

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

- 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.

- 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?

Sustainability issues

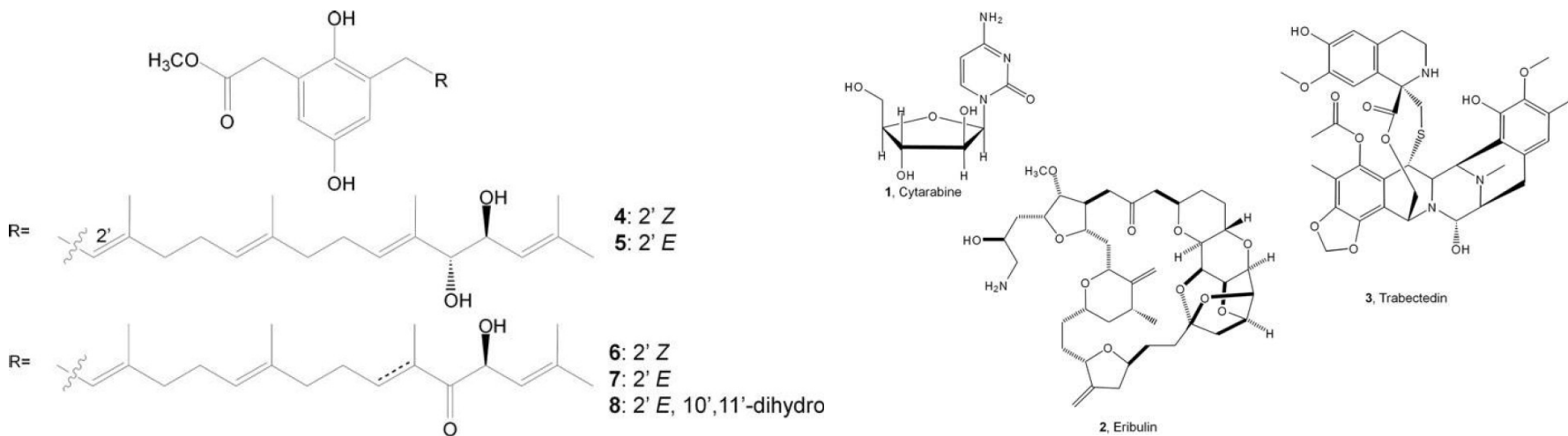
Little impact on health	Waste less Shop, cook and store efficiently
Synergy with health	Eat less food with limited nutritional value
Antagonistic with health	<u>Eat less fish</u> Only choose seasonal, locally produced F&V
BIG issue in synergy with health	Eat less meat and dairy products

More efficient use of marine harvests



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.



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”

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

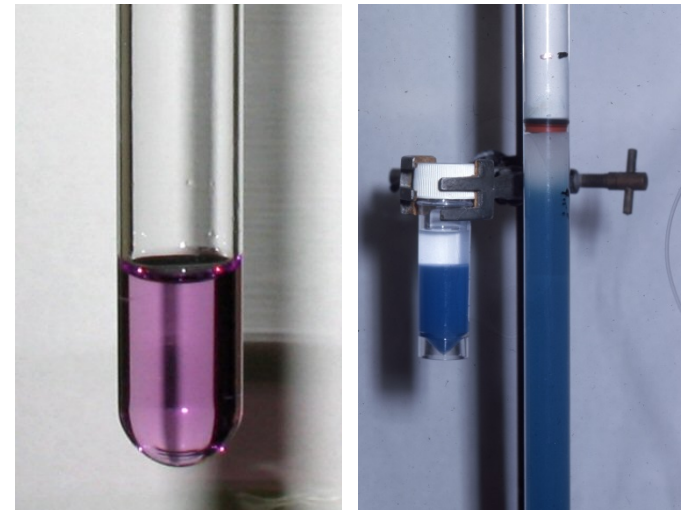
Sequences – some may be bioactive

Taste – “umami” versus “bitter peptides”!

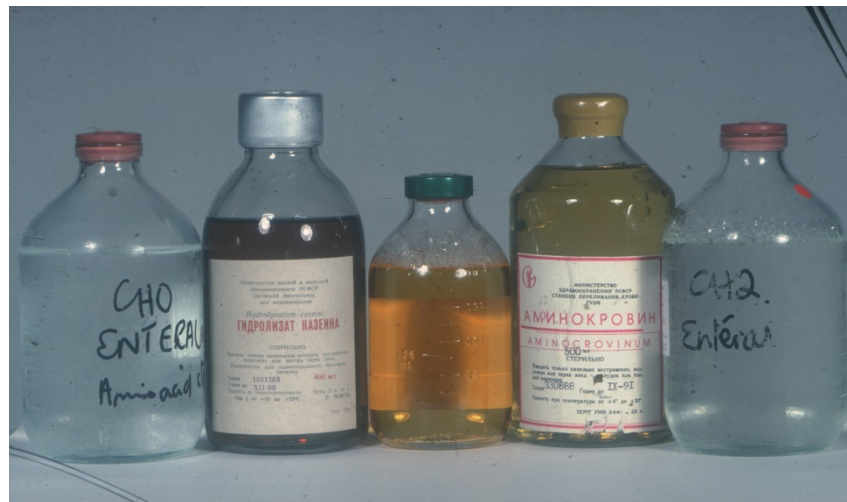
Solubility – more soluble than the parent protein

Surfactant – shampoos

Antioxidants – great chelators

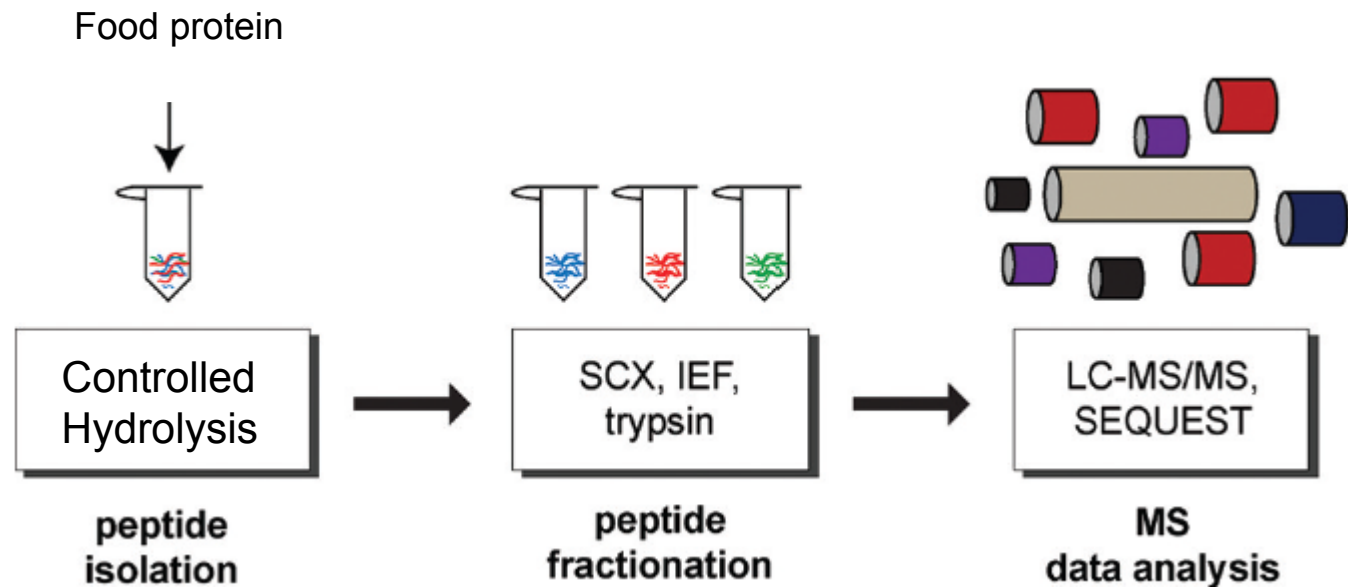
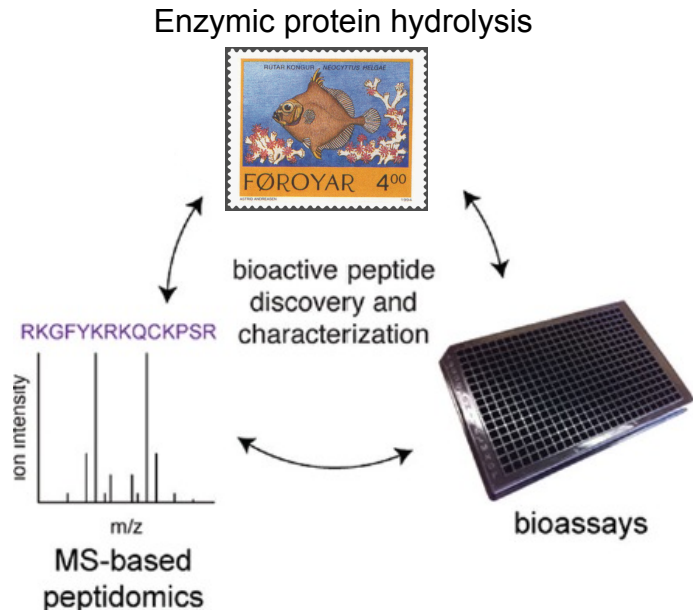


- For oral/tube feeding the patient with impaired intestinal absorption
- For intravenous feeding of patients with intestinal failure, as a substitute for intravenous amino acids
- As a source of bioactive peptides

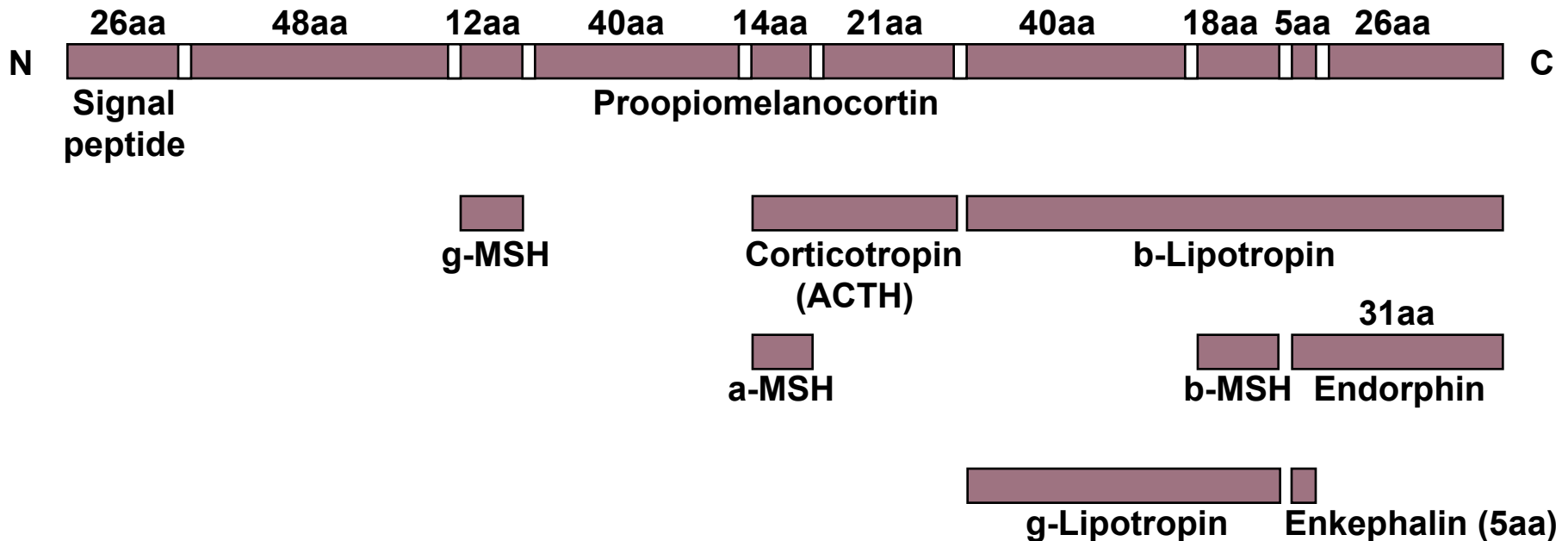


Finding bioactive peptides

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

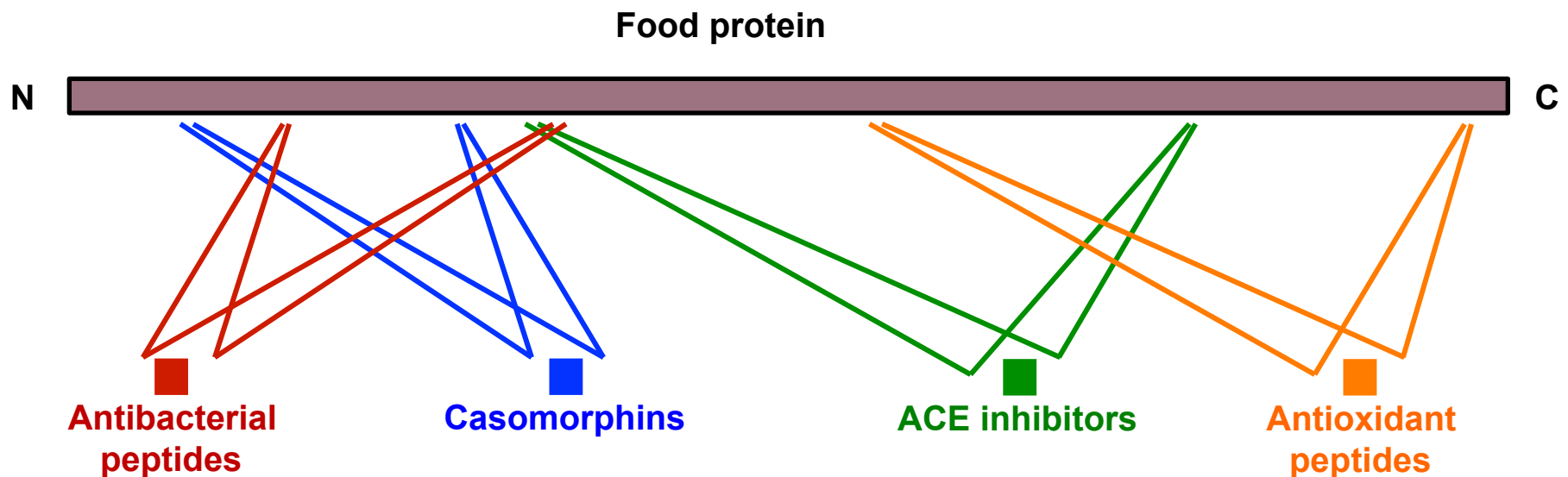


Multiple functional polypeptides from a single precursor
Processed in a cell-specific manner
Purposeful



Important in energy homeostasis

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.

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

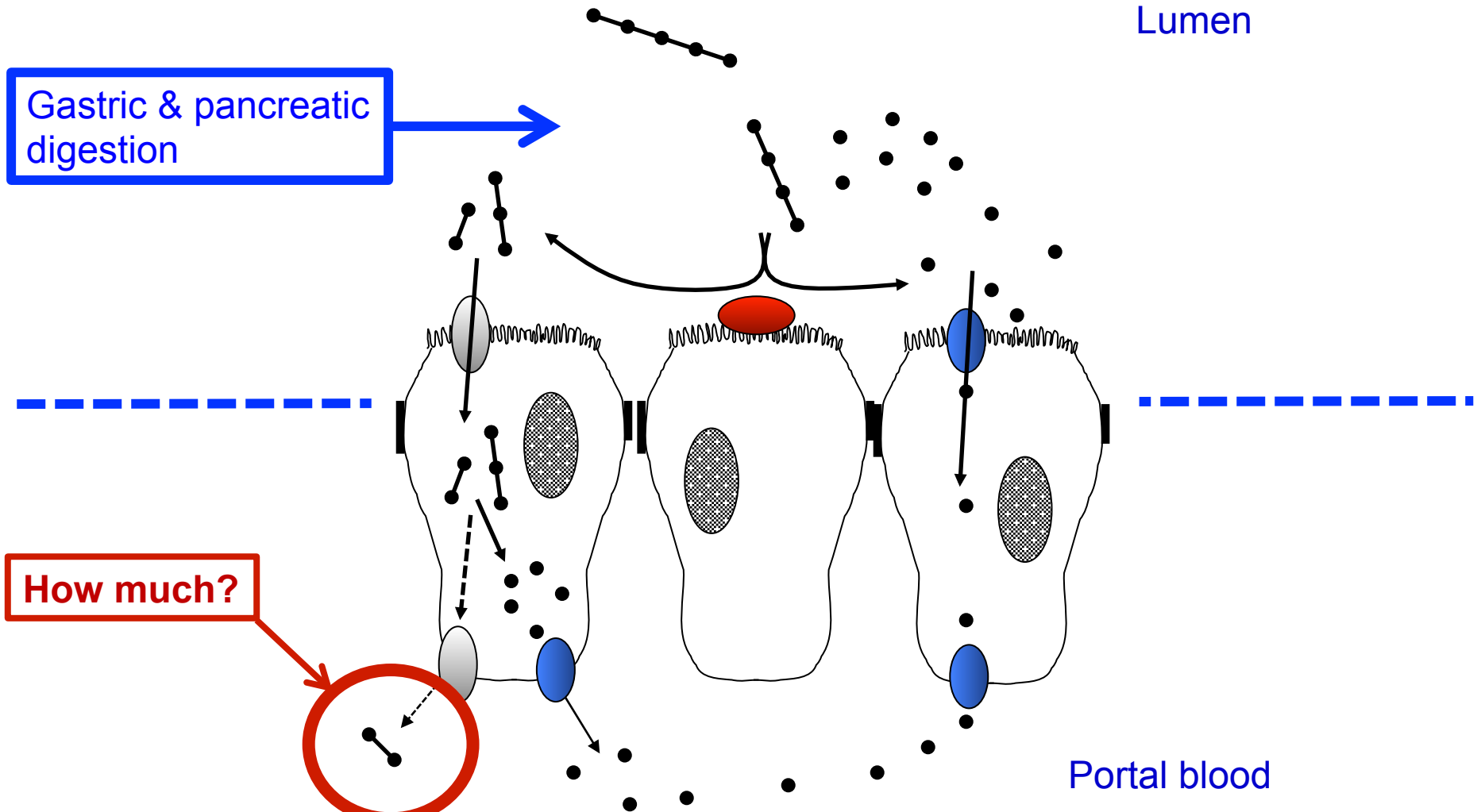
Macroalgae (phycobiliproteins), Microalgae, Fish muscle, Fish albumins, Mollusks, Crustaceans




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



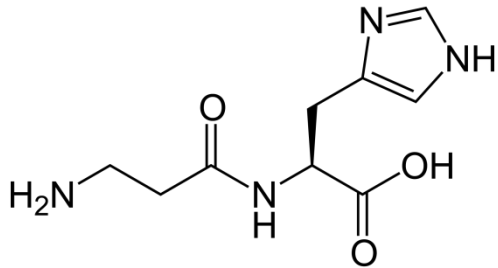
Are these peptides bioactive, in humans eating food?



-  Brush-border peptidase
-  PEPT1
-  Amino acid transporter

Some dipeptides get through

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



Dipeptide - β -alanine and histidine

QED. It was absorbed intact

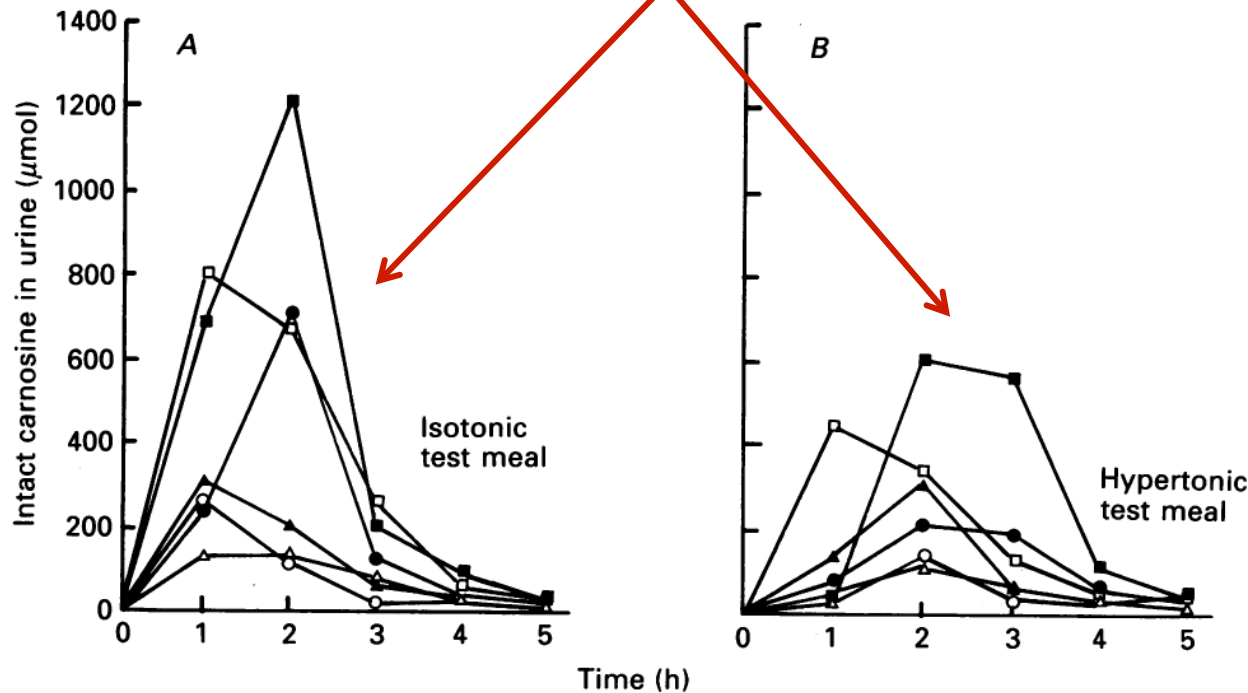
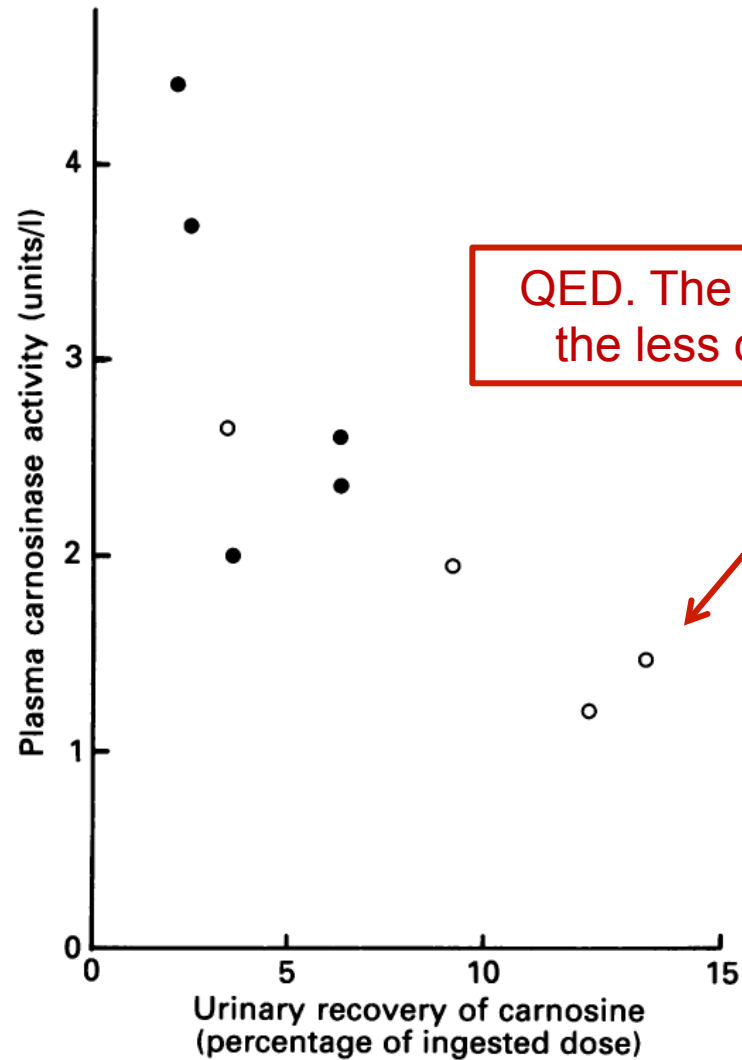


Fig. 1. Hourly urinary output of intact carnosine following ingestion of 4 g carnosine with an isotonic (A) or a hypertonic (B) test meal. Each symbol represents a different subject.

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



There are two pathways of protein breakdown

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

Endocytosis and phagocytosis

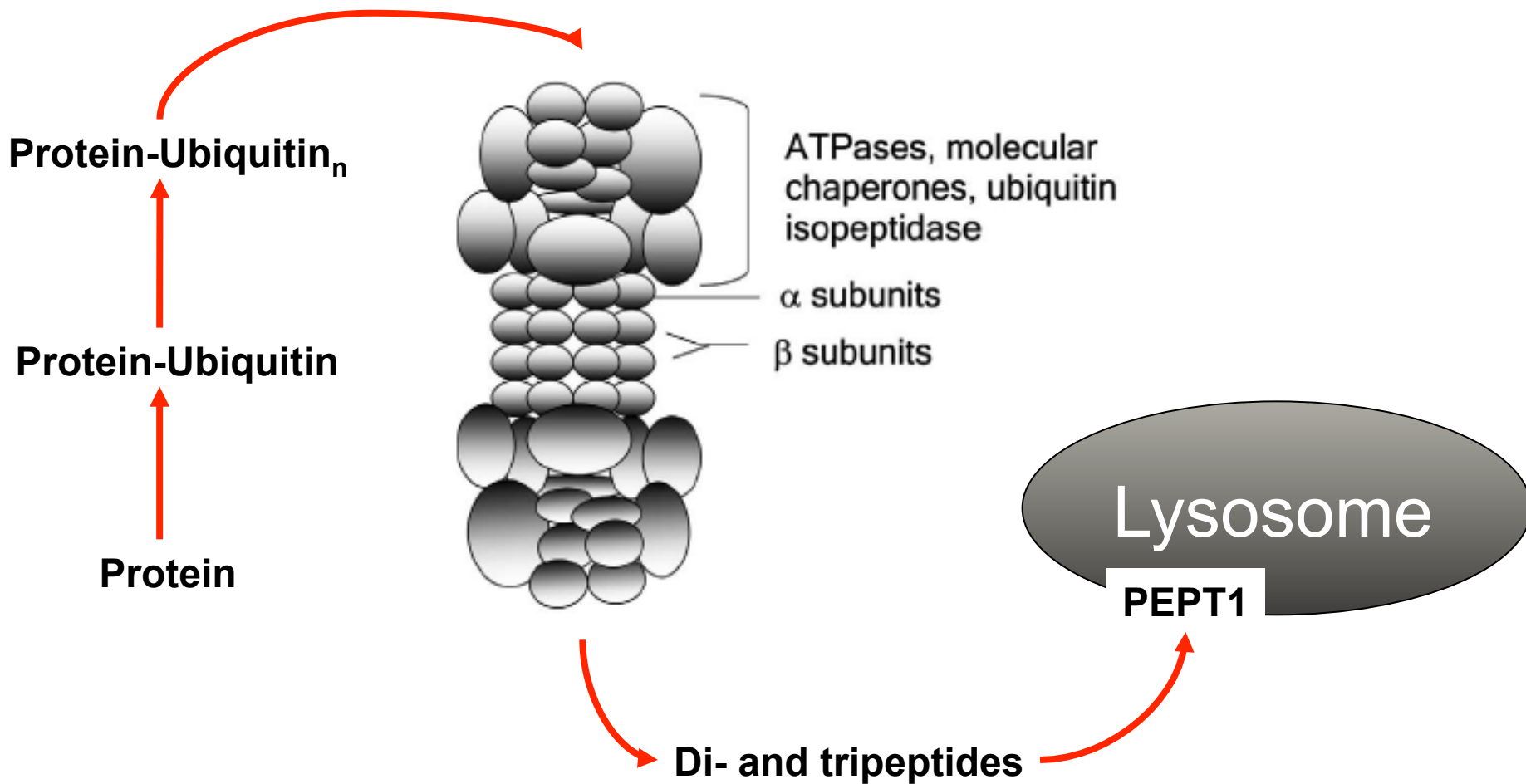
Not uncontrolled

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.

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

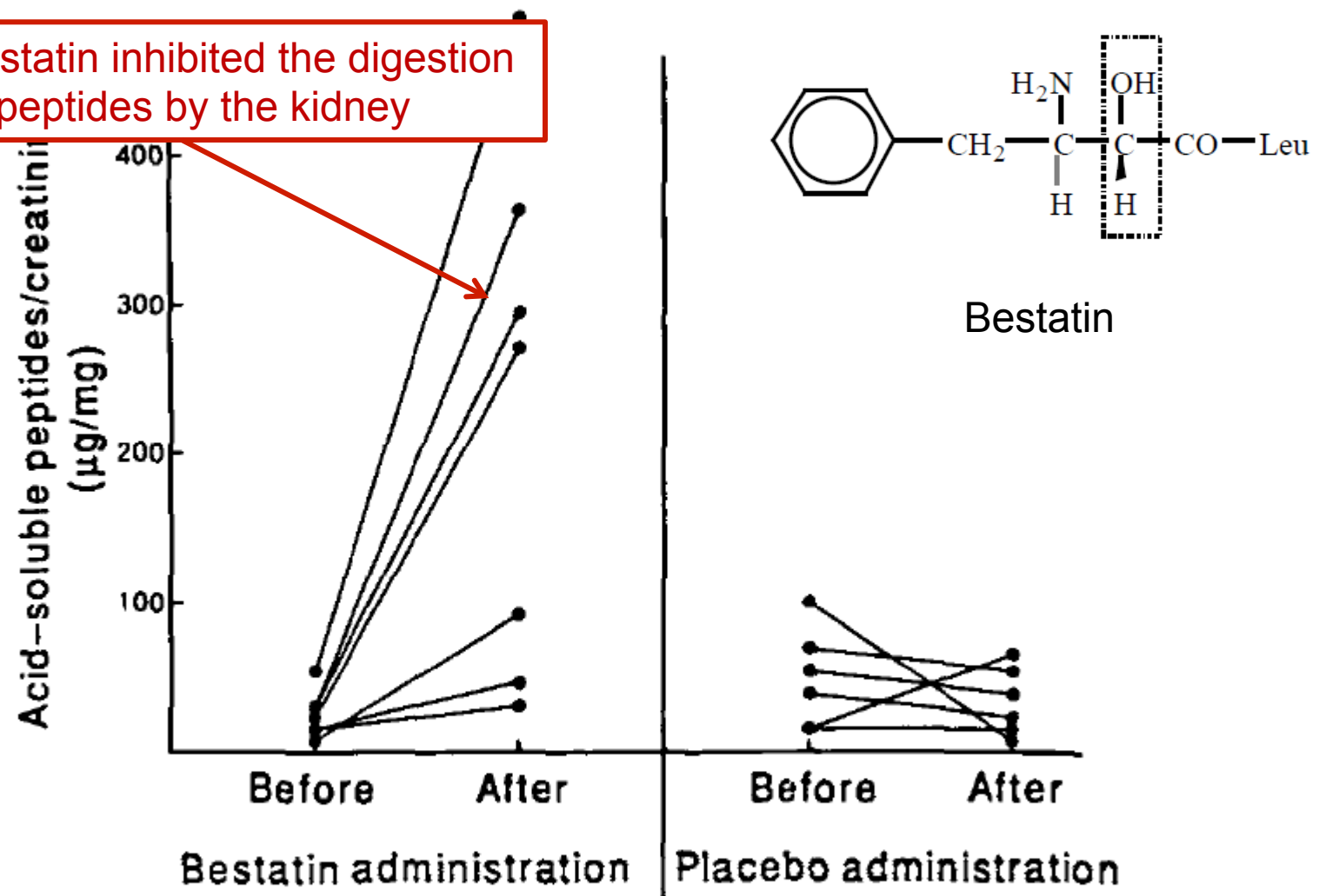
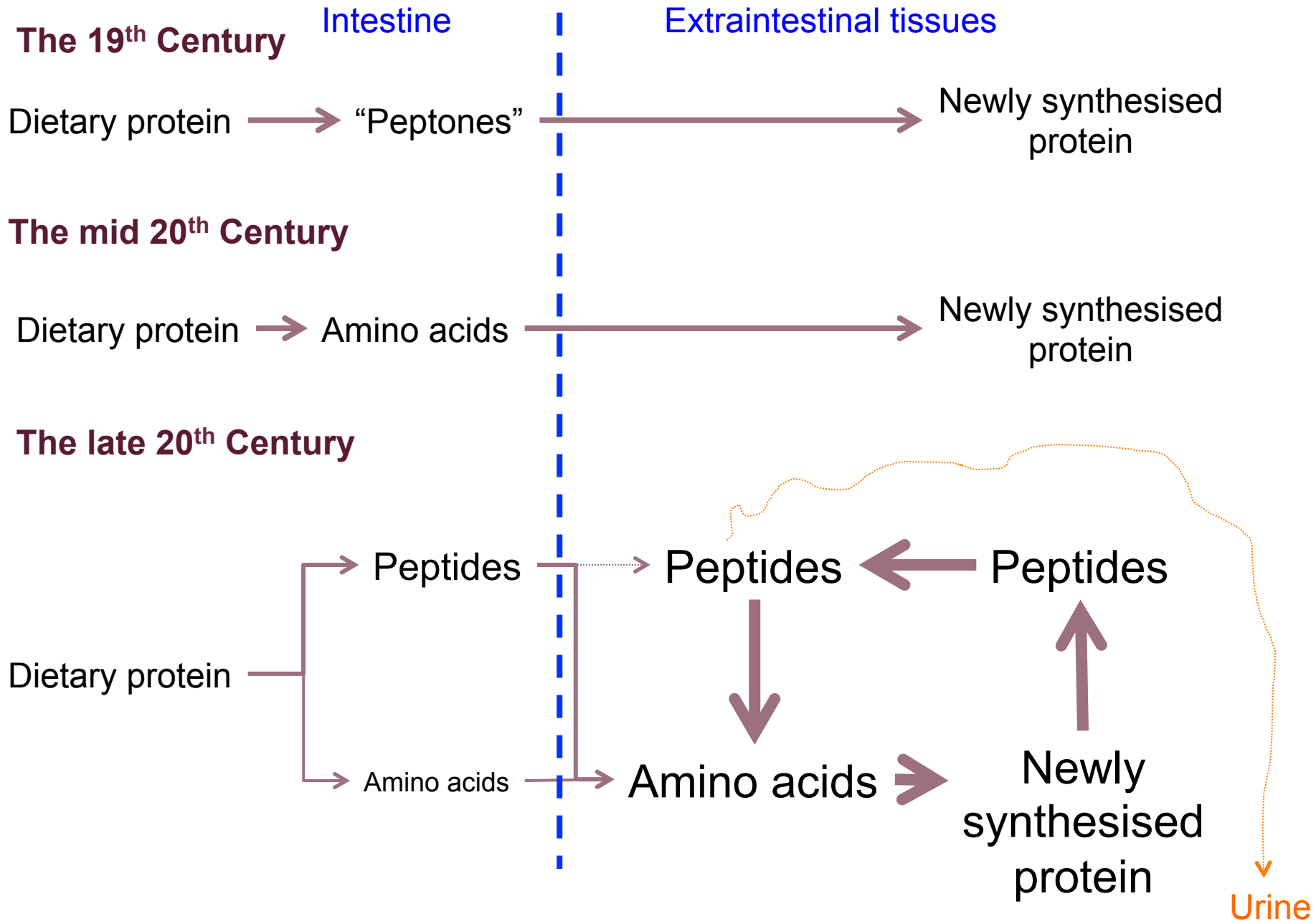
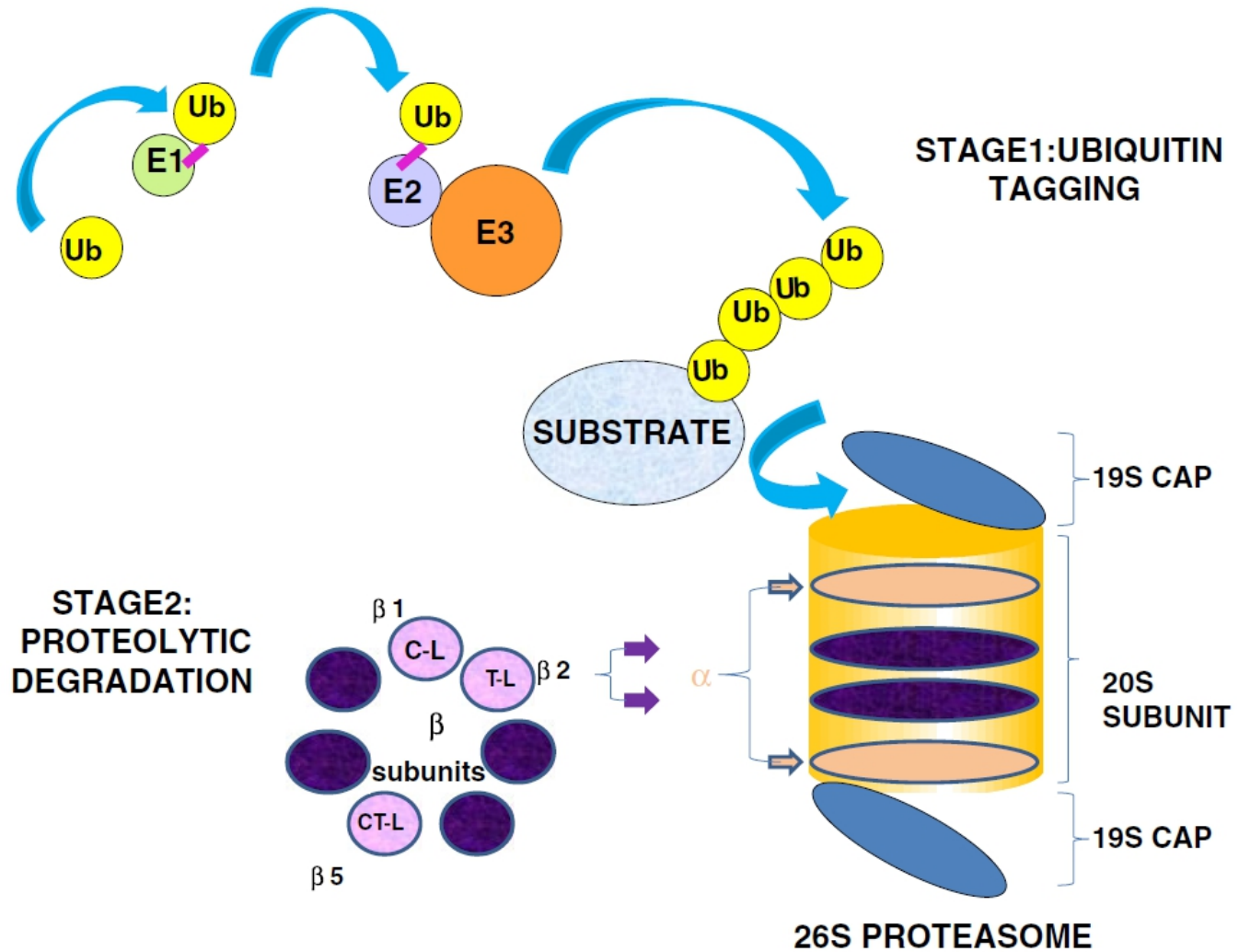


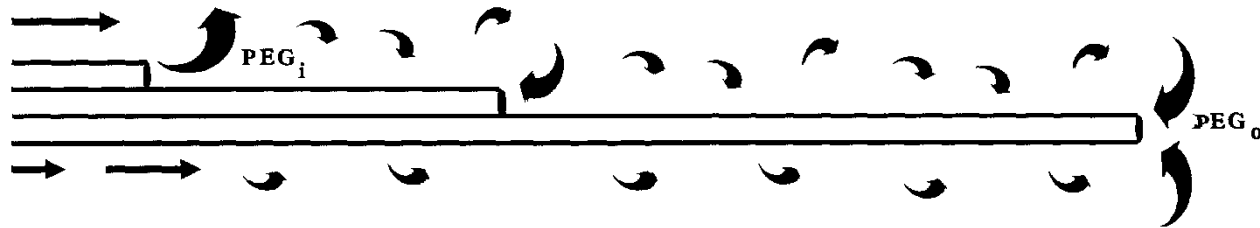
Fig. 4 Change in urinary acid-soluble peptide (ASP) ratio after oral administration of bestatin for 9 months.

Changing thoughts on peptides

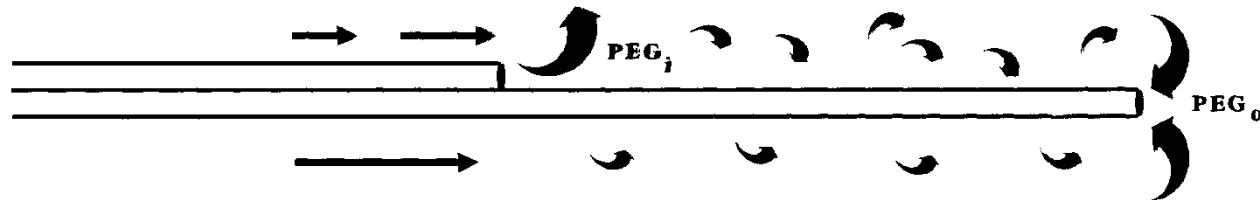




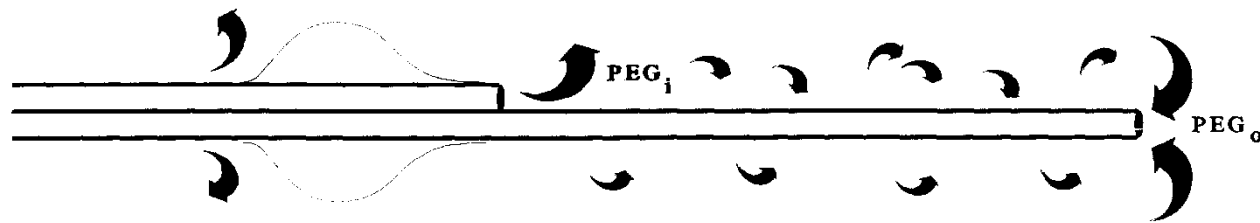
Triple-lumen Tube

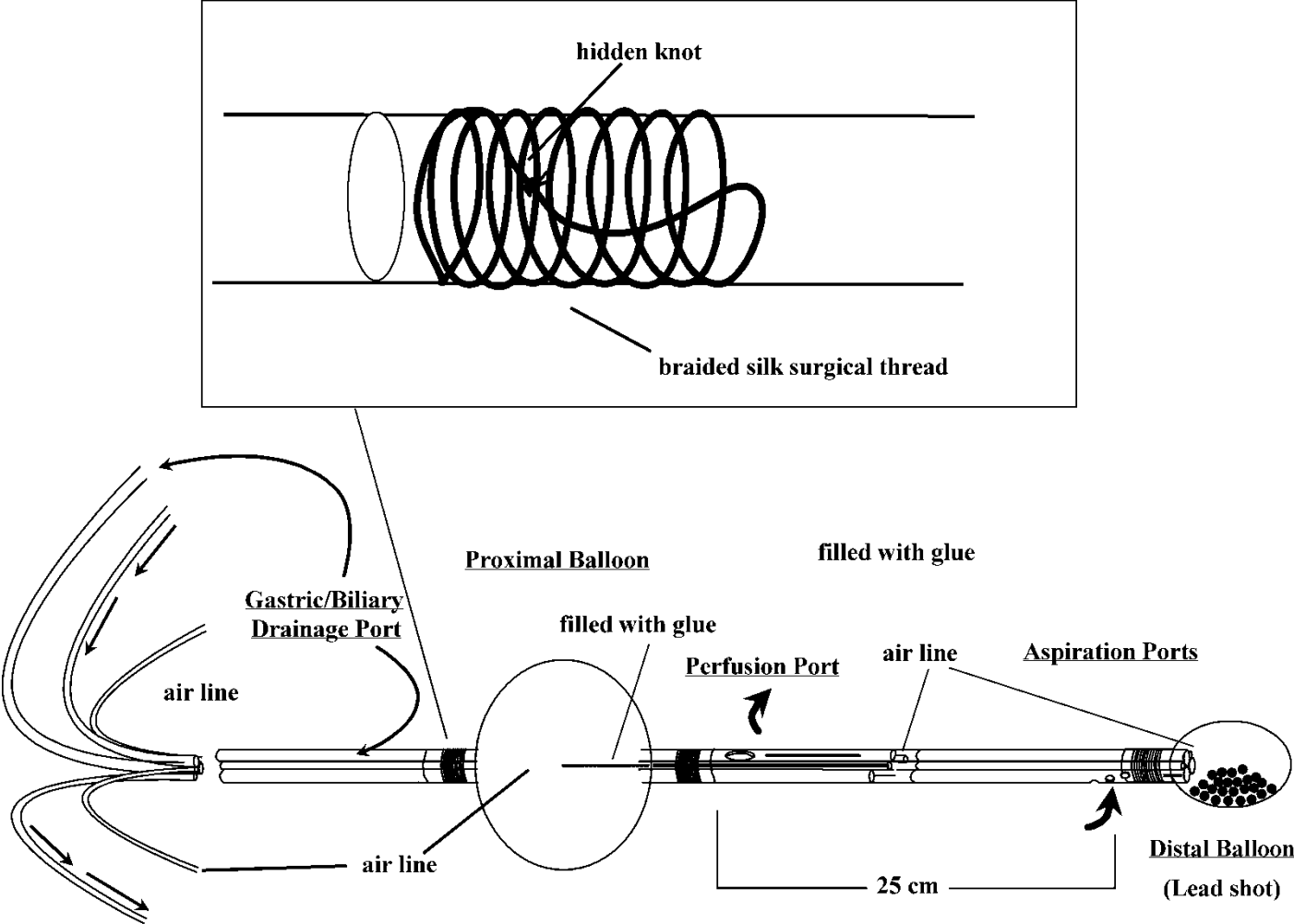


Double-lumen Tube



Double-lumen Tube (Occlusive Balloon)

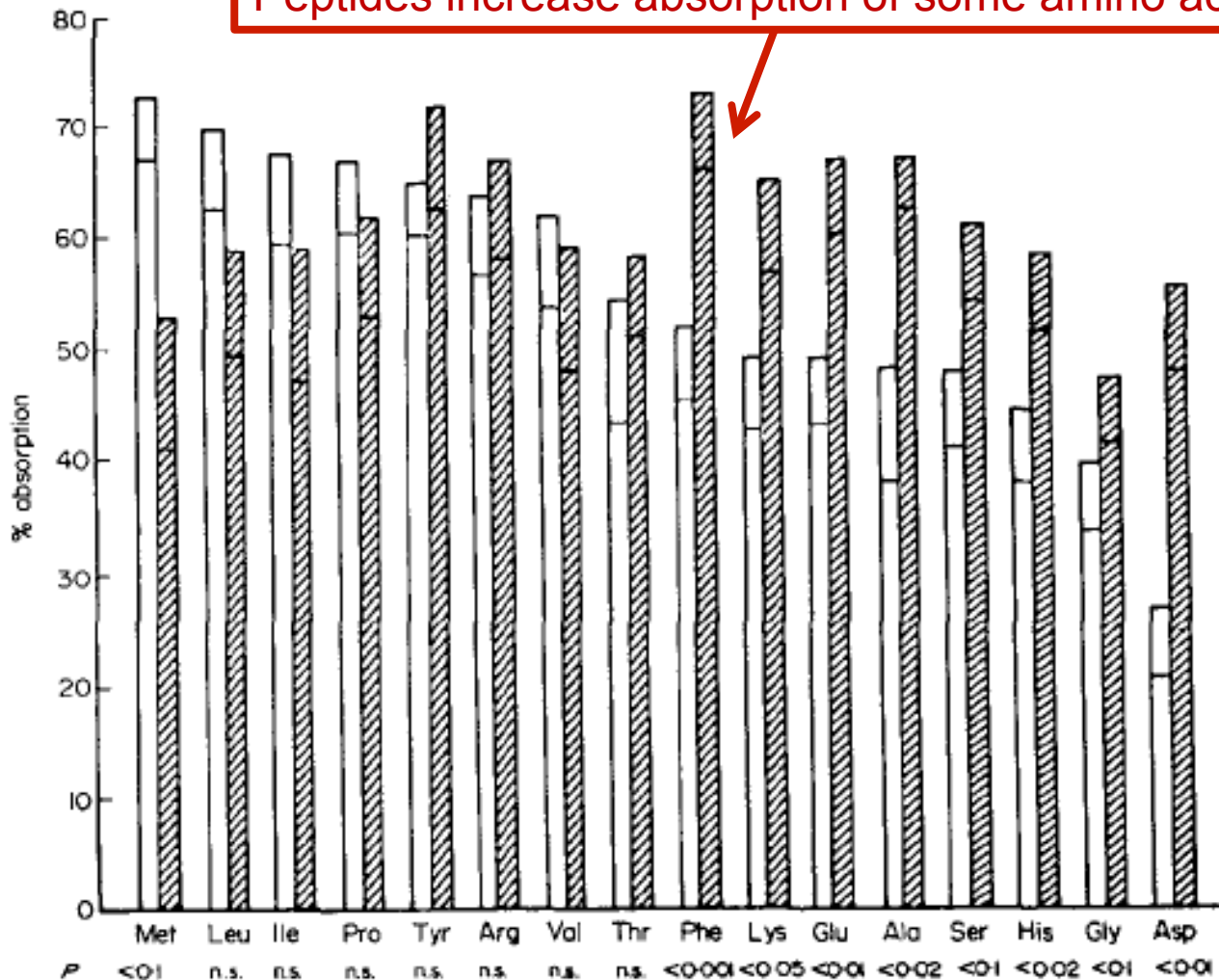


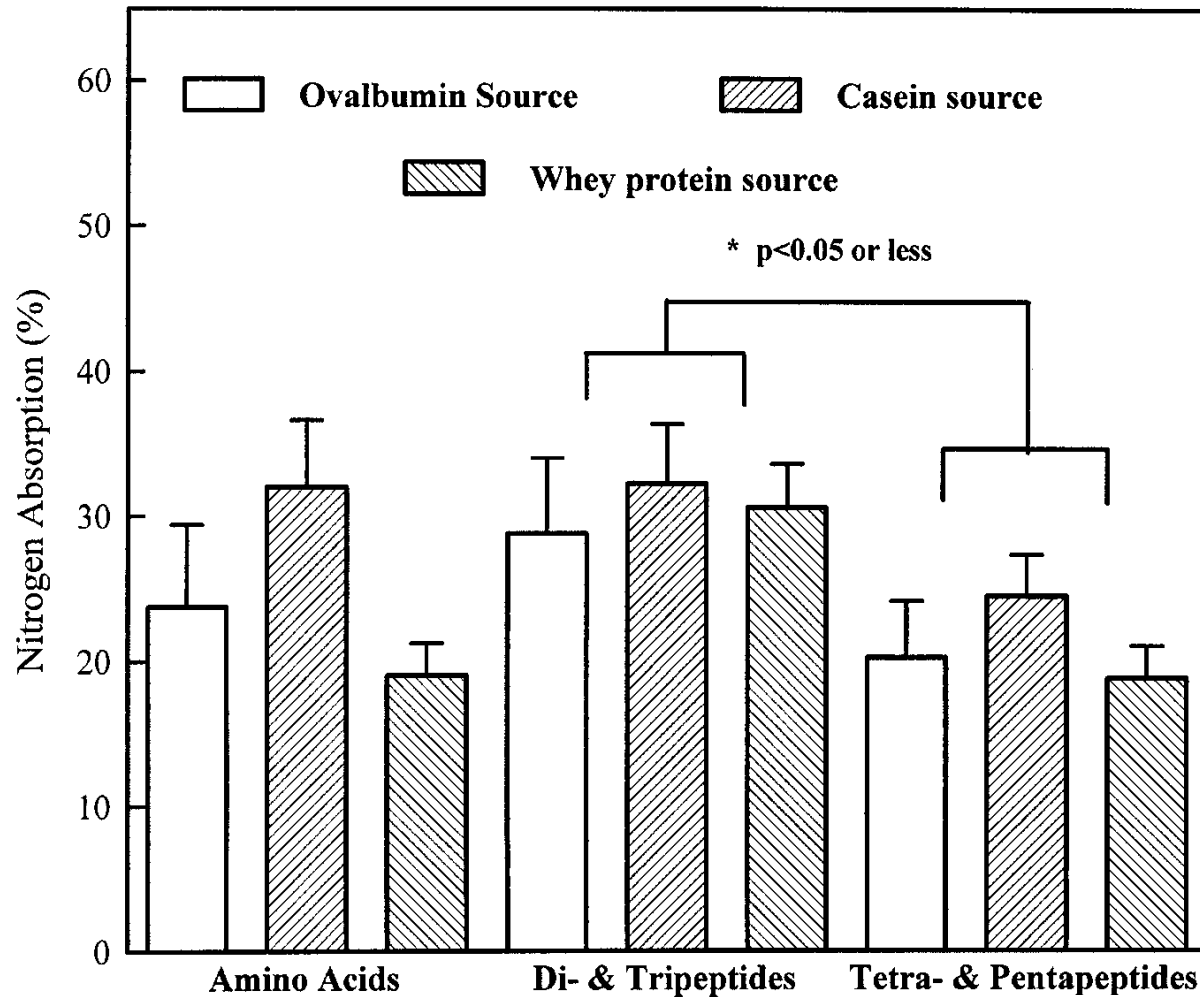


Kinetic advantage of peptides

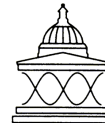
Silk et al. *J.Parent.Ent.Nutr.* 4 (6):548-553, 1980

Peptides increase absorption of some amino acids





Grimble et al 1987, 1988, 1994

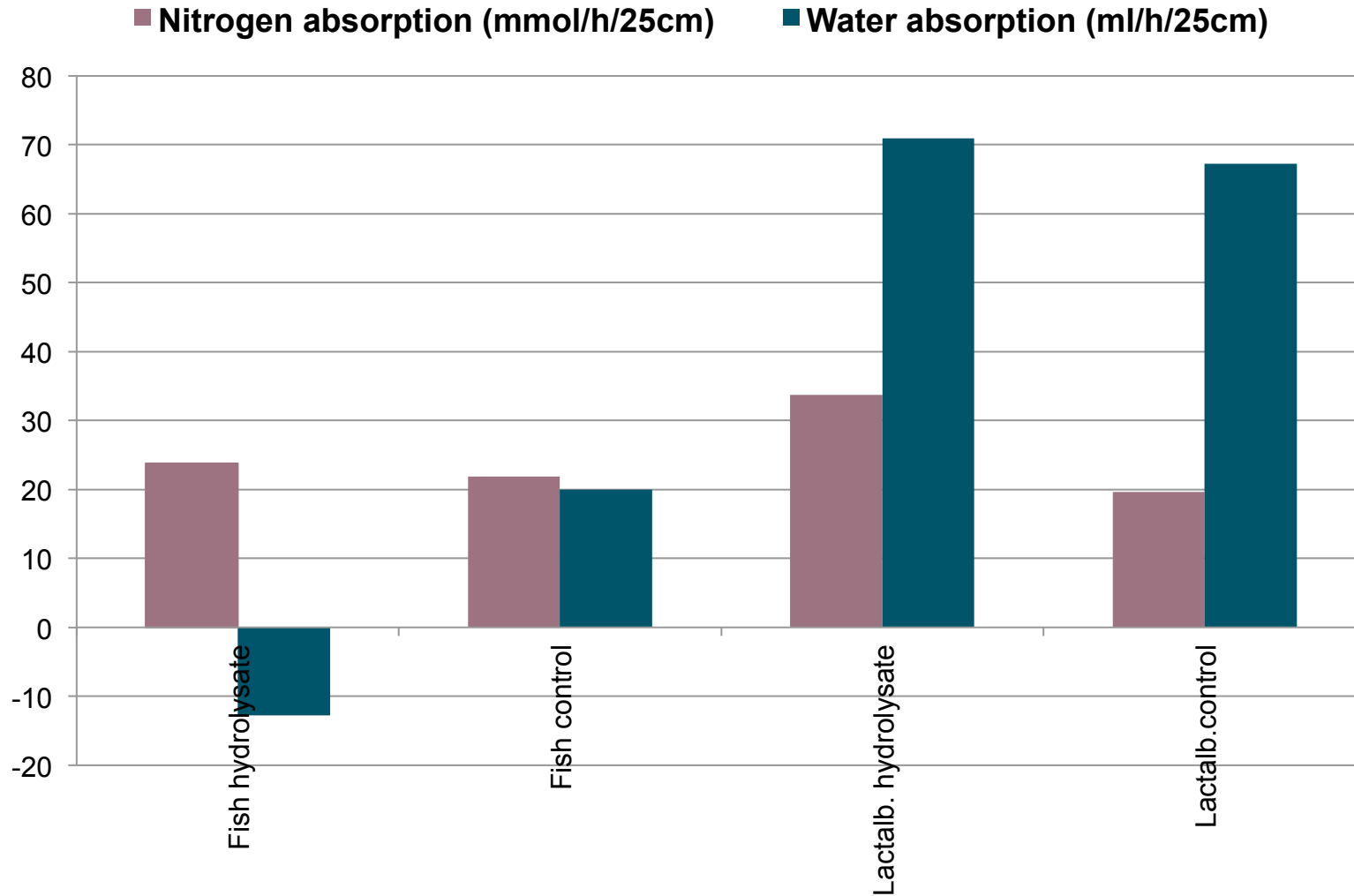


Helix Biotechnology

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

Something “fishy” going on!

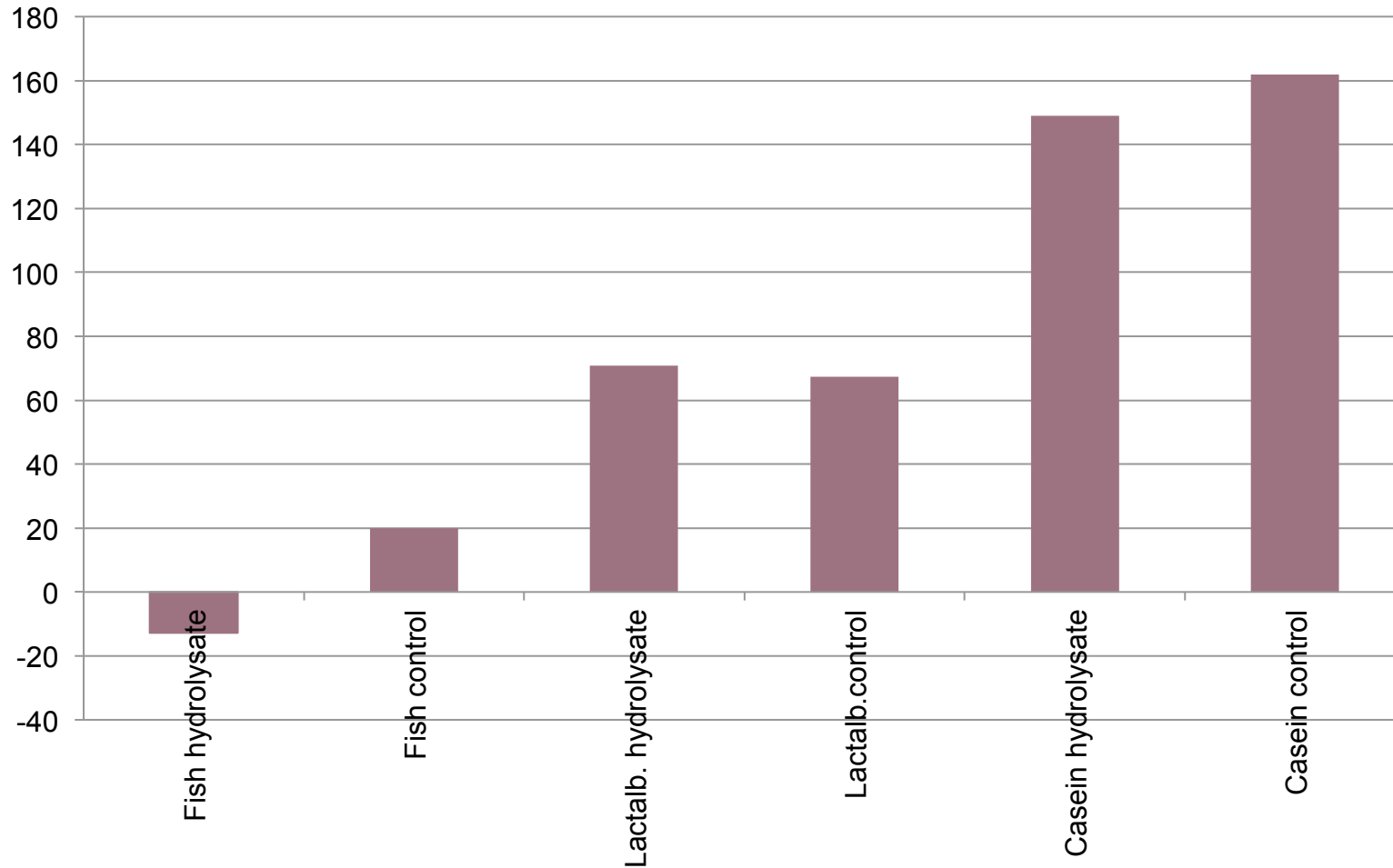
Fairclough et al. Comparison of the absorption of two protein hydrolysates and their effects on water and electrolyte movements in the human jejunum. Gut. 21:829-834, 1980.



Something “fishy” going on!

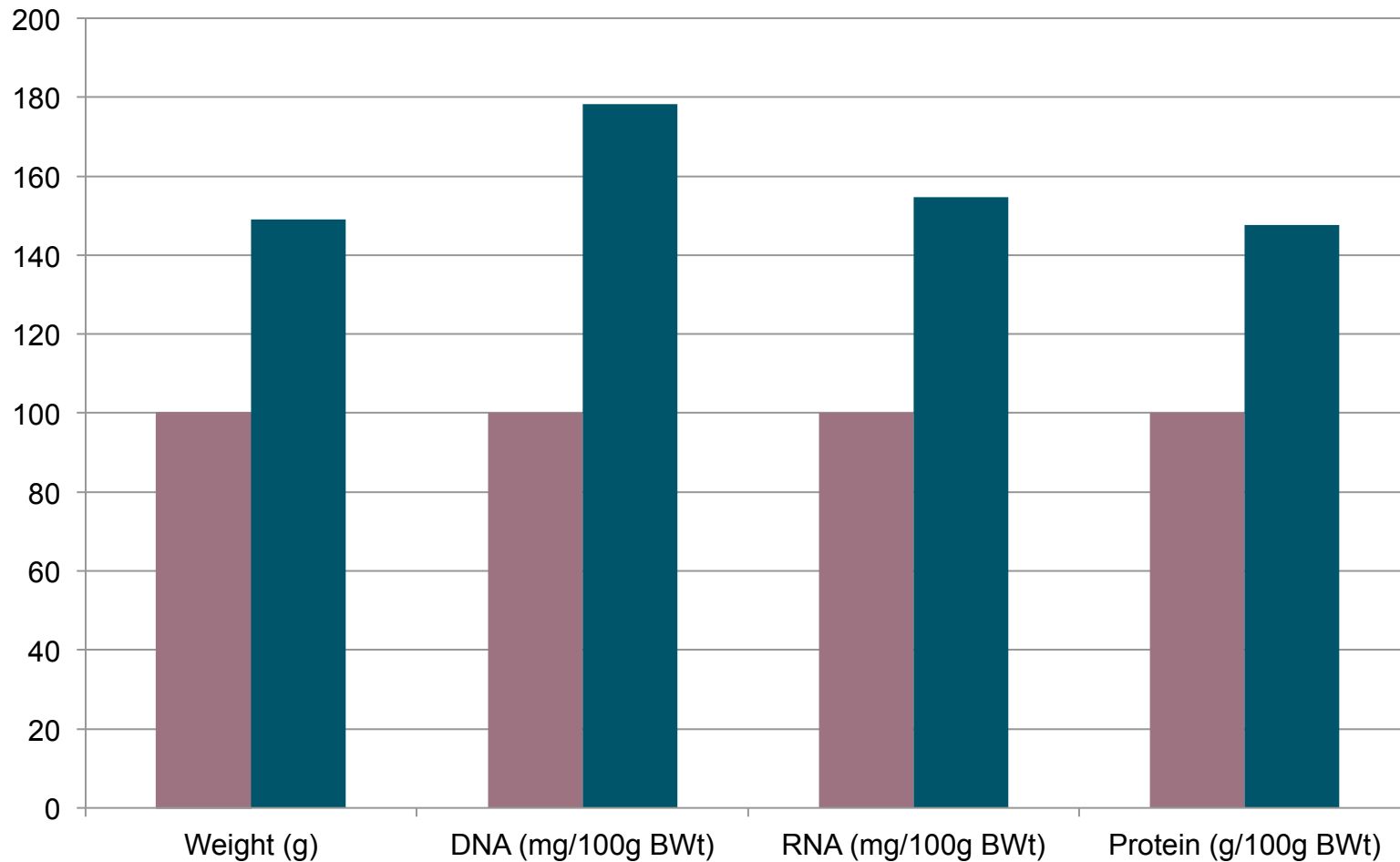
Silk et al. Use of a peptide rather than free amino acid nitrogen source in chemically defined "elemental" diets. J.Parent.Ent.Nutr. 4 (6):548-553, 1980.

Water absorption (ml/h/25cm)



CAECUM

■ Amino acid control ■ Milk hydrolysate

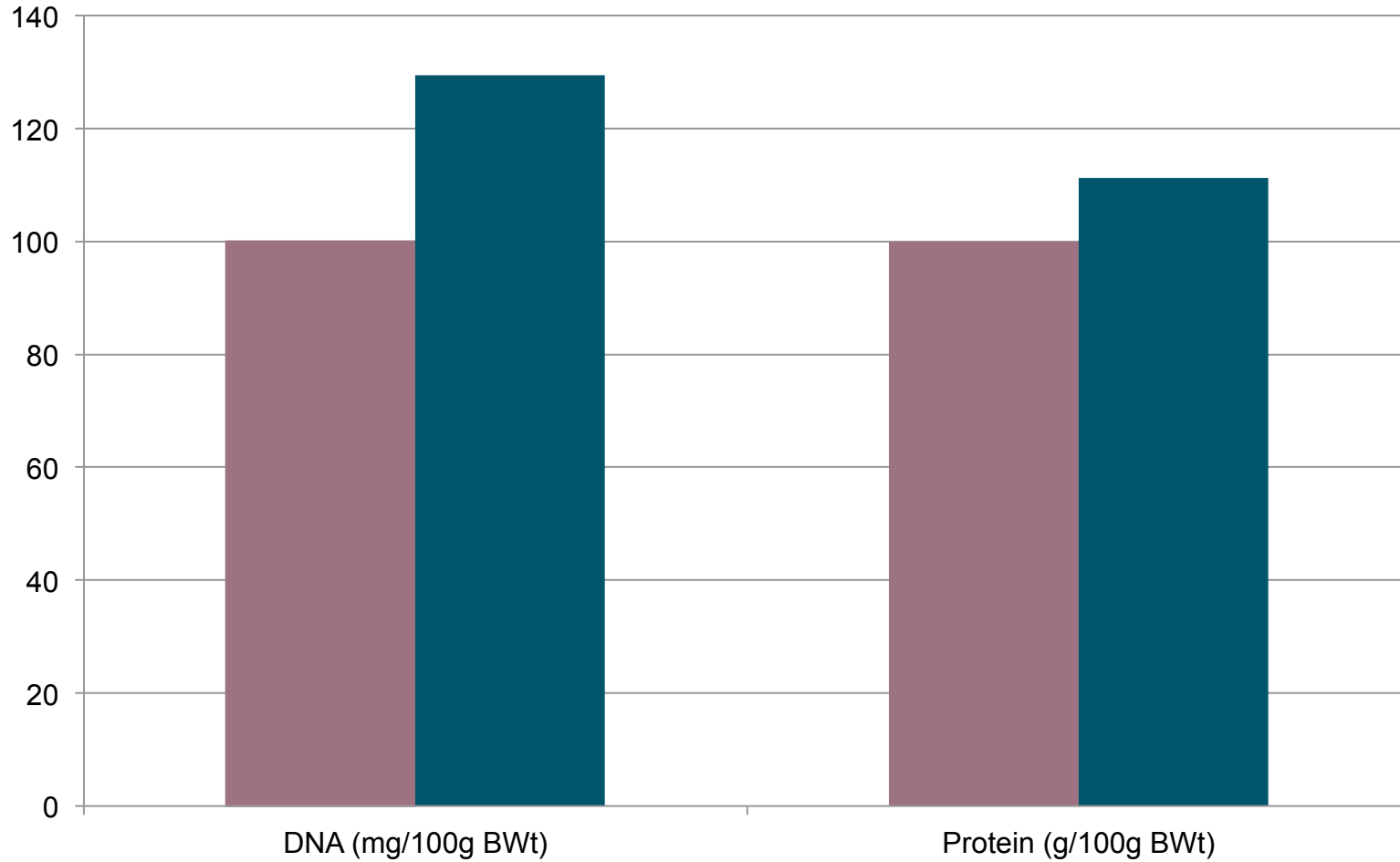


Bioactivity in protein hydrolysates

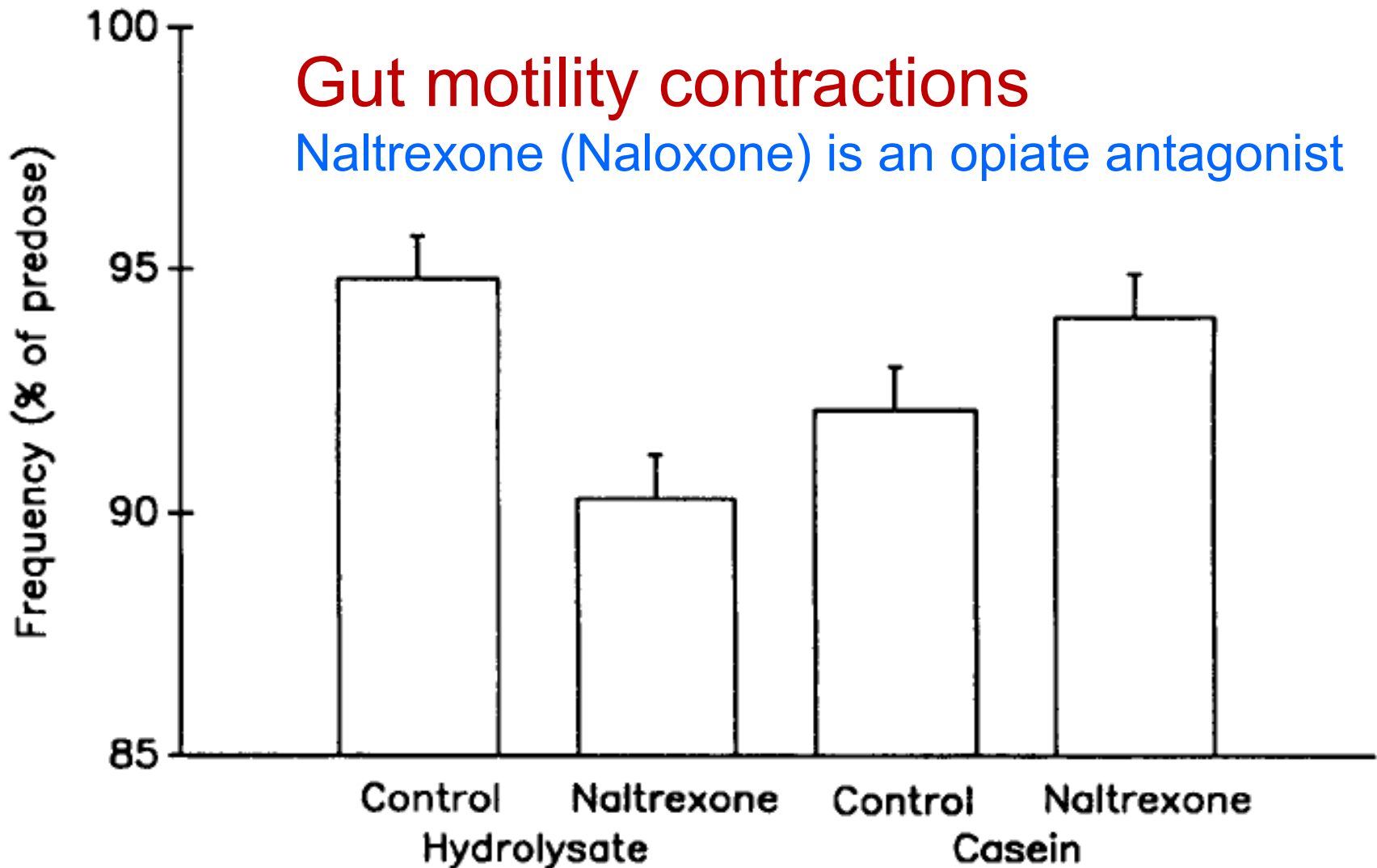
G. K. Grimble, V. Preedy, P. Garlick, and D. B. A. Silk. Trophic effects of dietary peptides on the rat intestinal tract. *Gut*. 30:A1454, 1989.

COLON

■ Amino acid control ■ Milk hydrolysate

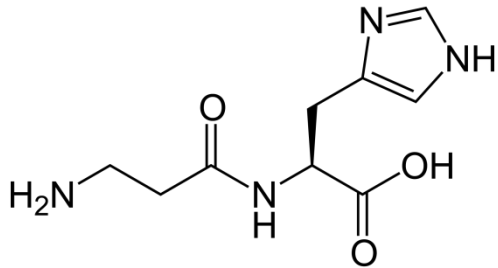


Froetschel. Bioactive peptides in digesta that regulate gastrointestinal function and intake. J.Anim.Sci. 74 (10):2500-2508, 1996.



Some dipeptides get through

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



Dipeptide - β -alanine and histidine

QED. It was absorbed intact

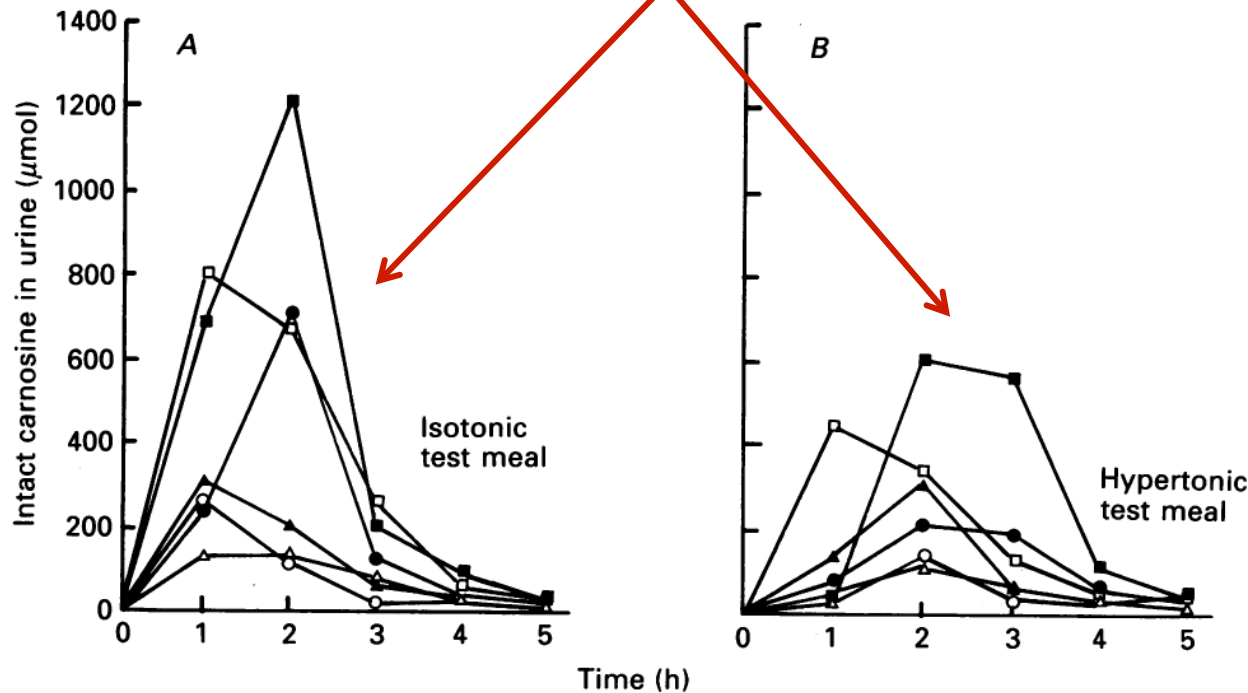
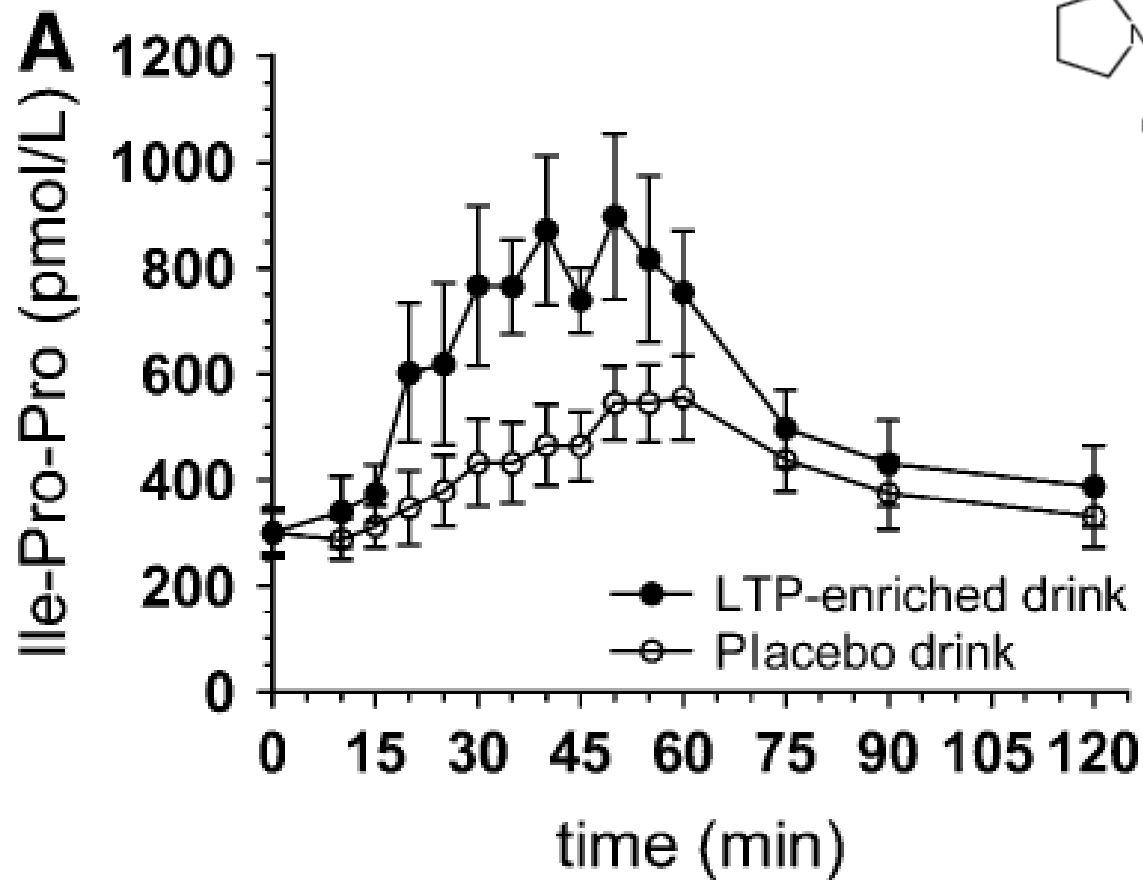
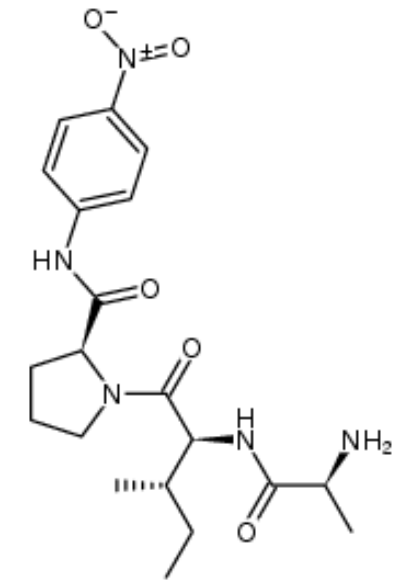


Fig. 1. Hourly urinary output of intact carnosine following ingestion of 4 g carnosine with an isotonic (A) or a hypertonic (B) test meal. Each symbol represents a different subject.

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

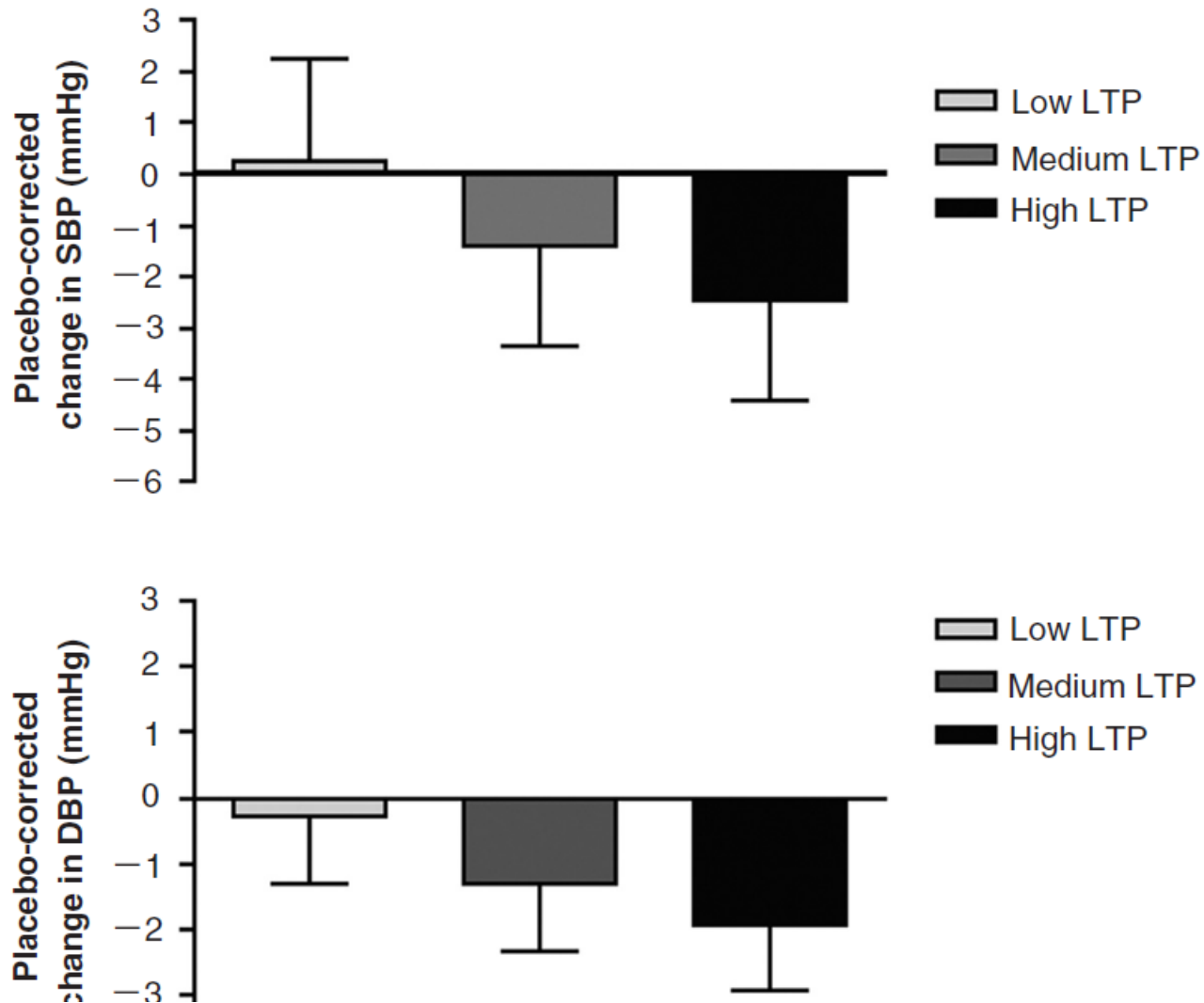


M. Foltz, P. C. van der Pijl, and G. S. Duchateau. Current in vitro testing of bioactive peptides is not valuable. *J.Nutr.* 140 (1):117-118, 2010.

“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

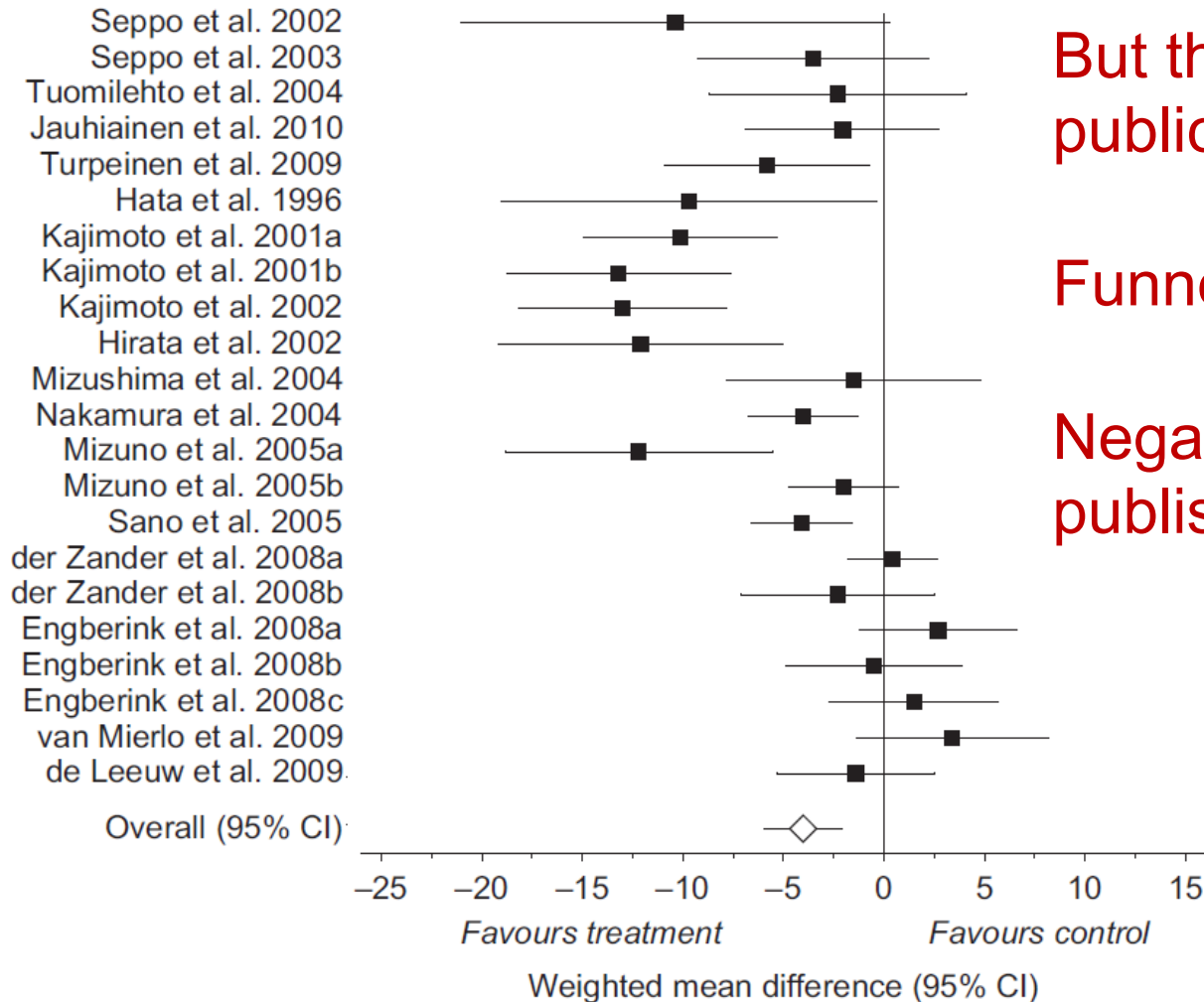
de Leeuw et al. Dose-dependent lowering of blood pressure by dairy peptides in mildly hypertensive subjects. *Blood Press* 18 (1-2): 44-50, 2009.



Summary of all studies

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

Systolic blood pressure (mm Hg)



But there was evidence of publication bias

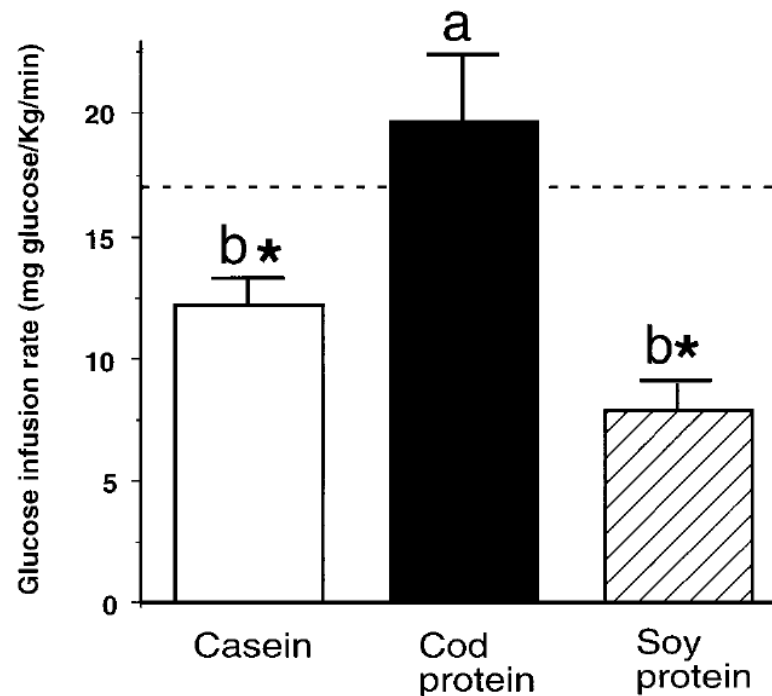
Funnel plot

Negative studies not published

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

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

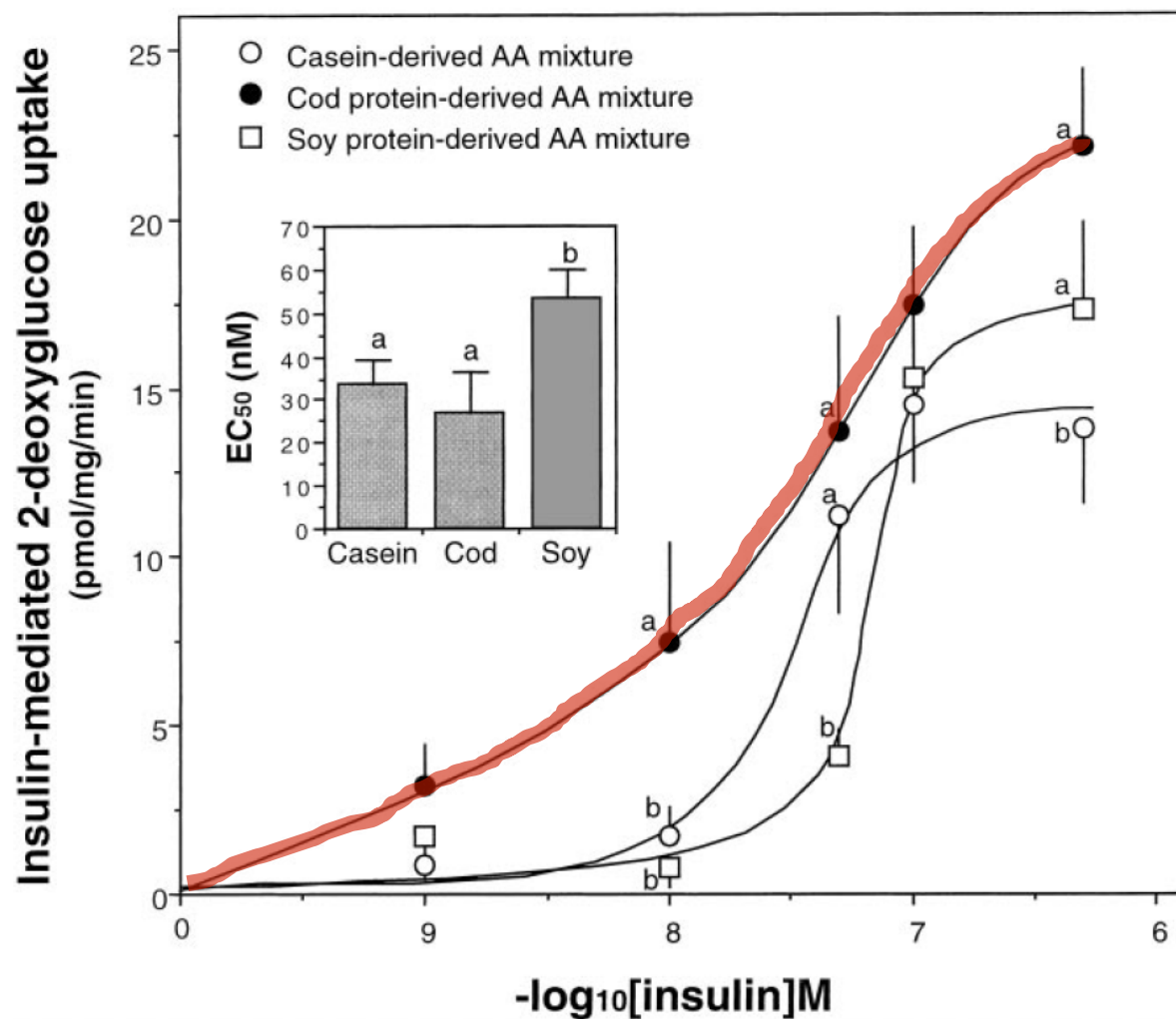
Table 1. Dietary protein-derived amino acid mixtures used for L6 culture conditions

	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 .

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

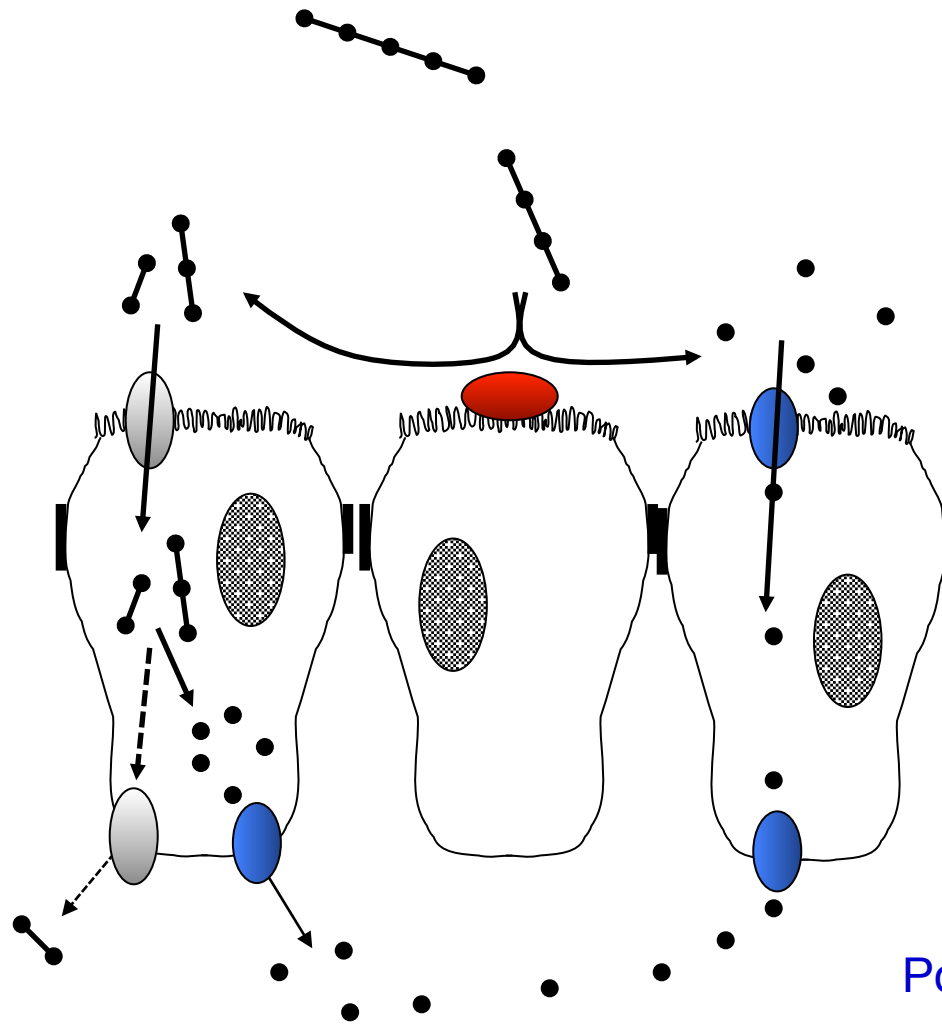
Incubated muscle cells
2-deoxyglucose is a non-metabolised glucose analogue



- 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

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