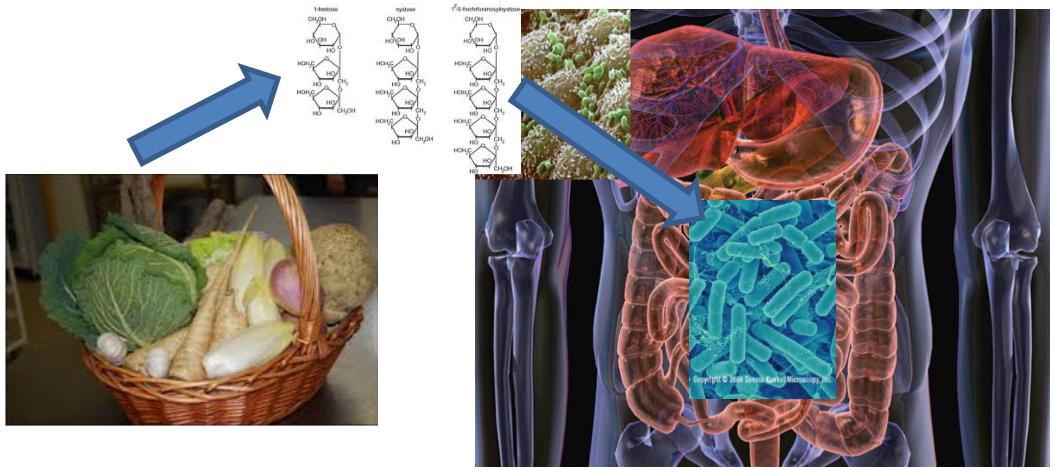
Metabolism and Nutrition Research Group



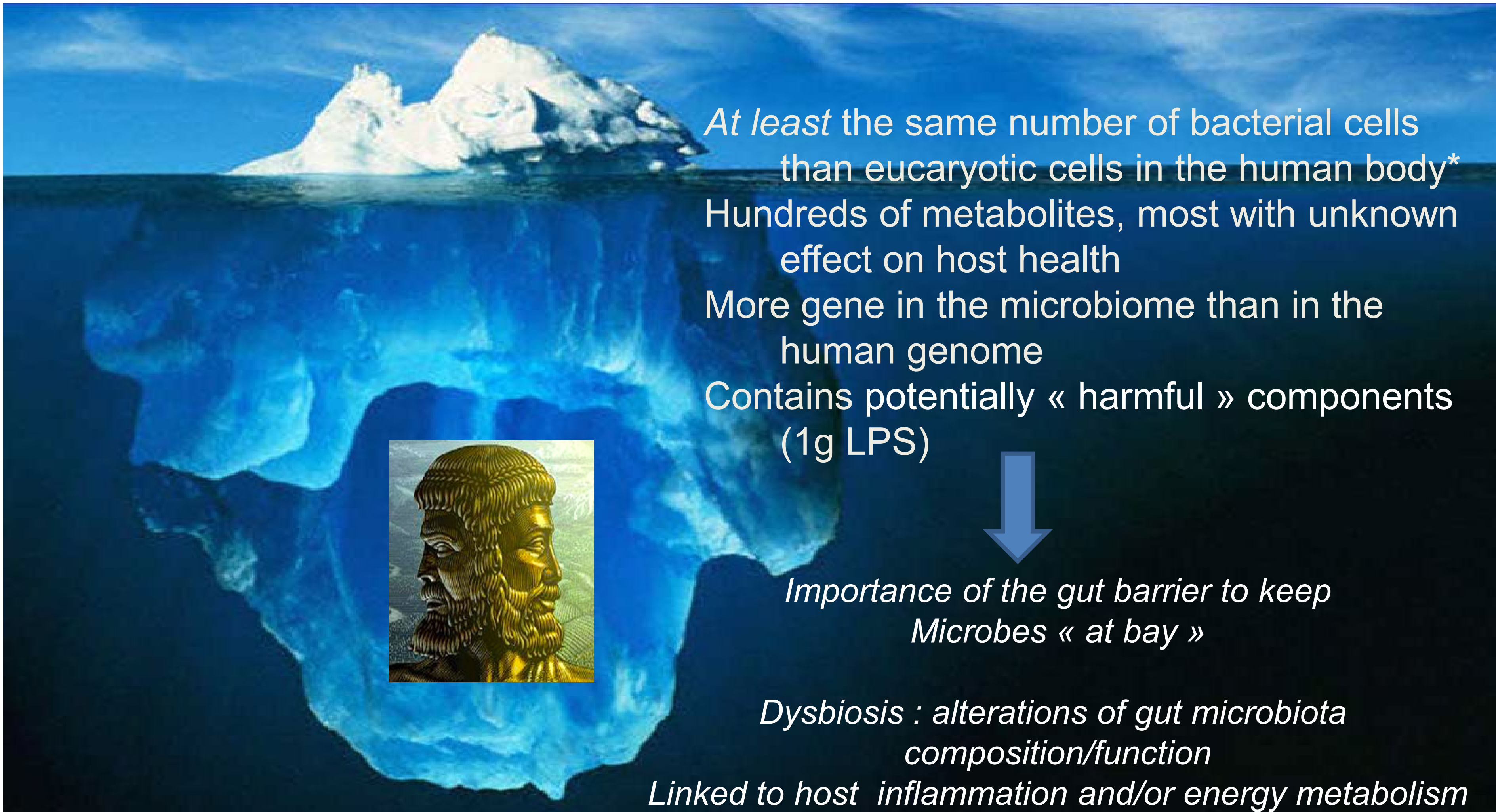
Egea 2018

Nutrition & Health: From science to practice

Modulating the microbiota by fiber-rich vegetables: a promising therapeutic approach in obesity?

I declare no conflict of interest related to this presentation

The Gut Microbiota: an internal organ we feed everyday



- *At least* the same number of bacterial cells than eucaryotic cells in the human body*
- Hundreds of metabolites, most with unknown effect on host health
- More gene in the microbiome than in the human genome
- Contains potentially « harmful » components (1g LPS)

Importance of the gut barrier to keep Microbes « at bay »

Dysbiosis : alterations of gut microbiota composition/function
Linked to host inflammation and/or energy metabolism

* *Abbott A Nature News 2016*

Dysbiosis associated with obesity, type 2 diabetes (T2D) and NAFLD in humans (Karlsonn et al Nature 2013, Tilg and Moschen Gut 2014, Delzenne et al Diabetologia 2015; Wiest et al J Hepatol 2017).

Characteristics of a « moderate » dysbiosis

- Decrease in bacterial diversity

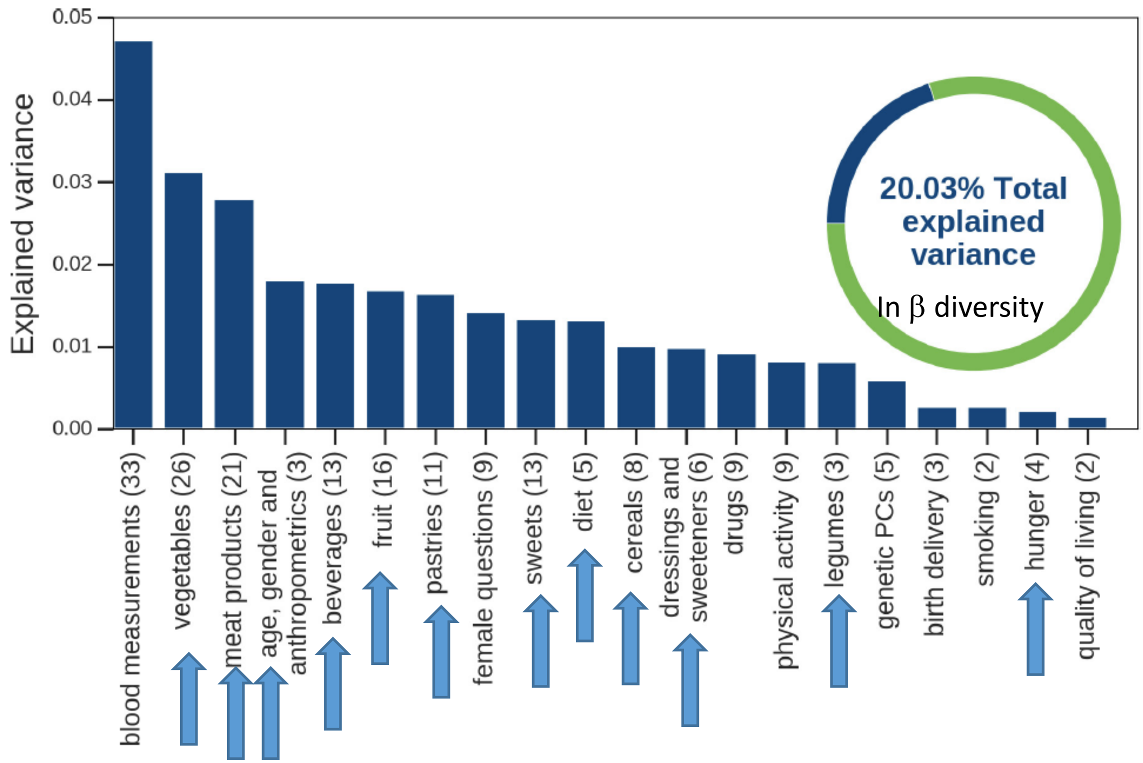
- Decrease in « beneficial » bacteria **Bifidobacteria** (immune system), *F. Prausnitzii* (anti-inflammatory), *Roseburia intestinalis or Clostridium* (butyrate producers), ***Akkermansia muciniphila*** (gut barrier)

- Increase in potentially harmful bacteria bacteria (i.e. *Desulfovibrio spp., beta-proteobacteria, Bacteroides-Prevotella*)

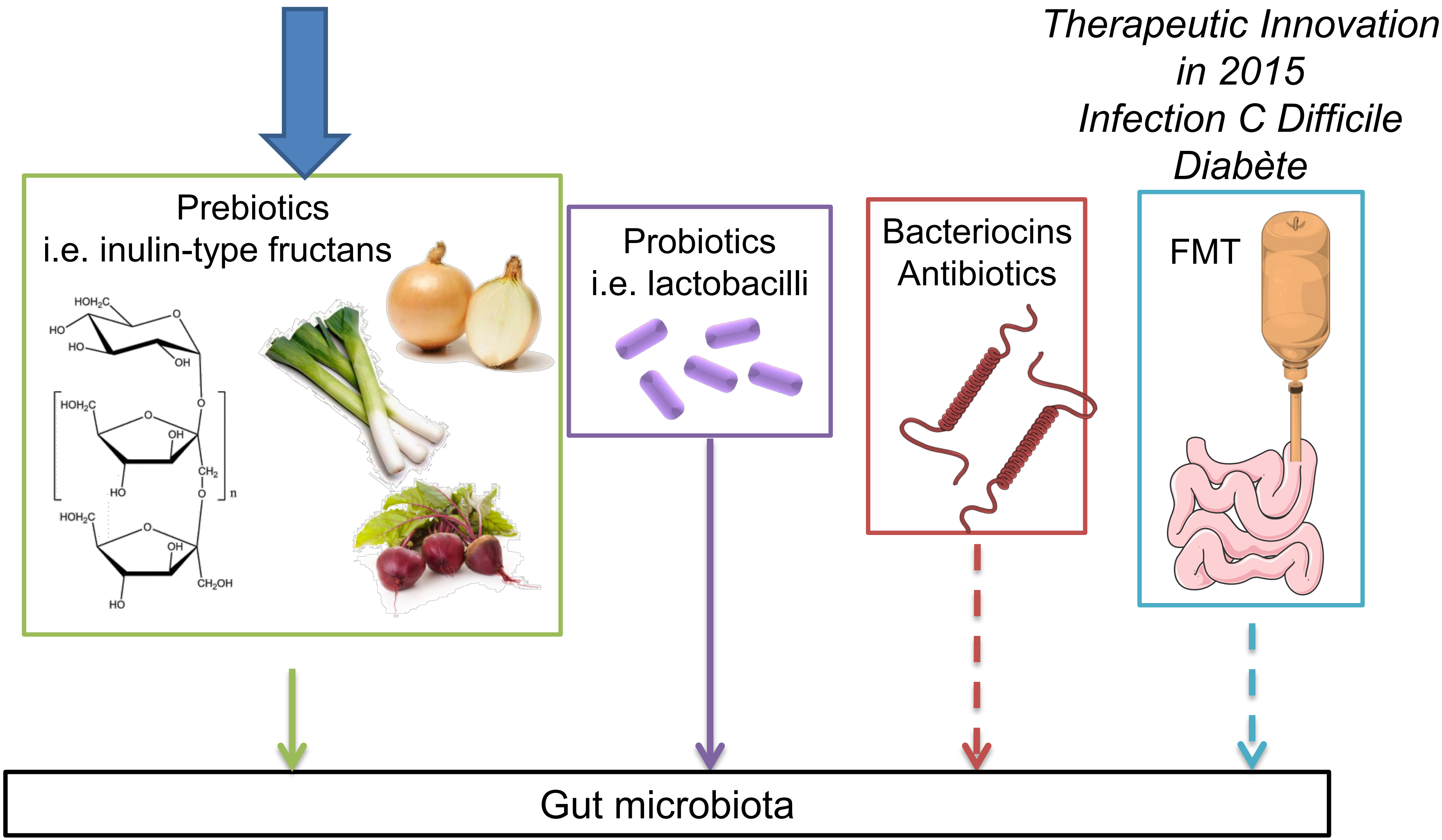
Environment dominates over host genetics in shaping human gut microbiota

Daphna Rothschild[1,2*], Omer Weissbrod[1,2*], Elad Barkan[1,2*], Alexander Kurilshikov[3], Tal Korem[1,2], David Zeevi[1,2], Paul I. Costea[1,2], Anastasia Godneva[1,2], Iris N. Kalka[1,2], Noam Bar[1,2], Smadar Shilo[1,2], Dar Lador[1,2], Arnau Vich Vila[3,4], Niv Zmora[5,6,7], Meirav Pevsner-Fischer[5], David Israeli[8], Noa Kosower[1,2], Gal Malka[1,2], Bat Chen Wolf[1,2], Tali Avnit-Sagi[1,2], Maya Lotan-Pompan[1,2], Adina Weinberger[1,2], Zamir Halpern[7,9], Shai Carmi[10], Jingyuan Fu[3,11], Cisca Wijmenga[3,12], Alexandra Zhernakova[3], Eran Elinav[5]§ & Eran Segal[1,2]§

Nature 2018



Available tools to modulate the gut microbiota



Adapted from Bindels & Delzenne, Int J Biochem Cell Biol 2013

Prebiotics: use of inulin type fructans (ITF) as modulators of the gut microbiota

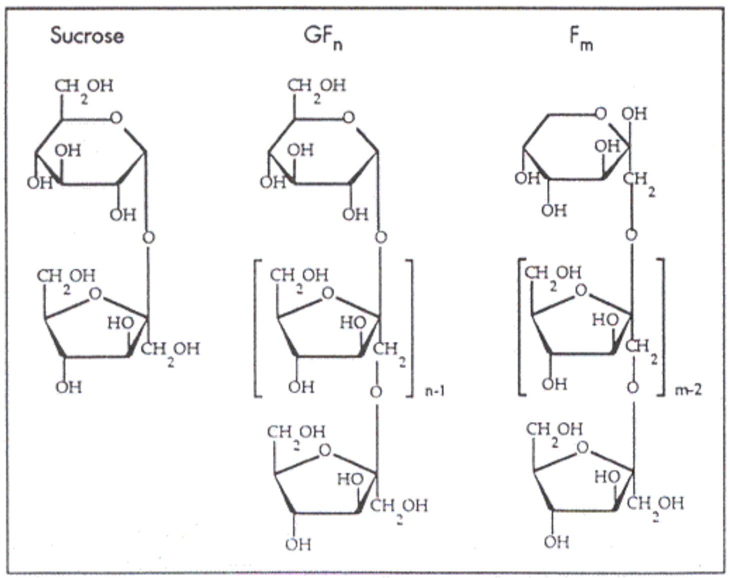
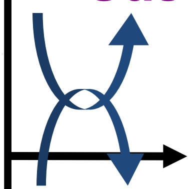


FIGURE 1 Chemical structure of the various fructo oligosaccharides. G, *glucose*; F, *fructose*; n or m indicate the number of fructose moieties in the molecules.

« Opportunistic » increase In Bacteria expressing β-fructosidase,such as Bifidobacteria

Gas



Prebiotic effect

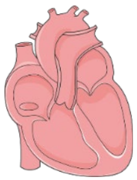
Short Chain Fatty Acids
Acetate
Propionate
Butyrate
Lactate

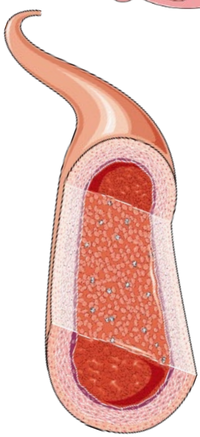
« cross-feeding »

ITF are also present in cereals and some vegetables …

Gibson et al, Nature Rev Gastroenterol 2017; Bindels et al Nature Reviews Gastroenterology and Hepatology 2015; Roberfroid and Gibson Br J Nutr 1995:

Inulin-type fructans modulates obesity and metabolic disorders in rodents





Improve vascular dysfunction

Catry et al. Gut. 2018

Glucose tolerance

Cani et al. Diabetologia. 2007; Everard et *al. Diabetes. 2011*

Triglycerides

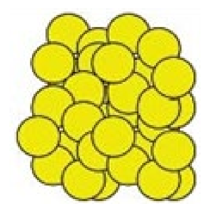
Kok et al br J Nutr 1995;
Everard et al. Diabetes. 2011; Hiel at al. Nutrients. 2018

Endotoxaemia, inflammation

Cani et al. Diabetologia. 2007 ; Cani et al. Gut. 2006)

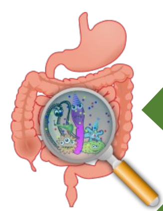
Improve hepatic steatosis
lipids content

Firdaliso et al Kok et al , Br J Nutr 1995
Everard et al. Diabetes. 2011;
Weitkunat et al. J Nutr Biochem. 2015;
Weitkunat et al. Sci. Rep. 2017



Fat mass accumulation

Dewulf et al. J.Nutr. Biochem 2011;
Weitkunat et al. Sci. Rep. 2017;
Everard et al. Diabetes. 2011



Gut barrier function

(Cani et al. Gut. 2009)

Gut endocrine function

Kok et al J Nutr 1998; Cani et al 2006

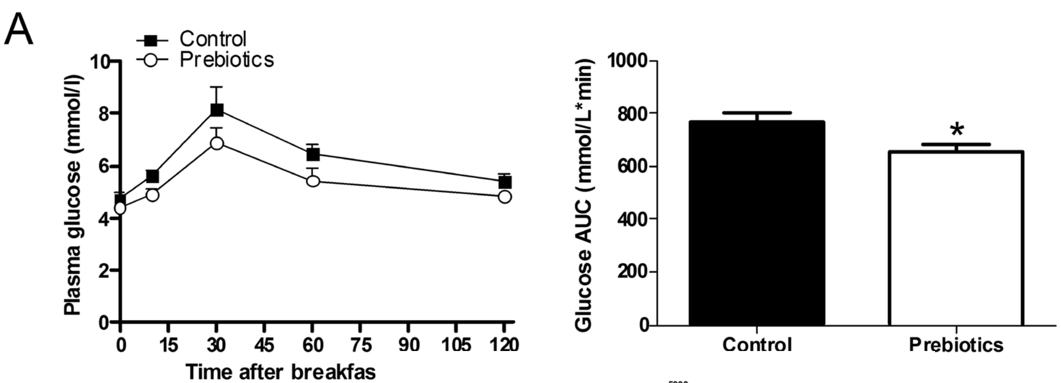
Nutrients digestion/absoption

Lipids (Hiel et al Nutrients 2018), sugars (Neyrinck et al 2017)

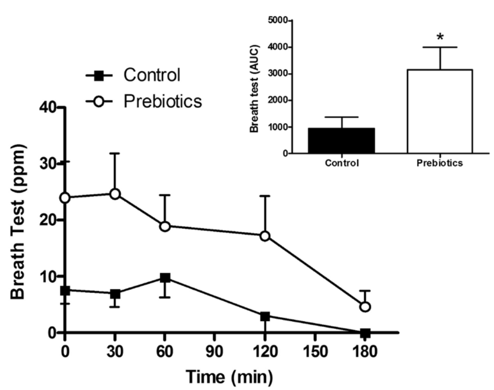
UCLouvain

Inulin-type fructans decrease post prandial glucose and modulate gut peptides after a high CHO breakfast in humans

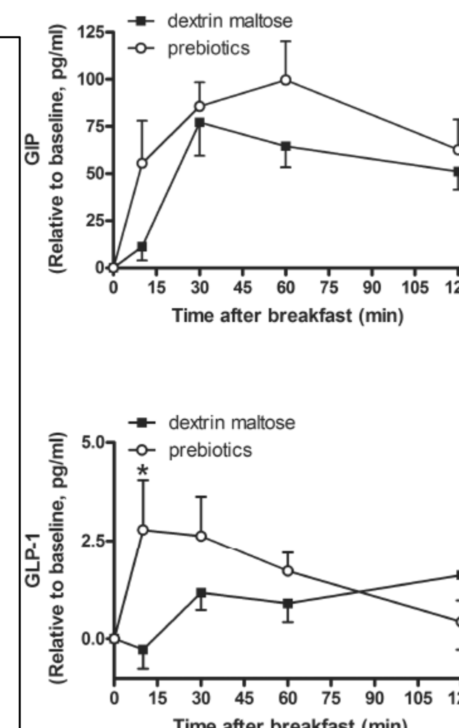
Randomized double blind parallel placebo controlled study
Healthy volunteers fed either maltodextrin (placebo) or Raftilose (short chain ITF)
16g per day for two weeks before standardized high carbohydrates breakfast test



Hydrogen Breath test (fermentation)



Gut hormones



Regulation of glycemia by Inulin accepted as health Claim by EFSA

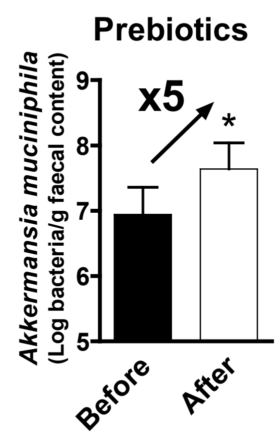
Cani et al Am J Clin Nutr 2009

Specific changes in gut microbiota induced by purified inulin-type fructans correlate with host physiology in obese women

- Double blind placebo controlled intervention study; 15 women per group; mean BMI 35 kg/m^2
- 2 groups : Synergy 1 (ITF) and maltodextrin (placebo)
- 3 months of treatment (8g twice a day)

	Placebo group			**Prebiotic group**			**Mann-Whitney test**
	T0	T3months	Δ	T0	T3months	Δ	p value
***Bifidobacterium* spp.**	9.36±0.16	9.05±0.20	-0.31±0.15	9.16±0.10	*9.79±0.12****	0.63±0.12	0.0001
B. adolescentis	6.39±0.37	6.57±0.39	0.18±0.22	6.52±0.23	*7.14±0.35***	0.62±0.17	0.0381
B. animalis	5.54±0.24	5.48±0.38	0.14±0.33	5.99±0.32	6.23±0.40	0.24±0.31	0.6943
B. bifidum	5.36±0.35	5.79±0.45	0.42±0.41	4.85±0.14	5.29±0.23	0.44±0.26	0.7107
B. breve	5.60±0.06	5.53±0.04	-0.08±0.05	5.62±0.05	5.55±0.03	-0.07±0.06	0.9085
B. longum	8.14±0.27	7.73±0.33	-0.45±0.16	7.94±0.17	*8.67±0.19****	0.73±0.12	<0.0001
B. pseudocatenulatum	7.33±0.43	6.79±0.45	-0.54±0.23	7.20±0.41	*8.49±0.41***	1.29±0.35	0.0005

B. Longum, Akkermansia muciniphila, and F. prausnitzii levels correlate with the **decrease in endotoxemia (LPS);** *B. Adolescentis* correlates with the **decrease in fat mass.**



Akkermansia muciniphila

UCLouvain

Dewulf et al Gut 2012, Salazar et al Clin Nutr 2014

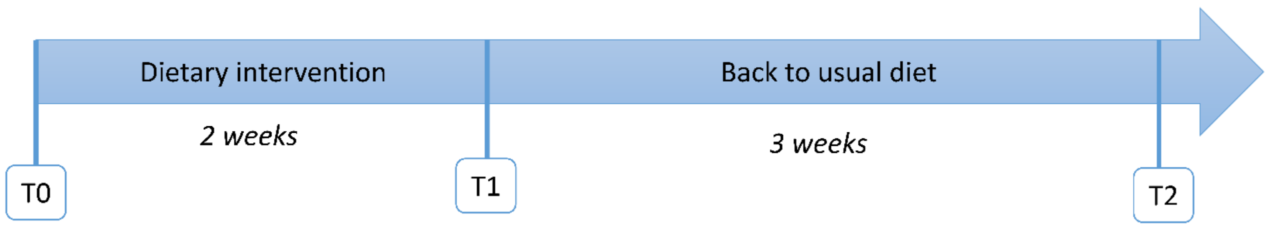
Everard A., Dewulf E., Thissen JP, Delzenne NM, Cani PD. Unpublished data

Can we provide enough inulin in food to get a prebiotic effect ?¨Proof of concept in healthy individuals

- Selection of foods (analytical data of vegetables cultivated in Wallonia) (Kalala et al 2017)
- Volunteers : 14 women, 12 men recruited; 25 completed the study (mean BMI 22,5 kg/m^2)
- Exclusion criteria : smoking; AB- pre- or probiotics for the last 6 weeks;

drugs (antidiabetics, proton pump inhibitors, laxatives..) , pregnancy, chronic diseases, psychiatric problems.

- Strict control of meals during the intervention period



Before T0, T1, T2 :

- 3-day fulfillment of food diary
- Fecal sample collection

During T0, T1, T2 :

- Hydrogen breath test
- Visual analogue scale for gastrointestinal symptoms and appetite feelings
- Sucrose detection treshold test
- Behavioural study

Every day during dietary intervention :

- Meal consumption containing vegetables rich in ITF (±15g ITF/day)
- Visual analogue scale for gastrointestinal symptoms ans appetite feelings
- Bristol stool scale

Pachikian , Bindels, Hiel et al, *in revision*

Data are expressed as mean±SEM and analyzed by a repeated measures one-way ANOVA followed by Tukey post test, or Friedman test followed by Dunn's post test: *** P<0.001 versus T0. # P<0.05, ### P<0.001 versus T1. n=25.

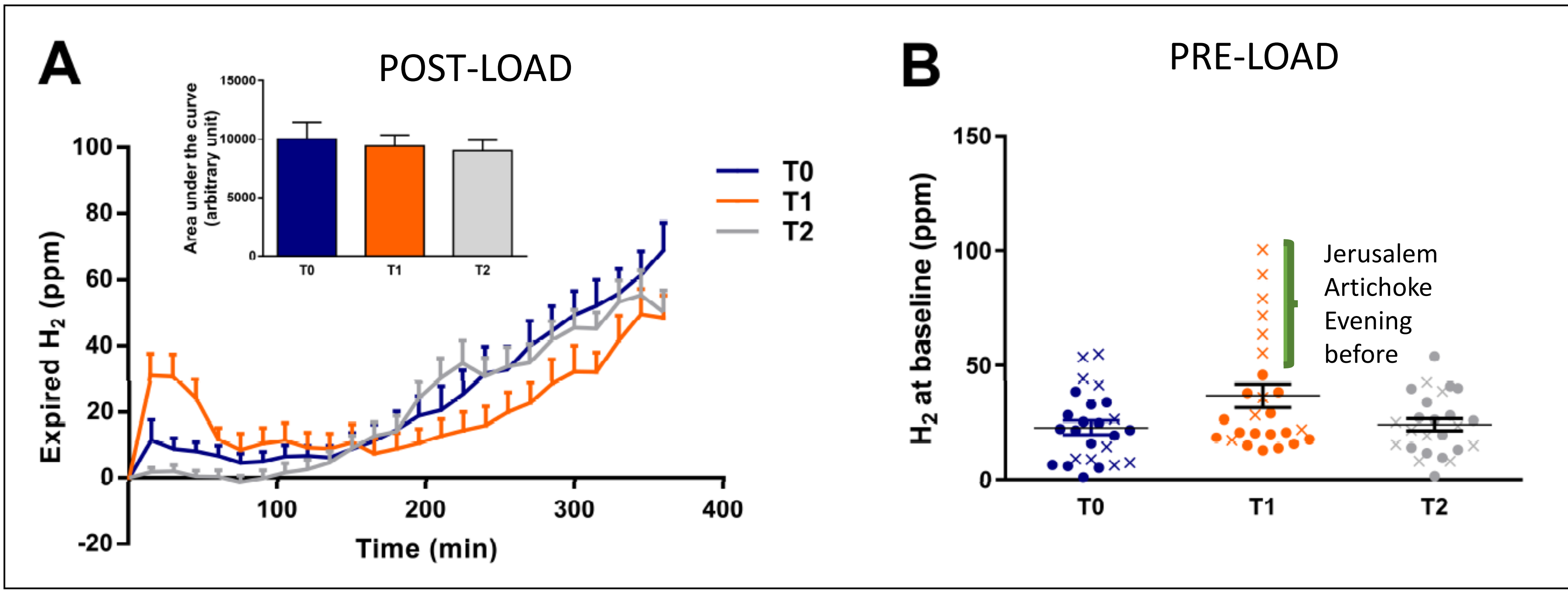
	T0	T1	T2
Energy (kcal/day)	1975 ± 77	1888 ± 70	1949 ± 75
Carbohydrates (g/day)	254.2 ± 10.4	221.6 ± 9.6	240.9 ± 10.1
Starch	139.9 ± 8.6	124.2 ± 7.2	$153.5^{\#}$ ± 10.6
Sugars	87.1 ± 5.8	83.6 ± 4.2	83.7 ± 6.2
Lipid (g/day)	67.3 ± 5.1	68.2 ± 3.8	70.7 ± 4.2
Protein (g/day)	74.8 ± 3.3	80.6 ± 3.1	74.6 ± 3.9
Fibres (g/day)	18.29 ± 1.61	31.46^{***} ± 1.08	$16.62^{\#\#\#}$ ± 1.45
Fructans	3.06 ± 0.51	15.67^{***} ± 0.31	$2.68^{\#\#\#}$ ± 0.26

Pachikian , Bindels, Hiel et al, *in revision*

Gastrointestinal fermentation and gastro-intestinal tolerance of inulin-type rich food

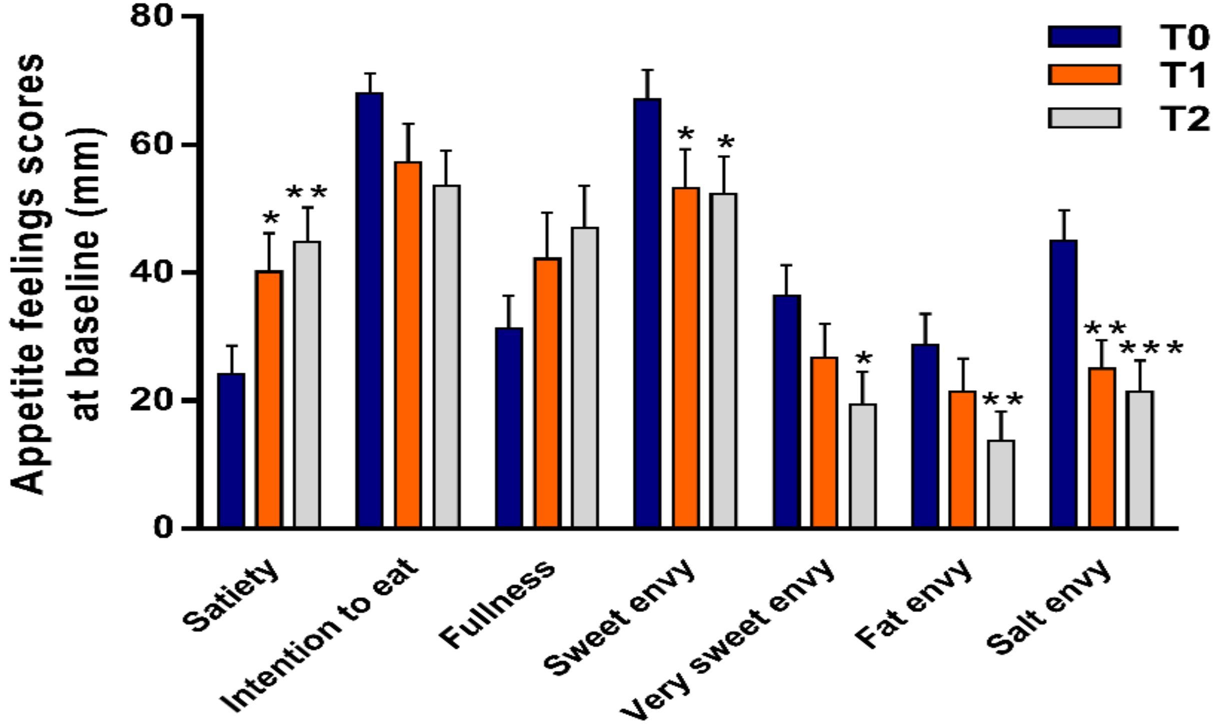
Purified native inulin load (15g)

Fasted volunteers (n=25) → Hydrogen breath test



- **Visual Analogue scale for gastrointestinal symptoms revealed a small increase in flatulence (T1), with no effect on burp, bloating, rumbling, cramp, discomfort and nausea**
- **GI tolerance towards inulin load (15g) was improved at T1 versus T0**

Persisting effect on food related behaviour by food intervention with inulin rich vegetables



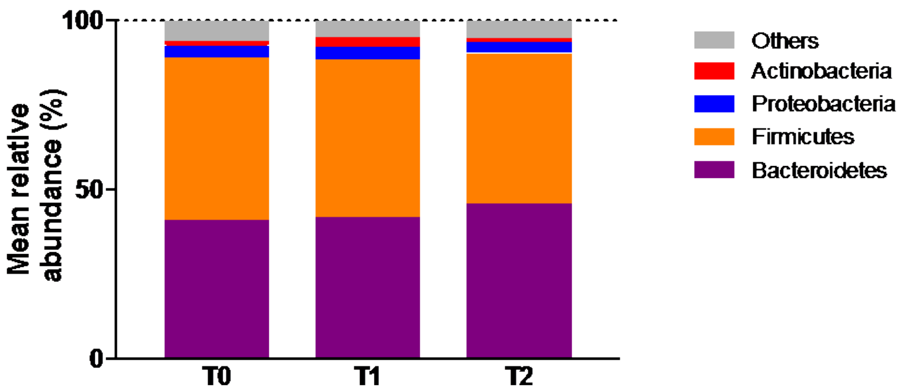
- Higher level of intrapersonal emotional competence at T2 versus T0
- No changes in perceived stress score
- Hedonic attitude for vegetables and leek consumption high before the study and stable throughout the intervention
- Intention to eat more vegetables, leek and salsify was not modified

Hiel, Bindels, Pachikian et al, *in revision*

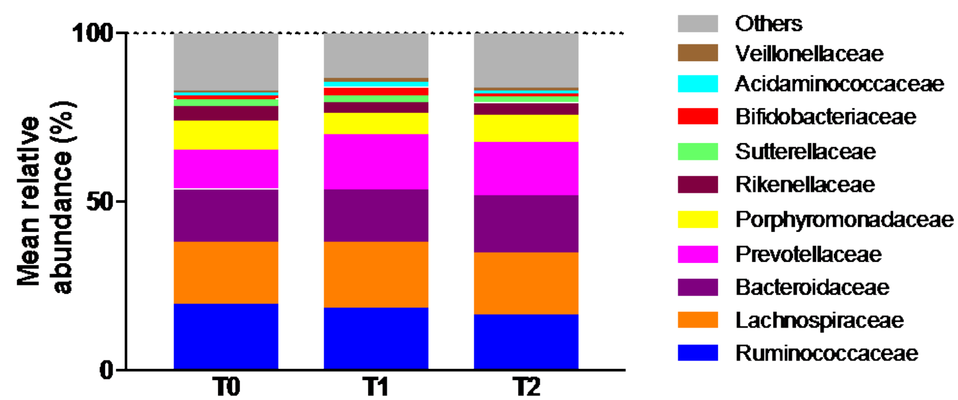
Gut microbiota composition

V5-V6 16SrDNA illumina sequencing

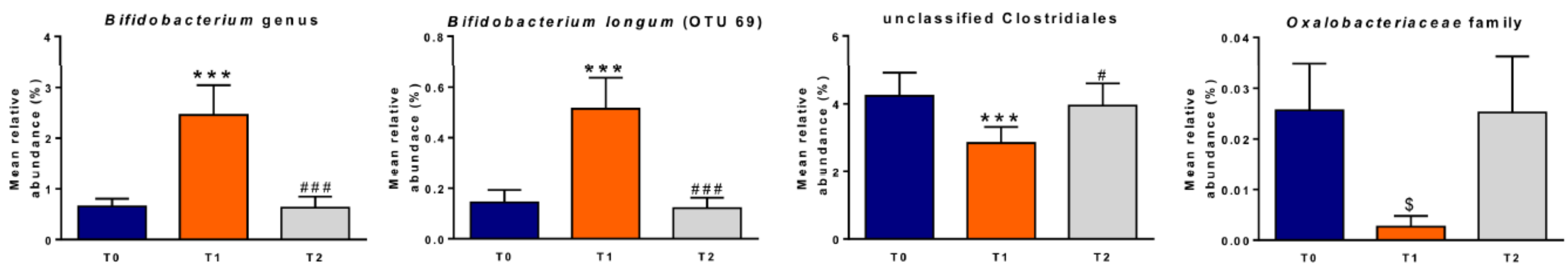
Phylum



Family



Taxa



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Conclusion of the study

- Inulin-rich vegetables are well tolerated, allow significant changes in the gut microbiota (reversible), can induce more persisting changes in food behaviour and emotional competences
- The increase in *Bifidobacterium* genus appears as a « signature » of inulin intake; an increase in Prevotella and a decrease in *Oscillibacter* sp., *Lachnospiraceae*, and *Alistipes* genus fits with observational studies relating vegetable intake and health.
- Subjects reported a reduced discomfort score following the food intervention; intestinal discomfort was negatively correlated with *Clostridium* cluster IV and *Ruminococcus callidus.*

Sophie Hiel, Barbara Pachikian, Julie Rodriguez,
Audrey Neyrinck
MD JP Thissen (UCL) N Paquot (Ulg), M. Cnop (ULB)

- To evaluate the impact of **native inulin** combined with **vegetables** rich in inulin on **weight management** and **metabolic alterations** in obese patients

- Randomized controlled trial, simple blind
- 150 patients (final objective)
- 3 months

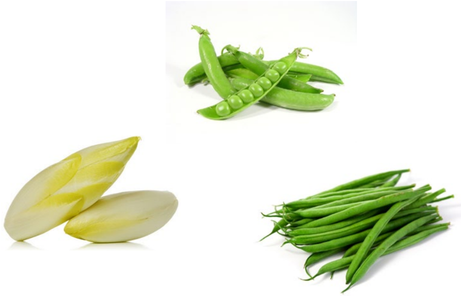
Sophie Hiel
PhD

Treated

- 16 g of purified inulin
- Recipes with vegetables containing high amount of ITF

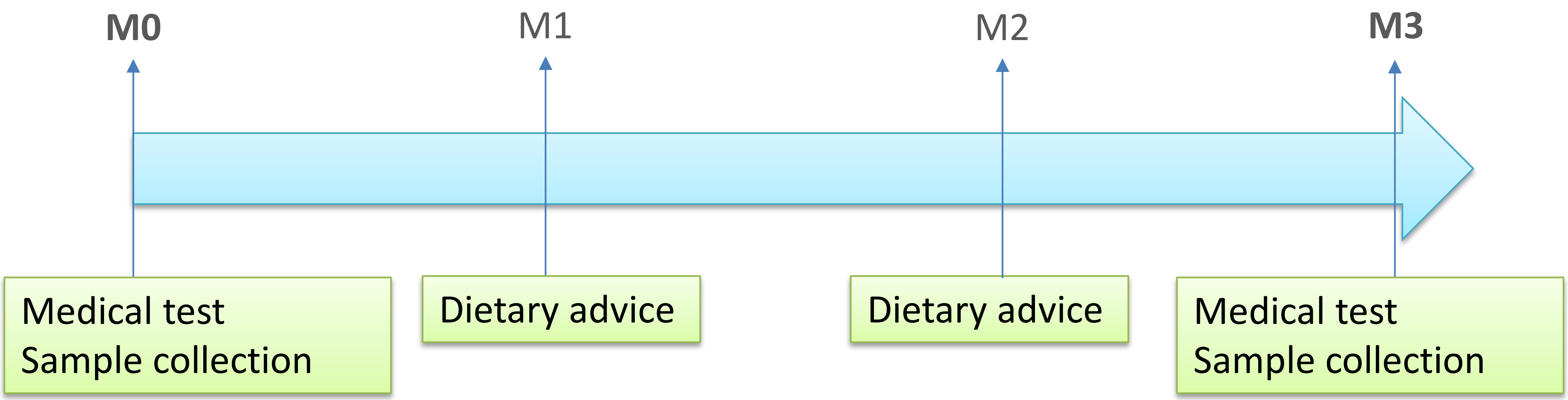
Placebo

- 16 g of maltodextrin
- Recipes with vegetables containing low amount of ITF



Clinical trial

FOOD 4 GUT
Programme d'excellence de la Region wallonne



M0 and M3 :

- Fibroscan
- Ct-scan
- Dietary habits evaluation
- Behaviour study (IPSY-PSYULB)
- Blood test and OGTT
- Anthropometry
- Sample collection

BMI (≥ 30 kg/m²)
Men and women
Age: between 18 and 65
Patient in pre-bariatric surgery
Obese patients with at least 1 comorbidity (treated or not)

- Pre-diabetes type 2 or diabetes type 2
- A dyslipidemia
- Hypertension
- Steatosis : level of transaminase or fibroscan

Pre-evaluation phase

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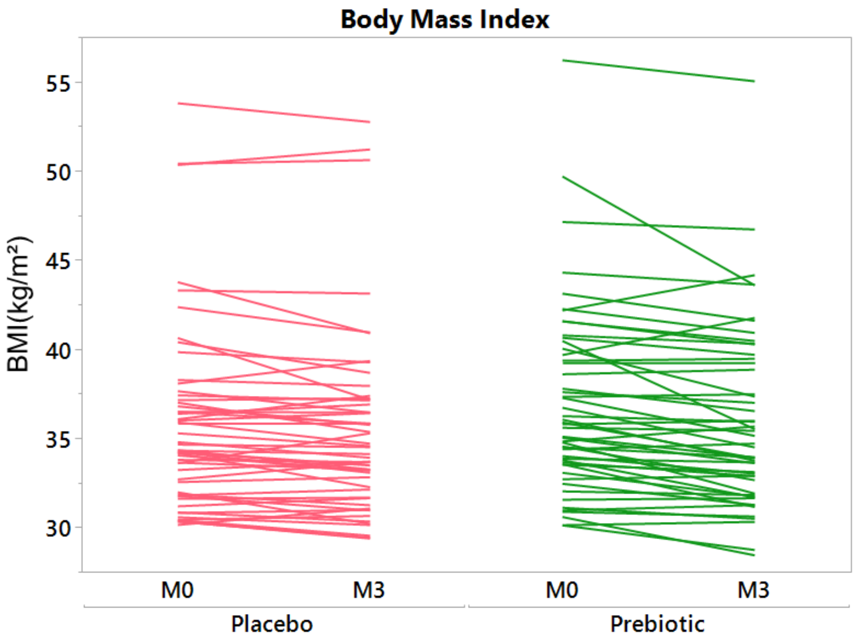
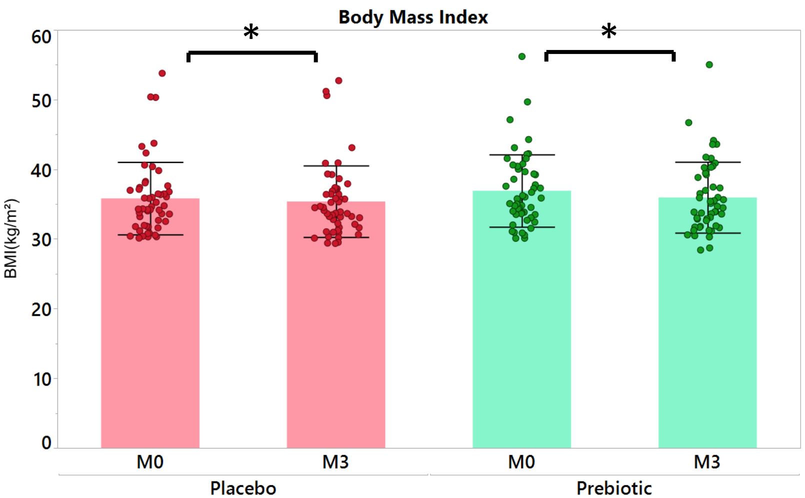


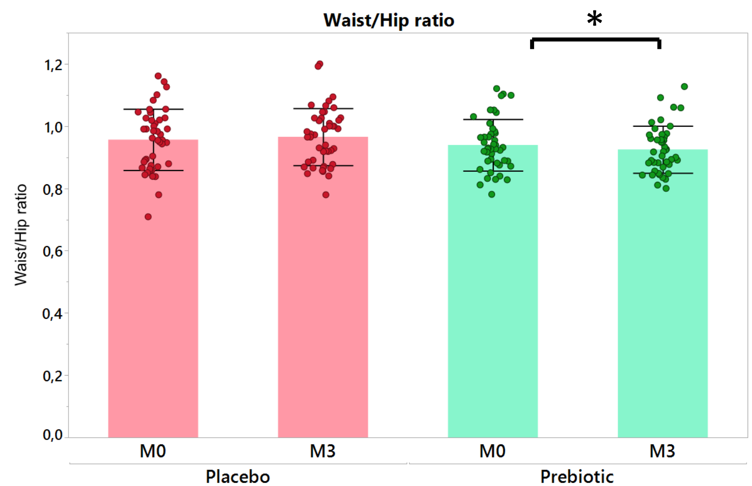
Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|
Intercept	36,010411	0,646432	1,786	55,71	0,0007*
Group[inuline]	0,5089714	0,498013	101,9	1,02	0,3092
Time[M0]	0,3472308	0,060125	101,1	5,78	<,0001*
Group[inuline]*Time[M0]	0,1325262	0,060125	101,1	2,20	0,0298*

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
inuline,M0	maltodextrine,M3	1,712404	1,003464	-0,90894	4,333744	0,3257
inuline,M0	maltodextrine,M0	1,282995	1,003056	-1,33728	3,903269	0,5783
inuline,M0	inuline,M3	0,959514	0,170903	0,51307	1,405963	<,0001*
inuline,M3	maltodextrine,M3	0,752890	1,003464	-1,86845	3,374230	0,8763
maltodextrine,M0	maltodextrine,M3	0,429409	0,169214	-0,01263	0,871444	0,0602
inuline,M3	maltodextrine,M0	0,323481	1,003056	-2,29679	2,943755	0,9883

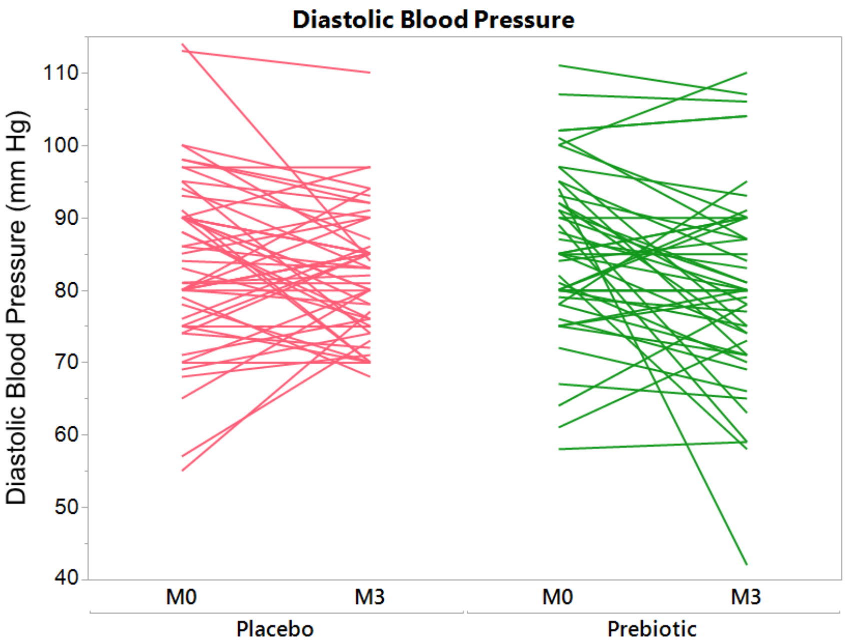
Level		
inuline,M0	A	
inuline,M3		B
maltodextrine,M0	A	B
maltodextrine,M3	A	B

Random Effect	Wald p-Value	Pct of Total
Hospital	0,7017	1,892
Patient	<,0001*	95,294
Residual		2,814
Total		100,000





Unpublished, preliminary data

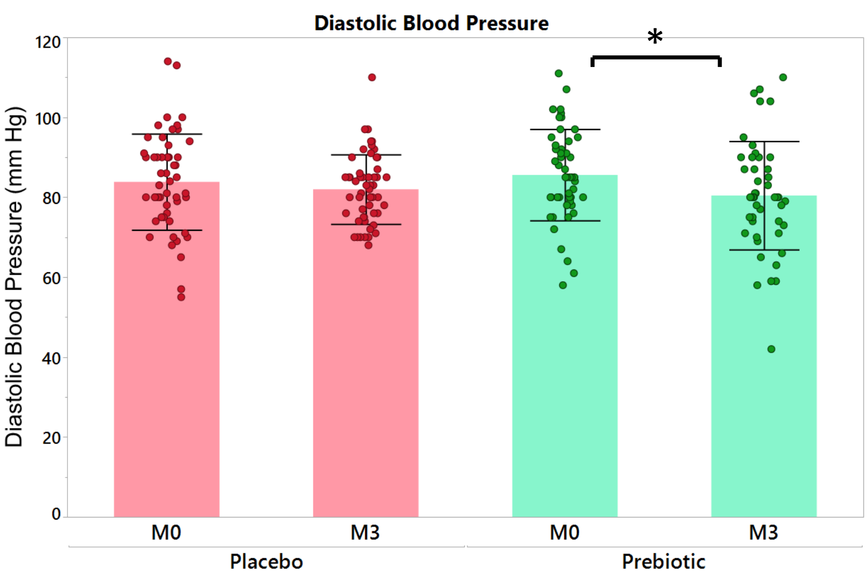


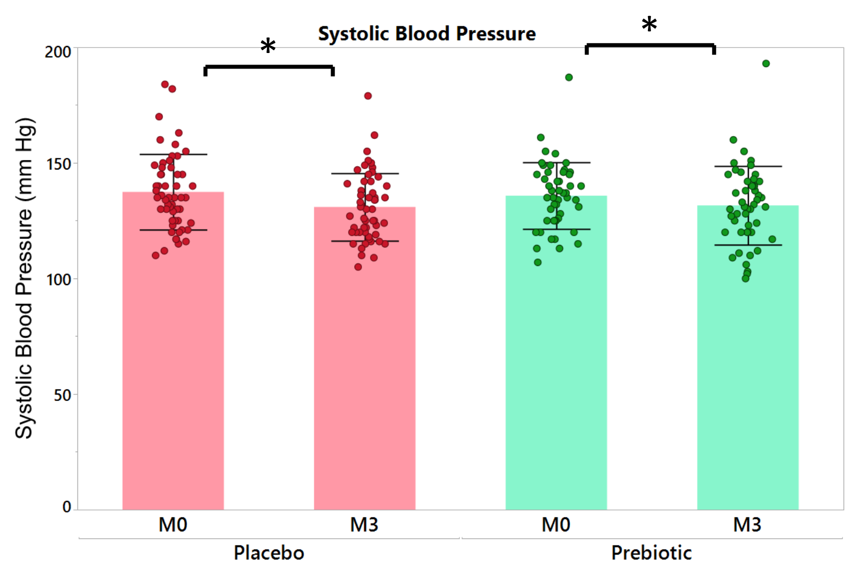
Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|
Intercept	82,809608	0,890058	1,397	93,04	0,0013*
Group[inuline]	-0,075448	0,990398	103,8	-0,08	0,9394
Time[M0]	1,8030293	0,557076	102,6	3,24	0,0016*
Group[inuline]*Time[M0]	0,8835355	0,557142	102,6	1,59	0,1159

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
inuline,M0	inuline,M3	5,373129	1,611697	1,16392	9,582338	0,0065*
maltodextrine,M0	inuline,M3	3,756955	2,258622	-2,14180	9,655712	0,3483
inuline,M0	maltodextrine,M3	3,455162	2,286570	-2,51659	9,426910	0,4347
maltodextrine,M3	inuline,M3	1,917968	2,265271	-3,99815	7,834090	0,8320
maltodextrine,M0	maltodextrine,M3	1,838988	1,538947	-2,18022	5,858198	0,6314
inuline,M0	maltodextrine,M0	1,616174	2,280113	-4,33871	7,571058	0,8934

Level		
inuline,M0	A	
maltodextrine,M0	A	B
maltodextrine,M3	A	B
inuline,M3		B

Random Effect	Wald p-Value	Pct of Total
Hospital	0,8138	0,000
Patient	<,0001*	51,470
Residual		48,530
Total		100,000





Unpublished preliminary data

Interindividual variability in the response to the dietary treatment

Does the gut microbiota prior intervention plays a role in the response to dietary intervention ?

- Illumina Sequencing of the gut microbiota (V5-V6 region-16SrDNA) of obese volunteers before the intervention
- Samples from 4 volunteers differing by the gut microbiota composition **at time 0 (before the treatment)**
- Transplantation in AB pretreated mice and evaluation of the response to high fat diet and inulin
- Comparison of the mice response versus human response to the dietary intervention for 3 months

Julie Rodriguez, Post Doc

UCLouvain

Unpublished data

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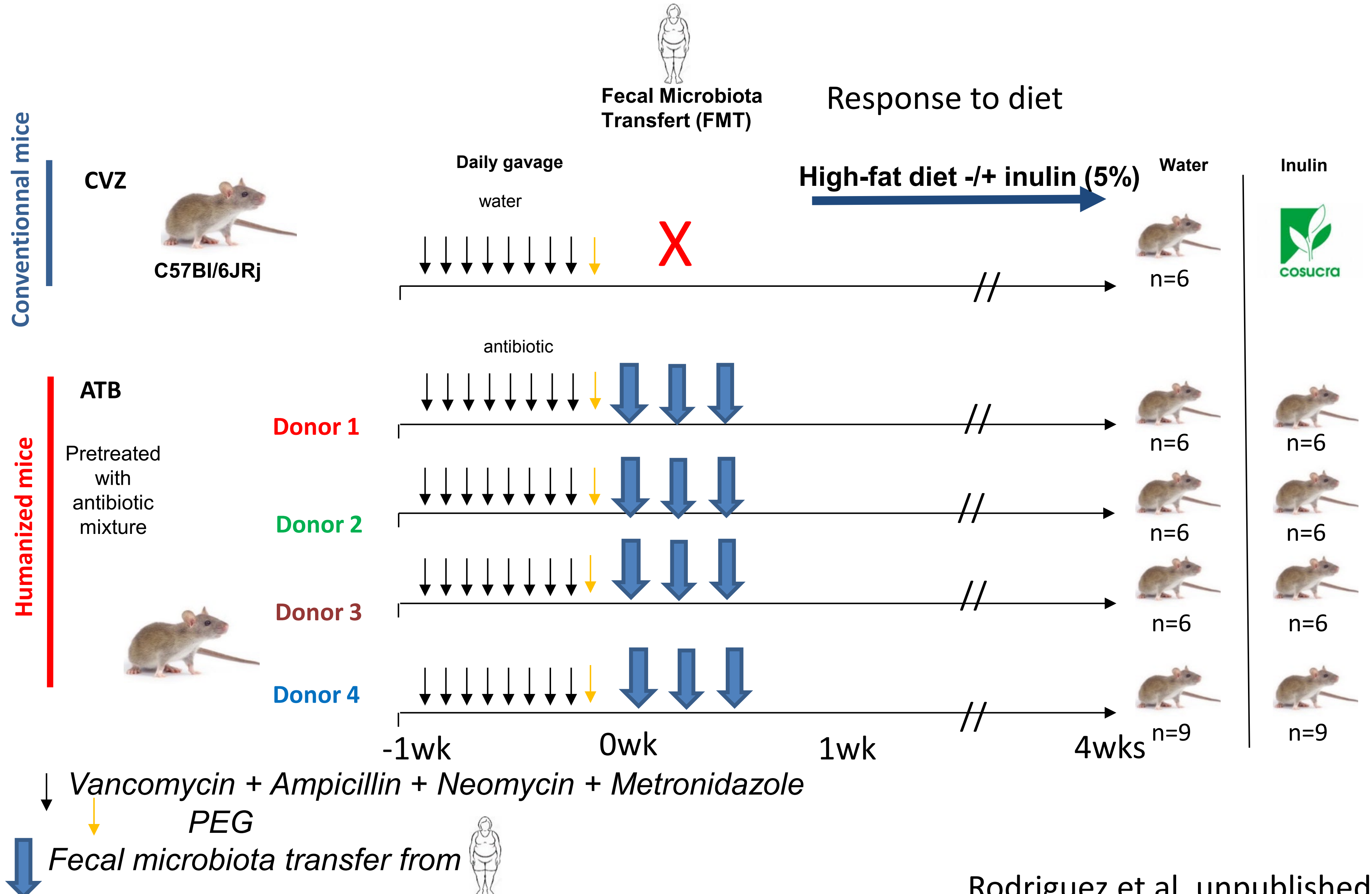
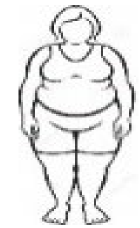
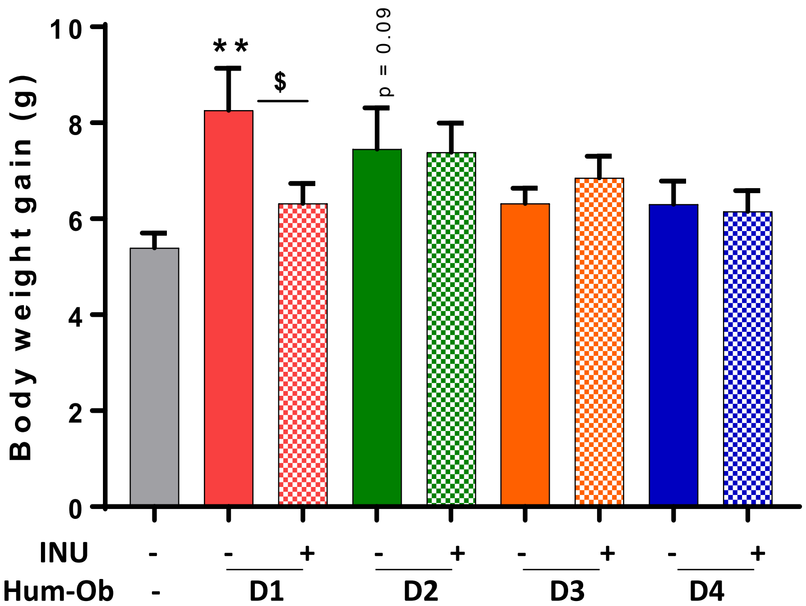
Experimental protocol



Rodriguez et al, unpublished

Individual response to dietary intervention is « transferable » to mice.



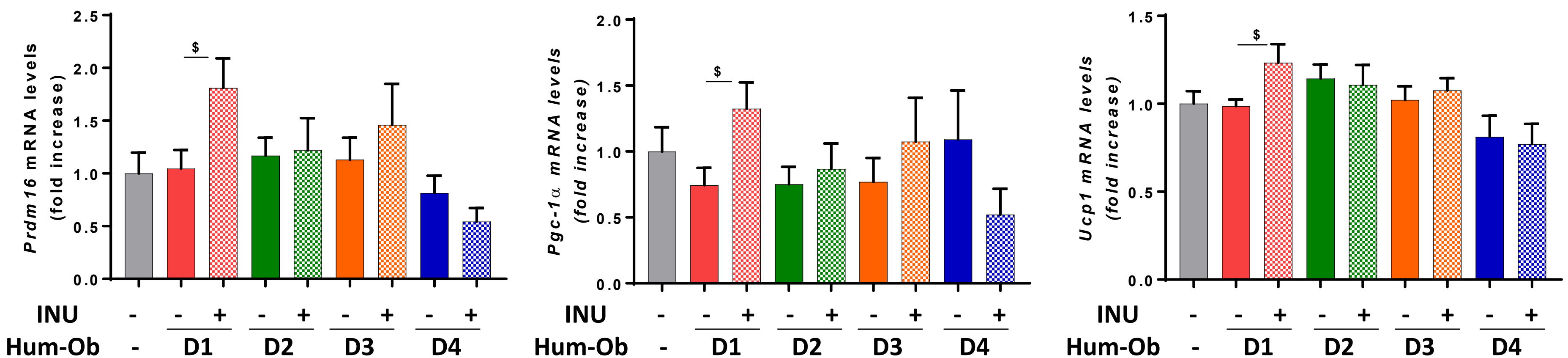


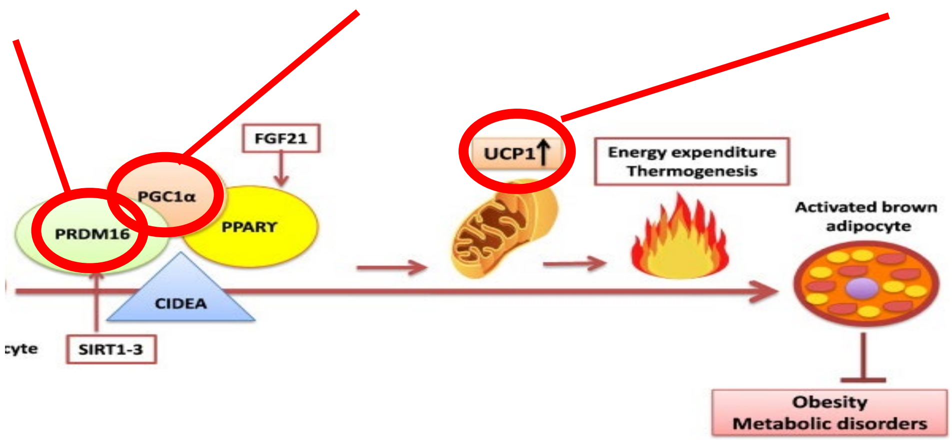
Body weight (kg)	M0	M3
D1	112.8	99
D2	113.6	110
D3	117.3	113.6
D4	108.6	113.7

*One-way ANOVA (vs CTRL, *p<0.05, **p<0.01) + t-test performed for each donor ($^{\$}$p<0.05).*

UCLouvain

Inulin stimulates the brown adipose tissue of D1 *Hum-ob* mice.





One-way ANOVA (vs CTRL) + t-test performed for each donor ($^{\$}p<0.05$).

UCLouvain

Take Home Messages and Future Prospects

- Dysbiosis modulates host metabolism through changes in phylogenic and metabolic profile
- Diet (vegetables rich in « prebiotics ») may reach the objective to modulate the gut microbiome significantlly : related health-effects ? Impact of gut microbiota on « personnalized » response to nutrition ?
- Intervention studies are in progress in humans to evaluate the health interest of fermentable fiber intake related to microbiota, and to validate biomarkers of fiber-microbiome-host interactions (fiberTAG project).

FiberTAG

TAGging dietary Fiber intake and their interest for health by measuring biomarkers related to the gut microbiota

ERA-HDHL Cofunded call for Joint Transnational
Research Proposals on "Biomarkers for Nutrition and Health"

FiberTAG

https://www.fibertag.eu/

Metabolism and Nutrition Research Group

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