CATÓLICA
ESCOLA SUPERIOR
DE BIOTECNOLOGIA

PORTO

IMPULSE

"IMpact of a PULSE-based partial replacement diet on metabolome and health"

by

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Porto | 09th of July 2019

Summary

- **Project context**
- **Pulses intake and health**
- **IMPULSE project**
  - I. Objectives
  - II. Study design
  - III. Data collection
  - IV. Preliminary results
  - V. Conclusions

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2016 INTERNATIONAL YEAR OF PULSES

TRUE

TRansition paths to sUstainable legume-based systems in Europe

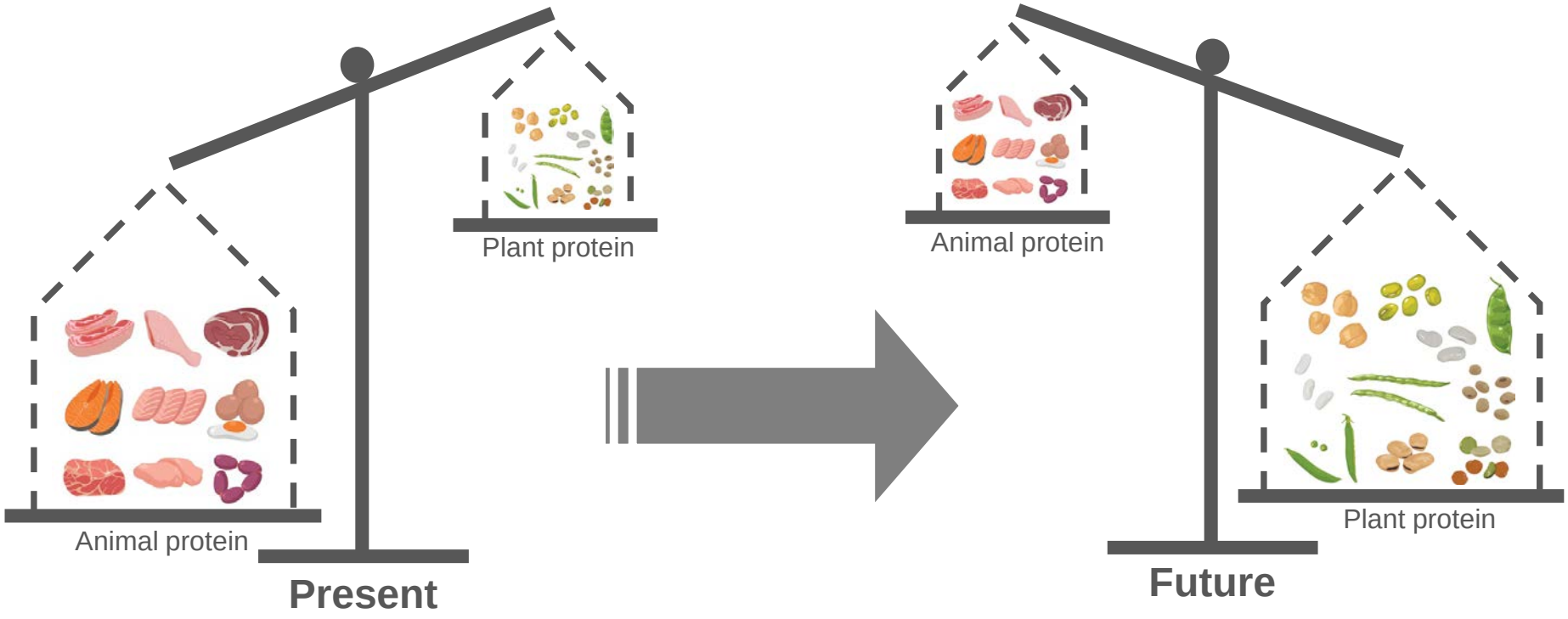
UMA INICIATIVA ASSOCIAÇÃO PORTUGUESA DOS NUTRICIONISTAS

UMA PORÇÃO LEGUMINOSAS POR DIA

European Commission

EU 2018: Report on the "Development of plant proteins in the European Union."

What we know…



↓ greenhouse-gas emissions
↓ land use
↓ water use

(Lancet. 2019 Feb 2;393(10170):447-492. doi: 10.1016/S0140-6736(18)31788-4. Epub 2019 Jan 16)

Health Hazards of red meat

J Intern Med. 2017 Feb;281(2):106-122. doi: 10.1111/joim.12543

Review — Journal of INTERNAL MEDICINE

doi: 10.1111/joim.12543

Potential health hazards of eating red meat

A. Wolk

From the Institute of Env

cohorts, summary results for the consumption of unprocessed red meat of 100 g day^{-1} varied from nonsignificant to statistically significantly increased risk (11% for stroke and for breast cancer, 15% for cardiovascular mortality, 17% for colorectal and 19% for advanced prostate cancer); for the consumption of 50 g day^{-1} processed meat, the risks were statistically significantly increased for most of the studied diseases (4% for total prostate cancer, 8% for cancer mortality, 9% for breast, 18% for colorectal and 19% for pancreatic cancer, 13% for stroke, 22% for total and 24% for cardiovascular mortality and 32% for diabetes). Potential biological mechanisms underlying the observed

> 100g/d unprocessed red meat
> 50g/d processed meat

- CVD mortality
- Stroke
- DM2
- Cancer

PORTUGAL

Prevalence of inadequate **red and processed meat** intake (>100g/day) – **22,5%**
Prevalence of inadequate **processed meat** intake (>50g/day) **– 6,3%**

Average daily **red meat** intake: **51,6g**
Average daily **processed meat** intake: **20,7g**

The National Food, Nutrition and Physical Activity Survey - Lopes C. *et al*, 2017

Benefits of plant-based diets

Crit Rev Food Sci Nutr. 2017 Nov 22;57(17):3640-3649. doi: 10.1080/10408398.2016.1138447

Vegetarian, vegan diets and multiple health outcomes: A systematic review with meta-analysis of observational studies

Monica
[a]Departr
Florence

with the risk of incidence from total cancer (RR 0.85; 95% CI, 0.75 to 0.95), despite obtained only in a limited number of studies. **Conclusions:** This comprehensive meta-analysis reports a significant protective effect of a vegetarian diet versus the incidence and/or mortality from ischemic heart disease (−25%) and incidence from total cancer (−8%). Vegan diet conferred a significant reduced risk (−15%) of incidence from total cancer.

- Ischemic heart disease
- Total Cancer

Effect of plant-based diets on obesity-related inflammatory profiles: a systematic review and meta-analysis of intervention trials

F. Eichelmann,[1] L. Schwingshackl,[2] V. Fedirko[3] and K. Aleksandrova[1]

Obesity reviews (2016) 17, 1067–1079

Association between plant-based diets and plasma lipids: a systematic review and meta-analysis

Yoko Yokoyama, Susan M. Levin, and Neal D. Barnard

Nutrition Reviews (2017) Vol. 75(9):683–698

Effect of vegetarian dietary patterns on cardiometabolic risk factors in diabetes: A systematic review and meta-analysis of randomized controlled trials

Effie Viguiliouk [a, b], Cyril WC. Kendall [a, b, c], Hana Kahleová [d, e], Dario Rahelić [f], Jordi Salas-Salvadó [g, h], Vivian L. Choo [a, b, i], Sonia Blanco Mejia [a, b], Sarah E. Stewart [a, b], Lawrence A. Leiter [a, b, j, k, l], David JA. Jenkins [a, b, j, k, l], John L. Sievenpiper [a, b, k, l, *]

Clinical Nutrition 38 (2019) 1133-1145

Circulation. 2019 Apr 9;139(15):1828-1845. doi: 10.1161/CIRCULATIONAHA.118.035225

Meta-Analysis of Randomized Controlled Trials of Red Meat Consumption in Comparison With Various Comparison Diets on Cardiovascular Risk Factors

Marta Guasch-Ferré | ,Ambika Satija | ,Stacy A. Blondin | ,Marie Janiszewski | ,Ester Emlen | ,Lauren E. O'Connor | ,

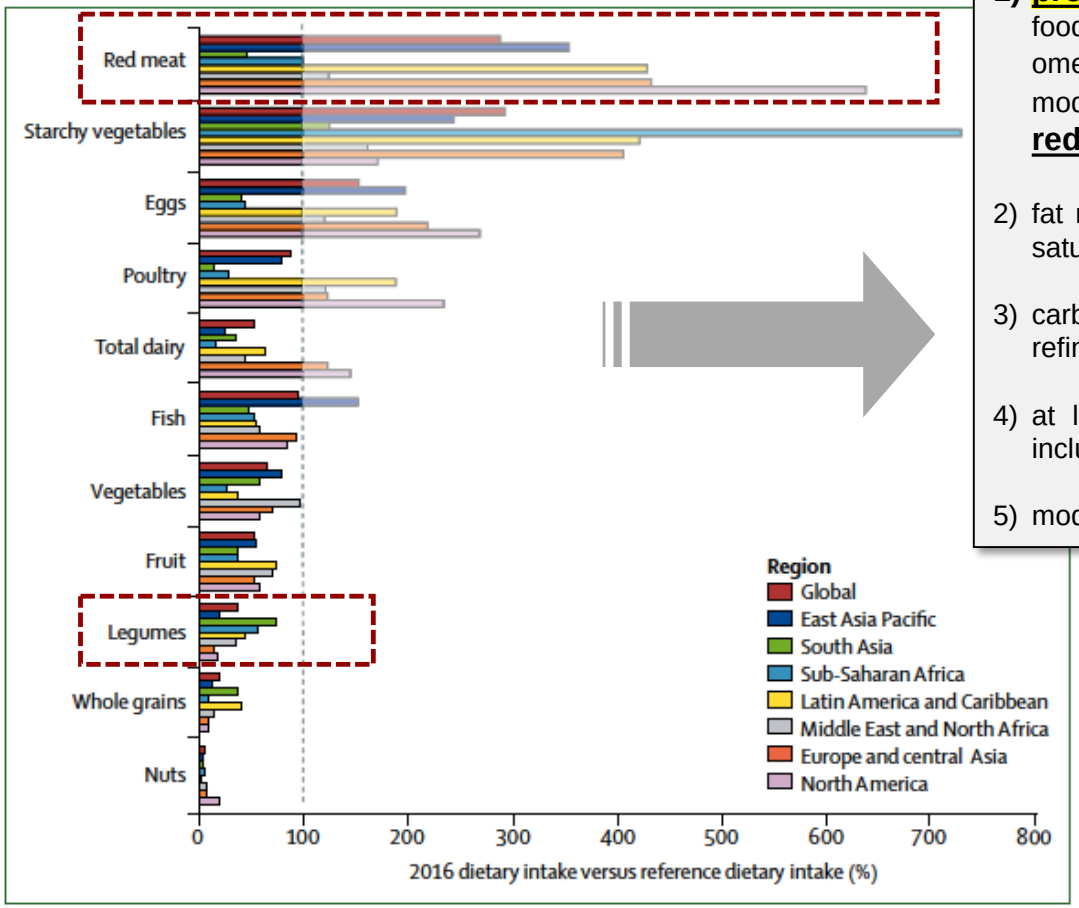
composition of the comparison diet. Substituting red meat with high-quality plant protein sources, but not with fish or low-quality carbohydrates, leads to more favorable changes in blood lipids and lipoproteins.

Food in the Anthropocene: the EAT–*Lancet* Commission on healthy diets from sustainable food systems

Walter Willett, Johan Rockström, Brent Loken, Marco Springmann, Tim Lang, Sonja Vermeulen, Tara Garnett, David Tilman, Fabrice DeClerck, Amanda Wood, Malin Jonell, Michael Clark, Line J Gordon, Jessica Fanzo, Corinna Hawkes, Rami Zurayk, Juan A Rivera, Wim De Vries, Lindiwe Majele Sibanda, Ashkan Afshin, Abhishek Chaudhary, Mario Herrero, Rina Agustina, Francesco Branca, Anna Lartey, Shenggen Fan, Beatrice Crona, Elizabeth Fox, Victoria Bignet, Max Troell, Therese Lindahl, Sudhvir Singh, Sarah E Cornell, K Srinath Reddy, Sunita Narain, Sania Nishtar, Christopher J L Murray

"Universal healthy reference diet"

(low risk of major chronic disease and overall wellbeing)



1) **protein sources primarily from plants**, including soy foods, other **legumes**, and nuts, fish or alternative sources of omega-3 fatty acids several times per week with optional modest consumption of poultry and eggs, and **low intakes of red meat, if any, especially processed meat;**

2) fat mostly from unsaturated plant sources, with low intakes of saturated fats, and no partly hydrogenated oils;

3) carbohydrates primarily from whole grains with low intake of refined grains and less than 5% of energy from sugar;

4) at least five servings of fruits and vegetables per day, not including potatoes; and;

5) moderate dairy consumption as an option.

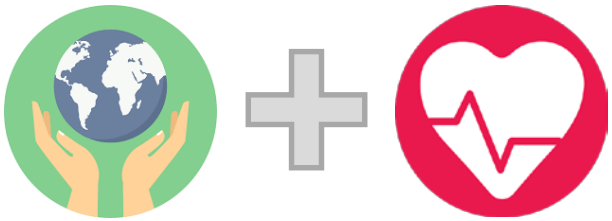


***Figure 1*: Diet gap between dietary patterns in 2016 and reference diet intakes of food**

(Lancet. 2019 Feb 2;393(10170):447-492. doi: 10.1016/S0140-6736(18)31788-4. Epub 2019 Jan 16)

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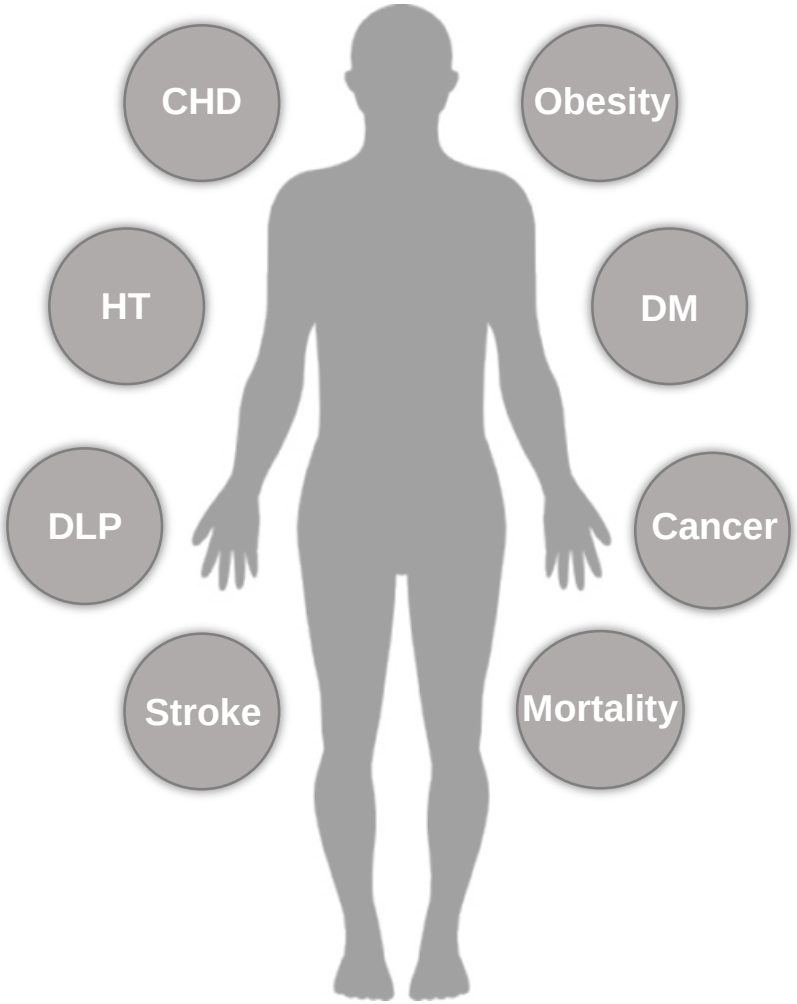
CHRONIC DISEASES



(Boye *et al*, 2010; Messina V., 2014; Mudryj NA. *et al*, 2014; Singh *et al*, 2016; Havemeier S. *et al*, 2017; Grela *et al*, 2017)

Healthy dietary guidelines

(examples)

Country	Food group	Serving[a]	Frequency
Brazil	"Beans"	**NP**	**NS**
Canada	"Meat alternatives"	**0.75 cup**	**NS**
USA	"Vegetables"; "Protein foods"	**0.5 cup**	**3 servings/wk[b]** **6 servings/wk[c]**
Greece	"Olives, pulses and nuts"	**100g**	**3-4 servings/wk**
Portugal	"Legumes"	**80g**	**1-2 servings/d**
Spain	"Lean meat, poultry, fish, eggs, legumes, nuts and seeds"	**~80g (raw)**	**≥2 servings/wk**
Kenya	"Legumes and pulses"	**0.5 cup**	**≥4 servings/wk**
Sierra Leone	"Pulses and legumes"	**0.5 cup**	**1 serving/d**
South Africa	"Dry beans, split peas, lentils and soya"	**NP**	**NS ("regularly")**
Cambodia[d]	"Fish, meat, eggs or beans"	**~60g (raw)**	**≥2-3 servings/d**
India	"Cereals, millets and pulses"	**~30g (raw)[e]**	**~2 servings/d**
Sri Lanka	"Fish, pulses, dried fish, egg, poultry and meat"	**3 tbsp**	**3-4 servings/d**
Australia	"Lean meats and poultry, fish, eggs, tofu, nuts and seeds and legumes/beans" "Vegetables and legumes/beans"	**1 cup (150g)** **0.5 cup (75g)**	**2.5-3 servings/d[f]** **5-6 servings/d[f]**
New Zealand	"Legumes, nuts, seeds, fish and other seafood, eggs, poultry or red meat with fat removed"	**0.75 cup**	**≥2 servings/d**

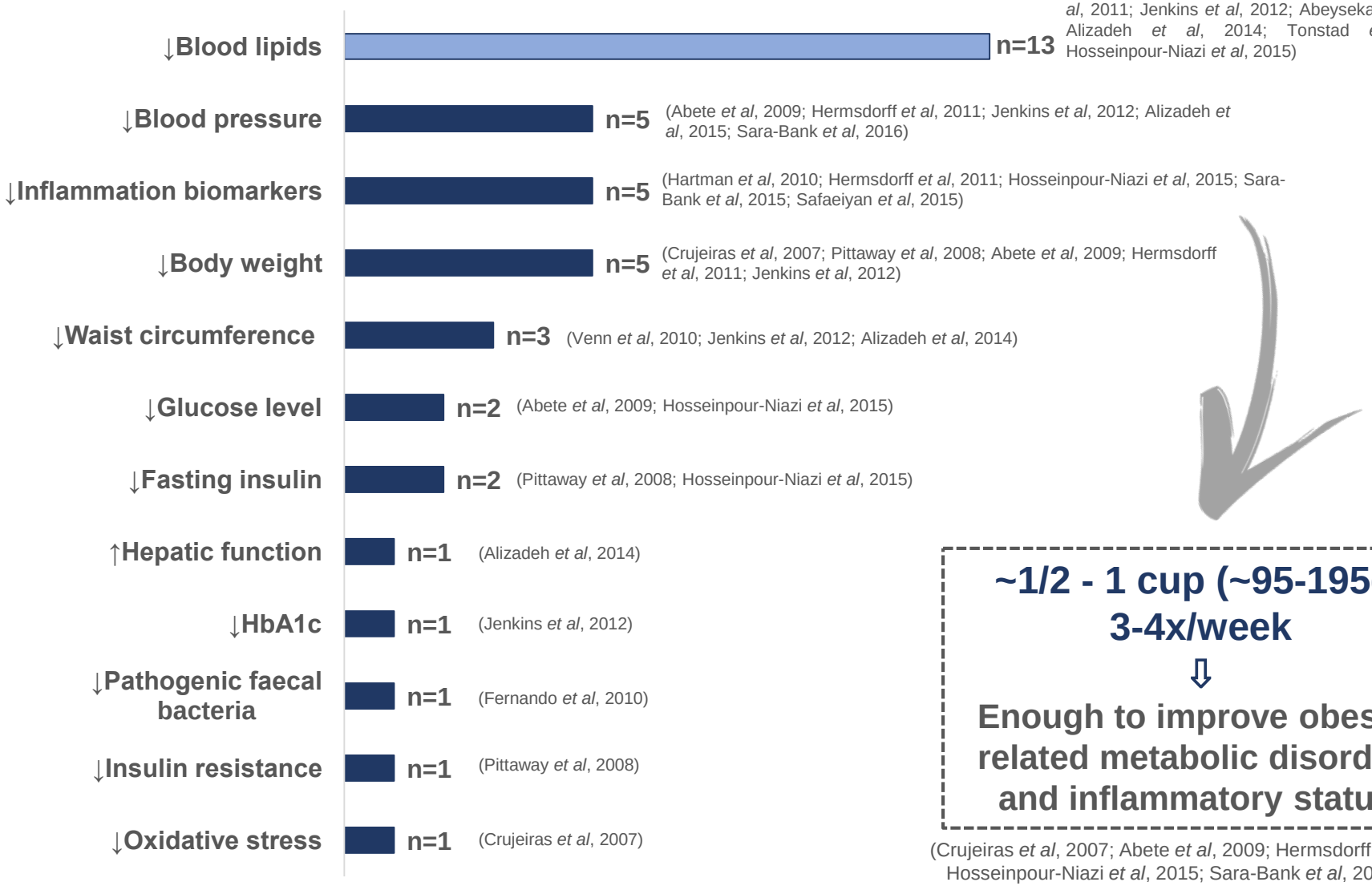
[a]Unless otherwise specified, serving sizes refer to cooked pulses; [b]2,000-calorie level non-vegetarian diet; [c]2,000-calorie level vegetarian diet; [d]School-aged children from 6 to 17 years; [e]Sedentary adult; [f]19-50 years. Abbreviations: d - day; NP – Not provided; NS – Non-specific; wk – week.

≥ 100g/day; cooked
(beans, lentils, chickpeas or peas)

Increase nutrient density of healthy diets!

(Marinangeli *et al,* 2017)

DIETS CONTAINING PULSES



~1/2 - 1 cup (~95-195g)
3-4x/week

⇩

Enough to improve obesity, related metabolic disorders and inflammatory status.

(Crujeiras *et al*, 2007; Abete *et al*, 2009; Hermsdorff *et al*, 2011; Hosseinpour-Niazi *et al*, 2015; Sara-Bank *et al*, 2015, 2016)

↑ **WEIGHT LOSS**
↑ **GLYCEMIC CONTROL**
↑ **LIPID PROFILE**

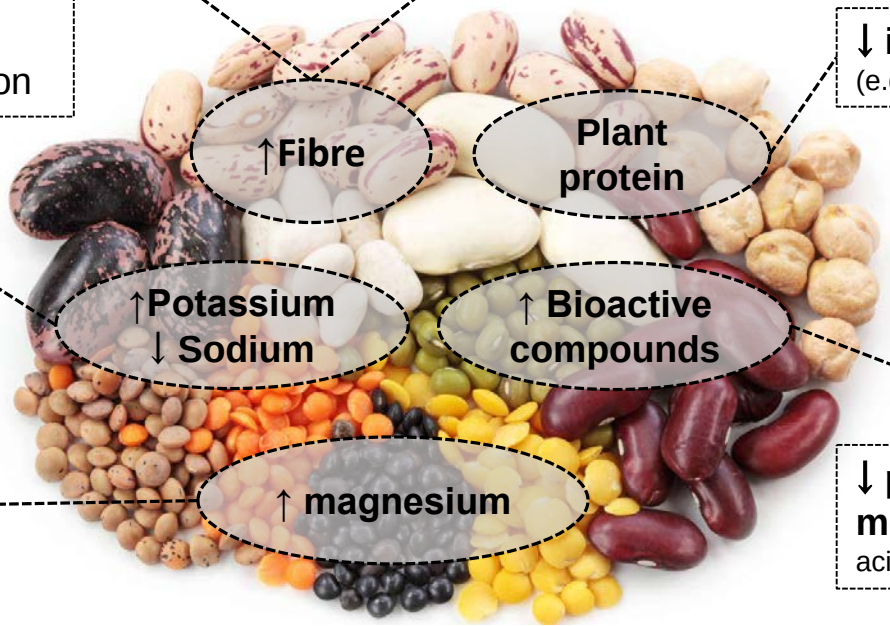
↓ **CVD RISK FACTORS**
↓ **INFLAMATORY STATUS**
↓ **OXIDATIVE STRESS**

Block gut absorption
- ↓ carbohydrate and fat uptake
- ↓ caloric intake
- ↓ Glycaemic index/load
- ↓ insulin
- ↓ insulin resistance
- ↑ mitochondrial oxidation
- ↓ cholesterol uptake
- ↑ biliary cholesterol secretion

Microbial fermentation - ↑ SCFAs
- ↓ hepatic cholesterol synthesis (animal models)
- ↑ *Bifidobacterium* spp.
- ↓ pathogenic and putrefactive bacteria

↓ inflammatory biomarkers (e.g. CRP, IL-6, TNF-***α***)

↓ blood pressure



↑ Down regulation of proinflammatory genes (e.g. TNF-related protein 9)

↓ proinflammatory metabolites (e.g. arachidonic acid, prostaglandins and leukotrienes)

CONCERNS

- ✓ Different control/intervention diets - e.g. energy restricted (weight loss effect);
- ✓ Different pulse amounts/frequency - e.g. weight vs cups, daily vs weekly;
- ✓ Lack of control over food intake - e.g. self-report, dietary advice;
- ✓ Health-impaired subjects - may cause overestimation of positive results;
- ✓ Indirect explanations – unclear biochemical mechanisms.

Best intake recommendations

- How?
- Who?
- How much?
- Frequency?
- **Animal protein replacement?** +

IMPULSE

"**IM**pact of a **PULSE**-based partial replacement diet on metabolome and health"

Classical nutritional research tools

Dietary intake

Body composition

Health biomarkers

Metabolomic approach

↓

Nuclear Magnetic Resonance (NMR)

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I. Objectives

Study the impact of replacing a **typical omnivorous meal** with a **vegetarian pulse-based meal** on...

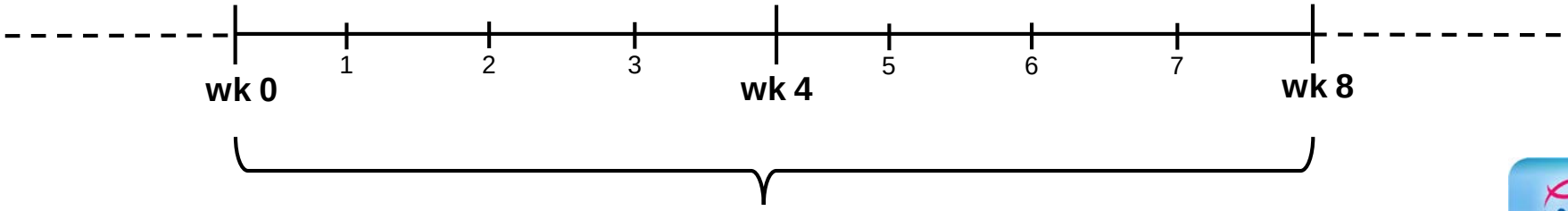
1) food and nutrient intake;
2) nutritional status and other health outcomes;
3) gut microbiota;
4) metabolic profile;
5) acceptability of pulses and pulse-based meals;
6) Portuguese household budgets.

II. Study design

- One-group comparison, quasi-experimental dietary intervention
- Volunteers:
  - **healthy male or female**
  - **18-45 years**
  - **not vegetarian or vegan**

n=27

March – May 2018 (n=19)
October – December 2018 (n=8)
October – December 2019 (still planning) – until n=40

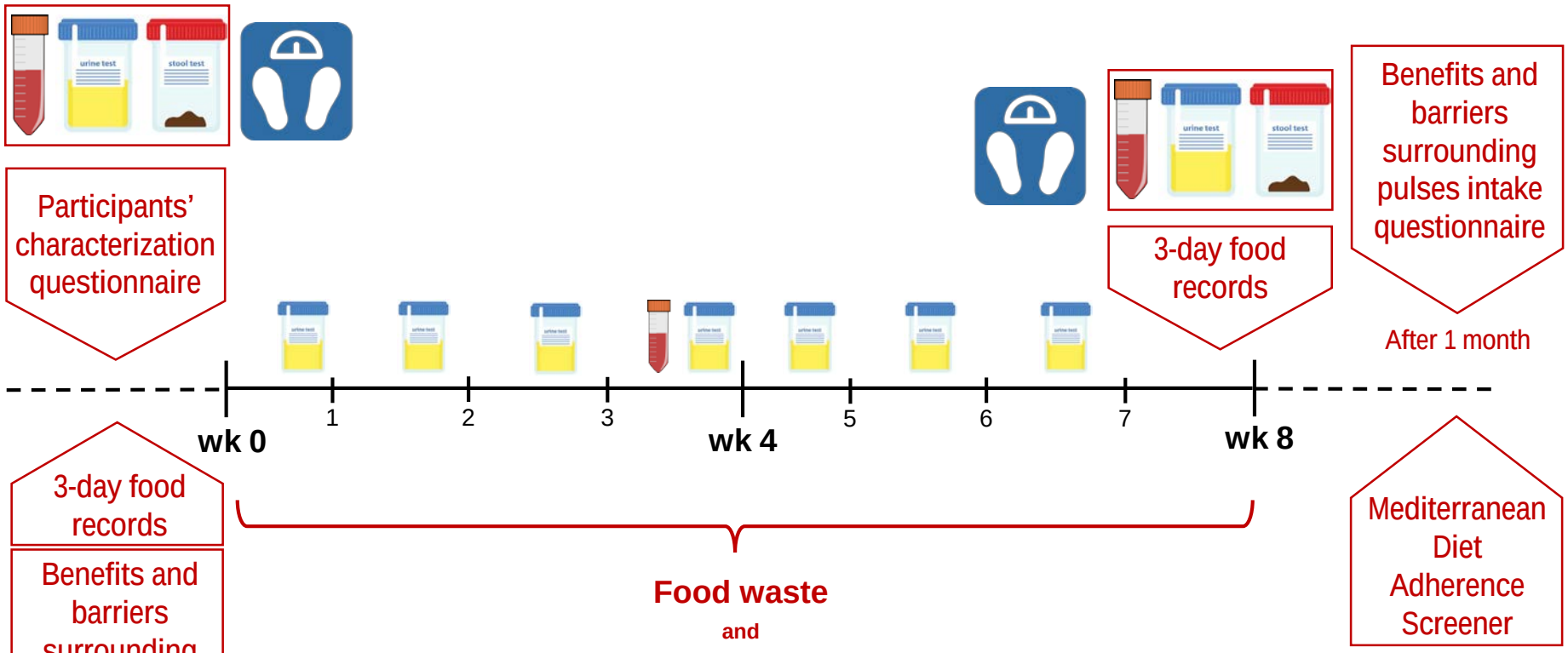



Meal room at CBQF

Vegetarian pulse-based meal
- Free lunch
- 8 consecutive weeks
- 5 days/week
- Monday to Friday


Meal example

III. Data collection

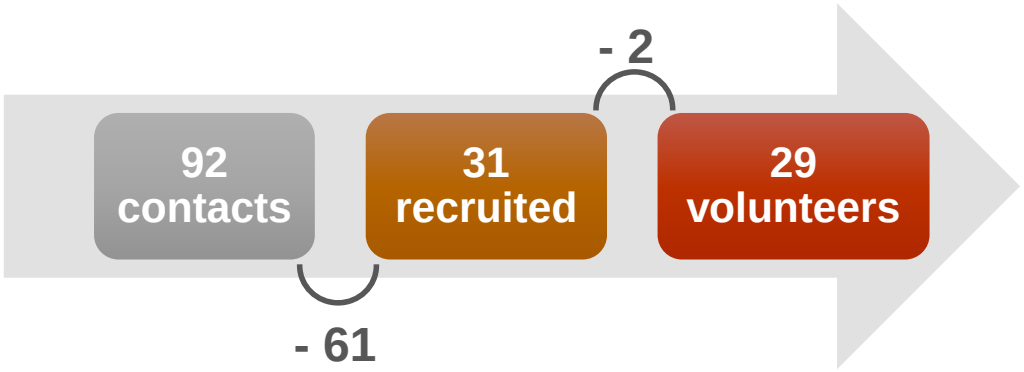


Prato	1	2	3	4	5	6	7	8	9
Temperatura									
Apresentação									
Sabor e tempero									
Apreciação global									

IV. Preliminary results

- ✓ **Study compliance**
- ✓ **Adverse effects**
- ✓ **Food frequency intake**
- ✓ **Food preference test**
- ✓ **Benefits and barriers questionnaire**
- ✓ **Anthropometric measurements**
- ✓ **Blood biochemistry**

Study compliance



Drop out: 2
6,9%

PARTICIPANTS

n=27

n=25 n=2

28,0 years - median
(min:18,0; max:43,0)

	IMPULSE meals	Vegetarian alternative	Vegetarian alternative with legumes
Total	**992**	33	**28**
Expected	1080		

91,9% compliance

94,4% compliance

Adverse effects

Reported - n=9 (33,3%)

Constipation (n=1)
Intestinal discomfort (n=1)

Abdominal bloating (n=1)

wk 0 1 2 3 wk 4 5 6 7 wk 8

Heartburn (n=2)

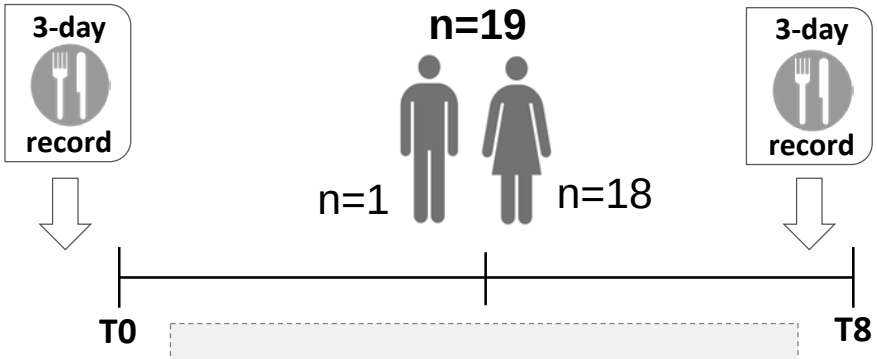
Increased flatulence (n=1)

Increased intestinal evacuation (n=1)

Increased headaches (n=1)

Hungry earlier after lunch (n=2)

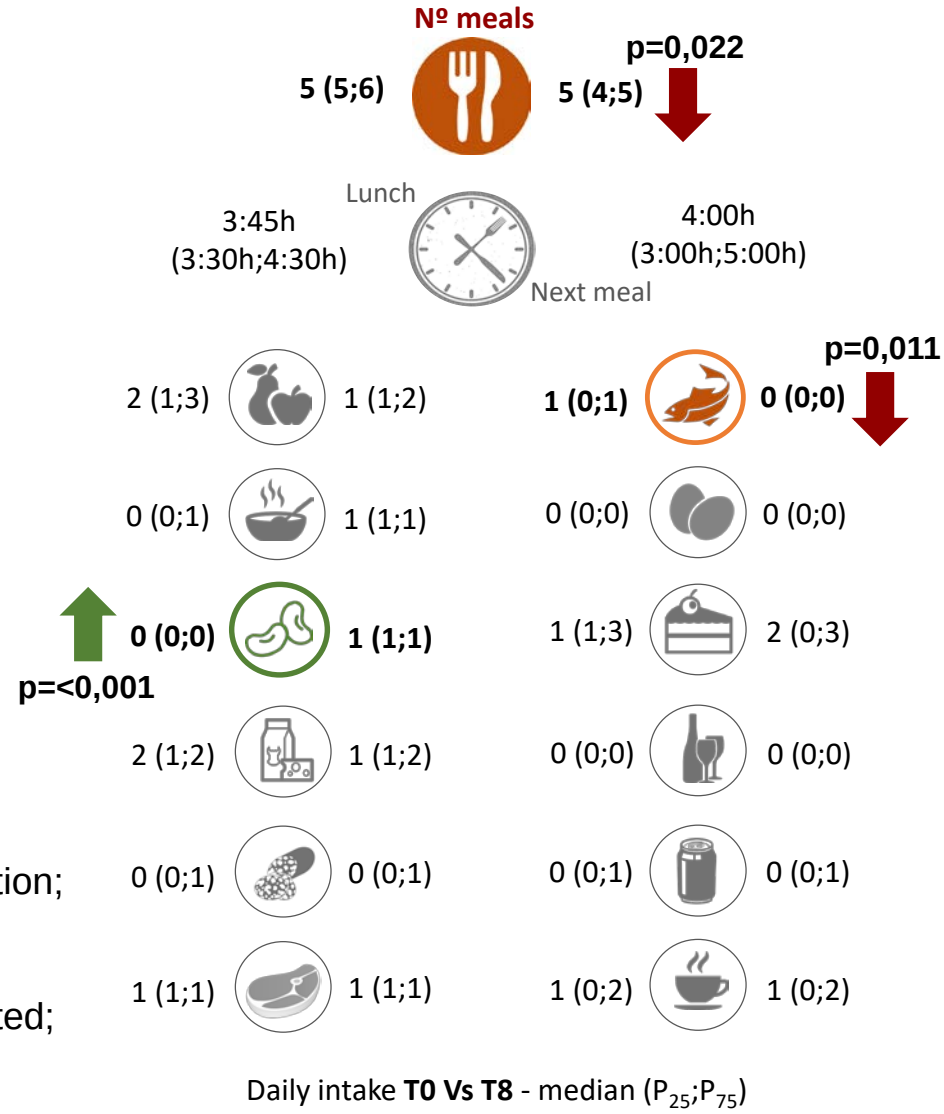
Food frequency intake



- **Nº of meals**
- **Meal time**
- **Foods/food groups:**
  - fruit; soup, legumes, dairy, meat products, meat, fish, eggs, "sweets", alcoholic beverages, juice and coffee.

Some considerations:

- Changes are consequences of the dietary intervention;
- Possible preference of meat over fish;
- No compensatory food behaviour (e.g. >"sweets");
- Overall food frequency intake appears barely affected;
- No food amounts were considered.

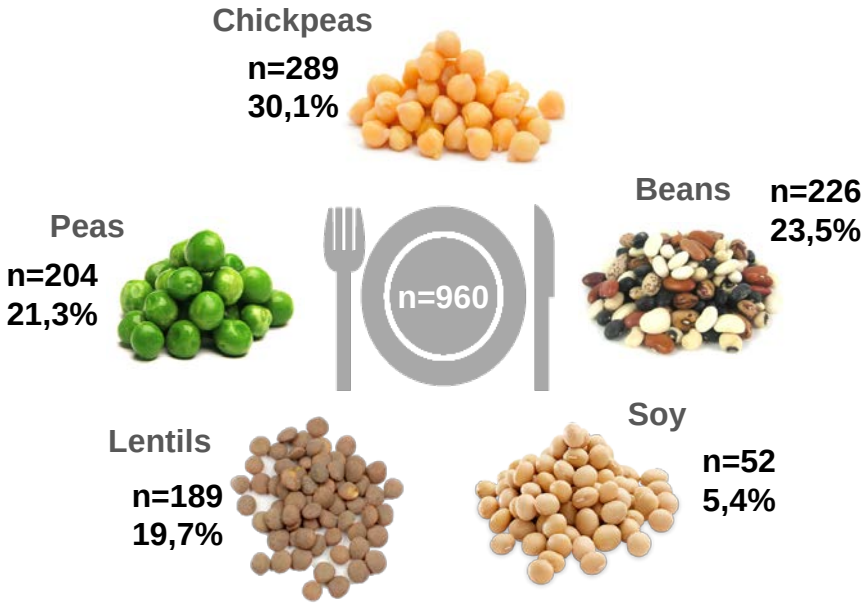


Daily intake **T0 Vs T8** - median (P_{25};P_{75})

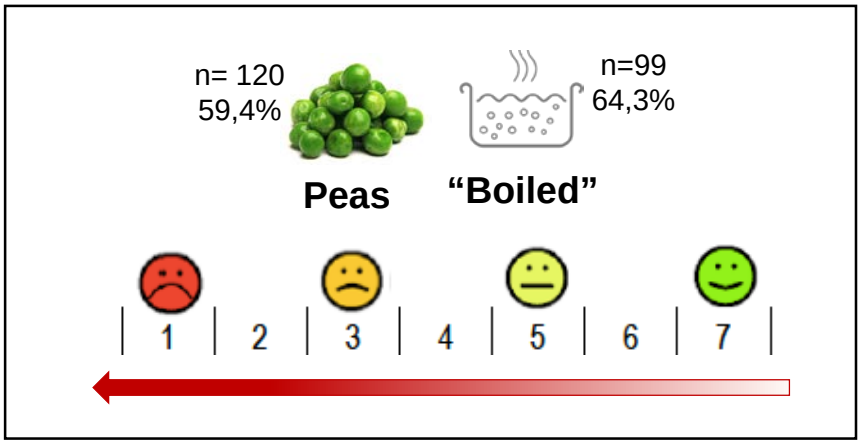
Food preference test

	1	2	3	4	5	6	7	8	9
Temperature									
Presentation									
Seasoning and taste									
Global appreciation									

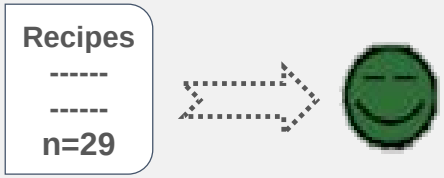
GLOBAL APPRECIATION



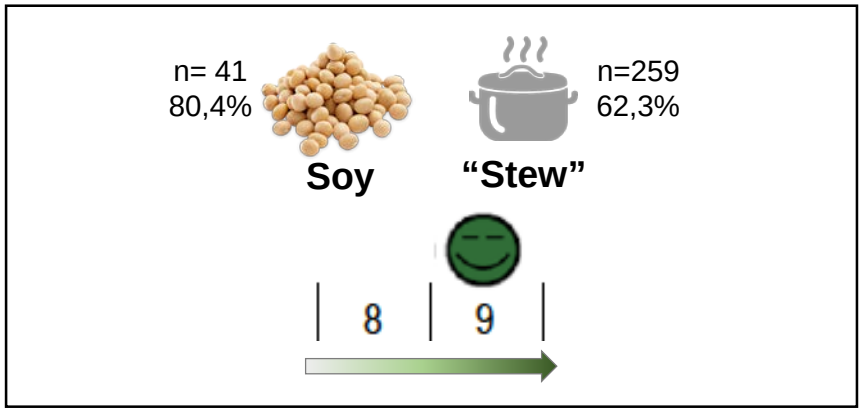
Recipes with **lower** global appreciation **[1-7]**



For most recipes (n=17,0; 58,6%), **> 50,0%** of participants reported a global appreciation of **8 and 9**.



Recipes with **higher** global appreciation **[8, 9]**



Benefits and barriers questionnaire

Negative beliefs/Barriers	Strongly disagree	Disagree	Not sure	Agree	Strongly Agree
Pulse-based meals or snacks are not available when I eat out.					
I believe it is too expensive to eat pulses.					
I would probably get indigestion, bloating or gas eating pulses.					
I don't know how to prepare pulses.					
I believe it takes too long to prepare pulses.					
I never think of using pulses when I cook.					
I believe that serving pulses helps me look more trendy' to my friends and family.					

Positive beliefs	Strongly disagree	Disagree	Not sure	Agree	Strongly Agree
I would buy a prepackaged pulse-based snack.					
I would eat (more) pulses if they had a more attractive appearance.					
I would buy a prepackaged pulses-based meal.					
I know how to cook pulses.					
I believe that pulse-based meals can help me save money.					
Pulses can be a part of a tasty diet.					
Pulses are part of my traditional diet.					
I believe pulses are a healthy food.					

Questionnaire adapted from: Phillips TNS. Master thesis: Diet Approaches to Increase Lentil Consumption in Youth - The D.A.I.L.Y. Project. Canada: University of Saskatchewan Saskatoon; 2011.

+ IMPULSE

LIN Greece
LIN Denmark

67,0%; n=65

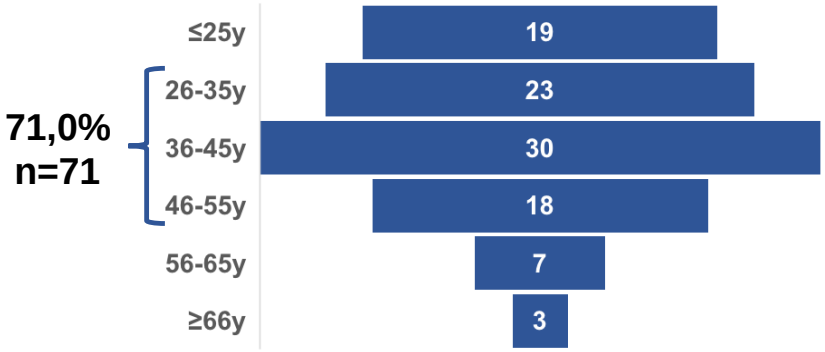
92,1%; n=93

COUNTRY

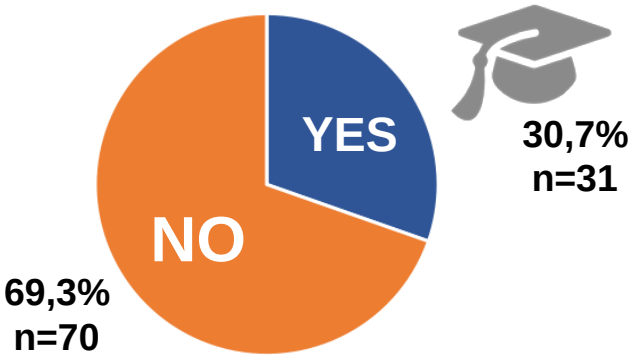
	n	%		n	%
Portugal	29	28,7	Brazil	1	1,0
Greece	19	18,8	France	1	1,0
Denmark	14	13,9	India	1	1,0
UK	13	12,9	Italy	1	1,0
Germany	8	7,9	Kenya	1	1,0
Ireland	3	3,0	Scotland	1	1,0
Croatia	2	2,0	The Netherlands	1	1,0
Hungary	2	2,0	Turkey	1	1,0
Slovenia	2	2,0			

TOTAL 101

Age



Health/nutrition qualifications



+ IMPULSE

"Agree" (n=101)

NEGATIVE	**Pulse-based meals or snacks are not available when I eat out.** (n=99)	**52,5%**
	I never think of using pulses when I cook. (n=100)	21,0%
	I believe that serving pulses helps me look more trendy' to my friends and family. (n=100)	20,0%
	I would probably get indigestion, bloating or gas eating pulses. (n=100)	17,0%
	I believe it takes too long to prepare pulses. (n=101)	17,8%
	I don't know how to prepare pulses. (n=101)	14,9%
	I believe it is too expensive to eat pulses. (n=100)	6,0%

POSITIVE	**I believe pulses are a healthy food.** (n=101)	**94,1%**
	Pulses can be a part of a tasty diet. (n=100)	92,0%
	Pulses are part of my traditional diet. (n=101)	60,4%
	I know how to cook pulses. (n=100)	60,0%
	I believe that pulse-based meals can help me save money. (n=99)	52,5%
	I would buy a prepackaged pulse-based snack. (n=101)	49,5%
	I would buy a prepackaged pulses-based meal. (n=101)	47,5%
	I would eat (more) pulses if they had a more attractive appearance. (n=101)	22,8%

Anthropometric measurements

n=27 median (P_{25}; P_{75})	T0	T8	p	
Weight (kg)	57,8 (54,4; 67,2)	58,2 (53,3; 66,8)	0,747	T0 >> T8
Body Mass Index (kg/m²)	21,9 (21,3; 24,5)	22,4 (21,3; 24,5)	0,948	Normal range
Body fat (%)	30,6 (25,6; 33,5)	30,2 (23,9; 33,7)	0,318	> Normal range
Lean mass (kg)	23,1 (20,7; 25,2)	23,0 (20,8; 24,6)	0,760	=
Waist circumference (cm)	71,5 (66,5; 75,2)	71,0 (66,4; 75,2)	0,509	Normal range

No changes
(isocaloric diet)

- **Recommended BMI*** - 18,50-24,99 kg/m²
- **Recommended Body Fat**** - Men: 10,0-20,0% | Women: 18,0-28,0%
- **Recommended WC*** – Men: <94 cm | Women: <80 cm

References:
***Direção Geral de Saúde (DGS). Orientação nº 017/2013 de 05/12/2013 - Avaliação antropométrica no adulto. 2013;
**Biospace. InBody720 - The precision body composition analyser - User's Manual. Biospace Co., Ltd.; 2004.

Blood biochemistry

n=27 median (P_{25}; P_{75})	T0	T8	T0-T8 p	Reference values*
Haemoglobin (g/dL)	13,6 (13,1; 14,2)	13,6 (13,1; 14,2)	0,990	W:12,0-16,0 M:13,0-18,0
Iron (µg/dL)	108,0 (92,0; 129,0)	99 (90,0; 142,0)	0,810	49-151
Transferrin (mg/dL)	276,0 (242,0; 315,0)	↑ **287,0** (252,0; 325,0)	**0,001**	200-360
Transferrin Saturation (%)	28,0 (22,0; 34,0)	27,0 (20,0; 34,0)	0,247	20-50
Ferritin (ng/mL)	46,8 (32,6; 85,8)	↓ **41,5** (33,6; 93,6)	**0,037**	20-250
Total cholesterol (mg/dL)	180,0 (154,0; 200,0)	↓ **179,0** (153,0; 193,0)	**0,029**	<200
HDL-C (mg/dL)	58,0 (50,0; 67,0)	57,0 (50,0; 65,0)	0,181	>60
LDL-C (mg/dL)	99,0 (91,0; 120,0)	↓ **92,0** (83,0; 107,0)	**0,002**	<130
Triglycerides (mg/dL)	89,0 (66,0; 114,0)	↑ **99,0** (55,0; 117,0)	**0,034**	<150
Fasting glucose (mg/dL)	85,0 (80,0; 89,0)	↑ **88,0** (81,0; 92,0)	**0,045**	75-110

Changes in iron dynamics

= Previous studies

≠ Previous studies

*Reference values from the laboratory.

NMR Metabolomics

university of aveiro
theoria poiesis praxis

ciceco
centre for research in ceramics & composite

PLASMA:
- Collected – 81 samples (n=27)
- Stored at -80ºC

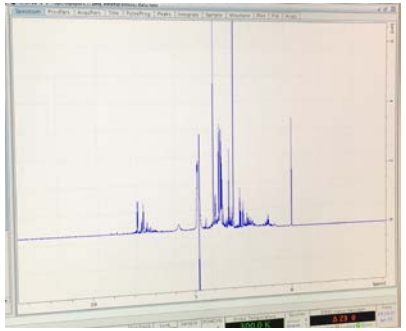
STOOL
- Collected – 54 samples (n=27)
- Stored at -80ºC

URINE
- Collected – 243 samples (n=27)
- Stored at -80ºC
- **NMR acquisition** – 171 samples **(n=19; 1st intervention group)**

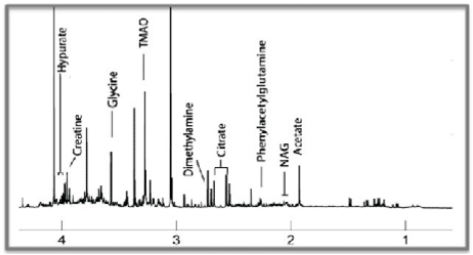
PRESENT TASK

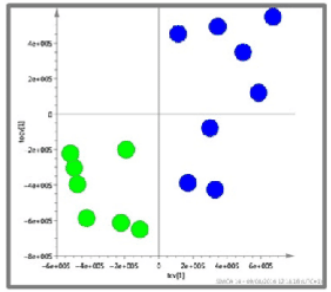
Data acquisition

Spectral processing

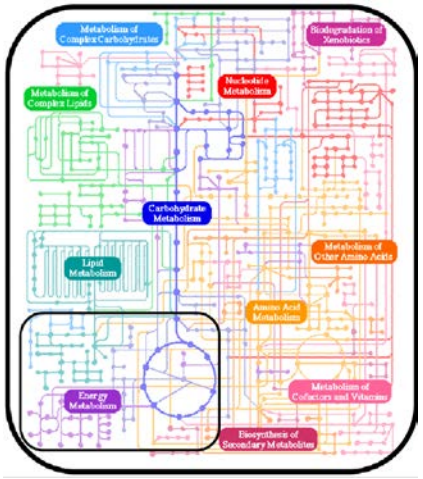


Metabolite identification and data analysis





Metabolic pathway analysis



Journal of proteome research
Article
pubs.acs.org/jpr

Urinary ^{1}H Nuclear Magnetic Resonance Metabolomic Fingerprinting Reveals Biomarkers of Pulse Consumption Related to Energy-Metabolism Modulation in a Subcohort from the PREDIMED study

Francisco Madrid-Gambin,[†] Rafael Llorach,[*,†,‡] Rosa Vázquez-Fresno,[†,§] Mireia Urpi-Sarda,[†,‡] Enrique Almanza-Aguilera,[†] Mar Garcia-Aloy,[†,‡] Ramon Estruch,[∥,⊥] Dolores Corella,[⊥,#] and Cristina Andres-Lacueva[*,†,‡]

Mediterranean diet

- Diabetes Mellitus 2 or
- ≥3 CVD risk factors

Olive oil vs Nuts

≥ 25,71g/day

Pulses

^{1}H-NMR

Pulses

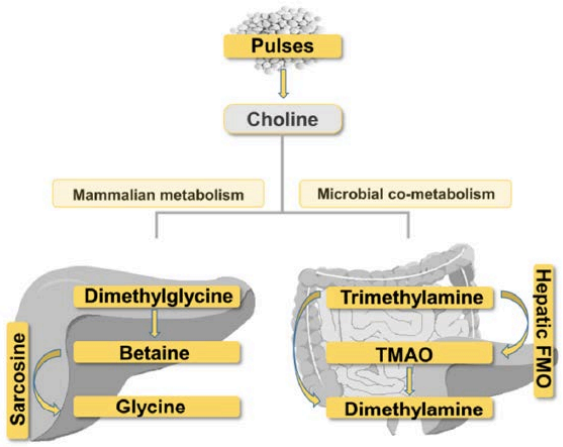
≤ 4,00g/day

choline metabolism

protein-related compounds

energy metabolism

↑ Choline
↑ Dimethylglycine
↑ TMAO
↑ Dimethylamine



↑ N-acetylglutamine
↑ Glutamine
↑ Phenylalanine
↑ Methylguanidine
↑ 3-methylhistidine

↑ Biomarkers of protein food sources

↓ Citric acid
↓ Cis-aconitic acid
↓ Glucose
↓ Acetoacetic acid
↑ Isovalerylglycine
↑ Leucine
↓ Isobutyric acid

↓ TCA cycle intermediates
↓ gluconeogenesis
↓
Glucose-lowering effect in Diabetic patients

(Adapted from Madrid-Gambin F. *et al*, 2017)

V. Conclusions (so far….)

- High diet compliance
- Low adverse effects – not drop out reasons!
- No relevant changes on eating habits
- High meal acceptance
- Positive behaviour surrounding pulses intake
- Anthropometric measures maintenance
- Iron status maintenance
- ”Slight” cholesterol level improvement

Data consolidation is needed!

Our results will…

…be presented in peer-reviewed **scientific journals**.

…contribute to the generation of **new health biomarkers** and insights on the health **benefits of pulses and plant-based diets**.

…provide **valuable input** for **public awareness** initiatives, including, nutritional education resources, workshops and seminars.

…help maximize the impact of **international and national campaigns** on the promotion of pulses and plant-based diets.

CATÓLICA
ESCOLA SUPERIOR
DE BIOTECNOLOGIA

PORTO

IMPULSE

"IMpact of a PULSE-based partial replacement diet on metabolome and health"

by

Helena Alexandra Gonçalves Ferreira

Supervision: Elisabete Pinto, PhD | Marta Vasconcelos, PhD | Ana Gil, PhD

Porto | 09th of July 2019