Using Nutritional Geometry to resolve the protein paradox in ageing and metabolic health

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Charles Perkins Centre

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The Protein Paradox

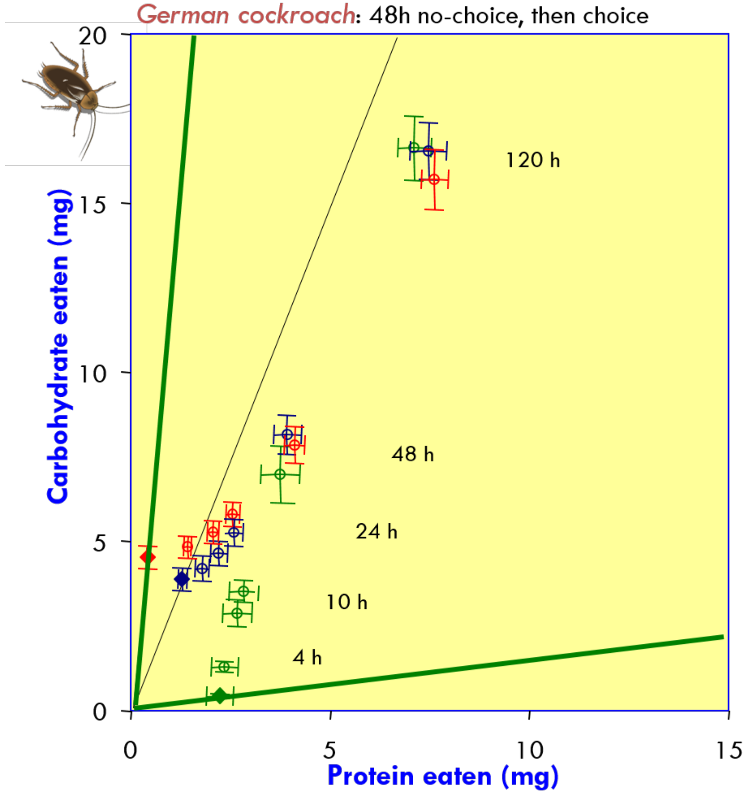
1. Reducing protein in the diet stimulates increased food intake, which can promote obesity, degrade cardiometabolic health and risk shortening lifespan.

2. But reducing protein intake (and that of key amino acids) also extends lifespan and improves mid-life metabolic health.

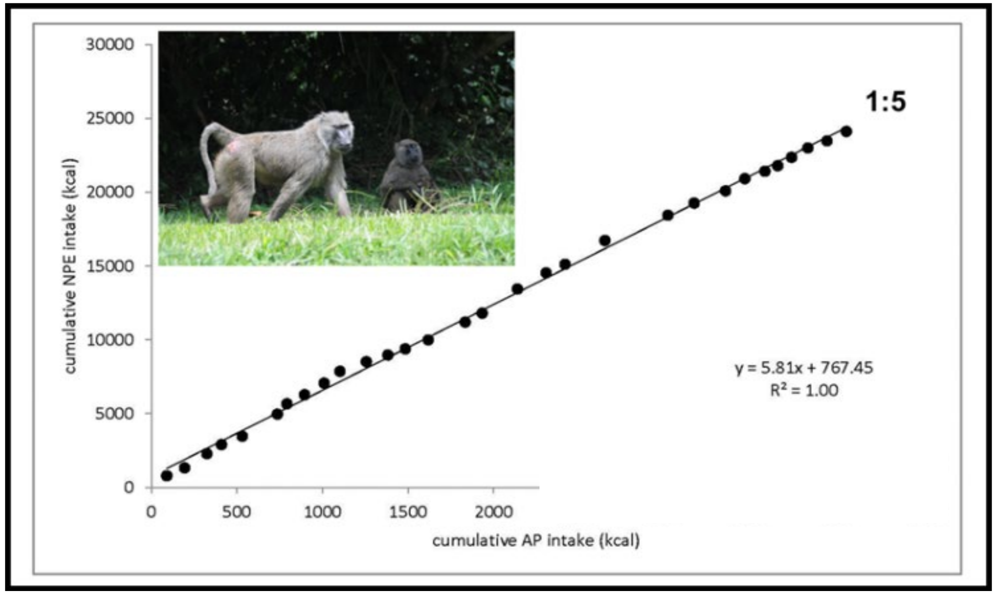
- Unpack the two components of the protein paradox
- Resolve the paradox - two parts to the solution
- Put the pieces together in a model of the human food environment

1. Reducing protein in the diet stimulates increased food intake .....

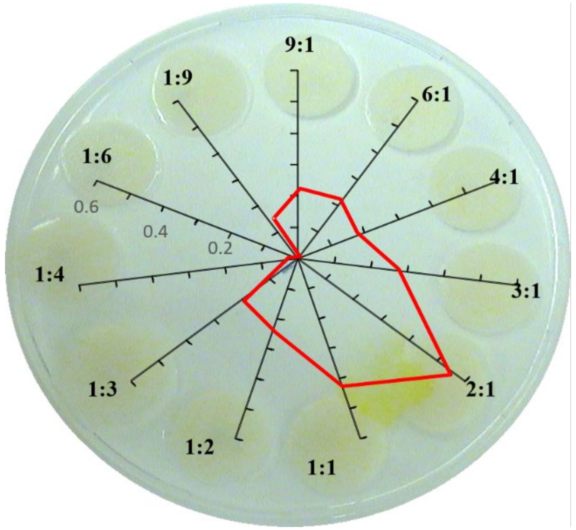
Animals have a specific appetite for protein (along with carb, fat, sodium and calcium) – guide nutrient balancing under appropriate conditions



Raubenheimer, D. & Jones S.A. 2006, *Animal Behaviour* 71: 1253-1262.

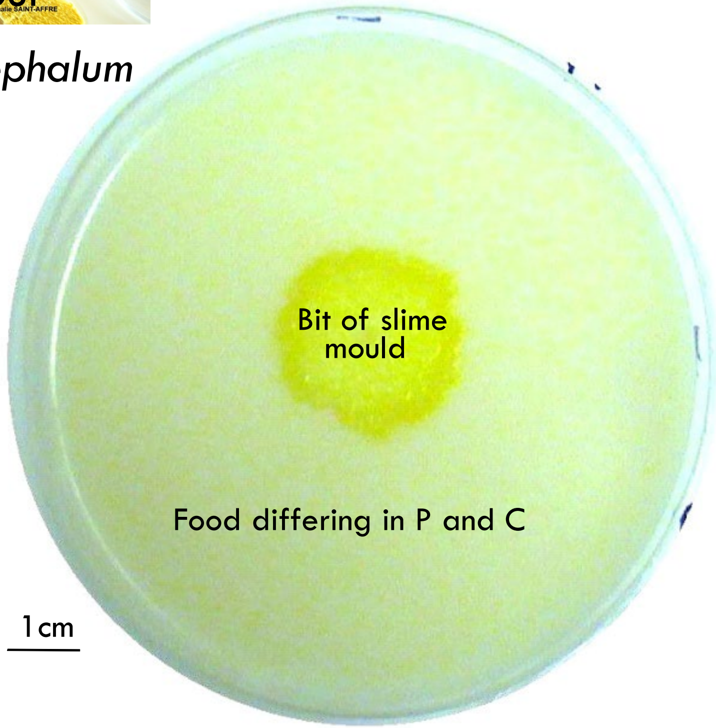


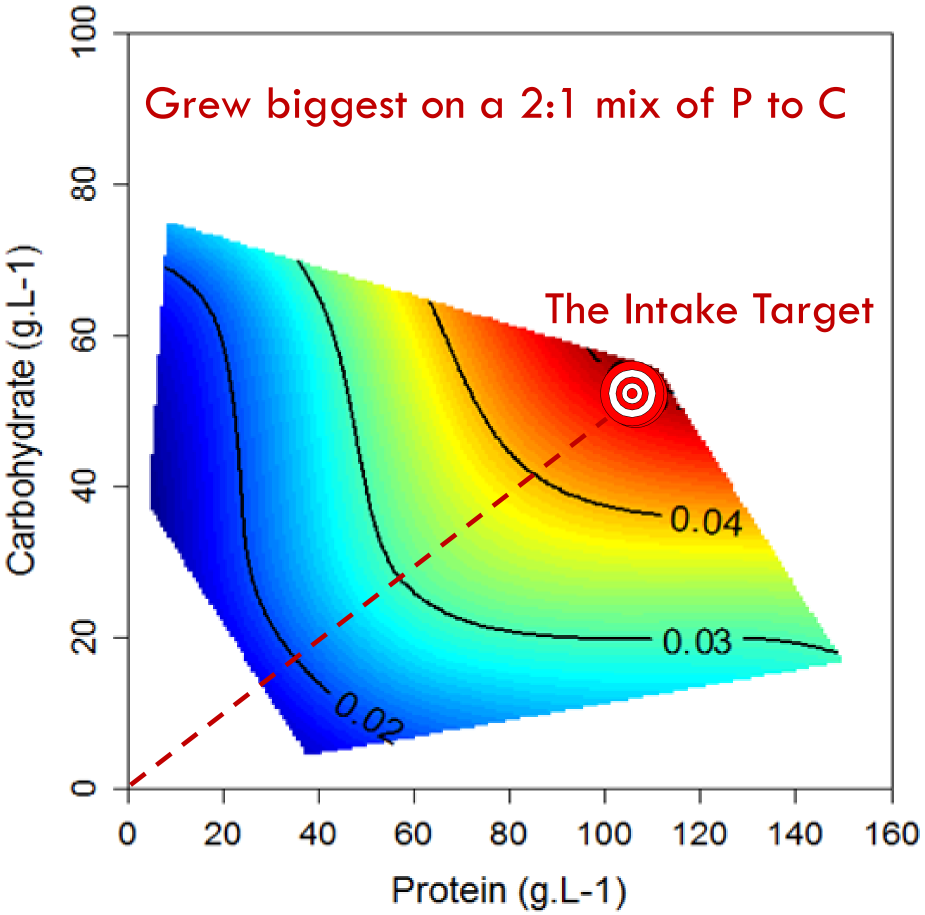
Johnson, C.A., Raubenheimer, D., Rothman, J.M., Clarke, D, & Swedell, R. 2013, *PLoS ONE* 8(7)



Dussutour, A., Latty, T., Beekman, M. & Simpson, S.J. 2010, *Proceedings of the National Academy of Sciences, USA* 107: 4607-4611.

Physarum polycephalum

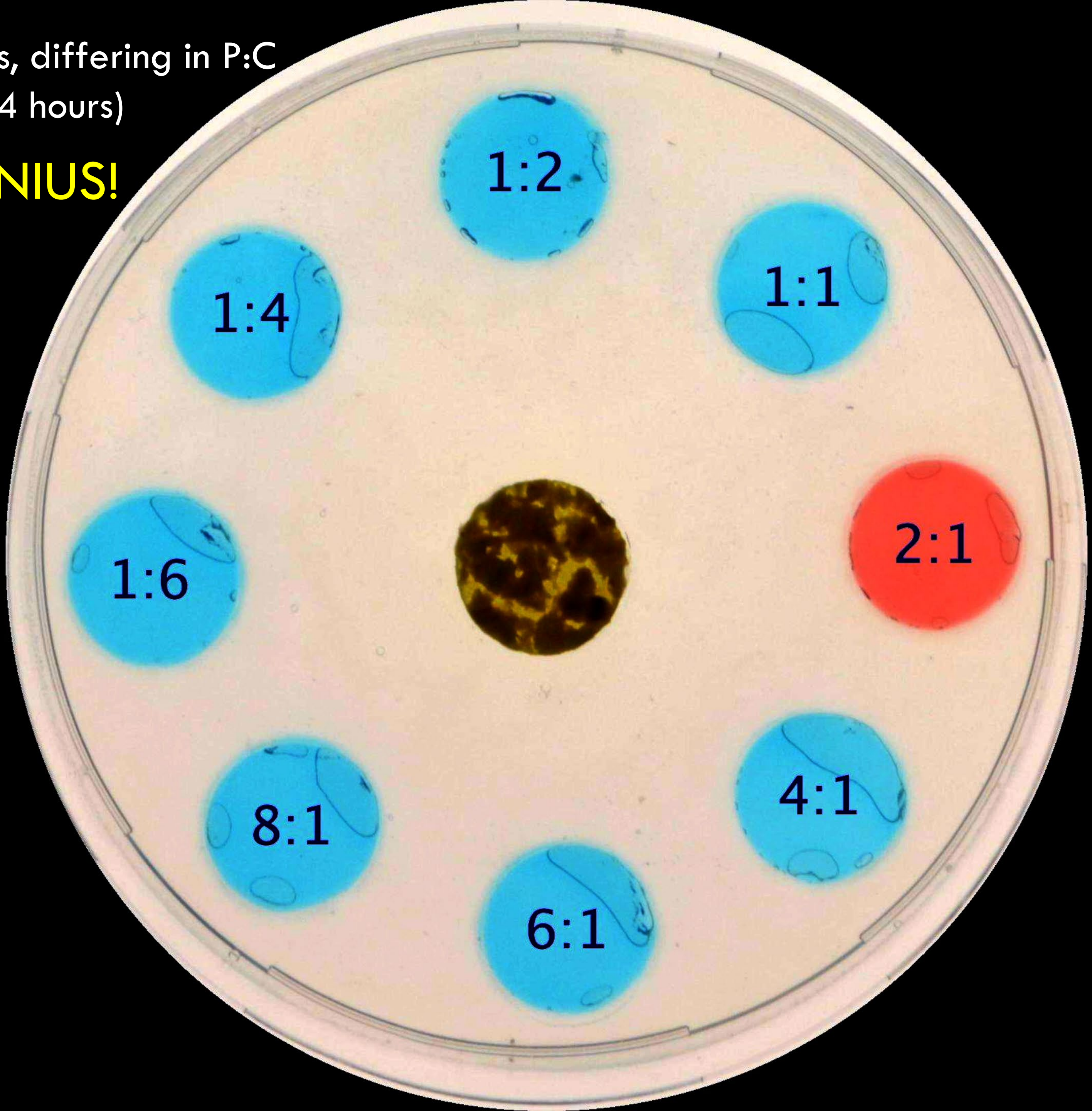




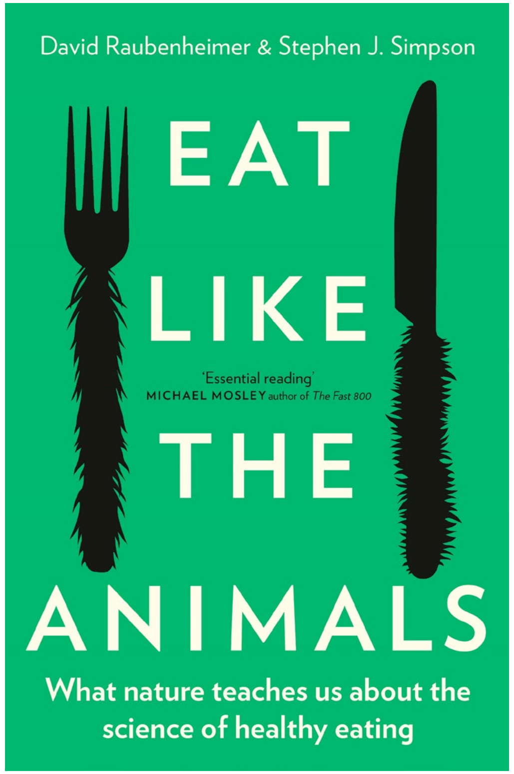
Dussutour, A., Latty, T., Beekman, M. & Simpson, S.J. 2010, *Proceedings of the National Academy of Sciences, USA* 107: 4607-4611.

Blob given 8 food choices, differing in P:C
(Time lapse video over 24 hours)

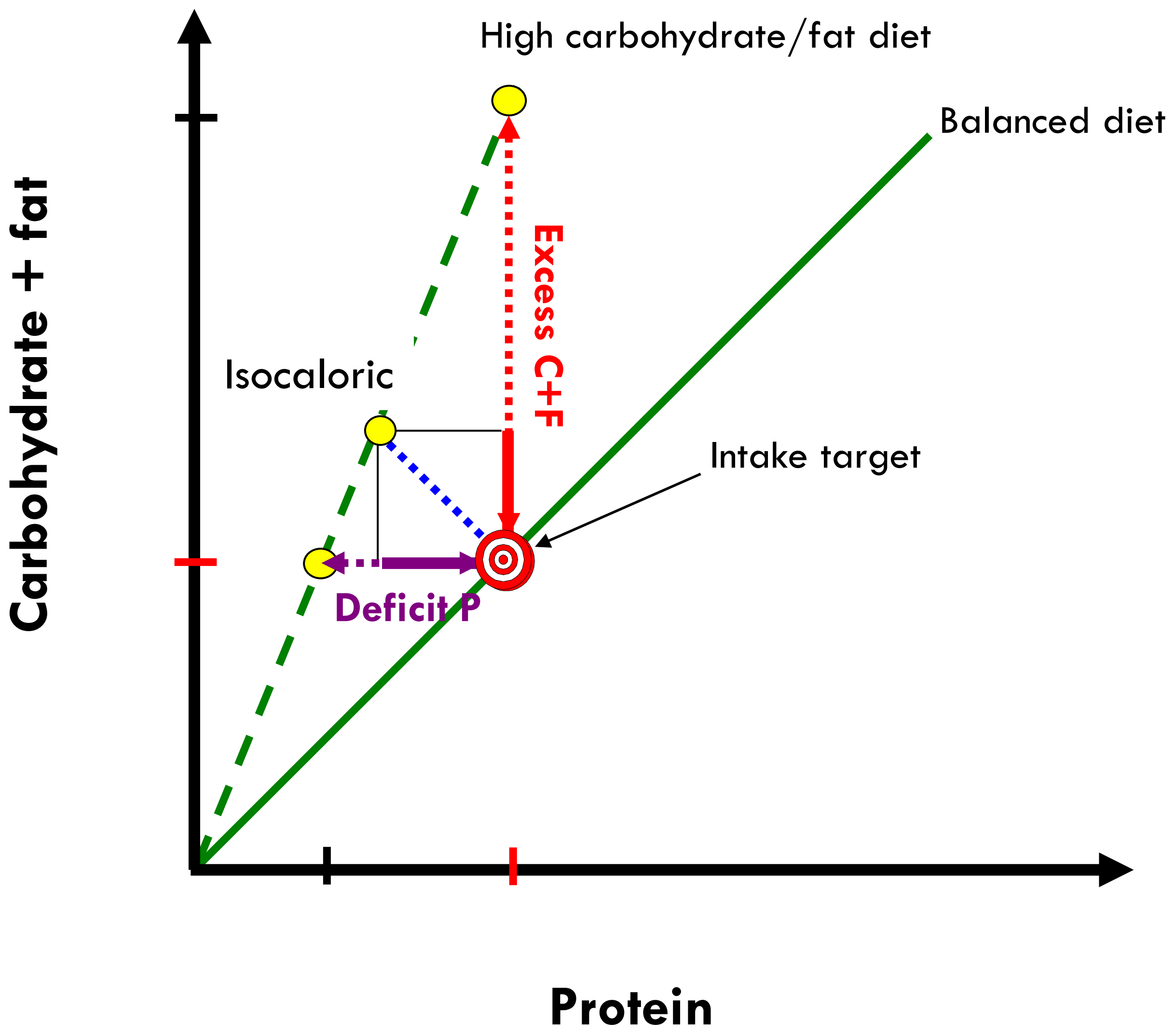
NUTRITIONAL GENIUS!



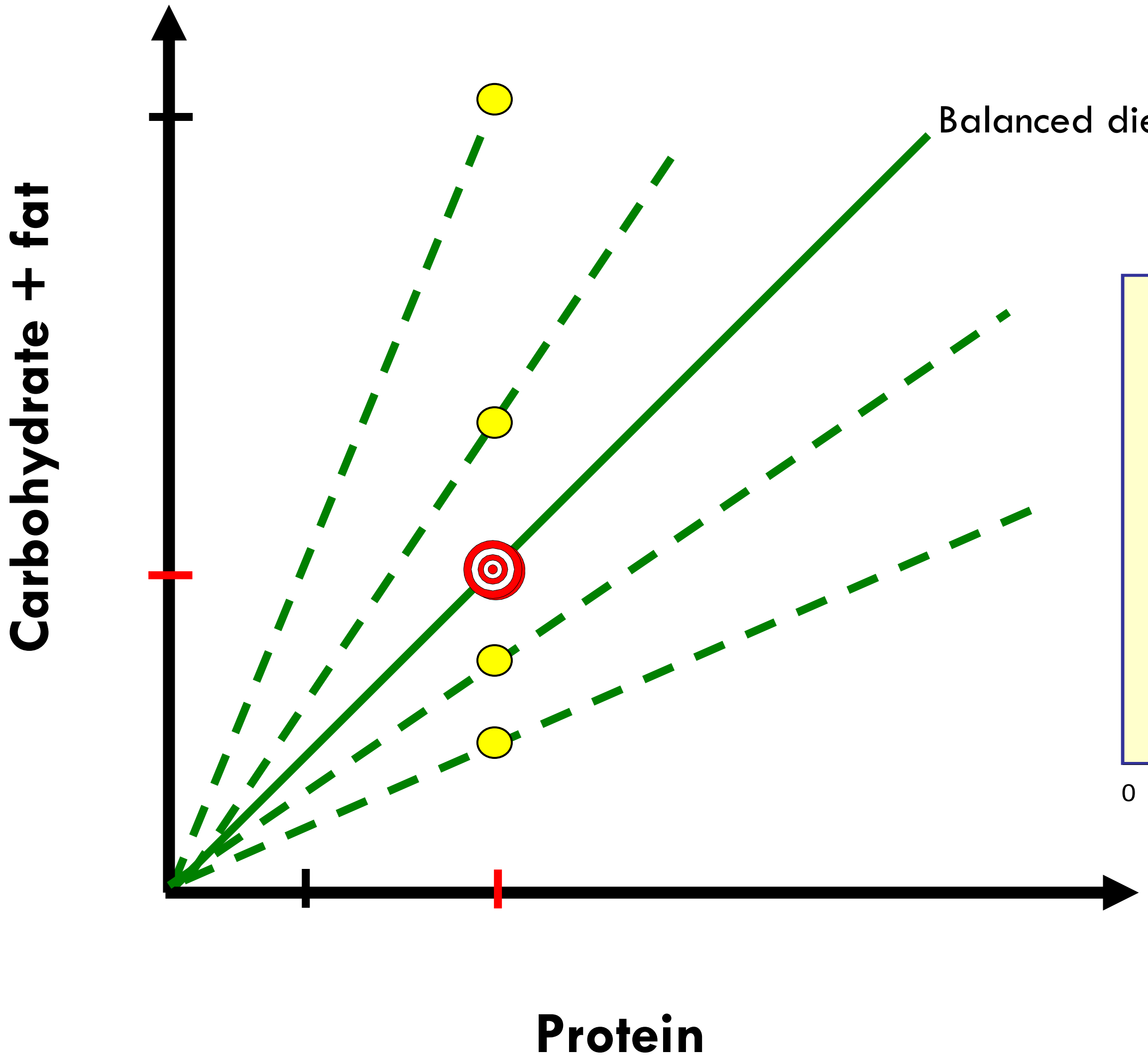
If a brainless slime mould can balance its diet, why can't we?

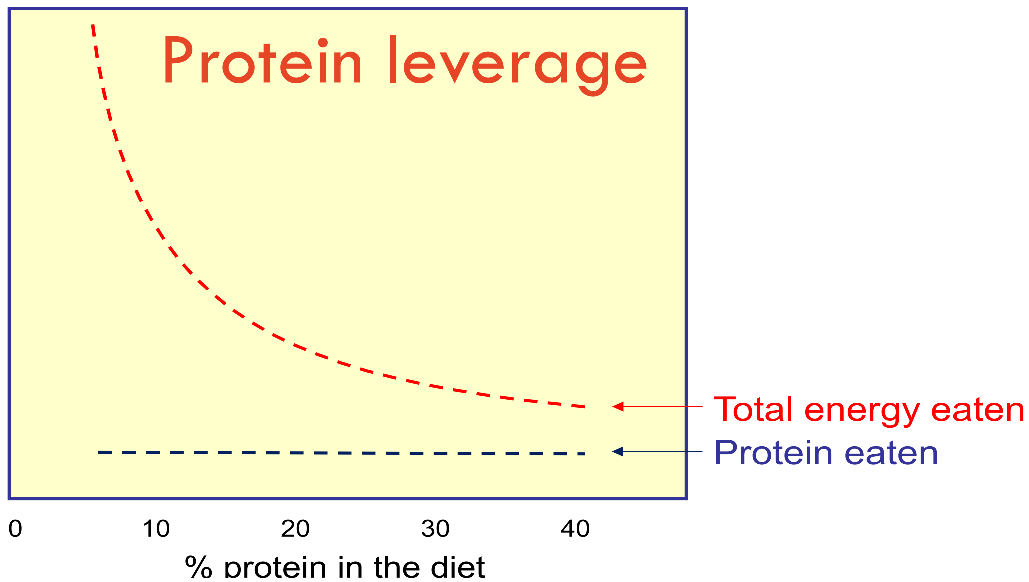


Nutrient-specific appetites *compete* when the diet is imbalanced



The solution in various (not all) species: prioritise protein





Simpson, S.J. & Raubenheimer, D. 2005, *Obesity Reviews* 6: 133-142; Raubenheimer, D. & Simpson, S.J. 2019, *Obesity* 27: 1225-1238.

Animal Behaviour 126 (2017) 195–208

Contents lists available at ScienceDirect

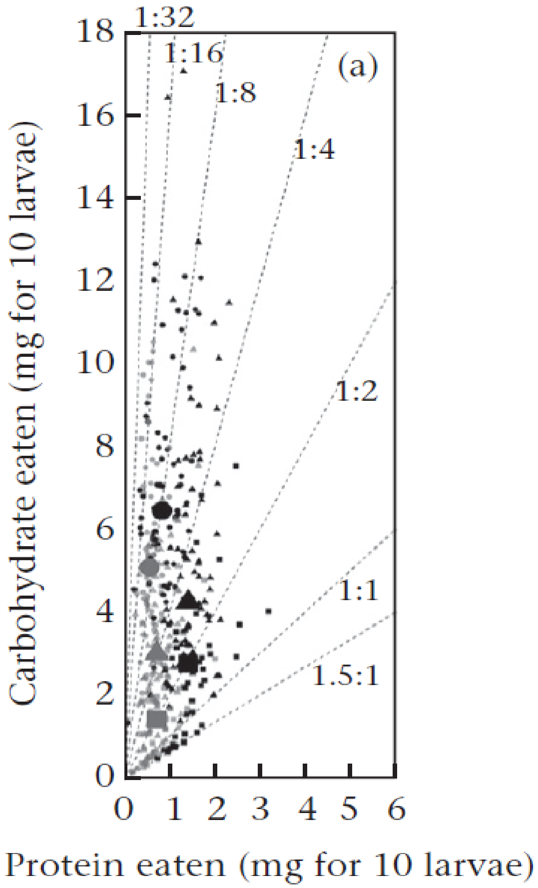
Animal Behaviour

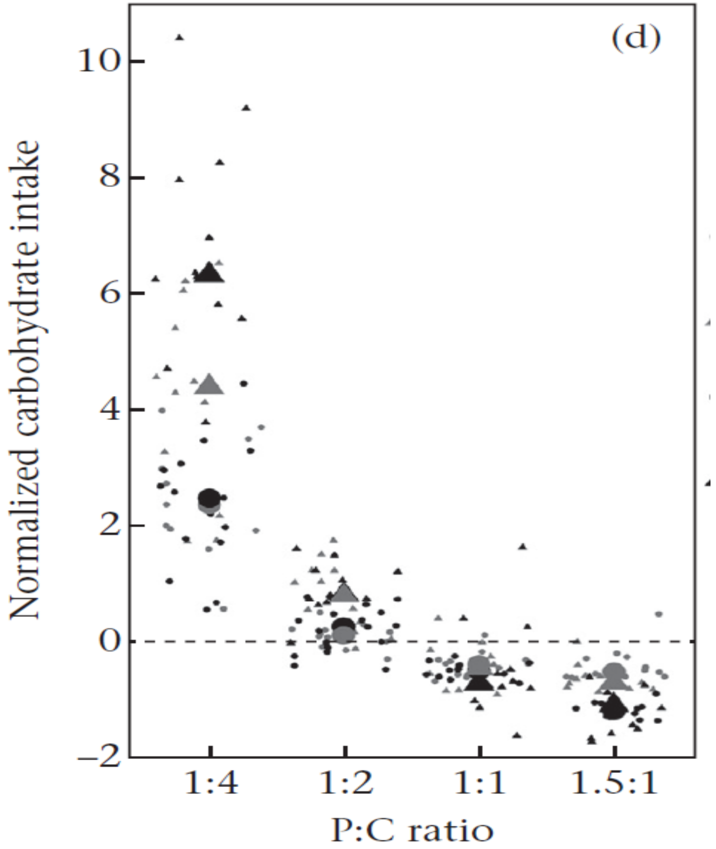
journal homepage: www.elsevier.com/locate/anbehav

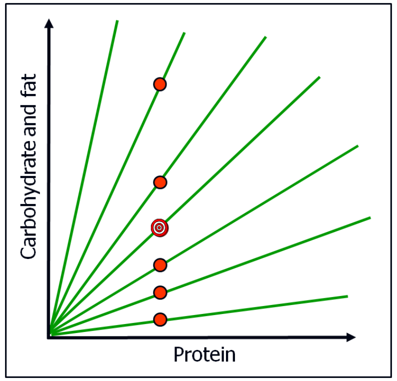
Food intake and food choice are altered by the developmental transition at critical weight in *Drosophila melanogaster*

Maria João Almeida de Carvalho [a], Christen Kerry Mirth [a, b, *]

[a] Development, Evolution and the Environment Laboratory, Instituto Gulbenkian de Ciência, Oeiras, Portugal
[b] School of Biological Sciences, Monash University, Clayton, VIC, Australia

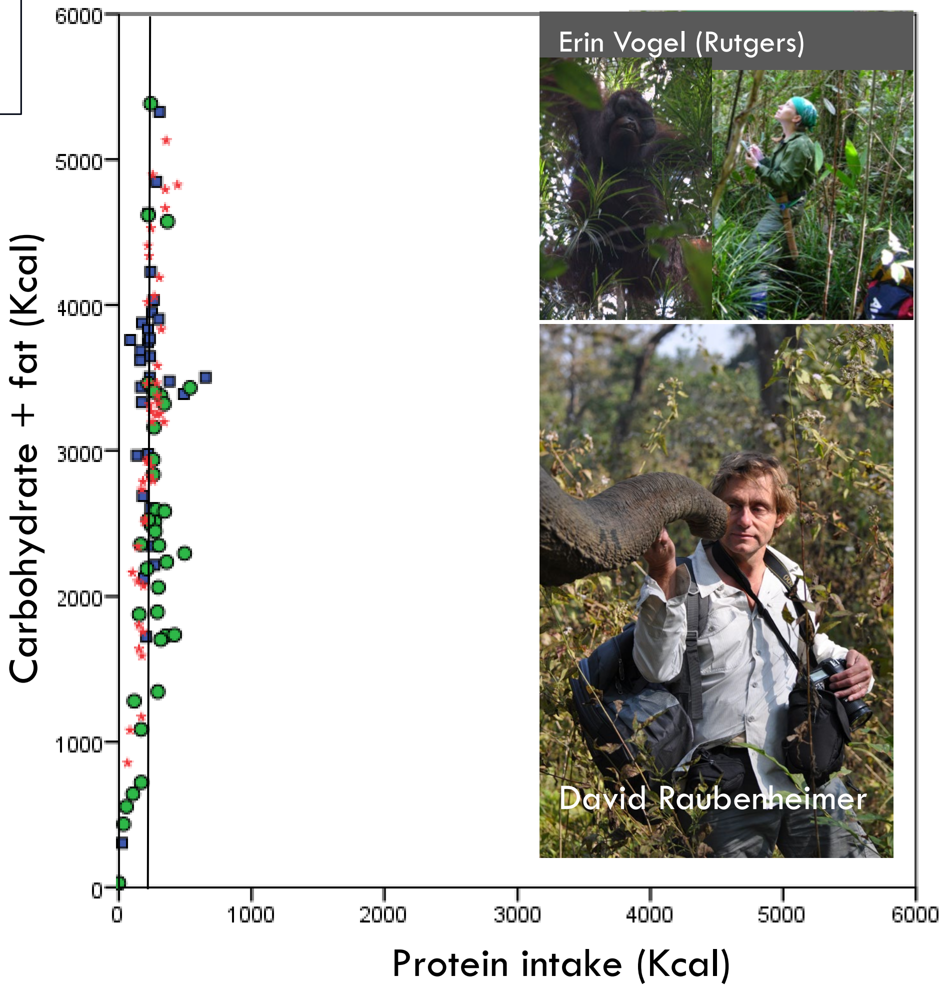




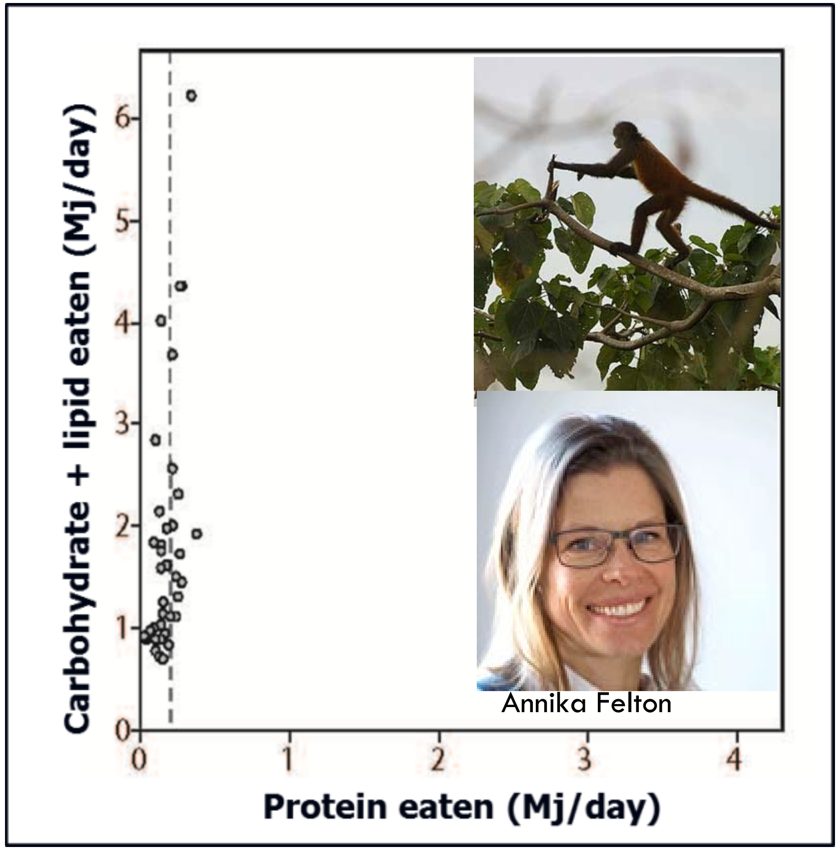


Protein leverage in primates

Orangutans (49 animals, >7,200 full day observations)



Spider monkeys

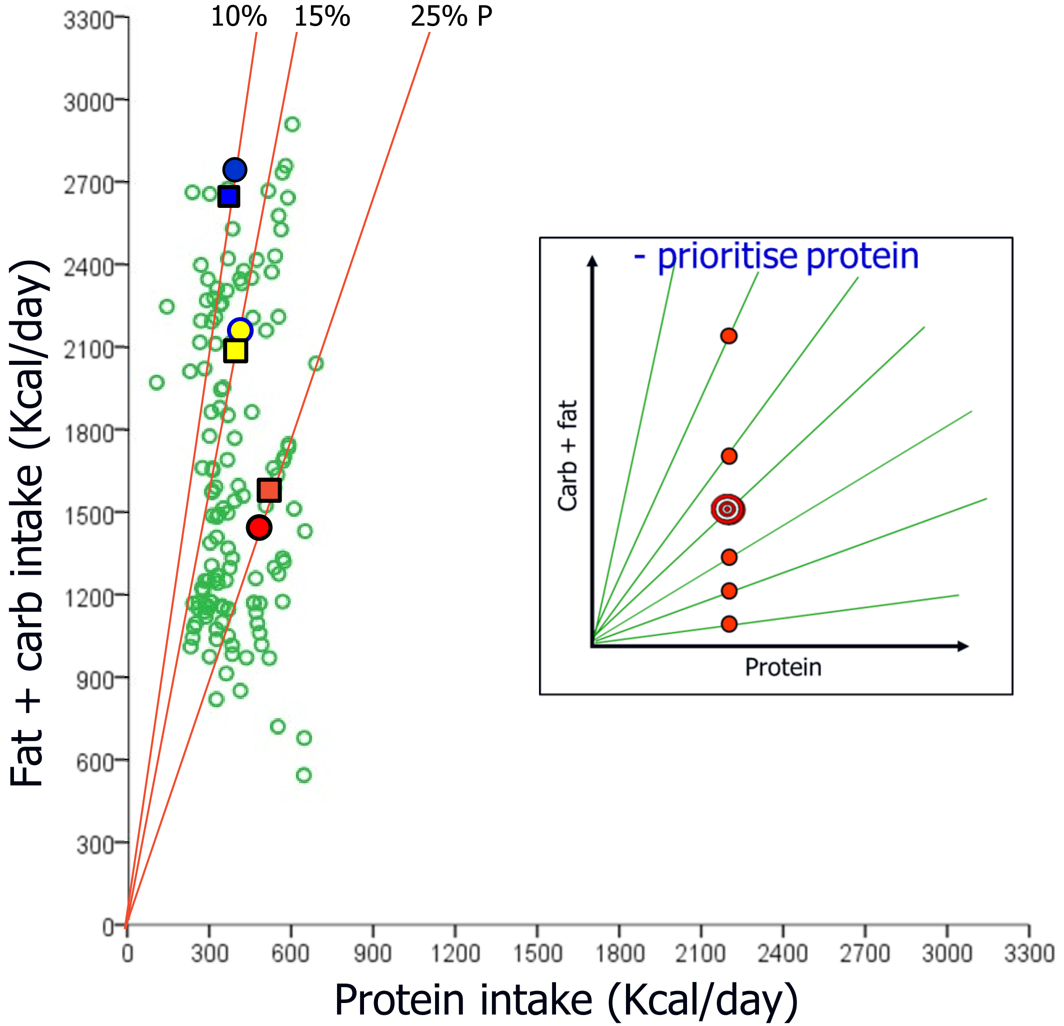


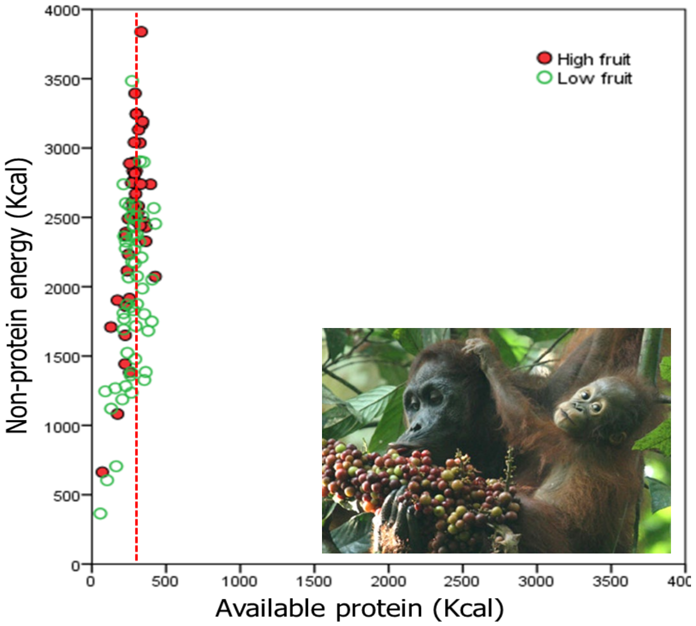
Vogel, Raubenheimer et al., unpublished data.

Felton, A.M. et al. 2009, *Behavioural Ecology* 20: 685-690.

Protein leverage in humans

i. Sydney   ii. Jamaica   ii. Literature data

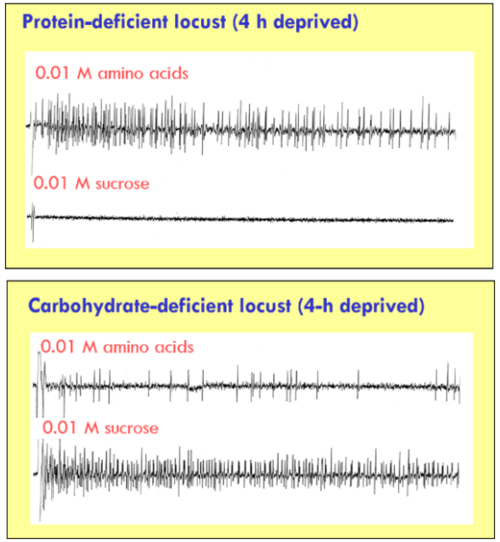




Gosby, A., Conigrave, A., Raubenheimer, D. & Simpson, S.J. 2014, *Obesity Rev.* 15: 183–191

Mechanisms of nutrient specific appetites – protein and carbs

State-dependant modulation of gustatory sensitivity



Simpson et al. (1991) *Appetite* 17: 141-154

Modulation of brain circuitry

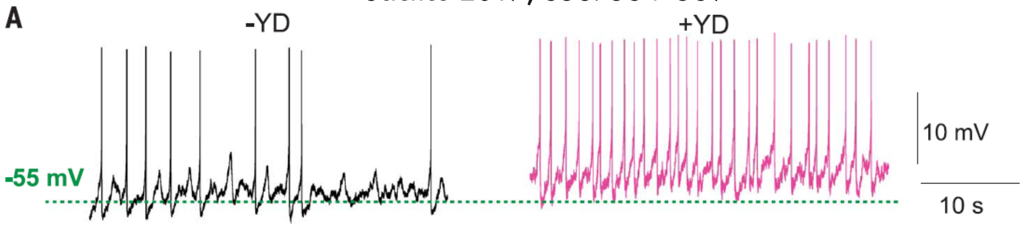
RESEARCH

NEUROSCIENCE

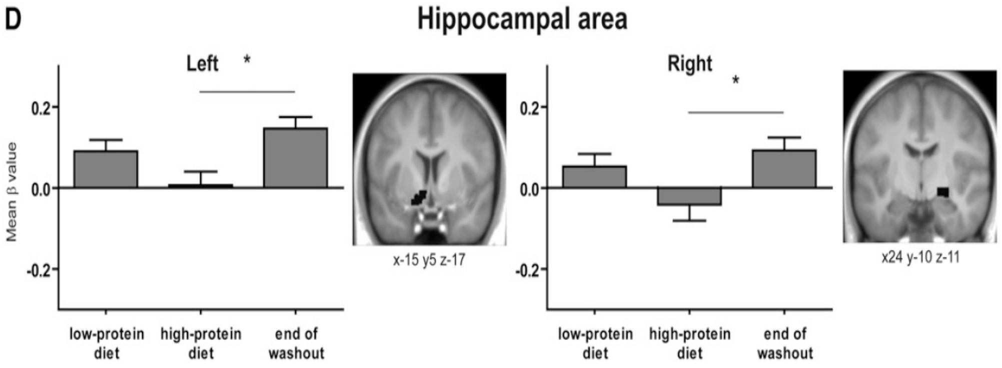
Branch-specific plasticity of a bifunctional dopamine circuit encodes protein hunger

Qili Liu,[1]* Masashi Tabuchi,[1]* Sha Liu,[1] Lay Kodama,[1] Wakako Horiuchi,[1] Jay Daniels,[1] Lucinda Chiu,[1] Daniel Baldoni,[1] Mark N. Wu[1,2]†

Science 2017, 356: 534–539



Human brain imaging

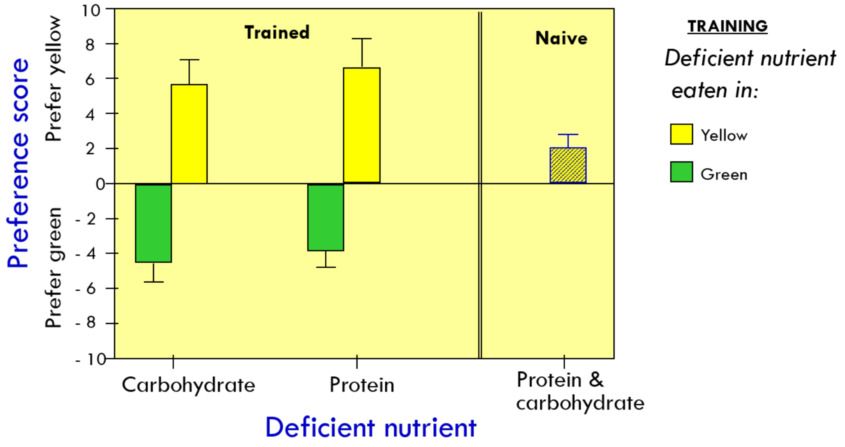


Human protein status modulates brain reward responses to food cues[1–3]

Sanne Griffioen-Roose, Paul AM Smeets, Emmy van den Heuvel, Sanne Boesveldt, Graham Finlayson, and Cees de Graaf

Am J Clin Nutr 2014; 100: 113-122

Nutrient-specific learning



Raubenheimer, D. & Tucker, D. (1997) *Animal Behaviour* 54: 1449-1459.

Available online at www.sciencedirect.com
ScienceDirect
Current Opinion in Neurobiology

Nutrient homeostasis — translating internal states to behavior

Daniel Münch, Gili Ezra-Nevo[1], Ana Patrícia Francisco[1], Ibrahim Tastekin[1] and Carlos Ribeiro

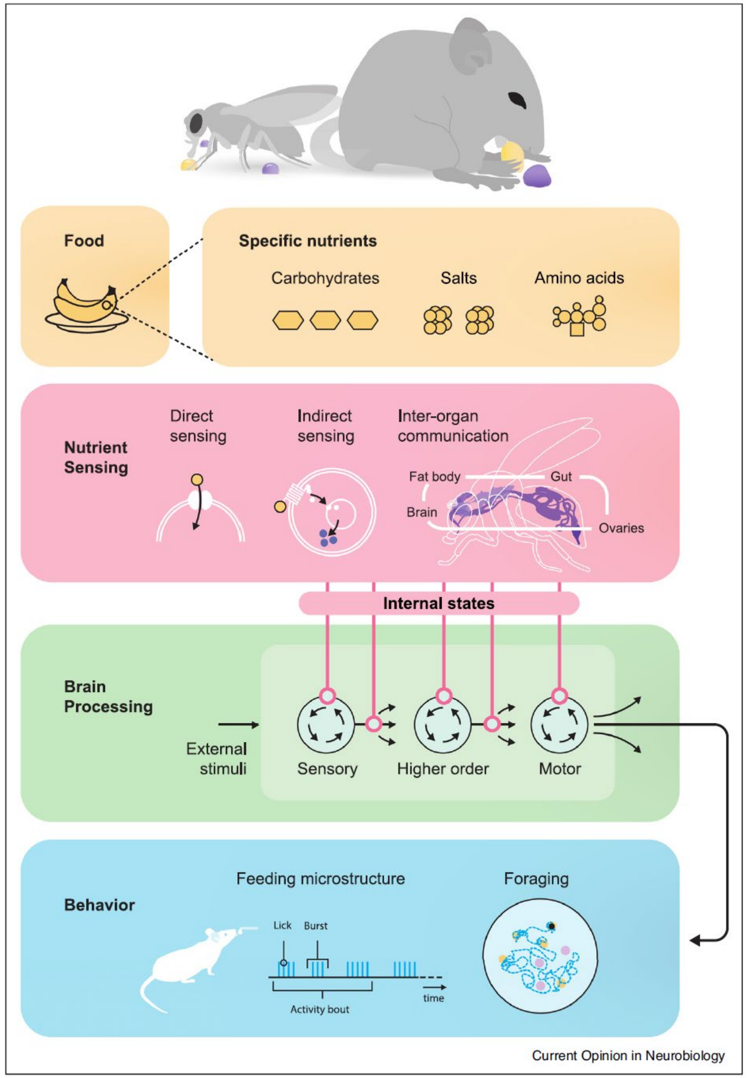
Article

The neuronal logic of how internal states control food choice

https://doi.org/10.1038/s41586-022-04909-5
Received: 8 June 2021
Accepted: 25 May 2022
Published online: 06 July 2022
Check for updates

Daniel Münch[1✉], Dennis Goldschmidt[1] & Carlos Ribeiro[1✉]

When deciding what to eat, animals evaluate sensory information about food quality alongside multiple ongoing internal states[1–10]. How internal states interact to alter sensorimotor processing and shape decisions such as food choice remains poorly understood. Here we use pan-neuronal volumetric activity imaging in the brain of *Drosophila melanogaster* to investigate the neuronal basis of internal state-dependent nutrient appetites. We created a functional atlas of the ventral fly brain and find that metabolic state shapes sensorimotor processing across large sections of the neuropil. By contrast, reproductive state acts locally to define how sensory information is translated into feeding motor output. These two states thus synergistically modulate protein-specific food intake and food choice. Finally, using a novel computational strategy, we identify driver lines that label neurons innervating state-modulated brain regions and show that the newly identified 'borboleta' region is sufficient to direct food choice towards protein-rich food. We thus identify a generalizable principle by which distinct internal states are integrated to shape decision making and propose a strategy to uncover and functionally validate how internal states shape behaviour.

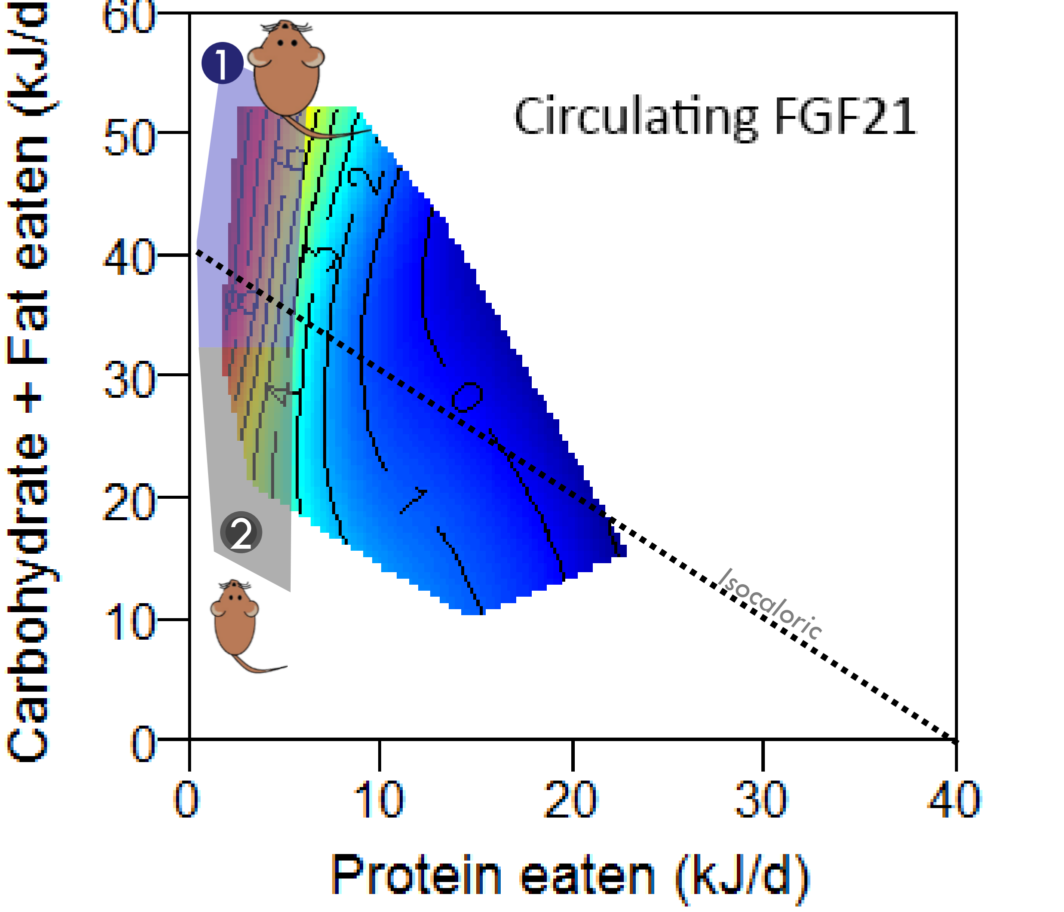


FGF21- the first known protein appetite hormone

Also linked to metabolic effects on insulin sensitivity and energy expenditure

(Laeger et al., 2014, *JCI*, 124, 3913-3922)

Phenotype 1: High FGF21, protein deprived, increased E intake, obese

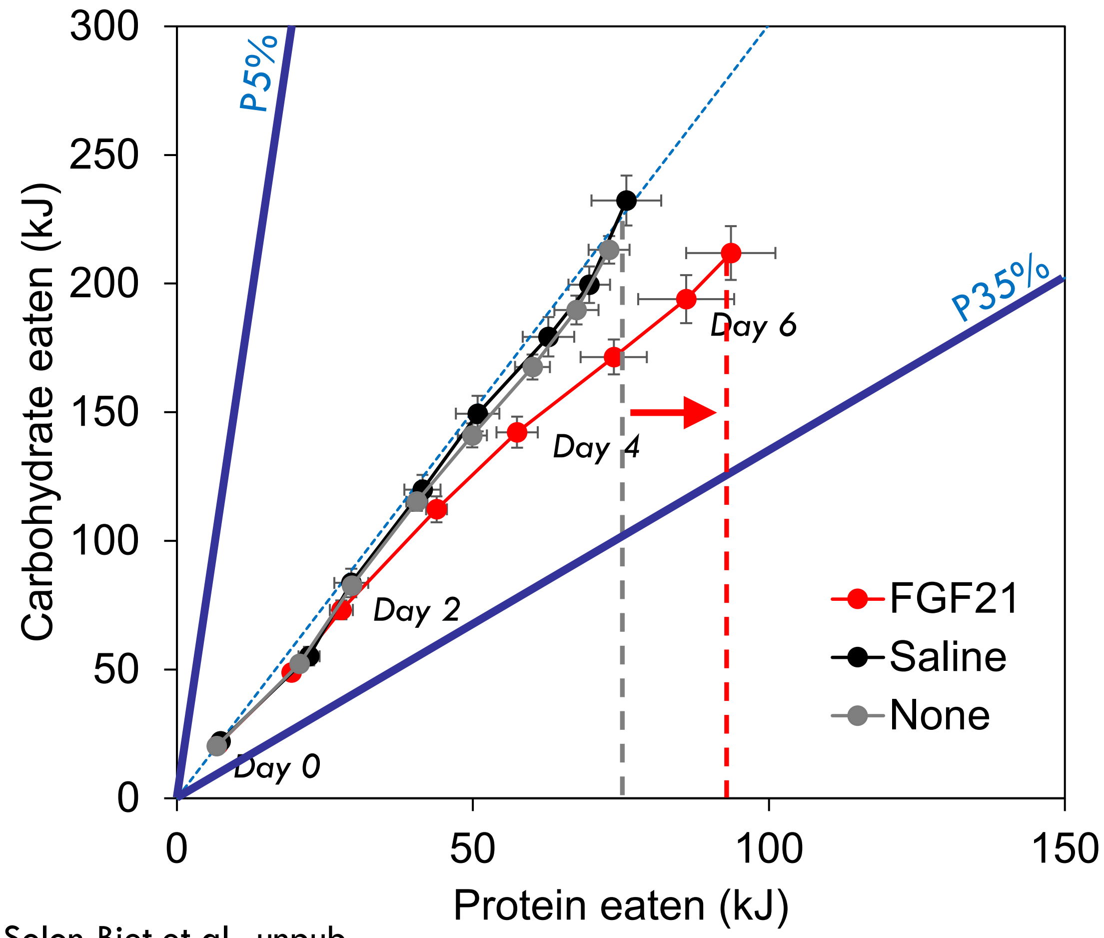


Phenotype 2: High FGF21, protein deprived, calorie restricted, lean

Solon-Biet et al., 2016, *Cell Metab.* 24: 555-565.

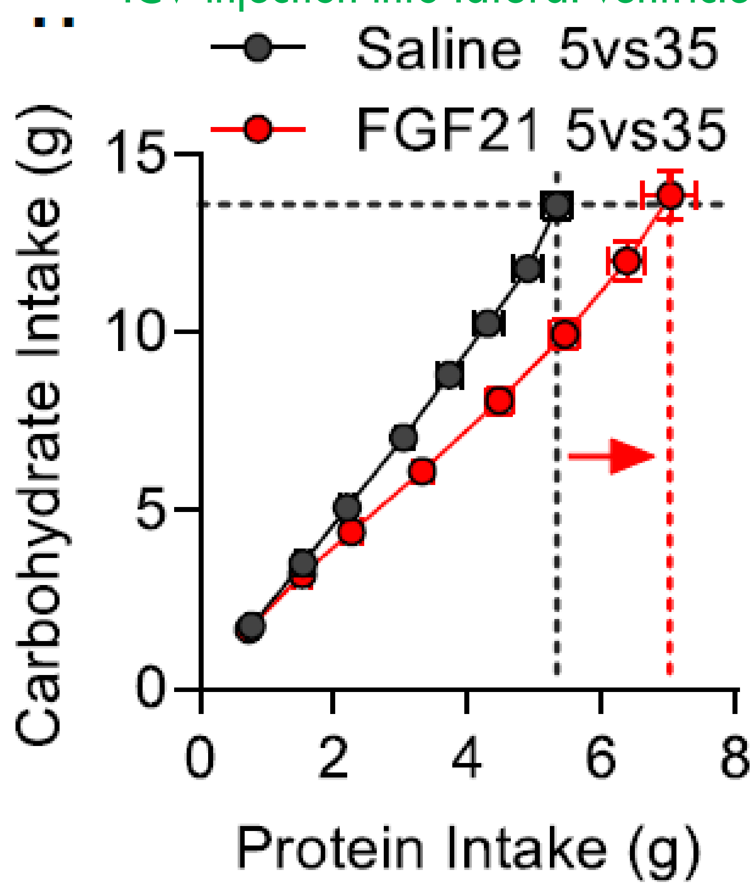
FGF21 perfusion stimulates protein-specific appetite independent of nutritional state

Subcutaneous mini-pump implantation



Solon-Biet et al., unpub.

ICV injection into lateral ventricle



Hill et al. 2019, *Cell Reports* 27: 2934–2947.

Central and peripheral FGF21 increases protein-specific appetite

Relevant to humans

RESEARCH ARTICLE

Raised FGF-21 and Triglycerides Accompany Increased Energy Intake Driven by Protein Leverage in Lean, Healthy Individuals: A Randomised Trial

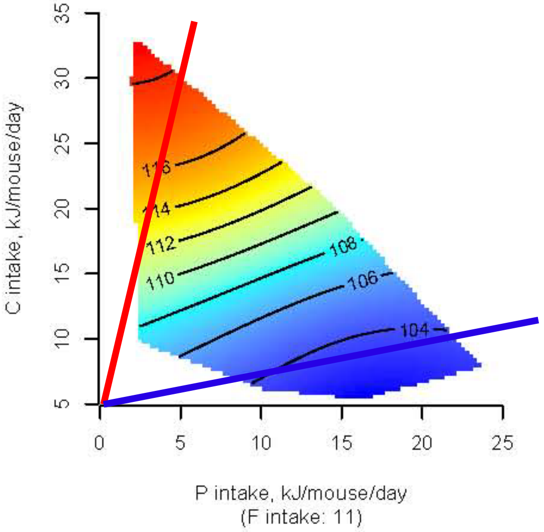
Alison K. Gosby[1,2]*, Namson S. Lau[1,3], Charmaine S. Tam[1,2,3], Miguel A. Iglesias[4], Christopher D. Morrison[5], Ian D. Caterson[1,2,3], Jennie Brand-Miller[1,2,3], Arthur D. Conigrave[1,2], David Raubenheimer[1,2,6], Stephen J. Simpson[1,2]

2. Lifespan is extended on low-P, high-CHO diets


Cell Metabolism
Article

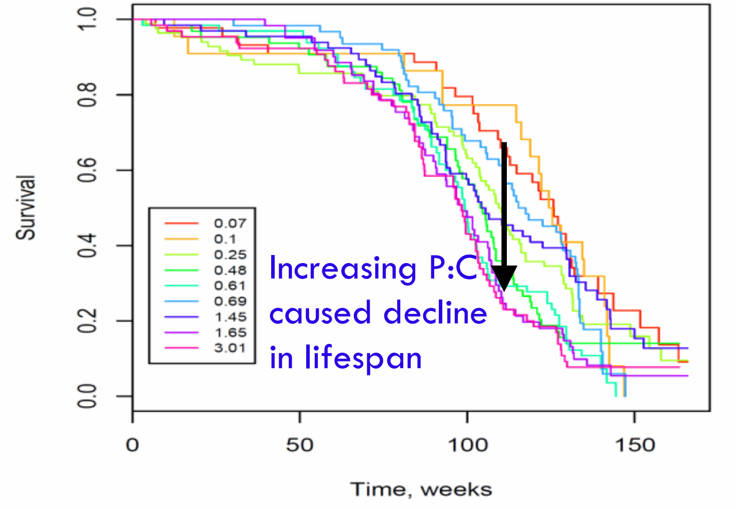
The Ratio of Macronutrients, Not Caloric Intake, Dictates Cardiometabolic Health, Aging, and Longevity in Ad Libitum-Fed Mice

Samantha M. Solon-Biet,[1,2,3,4,13] Aisling C. McMahon,[1,2,3,13] J. William O. Ballard,[5] Kari Ruohonen,[6] Lindsay E. Wu,[7] Victoria C. Cogger,[1,2,3] Alessandra Warren,[1,2,3] Xin Huang,[1,2,3] Nicolas Pichaud,[5] Richard G. Melvin,[8] Rahul Gokarn,[2,3] Mamdouh Khalil,[3] Nigel Turner,[8] Gregory J. Cooney,[9] David A. Sinclair,[7,10] David Raubenheimer,[1,4,11,12] David G. Le Couteur,[1,2,3,*] and Stephen J. Simpson[1,4,*]

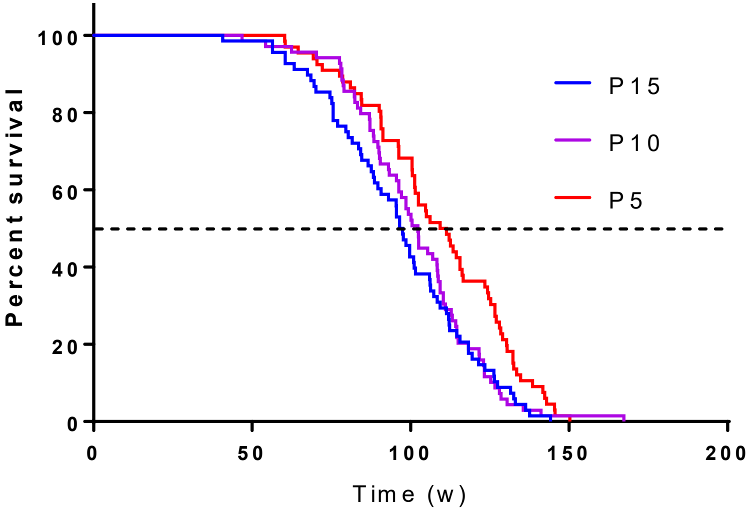


Lee, K.P. et al. (2008) *PNAS* 105, 2498-2503.

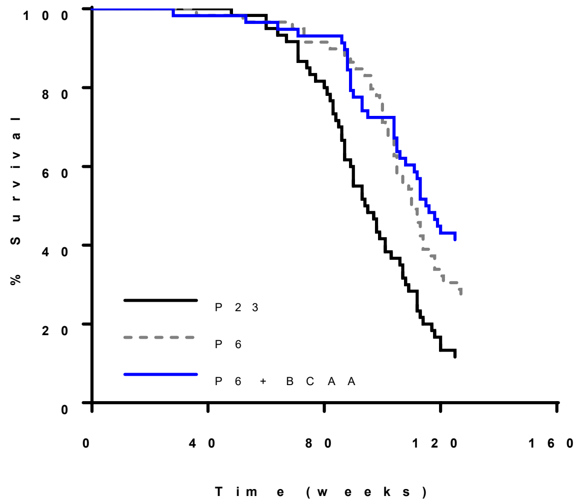
Actual lifespan



Solon-Biet et al. 2014, *Cell Metabolism*



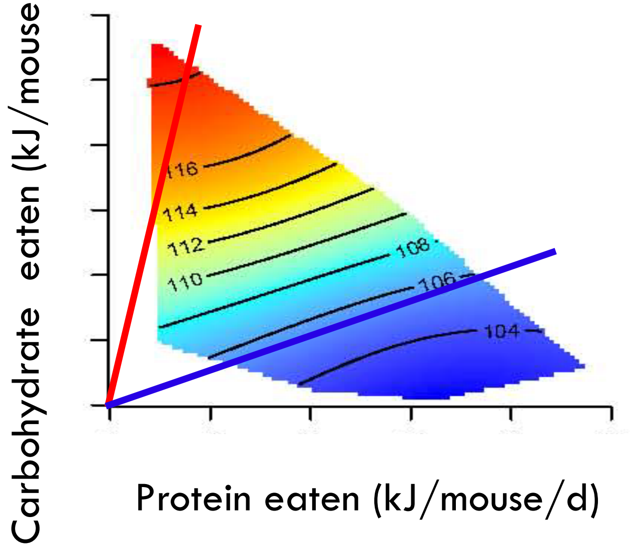
Independent experiment (unpub.)



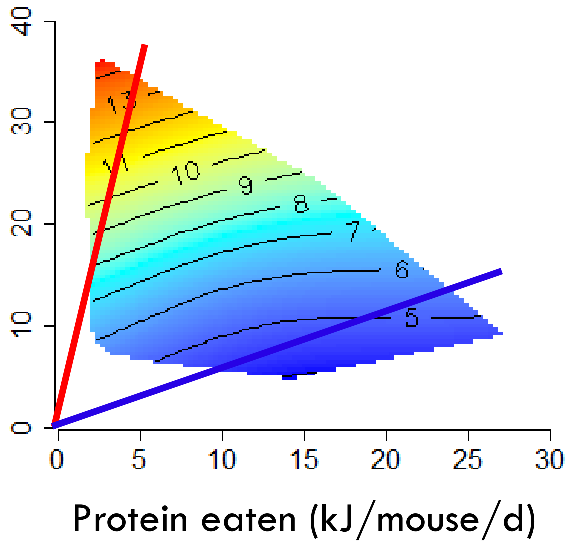
Data from Paula Juricic, Linda Partridge; in Solon-Biet et al. 2019 *Nature Metabolism,* 1: 532–545.

Mechanisms related to ageing biology

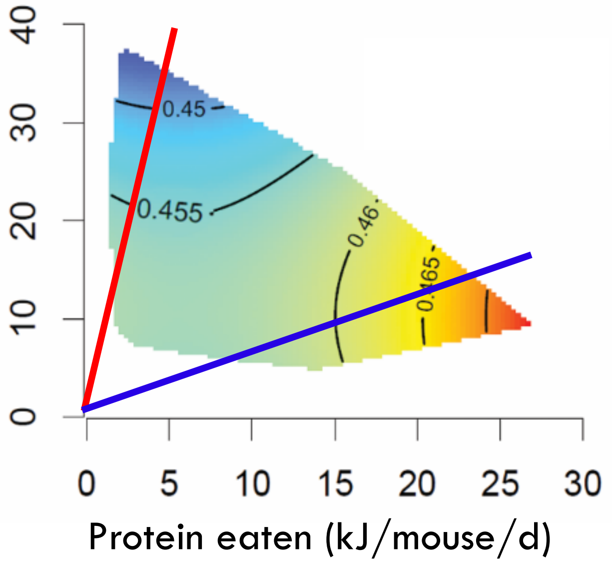
Median lifespan



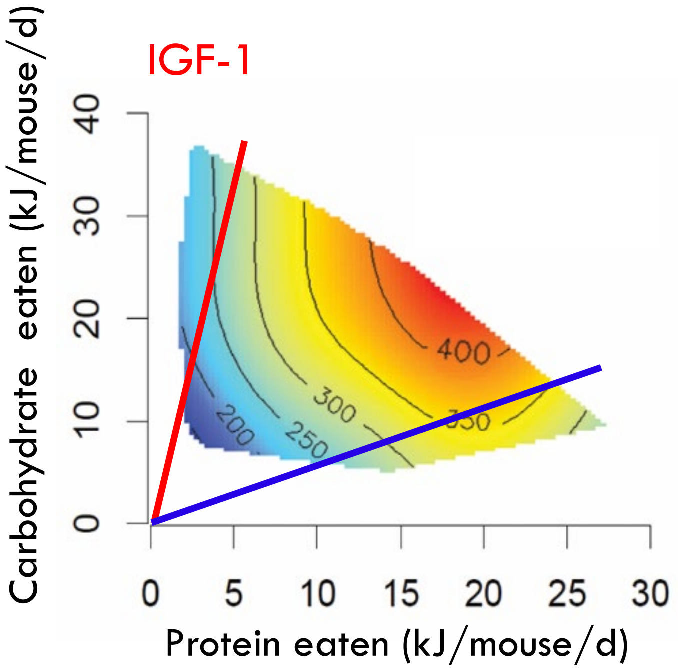
Telomere length



mTOR activation

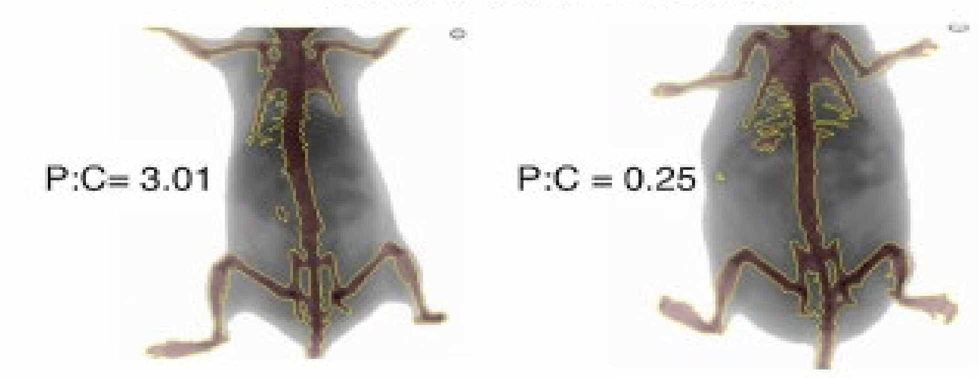


IGF-1

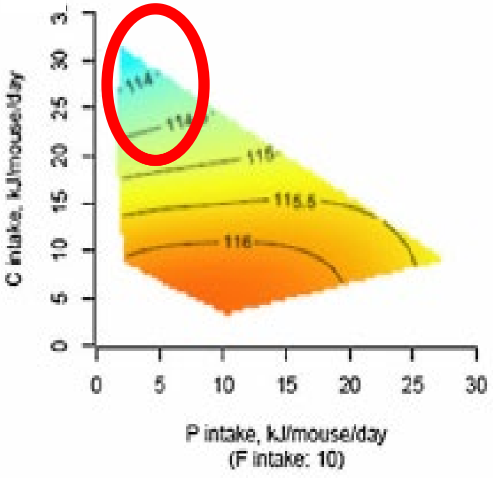


Gokarn et al., *J. Gerontol Biol. Sci.*, 2018, 73: 446-449.

Mid- and early-latelife cardiometabolic health best on low P:C diets
BUT body fat was high due to ‘protein leverage’ (weak in mice, but there)

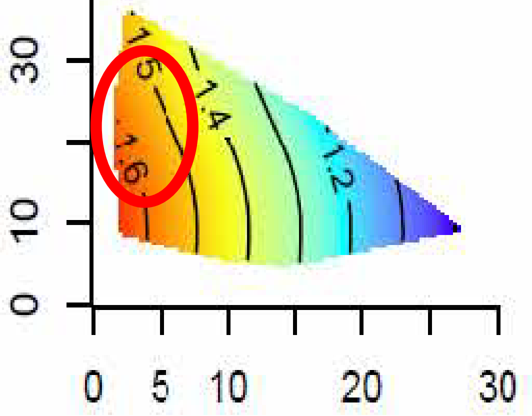


Blood pressure



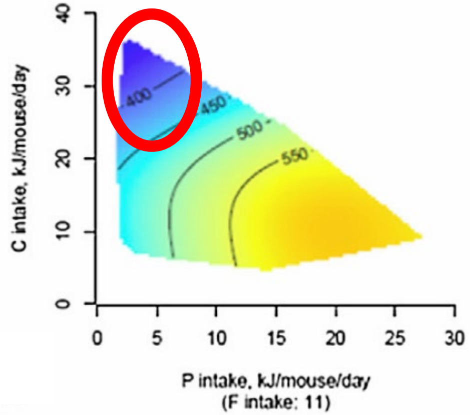
Immune markers

(spleen CD4: CD8)

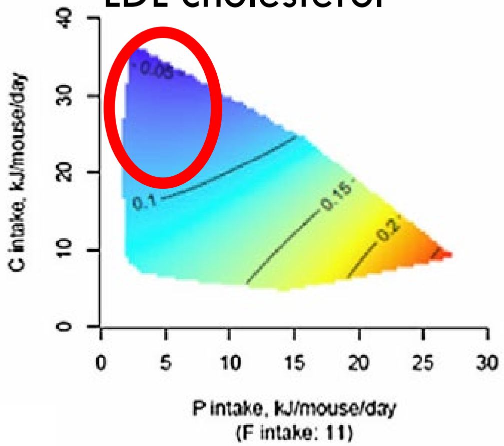


(Le Couteur et al. (2015). *J. Gerontol.*)

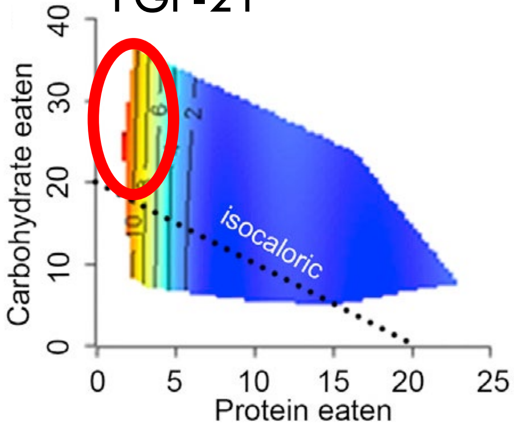
Glucose tolerance test



LDL cholesterol



FGF-21



(Solon-Biet et al. (2016). *Cell Metab.*)

Anti-ageing drugs interact with and act on the same pathways as nutrients

Cell Metabolism

CellPress

Article

Nutritional reprogramming of mouse liver proteome is dampened by metformin, resveratrol, and rapamycin

David G. Le Couteur,[1,2,3,*] Samantha M. Solon-Biet,[1] Benjamin L. Parker,[4] Tamara Pulpitel,[1] Amanda E. Brandon,[1,5] Nicholas J. Hunt,[2,3] Jibran A. Wali,[1] Rahul Gokarn,[1] Alistair M. Senior,[1] Gregory J. Cooney,[1] David Raubenheimer,[1,6] Victoria C. Cogger,[2,3] David E. James,[1,6] and Stephen J. Simpson[1,6,7,*]

[1]Charles Perkins Centre, University of Sydney, NSW 2006, Australia
[2]Centre for Education and Research on Ageing, Concord RG Hospital, NSW 2139, Australia
[3]ANZAC Research Institute, Sydney, NSW 2139, Australia
[4]Department of Anatomy and Physiology, University of Melbourne, VIC 3010, Australia
[5]School of Medical Sciences, University of Sydney, NSW 2006, Australia
[6]School of Life and Environmental Science, University of Sydney, NSW 2006, Australia
[7]Lead contact
*Correspondence: david.lecouteur@sydney.edu.au (D.G.L.C.), stephen.simpson@sydney.edu.au (S.J.S.)
https://doi.org/10.1016/j.cmet.2021.10.016

What about humans?

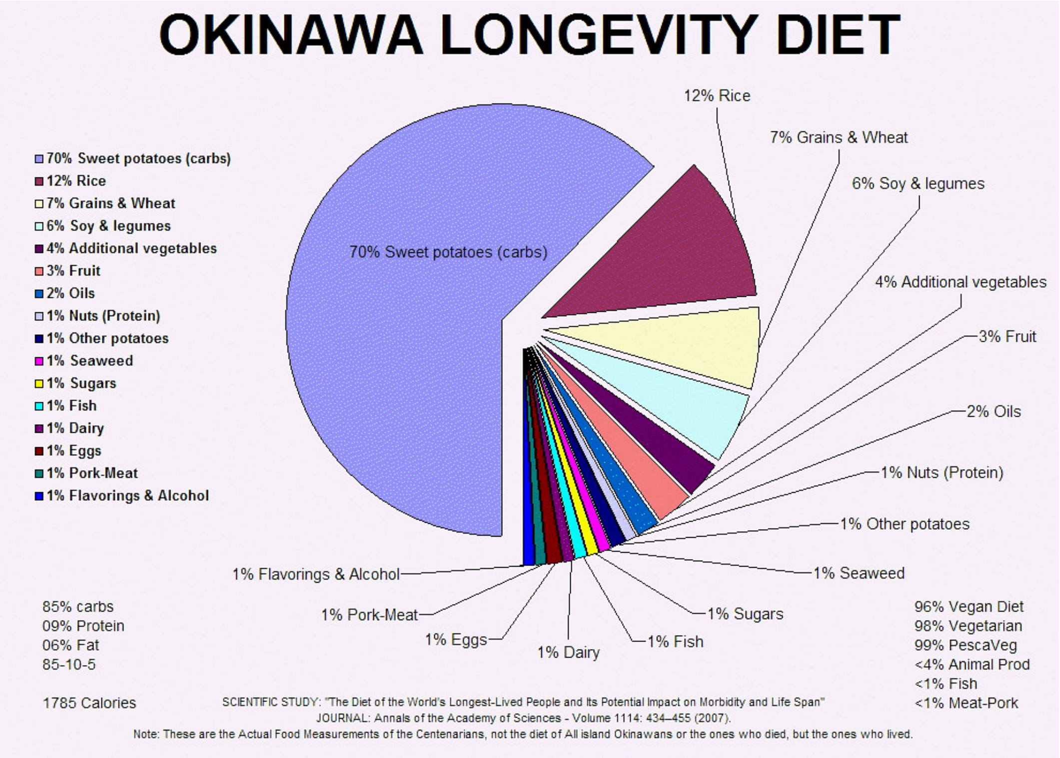
Longest lived populations have low-protein, high-carb, high-fibre diet

NEW HORIZONS

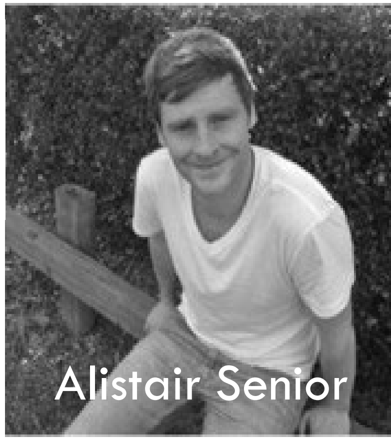
New Horizons: Dietary protein, ageing and the Okinawan ratio

David G. Le Couteur[1,2], Samantha Solon-Biet[1,3], Devin Wahl[1], Victoria C. Cogger[1,2], Bradley J. Willcox[4,5], D. Craig Willcox[4,5,6], David Raubenheimer[1,3], Stephen J. Simpson[1,3]

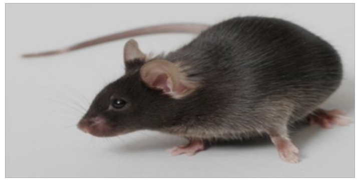
Tsimane from lowland Bolivia



Part 1 of a resolution to the Protein Paradox: Optimal macronutrient ratios vary with age



Benefits of a low-protein, high-carb diet arise during mid-life and early late-life



Dietary macronutrient content, age-specific mortality and lifespan

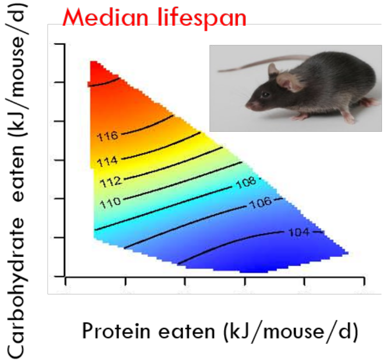
Alistair M. Senior[1,2], Samantha M. Solon-Biet[1,2], Victoria C. Cogger[1,3,4], David G. Le Couteur[1,3,4], Shinichi Nakagawa[5,6], David Raubenheimer[1,2] and Stephen J. Simpson[1,2] (2019, *Proc Roy Soc B*)

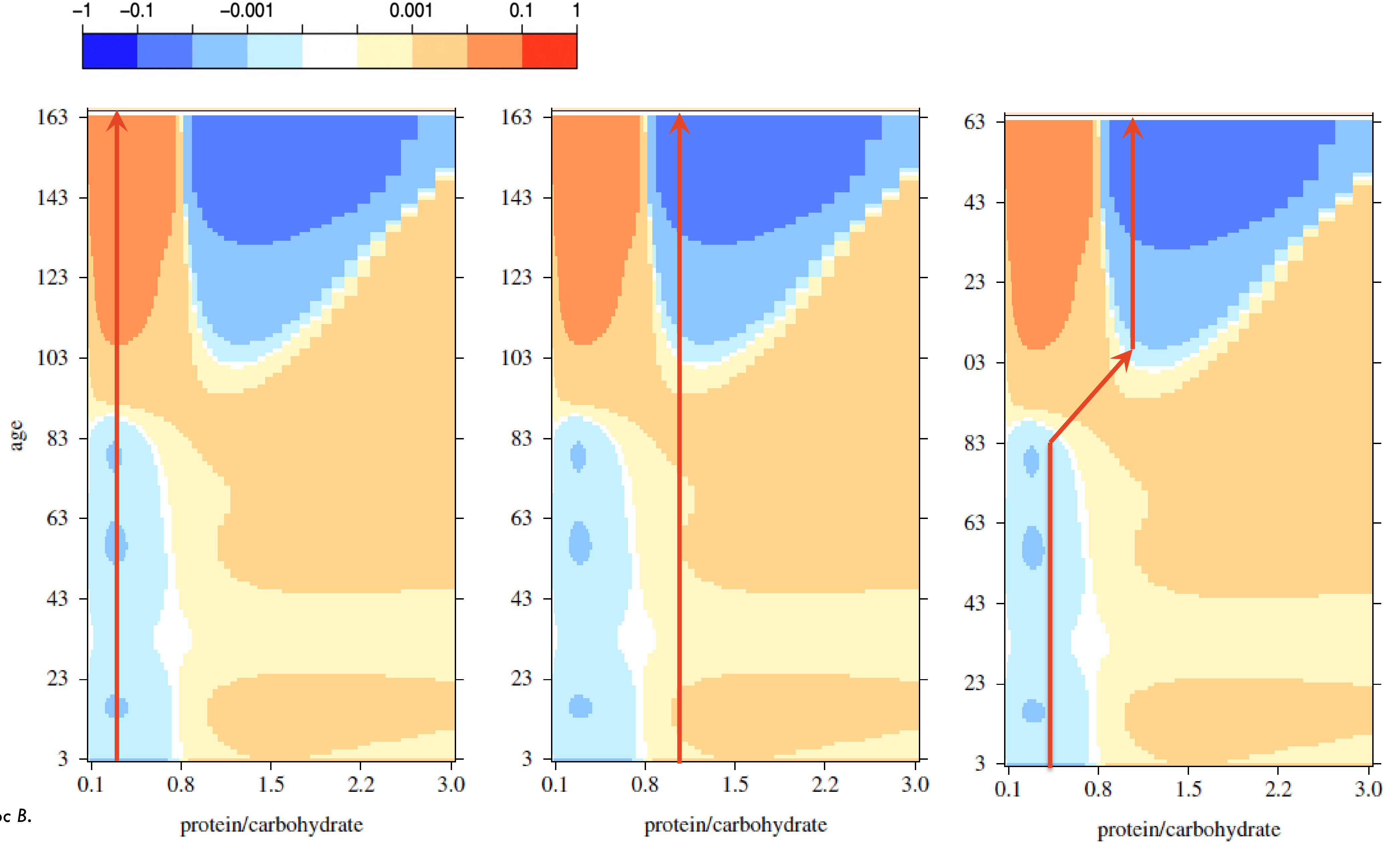
Global associations between macronutrient supply and age-specific mortality

PNAS 2020 117: 30824-30835

Alistair M. Senior[a,b,c,1], Shinichi Nakagawa[a,d,e], David Raubenheimer[a,b], and Stephen J. Simpson[a,b]

Change in age-specific mortality over lifecourse





Senior et al., 2019, *Proc Roy Soc B*.

Part 2 of the resolution to the Protein Paradox:
The *quality* of macronutrients matters

Protein amino acid balance is important

nature metabolism
ARTICLES
https://doi.org/10.1038/s42255-019-0059-2

Branched-chain amino acids impact health and lifespan indirectly via amino acid balance and appetite control

ARTICLE
https://doi.org/10.1038/s41467-020-16568-z OPEN

Restriction of essential amino acids dictates the systemic metabolic response to dietary protein dilution

Yann W. Yap[1,15], Patricia M. Rusu[1,15], Andrea Y. Chan[1], Barbara C. Fam[2], Andreas Jungmann[3,4], Samantha M. Solon-Biet[5], Christopher K. Barlow[6], Darren J. Creek[6,7], Cheng Huang[6], Ralf B. Schittenhelm[6], Bruce Morgan[8], Dieter Schmoll[9], Bente Kiens[10], Matthew D. W. Piper[11], Mathias Heikenwälder[12], Stephen J. Simpson[5], Stefan Bröer[13], Sofianos Andrikopoulos[2], Oliver J. Müller[4,14] & Adam J. Rose[1]✉

Cell Metabolism
Article

mino Acid Balance to the In Silico-Translated Exome Optimizes Growth and Reproduction without Cost to Lifespan

Matthew D.W. Piper,[1,8,9,*] George A. Soultoukis,[2,8] Eric Blanc,[3] Andrea Mesaros,[2] Samantha L. Herbert,[4] Paula Juricic,[2] Xiaoli He,[5] Ilian Atanassov,[2] Hanna Salmonowicz,[2] Mingyao Yang,[6] Stephen J. Simpson,[7] Carlos Ribeiro,[4] and Linda Partridge[1,2,10,*]

Carbohydrate type and digestibility

nature metabolism

ARTICLES

https://doi.org/10.1038/s42255-021-00393-9

Check for updates

Impact of dietary carbohydrate type and protein–carbohydrate interaction on metabolic health

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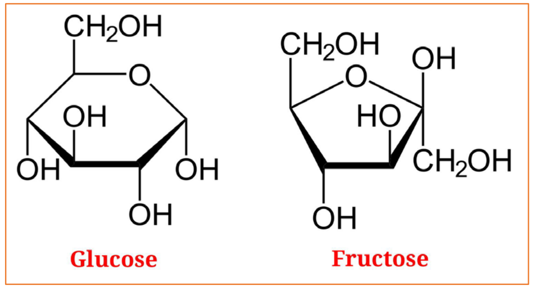
Reduced protein intake, through dilution with carbohydrate, extends lifespan and improves mid-life metabolic health in animal models. However, with transition to industrialised food systems, reduced dietary protein is associated with poor health outcomes in humans. Here we systematically interrogate the impact of carbohydrate quality in diets with varying carbohydrate and protein content. Studying 700 male mice on 33 isocaloric diets, we find that the type of carbohydrate and its digestibility profoundly shape the behavioural and physiological responses to protein dilution, modulate nutrient processing in the liver and alter the gut microbiota. Low (10%)-protein, high (70%)-carbohydrate diets promote the healthiest metabolic outcomes when carbohydrate comprises resistant starch (RS), yet the worst outcomes were with a 50:50 mixture of monosaccharides fructose and glucose. Our findings could explain the disparity between healthy, high-carbohydrate diets and the obesogenic impact of protein dilution by glucose–fructose mixtures associated with highly processed diets.

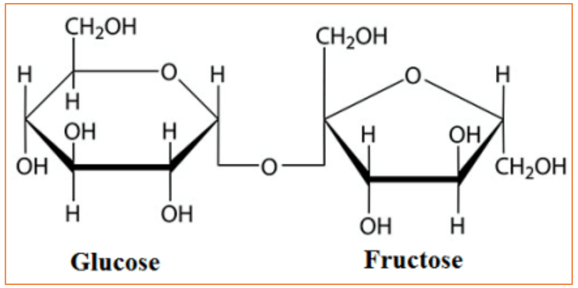
Major nutritive carbohydrates

High Fructose Corn Syrup



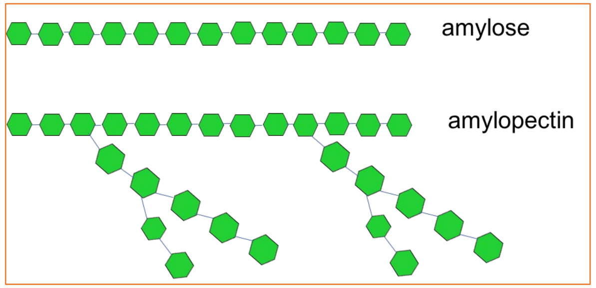
- *~1:1 mixture of gluc and fruc*
- *Major added sugar in US*

Sucrose



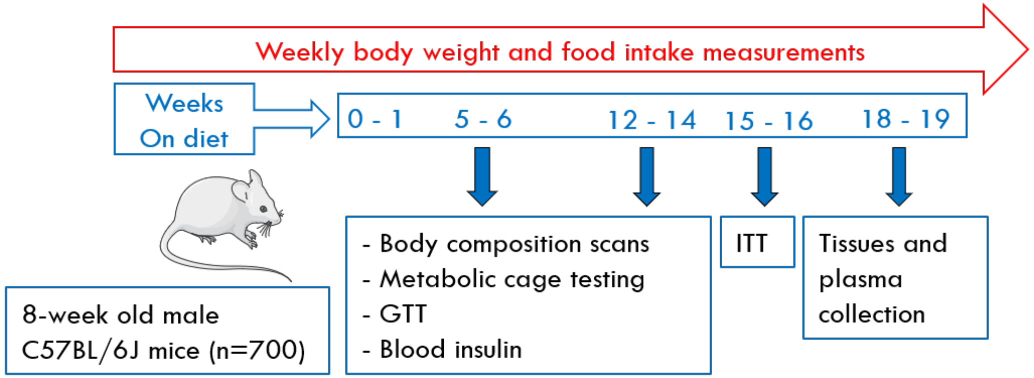
- *Disaccharide of gluc and fruc*
- *Major added sugar globally*

Starch



- *Polysaccharide of glucose*
- *Linear form (amylose)*
- *Branched form (amylopectin)*
- *Resistant starch has high amylose content than native starch which reduces the surface area for digestion*

Study timeline and design



33 isocaloric diets, which systematically varied:

- ➢ **Protein** (5, 10, 15, 20, 30%)
- ➢ **Carbohydrates** (50, 60, 65, 70, 75%):
- • Glucose + Fructose (0:100, 25:75, 50:50, 75:25; 100:0) vs Sucrose
- • Sucrose vs Native Starch (20:80; 35:65; 50:50; 65:35; 80:20)
- • Sucrose vs Resistant Starch (35:65; 80:20)
- ➢ **Fat** fixed (20%)

Wali et al. (2021) *Nature Metabolism*

Summary of results

Beneficial effects: ↑ ↓ **Harmful effects:** ↑ ↓ **Neutral effects:** ↔ **NS = Native Starch** **RS = Resistant Starch**

Protein %	5 %		10 %						20 %		30 %
Carb type	Sucrose	NS	Glucose	Fructose	HFCS	Sucrose	NS	RS	HFCS	Sucrose	HFCS
Energy Intake	↑	↑↑	↑	↑	↑↑	↑	↑↑	↑↑	↑	↔	↔
Fat Mass	↓↓	↓↓	↔	↔	↑↑	↓	↔	↓↓	↑	↑	↑
Liver Fat	↑	↑↑	↔	↔	↑↑	↔	↑	↔	↑	↔	↔
Plasma FGF-21	↑↑	↑↑	↑	↑	↑	↑	↑	↑↑	↔	↔	↓↓
Insulin Sensitivity	↑↑	↑↑	↓	↓	↓↓	↑	↓	↑↑	↓	↔	↓

- Metabolically healthy
- Sucrose ≈ Starch

- RS > Sucrose ≥ Starch ≈ Glucose ≈ Fructose > HFCS

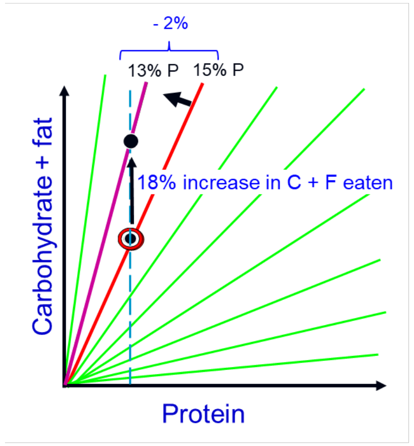
- Sucrose > HFCS

- Reduces harms of HFCS

- Decreasing P:C generally beneficial except when carbohydrate was HFCS
- Resistant starch + 10% protein = metabolically the best diet
- HFCS + 10% protein = metabolically the worst diet

Putting the pieces together in the modern human food environment

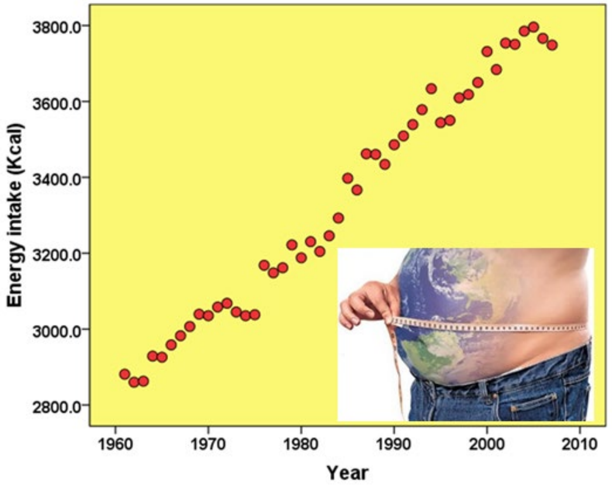
Strong P appetite



x

fat + carb-rich, fibre-poor

=



Is protein leverage the mechanism?

Dr Euri Martinez Steele

Prof Carlos Monteiro

USA diet survey

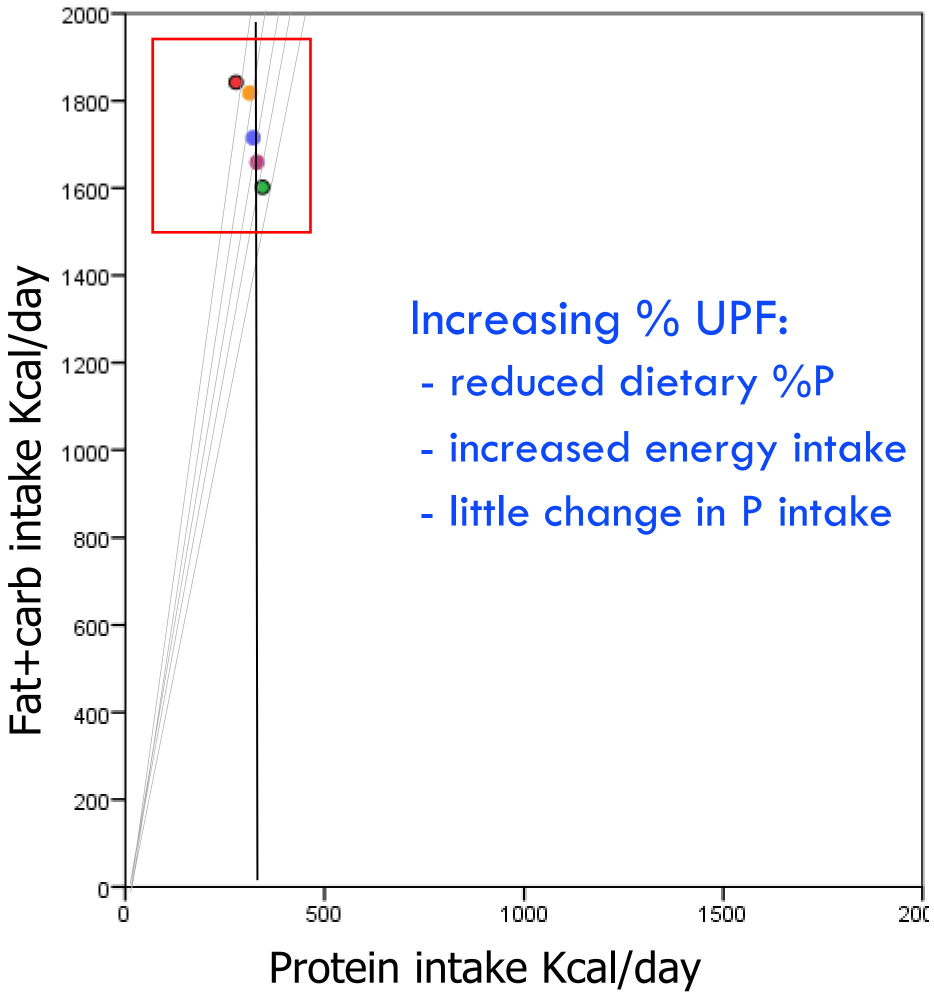
nhanes

National Health and Nutrition Examination Survey

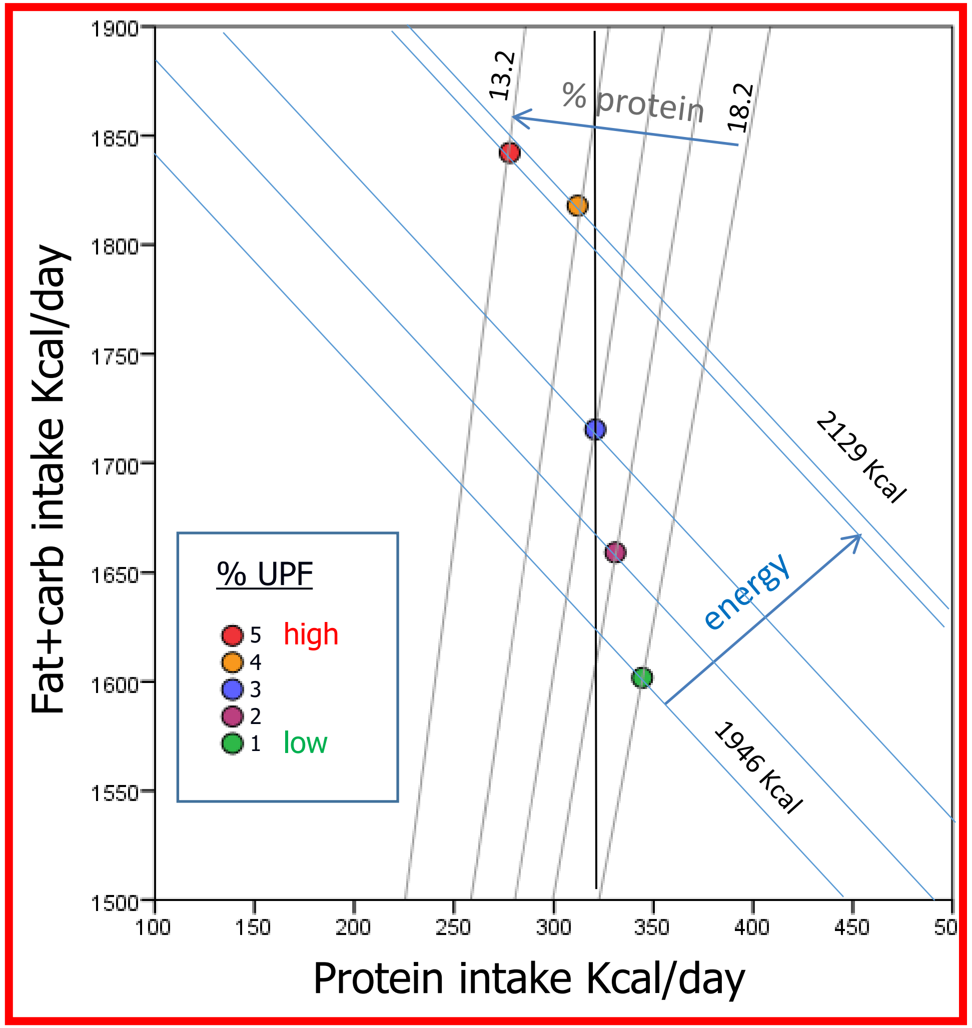
- diets of 9357 American people
- 5 groups based on % energy contributed by UPF

Martínez Steele, E., Raubenheimer, D., Simpson, S.J., Baraldi, L.G., Monteiro, C.A. (2018) *Public Health Nutrition* 21: 114–124.

What we found



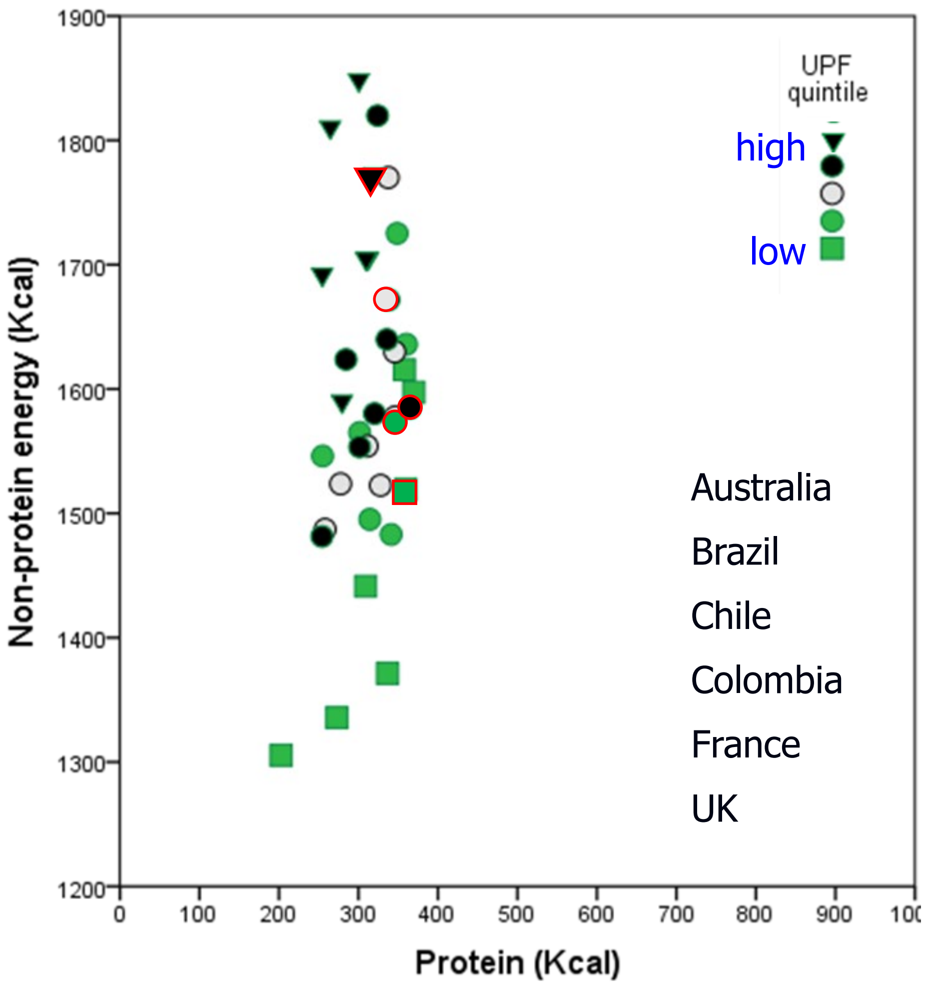
Increasing % UPF:
- reduced dietary %P
- increased energy intake
- little change in P intake

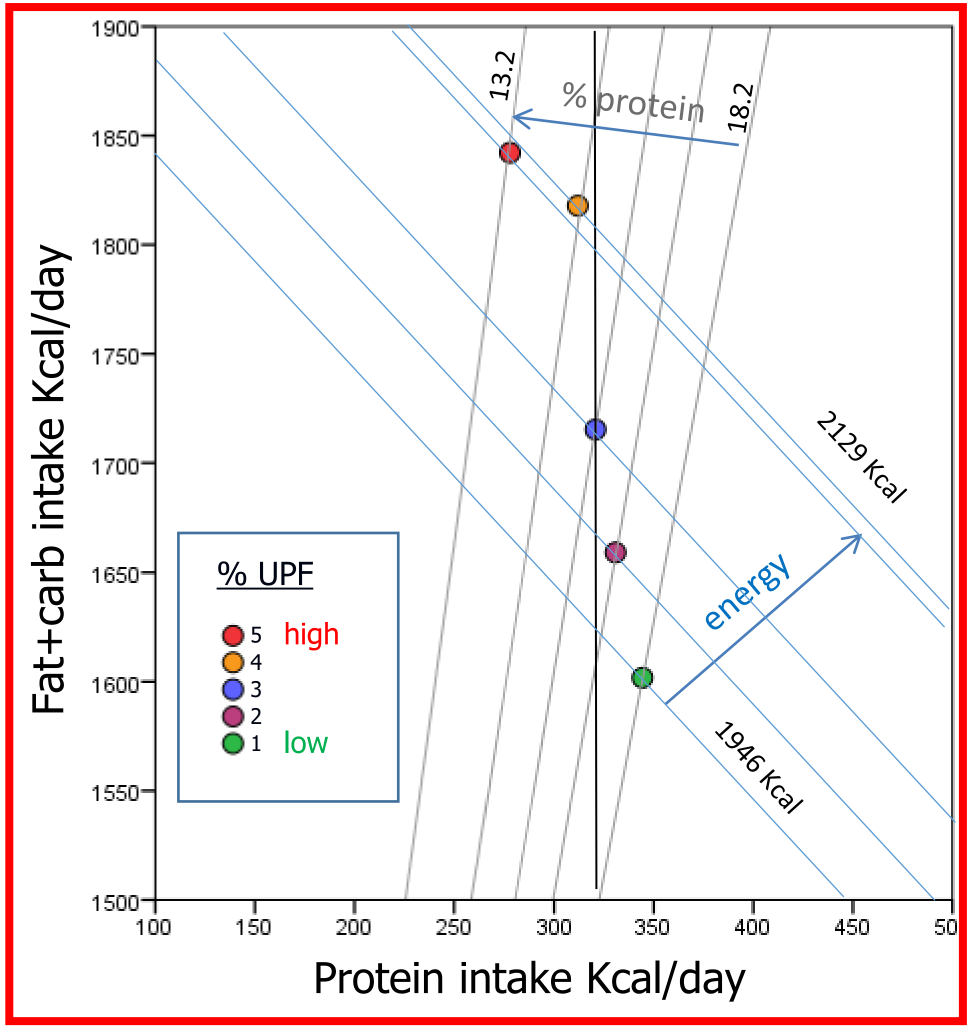


As predicted by PLH

Martinez et al. 2018 *Public Health Nutrition* 21:114-124

Not just the USA

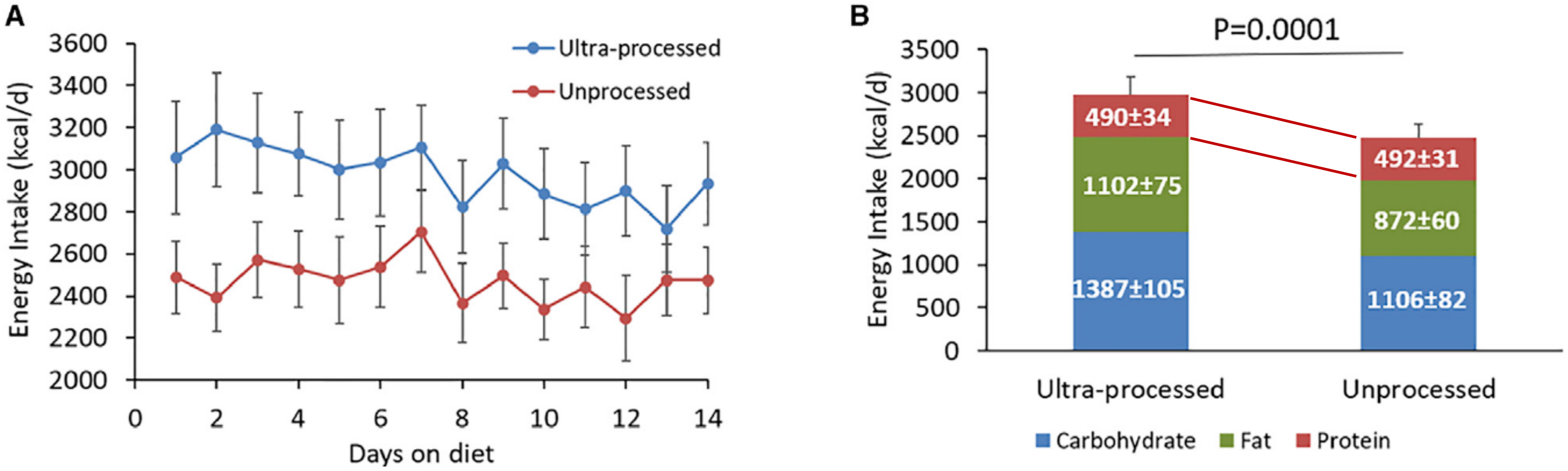


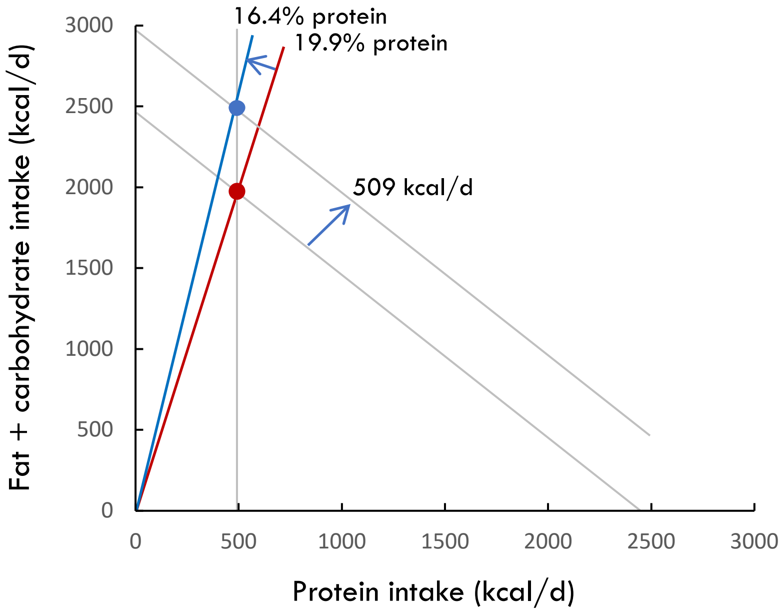


Martinez et al., in prep

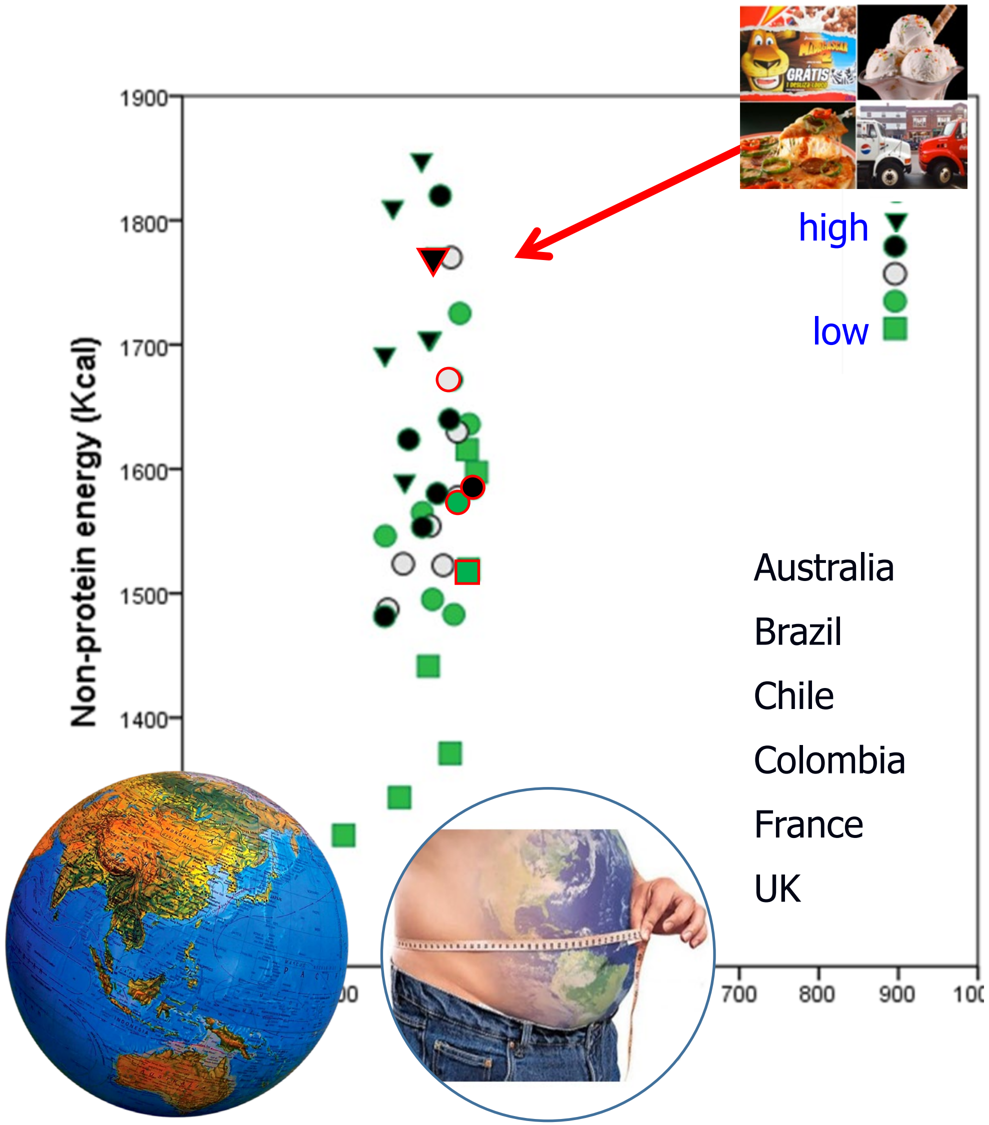
Ultra-Processed Diets Cause Excess Calorie Intake and Weight Gain: An Inpatient Randomized Controlled Trial of *Ad Libitum* Food Intake

Kevin D. Hall,[1,5,*] Alexis Ayuketah,[1] Robert Brychta,[1] Hongyi Cai,[1] Thomas Cassimatis,[1] Kong Y. Chen,[1] Stephanie T. Chung,[1] Elise Costa,[1] Amber Courville,[2] Valerie Darcey,[1] Laura A. Fletcher,[1] Ciaran G. Forde,[4] Ahmed M. Gharib,[1] Juen Guo,[1] Rebecca Howard,[1] Paule V. Joseph,[3] Suzanne McGehee,[1] Robert Ouwerkerk,[1] Klaudia Raisinger,[2] Irene Rozga,[1] Michael Stagliano,[1] Mary Walter,[1] Peter J. Walter,[1] Shanna Yang,[2] and Megan Zhou[1]

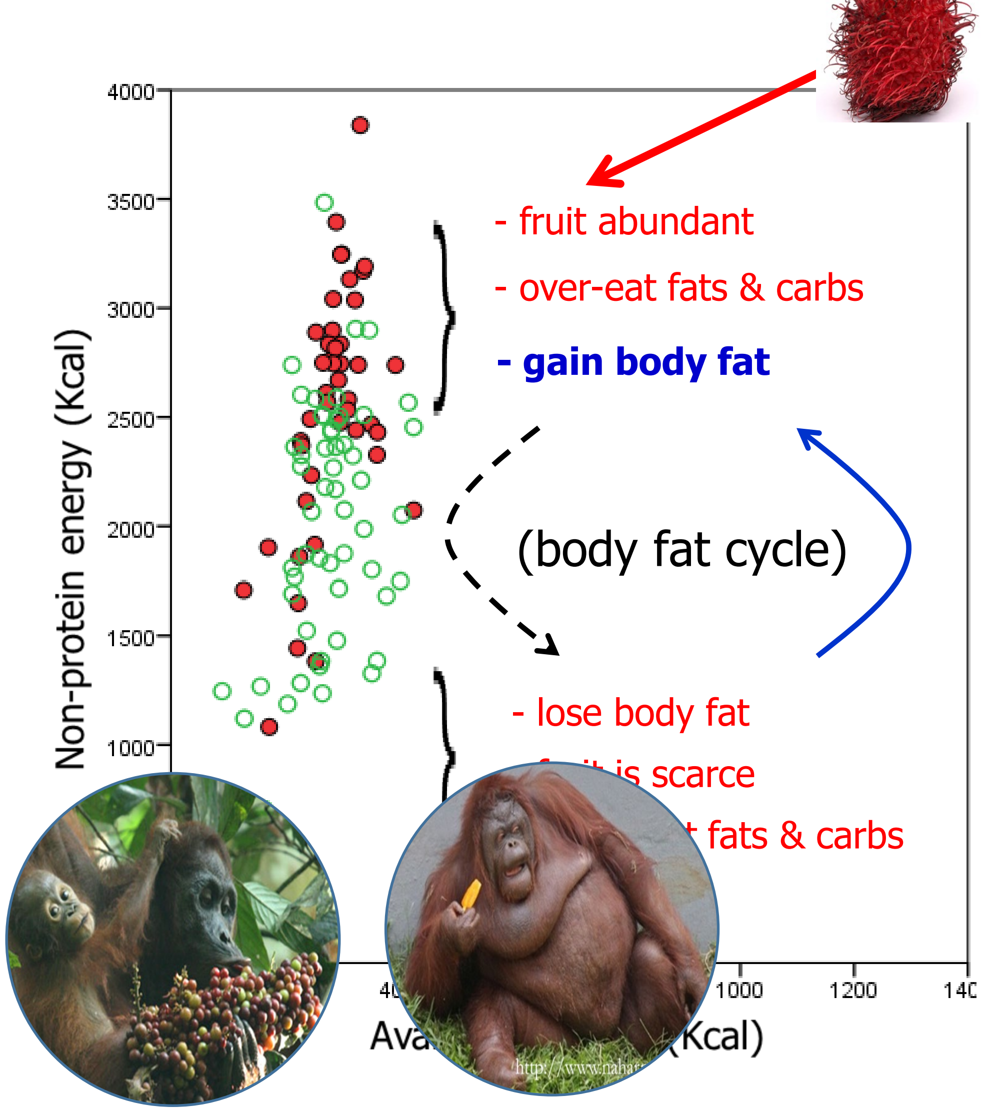




We're just like orangs ...

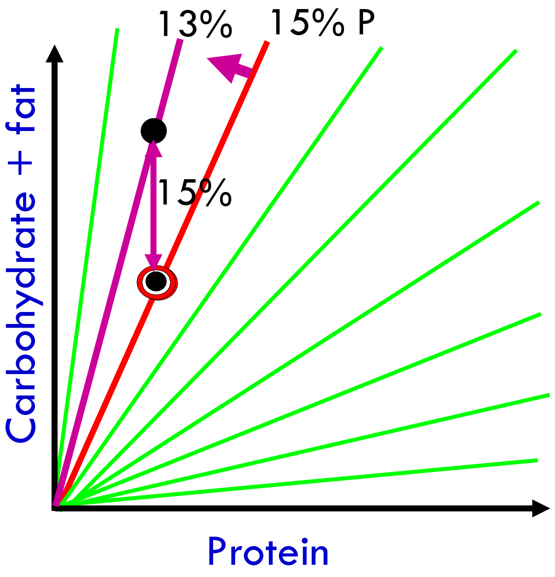


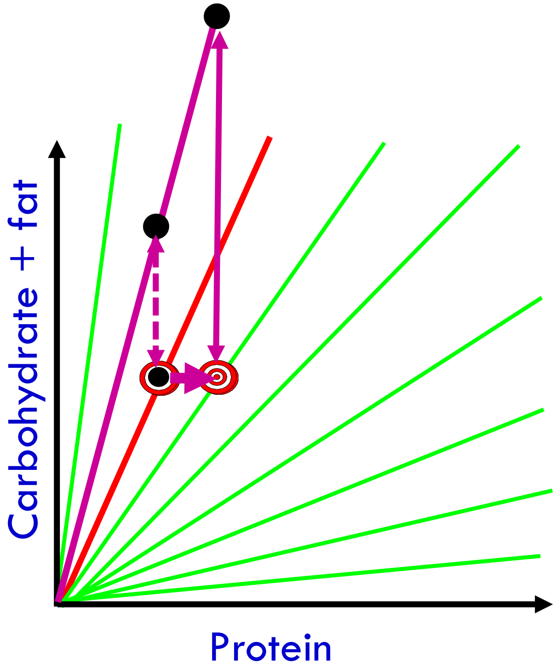
BUT: 1. UPF rather than fruit



2. humans never have a shortage

Made worse if protein target increases



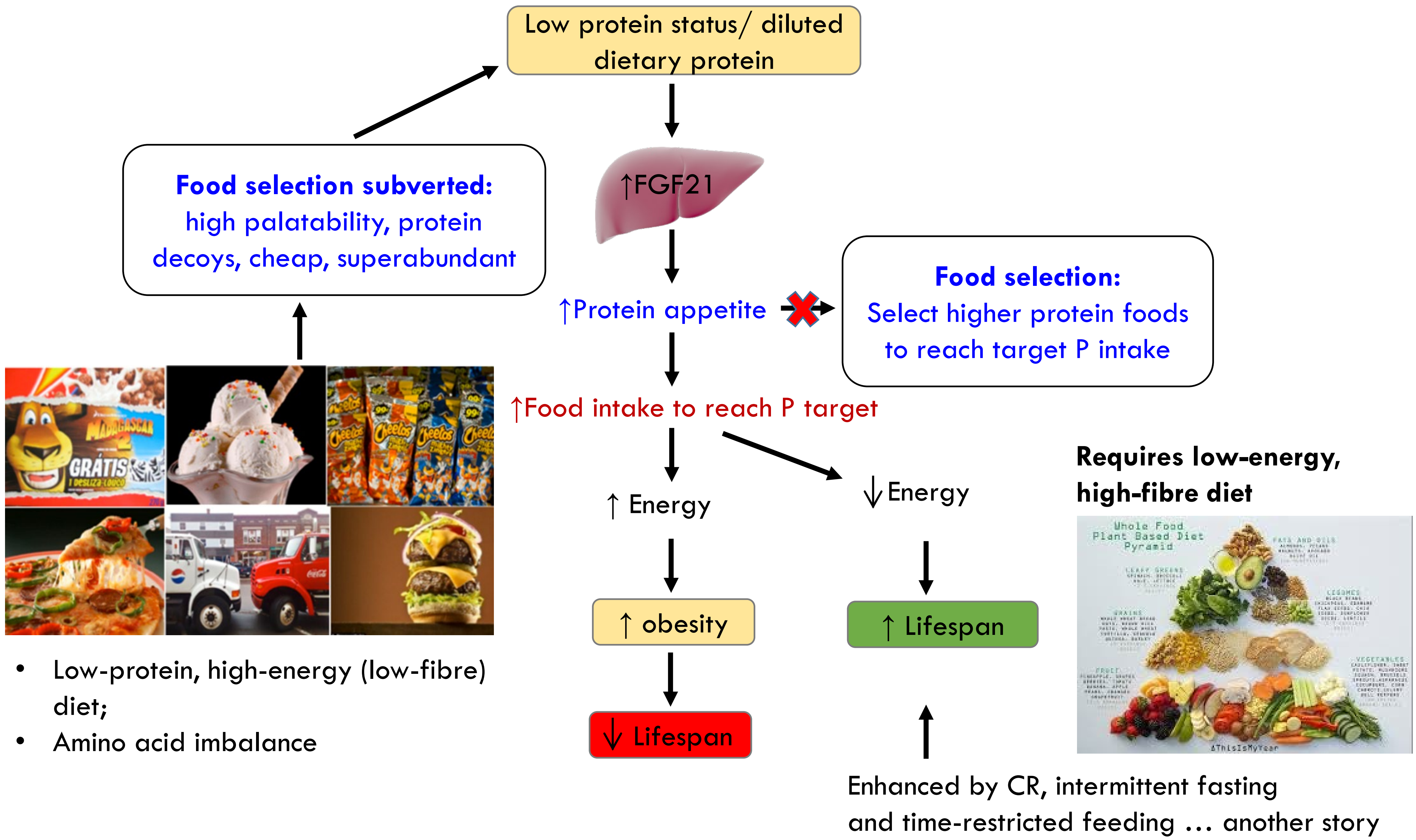


Why might P target increase?

- Developmental changes – lean growth, reproduction
- Decrease in protein efficiency – menopause, old age, insulin resistance
- Physiological adaptation to a higher P diet or anabolic regime
- Genetic adaptation to ancestral diet
- Epigenetic adaptation – paternal, in utero, early development

The GECKO
Gametic Epigenetics Consortium against Obesity

Simpson, S.J. and Raubenheimer, D. (2005) *Obesity Reviews* 6: 133-142.



Low protein status/ diluted dietary protein
↑FGF21
↑Protein appetite
Food selection:
Select higher protein foods to reach target P intake
↑Food intake to reach P target
↑ Energy
↑ obesity
↓ Lifespan
↓Energy
↑ Lifespan
Food selection subverted:
high palatability, protein decoys, cheap, superabundant
Low-protein, high-energy (low-fibre) diet;
Amino acid imbalance
Requires low-energy, high-fibre diet
Whole Food Plant Based Diet Pyramid
Enhanced by CR, intermittent fasting and time-restricted feeding … another story

Acknowledgements

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Kwang-Pum Lee
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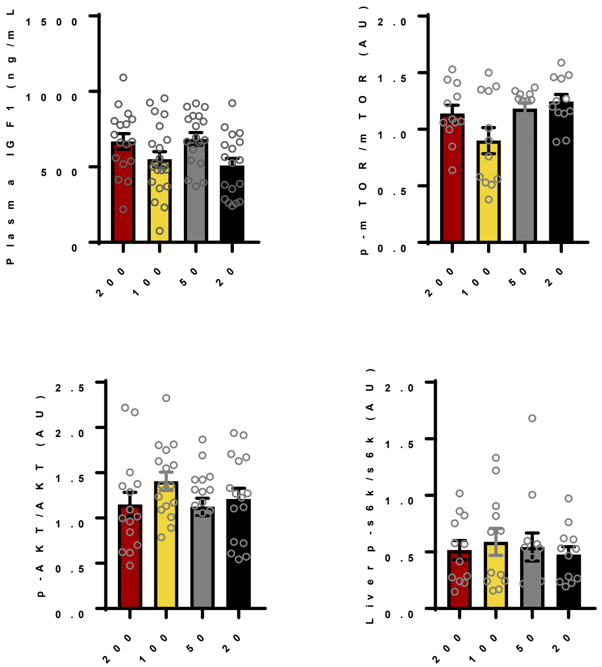
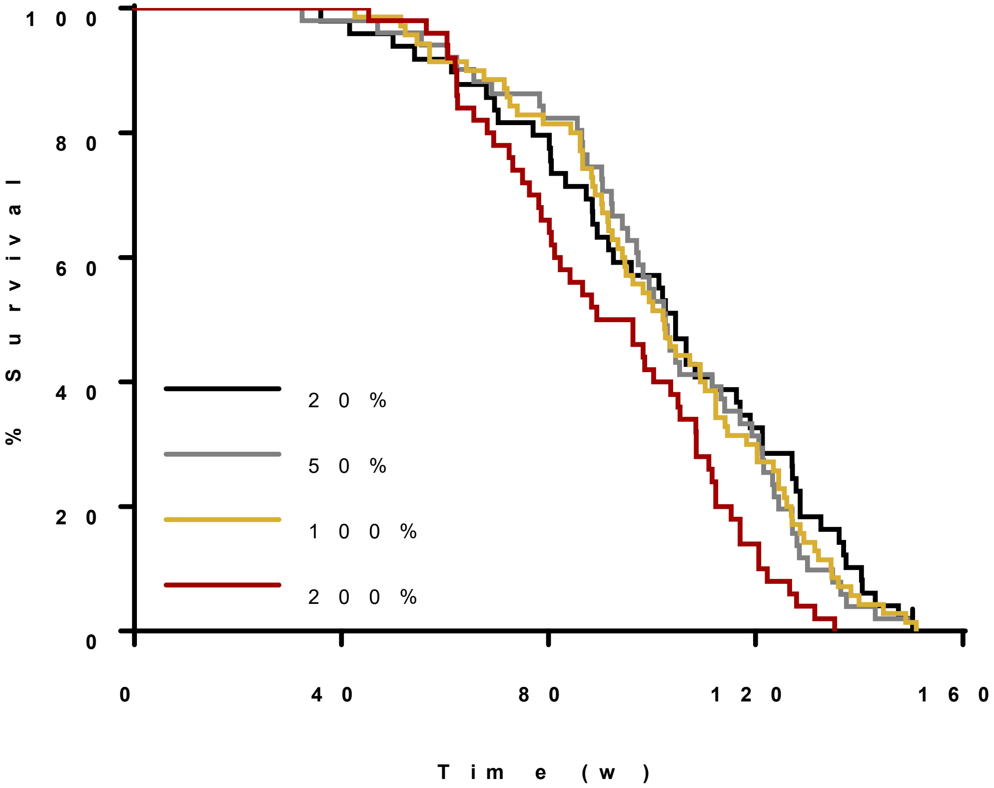
Adam Rose
Chris Morrison
Lindsay Wu
Andy Holmes
… and many others

THE UNIVERSITY OF SYDNEY
Australian Government
National Health and Medical Research Council
NHMRC
aarf Ageing and Alzheimer's Research Foundation
cera
ANZAC RESEARCH INSTITUTE
Australian Government
Australian Research Council

Lifespan reduced ca 10% on high-BCAA, moderate protein, high-carb diet

But not associated with mTOR, IGF-1

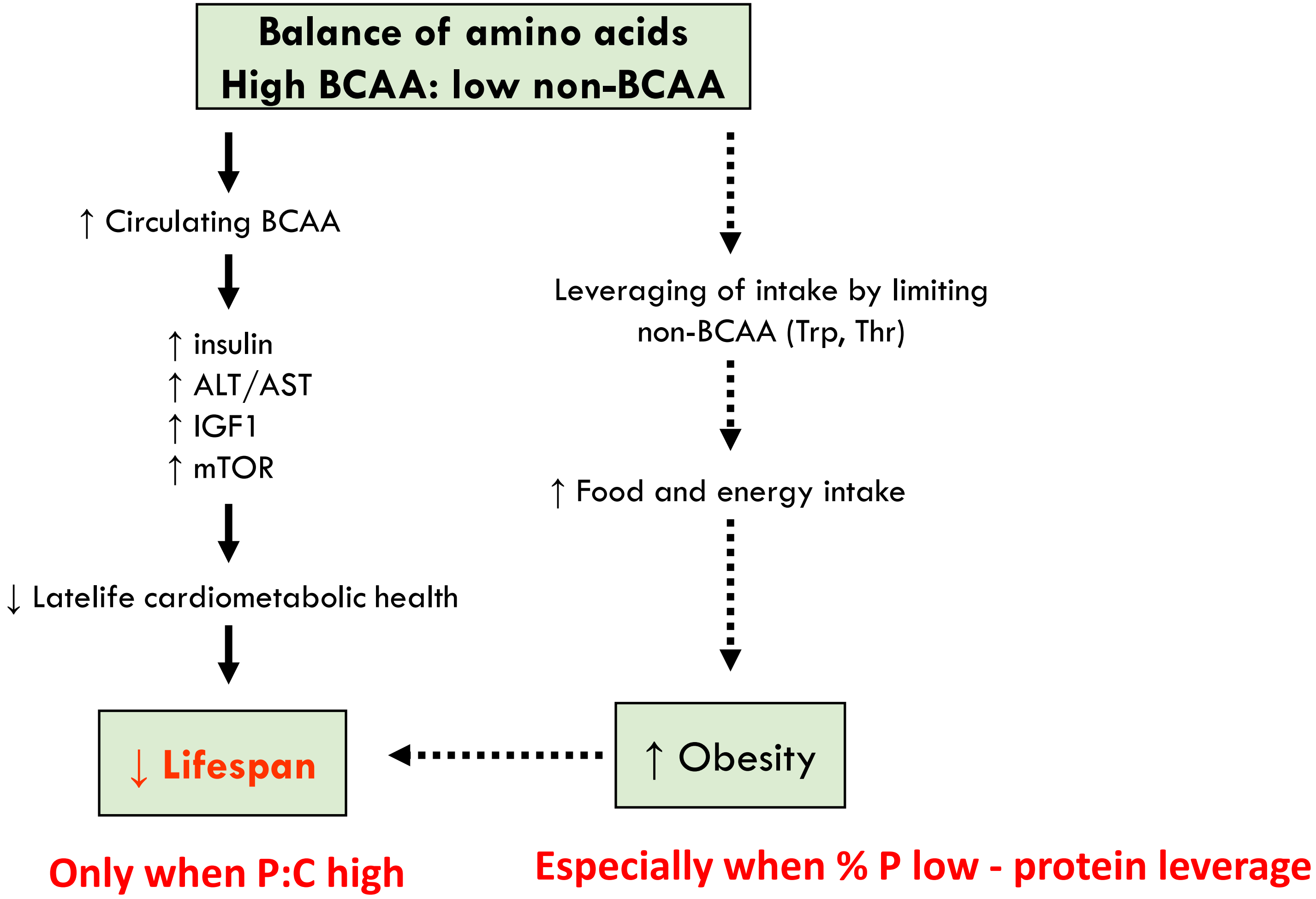




Due to obesity

Solon-Biet et al. 2019, *Nature Metabolism* 1: 532–545.

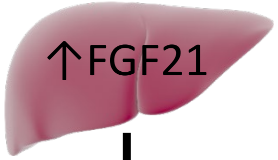
The double burden of BCAAs



Putting the pieces together in the modern human food environment

Low protein status/ reduced dietary protein

↓



↑FGF21

↓

↑Protein appetite → **Food selection:** Select higher protein foods to reach target P intake

↓

↑Food intake to reach P target

↓ ↑ Energy → ↑ EE (DIT) → ↑ obesity → ↓ Lifespan

↓ ↓Energy → ↓ EE → ↑ Lifespan

Requires low-energy, high-fibre diet

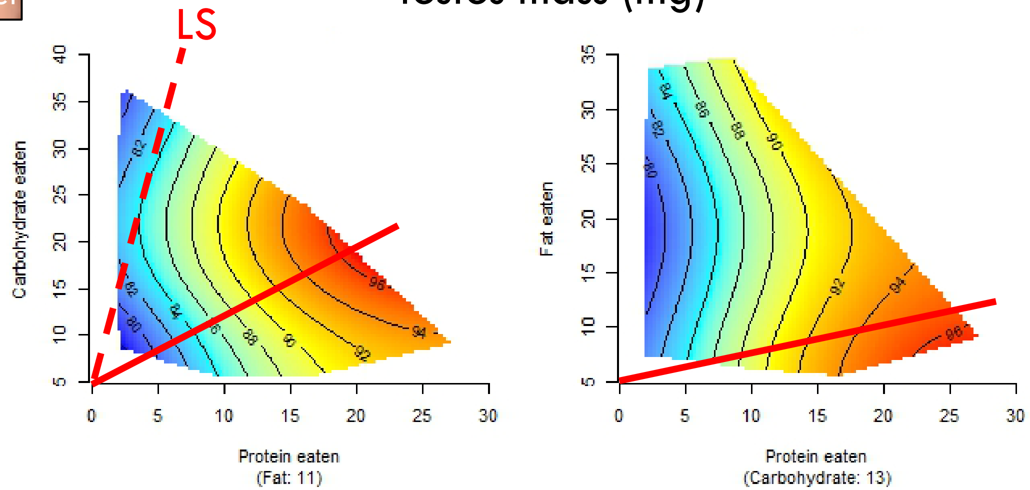
Food selection subverted – high palatability, cheap, superabundant

- **Prolonged low-protein, high-energy, high-sugar, low-fibre diet;**
- **Amino acid imbalance – e.g. high BCAA:Tryp**

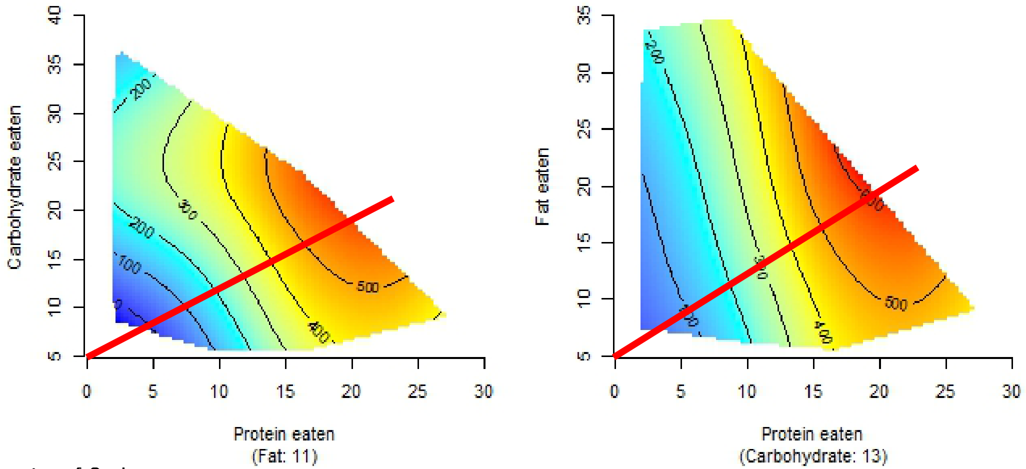
Optimal macronutrient ratios vary with age, e.g. reproduction vs lifespan

Males

Testes mass (mg)

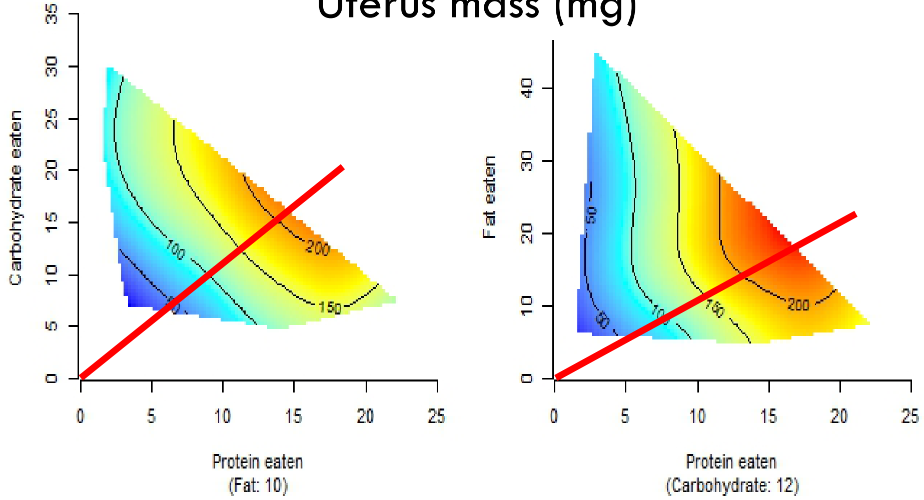


Seminal vesicle mass (mg)

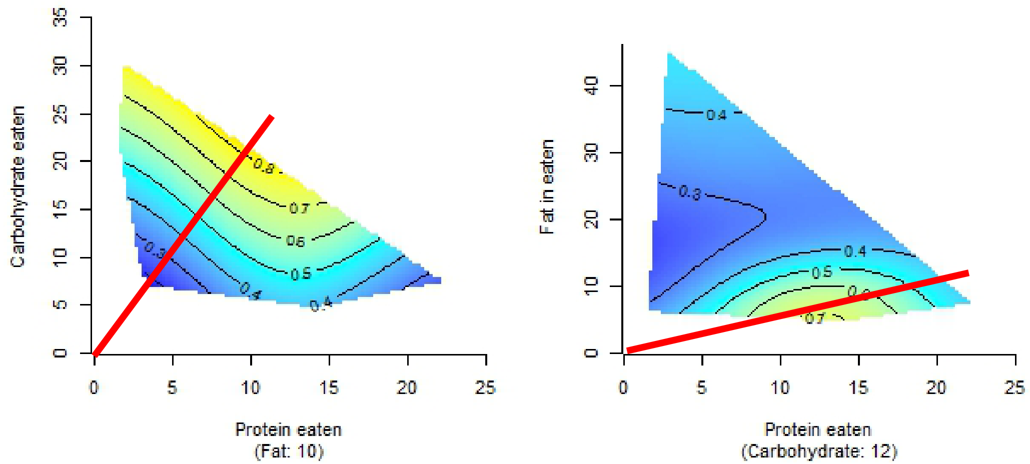


Females

Uterus mass (mg)



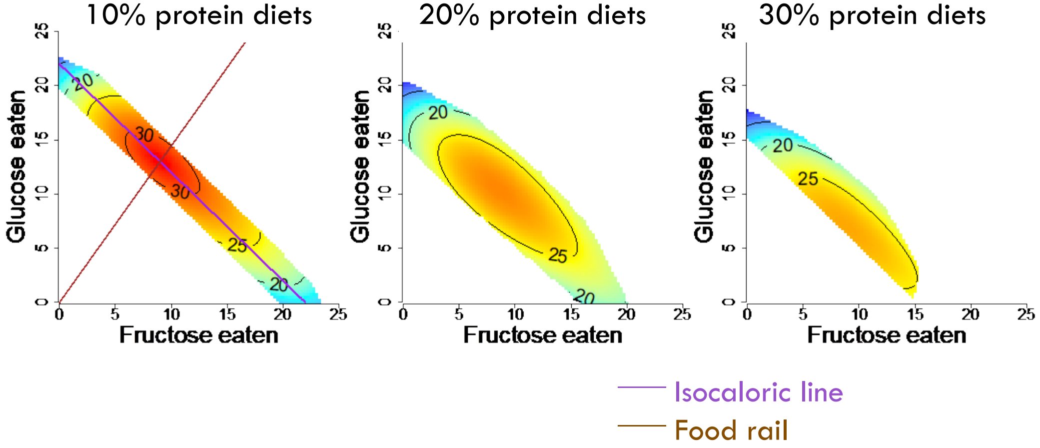
Corpora lutea (no. per section)



Solon-Biet, S.M., Walters, K.A., Simanainen, U., McMahon, A.C., Ruohonen, K., Ballard, J.W.O., Raubenheimer, D., Handelsman, D.J., Le Couteur, D.G., and Simpson, S.J. (2015) *PNAS* 112: 3481-3486.

1:1 mixture of glucose to fructose gave worst outcomes

Percent fat mass



Insulin tolerance test AUC