

Welcome to the webinar:

Pig models for human nutrition research

29 June 2021

Guest speaker: Knud Erik Bach Knudsen, Helle Nygaard Lærke, and Mihai Curtasu | Aarhus University, Denmark

- Many have signed up for this webinar from around the world and therefore all attendees are muted to avoid background noises, delays in sound, echoes etc.
- Please ask your questions in the questions/chat section and we will follow up in the Q&A sessions following the presentation.
- We encourage everyone to complete the survey after the webinar, so we can continue planning relevant, educational and insightful webinars.
- Presentation slides and a recording of the webinar will be shared within 1-2 days.
- Certificates of attendance are available upon request. Please email events@minipigs.dk

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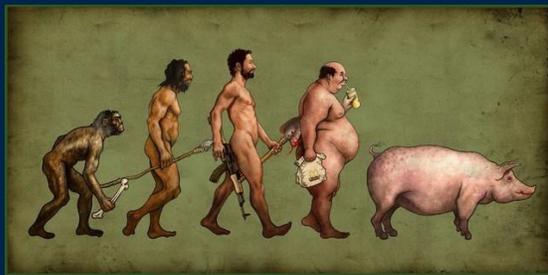


WeChat:



THE USE OF PIG MODELS TO STUDY THE ROLE OF CARBOHYDRATES IN HUMAN NUTRITION

Knud Erik Bach Knudsen, Professor, Ph.D.



POINTS TO BE ADDRESSED

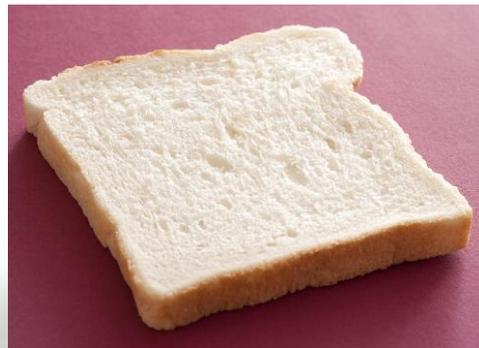
- Brief background on carbohydrates in nutrition and health
- Examples of cross species research projects with pigs selected for commercial production

CARBOHYDRATES IN NUTRITION AND HEALTH

Carbohydrates are the most important energy source in human nutrition contributing in the order of 45-55 % of total energy

Dietary carbohydrates are present as digestible (i.e. sugars, starch) and non-digestible (i.e. non-digestible oligosaccharides, resistant starch, non-starch polysaccharides); the last group also referred to as dietary fibre.

Refined carbohydrates foods



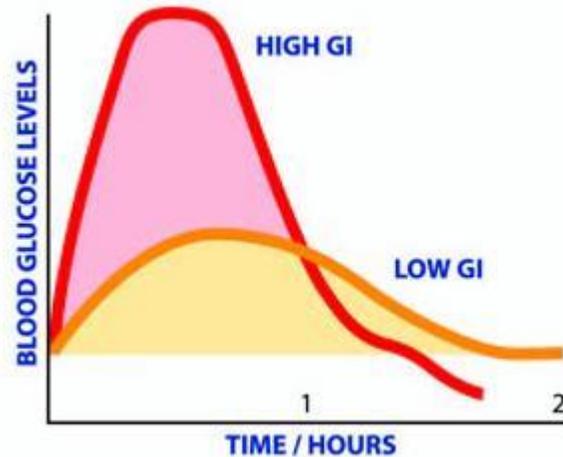
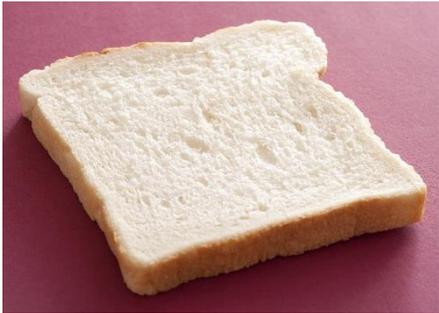
Dietary fibre rich foods



METABOLIC CONSEQUENCES OF READILY AVAILABLE CARBOHYDRATES

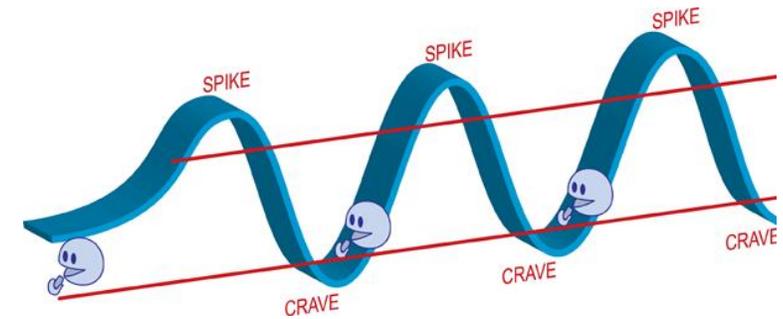
Easily digestible carbohydrates

- High starch/sugar contents
- Low dietary fibre (high GI)



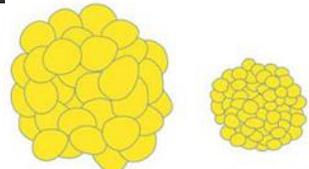
GI, Glycemic index

Fluctuation of blood glucose and insulin

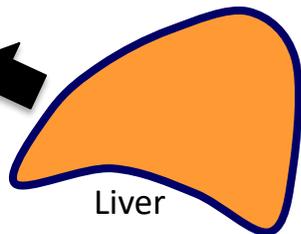


- Overconsumption
- Decrease insulin sensitivity
- Development of the metabolic syndrome

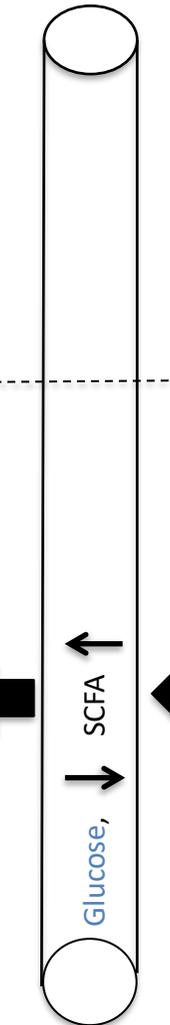
Physiological effects of dietary fibre



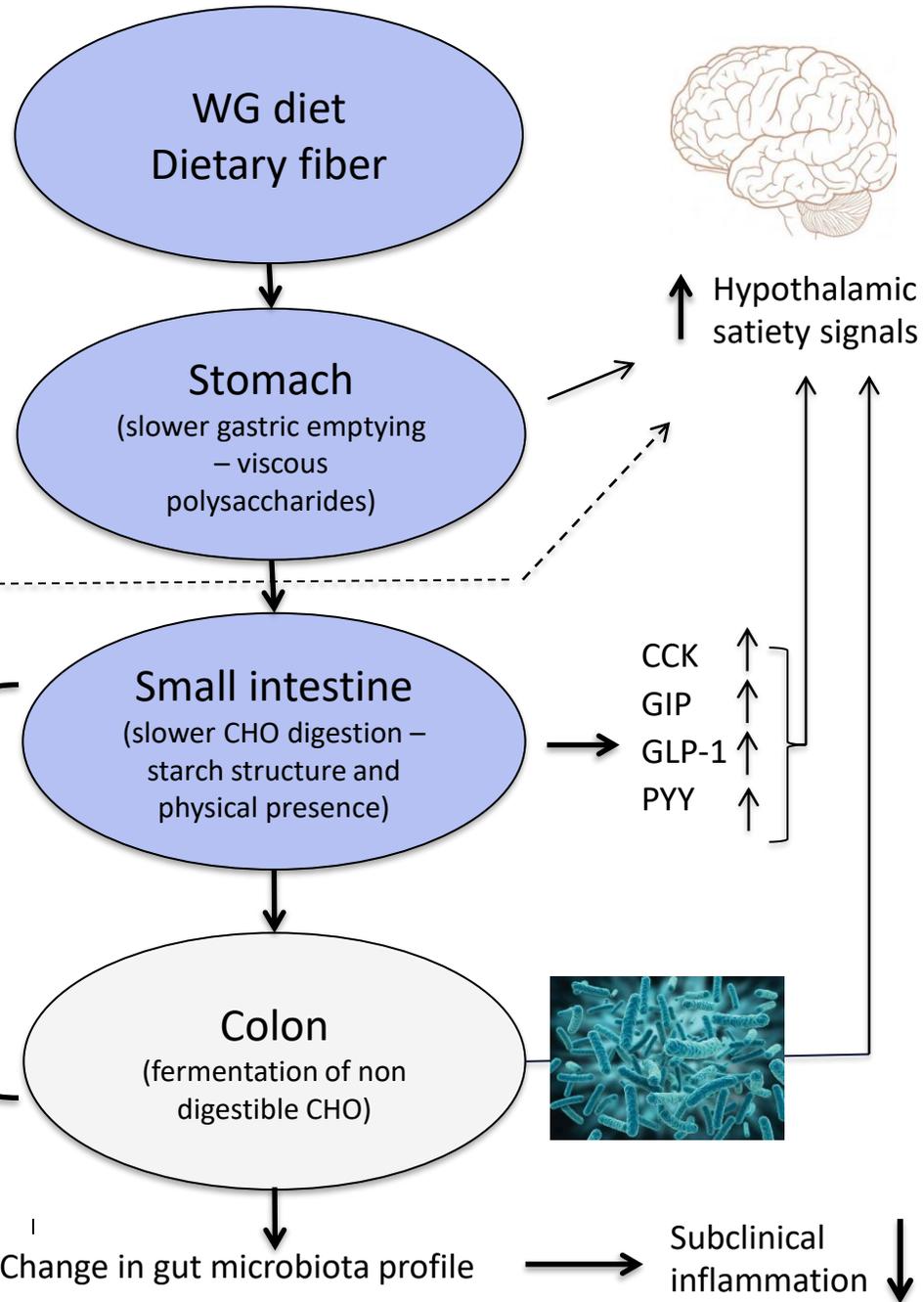
Fat storage ↓
Peripheral circulation and tissues



Liver



Portal vein

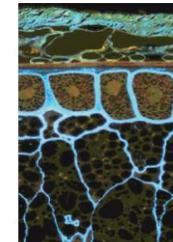
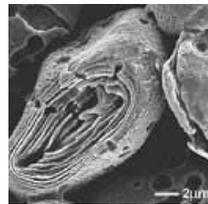
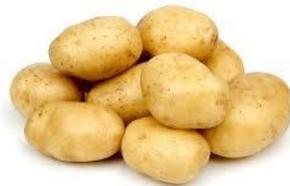


EXAMPLES FROM RESEARCH PROJECTS

- BioFunCarb: Biofunctional carbohydrates
 - Acute studies



- BUTCOINS: Concepts for enhanced butyrate production to improve colonic health and insulin sensitivity
 - Intervention studies

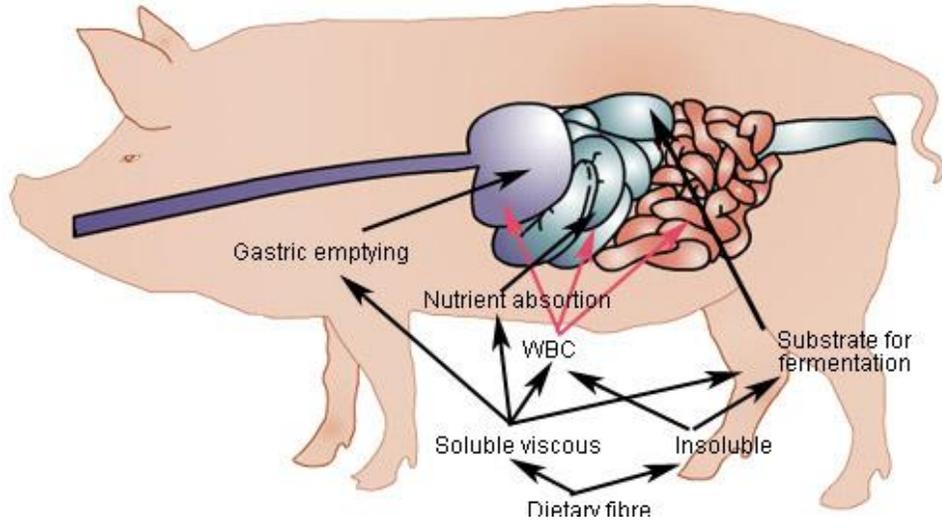
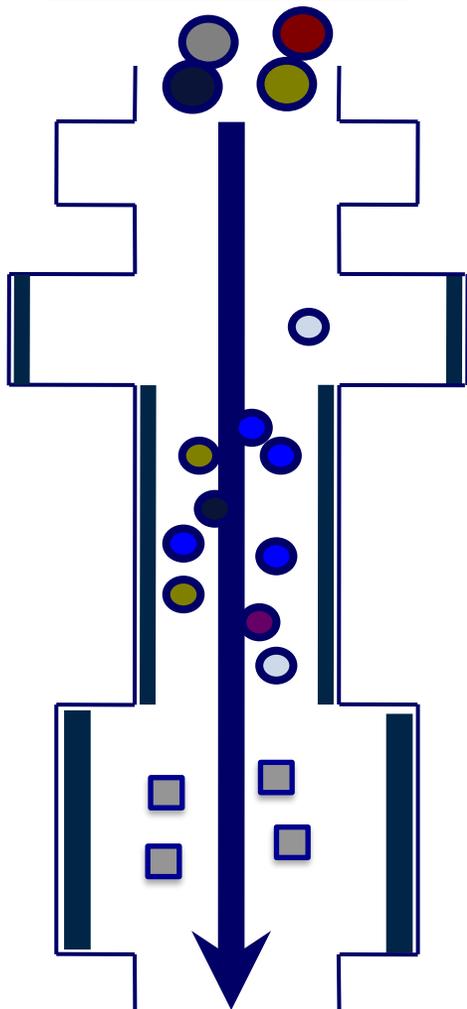
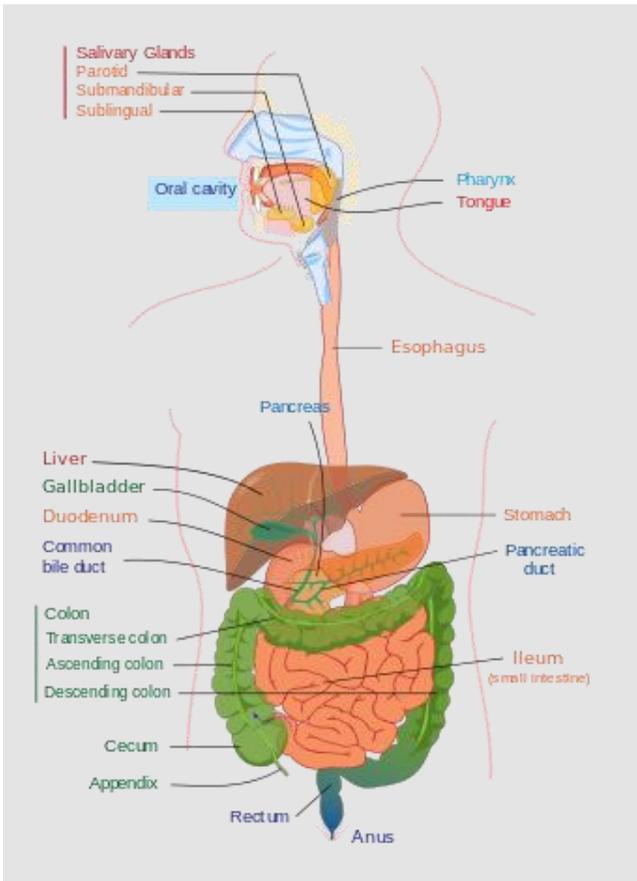
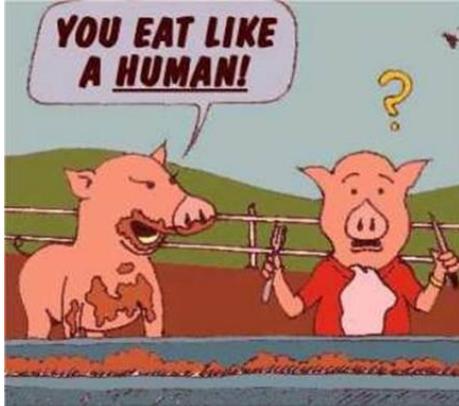
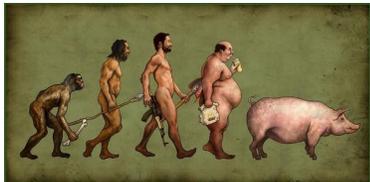


RS

ELLEGAARD MINIPIG WEBINAR | 29 JUNE 2021

AX

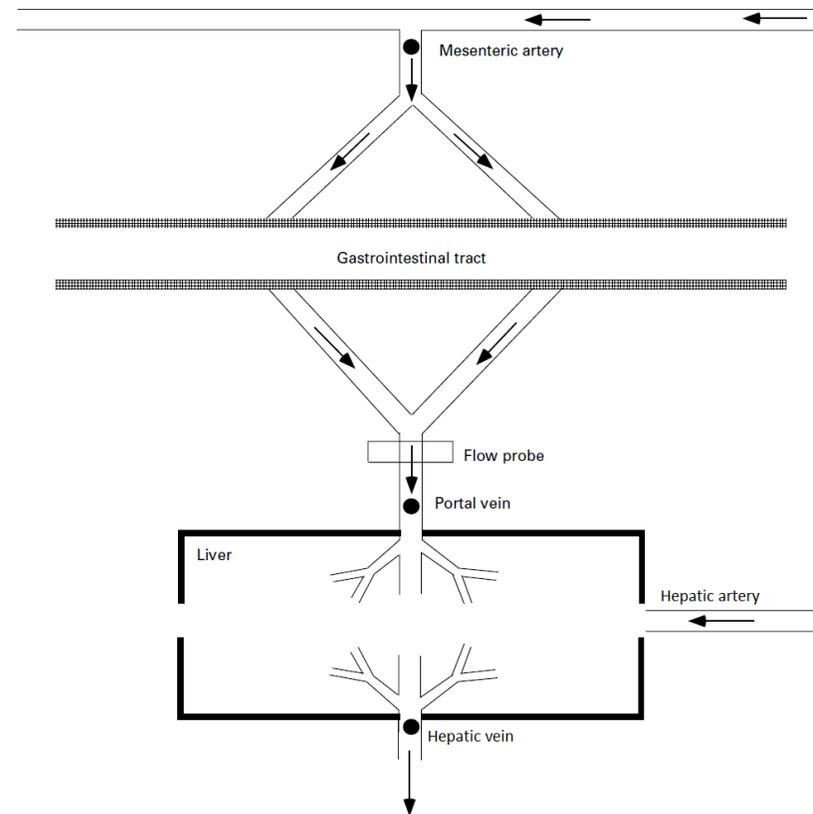
SPECIES AS EXPERIMENTAL OBJECTS



MULTI-CATHETERISATION MODEL

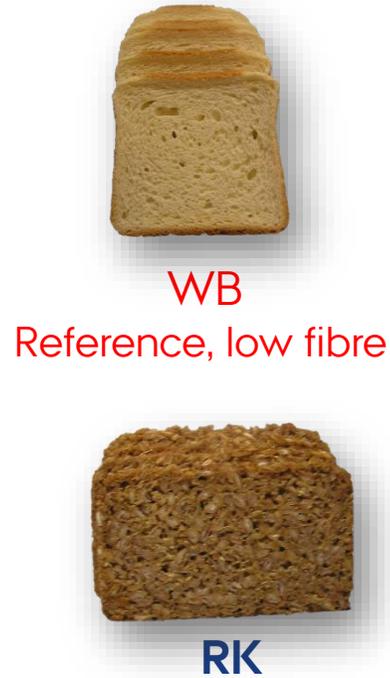
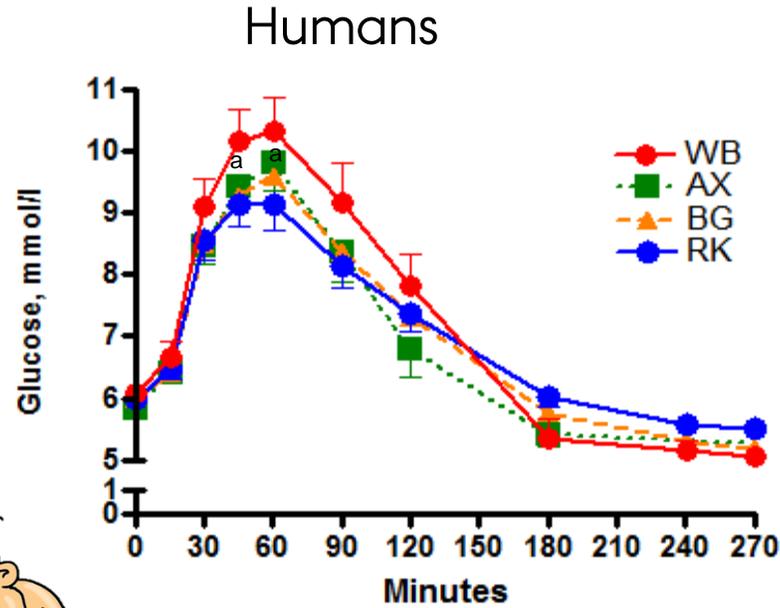
- Model with permanent catheters in the:
 - Mesenteric artery (peripheral levels)
 - Portal vein (gastrointestinal enrichment)
 - Vena Hepatica (extrahepatic supply)
- Flow-probe around the portal vein

Kinetics and quantitative determinations of net flux of metabolites and hormones across organs and to the body

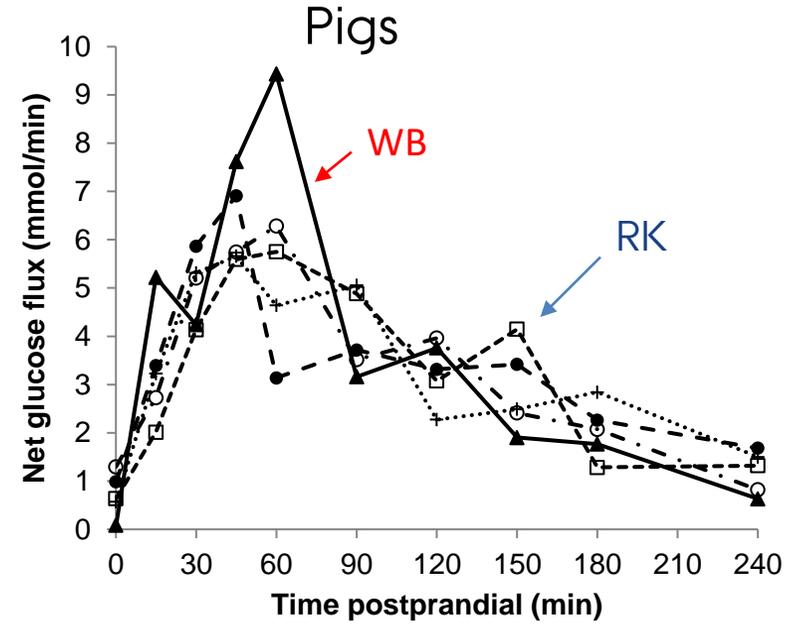


Modified from K. E. Bach Knudsen et al., Proc Nutr Soc. 2003, 62: 81-6.

GLYCEMIC RESPONSE IN HUMANS AND PIGS OF DIFFERENT BREAD TYPES



	GI	II
WB	100	100
RK	77	51



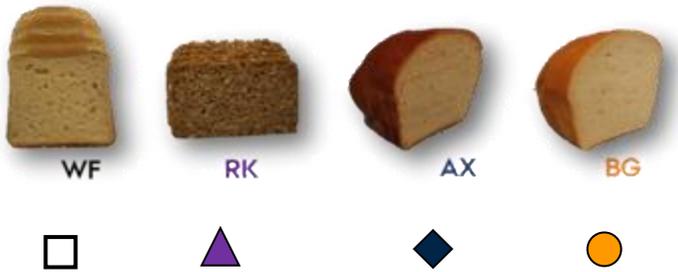
	GI	II
WB	100	100
RK	83	78

Hartvigsen et al (2014).

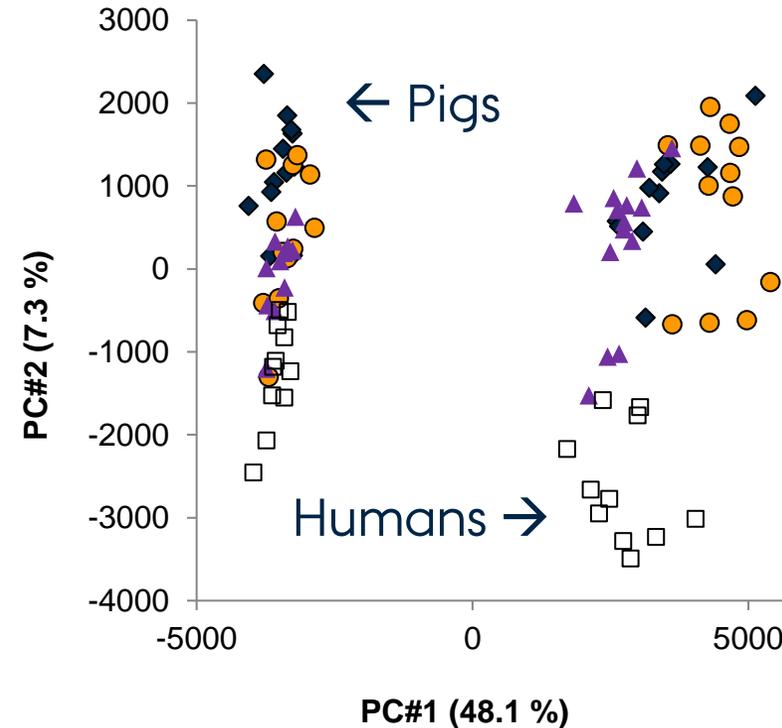
Christensen et al (2013).

INFLUENCE OF DIFFERENT BREADS ON THE PLASMA METABOLOME OF PIGS AND HUMAN SUBJECTS

- 30 min postprandial



- PCA-scores plot
 - Same “bread pattern” on pigs and humans in PC#2
 - Higher variation among the human subjects

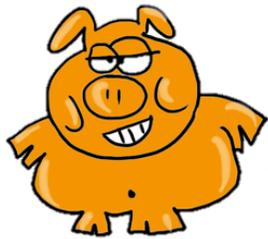
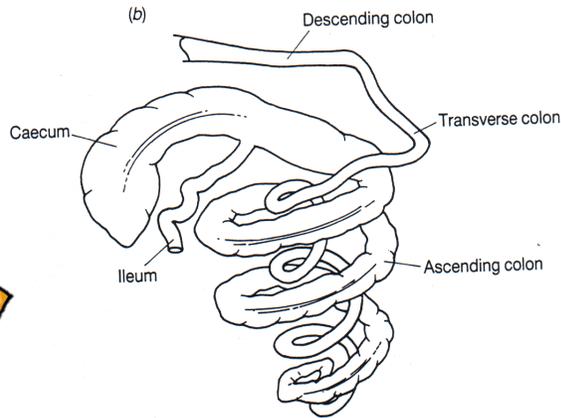


INFLUENCE OF RS AND AX ON MICROBIOTA AND SHORT-CHAIN FATTY ACIDS PRODUCTION AND ABSORPTION

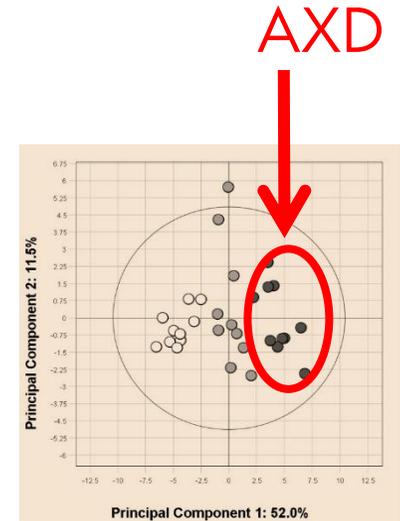
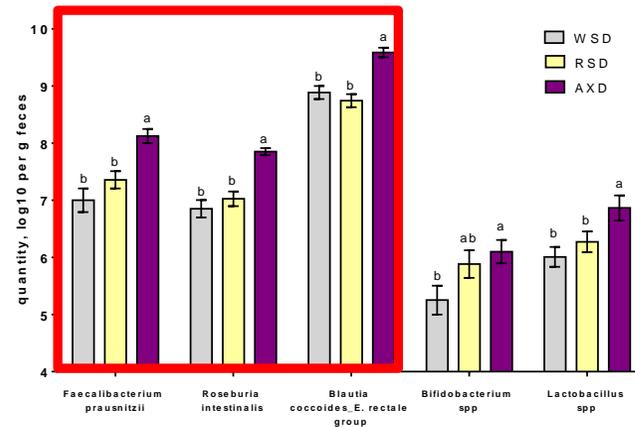


WSD **RSD** **AXD**
7 % fiber **19 %** **19 %**
 fiber **fiber**

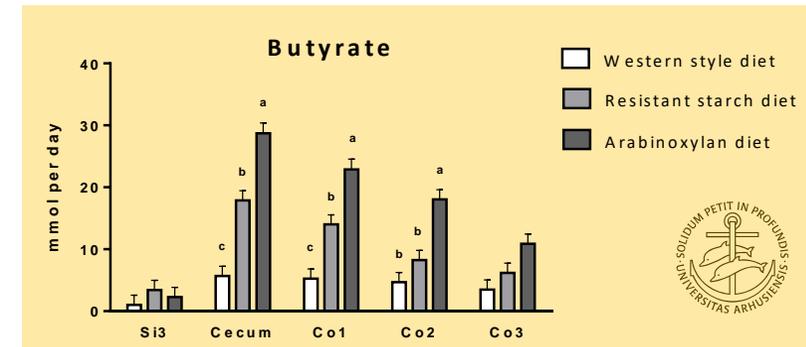
Fermentation in large intestine



Stimulation of butyrate producing microorganisms



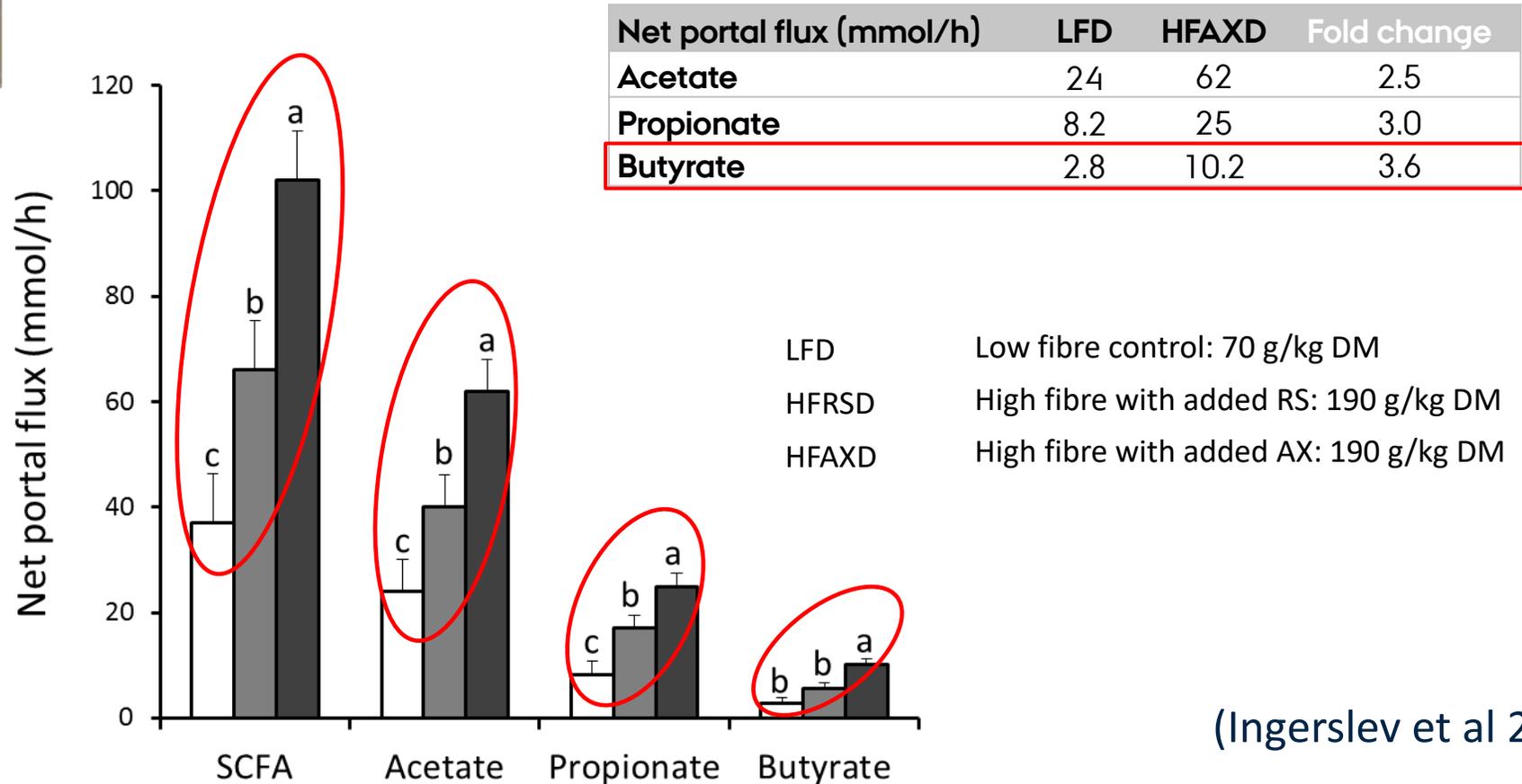
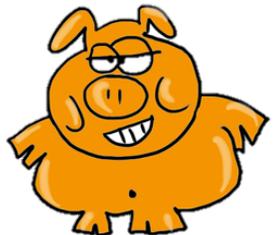
Stimulation of butyrate production



STIMULATION OF SCFA AND BUTYRATE ABSORPTION

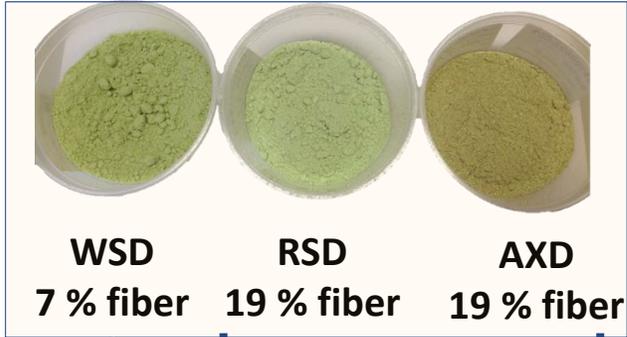


WSD **RSD** **AXD**
7 % **19 %** **19 %**
fiber **fiber** **fiber**

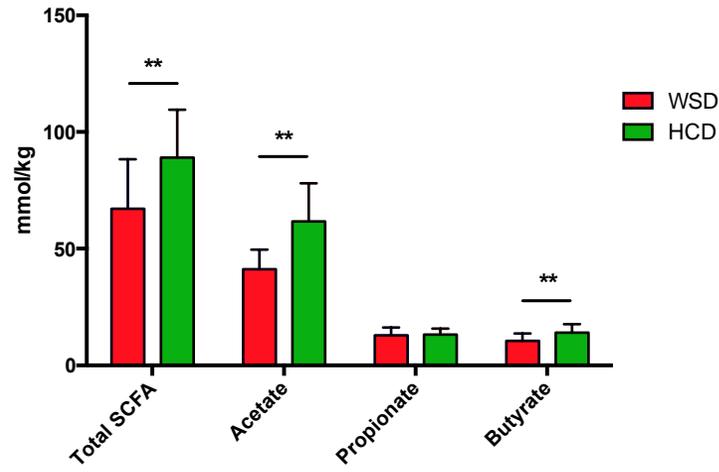


(Ingerslev et al 2014).

HUMAN EFFECTS



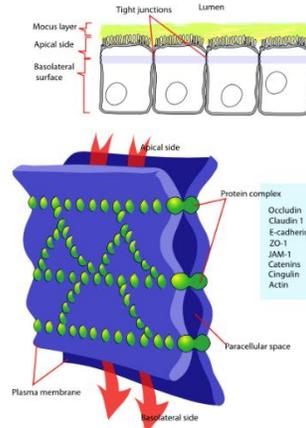
Feeds for pigs



Increased faecal butyrate



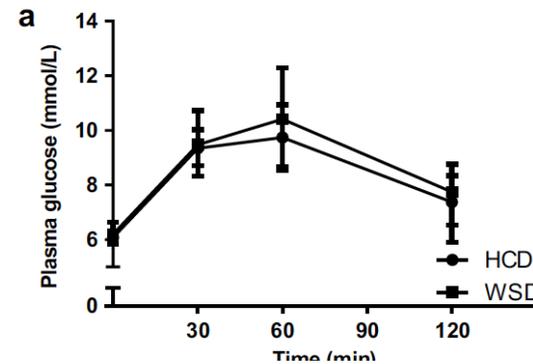
Foods for humans



Improved intestinal barrier function and reduced intestinal inflammation

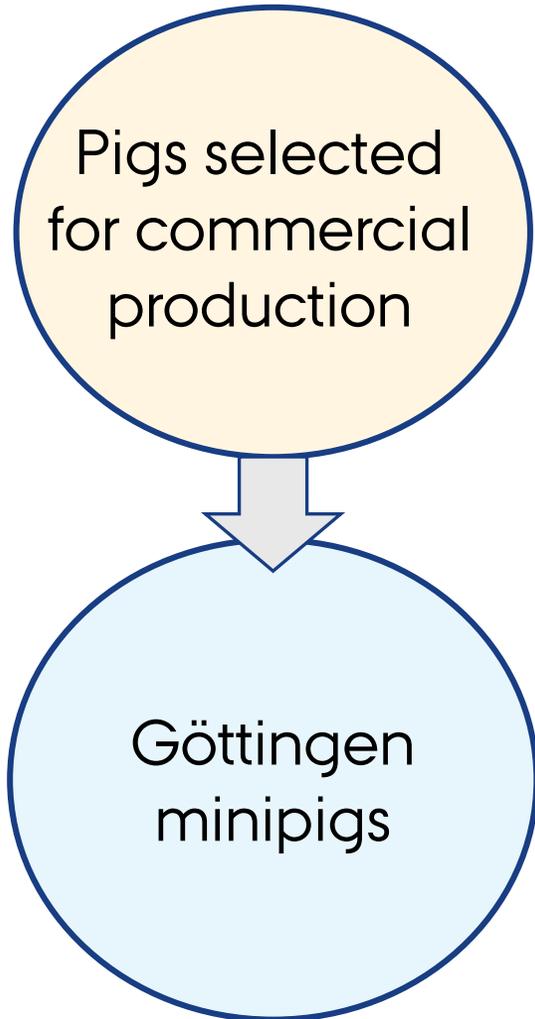
DF: 18

64 g/d



No significant effects on either peripheral inflammation markers or glucose response and insulin sensitivity

FROM PIGS SELECTED FOR COMMERCIAL PRODUCTION TO GÖTTINGEN MINIPIGS



Focus on:

- Digestion, absorption and metabolic response
- Providing in-depth understanding on the responses in humans

Focus on:

- Dietary strategies (carbohydrate quality) for the development of metabolic abnormalities in Göttingen minipigs
- Dietary strategies (protein and dietary fibre) to mitigate metabolic abnormalities in young Göttingen minipigs as model for human subjects with MetS

ROLE OF CARBOHYDRATES AND FEEDING REGIME IN THE DEVELOPMENT OF OBESITY AND METABOLIC SYNDROME IN GÖTTINGEN MINIPIGS

Mihai-Victor Curtasu, postdoc fellow, Ph.D.

Department of Animal Science , Laval University, Quebec, Canada

OBESOGENIC DIETS

Minipigs quickly become overweight and obese if diet is not well managed

Common referred to as High Energy Diets, High Fat High Carbohydrate diets, Western diets

In practice

→ **high carbohydrates** (sucrose, fructose, glucose, high starch content), 20-40%

→ **high fat** (lard, saturated fats), 12-25%

→ **addition of cholesterol**, cholate or other atherogenic compound, 0.5-2%

→ **lower protein content**

→ **low fiber content**

Calculated Analysis

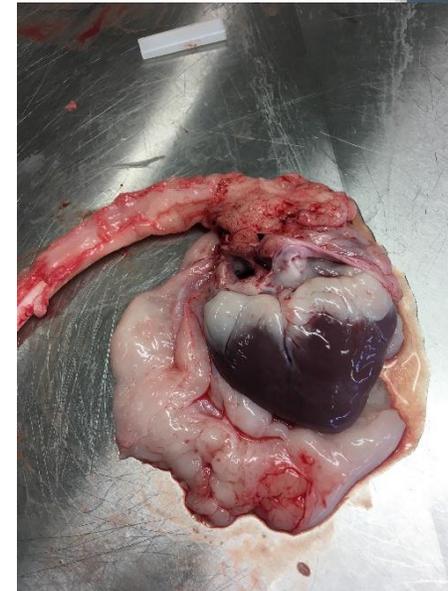
Mini-Pig

NUTRIENTS	Total	Supp (9)
Proximate Analysis		
Moisture (1)	%	10.00
Crude Oil	%	2.13
Crude Protein	%	13.03
Crude Fibre	%	14.52
Ash	%	7.35
Nitrogen Free Extract	%	52.41
Digestibility Co-Efficients (7)		
Digestible Crude Oil	%	1.20
Digestible Crude Protein	%	9.41
Carbohydrates, Fibre and Non Starch Polysaccharides (NSP)		
Total Dietary Fibre	%	29.66
Pectin	%	2.78
Hemicellulose	%	11.62
Cellulose	%	14.12
Lignin	%	2.85
Starch	%	27.12
Sugar	%	5.54
Energy (5)		
Gross Energy	MJ/kg	13.80
Digestible Energy (11)	MJ/kg	11.43
Metabolisable Energy (12)	MJ/kg	10.98
Atwater Fuel Energy (AFE) (8)	MJ/kg	11.74
AFE from Oil	%	6.82
AFE from Protein	%	18.55
AFE from Carbohydrate	%	74.62

- By comparison, typically the SDS (Special Diet Services) diet for minipigs:
 - > low levels of protein and carbohydrate (low energy diet)
 - > high levels of dietary fiber

OBESOGENIC DIET OUTCOMES

- Obese phenotype predisposing to Metabolic Syndrome:
 - Increased body weight
 - Increased insulin response
 - Early atherosclerosis
 - Elevated blood pressure
 - Elevated markers of dyslipidemia
 - Visceral fat and fatty organs
 - *Type 2 diabetes: impossible to establish in minipigs only based on diet*



EXAMPLES FROM PREVIOUS EXPERIMENTS WITH GÖTTINGEN MINIPIGS

Reference	Feeding method	Obesogenic diet description
Larsen et al., 2002	Restricted	High Fat High Energy
Christoffersen et al., 2013	Restricted	High Fat High Carbohydrate (+ Sucrose)
Bollen et al., 2005	Ad libitum	High Fat
Ludvigsen et al., 2015	Restricted	High Fat High Carbohydrate (+ Fructose + Cholesterol)
Hernández Hurtado et al., 2016	Restricted	High Fat High Energy (+ Sucrose)
Andreasen et al., 2018	Restricted	High Fat High Carbohydrate (+Fructose)
Haagensen et al., 2014	Restricted	Low Fat High Carbohydrate (+Sucrose) High Fat Low Carbohydrate (+2% cholesterol)
Pedersen et al., 2019	Restricted	High Fat High Cholesterol Choline-deficient, AA-deficient, High Fat + sucrose Choline-deficient, AA-deficient, High Fat + fructose
Christoffersen et al., 2013	Restricted	High Fat High Carbohydrate (+ sucrose)
Johansen et al., 2001	Restricted	High Fat High Energy
Renner et al., 2018	Restricted	High Fat High Energy (+ beet sugar, i.e. sucrose)
Bollen et al., 2005	Restricted vs ad libitum	Standard Diet
Bollen et al., 2005	Ad libitum	High Fat Diet
Xu et al., 2020	Ad libitum	High Fat High Carbohydrate (+ Fructose)
Curtasu et al., 2020	Ad libitum	High Fat High Carbohydrate (+ Fructose) High Fat High Carbohydrate (+ resistant starch)

In minipig studies carbohydrates are used as an important source of calories to promote obesity.

Examples:

➤ Sucrose 15 - 31%

➤ Fructose 18 - 20%

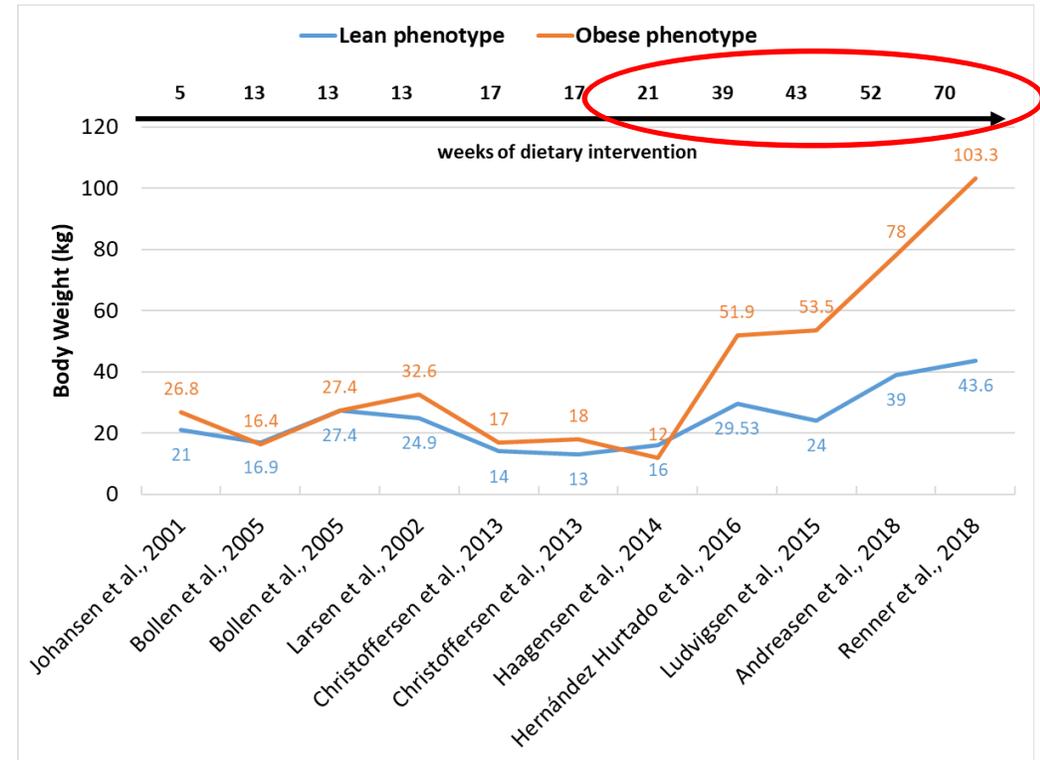
Excessive intake of dietary sugars is the major concern to obesity development and not necessarily the carbohydrate type

Fructose – main carbohydrate used for studies of fatty liver / NASH

OTHER ASPECTS INFLUENCING OBESITY DEVELOPMENT

Diet description	
High fat	high energy
High fat	high carbohydrate (+ sucrose)
High fat	high fructose high cholesterol
High fat	high energy (+ sucrose)
High fat	high carbohydrate (+ fructose)
High fat	low carbohydrate + cholesterol
High fat	diets
High fat	high fructose

When discussing carbohydrates in obesity models we need to be aware about the high levels of fat used to induce obesity



Period of dietary intervention (together with age) influences greatly obesity development

MERITS STUDY (2014-2018)

Metabolic changes by carbohydrate and protein quality in the development and mitigation of metabolic syndrome

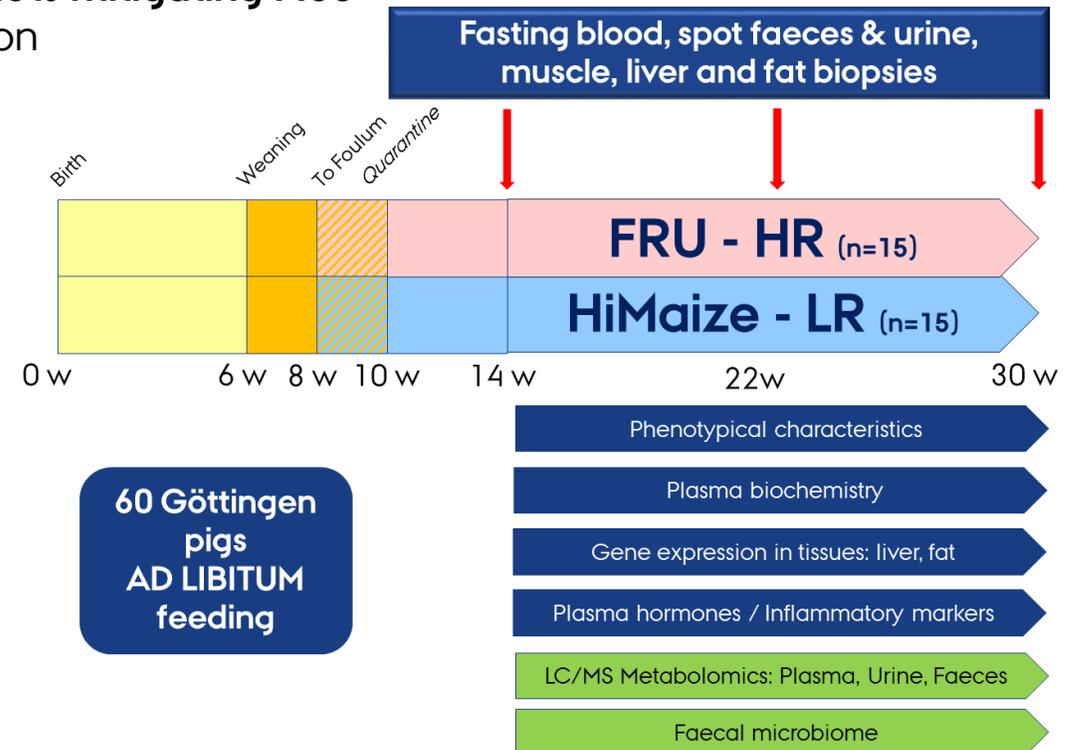
OBJECTIVE 1: Longitudinal study in a porcine miniature model of a diet predisposing to MeS

Development of a miniature pig model with specific signs of obesity and MetS, accelerated by ad libitum feeding of fructose

OBJECTIVE 2: Intervention in a porcine miniature model with diets mitigating MeS

→ further discussed by Helle Nygaard Laerke in next presentation

	High Risk	Lower Risk
Ingredients	g/kg	g/kg
Wheat starch	232.8	232.8
Whole grain wheat (milled)	150.0	150.0
Wheat bran (finely milled)	125.0	125.0
Wheat gluten	65.0	65.0
Fish meal	20.0	20.0
High amylose maize starch	0.0	200.0
Fructose	200.0	0.0
Lard 92/15	150.0	150.0
Vitamin/minerals premix/others*	57.2	57.2

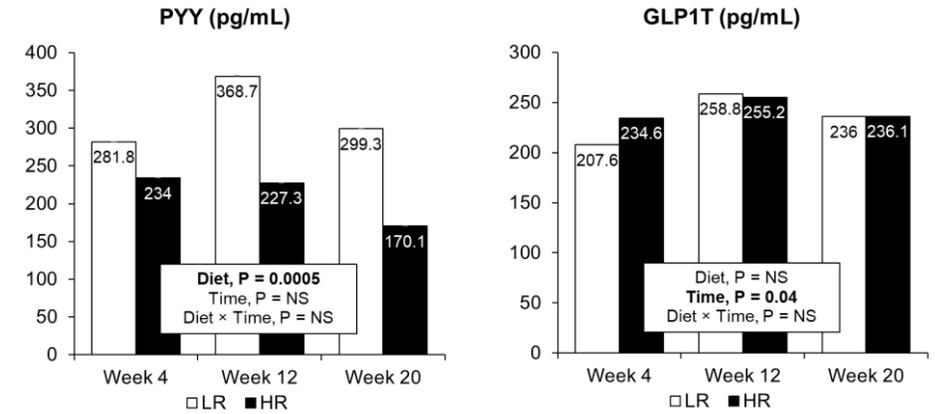
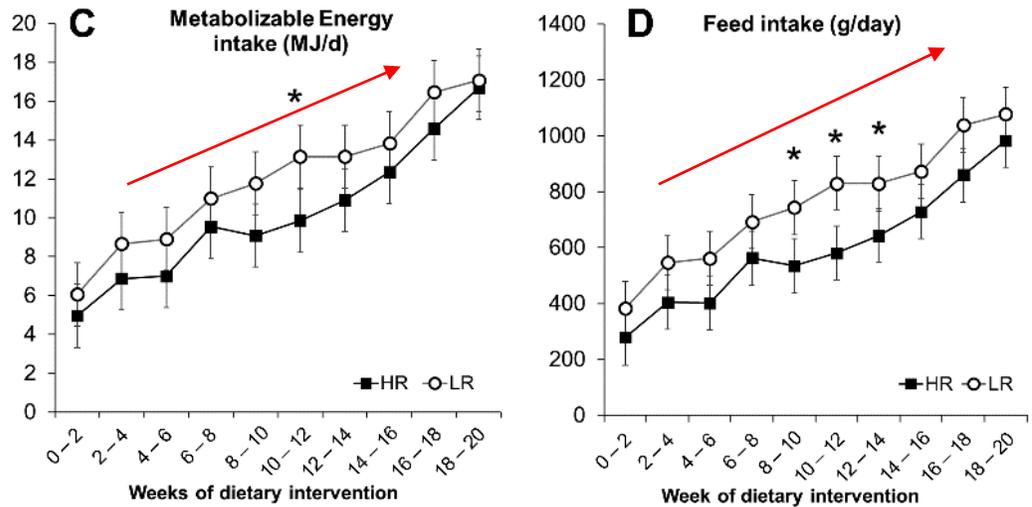
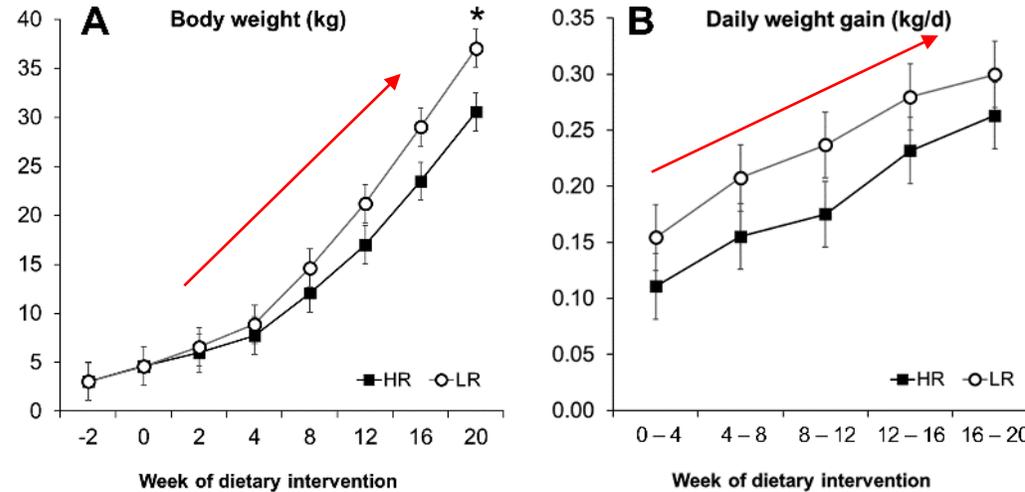


OBESITY DEVELOPMENT

Article

Obesity Development and Signs of Metabolic Abnormalities in Young Göttingen Minipigs Consuming Energy Dense Diets Varying in Carbohydrate Quality

Mihai Victor Curtasu ^{*}, Mette Skou Hedemann [†], Helle Nygaard Lærke [†] and Knud Erik Bach Knudsen [†]



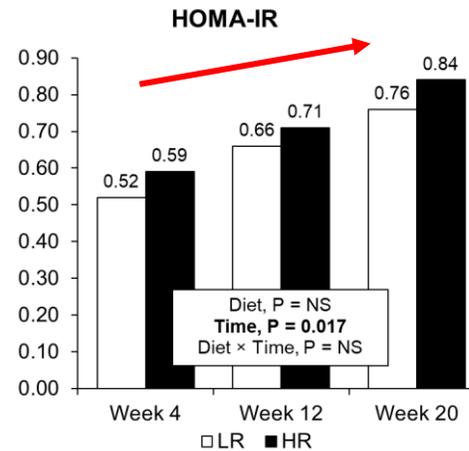
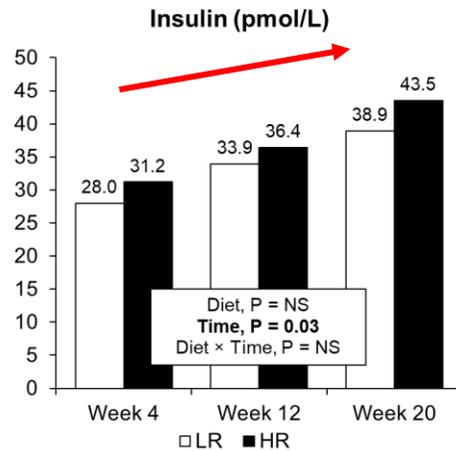
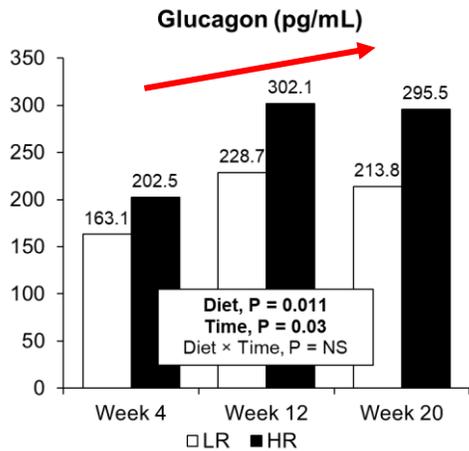
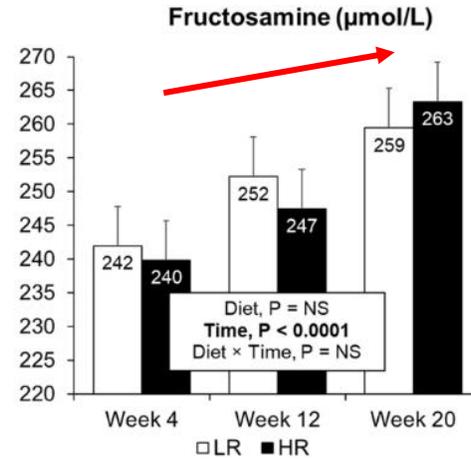
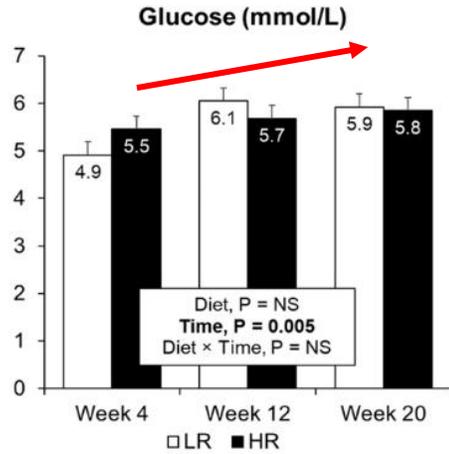
Fermentation of resistant starch (HiMaize) to SCFAs would influence satiety hormones (GLP-1, PYY)

PYY acts on increasing satiety, decreasing overall energy intake and delaying glucose absorption

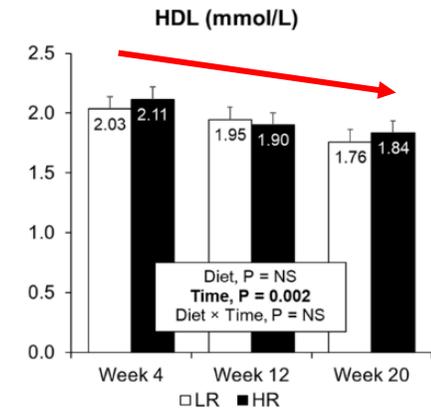
Ad libitum feed intake of HiMaize was higher despite the high release of PYY

OBESITY DEVELOPMENT

Glucose and insulin responses



Dyslipidemia

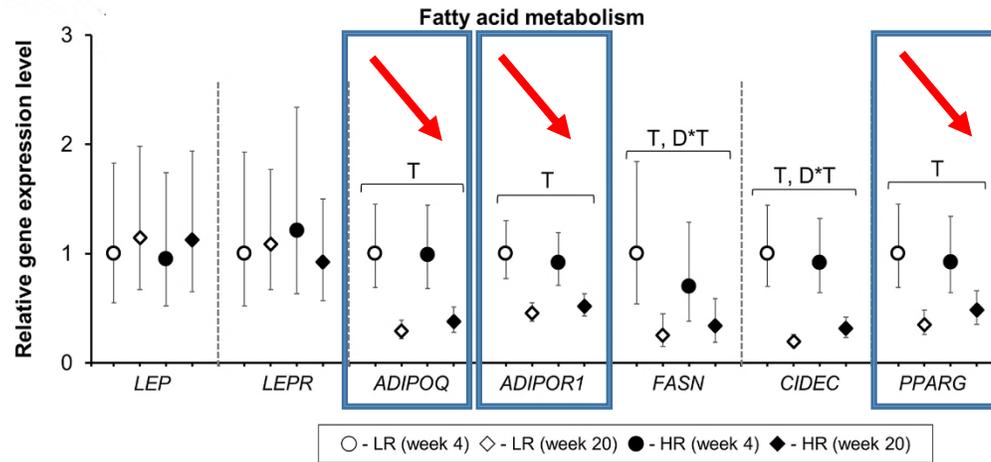


NO DIET OR TIME EFFECT:

- ✗ Total Cholesterol
- ✗ Triglycerides
- ✗ LDL-c
- ✗ LDL-c:HDL-c

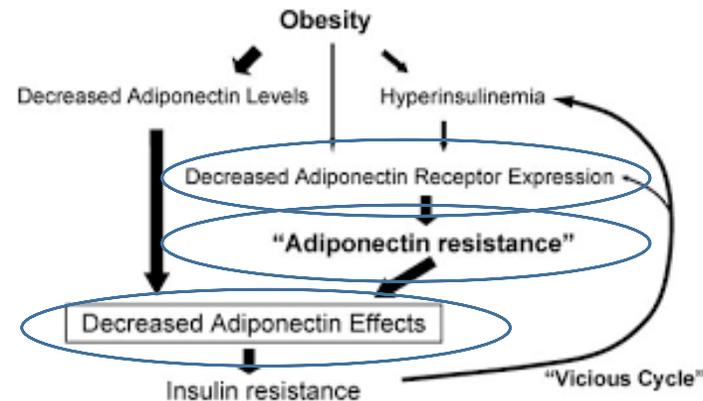
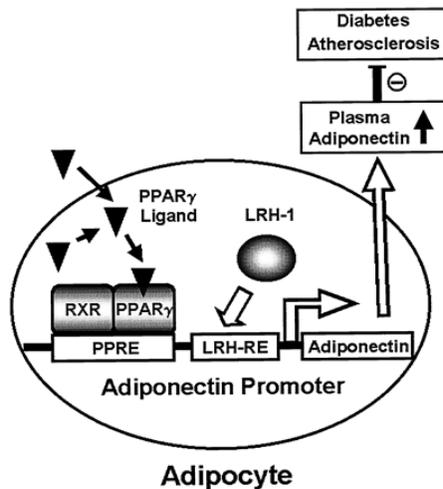
OBESITY DEVELOPMENT

Subcutaneous adipose tissue



Adiponectin is a known hormone to be strongly connected to obesity and a decrease of adiponectin is related to insulin resistance and hyperinsulinemia.

PPARG expression decrease results in decrease adiponectin expression which leads to higher risk of obesity

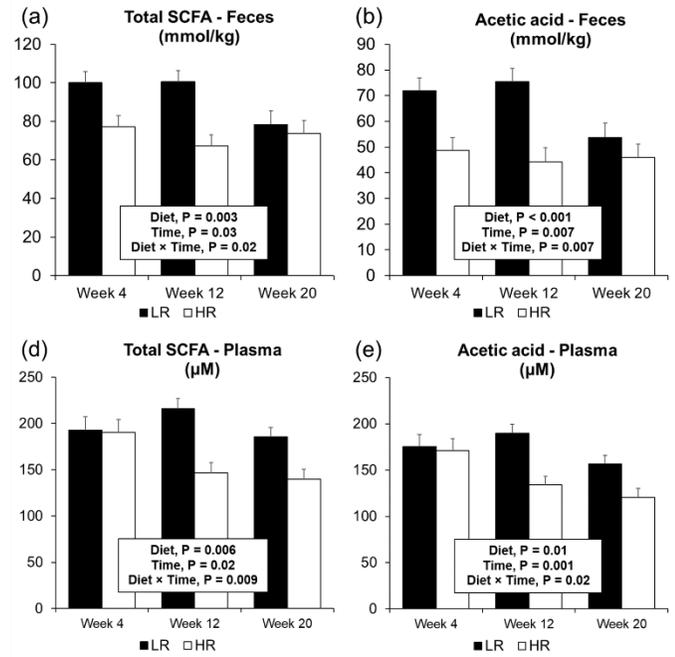
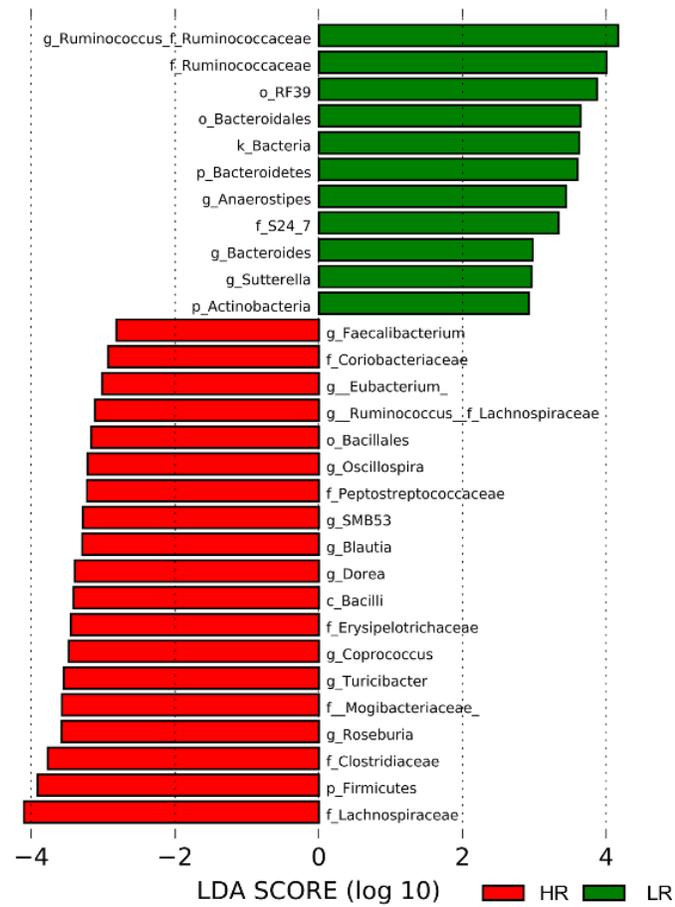


DIFFERENCES DUE TO CARBOHYDRATE SOURCE WERE MORE ACCENTUATED IN THE MICROBIOTA AND METABOLOME PROFILES

Article
Obesity-Related Metabolome and Gut Microbiota Profiles of Juvenile Göttingen Minipigs—Long-Term Intake of Fructose and Resistant Starch

Mihai V. Curtasu ^{1,*}, Valeria Tafintseva ², Zachary A. Bendiks ³, Maria L. Marco ³, Achim Kohler ², Yetong Xu ¹, Natalja P. Norskov ¹, Helle Nygaard Lærke ¹, Knud Erik Bach Knudsen ¹ and Mette Skou Hedemann ¹

Resistant starch increased concentrations of **total SCFA** and primarily **acetate**, in faeces and plasma



Ruminococcus species are excellent degraders of resistant starch and producers of acetate.

Ruminococcus, Bacteroidetes and *S24_7* consistent with studies of high intake of RS in humans, swine and rodents.

HR fructose

Lachnospiraceae, Firmicutes, Roseburia, Blautia, Dorea

have been found in other models of obesity fed high levels of fructose.

METABOLOME - MICROBIOME DATA INTEGRATION

LR – resistant starch diet

Ruminococcus (genus)
Bacteroidetes (phylum)
S24_7 (family)

Faecal Acetate
Faecal
Acetate+Propionate
+Butyrate

- Faecal metabolites**
- 2,4-dimethyladipic acid,
 - 3,3-methylglutaric acid
 - N-acetyl-L-glutamic acid
 - 2-hydroxy-glutarate
 - **succinic acid**
- Pantothenic acid

Positive correlations between variables



Increased bacterial production of acetate from RS sources related to the increased body weight?

Minimizing the effects on the satiety control mechanisms?
Previous studies in murine models:

Increased faecal succinate related to the increased body weight?

Published: 08 June 2016

Acetate mediates a microbiome–brain– β -cell axis to promote metabolic syndrome

Rachel J. Perry, Liang Peng, Natasha A. Barry, Gary W. Cline, Dongyan Zhang, Rebecca L. Cardone, Kitt Falk Petersen, Richard G. Kibbey, Andrew L. Goodman & Gerald I. Shulman [✉](#)

Nature **534**, 213–217 (2016) | [Cite this article](#)

Article | [Open Access](#) | Published: 12 February 2018

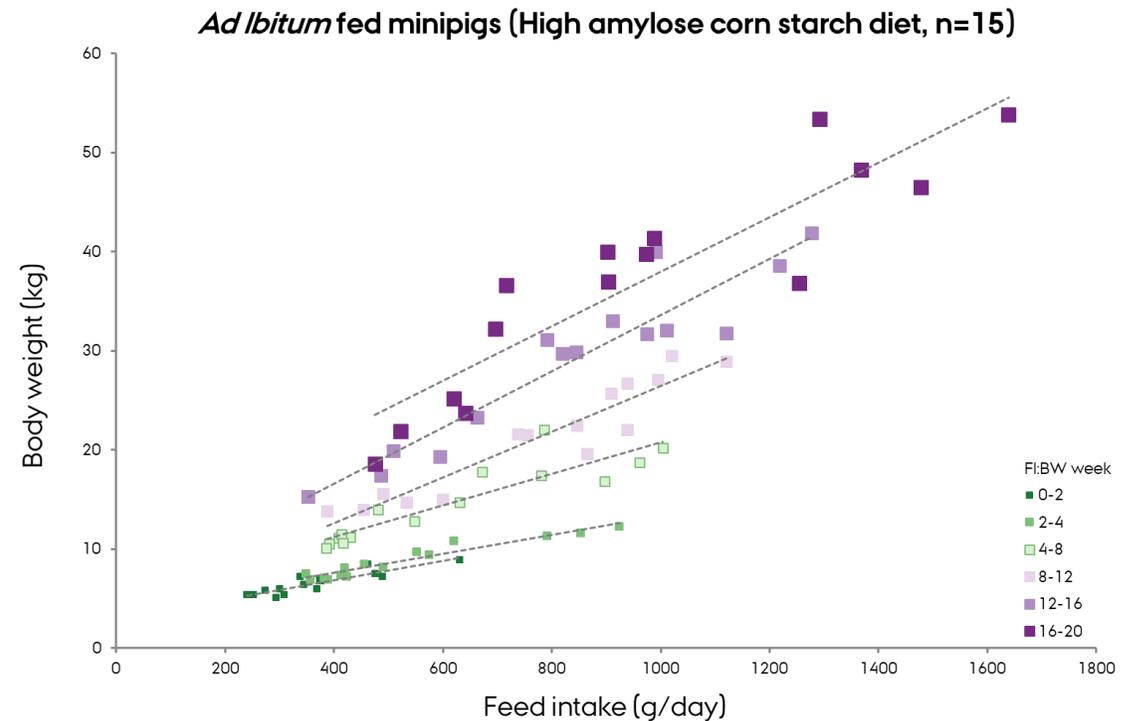
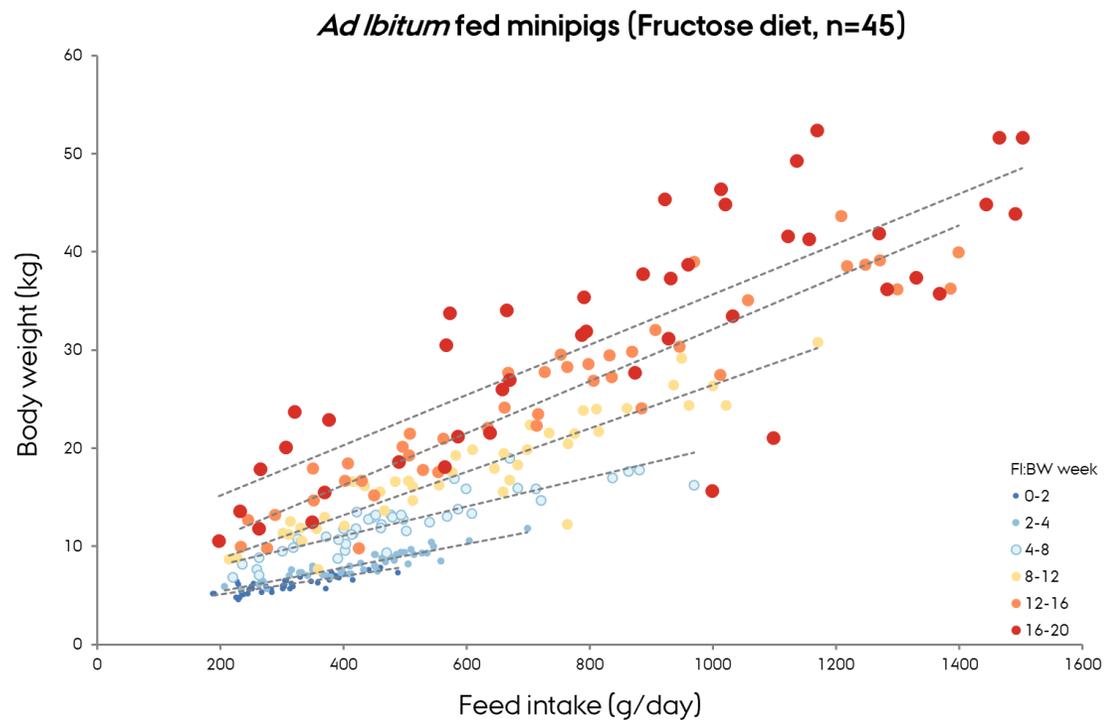
Elevated circulating levels of succinate in human obesity are linked to specific gut microbiota

Carolina Serena, Victoria Ceperuelo-Mallafré, Noelia Keiran, María Isabel Queipo-Ortuño, Rosa Bernal, Ricardo Gomez-Huelgas, Mireia Urpi-Sarda, Mónica Sabater, Vicente Pérez-Brocal, Cristina Andrés-Lacueva, Andres Moya, Francisco J Tinahones, Jose Manuel Fernández-Real, Joan Vendrell [✉](#) & Sonia Fernández-Veledo [✉](#)

The ISME Journal **12**, 1642–1657 (2018) | [Cite this article](#)

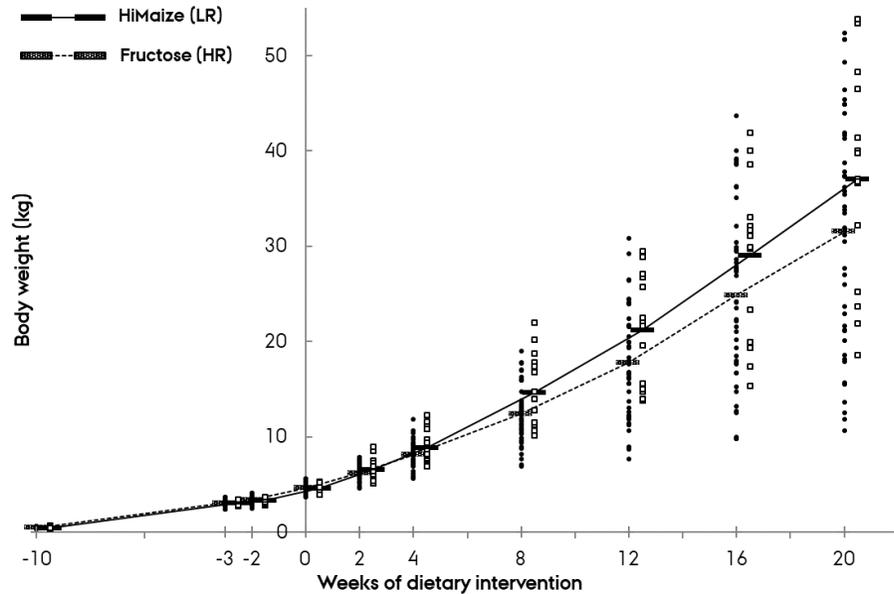
IMPORTANCE OF FEEDING REGIME IN OBESITY DEVELOPMENT

- Ad libitum feeding of high energy diets leads to a rapid development of obesity with visible external signs
- Restrictive feeding is a important to avoid obesity development in the Minipig model
- Gottingen minipigs do not restrain feed intake voluntarily



Ad libitum feeding induces a diversified response to the diet in terms of weight gain and feed intake

IMPORTANCE OF FEEDING REGIME IN OBESITY DEVELOPMENT

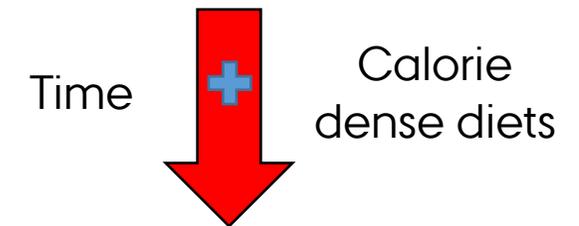


Is the ad libitum strategy advantageous?

- Rapid development of obesity phenotype
- Individual response (possibly genetically driven) kicks in and variation increases in metabolic responses
- Similar to human subjects having access to energy dense foods in “ad libitum” quantities
- Harder to implement certain procedures which are based on meal feeding or dose feeding. E.g. glucose tolerance tests – minipigs will not ingest a large portion of food in a single “sitting”

CONCLUSIONS OBJECTIVE 1

- Ad libitum feeding of high-fat-high-carbohydrate rapidly induces an obese phenotype with high individual response – similar to humans
- The time progression of obesity is more accentuated irrespective of carbohydrate type
- Systemic responses to carbohydrate quality are less obvious than expected
- Differences in carbohydrate quality are more clear at the microbiome + metabolome level



CAN DIETARY INTERVENTION MITIGATE THE DEVELOPMENT OF MetS?

Helle Nygaard Lærke, senior researcher, PhD.

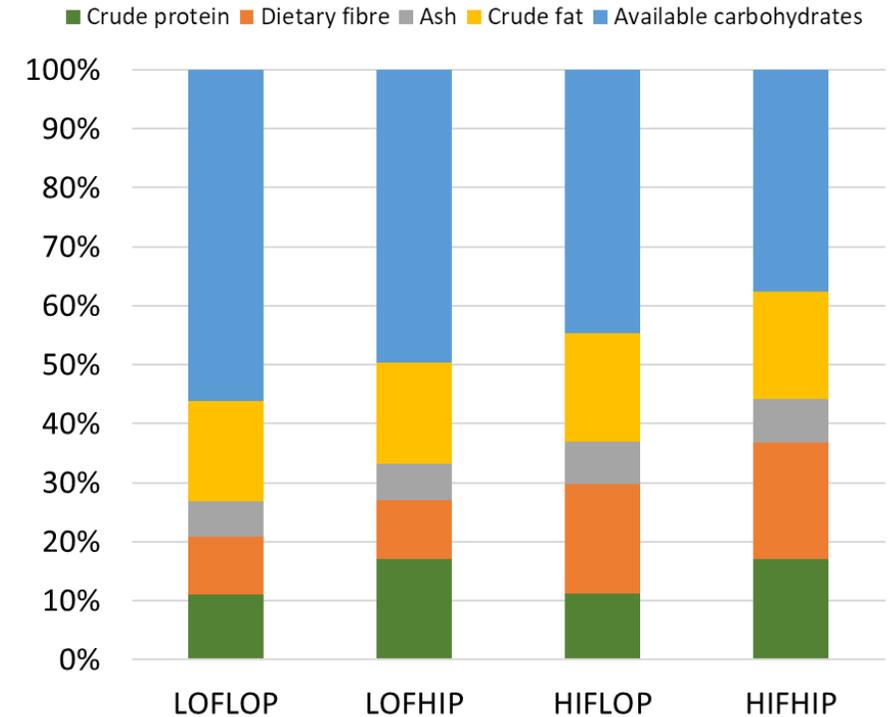
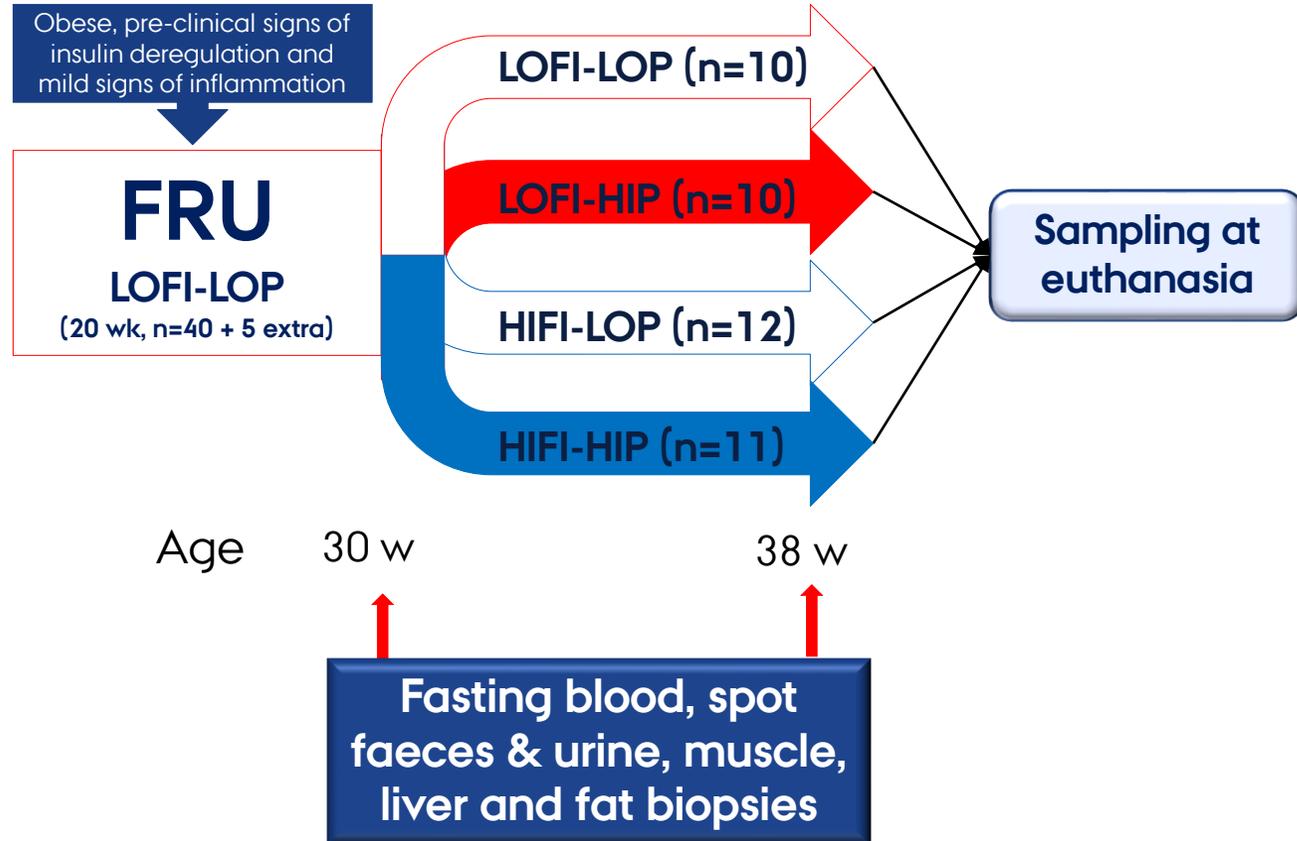
RESEARCH QUESTION

Can high dietary fiber from enzyme treated wheat bran and high protein including whey ameliorate the risk of MetS in young obese Göttingen Minipigs as a model for humans?

Hypotheses:

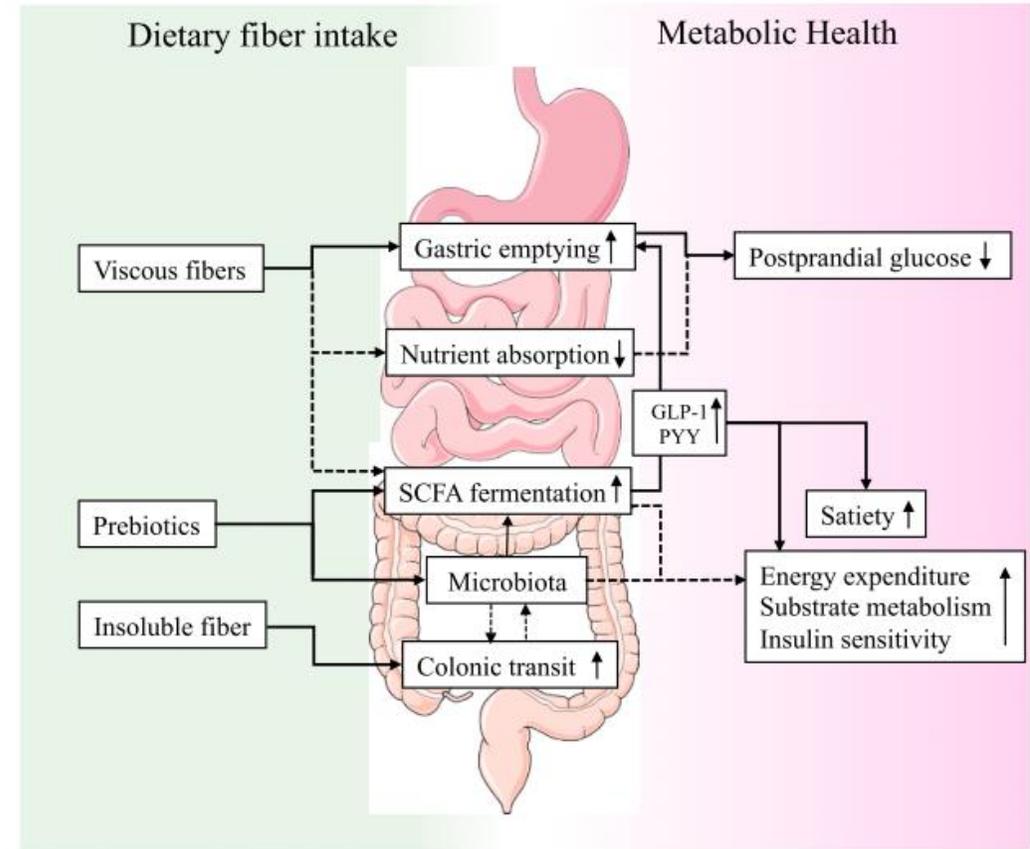
1. High **DF** and **protein** content or a combination hereof can **improve insulin response, lipid profile, inflammatory biomarkers** and tissue **gene expression** associated with **MetS** in a young obese Göttingen Minipig model. *(Xu et al. 2021, Br. J. Nutr., 125, 828-840).*
2. Dietary interventions will modulate the **degradation of DF** and **fermentation** profile in the gut, where high DF selectively will **stimulate beneficial bacteria, enhance SCFA production especially butyrate** and thereby **benefit systemic health** *(Xu et al. 2020, Food & Function, 11, 10758-10773).*

EXPERIMENTAL DESIGN



DIETARY FIBER

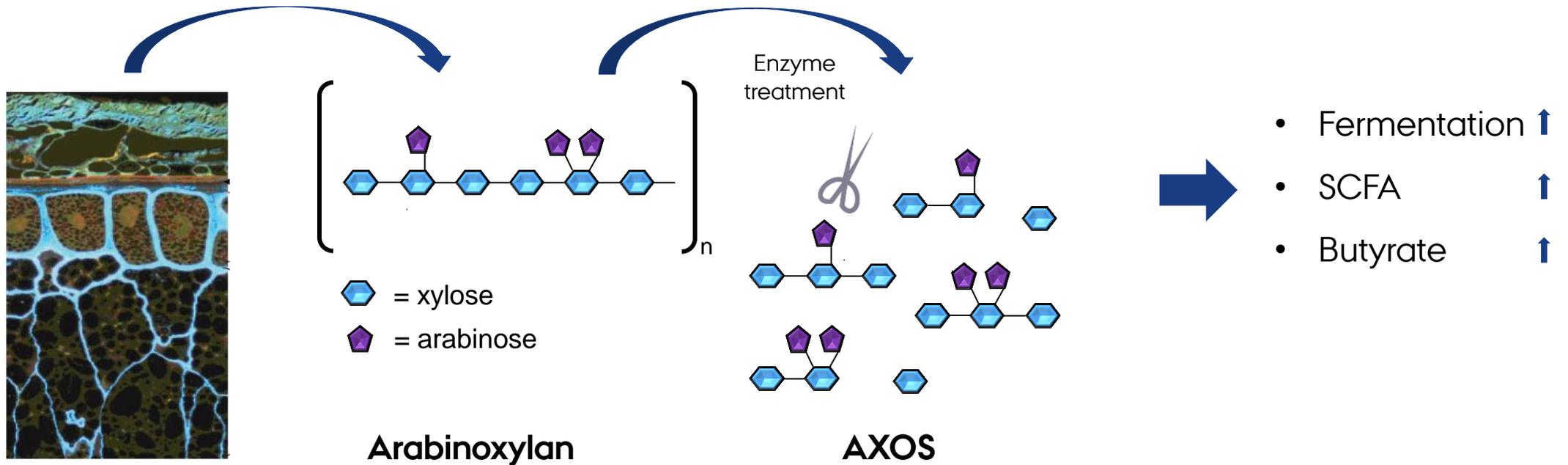
- The positive effect of fiber in obesity and MetS is mostly linked to reduced calorie intake and weight loss.
- Can DF modify metabolism in a weight stable model?
- Are specific metabolites involved?



Muller et al, 2018

DIETARY FIBER

Enzyme-treated wheat bran
Cellulose and arabinoxylan (AX)



DIETARY PROTEIN

- Insulinotropic → positive or negative effect on insulin resistance?

Table 1. Overview of the effects of high-protein diets on insulin action in human studies

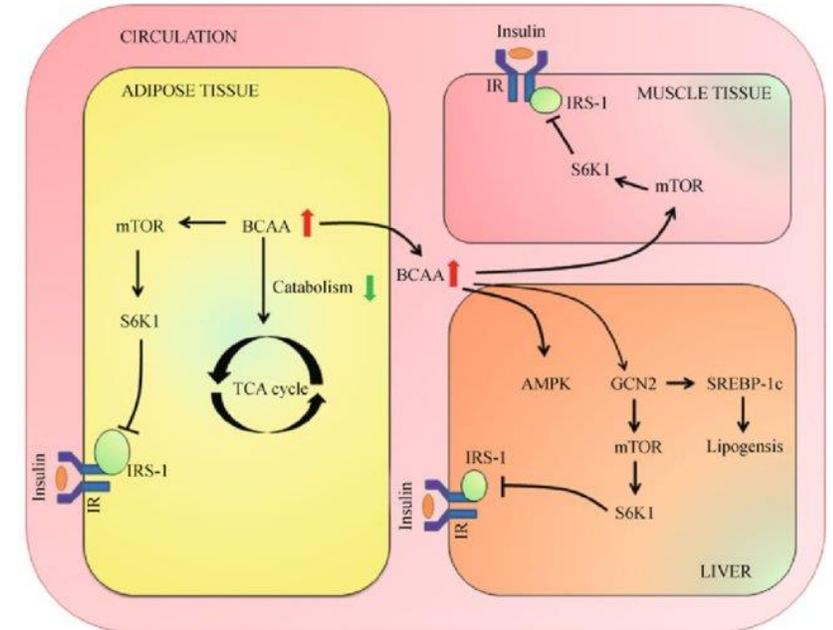
<i>Duration of the high-protein diet (> 20 En%)</i>	<i>Type of subjects</i>	<i>Effects on insulin resistance (IR)</i>
1 week to 6 months (intervention studies)	Healthy	No effect on IR ¹⁰⁻¹² Reduction in daily plasma insulin ¹³
	Obese	Improves IR ¹⁴ No effect on IR ^{15,16} Reduced IR ¹⁷ Improves IR if weight loss is present ²²⁻²⁴ No effect on IR if weight loss is present ²⁵⁻²⁷
	Insulin resistant, DM2	Improves IR ^{18,19} Decreased postprandial glucose concentration ²⁰ No effect on IR if weight loss is present ²⁸⁻³¹
> 6 months (longitudinal studies)	NA	Increased DM2 risk ^{4-6,33} Reduction in DM2 risk ³⁴

Abbreviations: DM2, type 2 diabetes mellitus; IR, insulin resistance.

Reitman et al., 2014

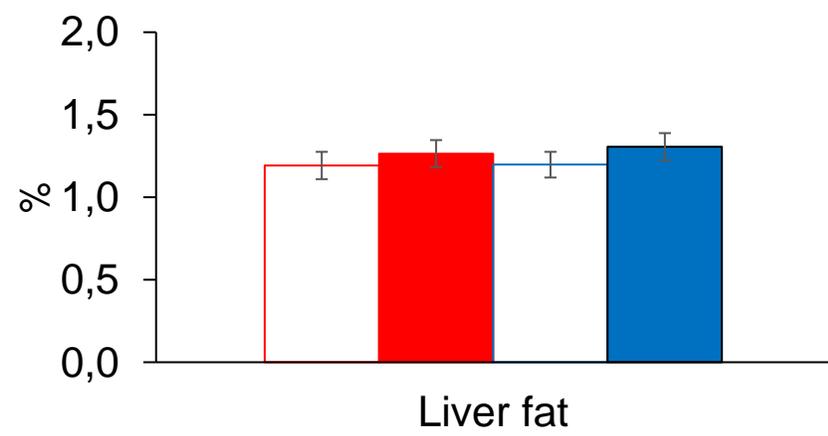
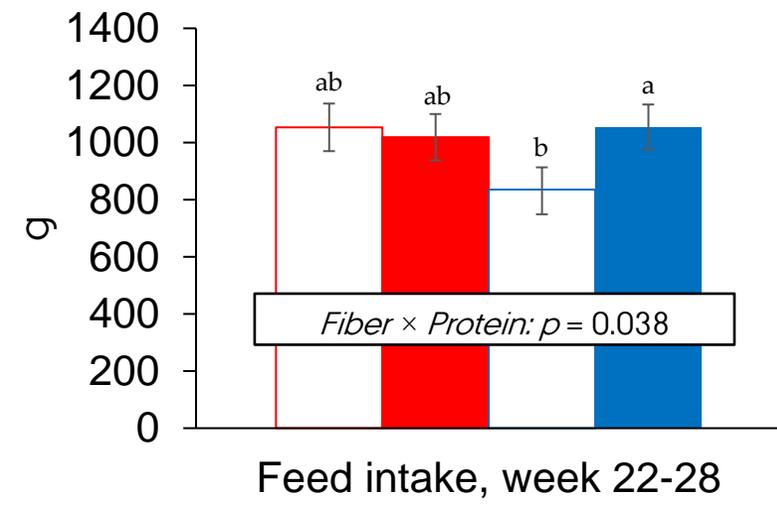
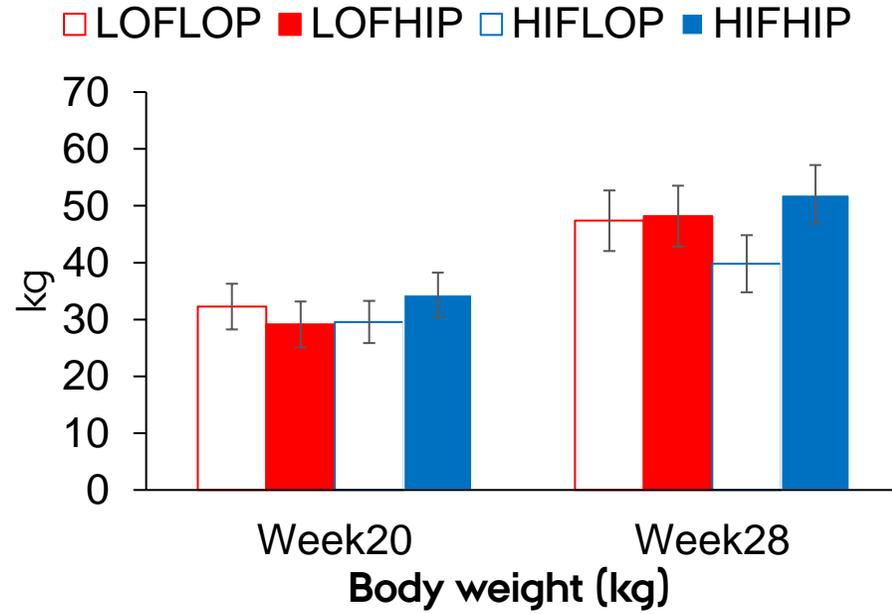
DIETARY PROTEIN AND BCAA

- The role of branched-chain amino acids (BCAA) is controversial
 - Plasma BCAA is elevated in obesity/diabetes
 - Whey protein consumption has a positive effect on the glycemic response and insulin sensitivity
 - BCAA-rich whey protein hydrolysate is rapidly absorbed and highly insulinotropic
- Can WPH independently or synergistically with DF affect MetS?

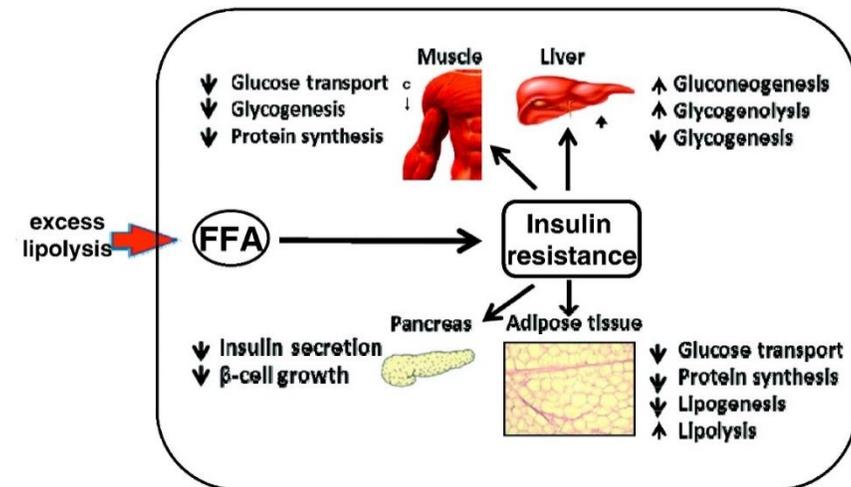
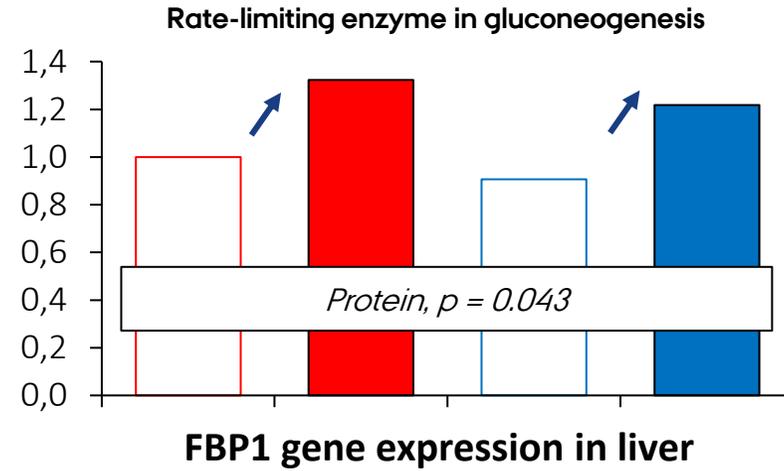
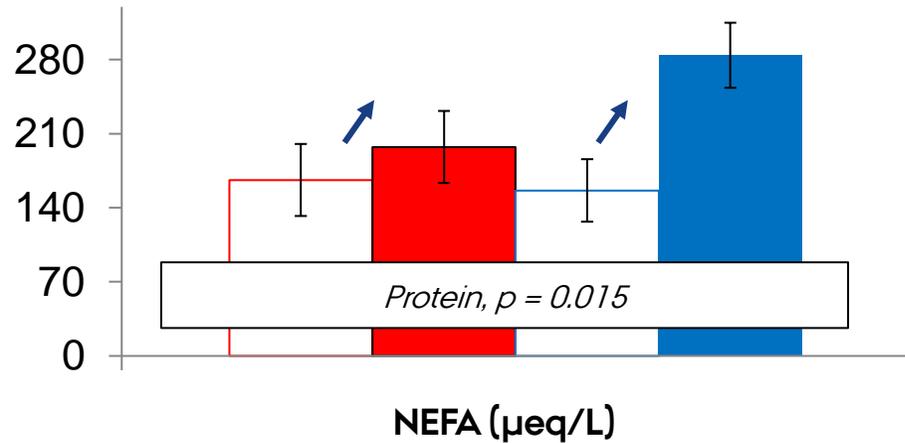
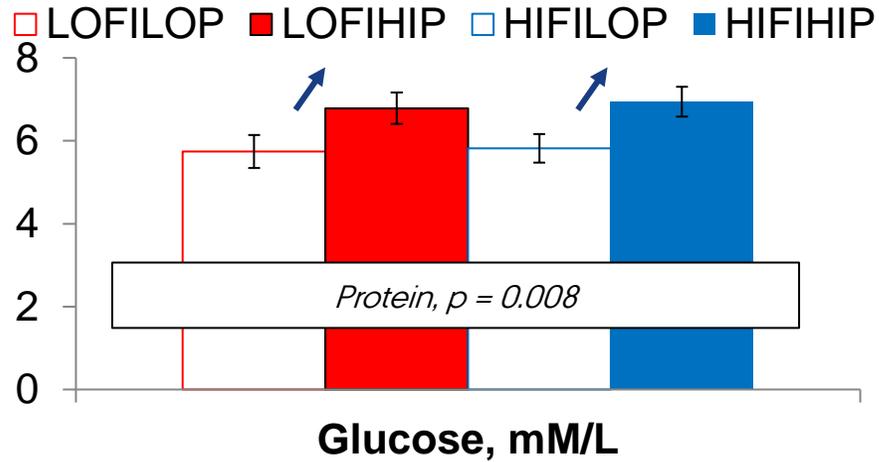


Adams et al., 2016

GAIN AND FEED INTAKE

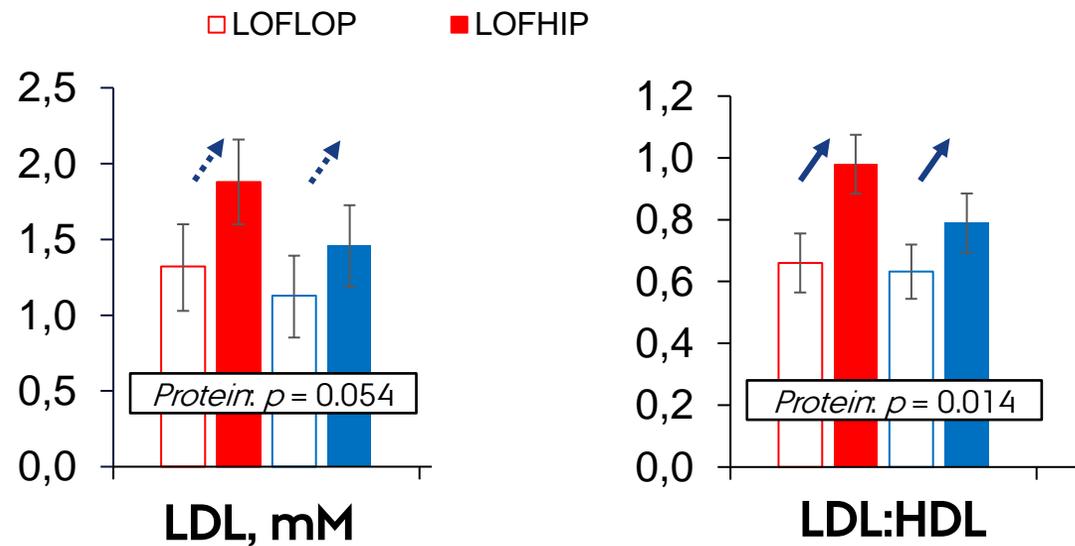


FASTING METABOLITE LEVELS



POSTPRANDIAL LIPID METABOLITES

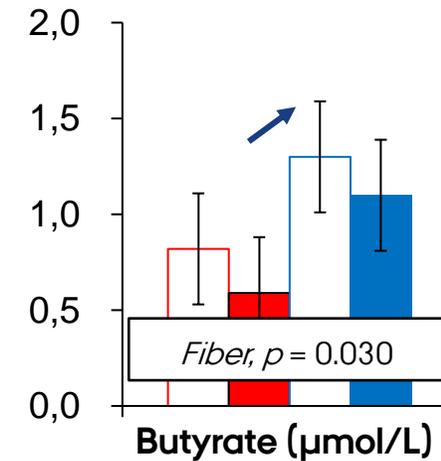
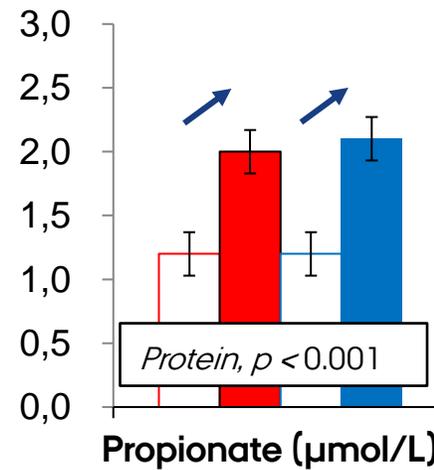
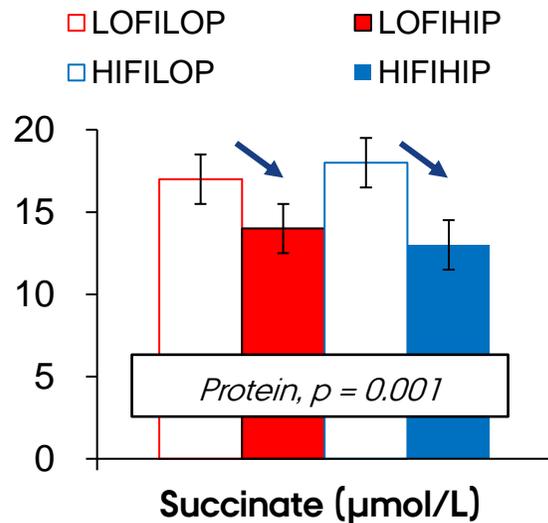
- Postprandial TG and cholesterol plasma levels were not affected by fiber or protein.
- High protein diets increased postprandial levels of dyslipidemic markers.



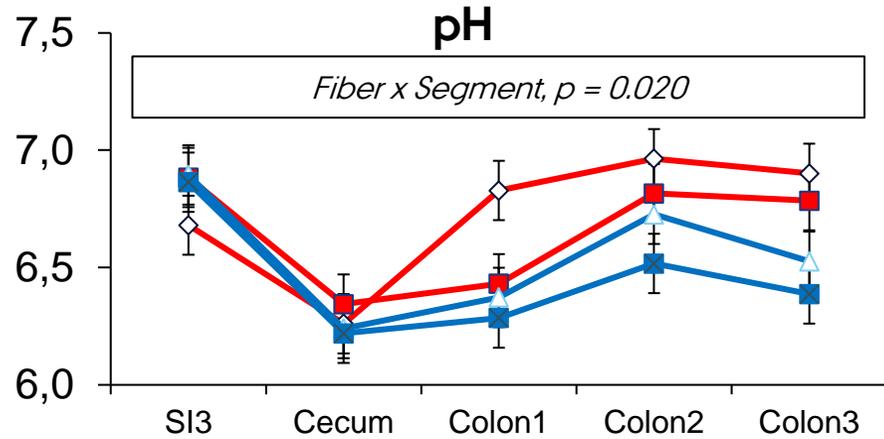
FATTY ACID METABOLITES IN PLASMA

Postprandial levels in the jugular vein

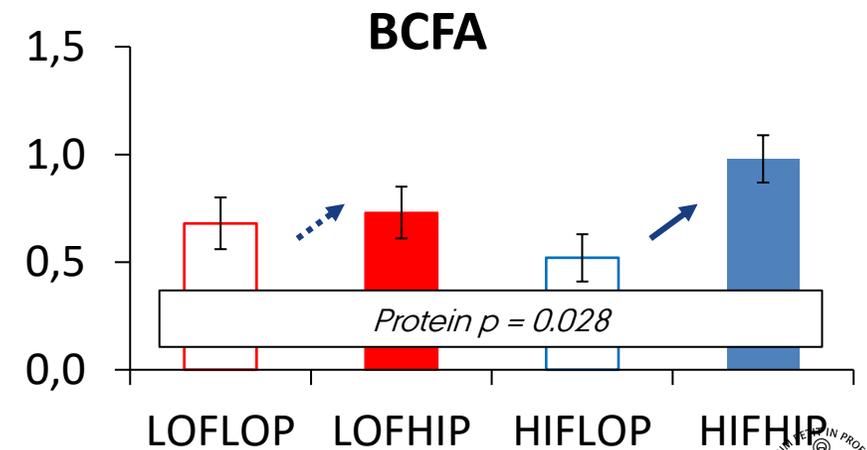
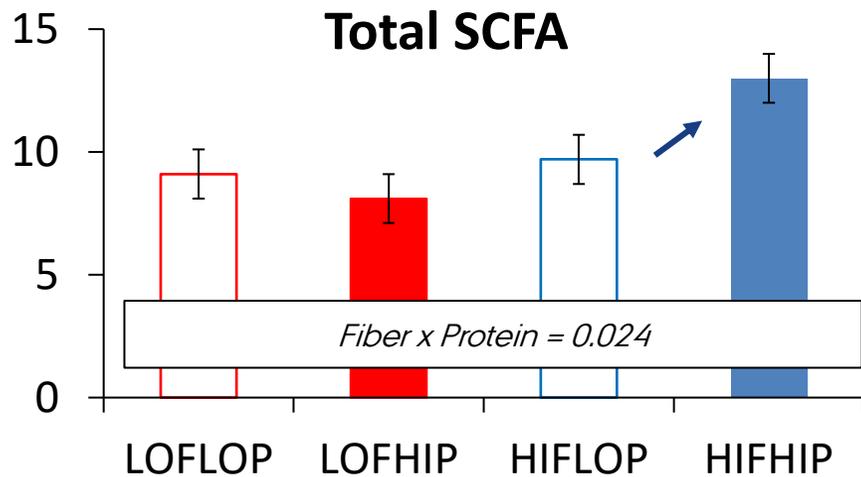
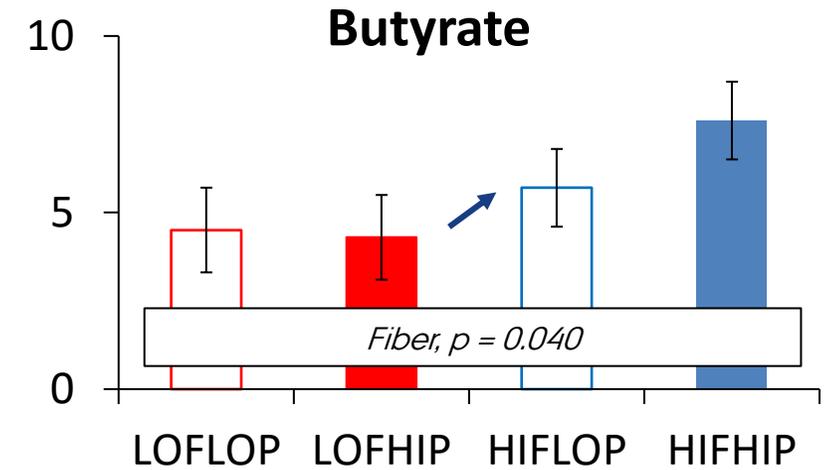
- Succinate is normally not accumulated in the plasma, but elevated levels are seen in obesity and MetS
- Also a metabolite of microbial carbohydrate and amino acid fermentation - main pathway of propionate production by primary fermenters.



INTESTINAL pH AND POOL SIZE OF SCFA IN THE LARGE INTESTINE (mmol)

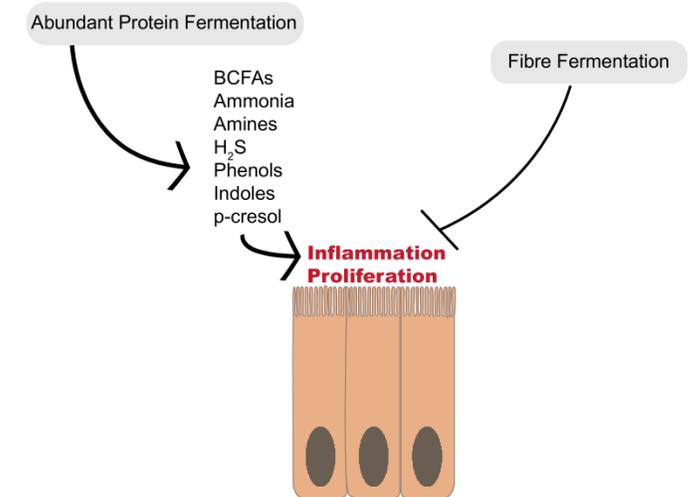
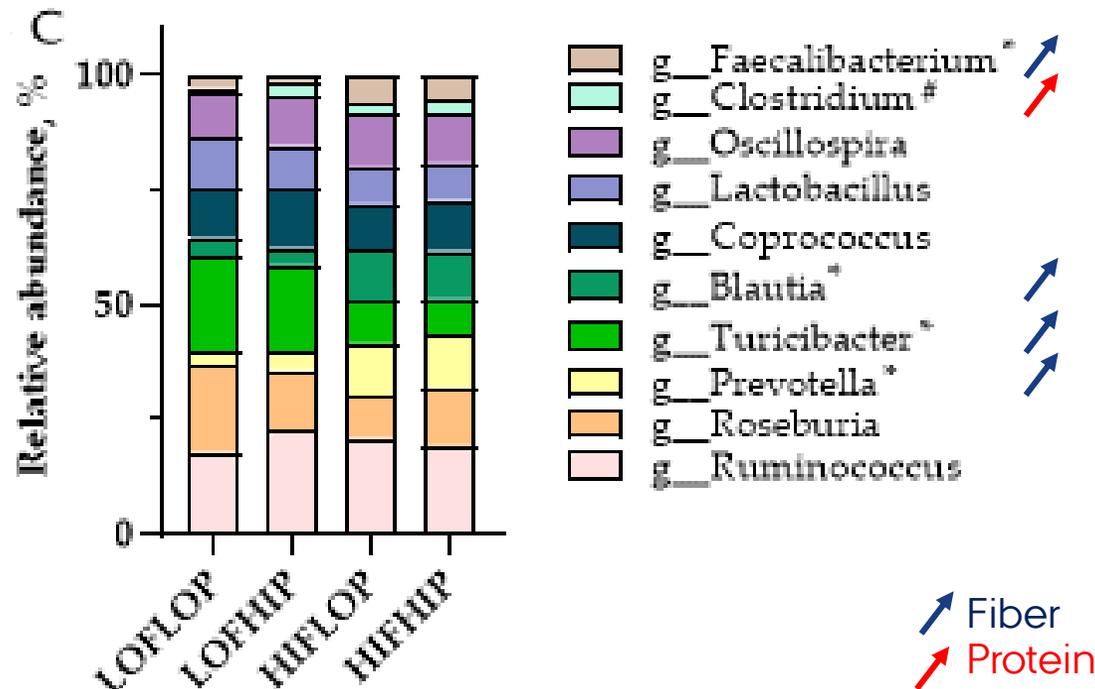


- High DF reduced pH
- High DF/High protein increased SCFA
- High DF increased butyrate production
- High protein increased BCFA.
- DF decreased BCFA concentration but pool size was higher in the high DF group fed high protein



MICROBIOTA, FAECES

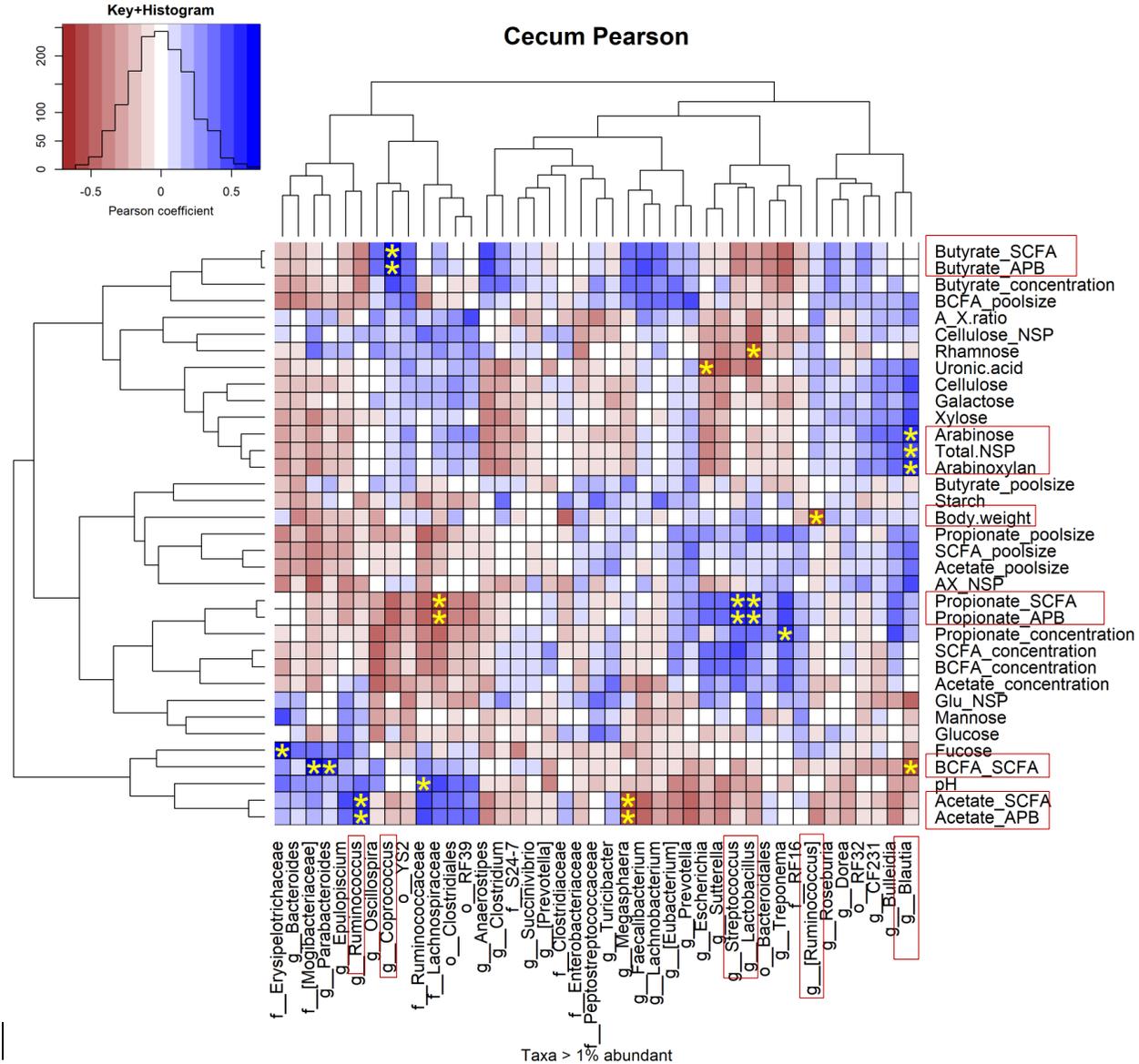
- **High fibre** increased the Bacteroidetes:Firmicutes ratio
- **High fibre** increased the abundance of butyrate-producing bacteria
- **High protein** increased the abundance of *Clostridium* → BCFA production



Diether and Willing, 2019

ASSOCIATIONS IN CAECUM

- Butyrate $\xrightarrow{+}$ Coprococcus
- AX NSP $\xrightarrow{+}$ Blautia $\xrightarrow{-}$ BCFA
- Acetate $\xrightarrow{+}$ Ruminococcus
- Propionate $\xrightarrow{+}$ Streptococcus, Lactobacillus
- Body weight $\xrightarrow{-}$ Ruminococcus ?



SUMMING UP ON THE INTERVENTION

- **High DF** improved **C-peptide** without effect on fasting responses
- **High protein** increased markers of **dyslipidemia**
- **High protein** increased **fasting glucose** and **insulin** but did not affect insulin sensitivity
- **High protein** increased **plasma propionate** and **glucose** but decreased **succinate** concentrations
- **High protein** intake increased **intestinal BCFA concentrations** which was reduced with **high DF**
- **High protein** had limited effect on microbial profile
- **High DF** increased **butyrate-producing bacteria**, modestly increased **butyrate production** and increased **circulating levels of butyrate**

Several indicators of insulin dysfunction and increased gluconeogenesis in the juvenile obese pig model

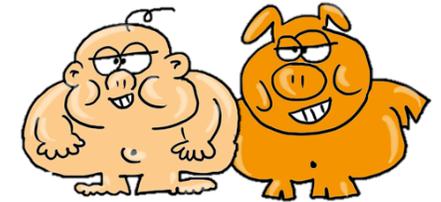
THE PIG AS MODEL FOR DIETARY INTERVENTION

- Juvenile vs. adult
- Obese but healthy?
- Duration of the intervention

- **Comparison to humans**



Data integration



Article

Whey Protein Combined with Low Dietary Fiber Improves Lipid Profile in Subjects with Abdominal Obesity: A Randomized, Controlled Trial

Elin Rakvaag^{1,*}, Rasmus Fuglsang-Nielsen^{1,†}, Knud Erik Bach Knudsen², Rikard Landberg³, Astrid Johannesson Hjelholt¹, Esben Søndergaard¹, Kjeld Hermansen^{1,4} and Søren Gregersen^{1,5}



European Journal of Clinical Nutrition (2021) 75:611–619
<https://doi.org/10.1038/s41430-020-00759-4>

ARTICLE

Clinical nutrition

Effects of whey protein and dietary fiber intake on insulin sensitivity, body composition, energy expenditure, blood pressure, and appetite in subjects with abdominal obesity

Rasmus Fuglsang-Nielsen^{1,2,3} · Elin Rakvaag^{1,4} · Bente Langdahl¹ · Knud Erik Bach Knudsen⁵ · Bolette Hartmann⁶ · Jens Juul Holst⁶ · Kjeld Hermansen^{1,7} · Søren Gregersen^{1,3}

THANK YOU TO OUR COLLEAGUES, PARTNERS AND FUNDERS



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