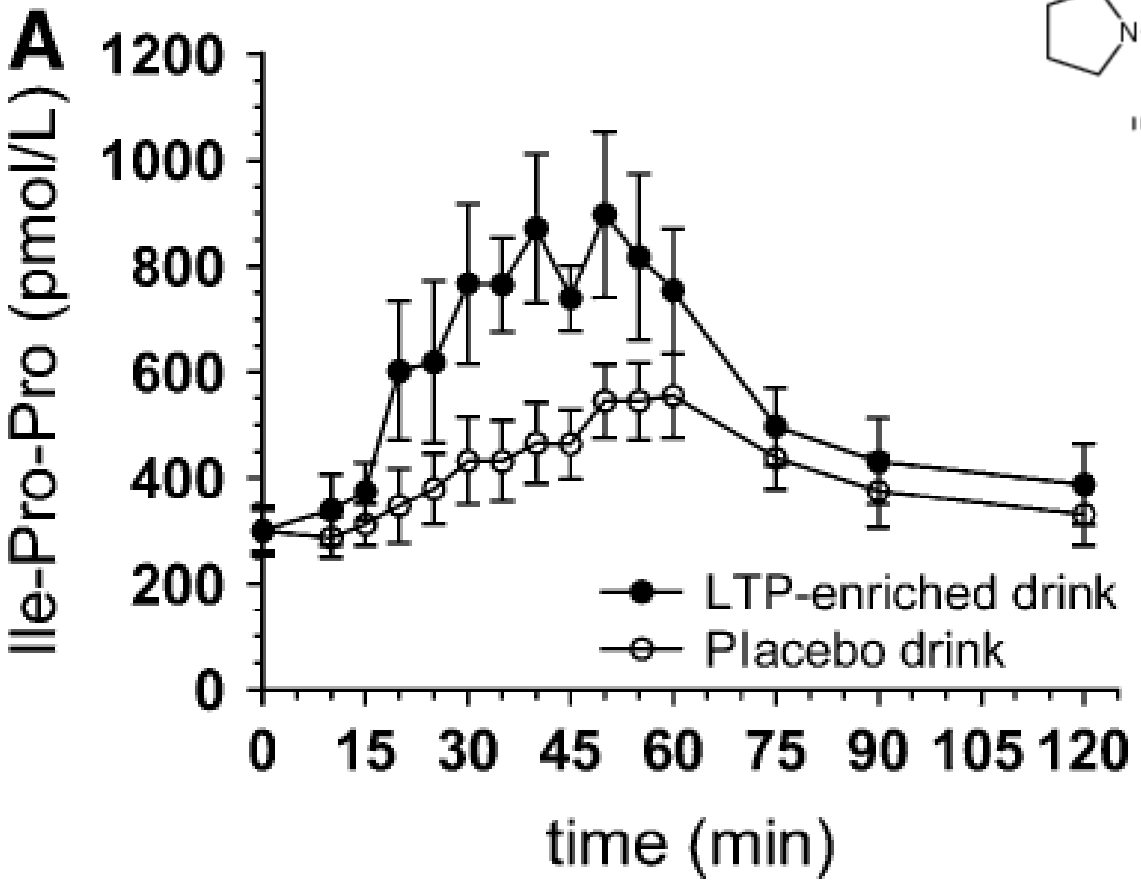
Fig. 1. Hourly urinary output of intact carnosine following ingestion of 4 g carnosine with an isotonic (*A*) or a hypertonic (*B*) test meal. Each symbol represents a different subject.

Evidence that small bioactive peptides get through

Foltz et al. *J.Nutr. 137 (4):953-958, 2007.*

Ile-Pro-Pro – tripeptide from partially hydrolysed casein.
Very resistant to peptidases (even ACE, dipeptidyl carboxypeptidase I)
Angiotensin Converting Enzyme inhibitor
Yogurt enrich with this material





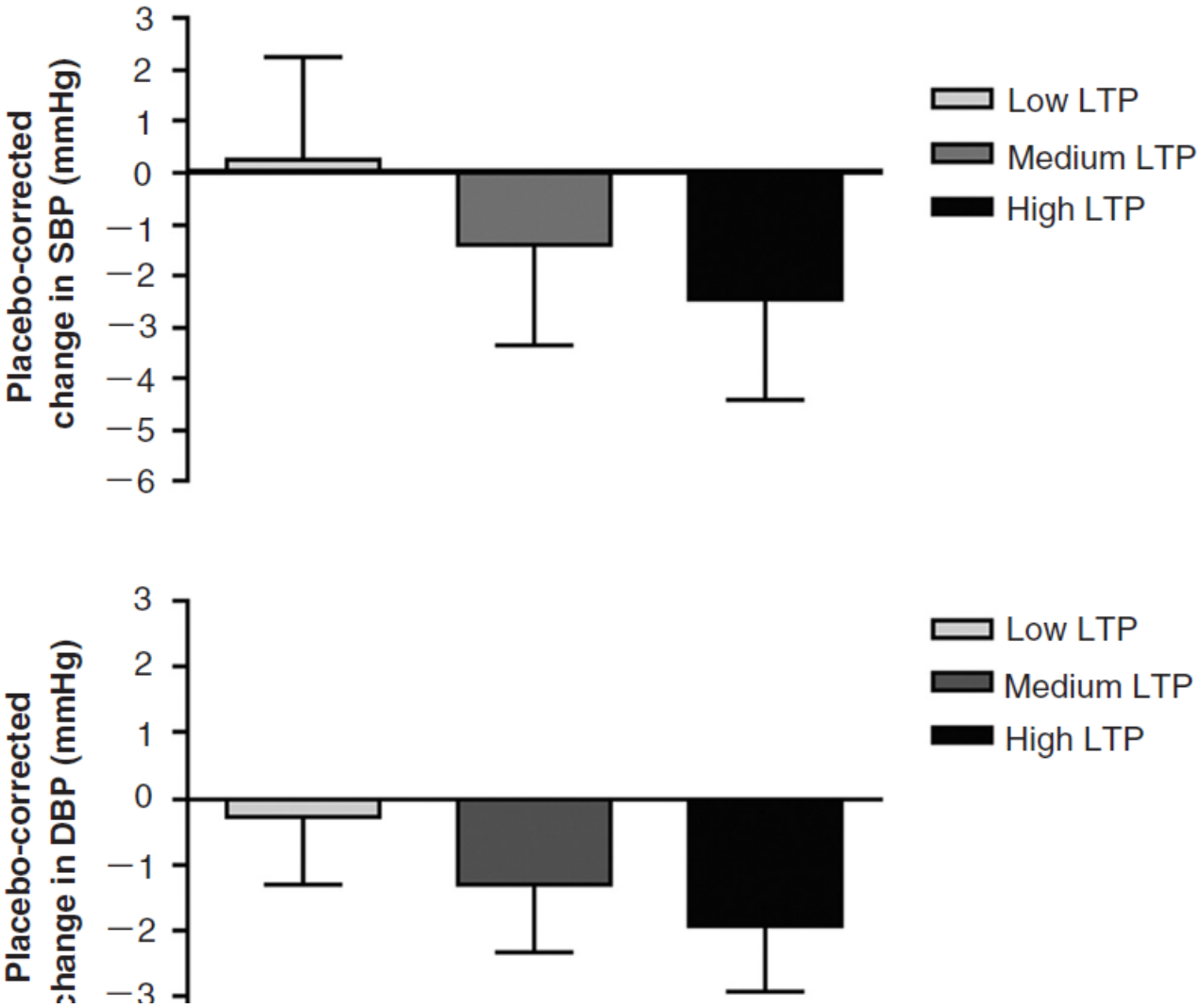
Evidence that small bioactive peptides get through?

M. Foltz, P. C. van der Pijl, and G. S. Duchateau. Current in vitro testing of bioactive peptides **is not valuable**. *J.Nutr. 140 (1):117-118, 2010.*

"At the moment, there is no scientific evidence that other small peptides, originating from dietary sources, have substantially improved absorption or plasma clearance profiles, which could result in an acceptable bioavailability or even very transiently high, free plasma concentrations."

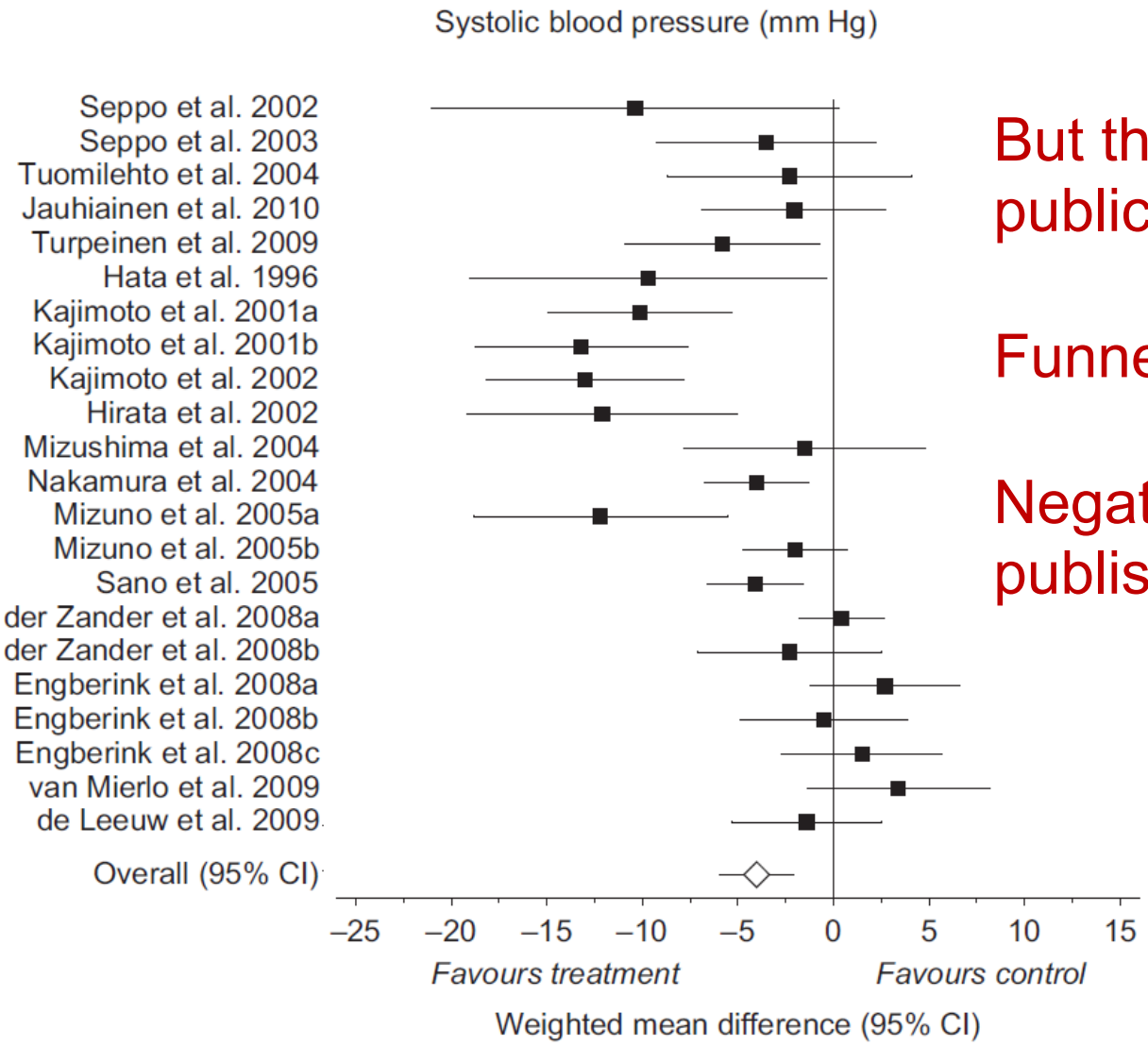
Clinical evidence for effectiveness

de Leeuw et al. Dose-dependent lowering of blood pressure by dairy peptides in mildly hypertensive subjects. *Blood Press 18 (1-2): 44-50, 2009.*



Summary of all studies

Turpeinen et al. Antihypertensive effects of bioactive tripeptides-a random effects meta-analysis. Ann.Med. 45 (1):51-56, 2013.



But there was evidence of publication bias

Funnel plot

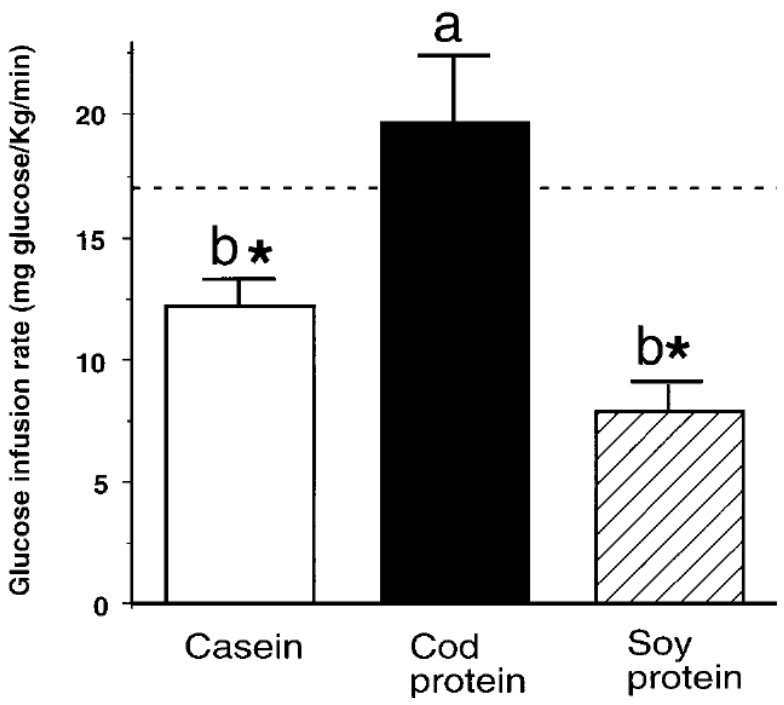
Negative studies not published

What about fish alone?

Lavigne et al. Am.J.Physiol.Endocrinol.Metab. 281 (1):E62-E71, 2001.

Insulin-resistant rats, high fat diet

This is not about fish oil



How much glucose rats can use

What about fish alone?

Lavigne et al. Am.J.Physiol.Endocrinol.Metab. 281 (1):E62-E71, 2001.

Table 1. *Dietary protein-derived amino acid mixtures used for L6 culture conditions*

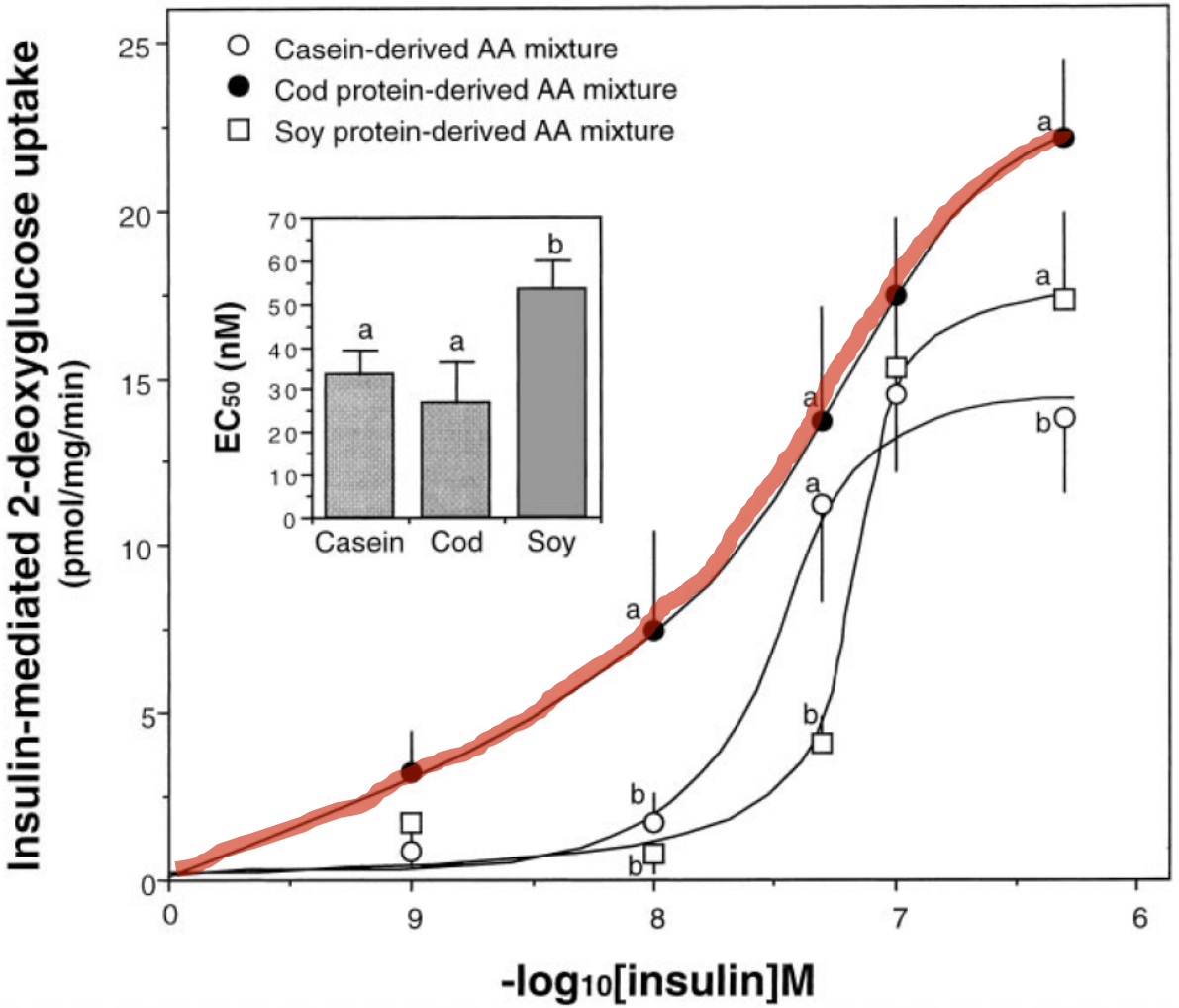
	Casein	Cod Protein	Soy Protein	Chow
L-Alanine	663	563	501	603
L-Arginine	150	194	176	137
L-Asparagine	129	111	121	108
L-Asparctic acid	26	21	20	19
L-Cysteine	23	29	24	15
L-Glutamic acid	110	114	211	104
L-Glutamine	1263	925	1027	1370
L-Glycine	215	255	272	382
L-Histidine	76	67	68	58
L-Isoleucine	115	90	98	89
L-Leucine	170	122	130	131
L-Lysine	470	441	386	373
L-Methionine	98	102	67	76
L-Phenylalanine	104	76	82	76
L-Proline	366	189	222	285
L-Serine	291	218	270	283
L-Threonine	366	261	277	386
L-Tyrosine	112	66	85	91
L-Valine	230	163	166	176

Amino acid mixtures are expressed in μM.

What about fish alone?

Lavigne et al. Am.J.Physiol.Endocrinol.Metab. 281 (1):E62-E71, 2001.

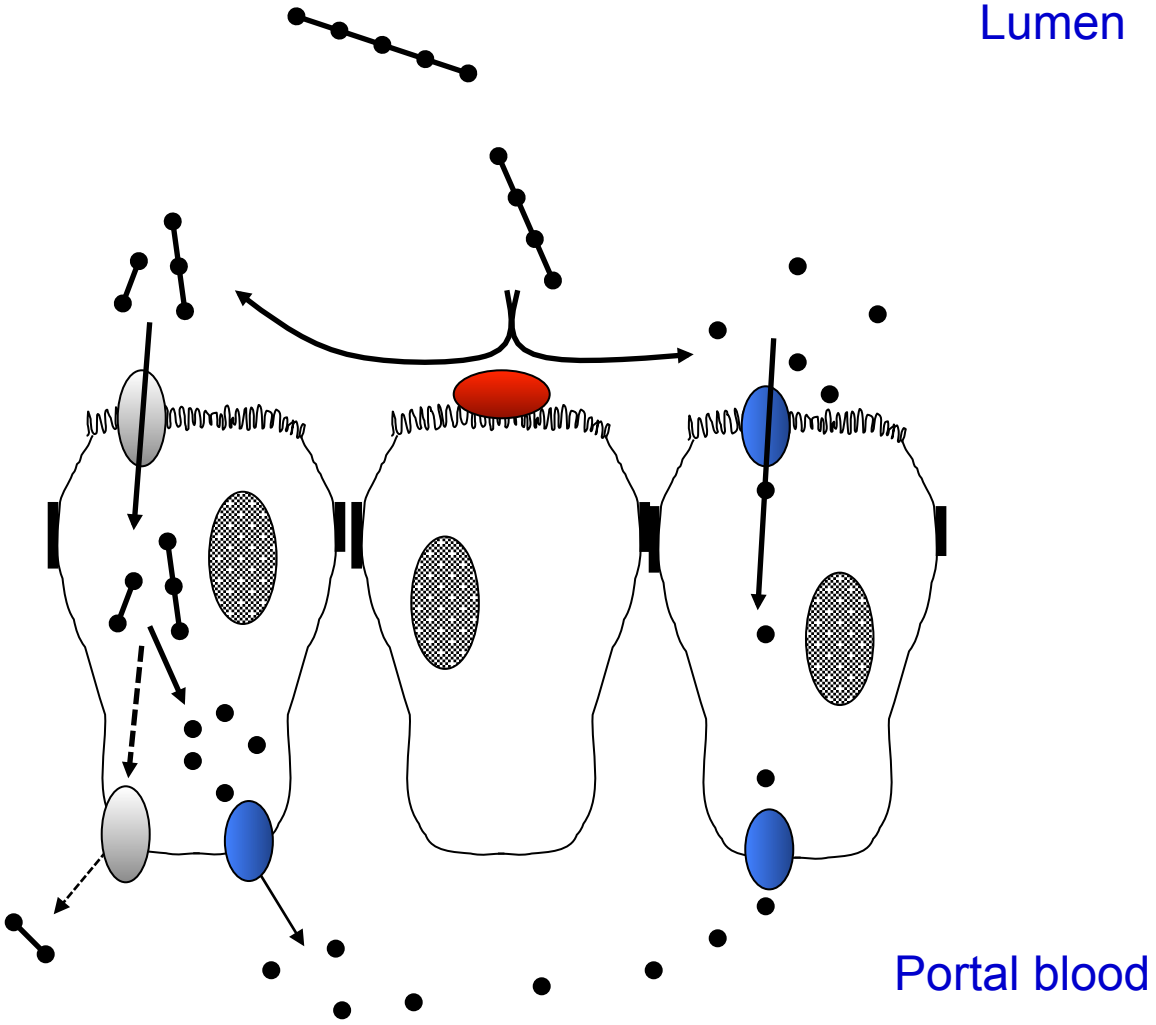
Incubated muscle cells
2-deoxyglucose is a non-metabolised glucose analogue



Conclusions

- Fish protein is good!
- It may contain bioactive peptides
- More solid research is needed
- Protein hydrolysis technology is the great leveller
- Development of new functional protein ingredients
- Environmentally friendly

Thank you for your attention


Lumen
Portal blood

What is a protein hydrolysate?

A partial hydrolysate of food protein

A huge range of properties

Amino acid composition

Chain-length profile

Sequences

Taste

Solubility

Surfactant

	Hydrolysing agent	Process
Old-fashioned stock cubes	Acids	lead tank, sulphuric acid, meat
Modern stock cubes	Enzymes	Fermenter, meat, specific enzymes to both hydrolyse and produce good flavour with no “bitter” taste