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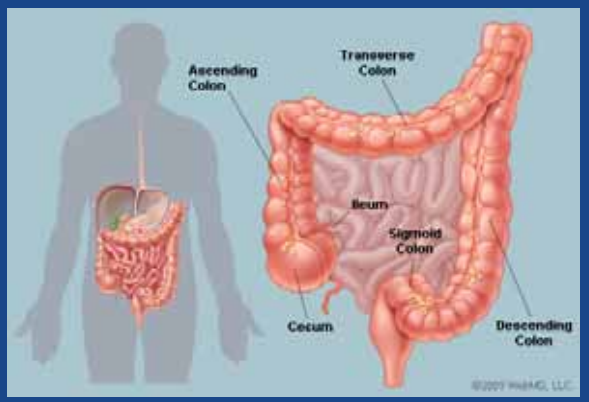
2 March 2016

Concepts for enhanced butyrate production to improve colonic health and insulin sensitivity – ButCoIns

Knud Erik Bach Knudsen
Department of Animal Science



Background

- It is generally recognized that an unhealthy dietary pattern – **high intake** of **fat** and **refined carbohydrates** and **low intake** of **dietary fibre** have been linked to several diseases of the affluent societies:
  - Inflammatory bowel disease
  - Colorectal cancer
  - Coronary heart disease
  - Type II diabetes, etc.

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Background: The large intestine – diet and health

- Dietary fibre is the limiting factor for maintaining a viable and diverse microbial community
- Dietary fibre controls the production of short chain fatty acids

Approximately 70 % of the incidences of colorectal cancer and inflammatory bowel diseases are related to dietary factors

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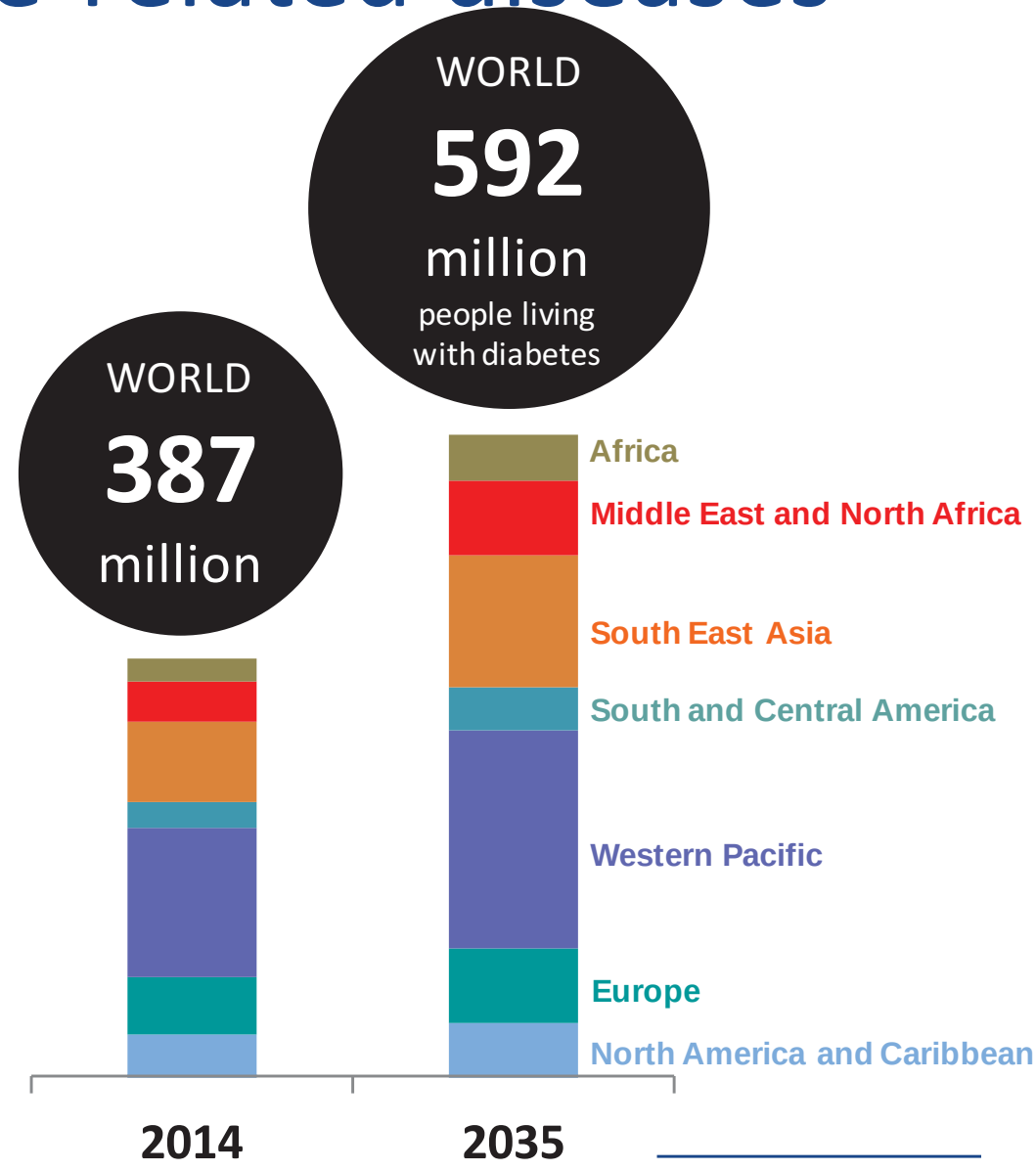
Background: Lifestyle-related diseases

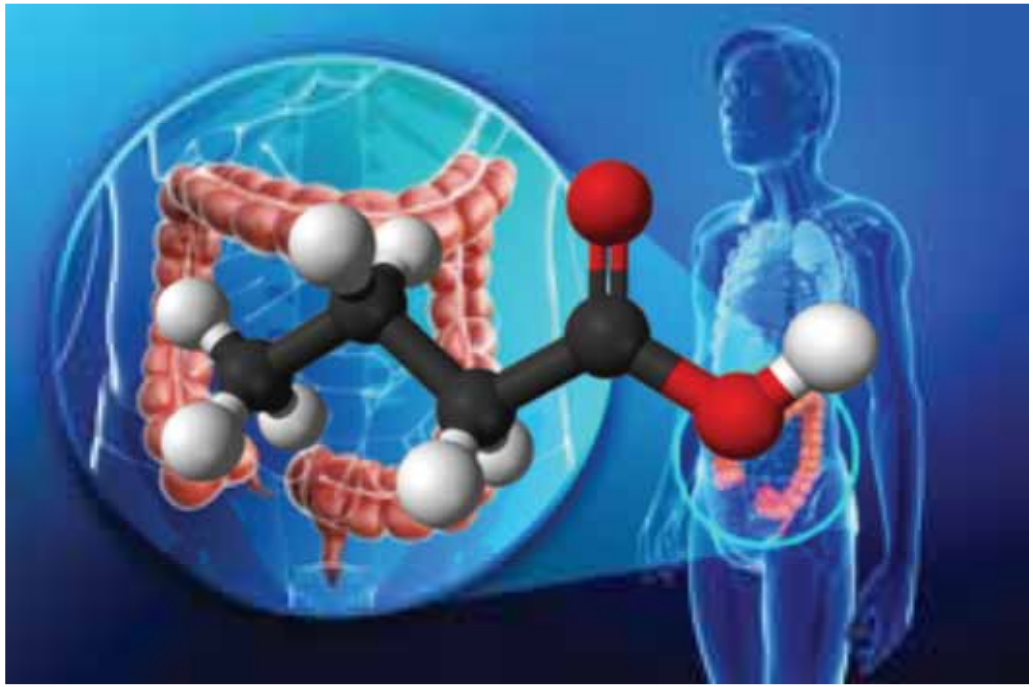
- Rapid economic growth
- Increased urbanization
- Access to diets rich in easily digestible carbohydrates and fat



Source: IDF Diabetes Atlas 2014

Background: Short-chain fatty acids

› It has increasingly been recognised that short-chain fatty acids, **butyrate** in particular, plays an important regulatory role for events in the large intestine and peripheral tissues



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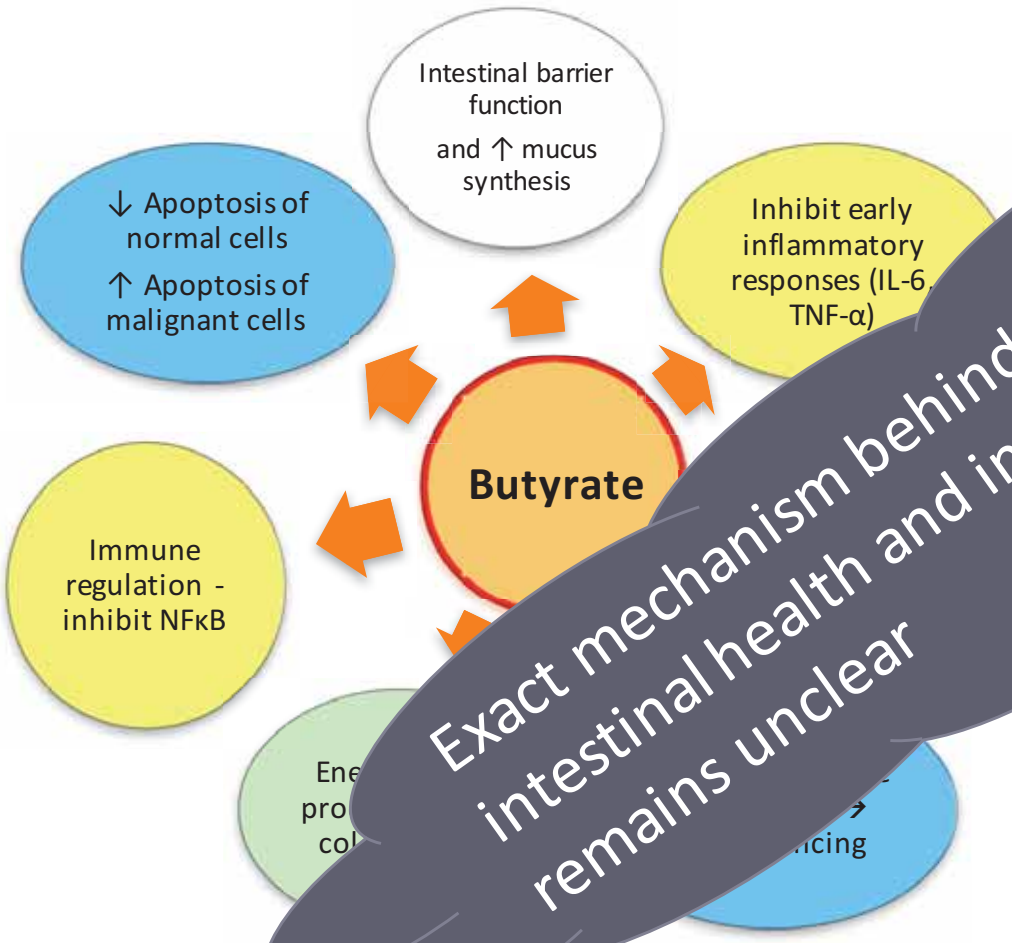
Background: Butyrate – *in vitro* and *in vivo*

Intestinal level



R. C. Canani et al., World … terol (2011), 17(12): 1519-28.

Syste… al

› ↑ …

…e-1 (GLP-1) secretion

…cts against diet induced obesity and …

…sistance in mice

…ving insulin sensitivity

→ Mechanism of action related to promotion of **energy expenditure** and **induction of mitochondria function**

(Z. Gao et al. (2009), Diabetes 58; 1509-17)

Exact mechanism behind improved intestinal health and insulin sensitivity remains unclear

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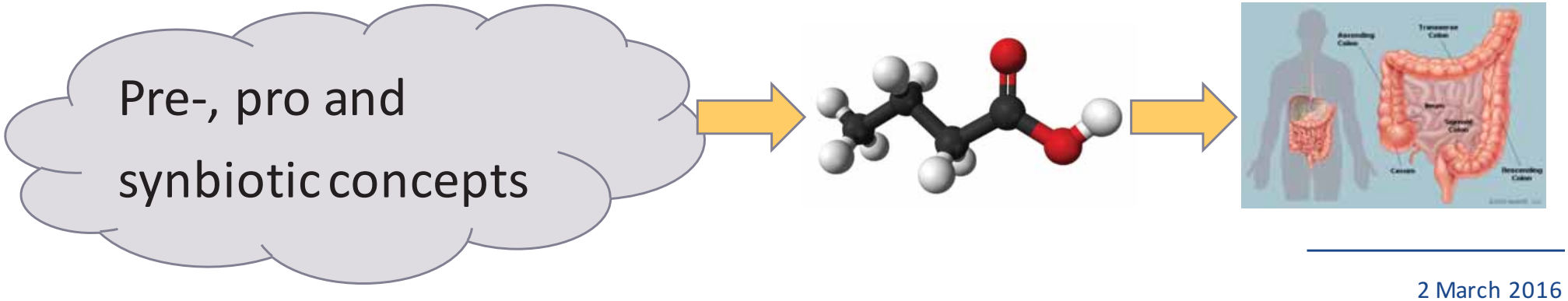
Concepts for enhanced butyrate production to improve colonic health and insulin sensitivity - **ButCoIns**

› The overall objective was to improve **colonic health**, peripheral **insulin sensitivity** and **glucose homeostasis** by increased colonic **butyrate production** brought about by **pre-, pro- and synbiotic concepts**



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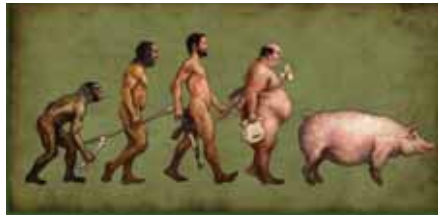
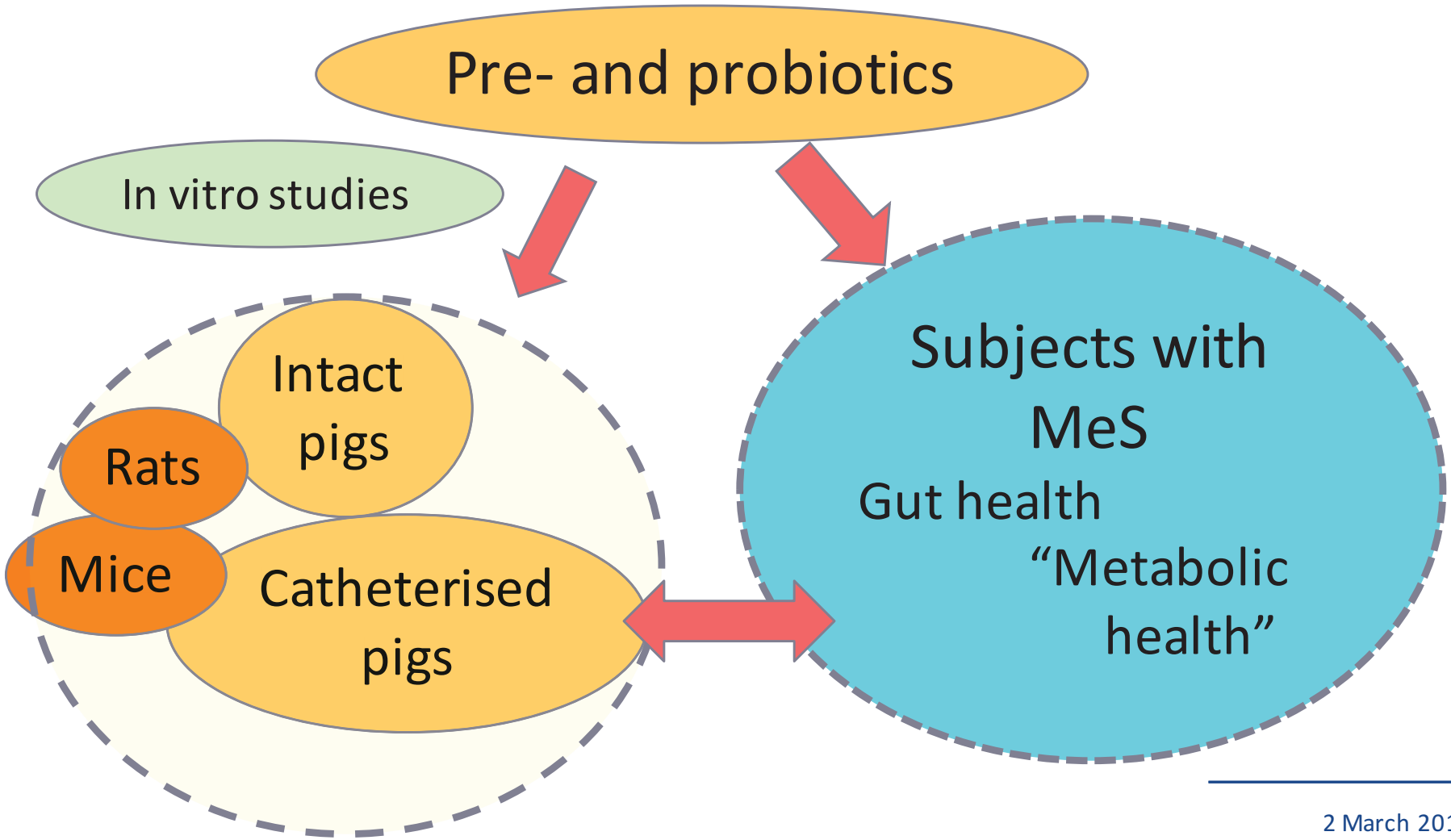


ButCoIns: Research elements



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ButCoIns – collaborating and funding bodies

- Aarhus University, Department of Animal Science
- Aarhus University Hospital, Department of Gastroenterology and Hepatology
- Aarhus University Hospital, Department of Endocrinology and Metabolism
- University of California, Davis
- Companies:
  - DuPont (formerly Danisco)
  - Lantmännen Foods
  - KMC

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UC DAVIS UNIVERSITY OF CALIFORNIA

DUPONT®

Lantmännen

Innovation Fund Denmark
RESEARCH, TECHNOLOGY & GROWTH

The Danish Council for Strategic Research

KMC

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Programme

9:00-9:30	Registration and coffee
Moderator:	Knud Erik Bach Knudsen
9:30-9:45	Welcome, background and introduction to the ButCoIns project *Knud Erik Bach Knudsen, Aarhus University, Dept. of Animal Science*
9:45-10:00	Arabinoxylan and resistant starch – two dietary fibre components with the potential to influence butyrate production *Helle Nygaard Lærke, Aarhus University, Dept. of Animal Science*
10:00-10:30	Butyrogenic effects of pre- and probiotics in vitro *Stig Purup, Aarhus University, Dept. of Animal Science*
10:30-11:00	Gut formation of butyrate and influence on gene expression parameters related to gut health – model studies with pigs and rats *Tina Skau Nielsen, Aarhus University, Dept. of Animal Science*
11:00-11:20	*Coffee break*
11:20-11:50	Dietary effects on butyrate absorption, insulin secretion and peripheral release *Peter Kappel Theil, Aarhus University, Dept. of Animal Science*
11:50-12:20	Beyond short-chain fatty acids – what complex arabinoxylan and resistant starch rich diets also deliver to the body *Mette Skou Hedemann, Aarhus University, Dept. of Animal Science*
12:20-13:20	Lunch

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Programme

12:20-13:20	Lunch
Moderator:	Kjeld Hermansen
13:20-13:50	Human subjects with the metabolic syndrome – why the target group for studying gut and metabolic health? Søren Gregersen and Jens Frederik Dahlerup, Aarhus University Hospital, Dept. Endocrinology and Metabolism and Dept. of Gastroenterology and Hepatology
13:50-14:30	Impact of arabinoxylan and resistant starch on gut health parameters in human subjects with metabolic syndrome Stine Hald, Dept. of Gastroenterology and Hepatology
14:30-15:00	*Coffee break*
15:00-15:40	Impact of microbial metabolites on the peripheral tissue and insulin sensitivity in human subjects with metabolic syndrome Anne Grethe Schioldan, Aarhus University Hospital, Dept. Endocrinology and Metabolism
15:40-16:00	General discussion
16:00-17:00	Meet the researchers - a chance for matchmaking under relaxed conditions

ARABINOXYLAN and RESISTANT STARCH

- two dietary fibre components with the potential to influence butyrate production

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DEPARTMENT OF ANIMAL SCIENCE

HELLE NYGAARD LÆRKE
SENIOR SCIENTIST

MARCH 2. 2016

WHY ARABINOXYLAN and RESISTANT STARCH?

Both escape digestion and absorption in the small intestine

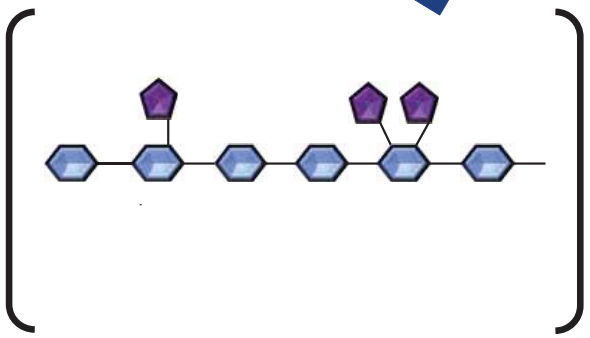
Both have been demonstrated to induce butyrate production by the commensal microbiota

ARABINOXYLAN - AX

Plant cell-wall component

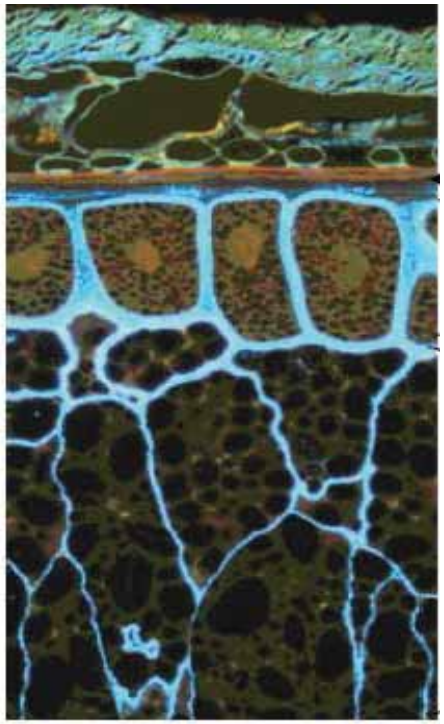
High in cereals

Particularly in the bran-fraction



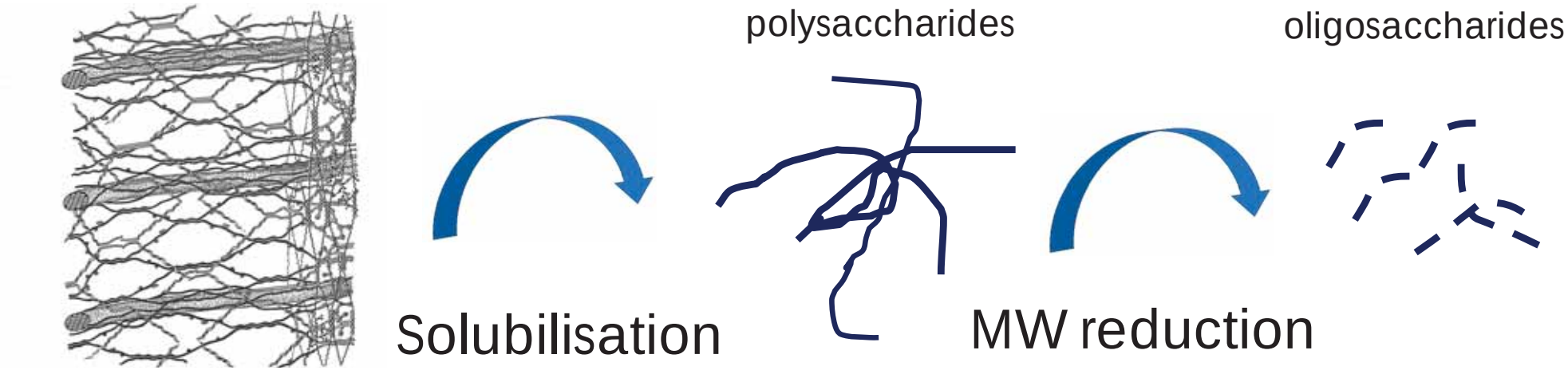
= xylose

= arabinose



Food & Nutrition Research 2009.
DOI: 10.3402/fnr.v53i0.1912

ENZYMATIC TREATMENT OF AX



Soluble and insoluble AX

Soluble AX

AXOS

ENZYMATIC TREATMENT OF WHEAT BRAN

Where is the arabinoxylan located?	Wheat bran No enzyme	Wheat bran Enzyme-treated
	g/kg dry matter	
Non-starch polysaccharides (NSP)	**148**	**114**
Soluble NSP	26	22
Insoluble NSP	**122**	**93**
Low molecular weight (AXOS)	**33**	**52**

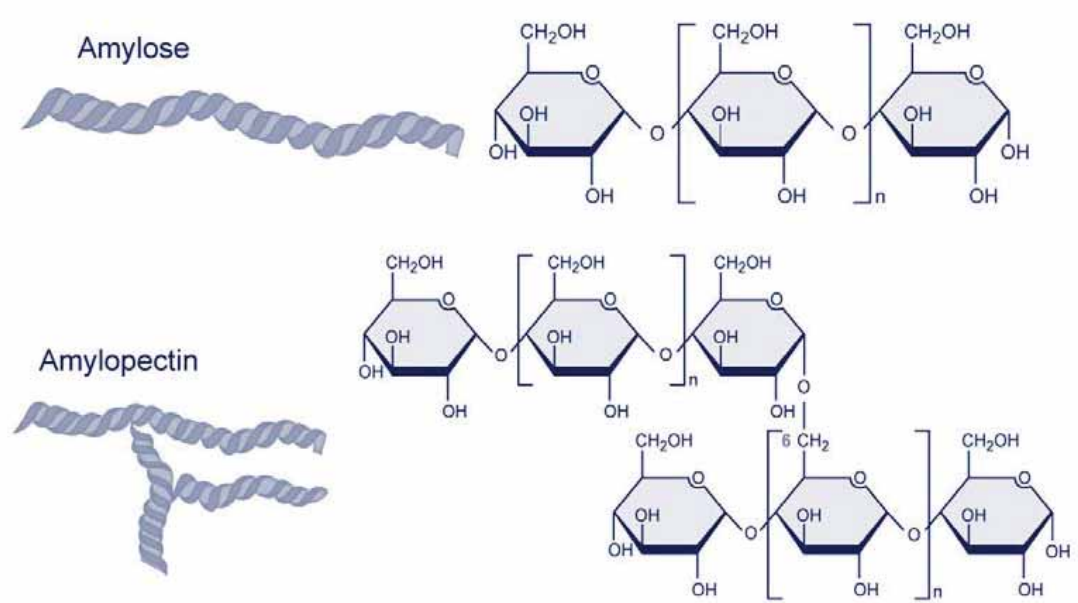
AXOS COMPOSITION (g/kg dry matter)

Enzyme treatment	No	Yes
AXOS	**33**	**52**
Arabinose	<0.1	0.8
Xylose	0	2.1
Xylobiose	0	0.8
Xylotriose	0.2	0.9
Xylotetraose	0.1	0.8
Xylopentaose	<0.1	0.4
Xylohexaose	0	0.4
Σ AXOS 1-6 units	0.3	**5.9**
AXOS > 6 units	27.7	**49.1**

STARCH

Glucose-units linked by α-1,4-linkages = amylose *or* α-1,4 and α-1,6 linkages = amylopectin

In different starch sources both polymers are present but in varying ratio



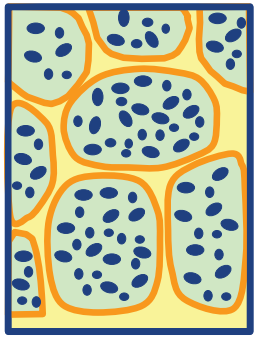
online.science.psu.edu

RESISTANT STARCH

- Humans – and all other monogastric animals- have the digestive enzymes required to degrade starch into glucose, which is then taken up in the small intestine
- SOME starch may escape digestion and absorption

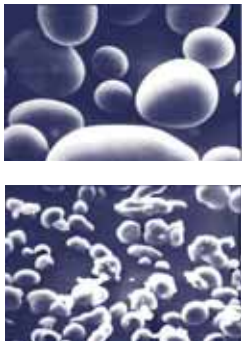
→ fermented to varying degree in the large instestine

CLASSES OF RESISTANT STARCH



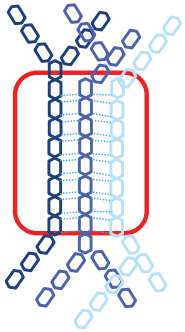
Physically inaccessible

RS1



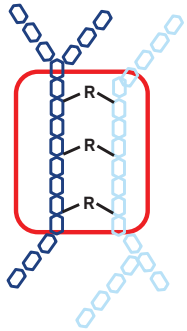
Native starch granules crystalinity

RS2



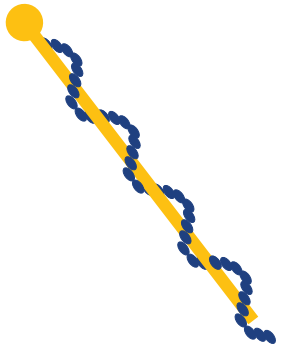
Retrograded amylose

RS3



Chemically modified

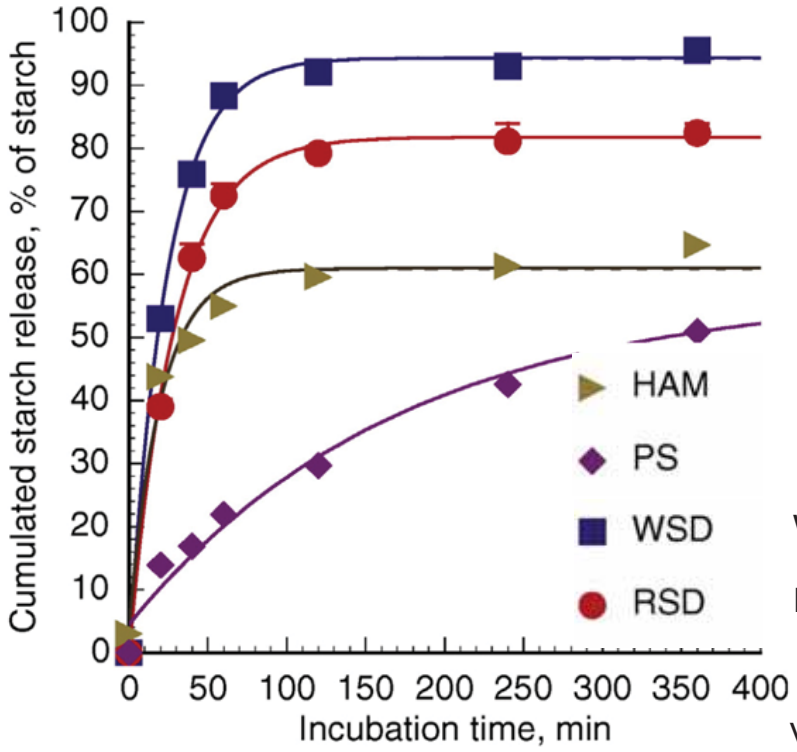
RS4



Amylose–lipid complexes

RS5

IN VITRO DIGESTION OF STARCHES



High Amylose Maize

Potato starch

Western style diet (pigs)

Resistant starch diet (pigs)

Vangsøe et al. 2016. Food. Res. Int. (in press)

TEST SETUP IN *IN VIVO* STUDIES

Pig studies

WSD Western style diet

RS Rich in resistant starch

AX Rich in arabinoxylan incl. AXOS

Human studies

WSD Western style diet

RS + **AX**

TEST SETUP IN *IN VIVO* STUDIES

Pig studies

WSD Western style diet

RS Rich in resistant starch

AX Rich in arabinoxylan incl. AXOS

Human studies

WSD Western style diet

HCD Healthy carbohydrate diet

THE PIG DIETS

WSD
Western style diet

RSD
Resistant starch diet

HI-Maize
Raw potato starch

AXD
Arabinoxylan diet

Rye flakes
Enzymatic treated wheat bran

COMPOSITION OF PIG THE DIETS

% of DM	WSD	RSD	AXD
Energy (kJ)	1970	2030	1930
Protein	20.7	19.1	15.4
Fat	15.2	15.0	13.5
Sugars	11.3	0.3	2.2
Starch	42.2	47.0	42.0

% of dry matter	WSD	RSD	AXD
Dietary fibre	7.2	18.6	19.6
AX	1.8	1.5	**7.2**
AXOS	0.2	0.2	**0.7**
RS	0.6	**11.3**	0.8

COMPOSITION OF PIG THE DIETS

% of energy	WSD	RSD	AXD
Energy (kJ)	1970	2030	1930
Protein	18	16	14
Fat	28	28	26
Sugars	52	49	53
Starch			

% of dry matter	WSD	RSD	AXD
Dietary fibre	2	7	7
AX	1.8	1.5	**7.2**
AXOS	0.2	0.2	**0.7**
RS	0.6	**11.3**	0.8

Intake of available carbohydrates in absorption studies			
g/meal	199	197	199

THE HUMAN DIETS

WSD

HCD

COMPOSITION OF FOOD ITEMS

% of dry matter

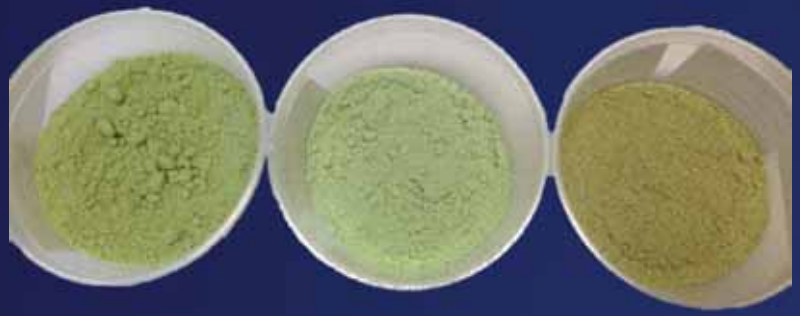
Key foods	Corn flakes	Rye flakes	Toast bread	Rye bread	Wheat pasta	Rye pasta	Spelt rolls	Combo rolls	Wheat pancake	Combo pancake	Smoothie	Smoothie + potato starch
Digestible carbohydrates	86.0	67.8	76.4	61.2	79.1	72.0	74.4	60.0	48.4	30.0	68.4	105.5
Dietary fibre	4.7	**19.2**	6.2	**20.3**	5.8	**13.8**	5.0	**17.2**	4.5	**20.3**	10.3	**71.8**
Resistant starch	1.8	0.4	1.2	2.1	0.9	1.1	0.6	**5.4**	0.7	**6.5**	0.0	**59.6**
Arabinoxylan	0.3	**9.1**	1.7	**8.4**	1.8	**5.6**	1.3	**3.4**	0.9	**3.3**	0.4	0.4
AXOS	0.1	0.0	0.3	0.0	0.4	0.0	0.3	**0.4**	0.2	**0.7**	0.2	0.5

INTAKE FROM KEY FOOD ITEMS/d

	WSD	HCD
Energy (KJ)	5280	**4722**
Protein (g)	40·4	**31·7**
Fat (g)	17·3	17·3
Digestible carbohydrates (g)	226	**181**
Sugars (g)	29.7	28.3
Starch (g)	196	**153**

INTAKE FROM KEY FOOD ITEMS/d

	WSD	HCD
Dietary fibre (g)	17·7	**63·9**
Non-digestible carbohydrates (g)	14·4	**58·9**
Resistant starch (g)	8·5	**32·3**
Arabinoxylan (g)	3·6	**16·0**



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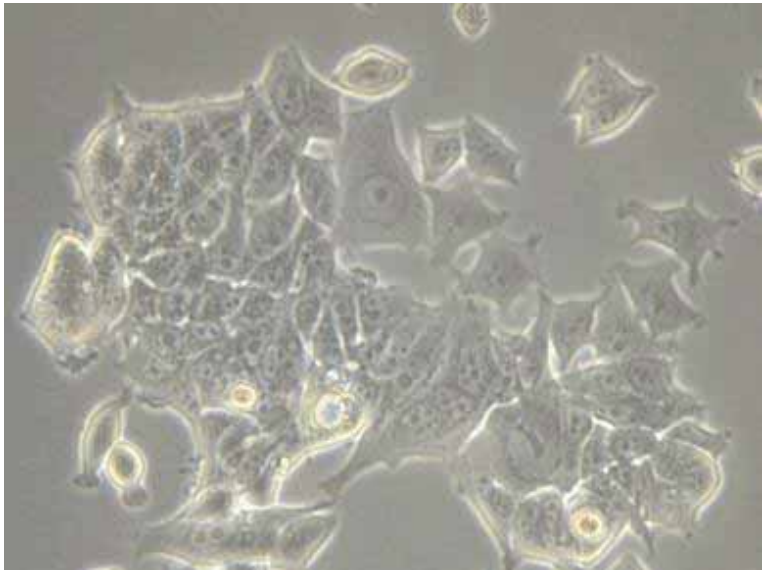
Butyrogenic effects of pre- and probiotics in vitro

Stig Purup, Ditte Søvsø G. Nielsen
Aarhus University
Department of Animal Science

Markku Saarinen
DuPont Nutrition and Health
Kantvik, Finland



Outline:

Fermentation products produced *in vitro* by pre- and probiotics:

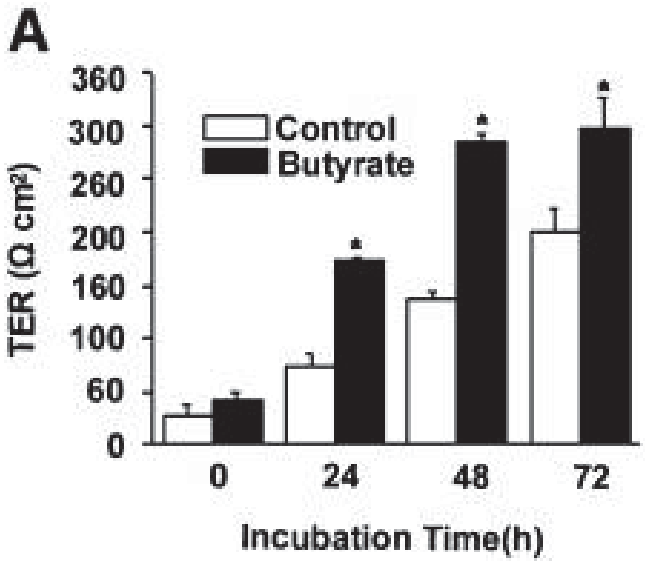
- Arabinoxylan products (AX)
  Butyrate producing bacteria
- DuPont food ingredients
  Human fecal slurry

Effect on colonic health *in vitro* (epithelial barrier function):

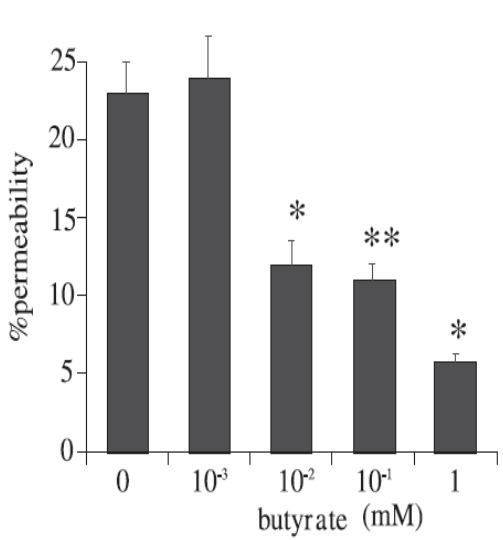
- Cellular parameters
  transepithelial electrical resistance (TEER)
  permeability
- Transcriptome analyses (gene expression)

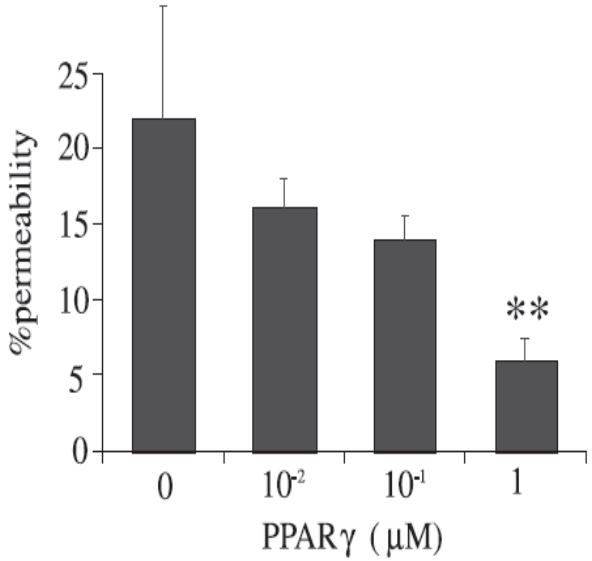
Does fermentation products with increased butyrate affect colon health positively?

› Positive effects of butyrate *in vitro:*



Caco-2 human colon cell monolayers treated with 2 mM butyrate.
(Peng et al., J Nutr 139, 2009)





HT-29 human colon cell monolayers
(Kinoshita et al., Biochem Biophys Res Comm 293, 2002)

› Intestinal barrier function

› Two-layered mucus (MUC2 and MUC5AC) protects the epithelium against pathogens.

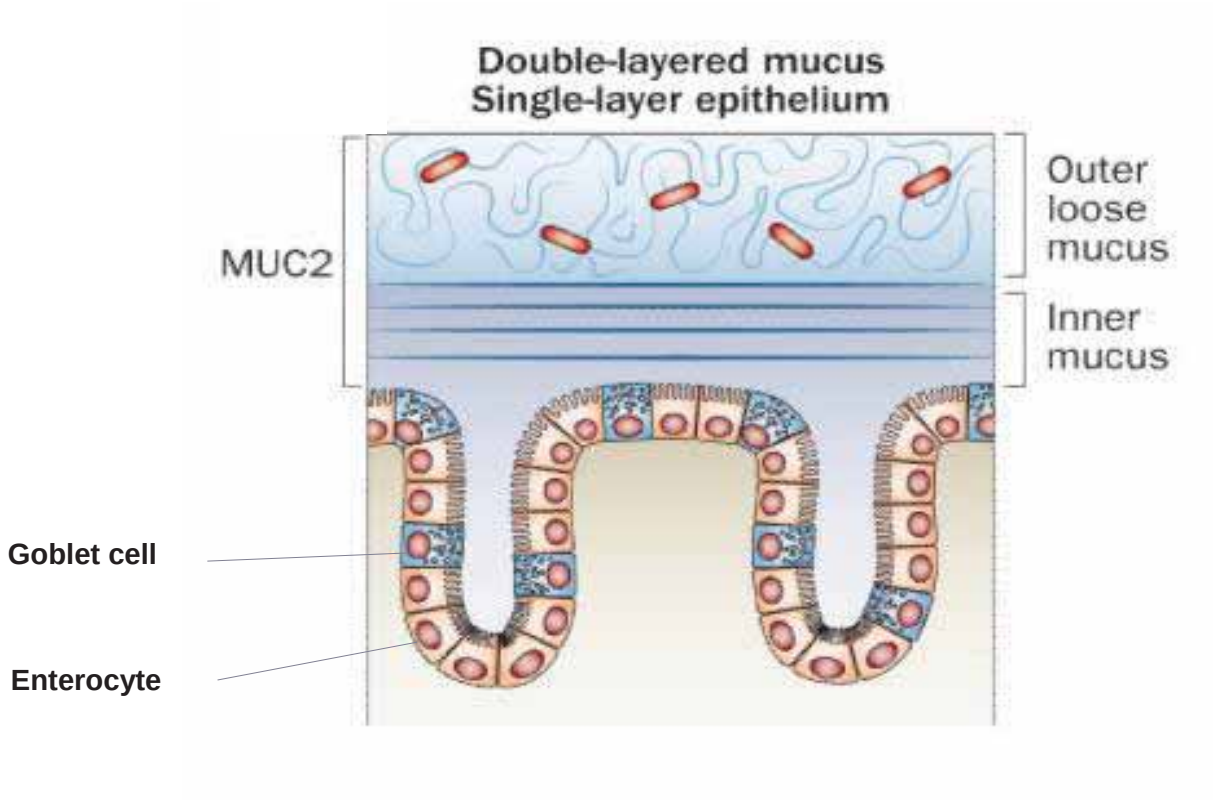
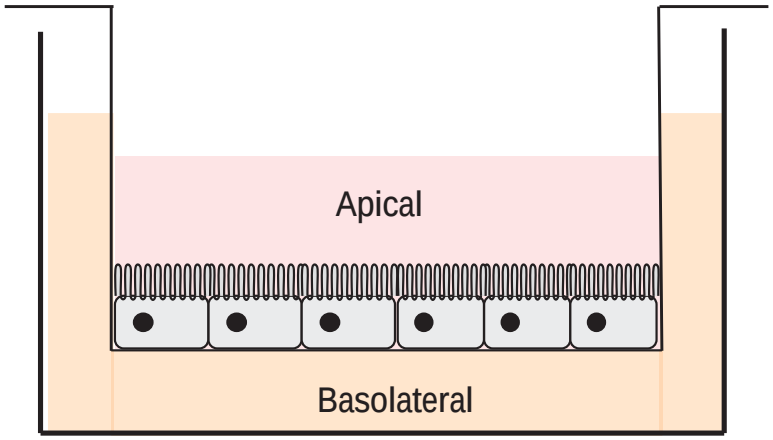


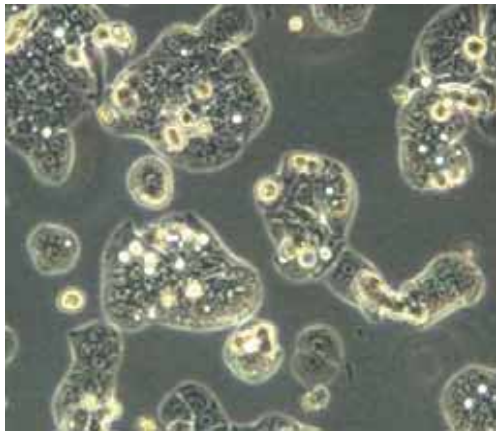
Figure modified from Johansson et al. 2013

› Which *in vitro* model to choose?

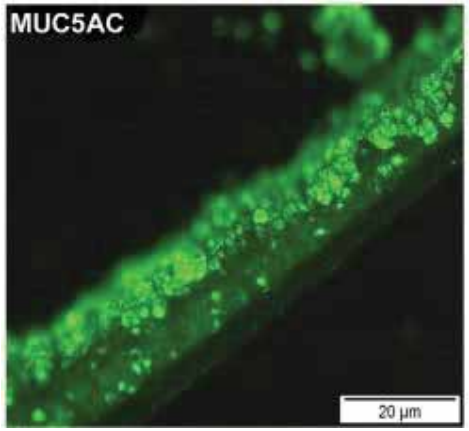
- 2-compartment model
  - Caco-2 or HT29 colon cells: epithelial cells (enterocytes)
  - HT29-MTX-E12 colon cells; 70-80 % mature goblet cells producing mucus layer



2-compartment model

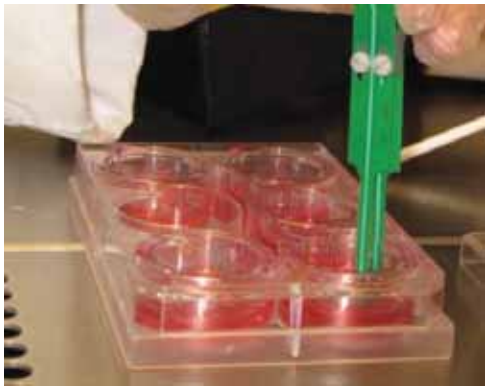


HT29-MTX-E12 human intestinal cells

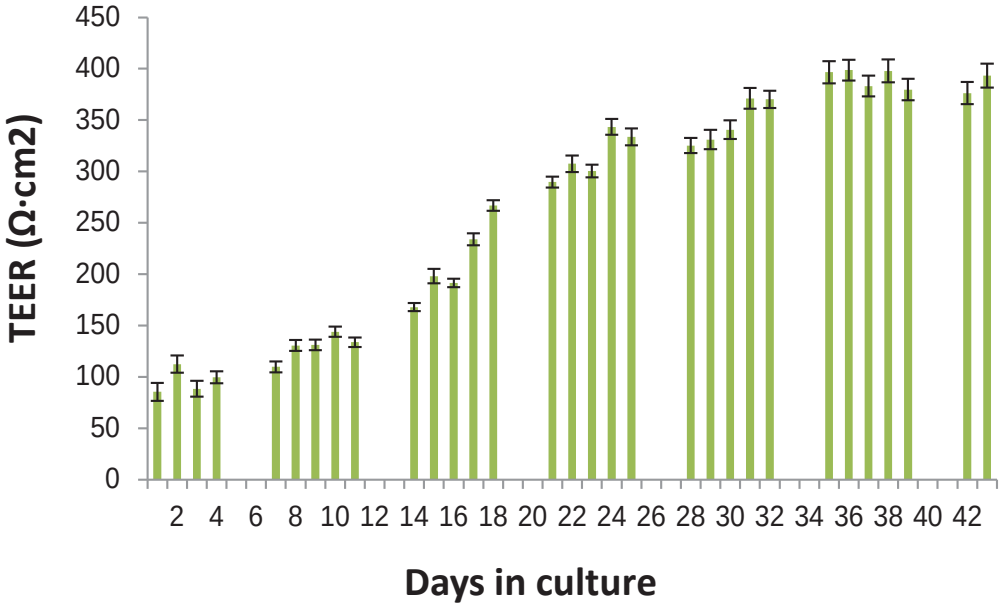


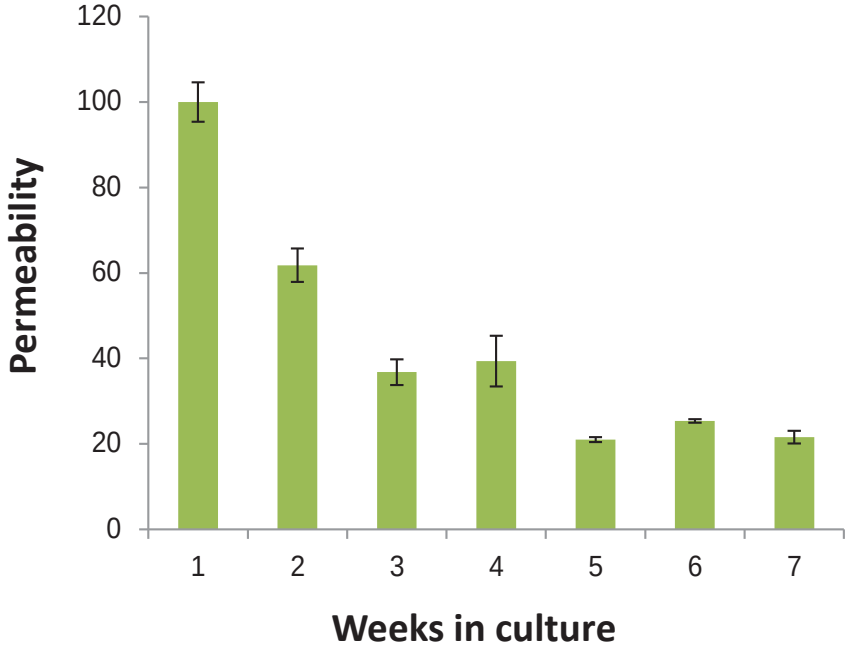
Mucus layer on HT29-MTX-E12 cells (*Dolan et al., 2012*)

› Intestinal barrier function in colon cells



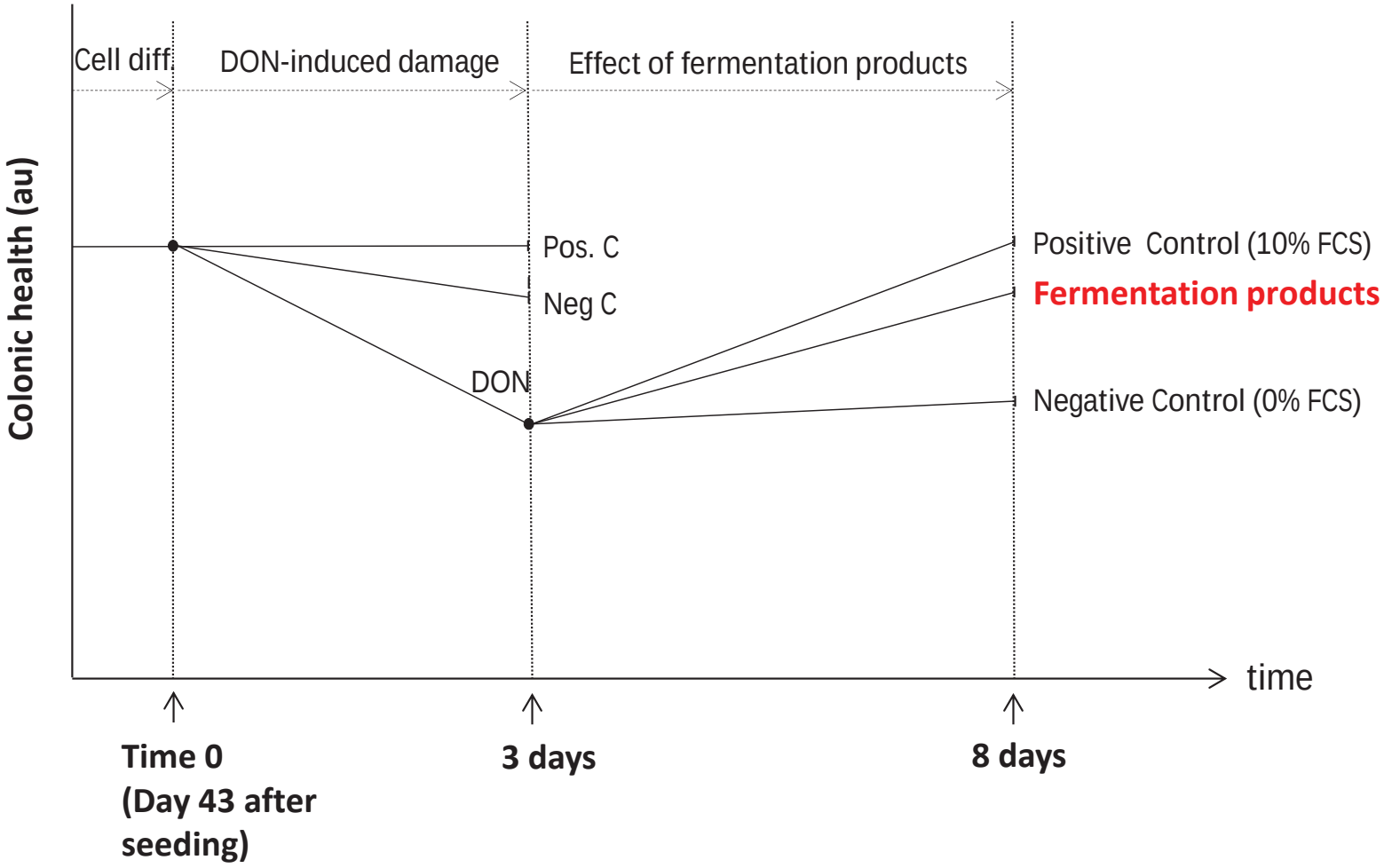
- › Trans-epithelial electrical resistance (TEER)
- › Paracellular flux of FITC-dextran (4 kDa)



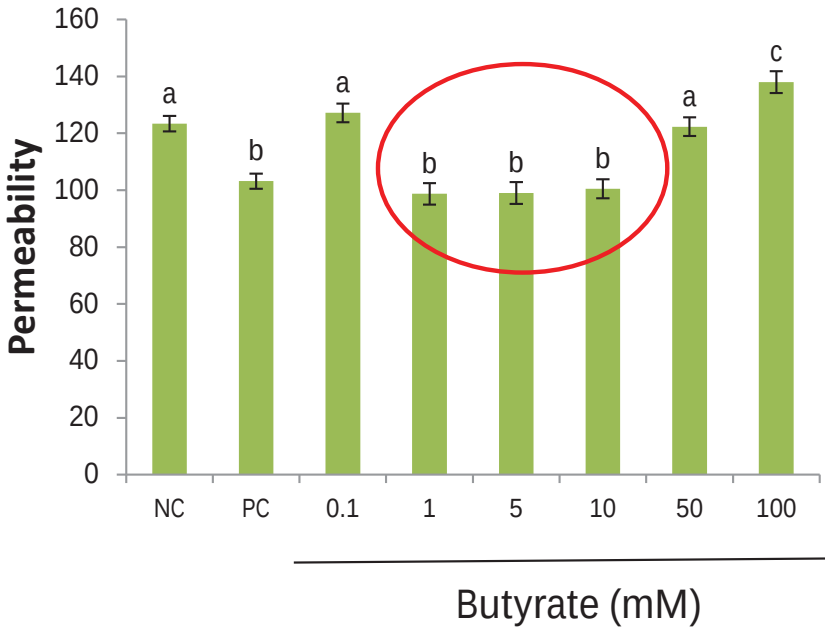


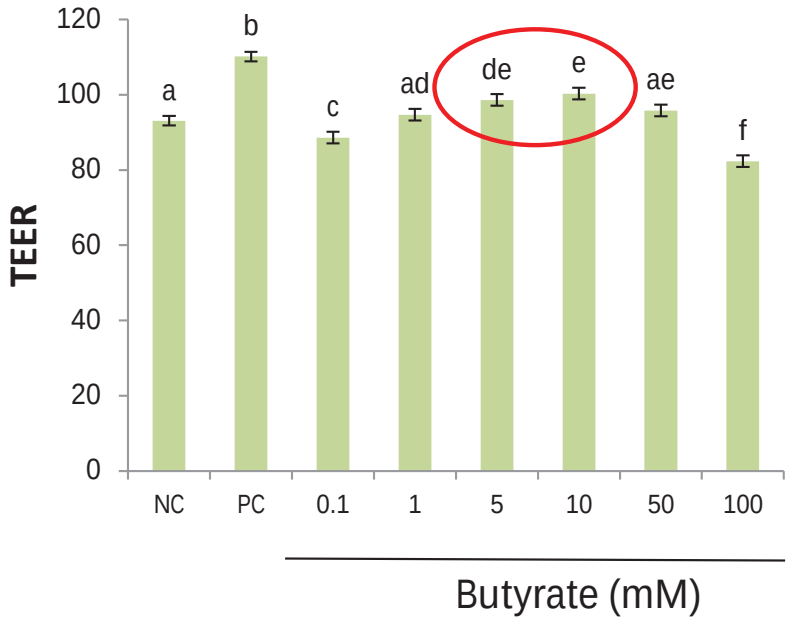
› Intestinal barrier function in colon cells

› Treatment with the mycotoxin deoxynivalenol (DON) for 72 h.



› Postive effect of butyrate





- 1-10 mM butyrate decreased the paracellular permeability (P<0.01)
- 5-10 mM butyrate increased TEER (P<0.05)
- 0.1-5 mM butyrate increased transcription of MUC2 (P<0.05; data not shown)
- 10-100 mM butyrate decreased transcription of MUC2 and MUC5AC (P<0.01; data not shown)
- 50-100 mM increased transcription of anti-oxidative stress genes superoxide dismutase 2 (SOD2) and catalase (CAT) (data not shown).

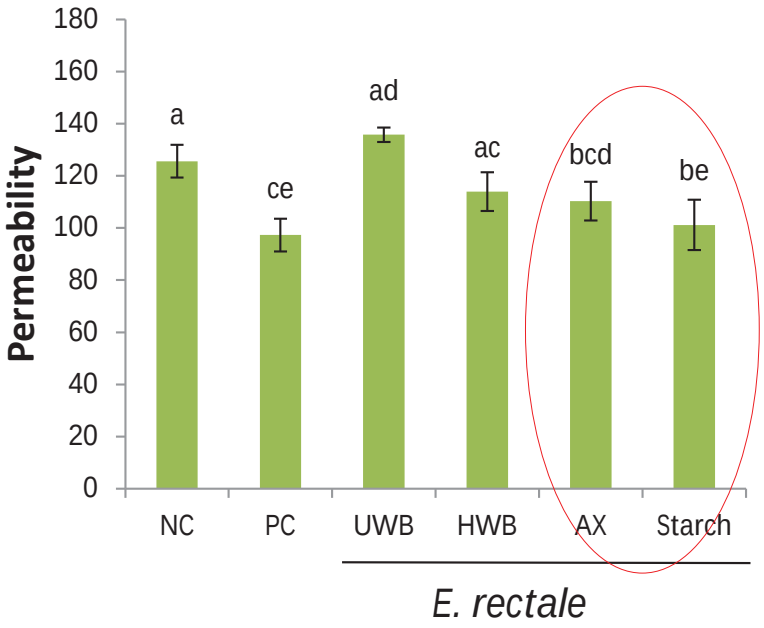
› Butyrate concentrations in fermentations products

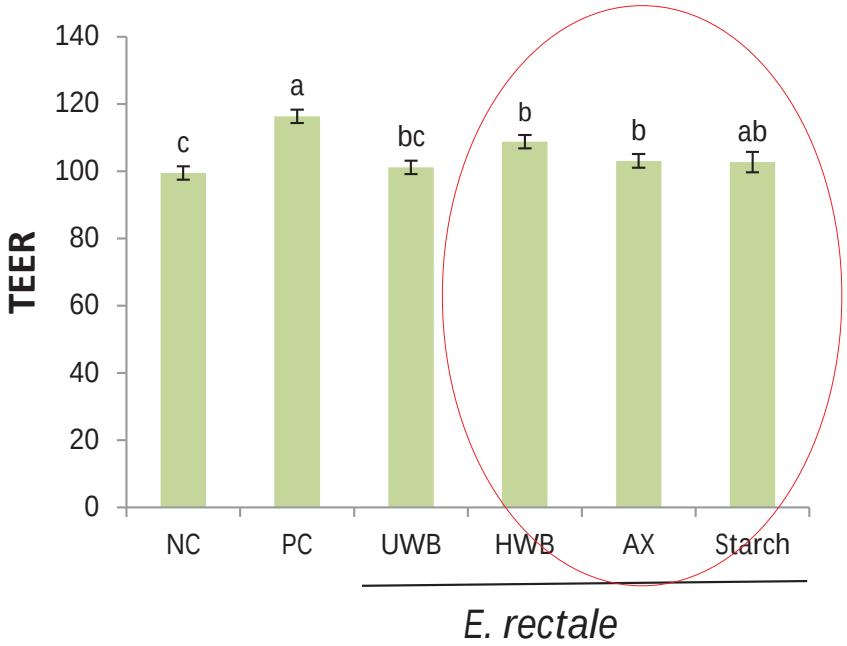
	Butyrate (mM)				
	Roseburia intestinalis	*Faecalibacterium prausnitzii*	*Eubacterium rectale*	*Butyrivibrio fibrisolvens*	*Control – no bacteria*
Unhydrolysed wheat bran	7.4*	7.2*	6.9*	10.8*	3.1*
Hydrolysed wheat bran	12.2*	13.5*	8.0*	17.2*	2.9*
Pure AX	3.5	3.5	6.7*	17.7*	3.2*
Starch	3.6	5.4	3.6*	3.9*	3.1*

*) Selected fermentation products for test on intestinal barrier function

› Positive effects of fermentation products

Butyrate (mM)	
UWB:	6.9
HWB:	8.0
AX:	6.7
Starch:	3.6





› No effects of *F. prausnitzii* & *R. intestinalis* (data not shown)

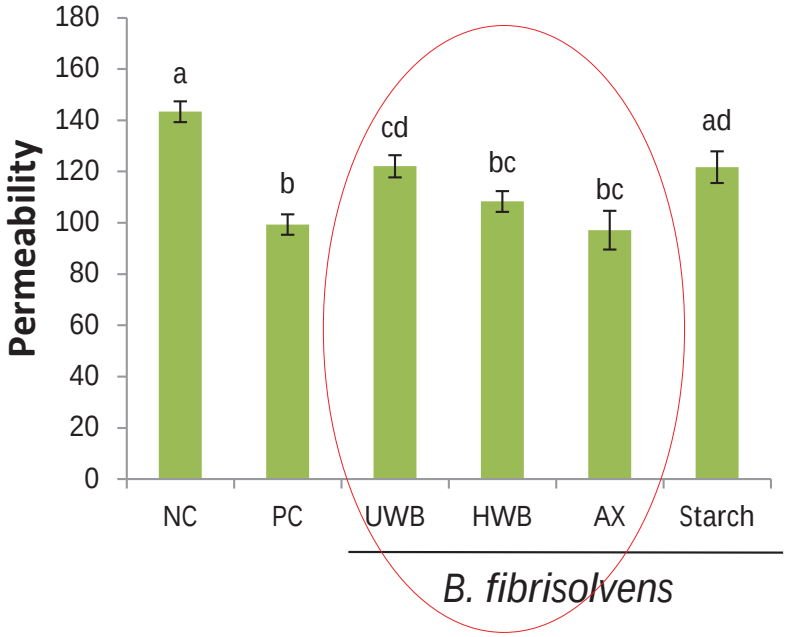
› *E. rectale* w. arabinoxylan or starch: decrease in permeability ($P<0.05$)

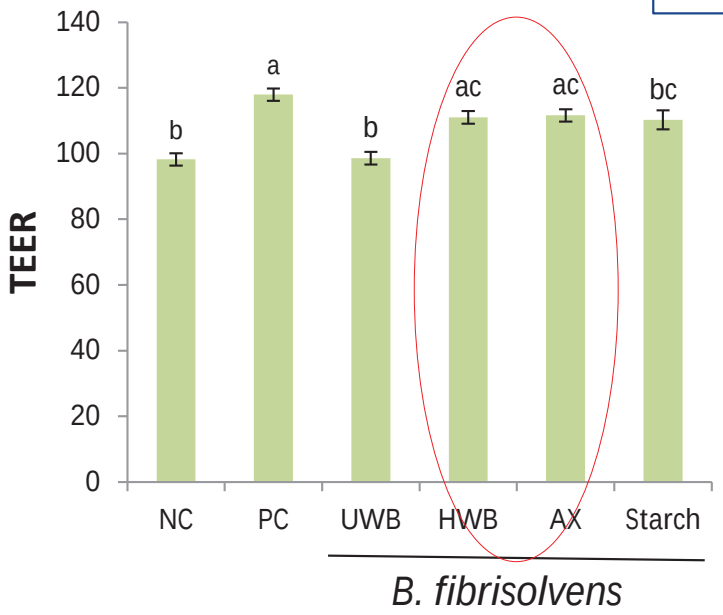
› *E. rectale* w. hydrolysed wheat bran, arabinoxylan or starch: increase in TEER ($P<0.05$)

› *E. rectale* alone increased transcription of MUC2 ($P<0.05$; data not shown)

› Effect of fermentation products

Butyrate (mM)	
UWB:	10.8
HWB:	17.2
AX:	17.7
Starch:	3.9





› *B. fibrisolvens* w. unhydrolysed- or hydrolysed wheat bran or arabinoxylan: decrease in permeability ($P<0.01$)

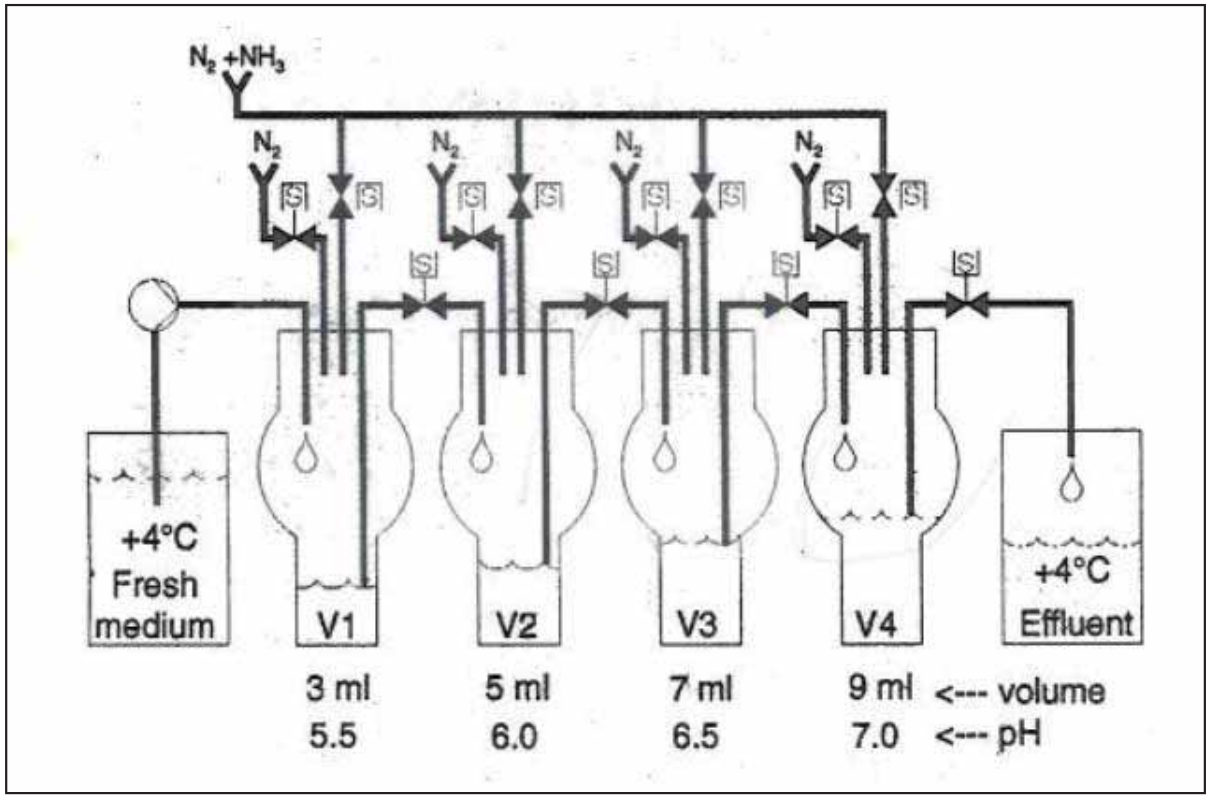
› *B. fibrisolvens* w. hydrolysed wheat bran or arabinoxylan: increase in TEER ($P<0.01$)

› *B. fibrisolvens* w. hydrolysed wheat bran or arabinoxylan tended to decrease transcription of MUC2 ($P<0.1$)

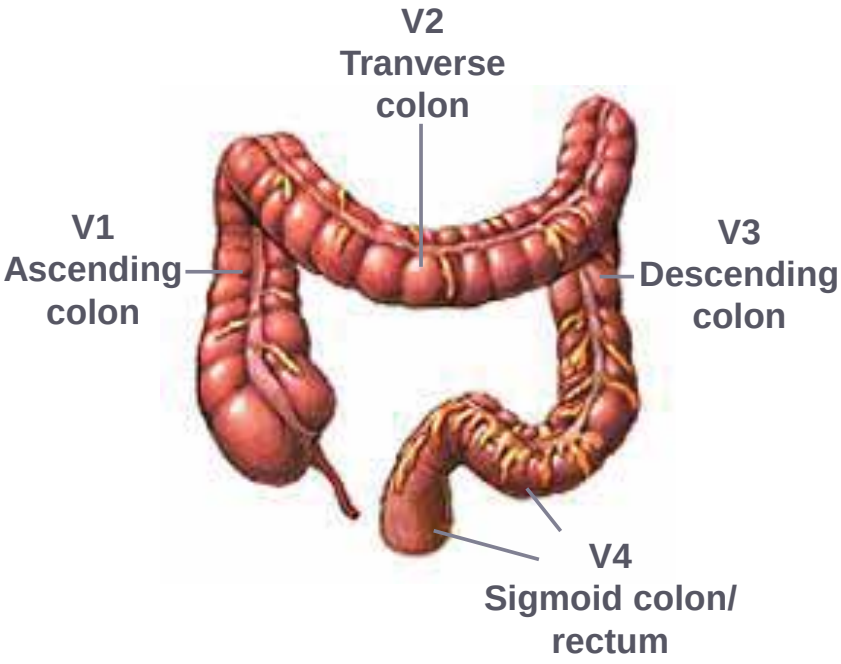
Summary – fermentation products with AX-containing substrates

- Butyrogenic bacteria and substrates containing AX increased the production of butyrate.
- Fermentation products had varying effect on epithelial barrier function.
- Some genes related to epithelial integrity were affected by fermentation products.
- *B. fibrisolvens* are of specific interest (rumen bacterium).
- Hydrolysed wheat bran and pure arabinoxylan are of specific interest.

› Enteromix colon simulation model (DuPont, Kantvik)



Rautonen et al. 2005



› Substrates for Enteromix colon simulation model

› **Prebiotics**
  › **Lactitol:** sugar alcohol, increases acetate, butyrate and total SCFA in colon.

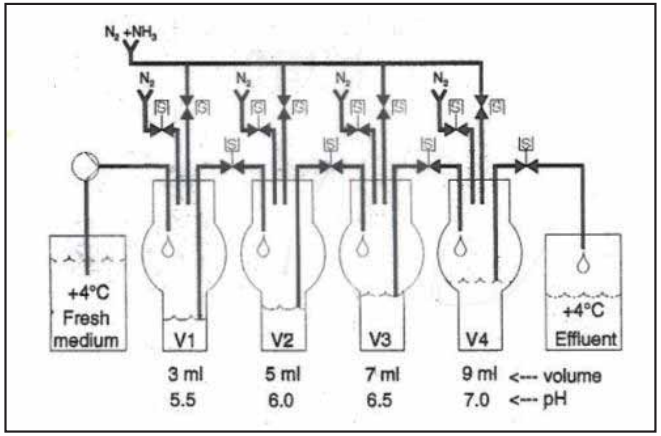
  › **PDX:** polydextrose, polymer of glucose, increases propionate and butyrate in colon.

› **Xylitol:** sugar alcohol, increases butyrate in colon.

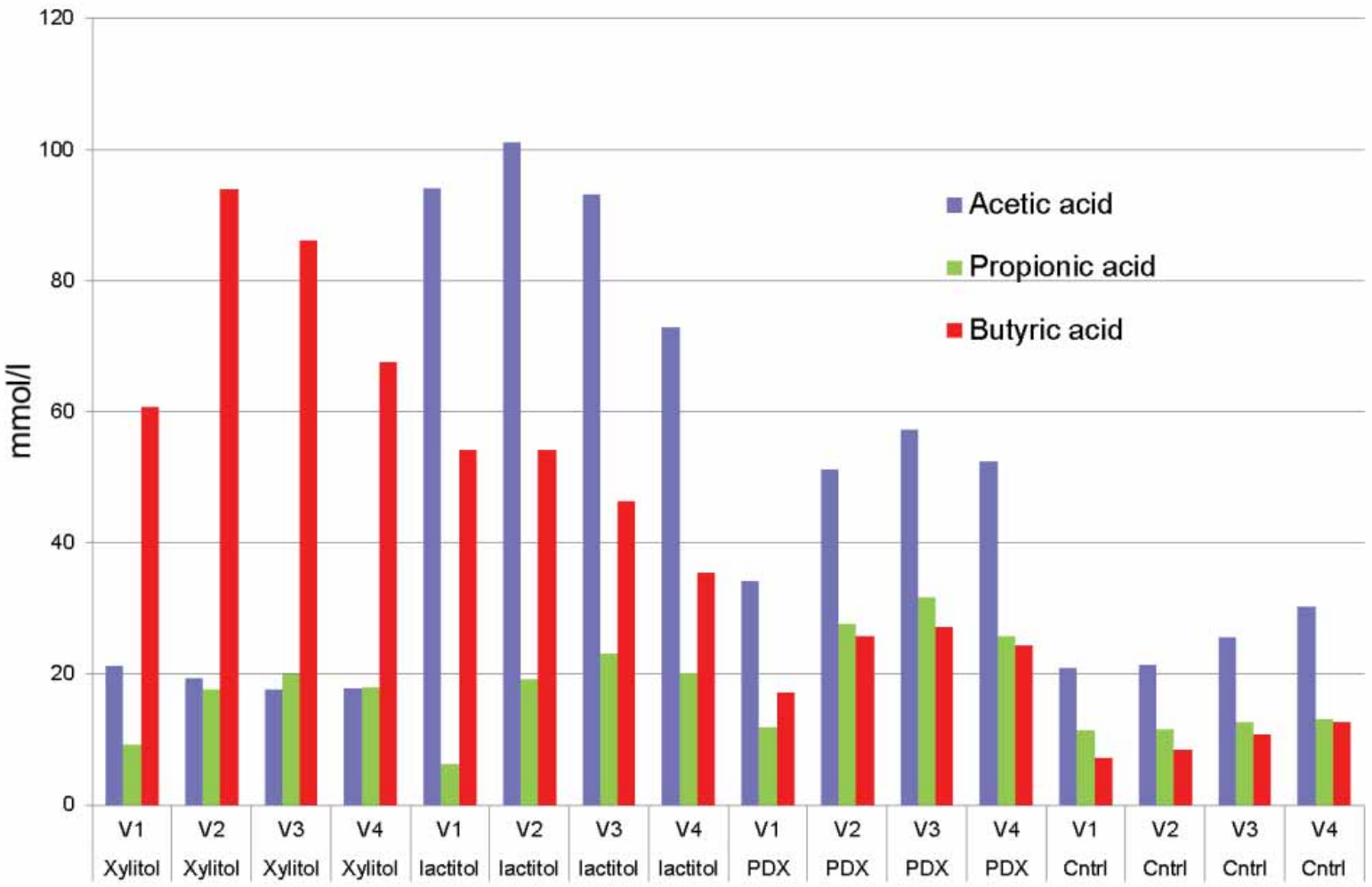
› **Control:** synthetic medium mimicking the content of the small intestine.

Inoculation: Faecal sample from healthy donor.

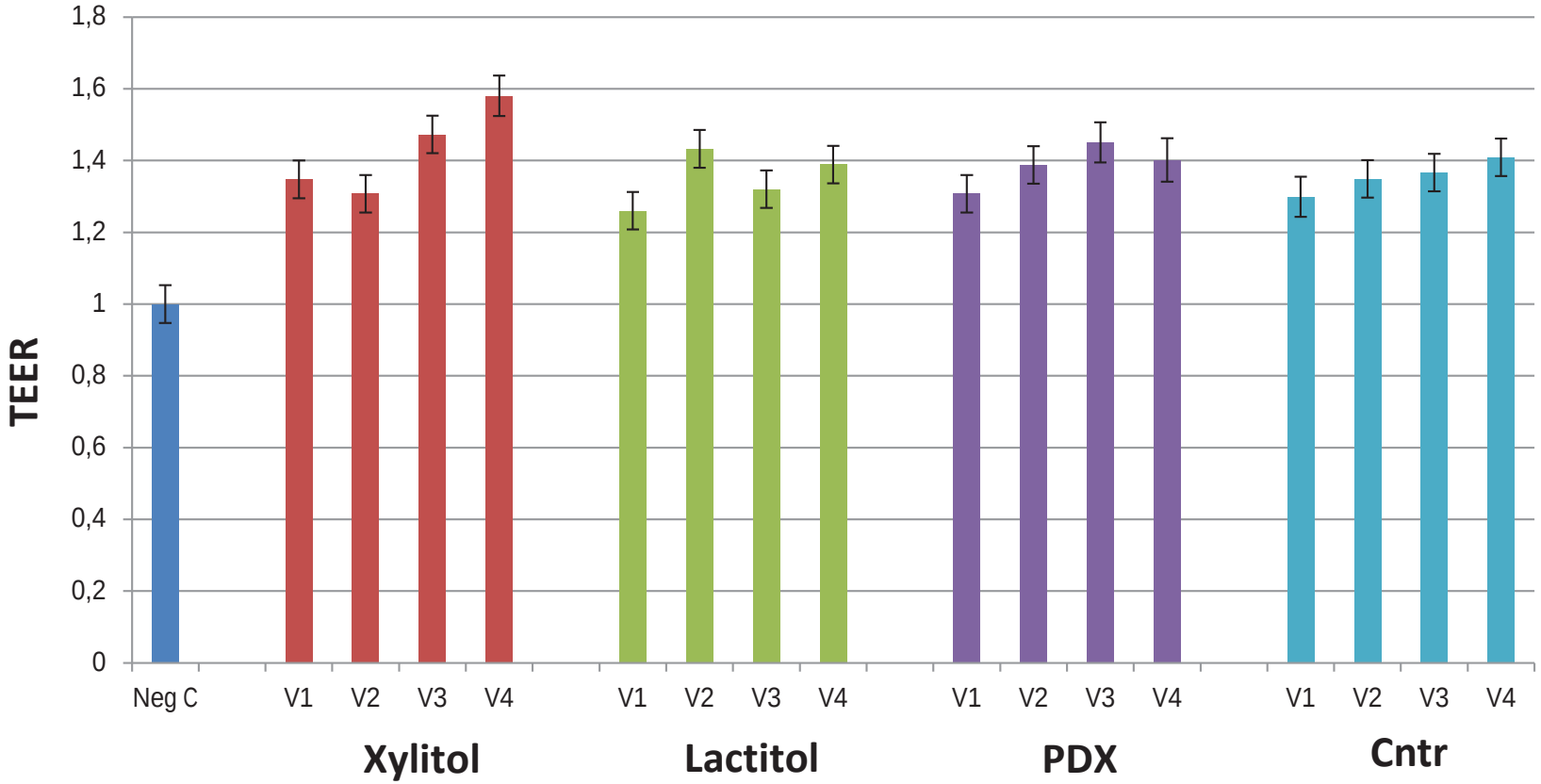
Simulation: 48 h incubation.



› Enteromix colon simulation – SCFA content

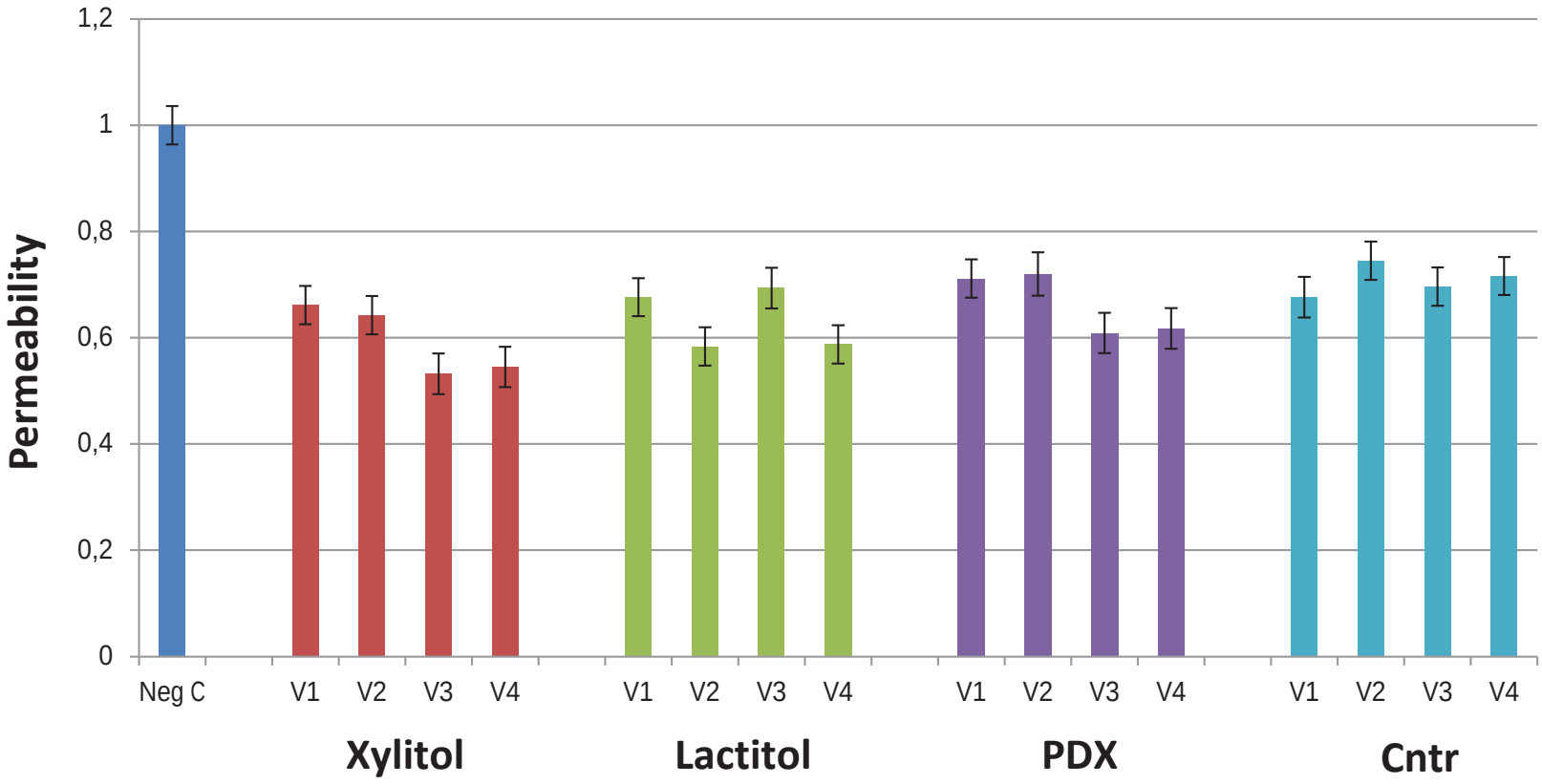


› Enteromix colon simulation – epithelial barrier function



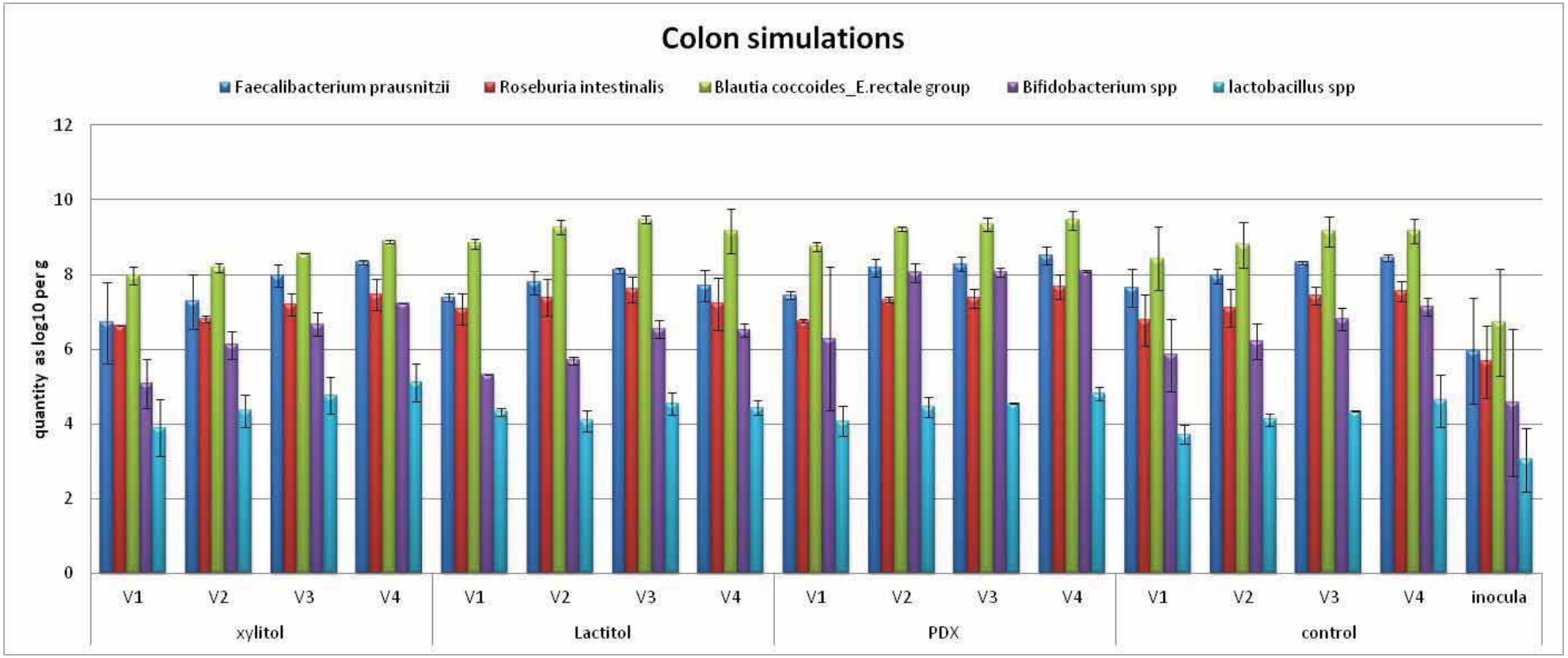
	Xylitol	Lactitol	PDX	Cntr
Butyric acid	77,1	47,5	23,6	9,8
Acetic acid	19,0	90,3	48,8	24,5
Propionic acid	16,2	17,2	24,2	12,2
Total (mM)	112	155	97	46

› Enteromix colon simulation – epithelial barrier function



	Xylitol	Lactitol	PDX	Cntr
Butyric acid	77,1	47,5	23,6	9,8
Acetic acid	19,0	90,3	48,8	24,5
Propionic acid	16,2	17,2	24,2	12,2
Total (mM)	112	155	97	46

› Enteromix colon simulation – microbial analysis



Conclusion

- We have developed an *in vitro* model with mucus producing intestinal cells to investigate the epithelial barrier function with butyrate or fermentation products – an important step before proceeding to animal or clinical trials with the most promising candidates.
- The Enteromix simulator is an effective tool for modeling prebiotic fermentation of the human colon representing different compartments.
- Combining butyrogenic bacteria and substrates - specifically *B. fibrisolvens* with hydrolysed wheat bran (HWB) and pure arabinoxylan (AX) – increase production of butyrate and positively affects the epithelial barrier function.
- Fermentation products from the Enteromix simulation suggest that the proportion of SCFA rather than butyrate alone might explain the effects observed on intestinal barrier function.

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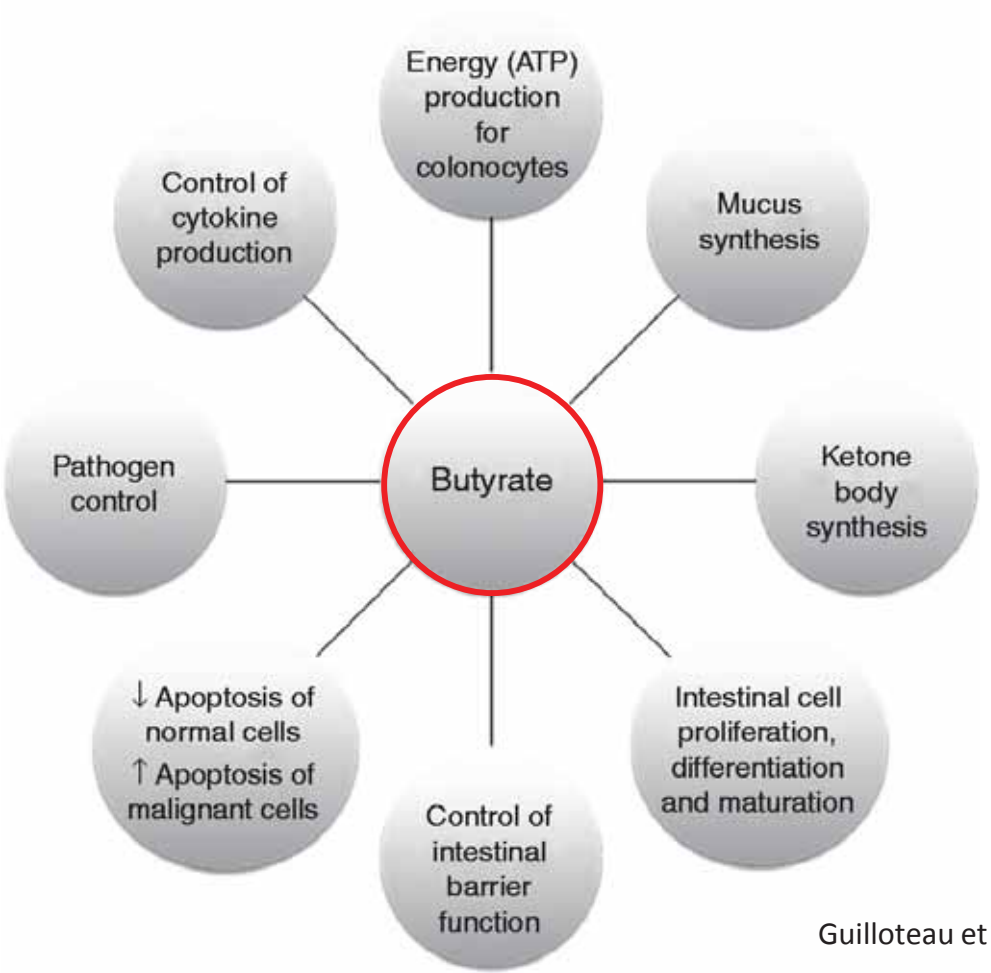
AARHUS UNIVERSITY
DEPARTMENT OF ANIMAL SCIENCE

Gut formation of butyrate and large intestinal gene expression as indicators of intestinal health - animal studies

Tina Skau Nielsen, post doc

Multiple (positive) effects of butyrate locally in the intestine

…also potential positive systemic effects.



Guilloteau et al., Nutr. Res. Rev. 23, 2010

How can we increase the production of butyrate in the gut by dietary means ?

Resistant starch (RS)

Arabinoxylan (AX)

Butyrogenic substrates

+

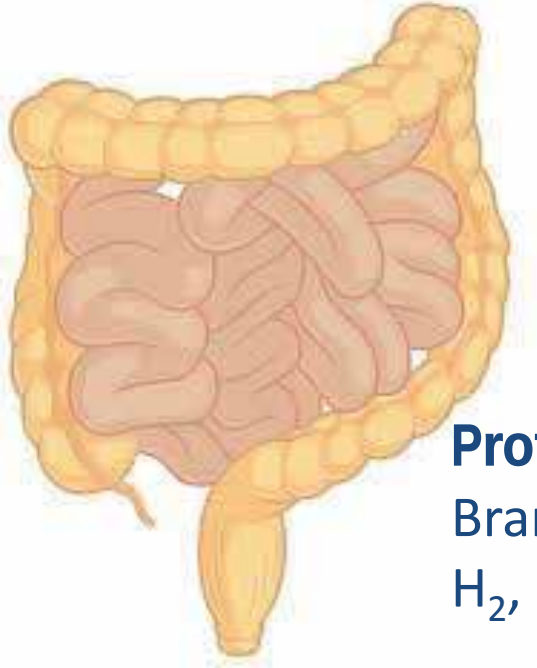
Butyrogenic bacteria

= Butyrate

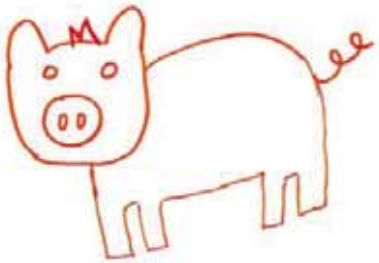
Butyrate production:

Cecum > proximal colon > mid colon > distal colon

Saccharolytic bacteria:
linear SCFA´s, CO_2, H_2



Proteolytic bacteria:
Branched SCFA´s, CO_2, CH_4, H_2, phenols, amines



Experimental design

Western style diet WSD

Resistant starch diet RSD

Arabinoxylan diet AXD

7 % fiber

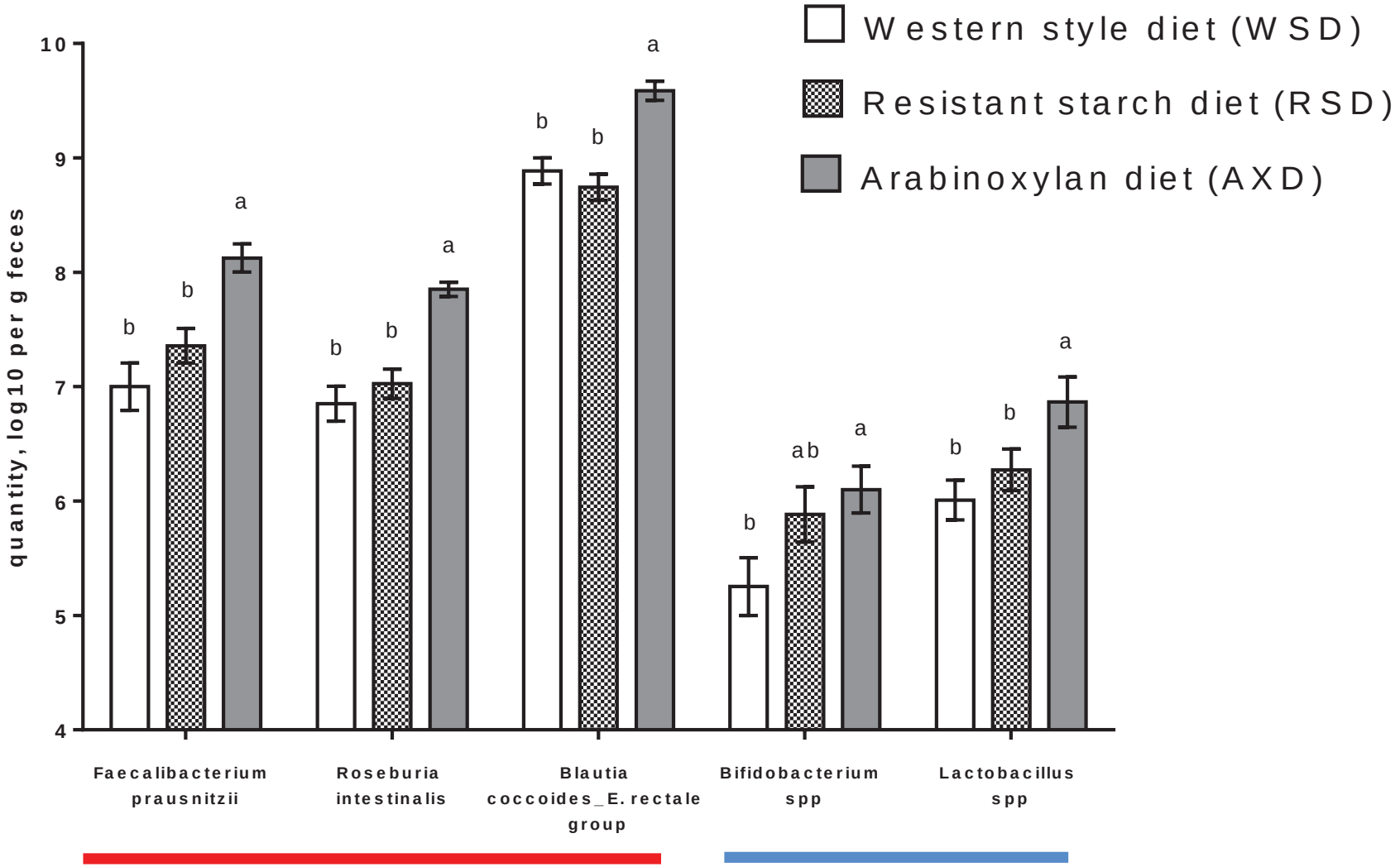
19 % fiber

19 % fiber

- High in refined carbohydrates
- Equal and high in fat
- Equal in protein

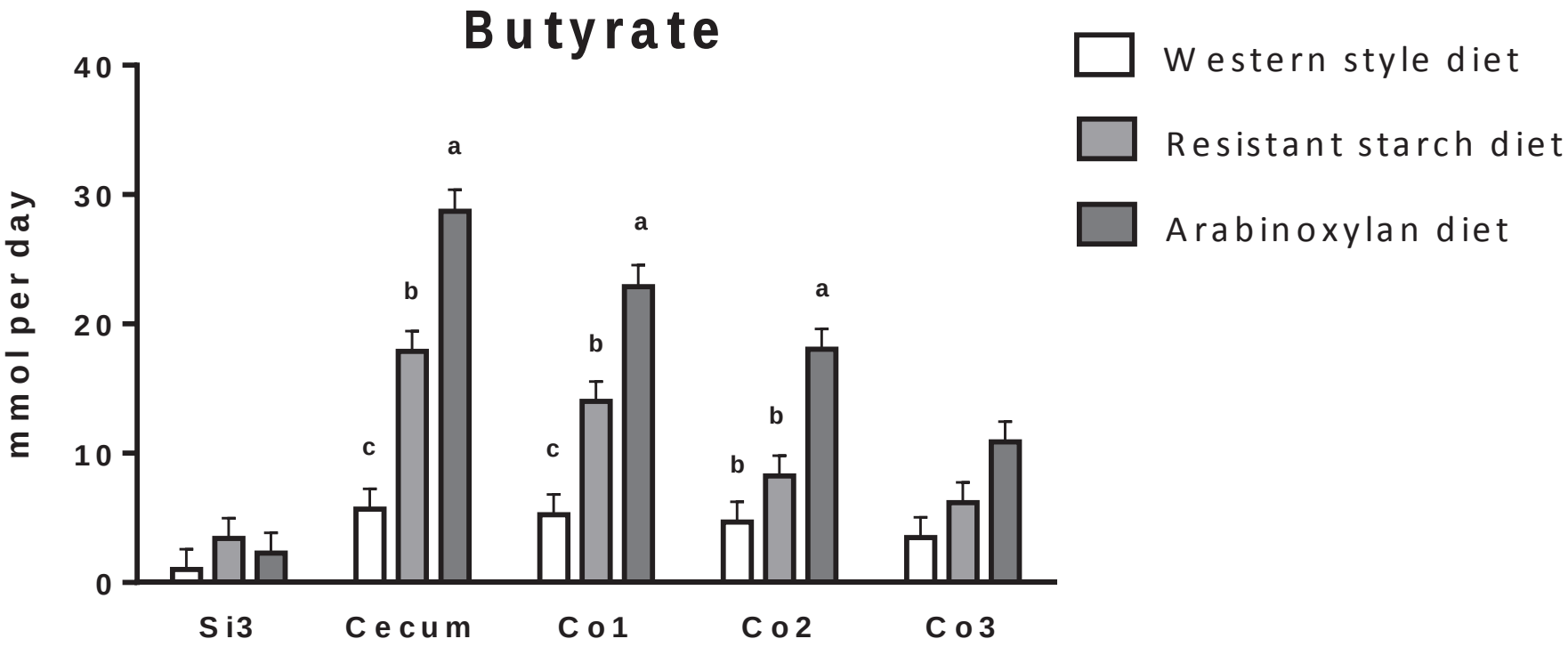
10 pigs per diet
3 week experimental period

Microbial composition in faeces after 3 weeks



AXD = higher number of butyrate producing and commensal beneficial bacteria

Butyrate production

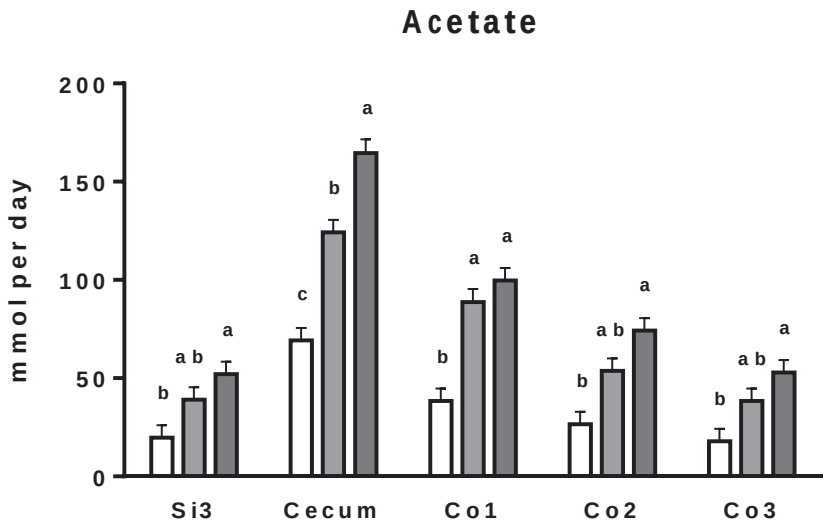


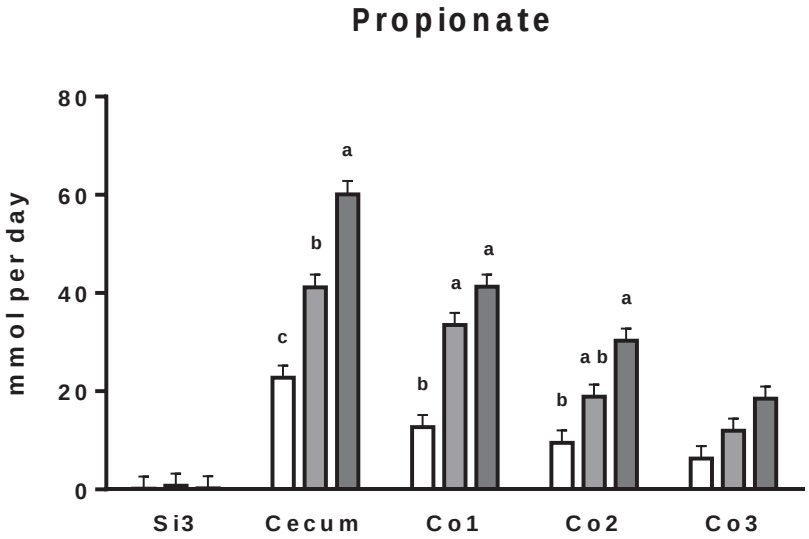
Cecum, Co1 and Co2: **AXD vs. WSD = 3 to 5 fold increase**

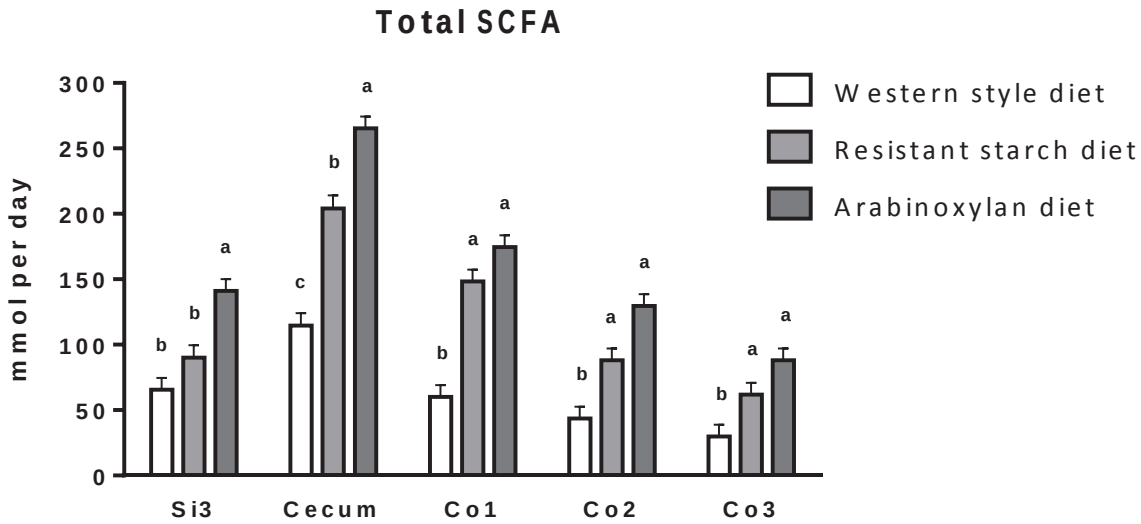
Cecum, Co1 and Co2: **AXD vs. RSD = 1.6 to 2.2 fold increase**

Tendency for increased butyrate production in the distal colon

SCFA production







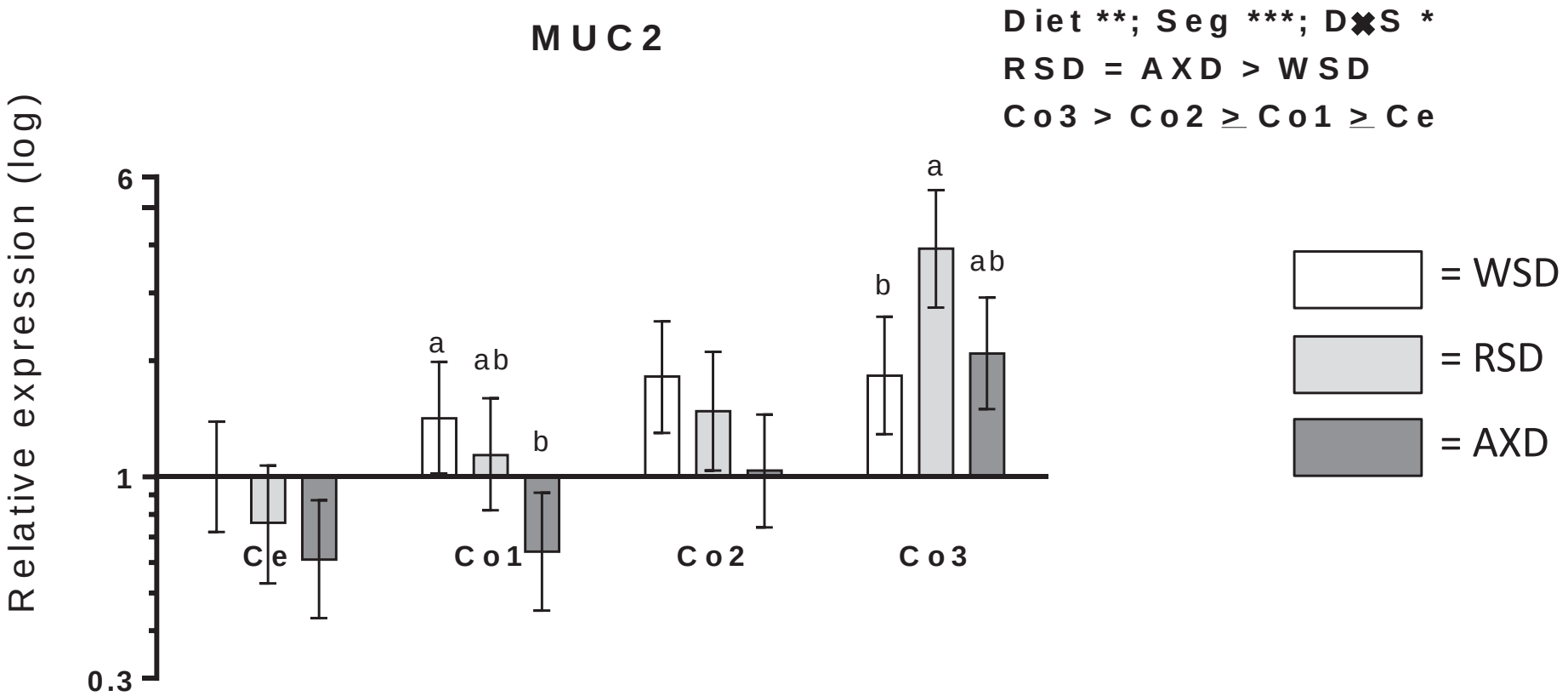
Expression of genes as indicators of large intestinal health
10 selected genes potentially affected by SCFA´s (butyrate)

	Cecum	Colon1	Colon2	Colon3
Butyrate transport				
MCT1	X	X	X	X
SCFA sensing				
GPR41	X	X	X	X
GPR43	X	X	X	X
Immunity/inflammation				
MCP1	X	X	X	X
TNF-α	X	X	X	X
NF-κβ	X	X	X	X
PPARγ	X	X	X	X
Epithelial permeability				
MUC2	X	X	X	X
ZO1	X	X	X	X
OCLN	X	X	X	X

6 of 10 genes regulated by **diet** or **diet × segment**

	Cecum	Colon1	Colon2	Colon3	
Butyrate transport					
MCT1	X	X	X	X	
SCFA sensing					
GPR41	X	X	X	X	G-protein coupled receptor 41
GPR43	X	X	X	X	G-protein coupled receptor 43
Immunity/inflammation					
MCP1	X	X	X	X	Monocyte chemoattractant protein 1
TNF-α	X	X	X	X	Tumor necrosis factor-alpha
NF-κβ	X	X	X	X	Nuclear transcription factor kappa-beta
PPARγ	X	X	X	X	
Epithelial permeability					
MUC2	X	X	X	X	Mucin 2
ZO1	X	X	X	X	
OCLN	X	X	X	X	

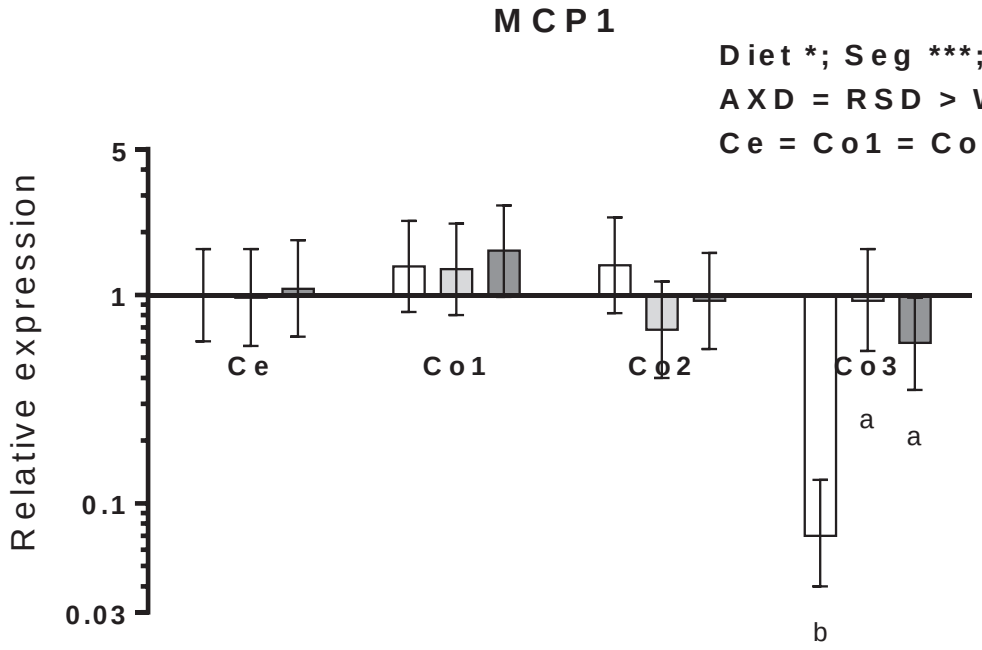
Epithelial permeability - mucus secretion (positive if expression is high)

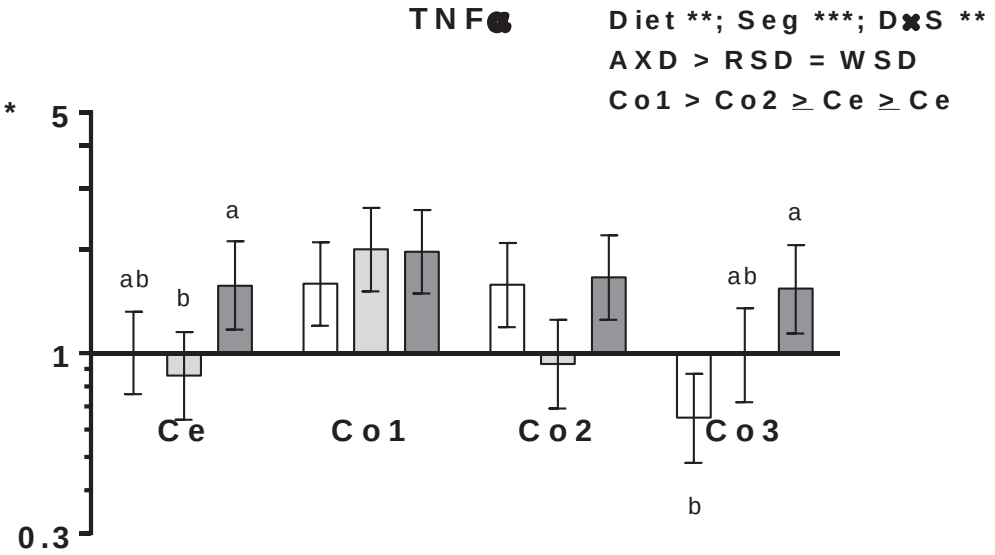


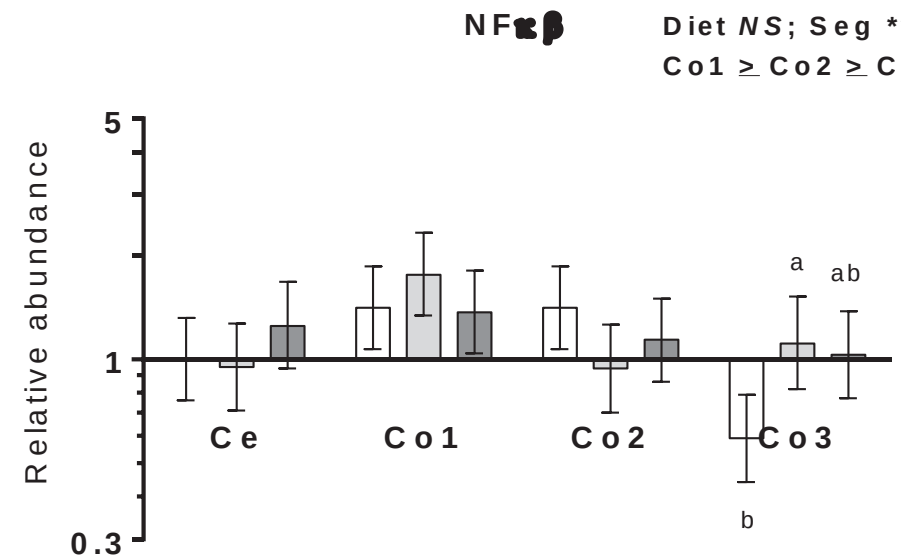
Expression increases throughout the large intestine

Effect of diet depends on segment
(WSD highest in the proximal colon, RSD highest in the distal colon)

Pro-inflammatory markers (positive if expression is low)





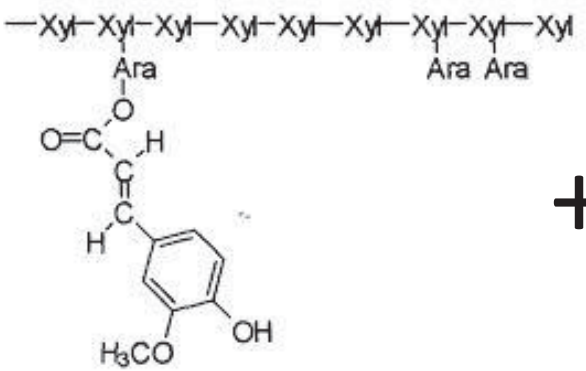


Conclusions

- AX most efficiently shifted the microbial composition towards butyrogenic species, more commensal bacteria and increased butyrate production throughout the large intestine
- AX and RS affected the expression of a number of genes in the large intestine
- Gene expression changes was not unambigously health-promoting, could not directly be related to SCFA

Difficult to show a ”health-promoting effect” in the large intestine of an already healthy animal (human)

Question: Can large intestinal butyrate production be maximized through a synbiotic concept ?



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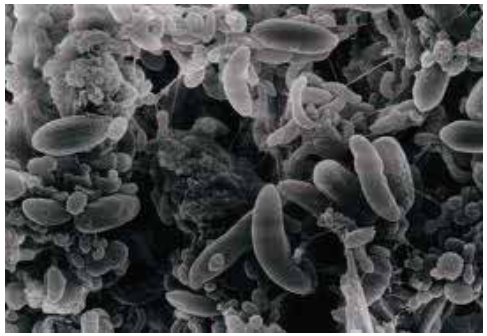


= Butyrate

Prebiotic (AX)

Probiotic (Butyrate producer)

In vitro **fermentation:**

B. fibrisolvens **+ AX = maximum butyrate conc.**

In vitro

In vivo

The diets

AXD: 15% dietary fibre

Both high fat and protein

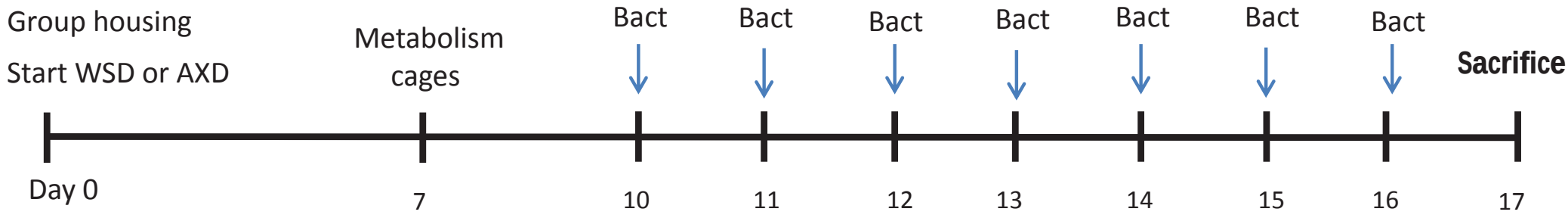
WSD: 5% dietary fibre

Design

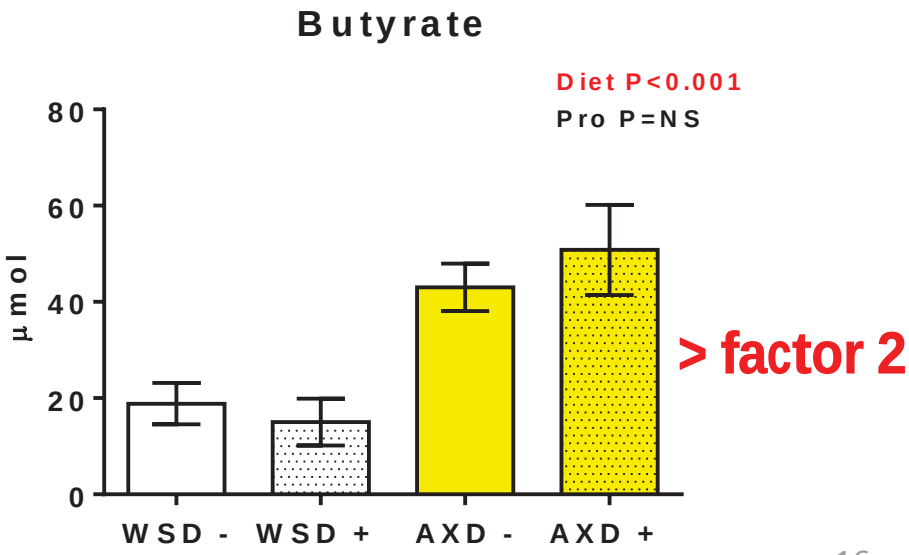
4 groups (10 rats per group):

- WSD **+/÷** *B. fibrisolvens (*live intact cells, oral gavage)

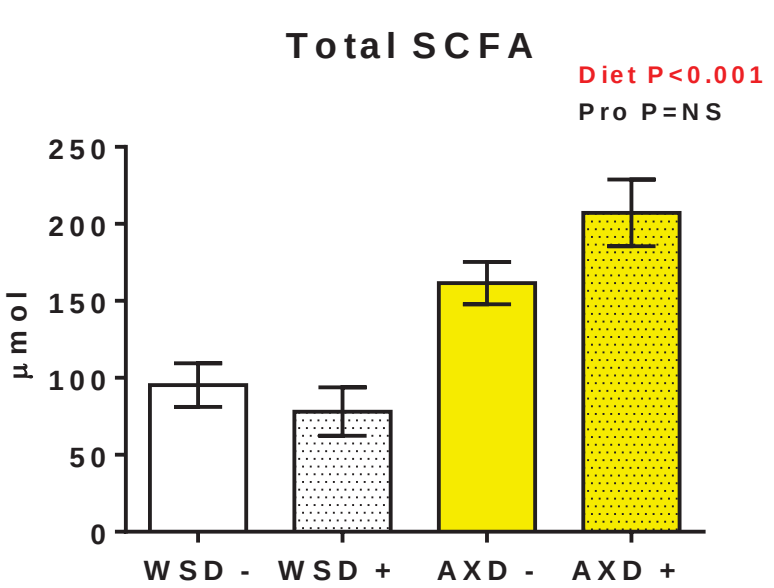
- AXD **+/÷** *B. fibrisolvens*

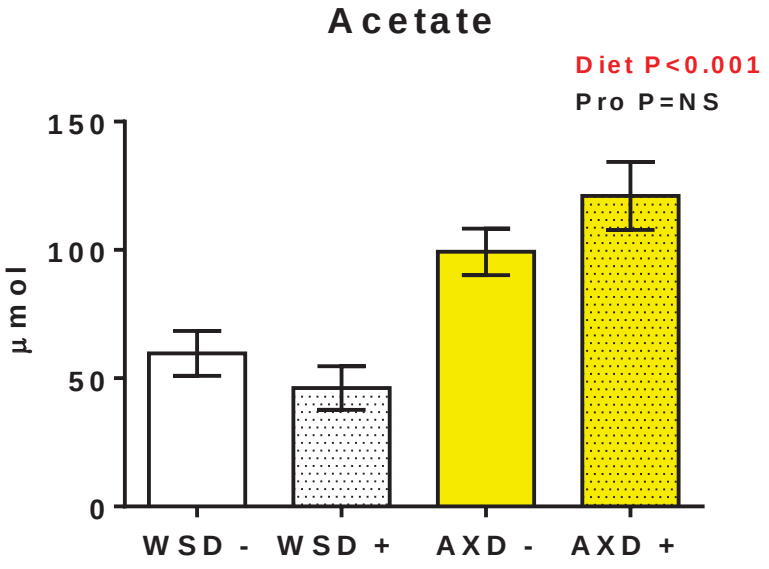


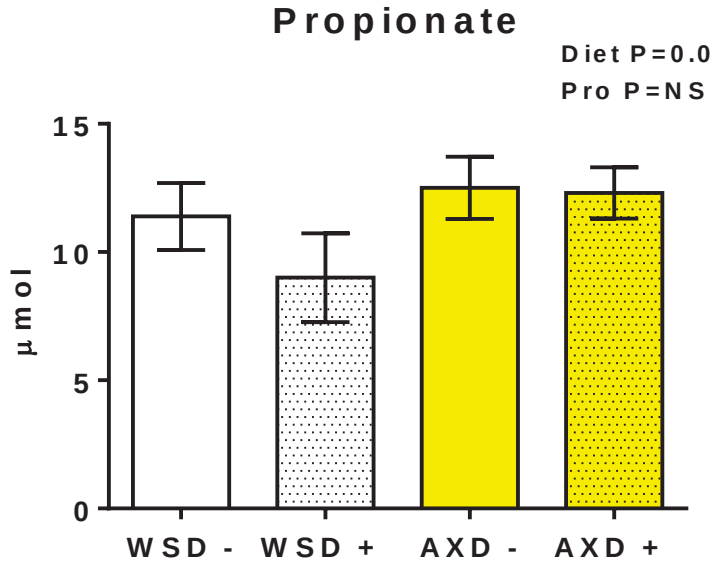
Results: Pool of SCFA (µmol) in cecum digesta

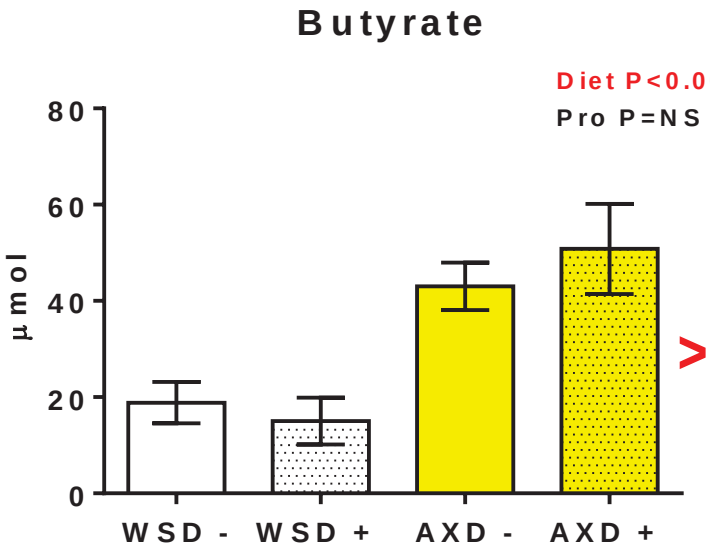


Results: Pool of SCFA (µmol) in cecum digesta





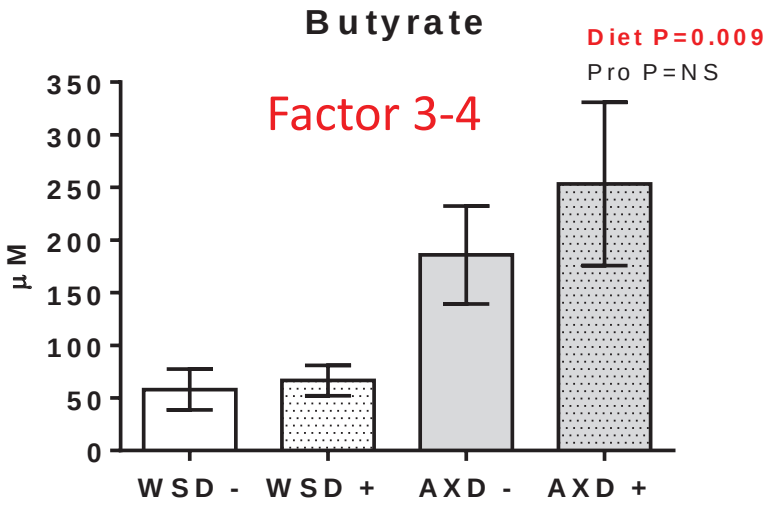




> factor 2

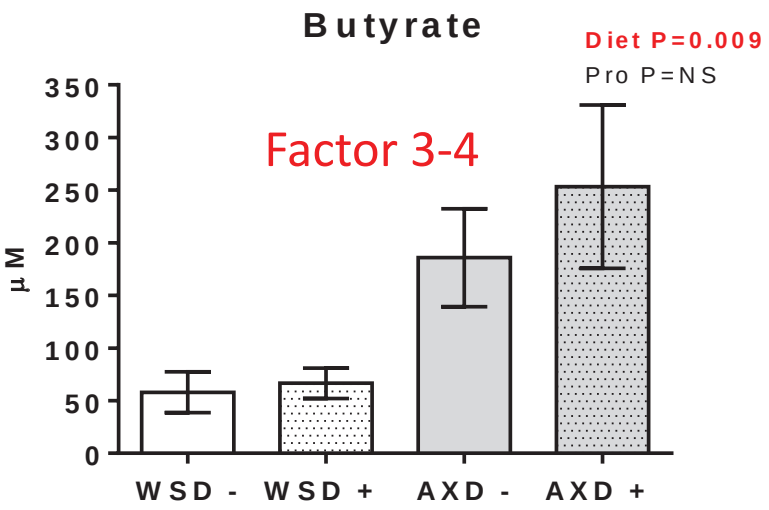
SCFA concentrations in blood

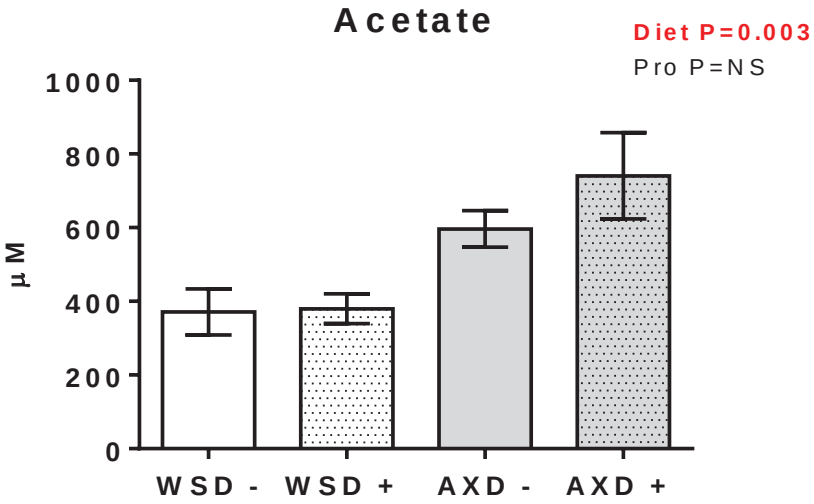
Portal blood

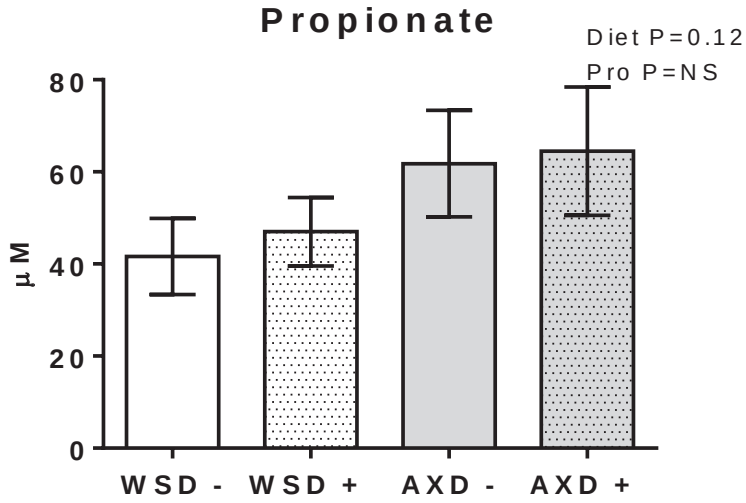


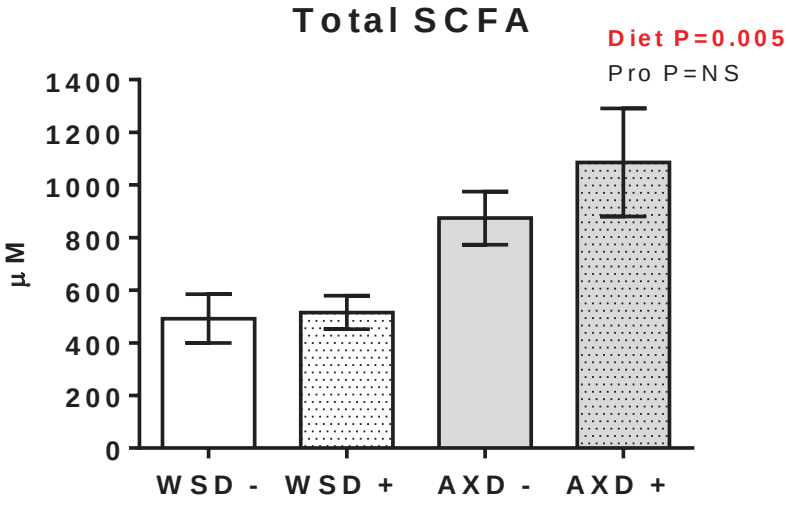
SCFA concentrations in blood

Portal blood



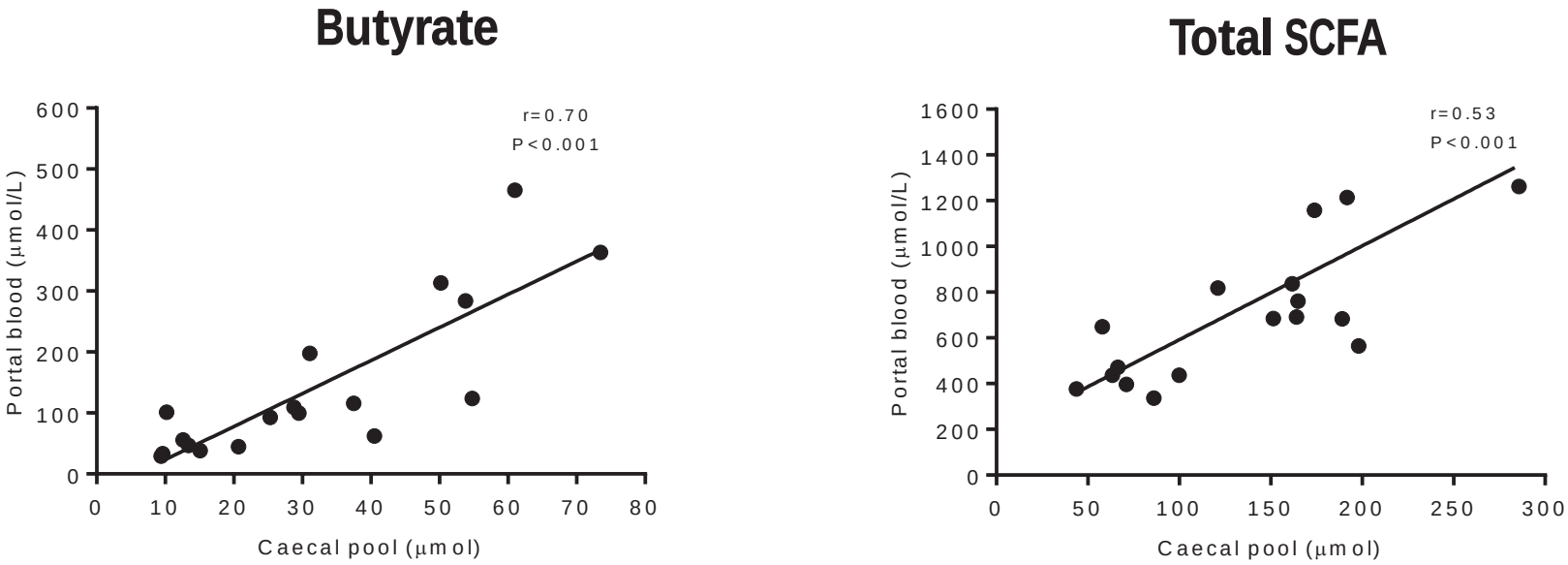






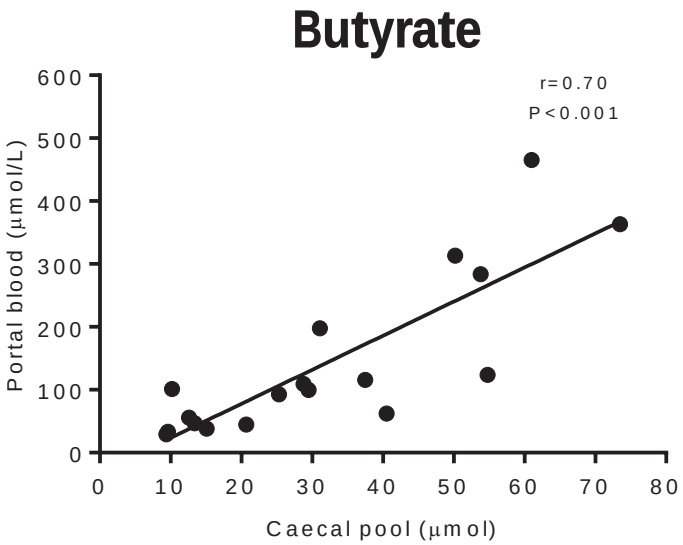
Correlations between A) Caecal pool and **portal** blood SCFA conc.

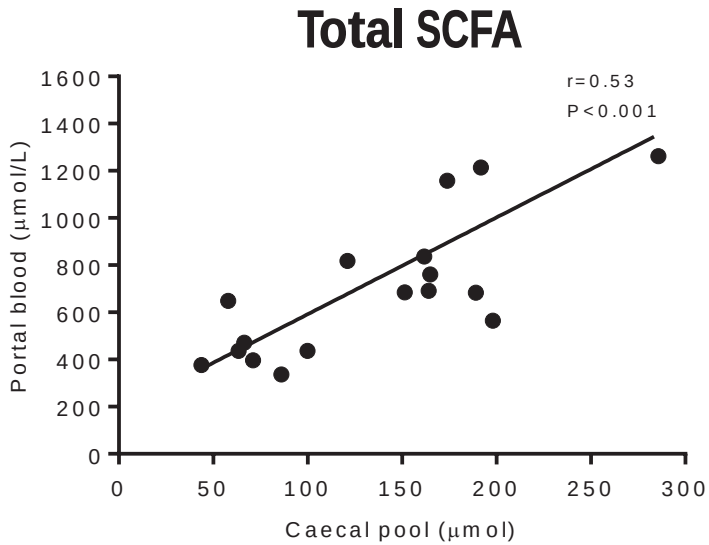
A



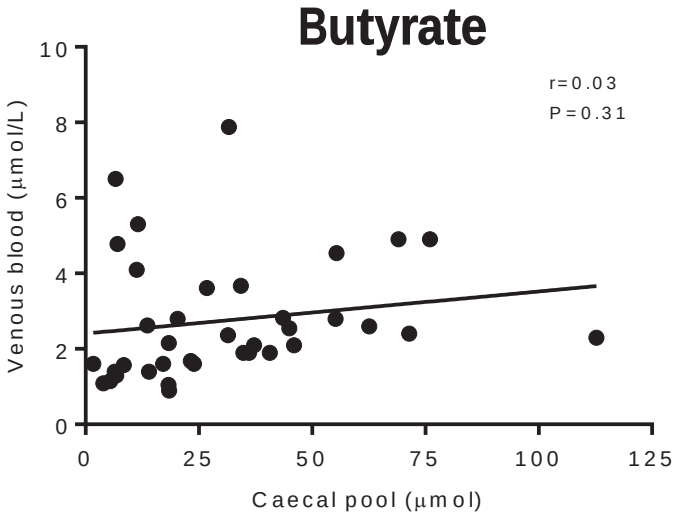
Correlations between A) caecal pool and **portal** blood SCFA conc. and B) caecal pool and **venous** blood SCFA conc.

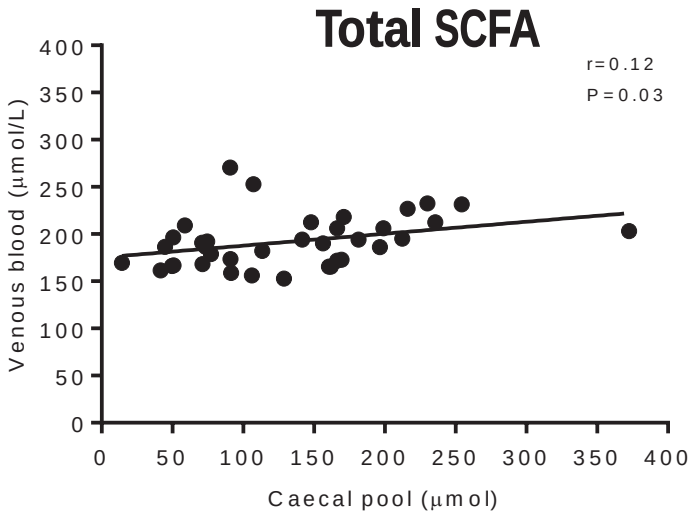
A





B





Conclusions:

- AX efficiently increased large intestinal butyrate production and portal blood butyrate concentration

- Increased caecal butyrate production was not reflected as an increased peripheral blood butyrate concentration

- No effect of *B. fibrisolvens* - no synbiotic effect on butyrate production in the cecum and portal blood butyrate concentration

British Journal of Nutrition (2014), **112**, 1837–1849
doi:10.1017/S000711451400302X
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Diets high in resistant starch and arabinoxylan modulate digestion processes and SCFA pool size in the large intestine and faecal microbial composition in pigs

Tina S. Nielsen[1]*, Helle N. Lærke[1], Peter K. Theil[1], Jens F. Sørensen[2], Markku Saarinen[3], Sofia Forssten[3] and Knud E. Bach Knudsen[1]

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
DOI: 10.1021/acs.jafc.5b03372
J. Agric. Food Chem. 2015, 63, 10418−10430
Article
pubs.acs.org/JAFC

Effects of Resistant Starch and Arabinoxylan on Parameters Related to Large Intestinal and Metabolic Health in Pigs Fed Fat-Rich Diets

Tina Skau Nielsen,* Peter Kappel Theil, Stig Purup, Natalja P. Nørskov, and Knud Erik Bach Knudsen

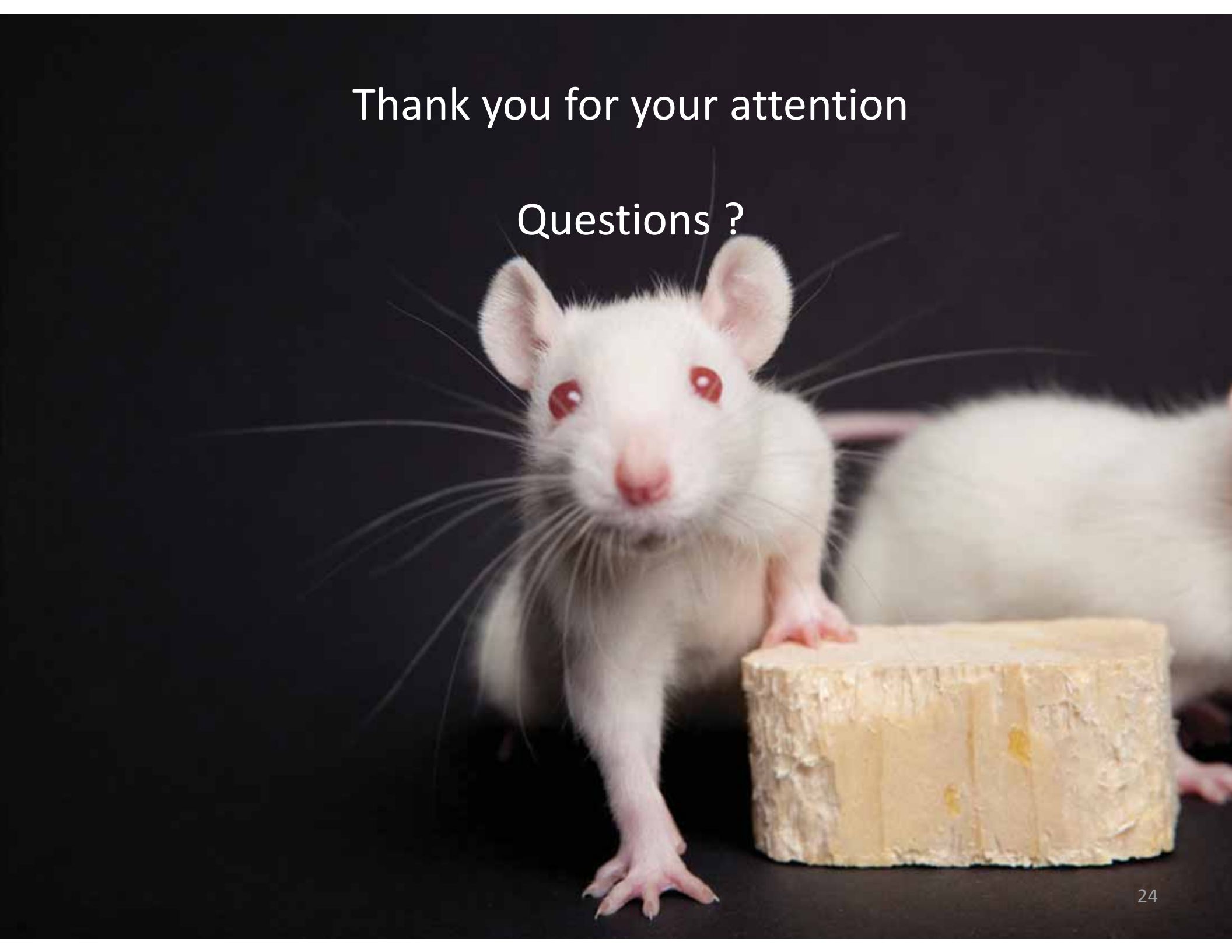
Searching for synbiotics: Effects of enzymatically modified arabinoxylan and *Butyrivibrio fibrisolvens* on short-chain fatty acids in cecum content and plasma of rats

Tina S. Nielsen[a*], Bent B. Jensen[a], Stig Purup[a], Stephanie Jackson[b], Markku Saarinen[c], Anna Lyra[c], Jens F. Sørensen[d], Peter K. Theil[a] & Knud E. Bach Knudsen[a]

Submitted to “Food & Function”

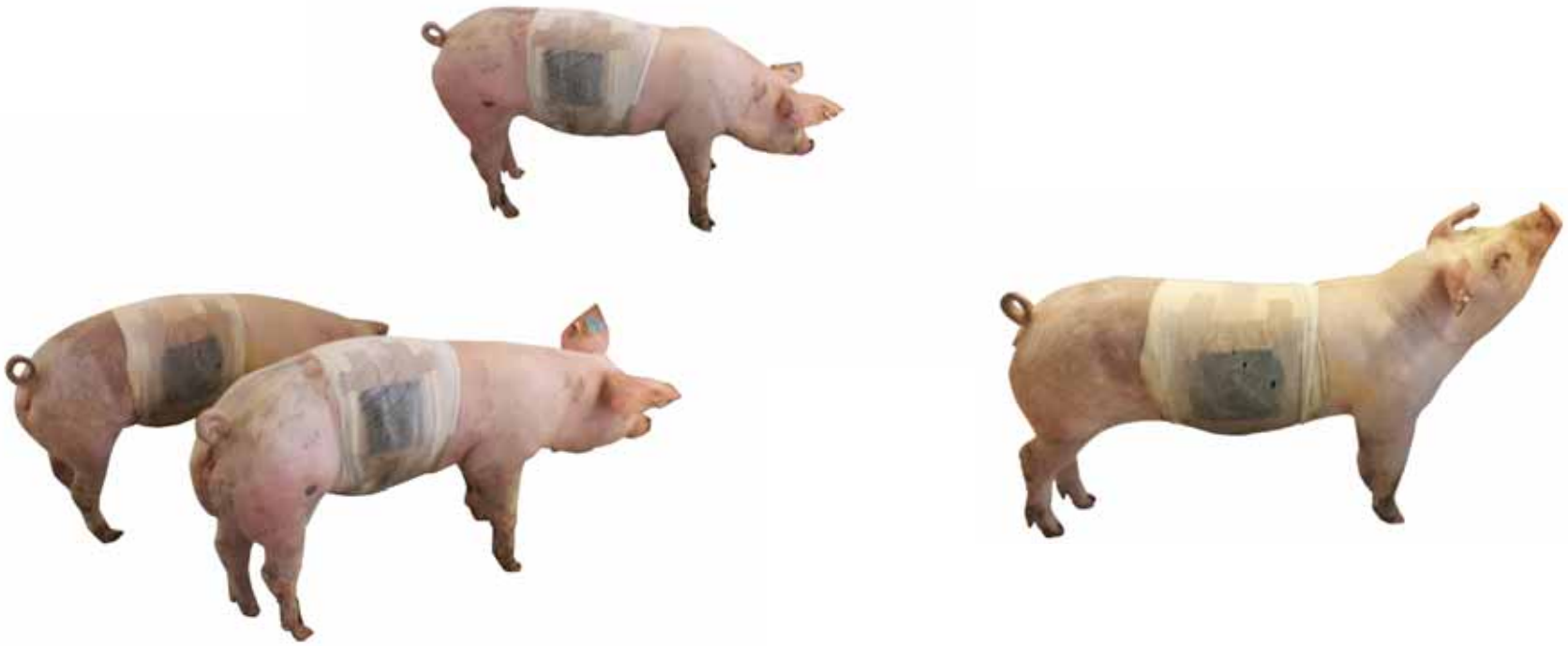
Thank you for your attention

Questions ?



Dietary effects on butyrate absorption, insulin secretion and peripheral release

Peter K. Theil
Department of Animal Science
Aarhus University



Life style related disorders

What is the real problem?

- **Calorie intake (appetite regulation)?**
- **Calorie density (Fat ↑, fiber ↓)?**
- **Fiber intake / butyrate production daily?**
- **Insulin (Available carbohydrates, glycaemic index)?**
- **Too low physical activity?**

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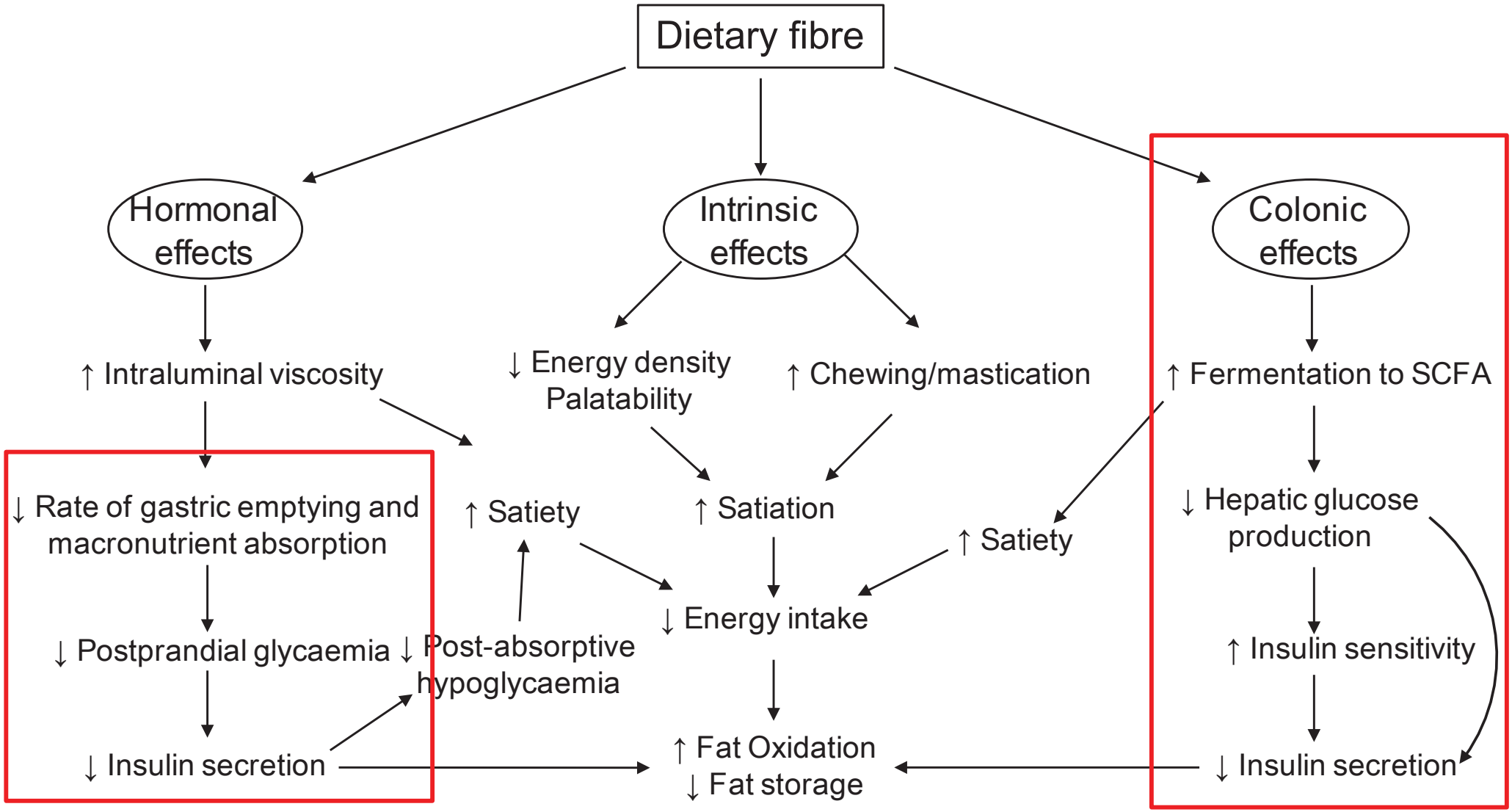
Dietary fibre effects



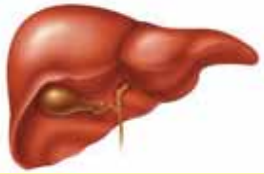
Modified from J. L. Slavin., J Nutr. 2005, 21(3): 411-18.

SCFA: short-chain fatty acids

Postprandial glucose homeostasis

↑ Calorie intake → ↑ Plasma glucose →  → ↑ Plasma insulin

Glucose synthesis



↑ Glycogen synthesis
↓ Gluconeogenesis

↑ Glucose uptake
↑ Triglyceride storage
↓ Lipolysis

Glucose uptake

↑ Glucose uptake

↓ Plasma glucose
↓ Plasma NEFA

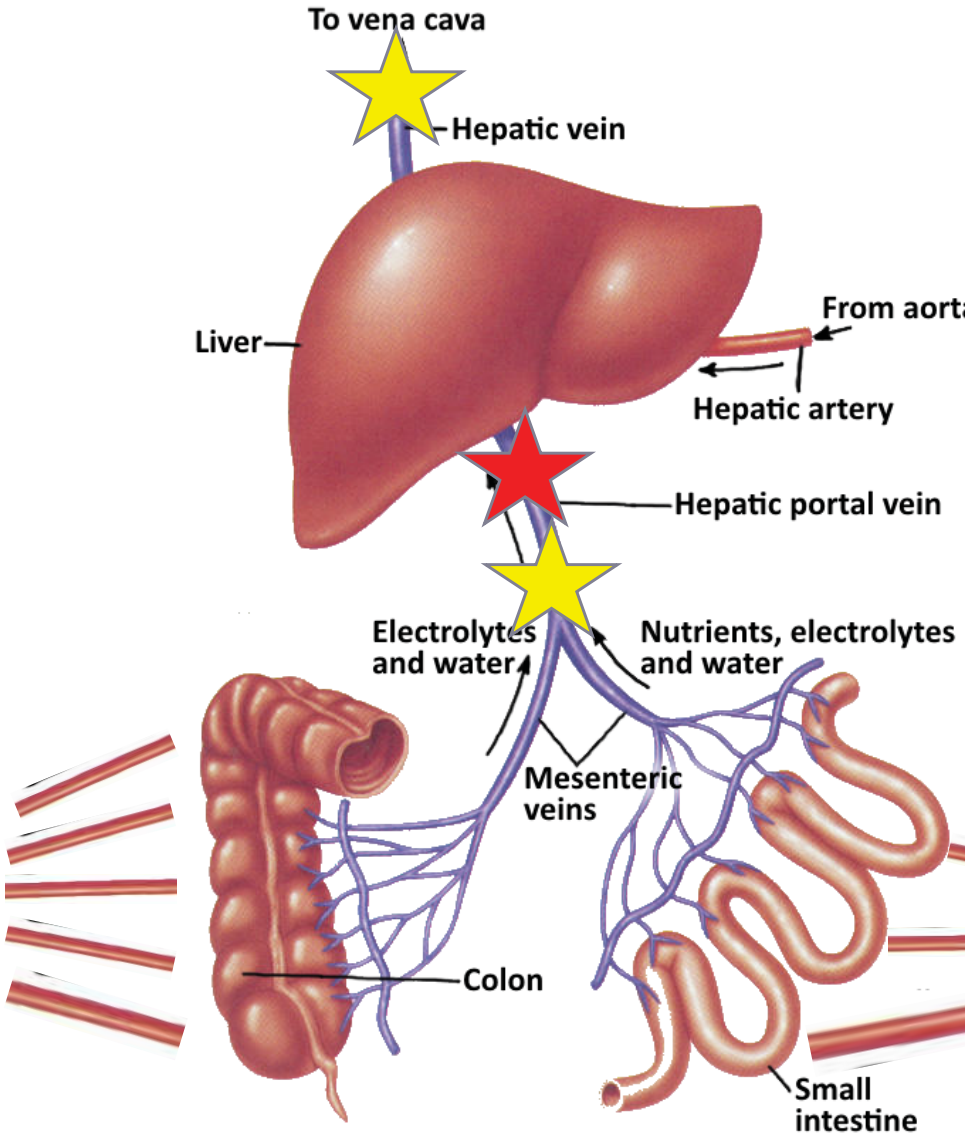
NEFA; non-esterified fatty acid

Hypotheses:

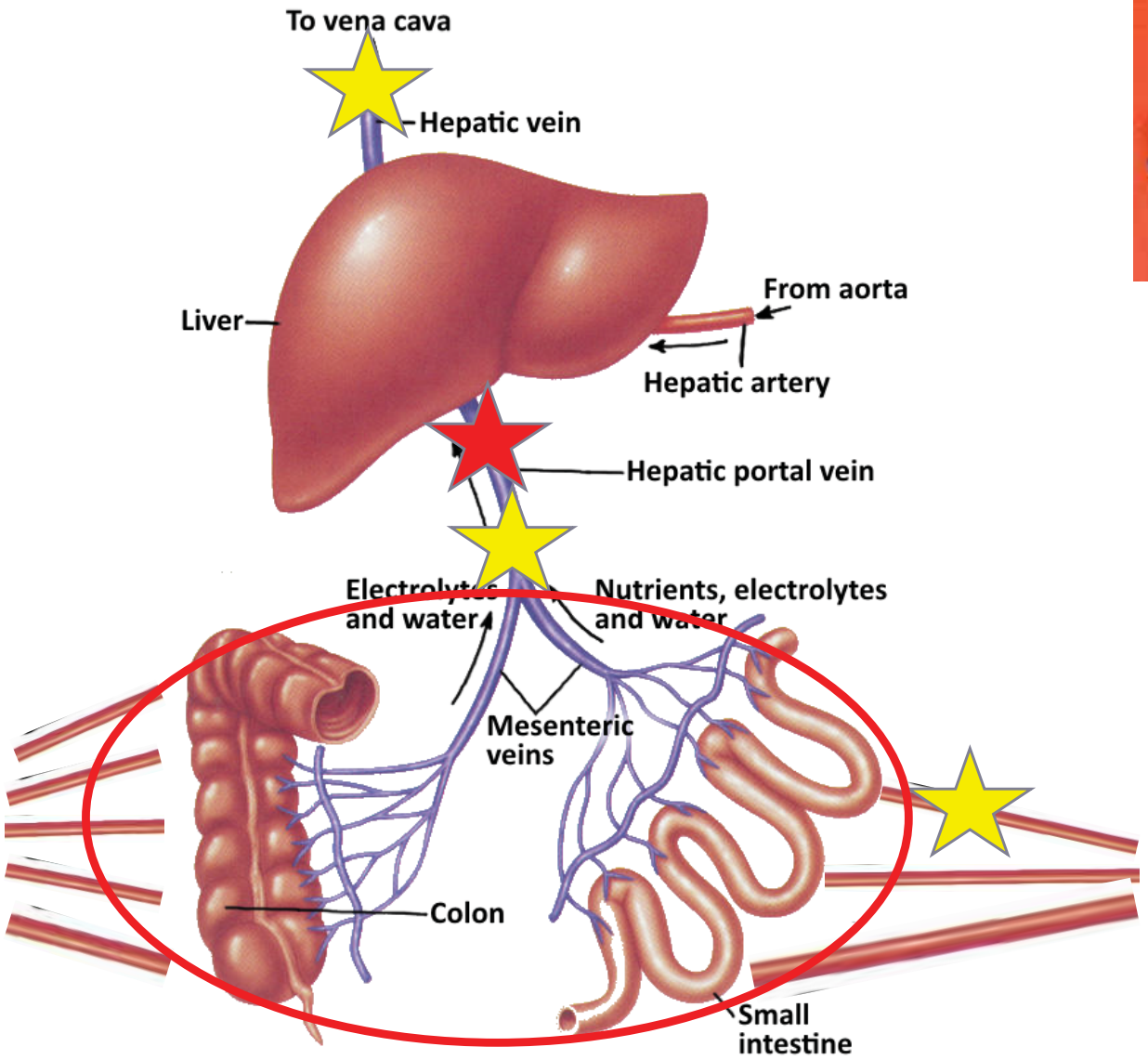
High intake of fiber increases butyrate production and alleviates negative consequences of western style diets (high fat, protein and refined sugar)

Short chain fatty acids (especially butyrate) may be implicated in lowering postprandial insulin secretion

Animal experiment with multi-catheterised pigs - a mechanistic study



Absorption of nutrients from gastrointestinal tract
Insulin secretion from pancreas

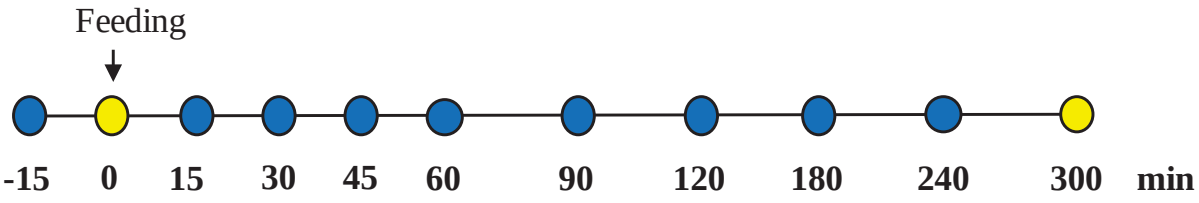


Experimental design – catheterised pigs

› 3 different diets
  › WSD
  › RSD
  › AXD

› 6 pigs
› One diet per week – repeated 3x3 Latin square design
› 3 meals per day

› 200 g available carbohydrates per meal
› Sampling 5 h postprandial on day 7



Experimental diets

Western-style WSD (Control)

RSD

AXD

Available carbohydrates (g/meal)

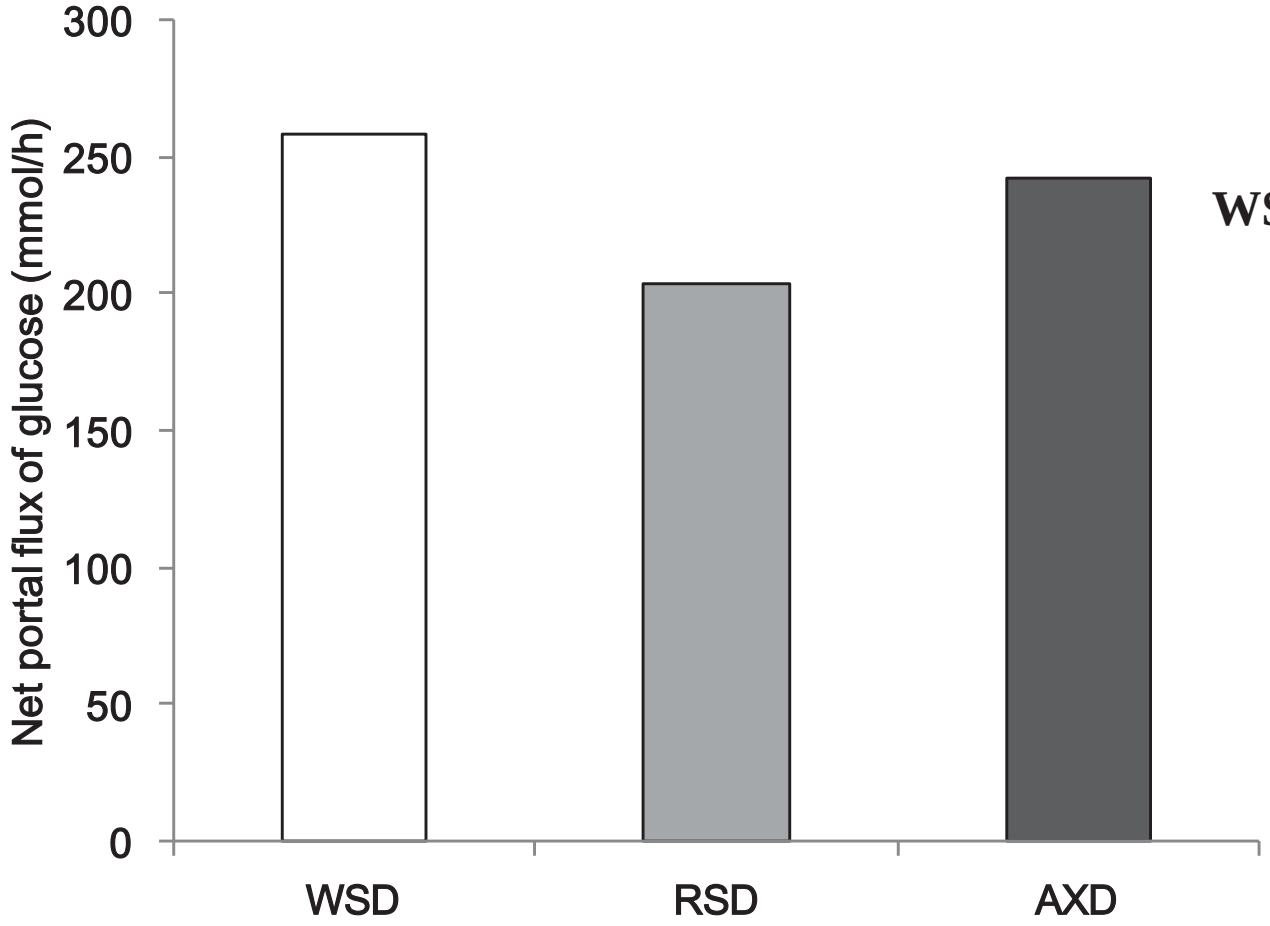
199	197	199

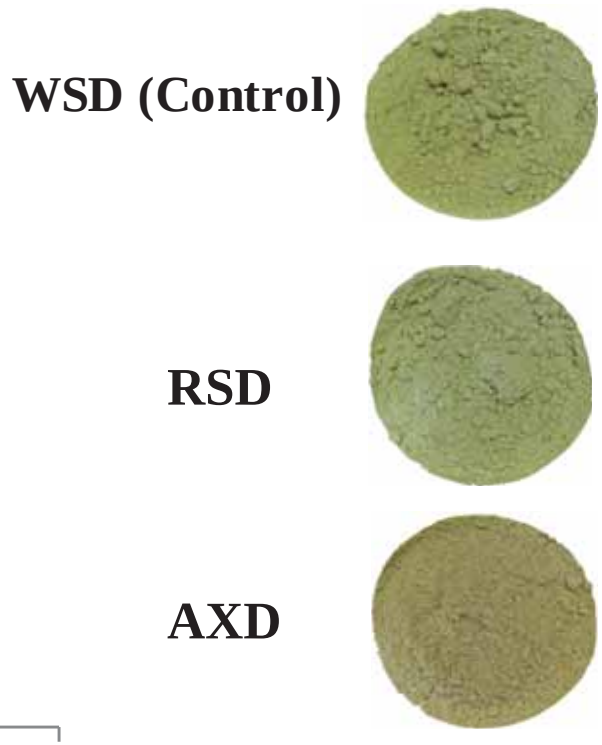
Dietary fiber content (g/kg dry matter)

70	192	189 (~2.7xWSD)

Absorption of glucose

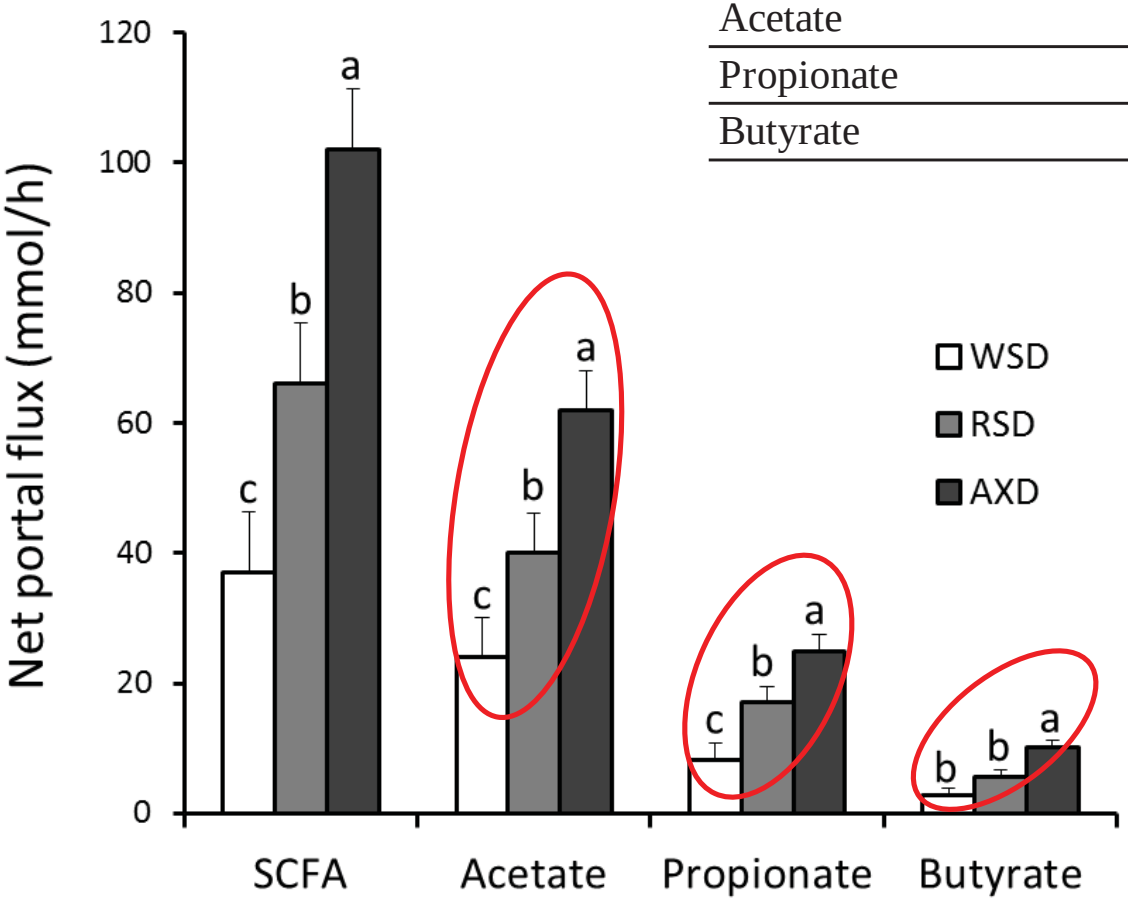
Effect of diet (Not significant)





Absorption of short chain fatty acids
Effect of diet (P ≤ 0.001)

Net portal flux (mmol/h)	WSD	AXD	Fold change
Acetate	24	62	2.5
Propionate	8.2	25	3.0
Butyrate	2.8	10.2	3.6



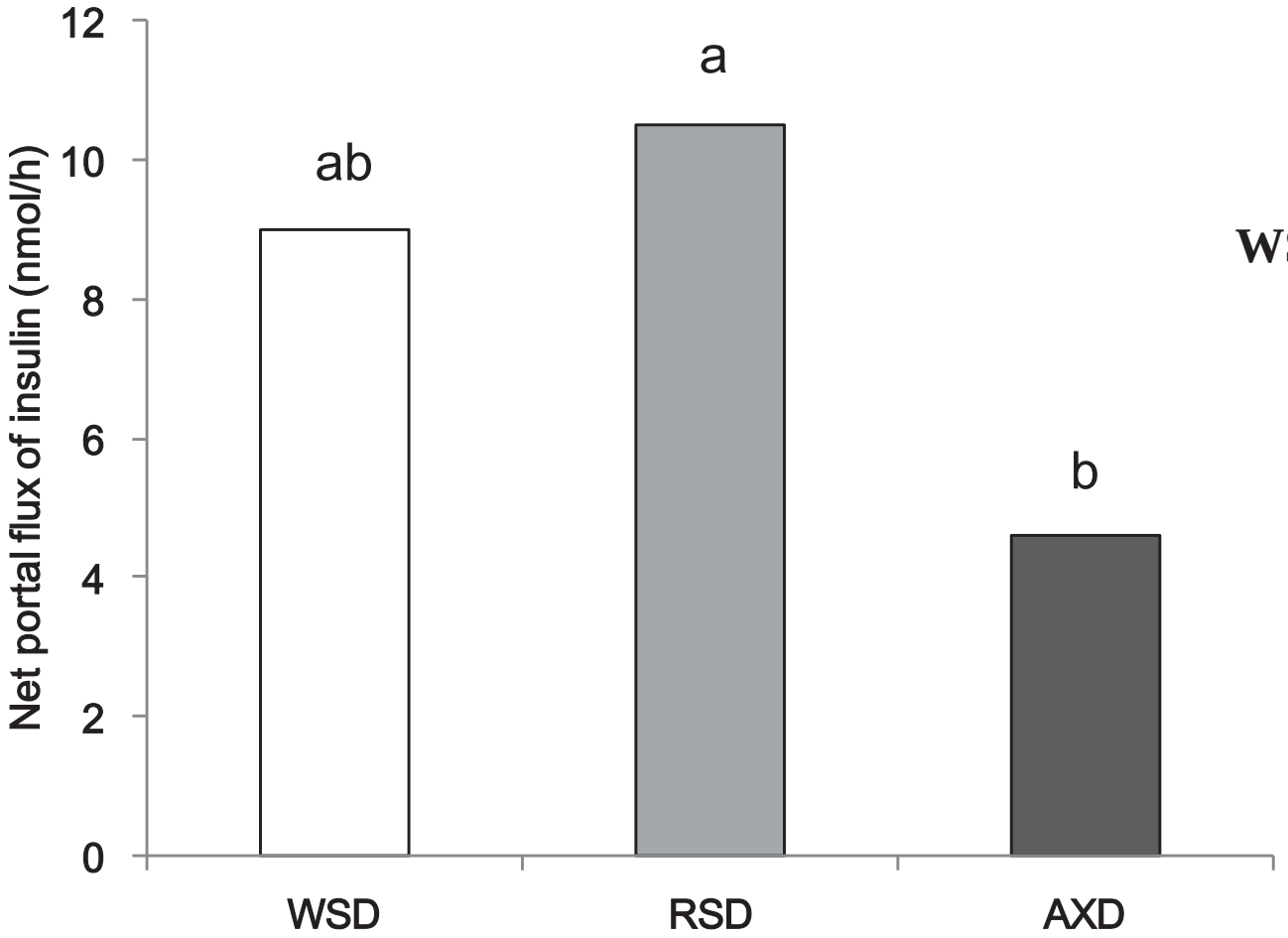
WSD (Control)

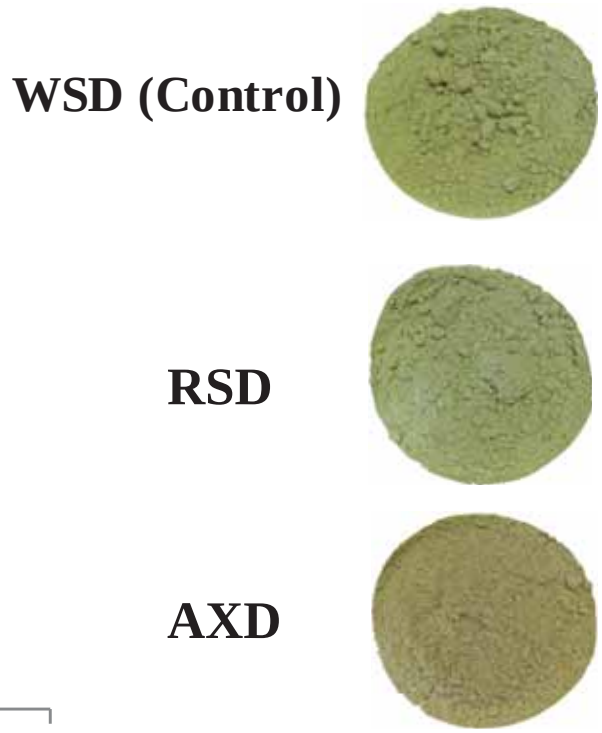
RSD

AXD

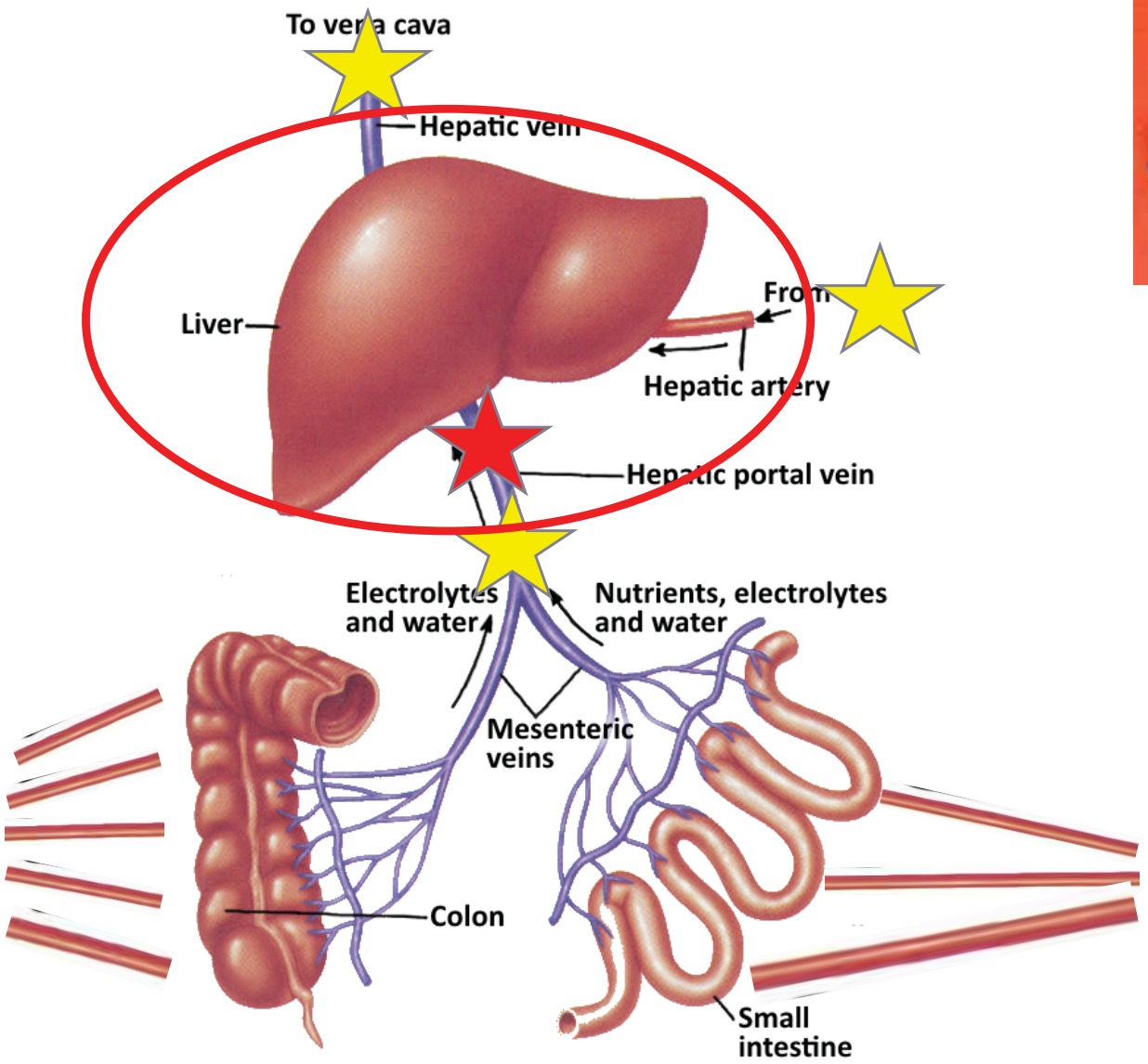
Secretion of insulin

Effect of diet (P = 0.09)

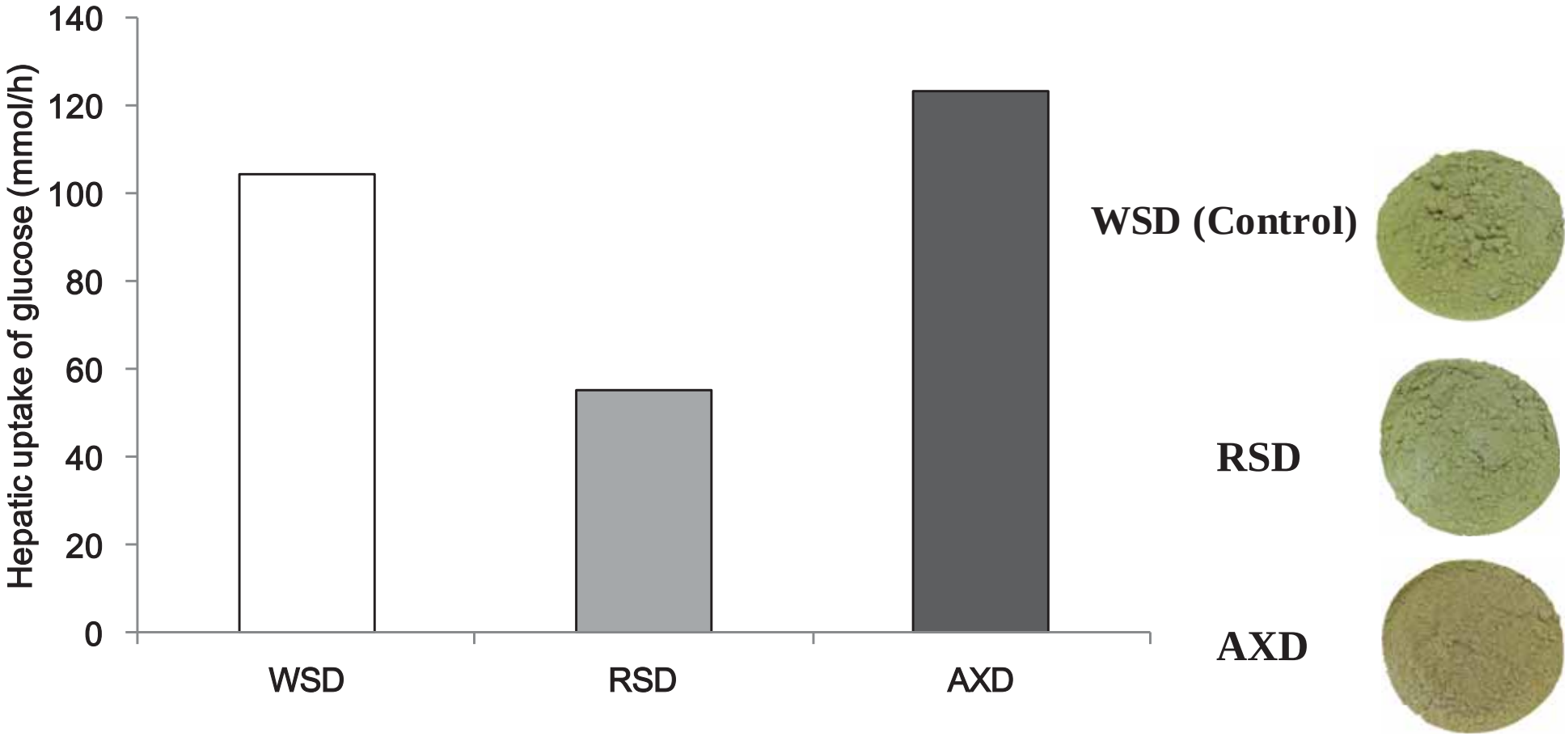




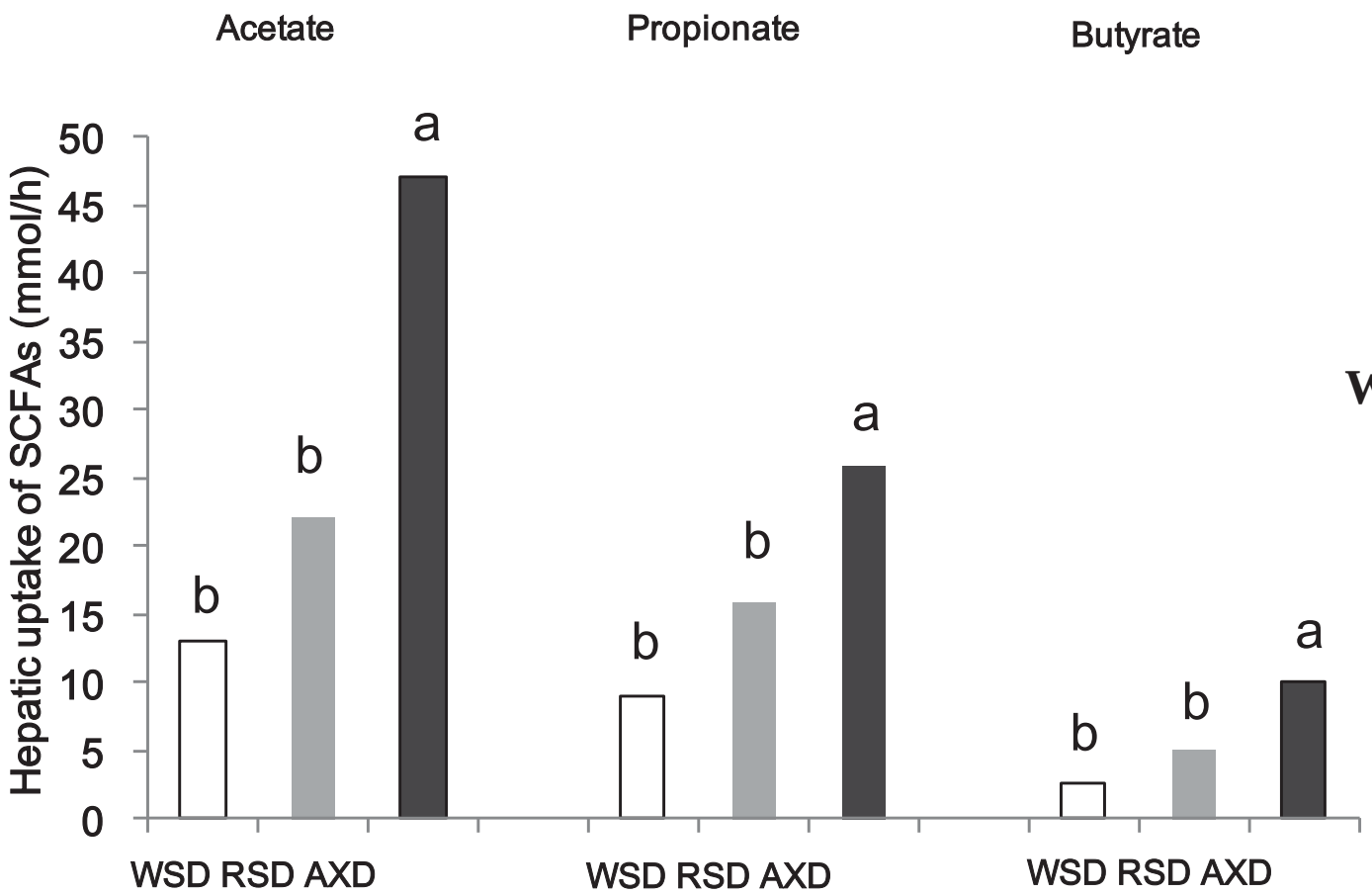
Hepatic uptake of nutrients
Hepatic clearance of insulin



Hepatic uptake of glucose (i.e. removal from blood) Effect of diet (Not significant)



Hepatic uptake of acetate, propionate and butyrate (i.e. removal from blood) Effect of diet (P < 0.05)

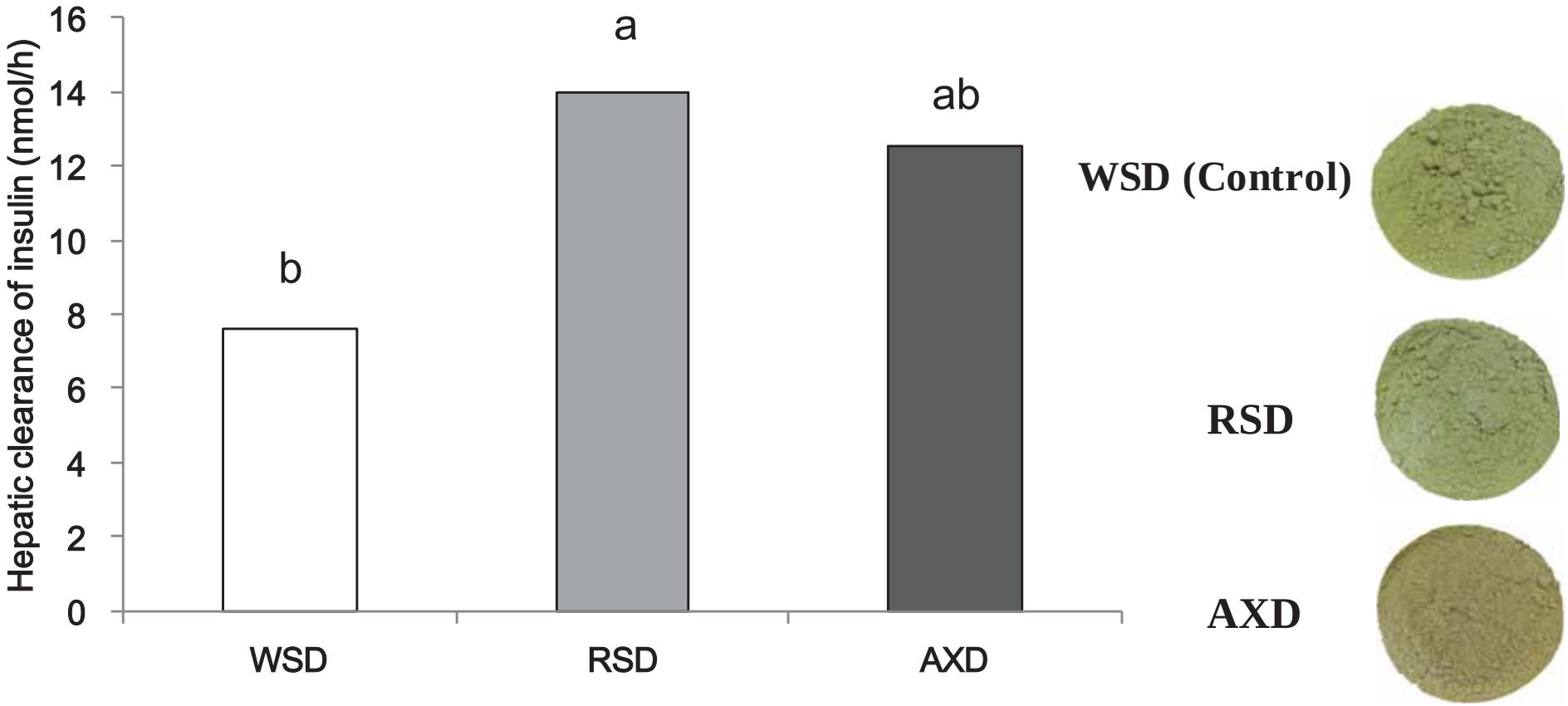


WSD (Control)

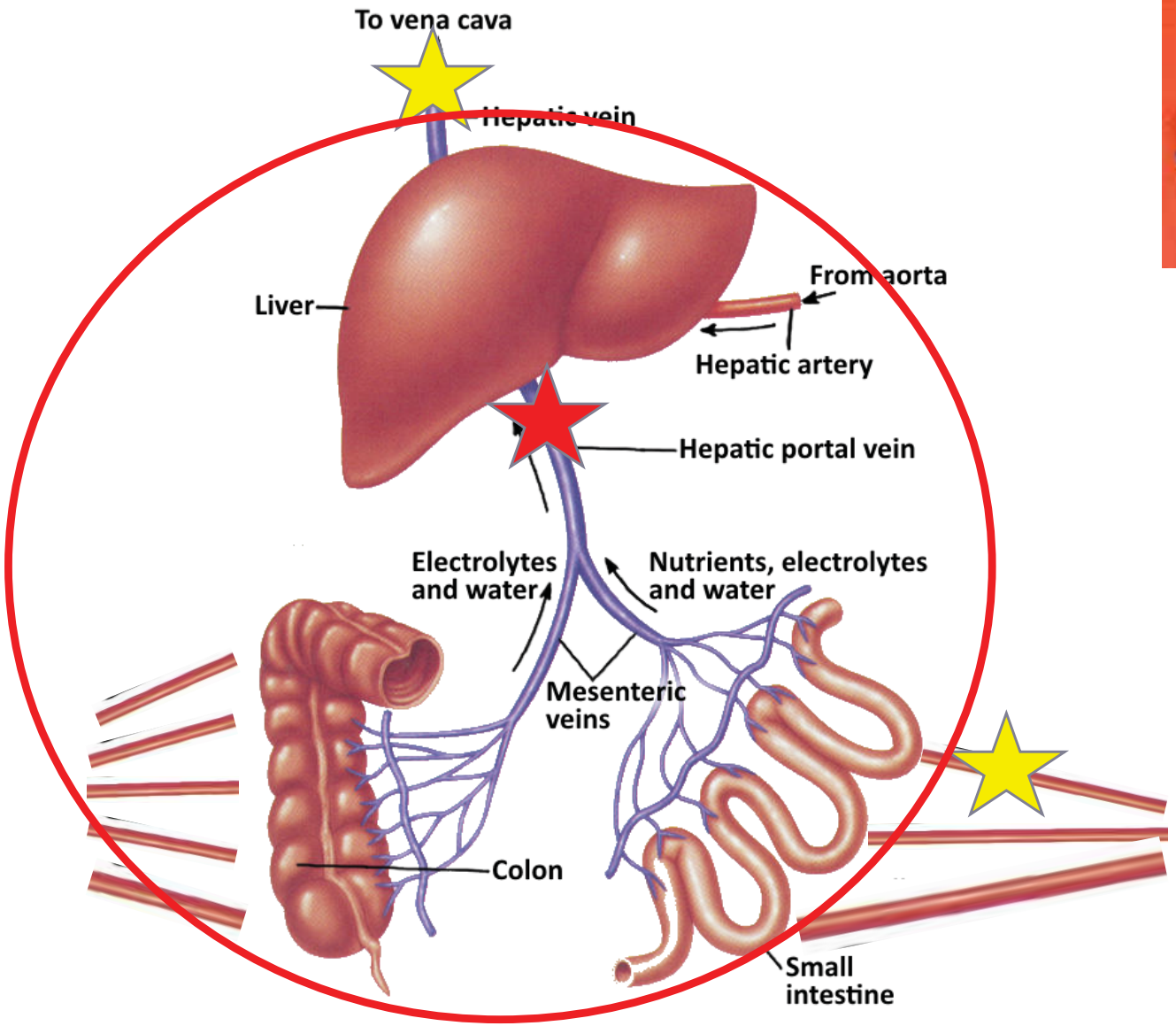
RSD

AXD

Hepatic clearance of insulin (removal from blood) Effect of diet (P = 0.05)

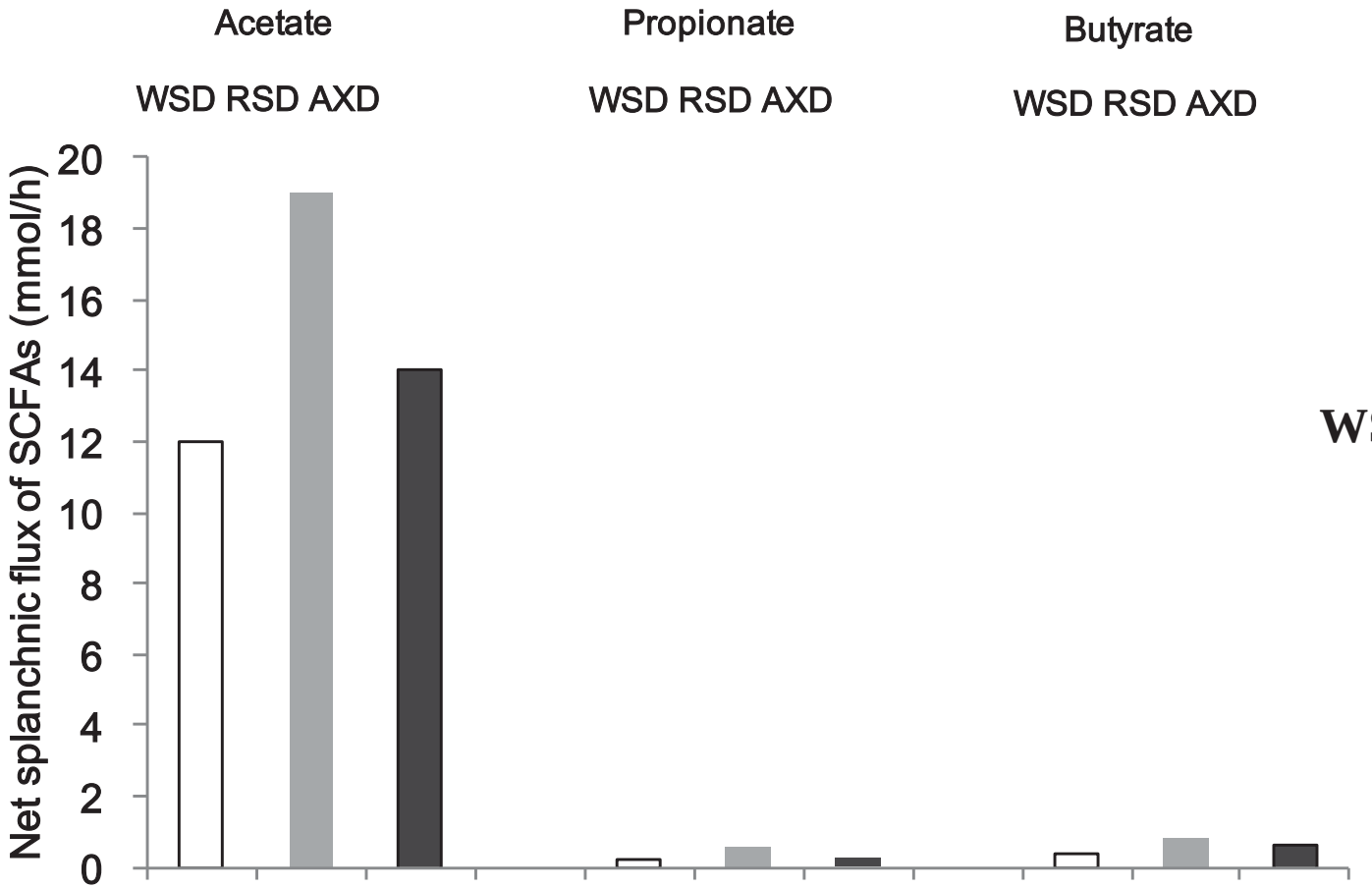


Peripheral release of acetate, propionate and butyrate (exposure to peripheral tissues)



Peripheral release of acetate, propionate and butyrate from gastrointestinal tract and liver

Effect of diet (Not significant)



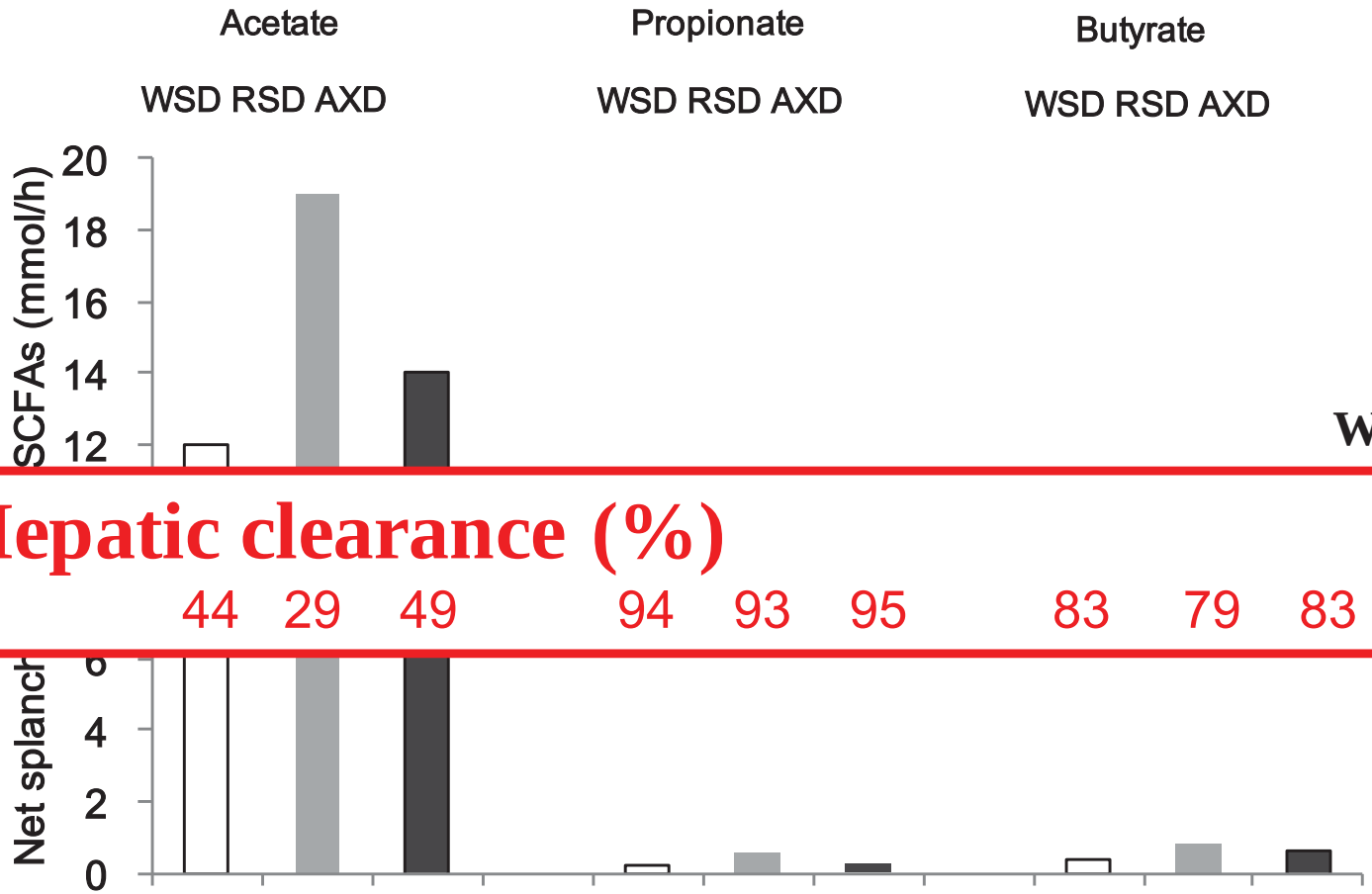
WSD (Control)

RSD

AXD

Peripheral release of acetate, propionate and butyrate from gastrointestinal tract and liver

Effect of diet (Not significant)



Hepatic clearance (%)

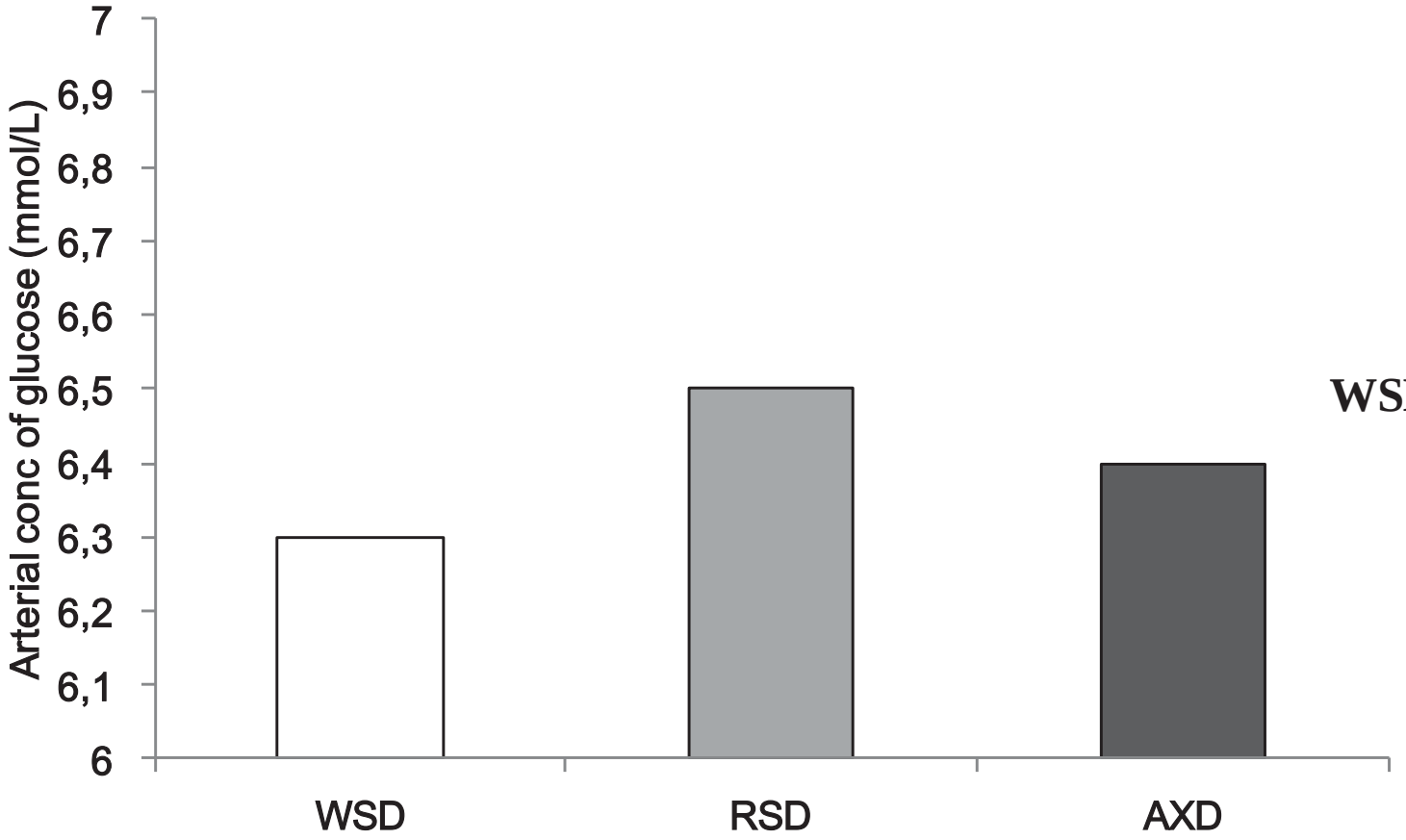
Acetate			Propionate			Butyrate		
WSD	RSD	AXD	WSD	RSD	AXD	WSD	RSD	AXD
44	29	49	94	93	95	83	79	83

WSD (Control)

RSD

AXD

Arterial concentration of glucose
Effect of diet (Not significant)

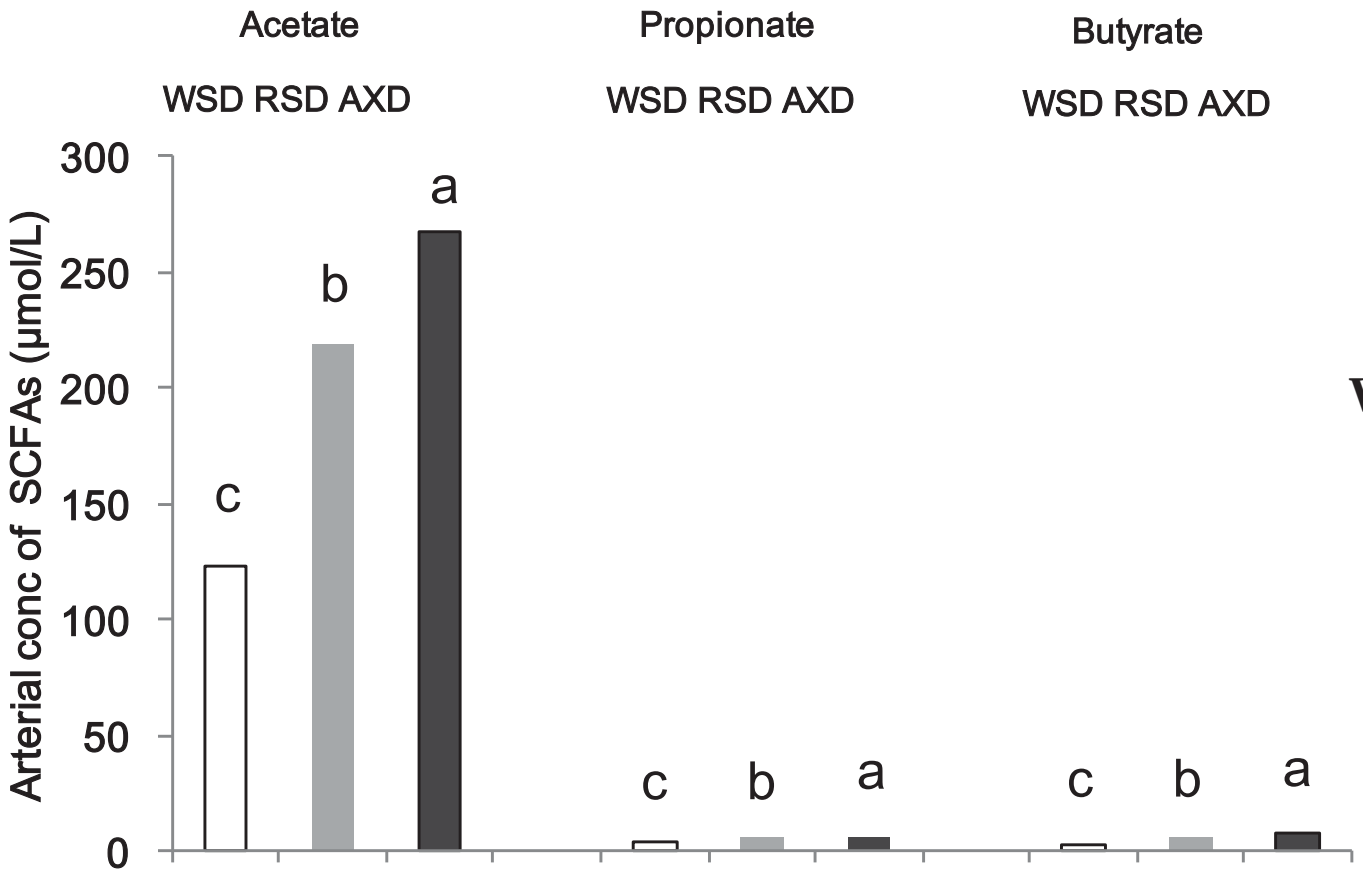


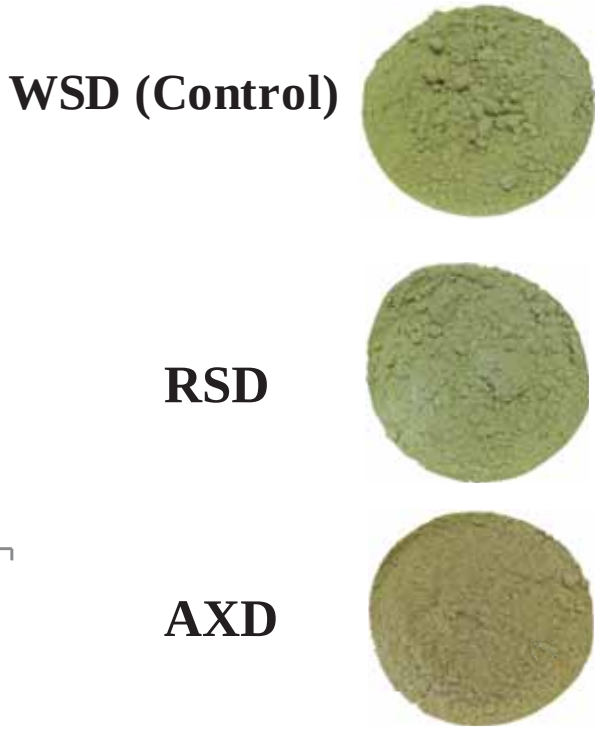
WSD (Control)

RSD

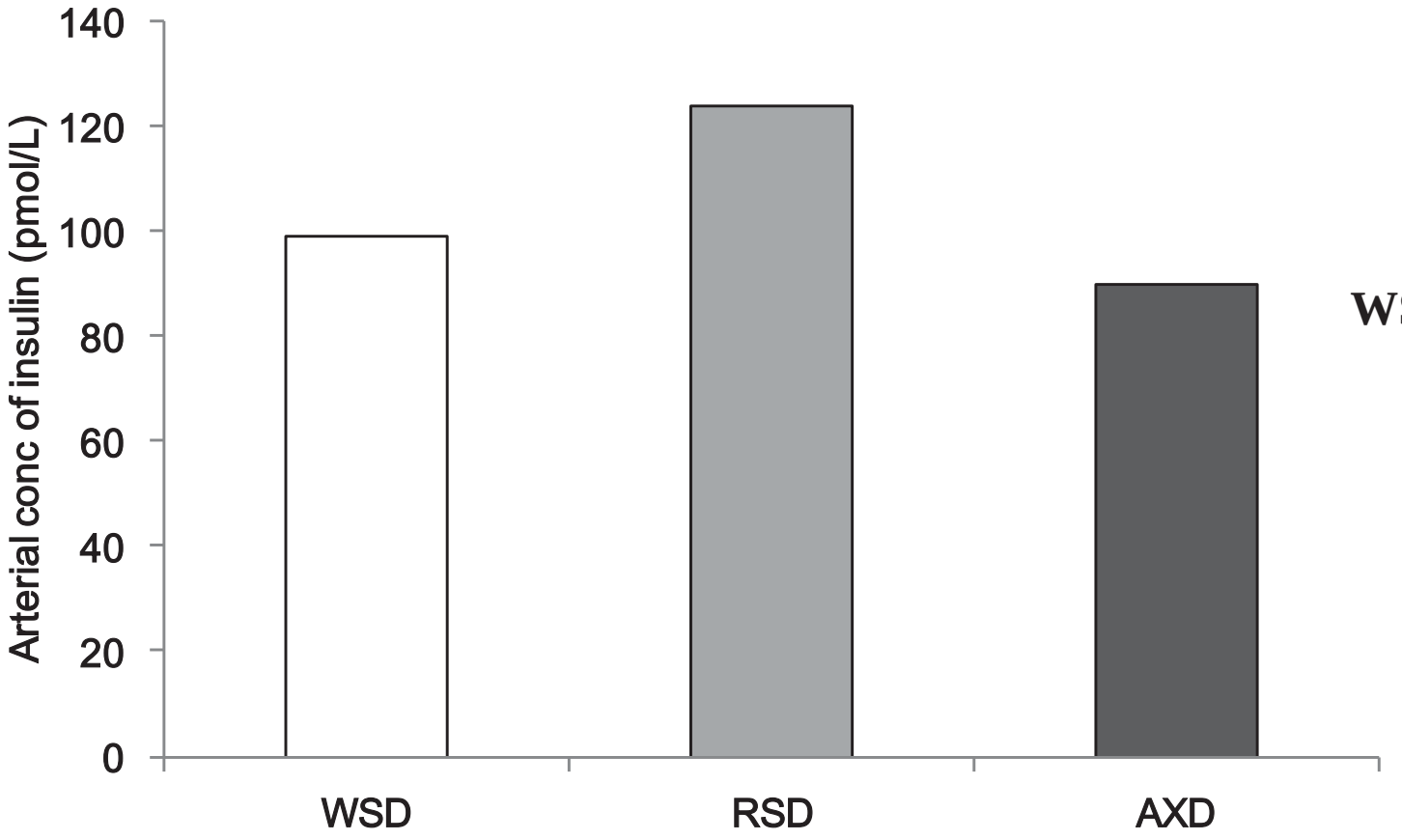
AXD

Arterial concentration of SCFAs
Effect of diet (P < 0.01)





Arterial concentration of insulin
Effect of diet (Not significant)

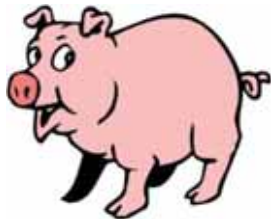


WSD (Control)

RSD

AXD

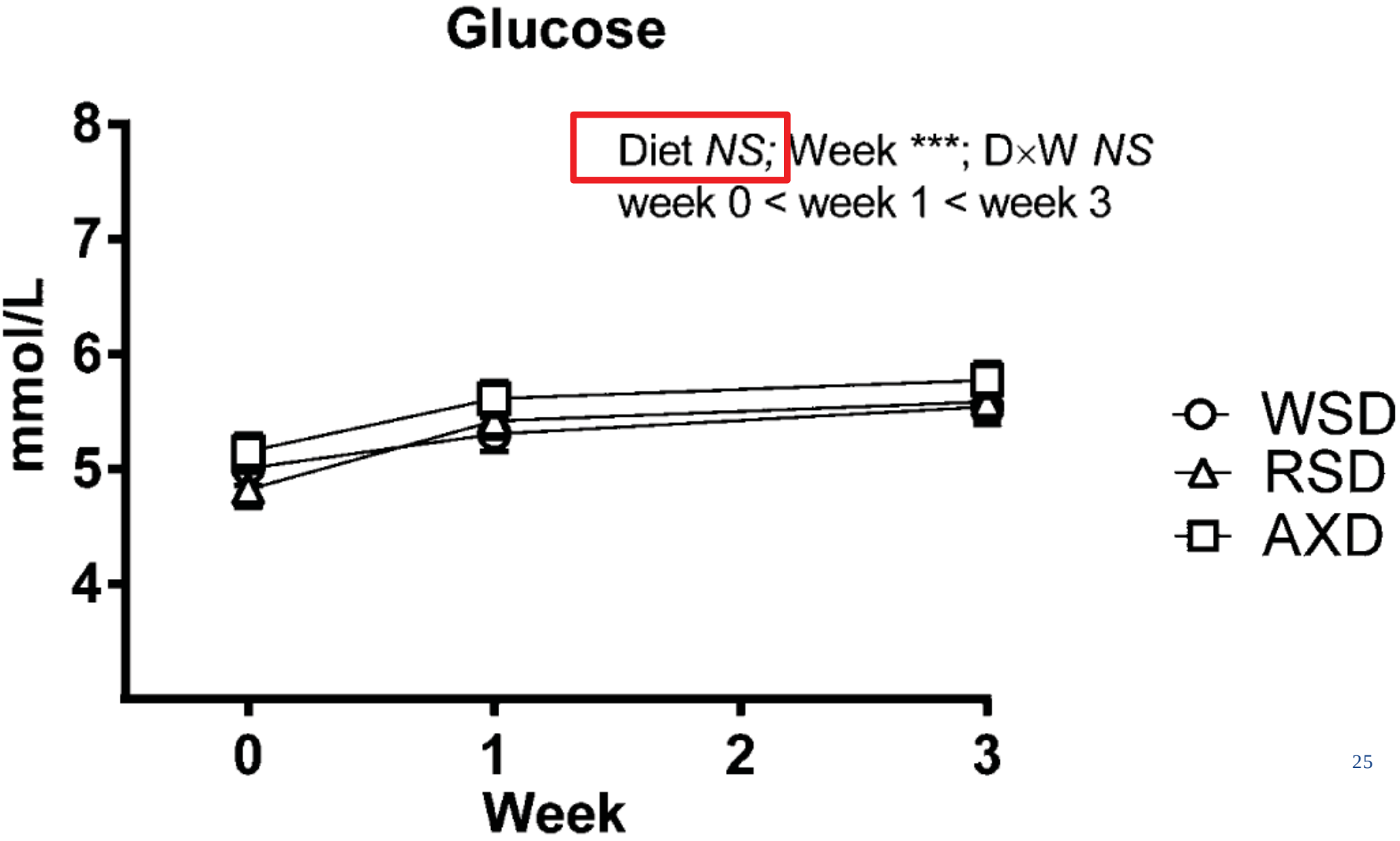
Experimental design - intact pigs



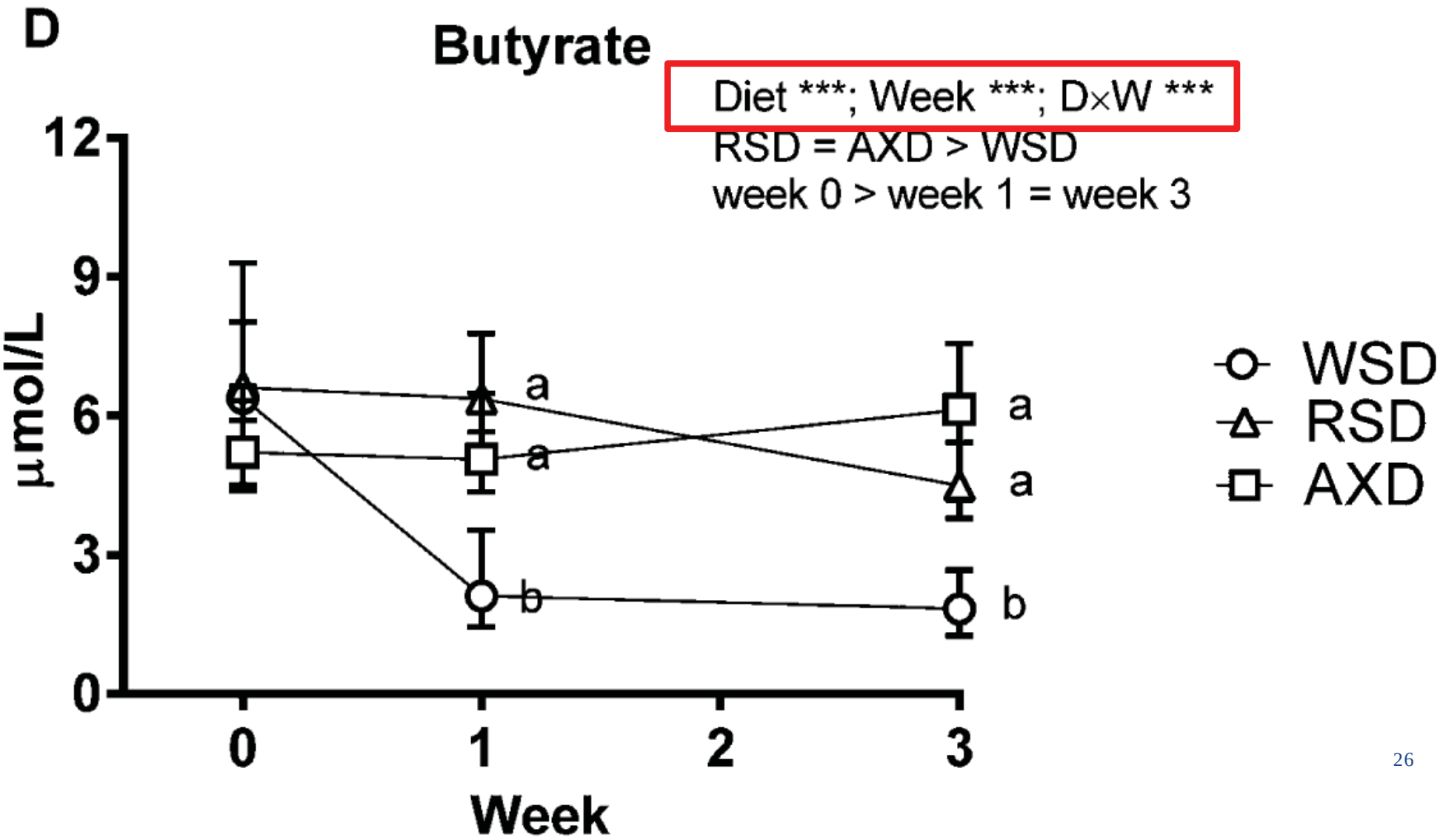
- 3 diets
  - WSD
  - RSD
  - AXD
- 10 pigs per diet
- 3 weeks experimental period

Week 0 (fasting)	Week 1 (fasting)	Week 3 (fasting)
Plasma	Plasma	Plasma

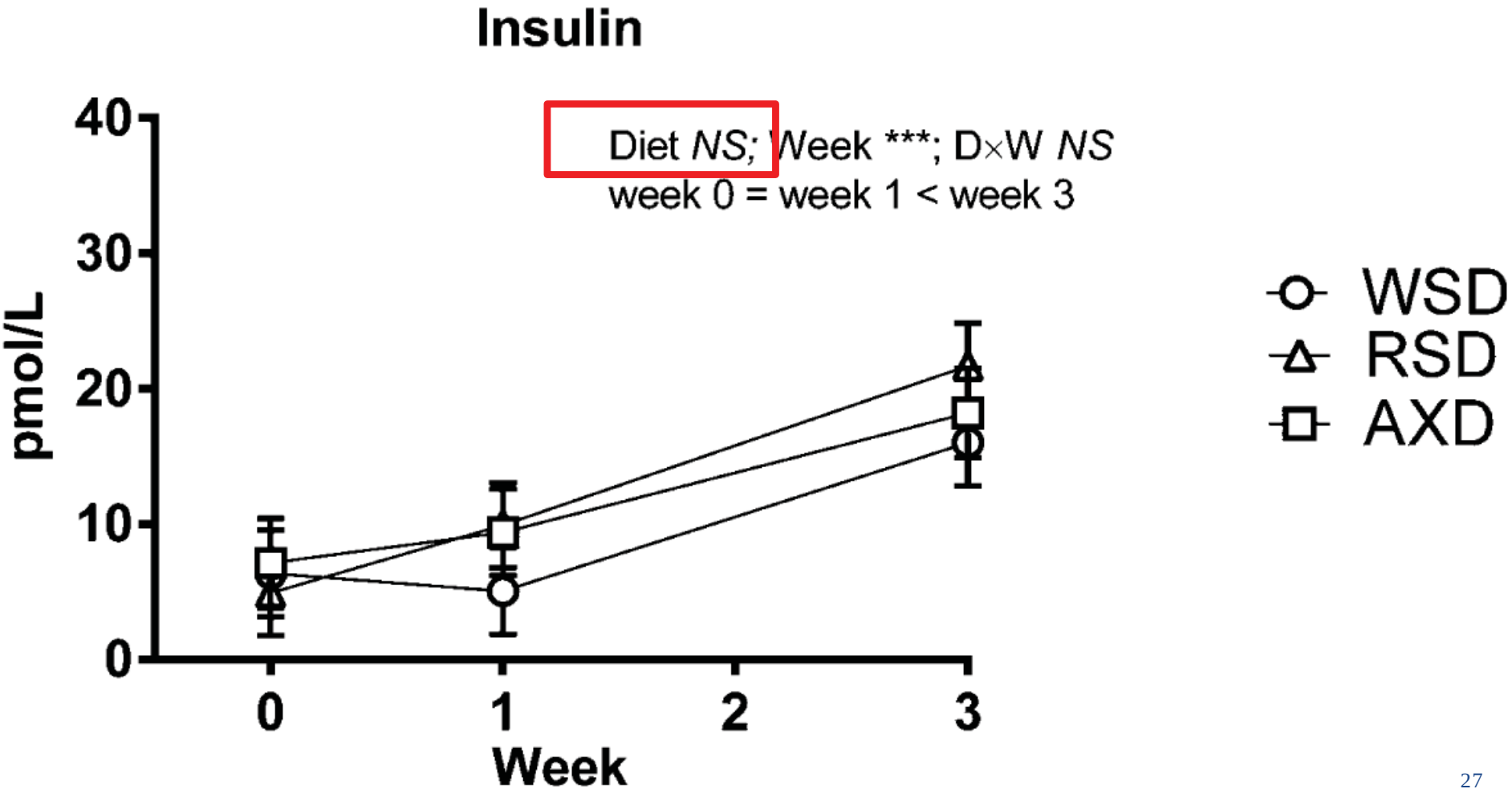
Peripheral concentration of glucose after 0, 1, or 3 weeks of intervention



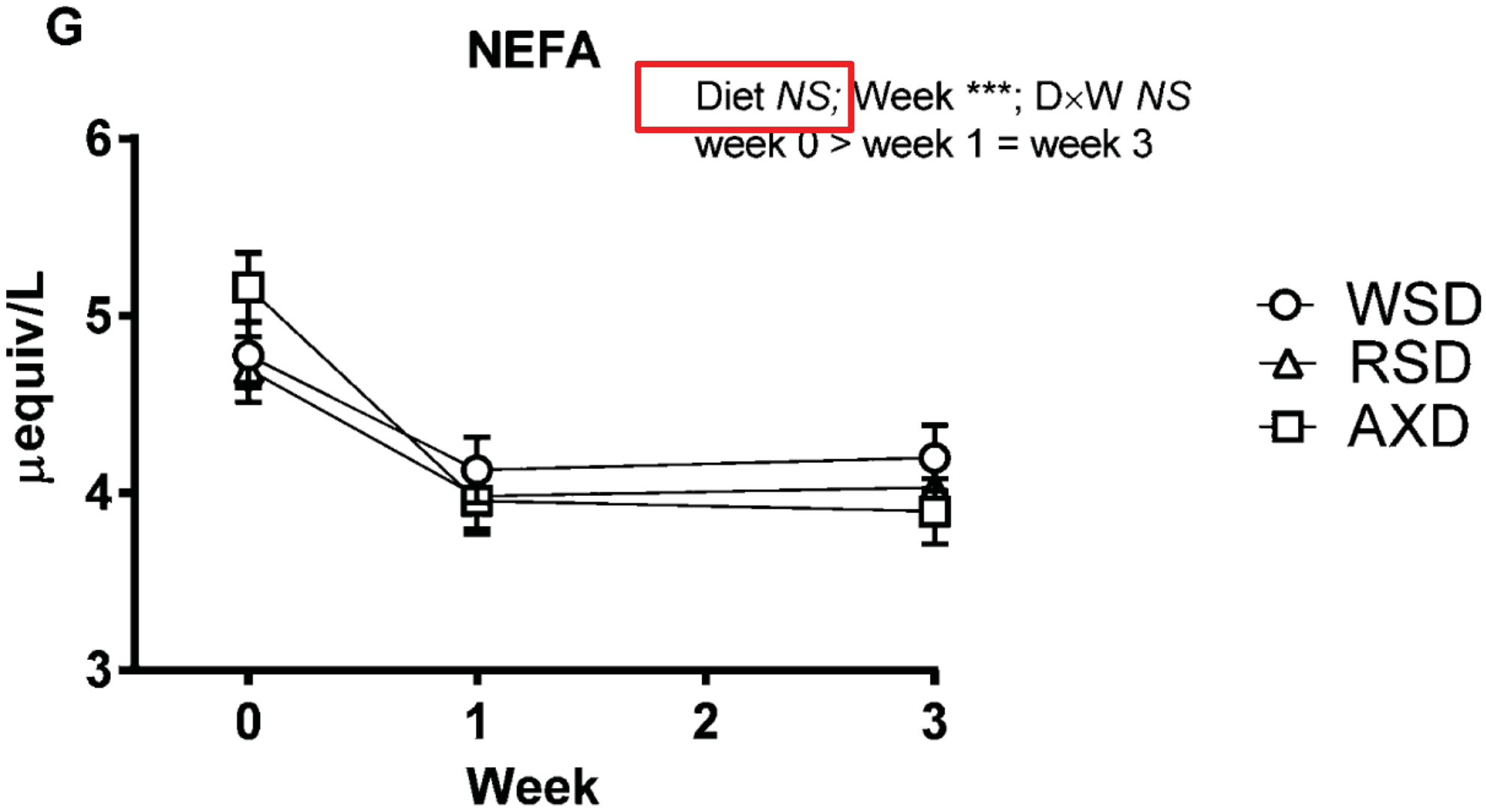
Peripheral concentration of butyrate after 0, 1, or 3 weeks of intervention



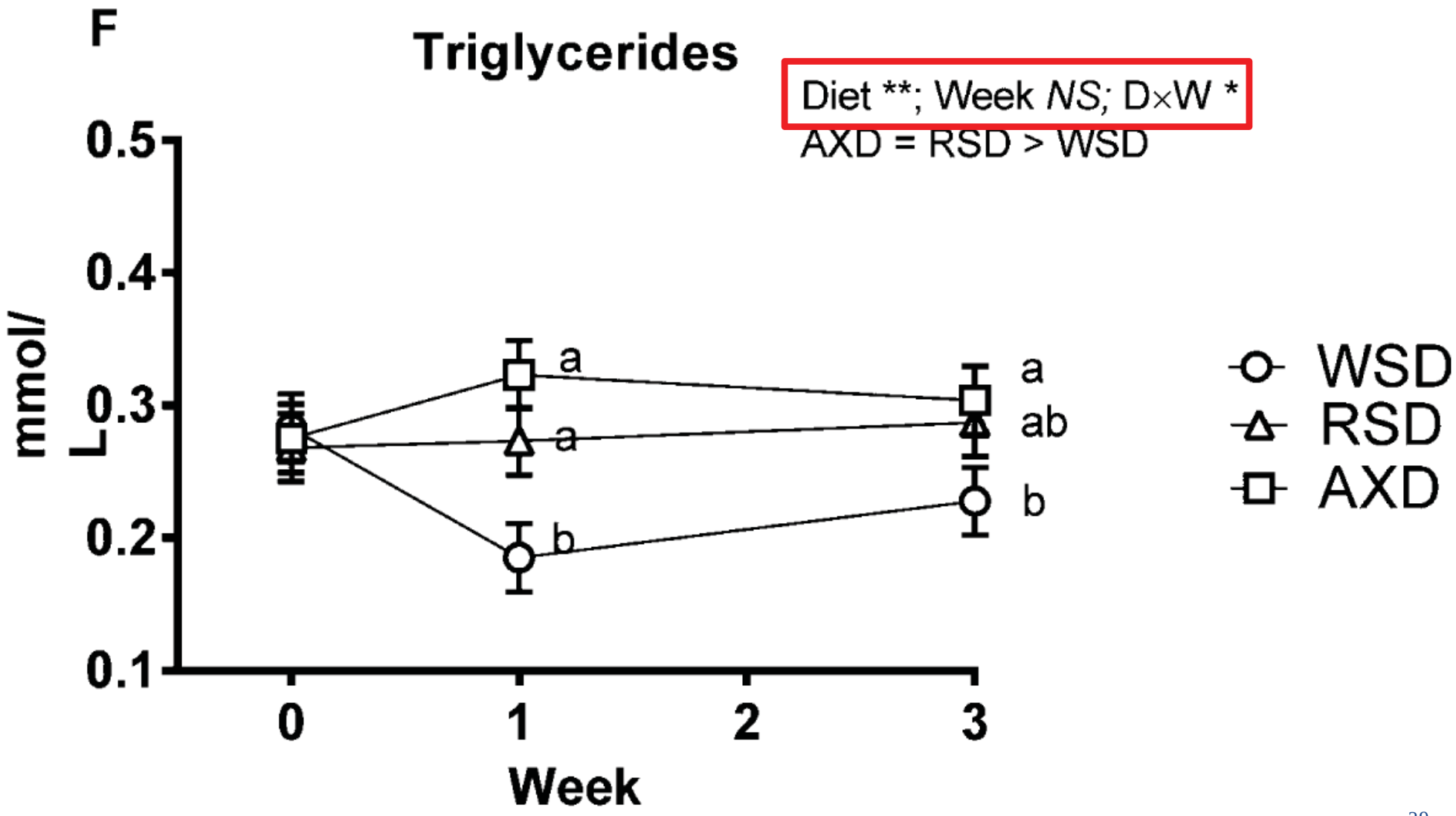
Peripheral concentration of insulin after 0, 1, or 3 weeks of intervention



Peripheral concentration of non-esterified fatty acids (NEFA) after 0, 1, or 3 weeks of intervention



Peripheral concentration of Triglycerides after 0, 1, or 3 weeks of intervention



Conclusions

- **Dietary fibres increased absorption of short chain fatty acids**
  - AXD > RSD > WSD
- **Dietary fibres stimulated butyrate production**
  - AXD > RSD > WSD
- **Dietary fibres affected Insulin economy**
  - AXD tended to reduce apparent insulin secretion
  - AXD and RSD increased hepatic clearance of insulin
  - No clear relation between insulin economy and butyrate absorption
- **Fate of short chain fatty acids**
  - Liver clearance: Propionate (95%) > Butyrate (80%) > Acetate (29-49%)
  - Peripheral release: Acetate > Propionate = Butyrate
- **3-week intervention study with slaughter pigs:**
  - Plasma SCFAs reflect pattern of SCFA absorption
  - Plasma insulin increased from week 0 to week 3, no diet effect
  - Surprisingly, the plasma triglycerides was reduced by the WSD diet!

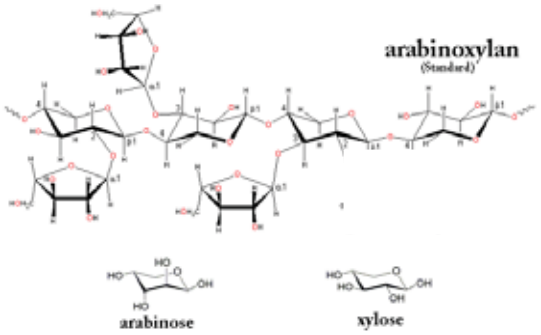
BEYOND SHORT-CHAIN FATTY ACIDS – WHAT COMPLEX ARABINOXYLAN AND RESISTANT STARCH RICH DIETS ALSO DELIVER TO THE BODY

A U AARHUS UNIVERSITY
SCIENCE AND TECHNOLOGY

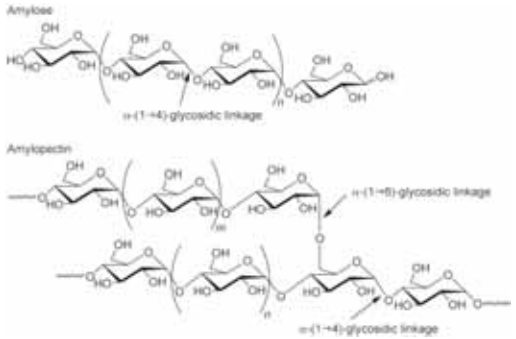
METTE SKOU HEDEMANN
SENIOR SCIENTIST

BUTCOINS OPEN SEMINAR
02 MARCH 2016

ARABINOXYLAN AND RESISTANT STARCH



Rye and wheat bran (enzymatically hydrolysed)



High amylose maize and raw potato starch

Bran

- Insoluble dietary fibre (xylans, cellulose, lignin)
- Antioxidants bound to cell walls (phenolic acids)

Outer and Inner pericarp (4-5%)

- Soluble & insoluble dietary fibre (xylans, β-glucans)
- Proteins
- Antioxidants (phenolic acids)
- Vitamin E
- B vitamins
- Minerals
- Phytic acids
- Enzymes

Testa (1%)

- Alkylresorcinols

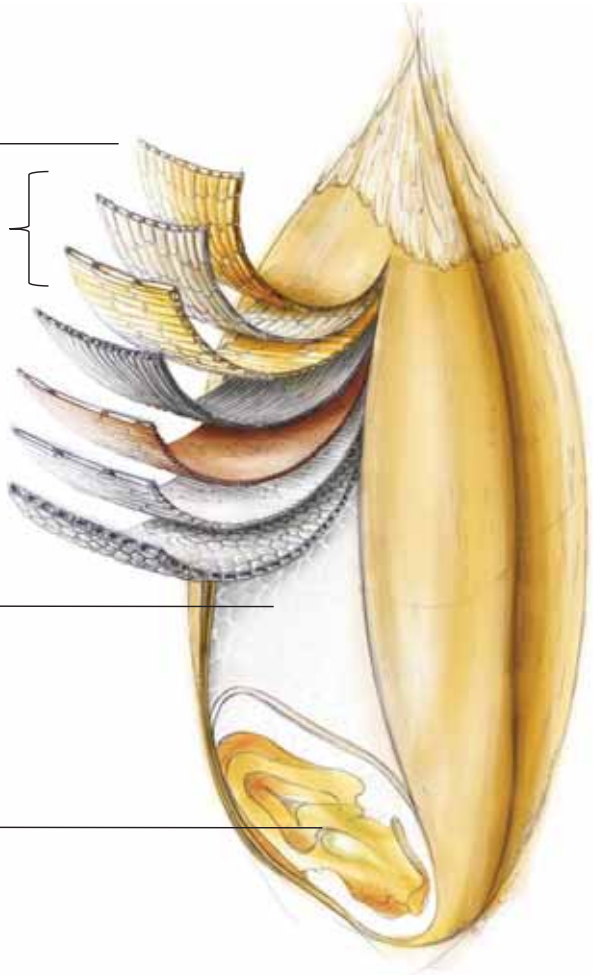
Aleurone layer (6-9%)

Starchy endosperm (80-85%)

- Starch and proteins

Germ (3%)

- Lipids
- Antioxidants
- Vitamin E
- Minerals
- Plant sterols
- Enzymes

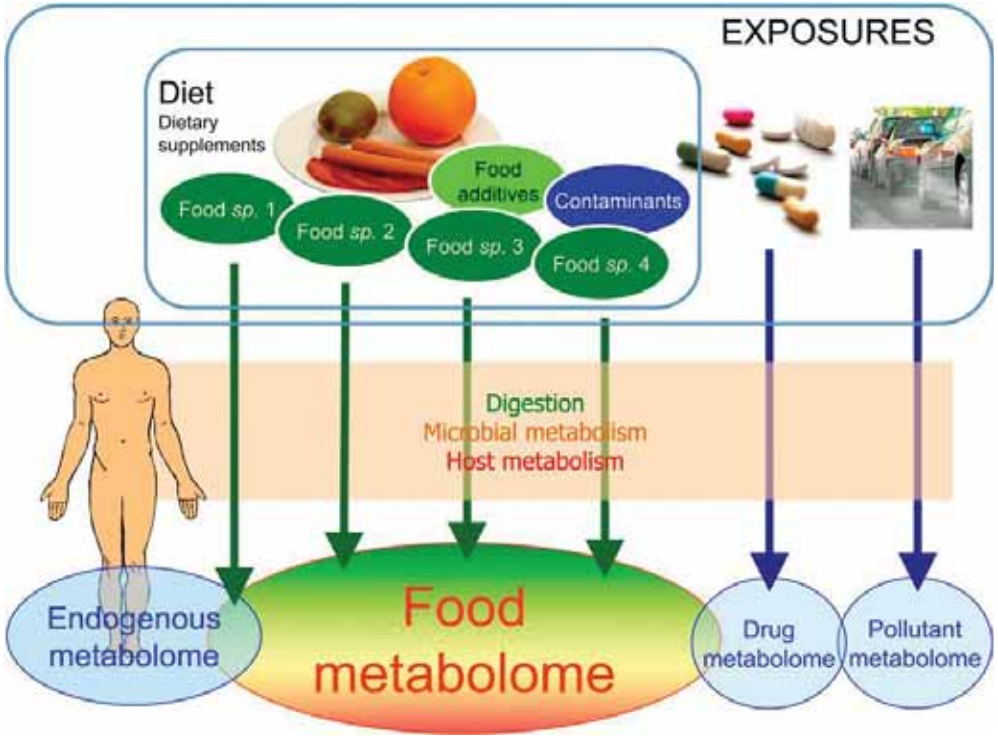


HOW TO MEASURE WHAT'S BEYOND .....

Characterizing the metabolome – the small molecules associated with metabolism

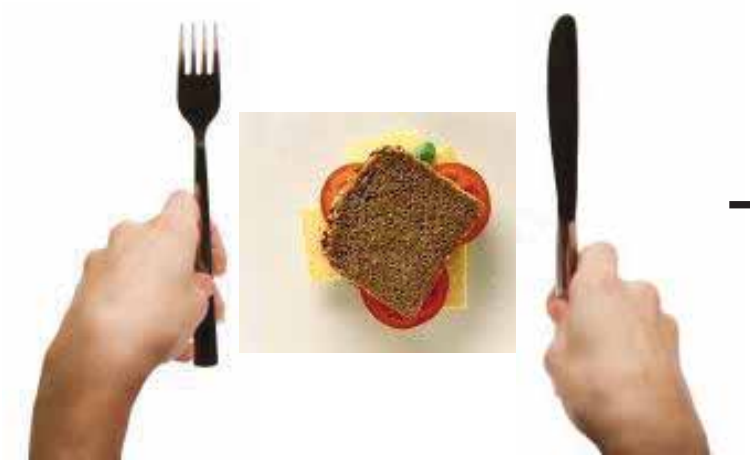
Non-targeted metabolomics – a non-biased technology that allows measurement of hundreds or even thousands metabolites at a time

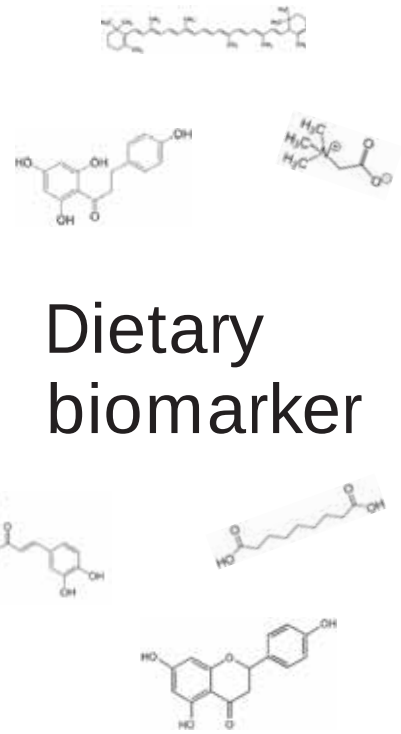
THE METABOLOME



Scalbert et al., Am. J. Clin. Nutr. 2014; 99: 1286-1308

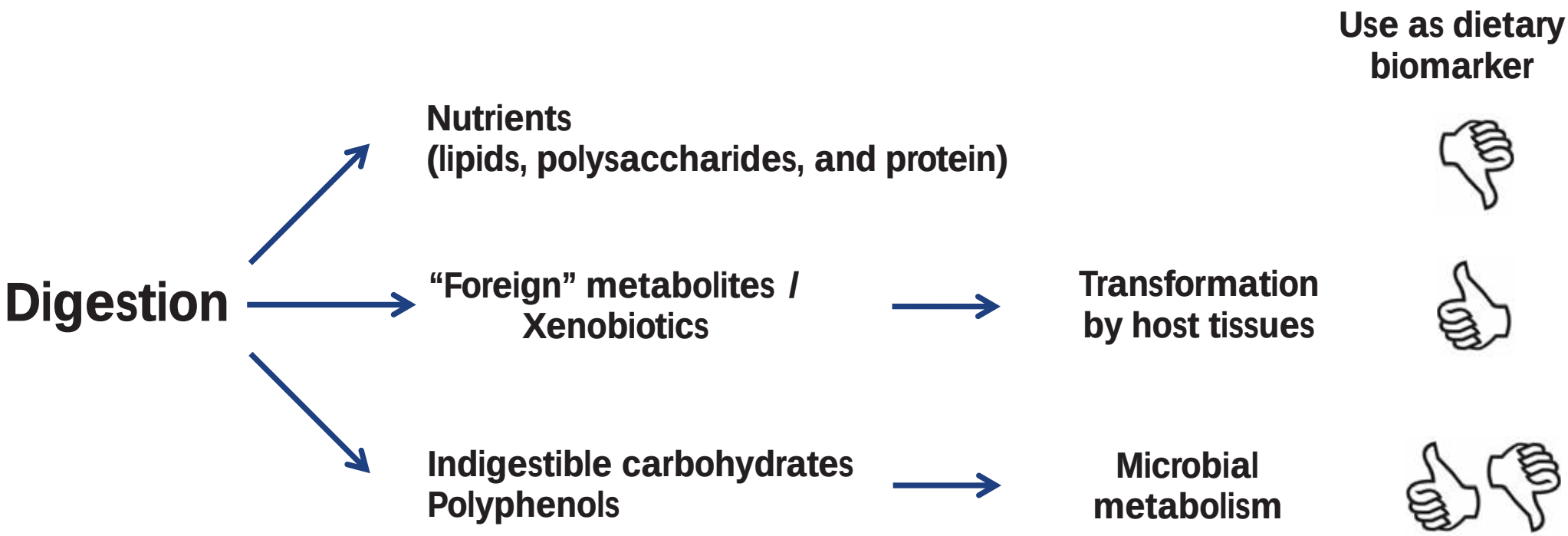
DIETARY BIOMARKERS:





Dietary biomarker

Metabolism of food constituents

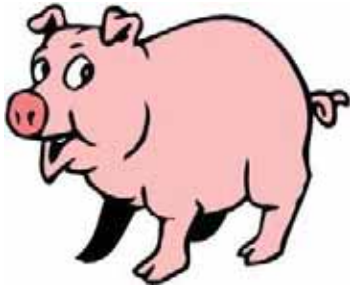


BIOMARKERS USED AS SURROGATE INDICATORS OF CONSUMPTION OF FOOD AND FOOD GROUPS

Food category	Biomarkers
Fruit and vegetables	Polyphenols, carotenoids, vitamin C
Whole-grain cereals	Alkylresorcinols
Soy	Isoflavones
Meat	Amino acids, fatty acids
Dairy products and fish	Fatty acids
Tea and wine	Polyphenols

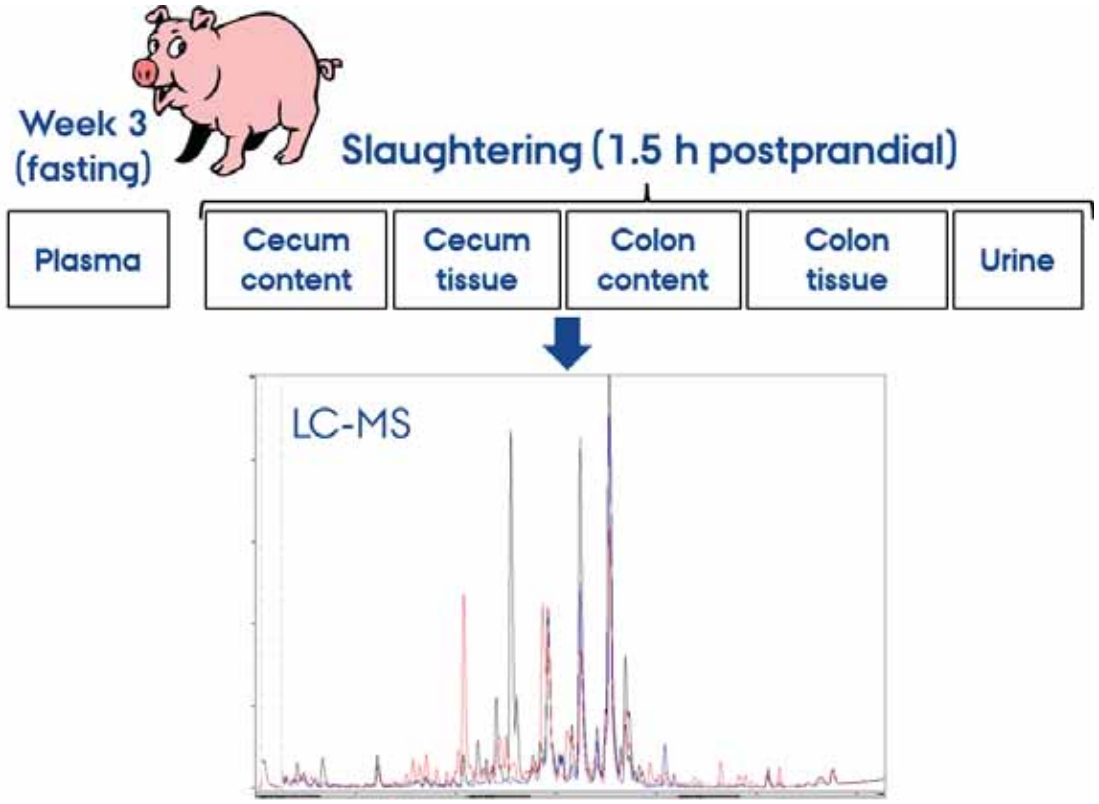
STUDIES IN BUTCOINS WHERE THE METABOLOME WAS INVESTIGATED

Healthy, lean pigs fed diets with high content of arabinoxylan and resistant starch



Zucker Diabetic Fatty rats (ZDF rats) fed diets based on starch or resistant starch

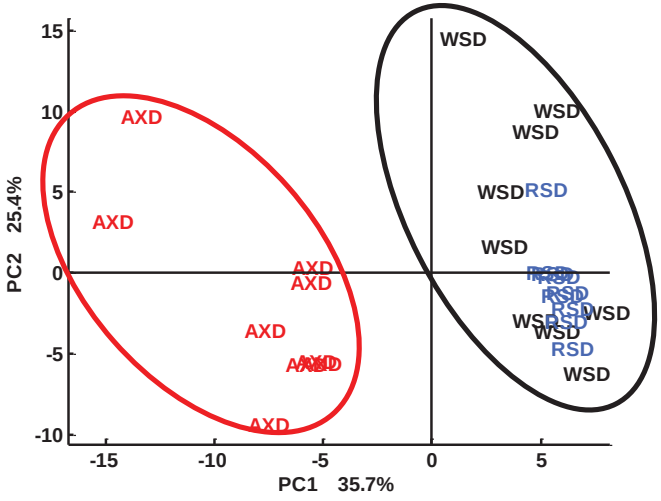
METABOLITES OF DIETARY ORIGIN WHEN FEEDING HEALTHY PIGS ARABINOXYLAN AND RESISTANT STARCH RICH DIETS



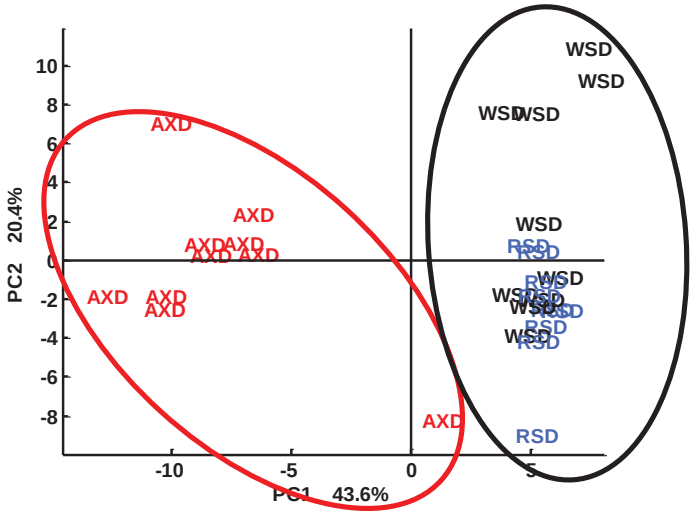
Ingerslev et al., J. Proteome Res. 2015; 14: 3095-3110

SEPARATION IN GASTROINTESTINAL CONTENTS AND URINE (PCA)

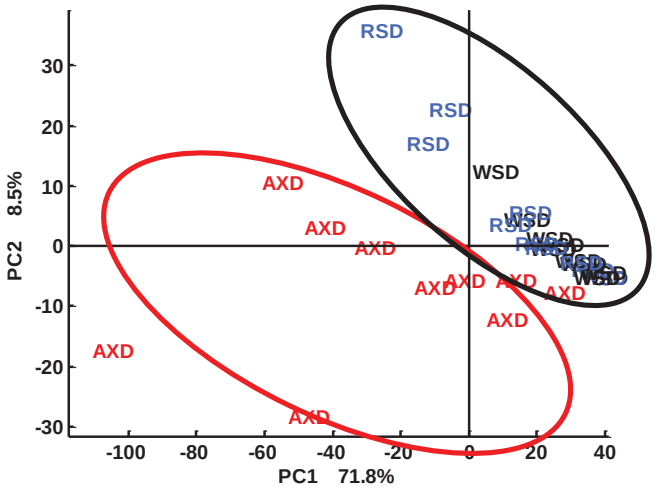
Cecum content



Colon content

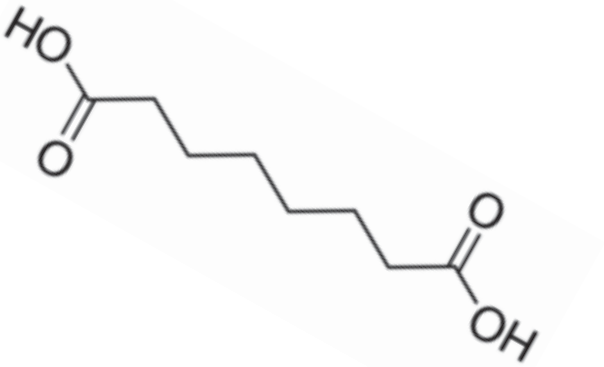


Urine



METABOLITES IN DIGESTA CAUSING SEPARATION BETWEEN DIETS

Suberic acid
Azelaic acid } Dicarboxylic acids increased after consumption of AXD
Sebacic acid

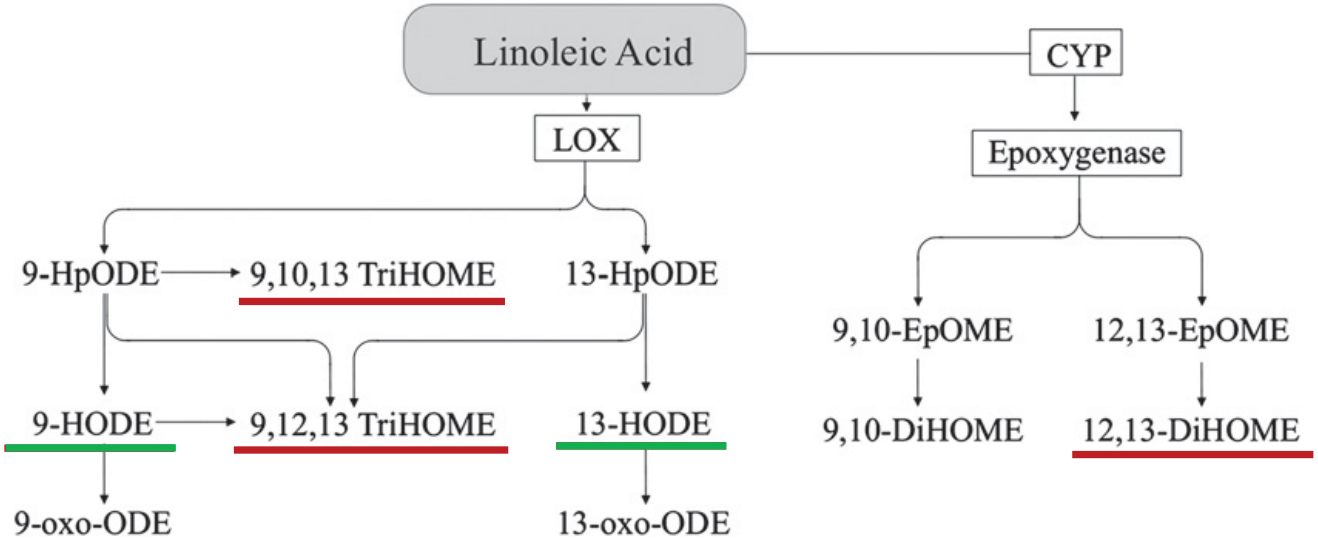


- Azelaic acid is naturally occurring in wheat and rye
- Oleic acid is a precursor for dicarboxylic acids
- Dicarboxylic acids are suggested as marker for feeding of medium-chain triglycerides*

*Brass et al., Am. J. Clin. Nutr. 1990; 52: 923-6

OXYLIPINS IN DIGESTA

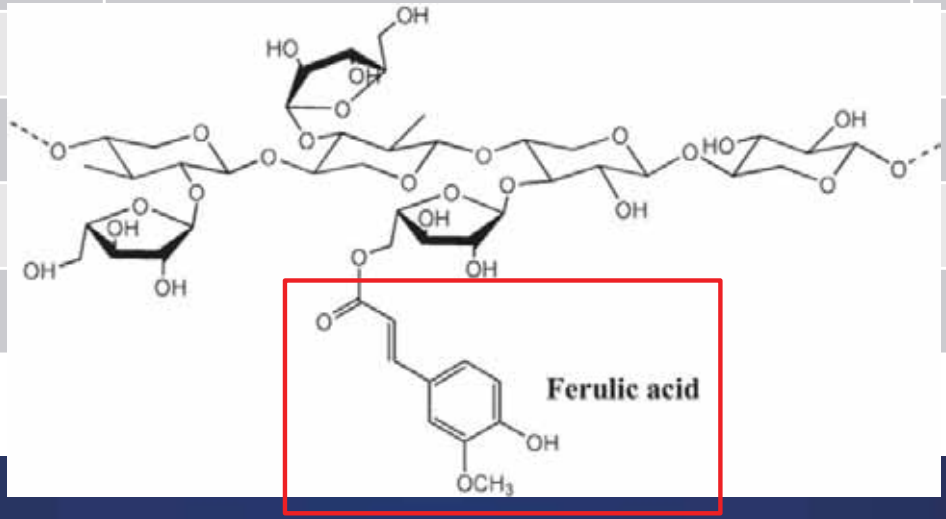
The main metabolites causing the separation between diets where oxylipins of the linoleic acid cascade



Gabbs et al., Adv. Nutr. 2015; 6: 6513-40

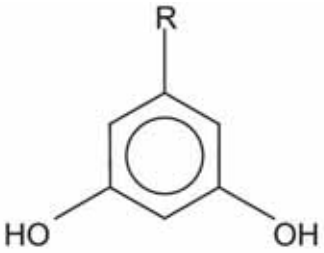
METABOLITES IN URINE CAUSING SEPARATION BETWEEN DIETS (AXD VS. WSD)

Metabolite	Pathway	Fold change
Hippuric acid	Phenolic acid metabolism	8.3
p-Cresol sulfate		3.5
4-Pyridoxic acid		3.5
n-feruloylglycine		42.3
DHPPA glucuronide		34.1



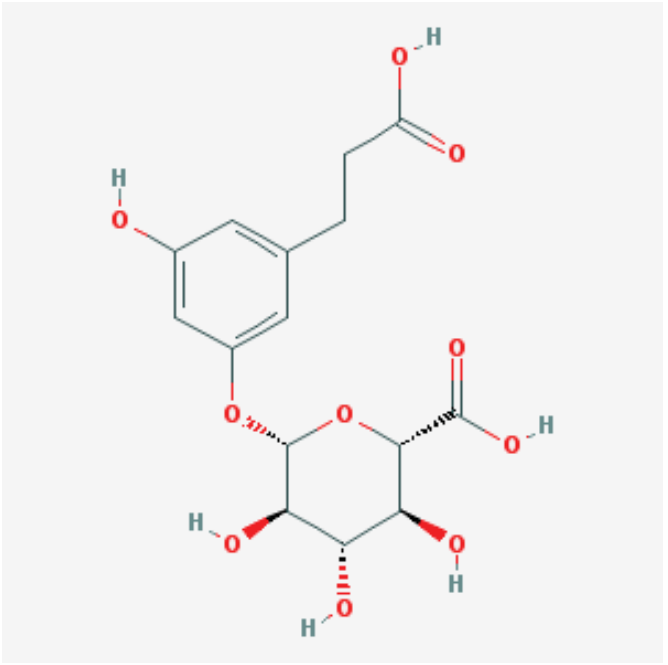
METABOLITES IN URINE CAUSING SEPARATION BETWEEN DIETS (AXD VS WSD)

Metabolite	Pathway	Fold change
Hippuric acid	Phenolic acid metabolism	8.3
p-Cresol sulfate	Microbial fermentation	3.5
4-Pyridoxic acid	Vitamin B6 metabolism	3.5
n-feruloylglycine	Phenolic acid metabolism	42.3
DHPPA glucuronide	Alkylresorcinol metabolism	34.1



Alkylresorcinol	Abbreviation used	R	Molecular weight (g/mol)
5-n-Heptadecylresorcinol	(C17:0)	$C_{17}H_{35}$	348
5-n-Nonadecylresorcinol	(C19:0)	$C_{19}H_{39}$	376
5-n-Heneicosylresorcinol	(C21:0)	$C_{21}H_{43}$	404
5-n-Tricosylresorcinol	(C23:0)	$C_{23}H_{47}$	432
5-n-Pentacosylresorcinol	(C25:0)	$C_{25}H_{51}$	460

Metabolism, glucuronidation and excretion in urine



3-(3,5-Dihydroxyphenyl)-1-Propanoic acid glucuronide

Bran

- Insoluble dietary fibre (xylans, cellulose, lignin)
- Antioxidants bound to cell walls (phenolic acids)

Outer and Inner pericarp (4-5%)

- Soluble & insoluble dietary fibre (xylans, β-glucans)
- Proteins
- Antioxidants (phenolic acids)
- Vitamin E
- B vitamins
- Minerals
- Phytic acids
- Enzymes

Testa (1%)

- Alkylresorcinols

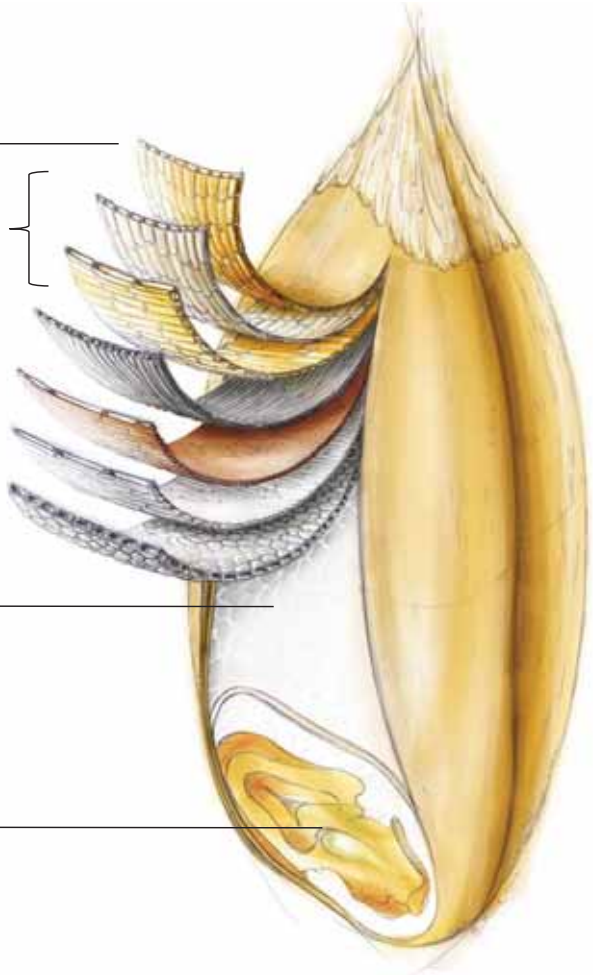
Aleurone layer (6-9%)

Starchy endosperm (80-85%)

- Starch and proteins

Germ (3%)

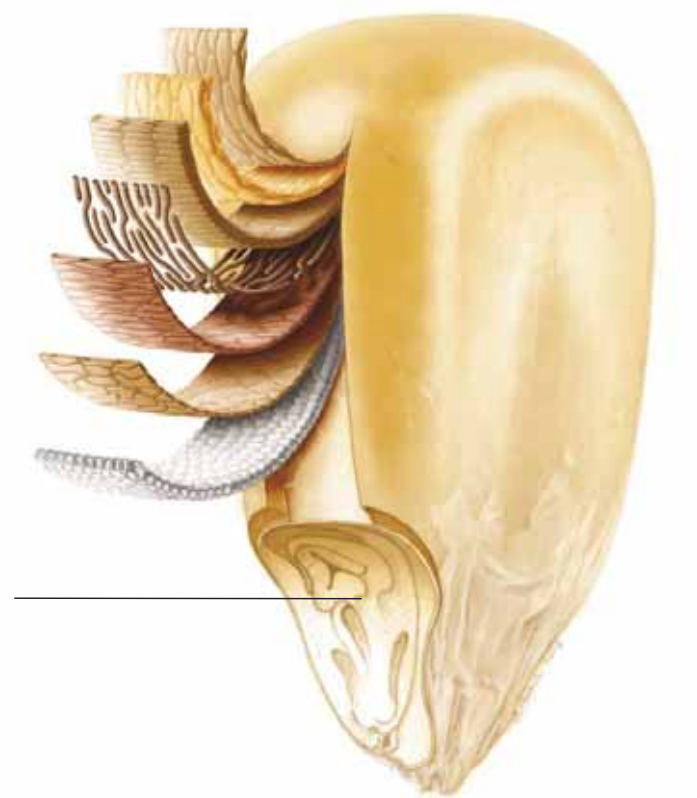
- Lipids
- Antioxidants
- Vitamin E
- Minerals
- Plant sterols
- Enzymes



RESISTANT STARCH FROM CORN AND POTATO

Starchy endosperm
(80-85%)

- Starch and proteins



Journal of proteome research

Article

pubs.acs.org/jpr

Whole Grain Consumption Increases Gastrointestinal Content of Sulfate-Conjugated Oxylipins in Pigs – A Multicompartmental Metabolomics Study

Anne Krog Ingerslev,[*,†] Ibrahim Karaman,[‡] Murat Bağcıoğlu,[§] Achim Kohler,[§,⊥] Peter Kappel Theil,[†] Knud Erik Bach Knudsen,[†] and Mette Skou Hedemann[*,†]

DOI: 10.1021/acs.jproteome.5b00039
J. Proteome Res. 2015, 14, 3095−3110

CONCLUSION: WHAT'S BEYOND SHORT-CHAIN FATTY ACIDS?

Arabinoxylans:

- Specific markers for intake of whole-grain – alkylresorcinols and phenolic acids
- Oxylipins – importance in relation to whole-grain intake
- Changed metabolism – phenolic acids and vitamins
- Other changes to metabolism not evident – healthy, lean pigs

A U AARHUS UNIVERSITY

Human subjects with the metabolic syndrome –

why the target group for studying gut and metabolic health?

Søren Gregersen, MD, PhD
Department of Endocrinology and Internal Medicine
Aarhus University Hospital

ButCoIns meeting 2.3.2016

After the ButCoIns party

Reflection

2013 → 2016

Objective and aims

- The **overall objective** is to improve colonic health, peripheral insulin sensitivity and glucose homeostasis by increased colonic butyrate production brought about by pre-, pro- and synbiotic concepts

- Specific aims:
  - **Quantify** the implication of pre- and probiotics on colonic butyrate production
  - **Develop** novel synbiotic concepts for improved butyrate production
  - **Document** the impact of enhanced butyrate production on colonic health parameters
  - **Document** the impact of increased butyrate production on insulin sensitivity and glucose homeostasis

Study design considerations

What exactly do we aim to study (outcome) ?

- and in what population –

Healthy
Pre-disease
Diseased

± disposition for disease

Gender, age etc.

Compliance – impact of medication
Lifestyle

Extrapolation

many more…..

Based on our aim we choosed to study subjects who were

”metabolically challenged”

Metabolic syndrome

Reaven 1988 –

A condition consisting of metabolic risk factors for type 2 diabetes and cardiovascular disease

Abdominal obesity → **insulin resistance** (reduced glucose uptake and fat oxidation)

→ increased insulin levels and elevated glucose
cytokines
endothelial dysfunction / inflammation
elevated blood pressure
dyslipidemia

↓

CARDIOVASCULAR DISEASE

Metabolic syndrome

”Cluster” of important cardiovascular risk factors

Prevalence: approx. 20-25 % of the adult population has metabolic syndrome

The metabolic syndrome is a strong predictor for type 2 diabetes

Subjects with the metabolic syndrome have a
5 x increased risk of type 2 diabetes
2-3x increased risk of CVD

The number of individual elements potentiates the risk for cardiovascular disease

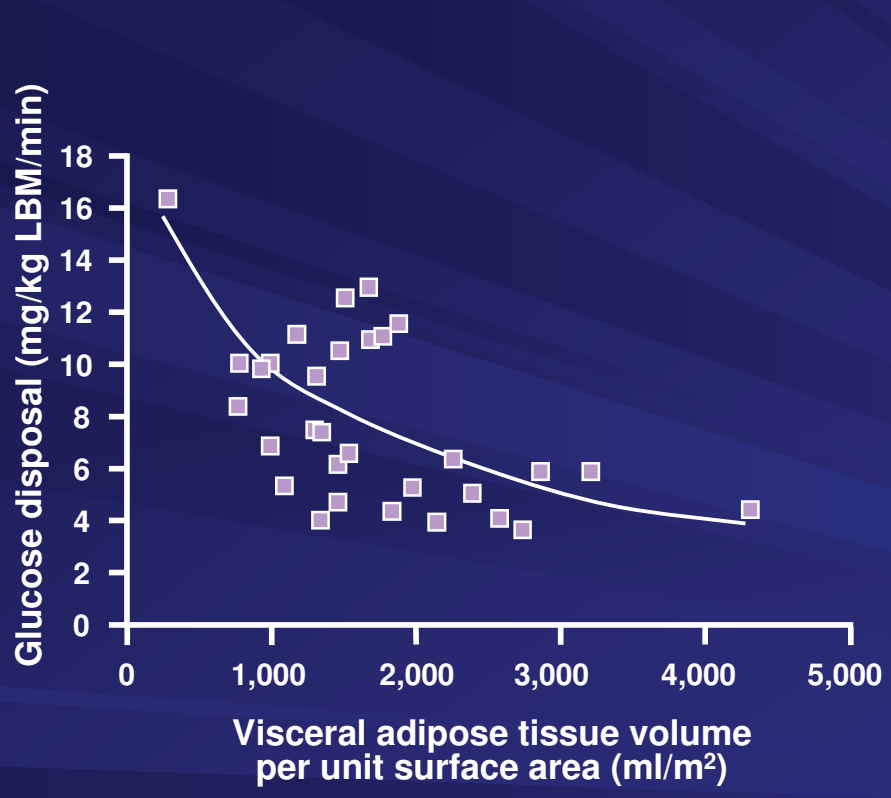
Causal elements : obesity and insulin resistance

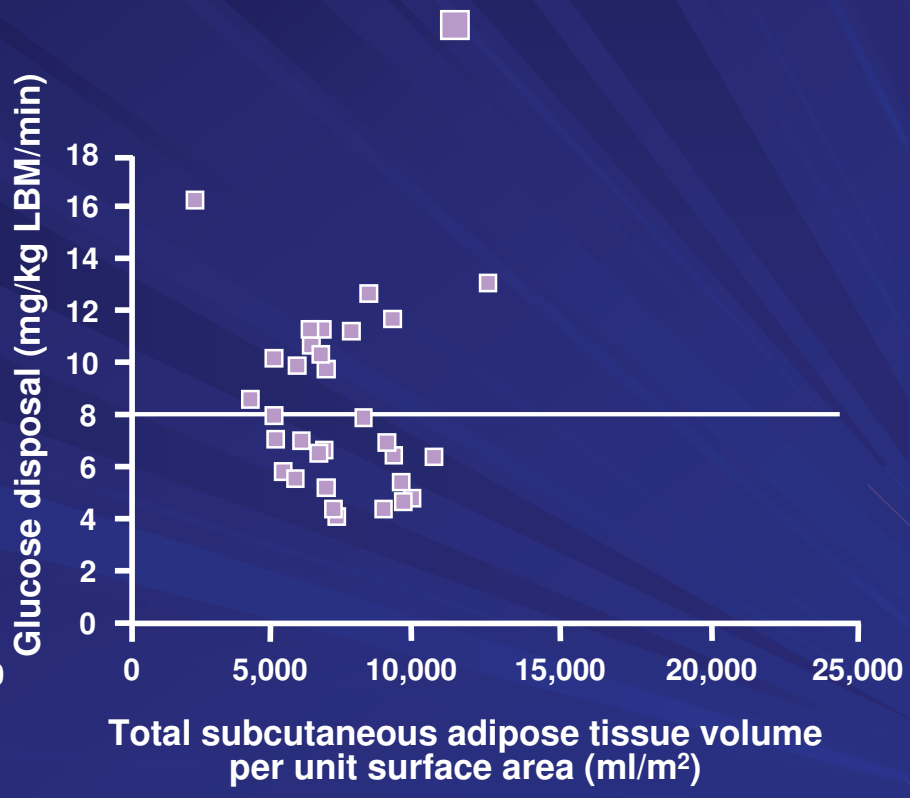
IDF

Table 2 | **The association of VAT with metabolic risk factors in the FHS[9]**

Risk factors	Women		Men		Sex interaction (*P*)
	Effect size or odds ratio	*P*	Effect size or odds ratio	*P*	
Continuous risk factors*					
Systolic blood pressure[‡]	4.8 ± 0.4	<0.0001	3.3 ± 0.4	<0.0001	<0.0001
Diastolic blood pressure[‡]	2.6 ± 0.3	<0.0001	2.6 ± 0.2	<0.0001	0.01
Fasting plasma glucose[§]	4.8 ± 0.4	<0.0001	3.1 ± 0.5	<0.0001	<0.0001
Log[triglycerides][‖]	0.23 ± 0.01	<0.0001	0.22 ± 0.01	<0.0001	0.0002
HDL cholesterol[‖]	−5.9 ± 0.4	<0.0001	−4.5 ± 0.7	<0.0001	<0.0001
Dichotomous risk factors[¶]					
Hypertension	2.1 (1.8–2.4)	<0.0001	1.9 (1.6–2.1)	<0.0001	0.01
Impaired fasting glucose	2.5 (2.1–2.9)	<0.0001	1.8 (1.6–2.0)	<0.0001	<0.0001
T2DM	2.1 (1.6–2.6)	<0.0001	1.6 (1.3–2.0)	<0.0001	0.03
Metabolic syndrome	4.7 (3.9–5.7)	<0.0001	4.2 (3.5–5.0)	<0.0001	0.002

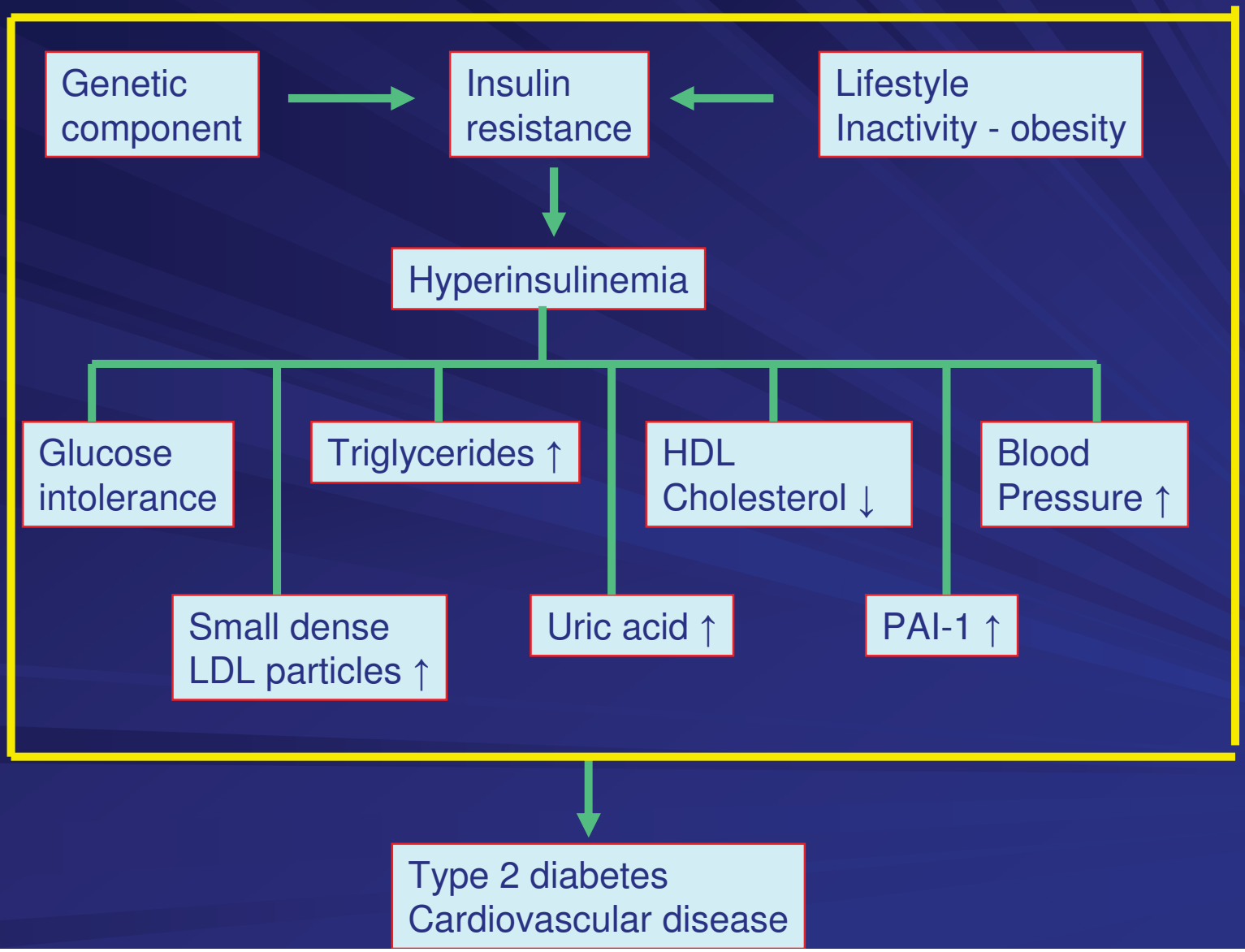
Abdominal fat = insulin resistance





Banerji MA, *et al. Am J Physiol* 1997; 273:E425–E432.

Metabolic syndrome



Metabolic syndrome

Associated to

Fatty liver (non-alcoholic fatty liver disease)

Cirrhosis – hepatocellular carcinoma

Chronic kidney disease

Polycystic ovary syndrome (PCOS)

Sleep apnoea

Gout

Dementia – cognitive impairment

Cancer, e.g. colo-rectal

…… and more……

Metabolic syndrome

Several definitions of the metabolic syndrome (WHO, EGIR, NCEP ATPIII).

Most clinical studies uses the IDF definition with its central components

- **Obesity**
- **Insulin resistance**
- **Dyslipidemia**
- **Raised blood pressure**

Definition Metabolic syndrome

Table 1: The new International Diabetes Federation (IDF) definition

According to the new IDF definition, for a person to be defined as having the metabolic syndrome they must have:

Central obesity (defined as waist circumference* with ethnicity specific values)

plus any two of the following four factors:

Raised triglycerides	≥ 150 mg/dL (1.7 mmol/L) **or specific treatment for this lipid abnormality**
Reduced HDL cholesterol	< 40 mg/dL (1.03 mmol/L) in males < 50 mg/dL (1.29 mmol/L) in females **or specific treatment for this lipid abnormality**
Raised blood pressure	systolic BP ≥ 130 or diastolic BP ≥ 85 mm Hg **or treatment of previously diagnosed hypertension**
Raised fasting plasma glucose	(FPG) ≥ 100 mg/dL (5.6 mmol/L), **or previously diagnosed type 2 diabetes** If above 5.6 mmol/L or 100 mg/dL, OGTT is strongly recommended but is not necessary to define presence of the syndrome.

Table 2: Ethnic specific values for waist circumference

Country/Ethnic group		Waist circumference
Europids* In the USA, the ATP III values (102 cm male; 88 cm female) are likely to continue to be used for clinical purposes	Male	≥ 94 cm
	Female	≥ 80 cm

IDF 2006

Metabolic syndrome

Negative

It is unknown if ”metabolic syndrome” confers a higher risk than the risks associated with the individual components of the syndrome

The syndrome does not include the (maybe most important) determinants
i.e. age, family history, physical activity[1]

Positive

Identification of the metabolic syndrome in a subject followed by a ”management plan” can reduce the risk for type 2 diabetes and cardiovascular disease

Easy risk prediction model identifying subjects at risk

1. Knut Borch-Johnsen 2013

Metabolic syndrome

The concept has opened for numerous studies on the pathogenesis of type 2 diabetes and for preventive measures to reduce the risk of related disorders

It has thus become an important research tool for clinicians

Metabolic syndrome

Table 3: Additional metabolic measurements for research

Abnormal body fat distribution	General body fat distribution (DEXA) Central fat distribution (CT/MRI) Adipose tissue biomarkers: leptin, adiponectin Liver fat content (MRS)
Atherogenic dyslipidaemia (beyond elevated triglyceride and low HDL)	ApoB (or non-HDL-c) Small LDL particles
Dysglycaemia	OGTT
Insulin resistance (other than elevated fasting glucose)	Fasting insulin/proinsulin levels HOMA-IR Insulin resistance by Bergman Minimal Model Elevated free fatty acids (fasting and during OGTT) M value from clamp
Vascular dysregulation (beyond elevated blood pressure)	Measurement of endothelial dysfunction Microalbuminuria
Proinflammatory state	Elevated high sensitivity C-reactive protein Elevated inflammatory cytokines (eg TNF-alpha, IL-6) Decrease in adiponectin plasma levels
Prothrombotic state	Fibrinolytic factors (PAI-1, etc) Clotting factors (fibrinogen, etc)
Hormonal factors	Pituitary-adrenal axis

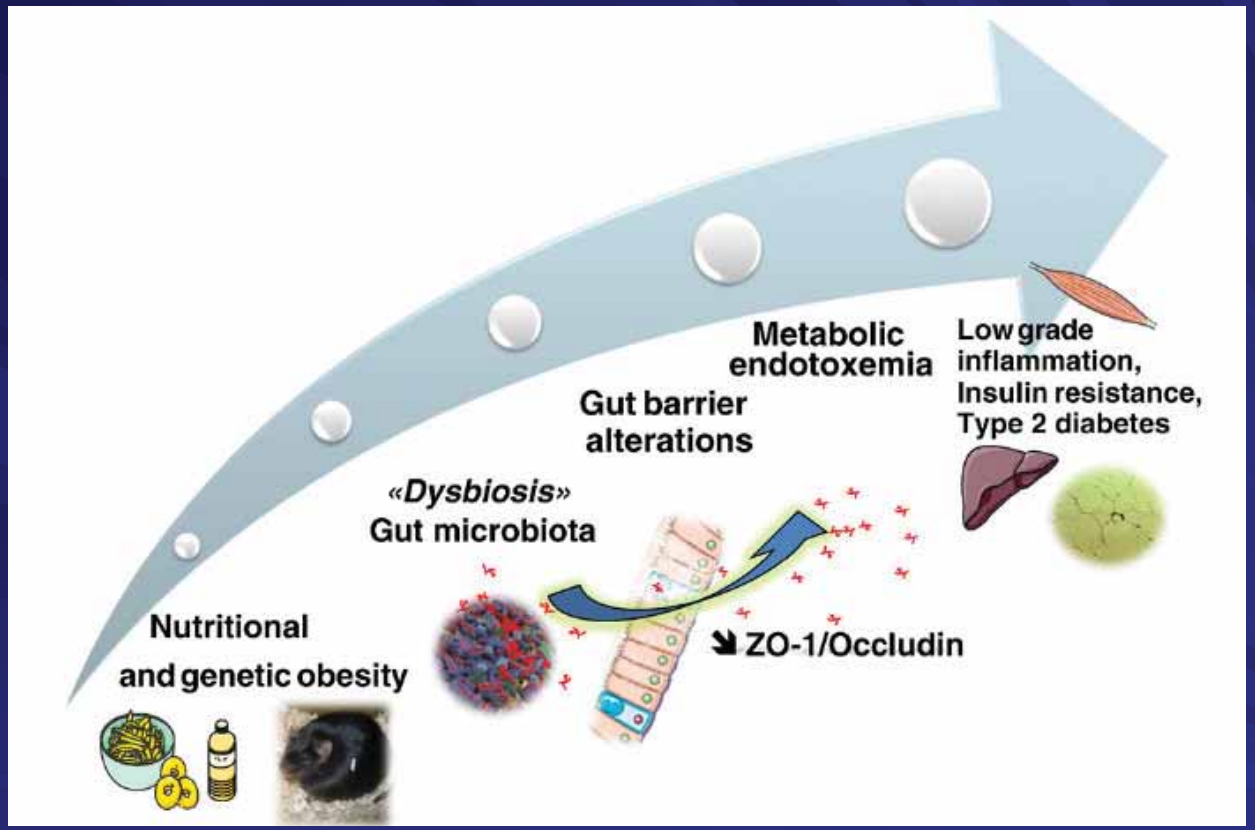
Metabolic syndrome

We aimed to see if a combination of dietary fibers in subjects with the metabolics syndrome improves

- key metabolic parameters

- and colon health

Note: Risk markers only
Short intervention period
Metabolic challenge – study population



P.D. Cani, N.M. Delzenne / Pharmacology & Therapeutics 130 (2011) 202–212

Thank you

Human subjects with the metabolic syndrome

– Why the target group for studying gut and metabolic health

Gut feelings and number of participiants needed?

Jens F. Dahlerup,

Aarhus University Hospital, Department of Hepatology and Gastroenterology

Metabolic syndrome

abdominal fat + 2 risk factors (type 2 diabetes and cardiovascular disease)

Waist circumference	≥ 94 cm (men) ≥ 80 cm (women)
- *Plus any two of the following:*	
Triglyceride concentration	≥ 1.17 mmol/L
High density lipoprotein cholesterol	< 1.0 mmol/L (men) < 1.3 mmol/L (women)
Blood pressure	Systolic > 130 mmHg and / or Diastolic > 85 mmHg
Fasting plasma glucose	> 5.6 mmol/L

Alberti et al. *Lancet* 2005

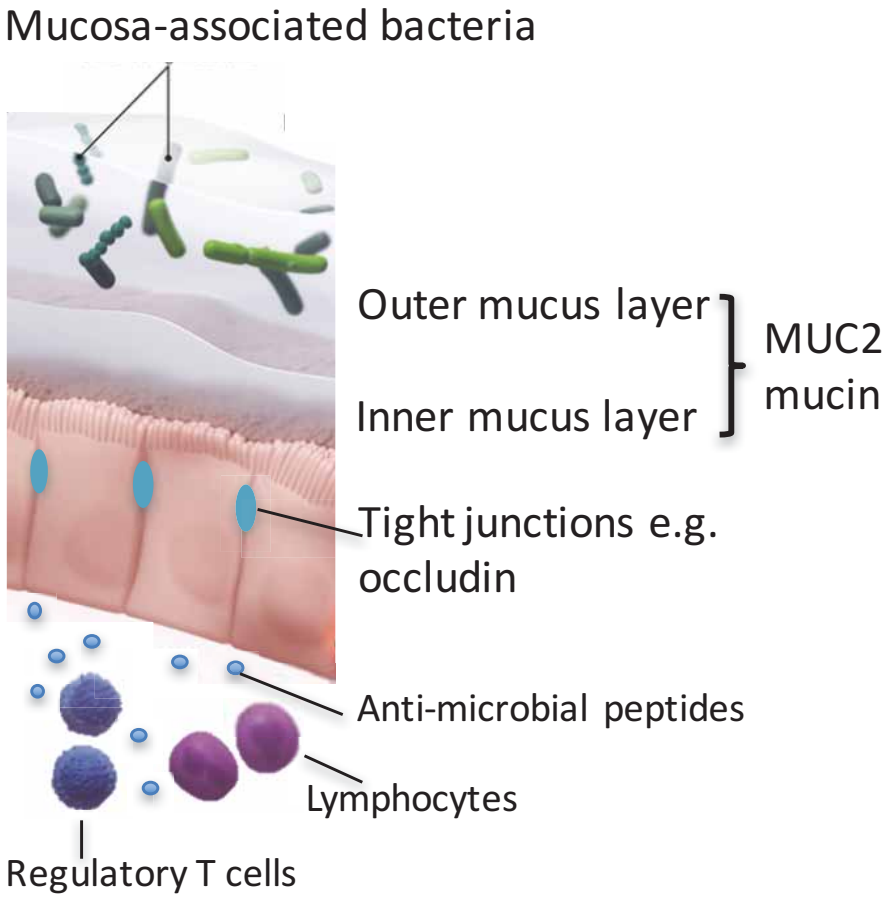
Gut barrier – structure and function

Physical barrier

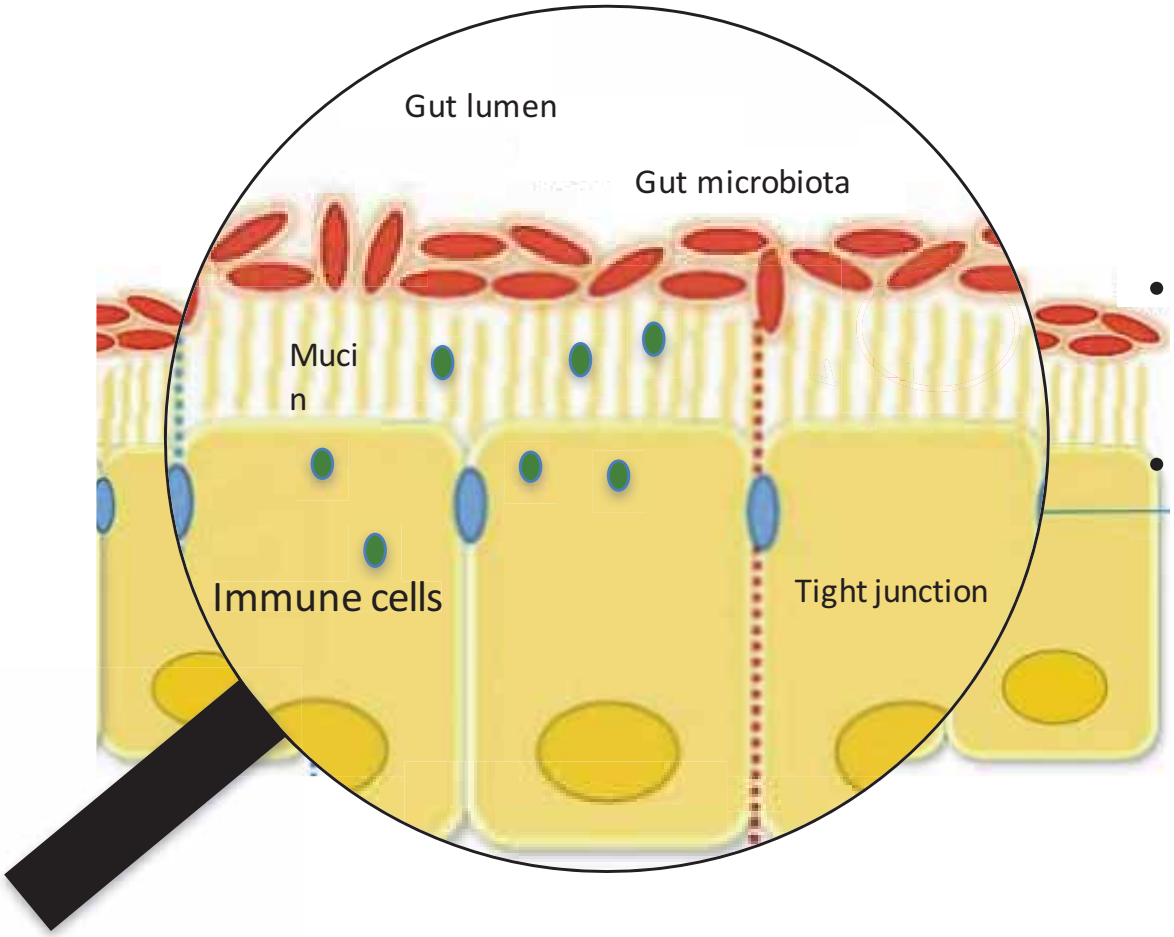
- Prevents bacterial adhesion
- Regulates paracellular diffusion

Functional barrier

- Maintains tolerance towards commensal bacteria
- Initiates immune responses against pathogens



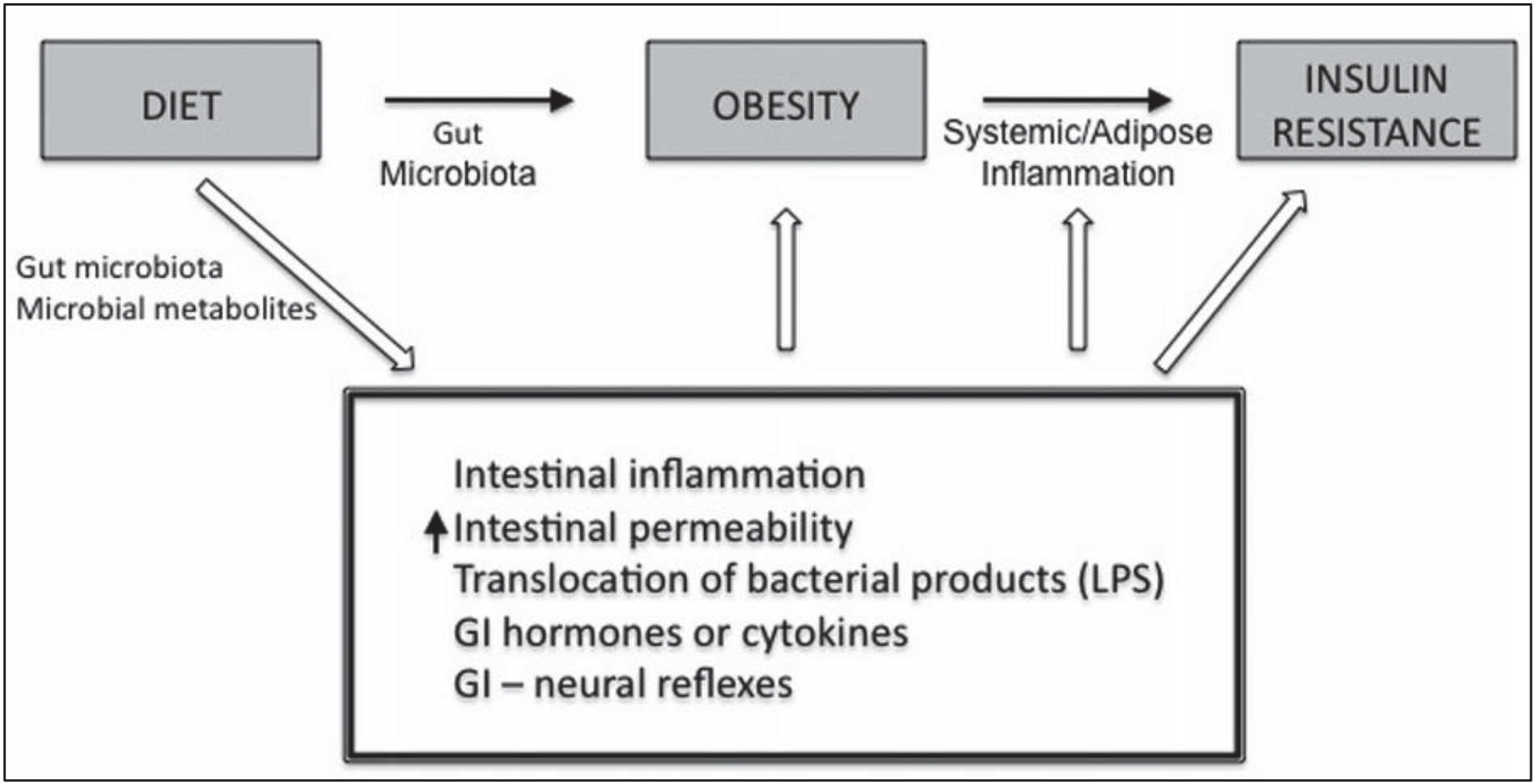
Intestinal inflammation in the metabolic syndrome?



- Elevated faecal-calprotectin in obese subjects (Verdam et al. *Obesity*, 2013)
- Pro-inflammatory shift in T cells in obese subjects (Luck et al. *Cell metabolism*, 2015)

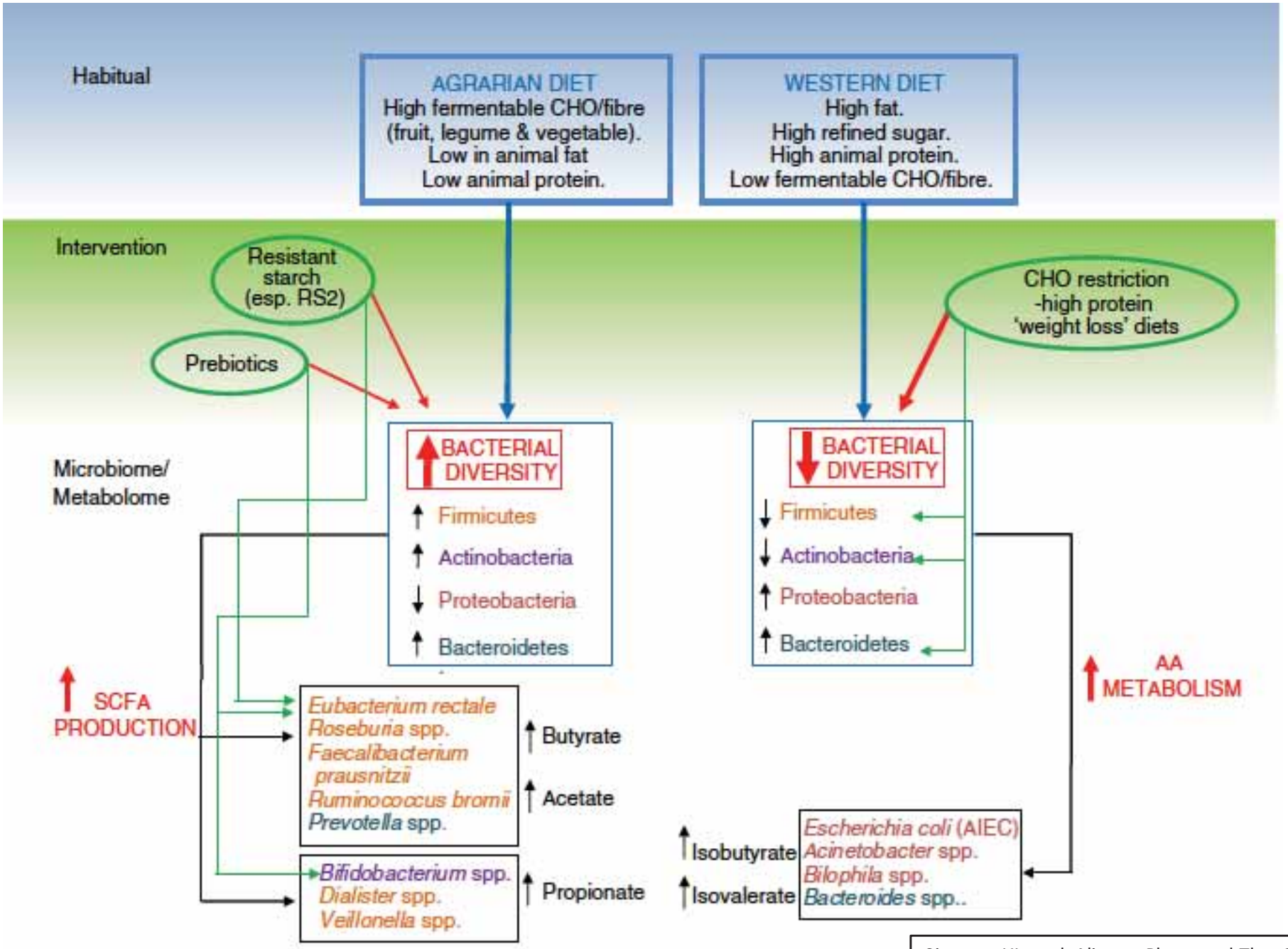
Modified, Fonvig et al. *Ugeskr Laeger* 2014

Diet – Microbiota – Gut – Obesity



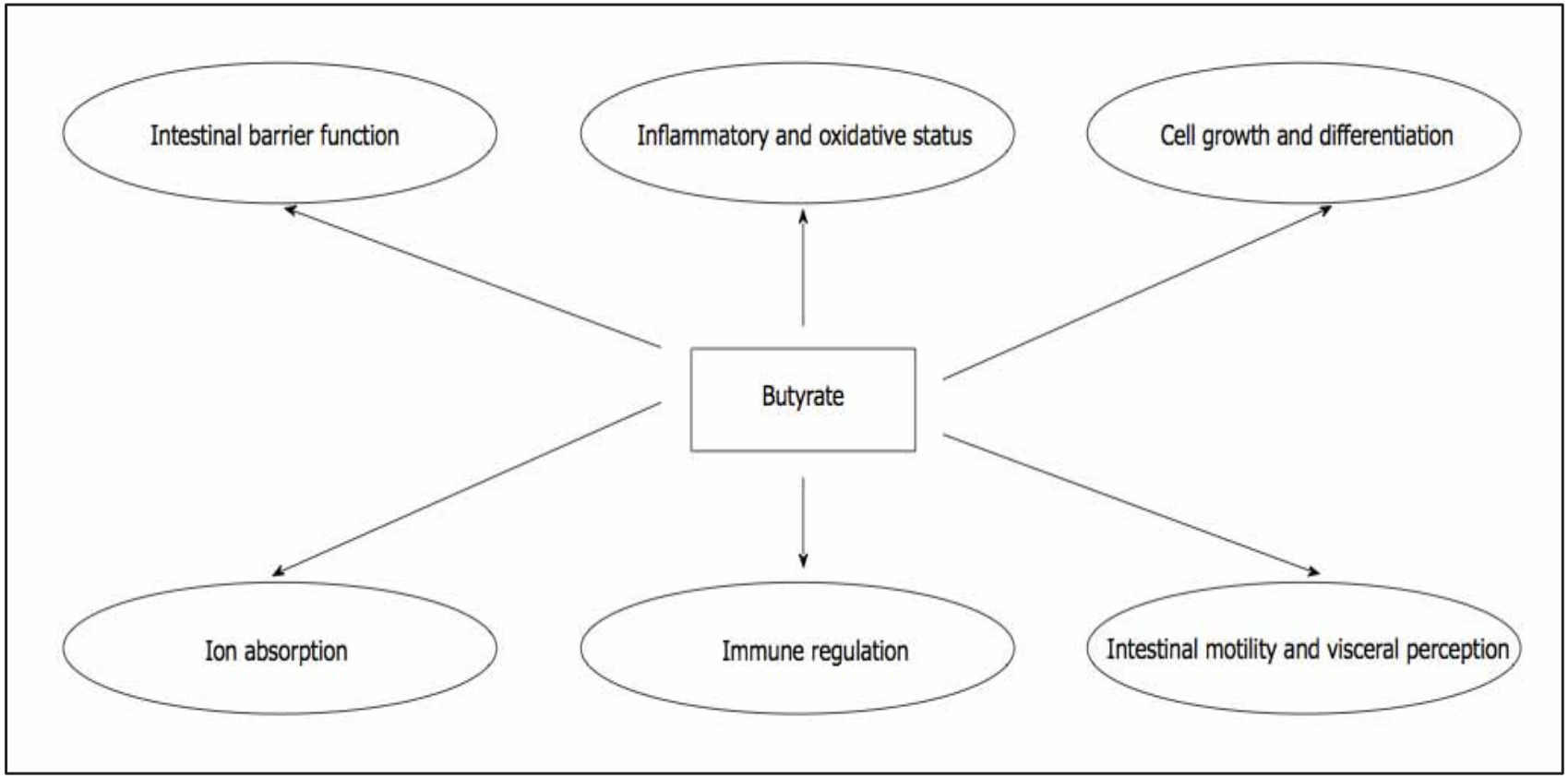
Shengli et al, *Curr Opin Clin Nutr Metab Care 2011*

What to expect – *Healthy Carbohydrate Diet* (HCD) versus *Western Style Diet (WSD)*



Simpson HL et al, Aliment Pharmacol Ther 2015

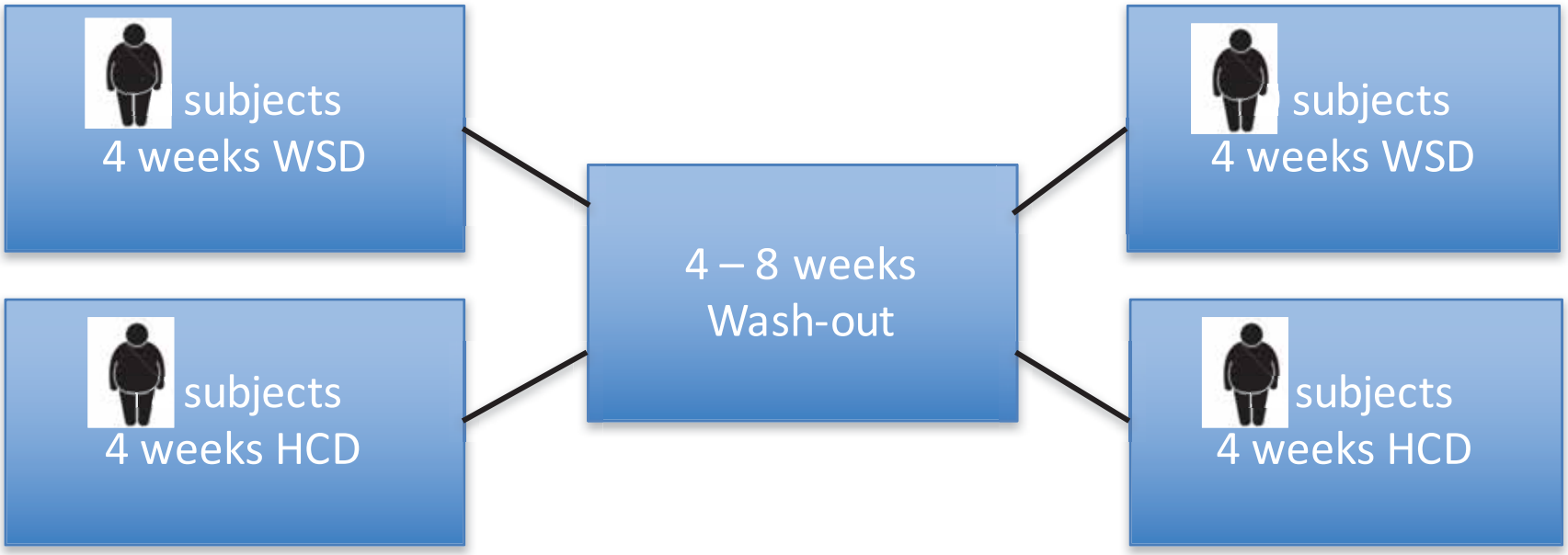
Butyrate and intestinal ”health”



Canani RB et al. *World J Gastroenterol 2011*

Design – cross-over - but how many participiants?

A single blinded randomized cross-over study



WSD - Western Style Diet

HCD - Healthy Carbohydrate Diet

Number of participiants needed in human BUTCOINS?

Is HCD in relation to WSD able to increase faecal butyrate concentration?

What minimal difference in faecal butyrate concentration is important – and should not be overlooked?

Goal is to: have sufficient power to choose between two simple hypotheses

- Variability of the "building block" response (σ^2)
- Type I error (α), significant level
- Type II error (β), power = 1- β

- Size of minimal difference considered important (Δ)

Number of participiants needed in human BUTCOINS?

Is HCD in relation to WSD able to increase faecal butyrate concentration?

What minimal difference in faecal butyrate concentration is important – and should not be overlooked?

- From the results of a Danish study population treated with a fiber diet of 18 g of dietary fibre (*Plantago ovata* seeds) for 4 weeks versus control diet could be calculated:
- the standard deviation of the mean difference in faecal butyrate concentrations between the diets in that study were 7.5 mmol/l
- We choosed that a minimal relevant difference between means would be 7.5 mmol/l
- Thus we used a standardized minimal relevant difference (d) of 1 (7,5/7.5) in a crossover design

Nordgaard I et al. *Scand J Gastroenterol 2011*

Julious SA et al. *J Biopharm Statistics 1999*

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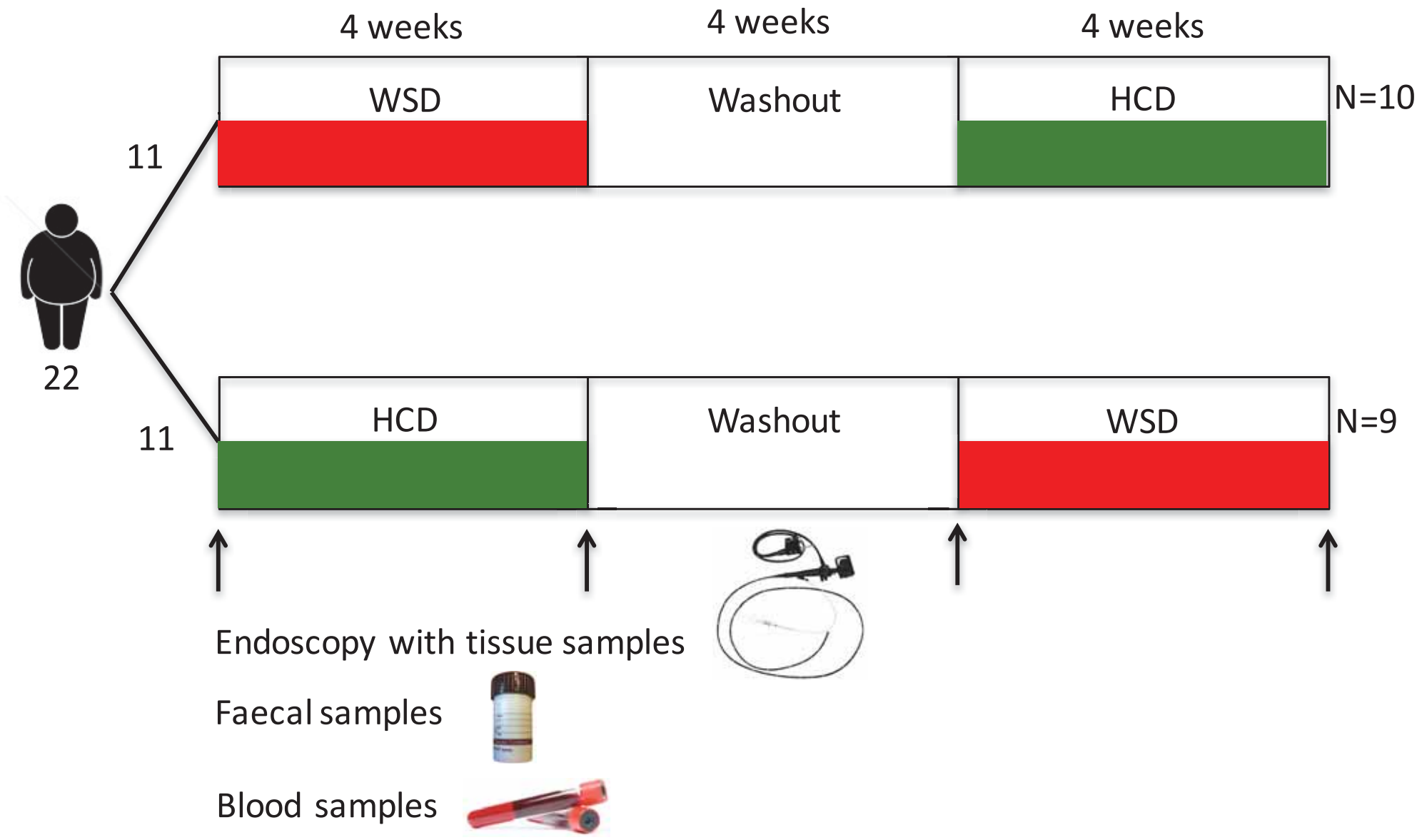
- Total number needed = $(10.5/d^{2)} + 2$ (type I error of 5% and power of 90%)
- The power calculation was also done using software http://hedwig.mgh.harvard.edu/sample_size/size.html#cross
- The total number of subjects needed was 13 (type I error of 5% and power of 90%)
- The anticipated dropout rate was set to 33%
- We included 22 participiants in the human BUTCOINS project

Nordgaard I et al. *Scand J Gastroenterol 2011*

Julious SA et al. *J Biopharm Statistics 1999*

22 participiants was enrolled in the human BUTCOINS project

Randomized crossover study with two diet interventions



GUT and Fecal Microbiota might rule local and systemic inflammation

(BUTCOINS certainly ruled our sparetime)

Mission accomplished !!

After more than 3600 colonic biopsies

THE final biopsy in ButCoIns project

Thank you for your attention!

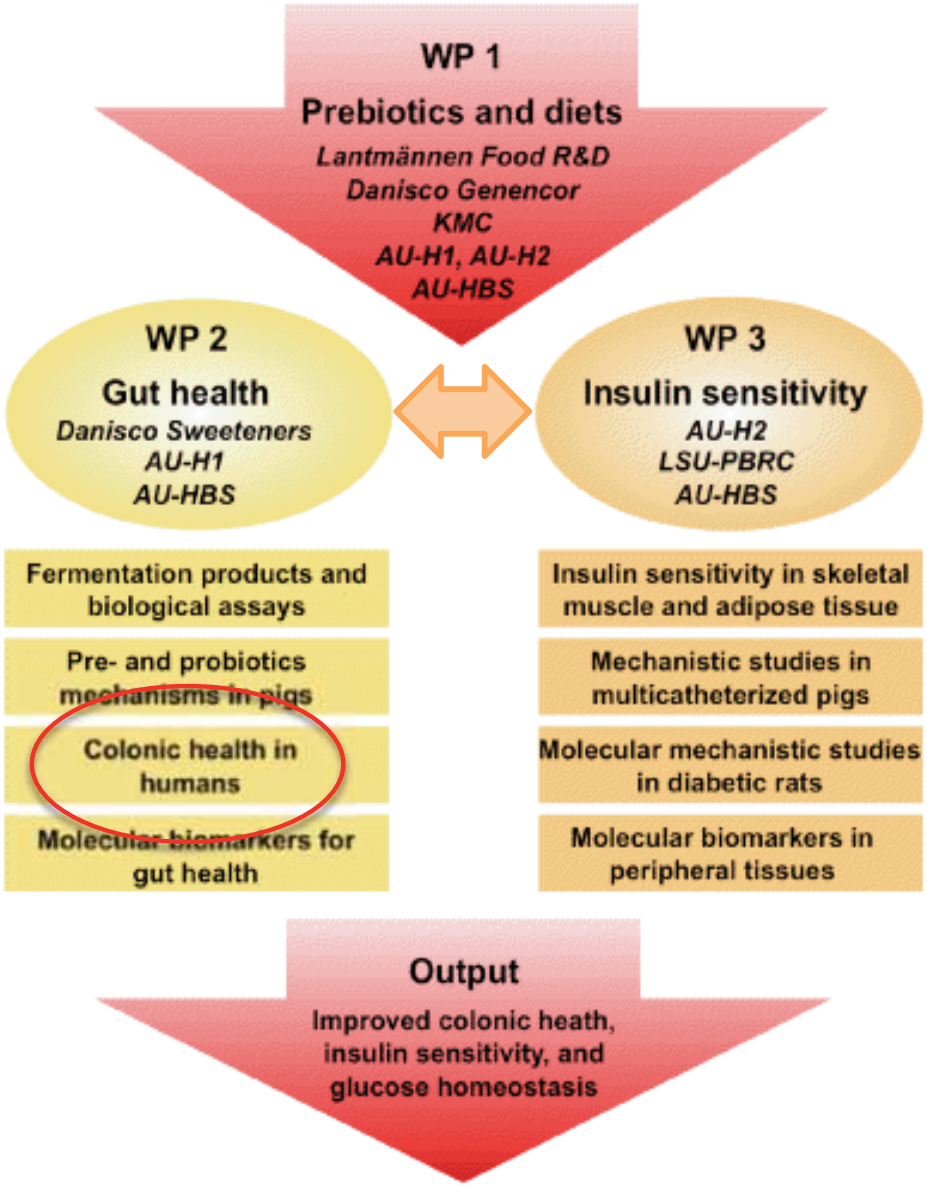
Impact of Arabinoxylan and Resistant Starch on The Gut Microbiome and Gut Health Parameters in Subjects with Metabolic Syndrome

Stine Hald
PhD, MD

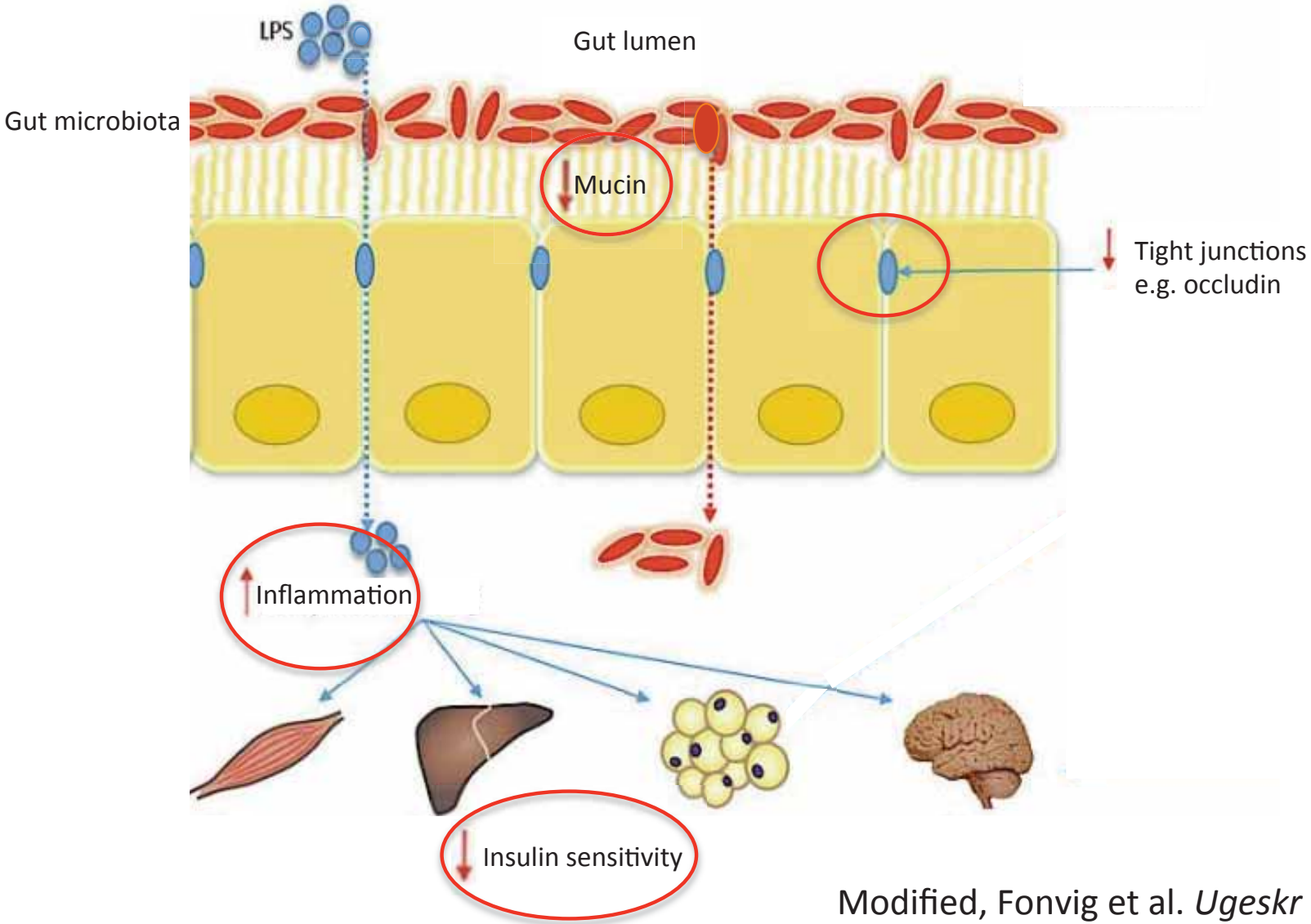
AARHUS UNIVERSITY

Aarhus University Hospital

ButCoIns

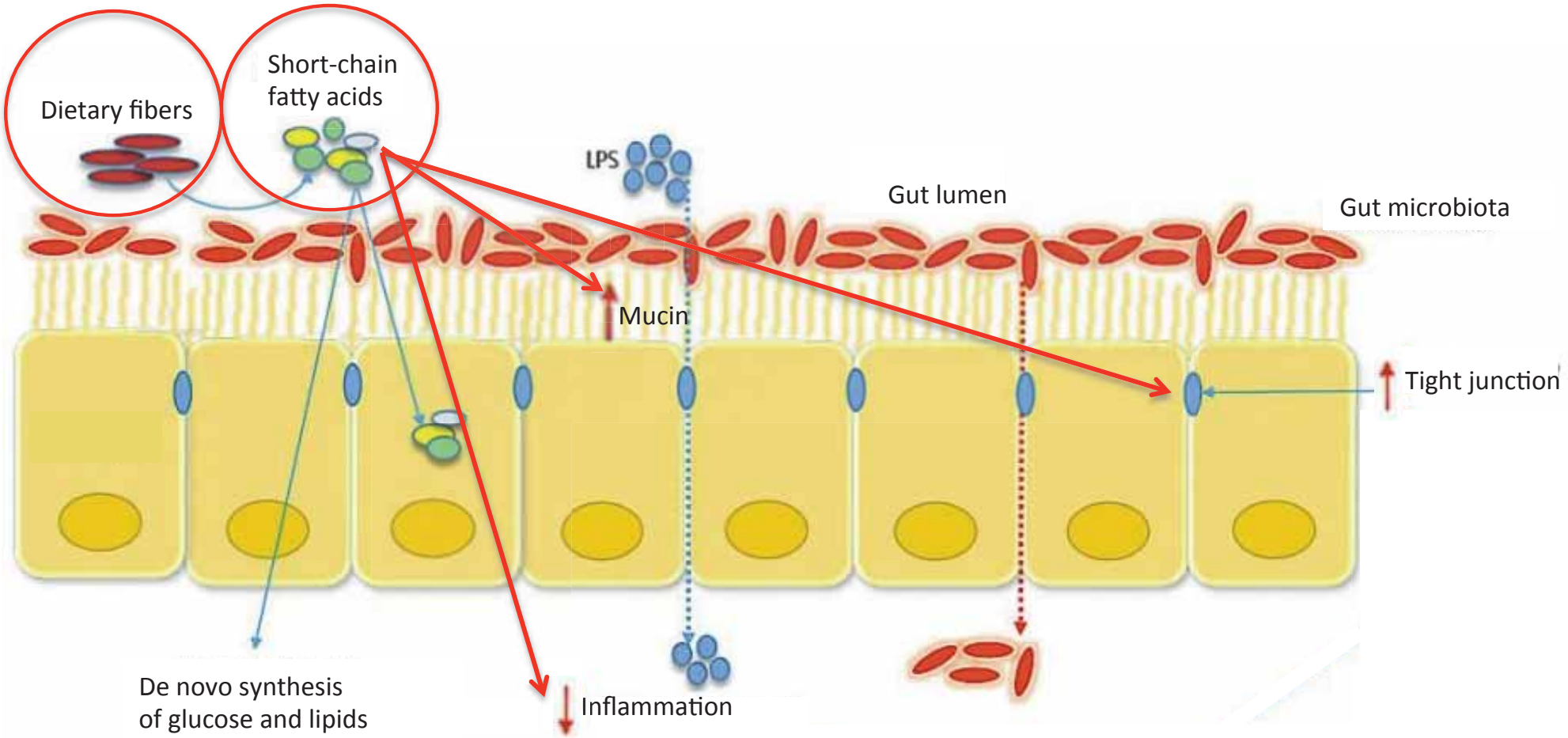

WP 1
Prebiotics and diets
Lantmännen Food R&D
Danisco Genencor
KMC
AU-H1, AU-H2
AU-HBS
WP 2
Gut health
Danisco Sweeteners
AU-H1
AU-HBS
WP 3
Insulin sensitivity
AU-H2
LSU-PBRC
AU-HBS
Fermentation products and biological assays
Pre- and probiotics mechanisms in pigs
Colonic health in humans
Molecular biomarkers for gut health
Insulin sensitivity in skeletal muscle and adipose tissue
Mechanistic studies in multicatheterized pigs
Molecular mechanistic studies in diabetic rats
Molecular biomarkers in peripheral tissues
Output
Improved colonic heath, insulin sensitivity, and glucose homeostasis

The metabolic syndrome
- from a gut perspective



Modified, Fonvig et al. *Ugeskr Laeger* 2014

Dietary fibres and the metabolic syndrome



Modified, Fonvig et al. *Ugeskr Laeger* 2014

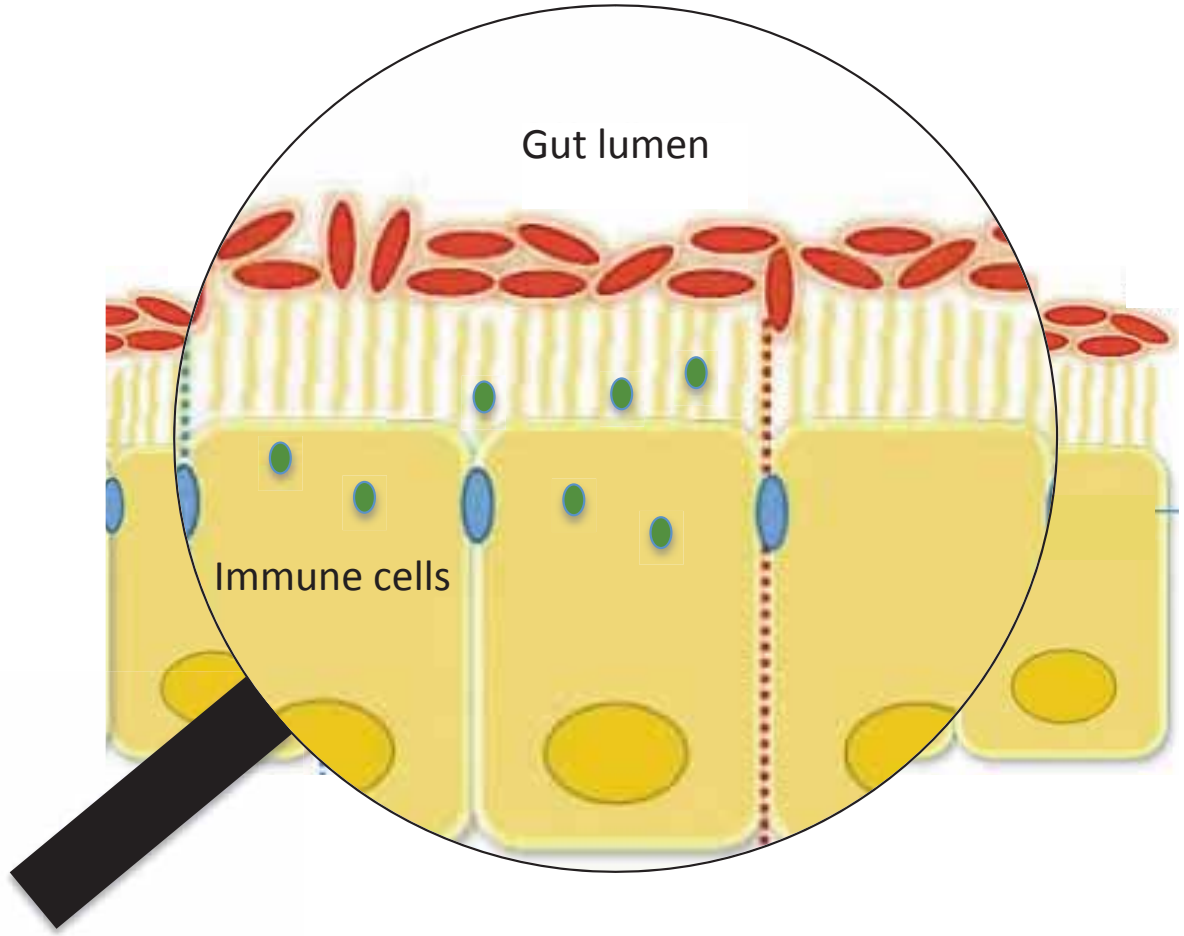
Study I

Hypothesis

Subjects with metabolic syndrome have a low degree of intestinal inflammation

Aim

Examine the colonic mucosal immune activity in subjects with metabolic syndrome compared to healthy controls



Modified, Fonvig et al. *Ugeskr Laeger* 2014

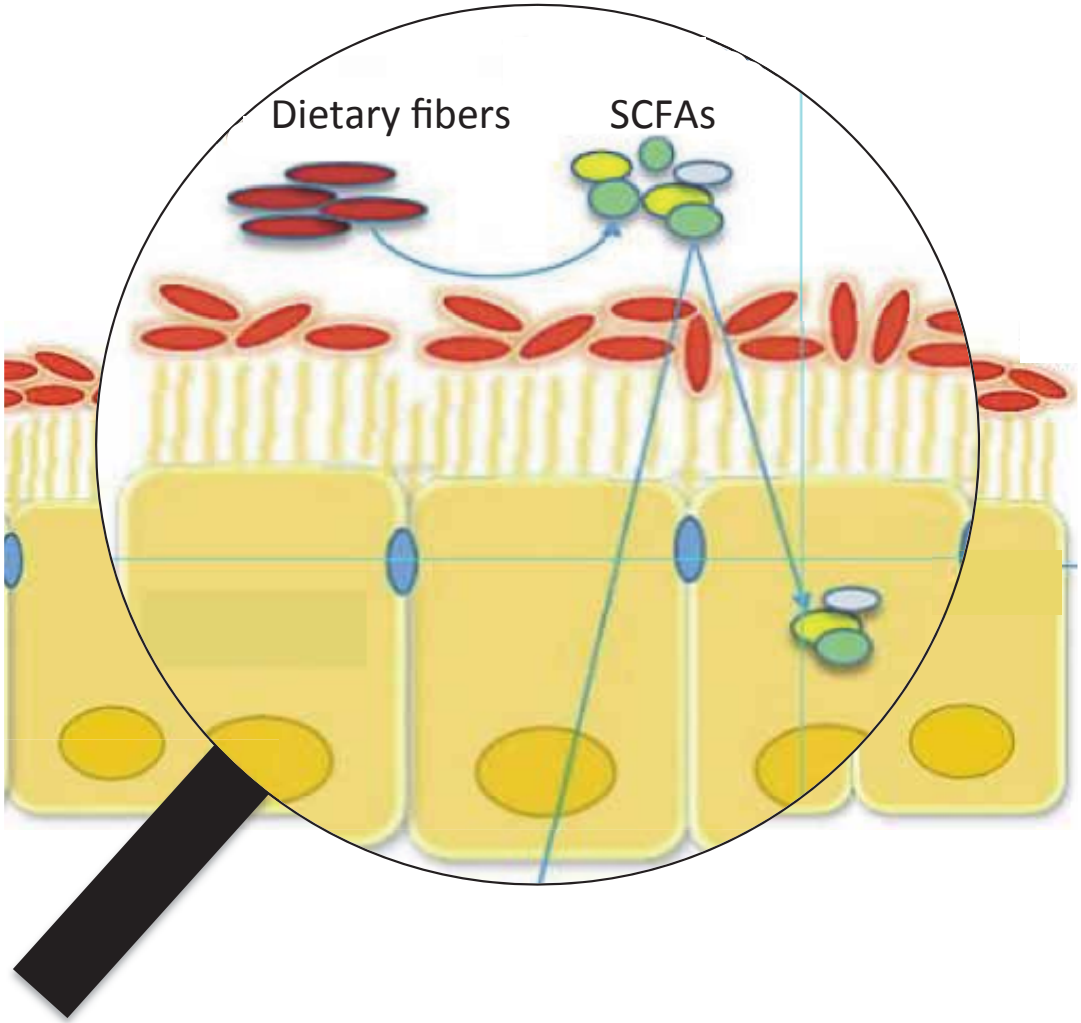
Study II

Hypothesis

A diet rich in AX and RS modulates the gut microbiota and increases SCFAs

Aim

Investigate the ability of AX and RS to modulate the gut microbiota and to affect the production of SCFAs



Modified, Fonvig et al. *Ugeskr Laeger* 2014

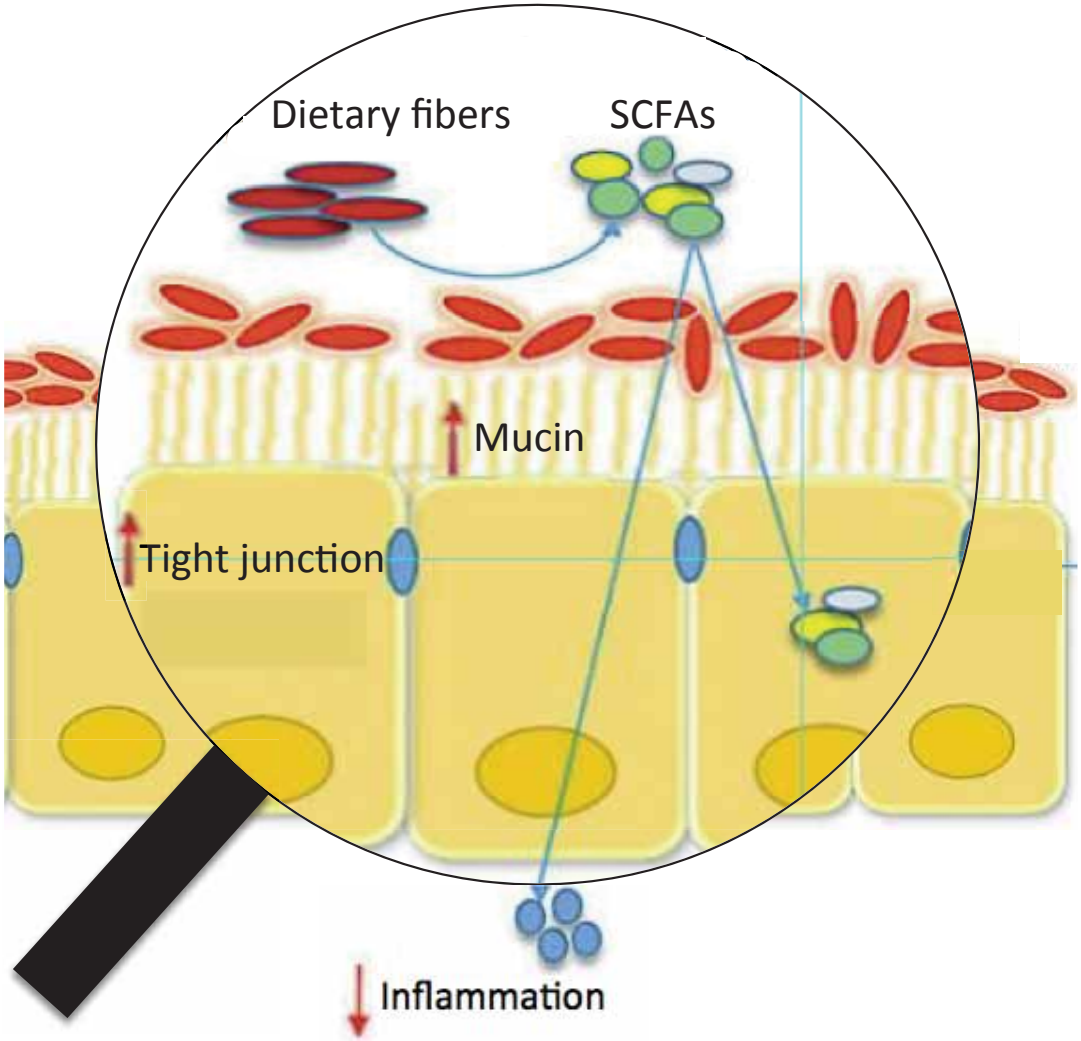
Study III

Hypothesis

AX and RS strengthen the gut barrier and reduce intestinal inflammation

Aim

Study the the mucosal expression of MUC2 and occludin and the degree of intestinal inflammation



Modified, Fonvig et al. *Ugeskr Laeger* 2014

Study I

Participants

22

12

Materials

Endoscopy with tissue samples

Faecal samples

Blood samples

Analyses

- Histology
- T-cell phenotype and activation (flow-cytometry)
- Inflammation-associated genes (qPCR)

Mucosal inflammation

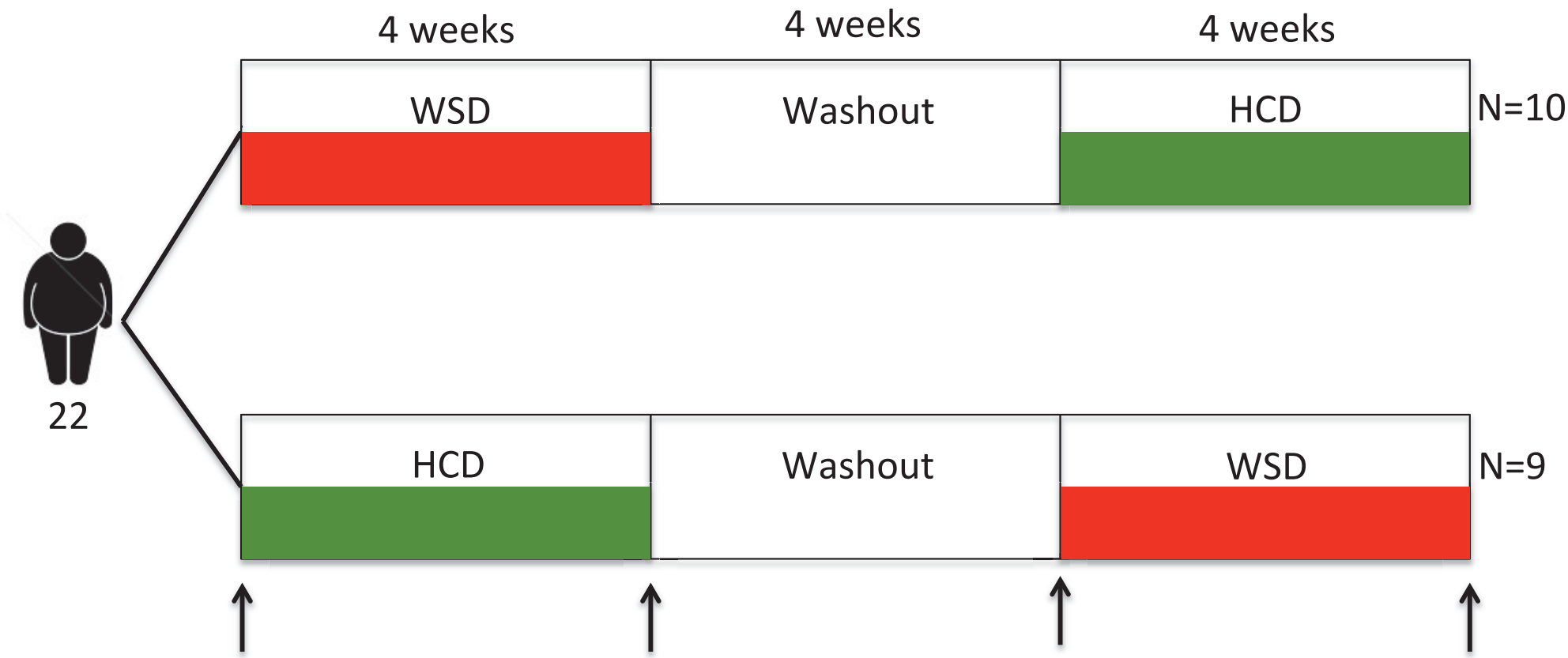
- Faecal calprotectin (ELISA)
- SCFAs (gas chromatography)

- Hs-CRP, IL-1Ra, IL-6, Adiponectin (ELISA)

Systemic inflammation

Studies II - III

Randomized crossover study with two diet interventions



Faecal samples (study II – III)

Endoscopy with tissue samples (study III)

Studies II - III

Healthy Carbohydrate Diet (HCD) and Western Style Diet (WSD)

~ 50% of the subjects' calculated daily energy needs

Studies II - III

Dietary composition of the diets

Daily intake from key foods	**WSD**	**HCD**
Energy (kJ)	5280	4722
Protein (g)	40	32
Fat (g)	17	17
Dietary fiber (g)	18	64
- Arabinoxylan (g)	4	16
- Resistant starch (g)	3	21

Methods - endoscopy

Perspective

Where do I want to go from here

Further investigate the possible immune modulatory effects of AX and RS

- Gene chip analysis

Correlate changes in gut microbiota with SCFA concentrations and inflammatory markers

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Conflict of interest
None

IMPACT OF MICROBIAL METABOLITES ON THE PERIPHERAL TISSUE AND INSULIN SENSITIVITY IN HUMAN SUBJECTS WITH METABOLIC SYNDROME

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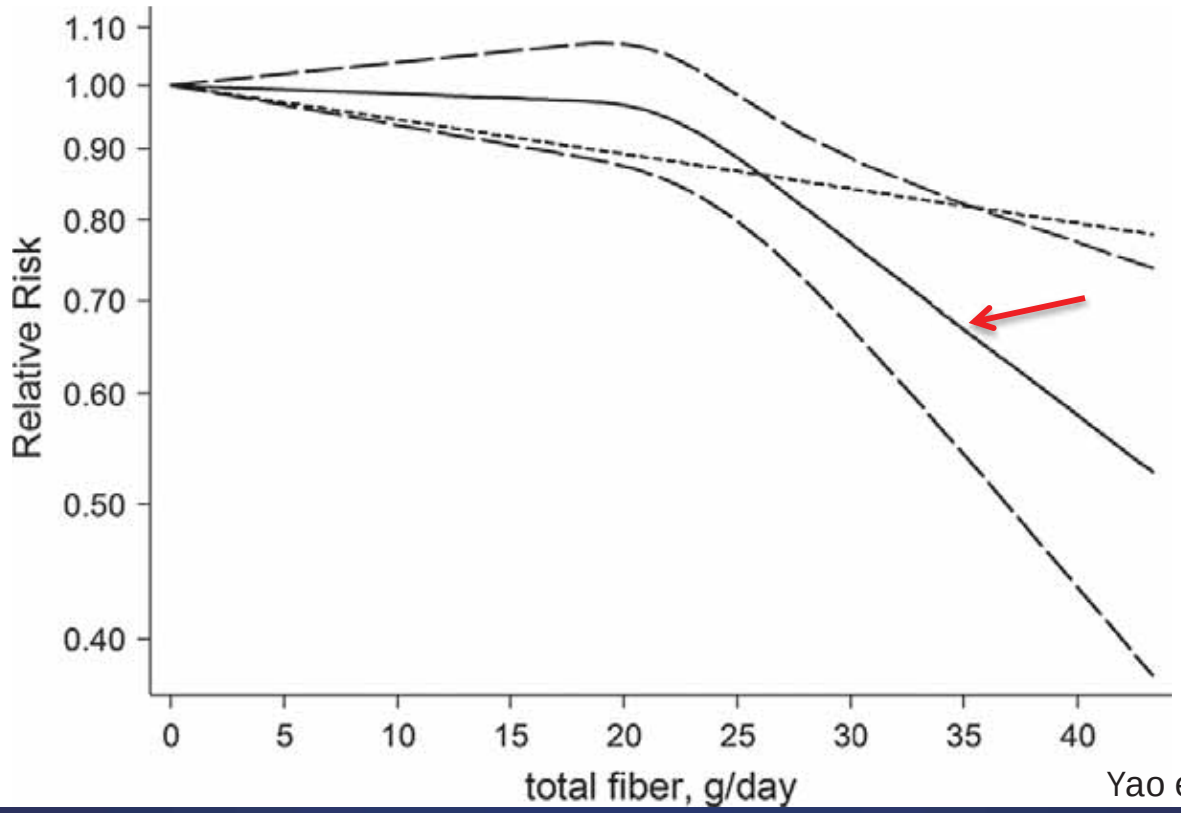
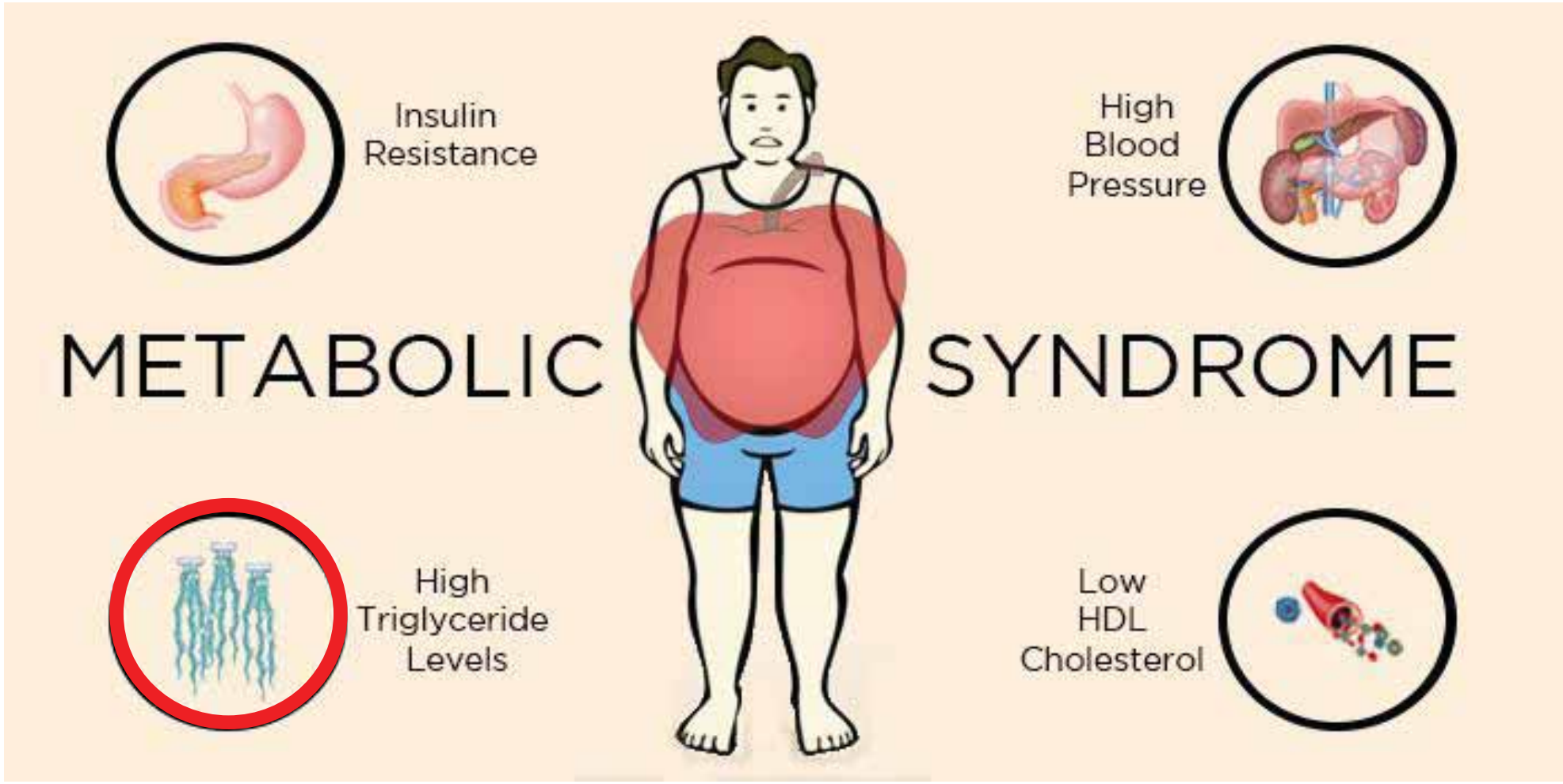
ANNE GRETHE SCHIOLDAN
LÆGE, PH.D.-STUDERENDE

BUTCOINS
2. MARTS 2016

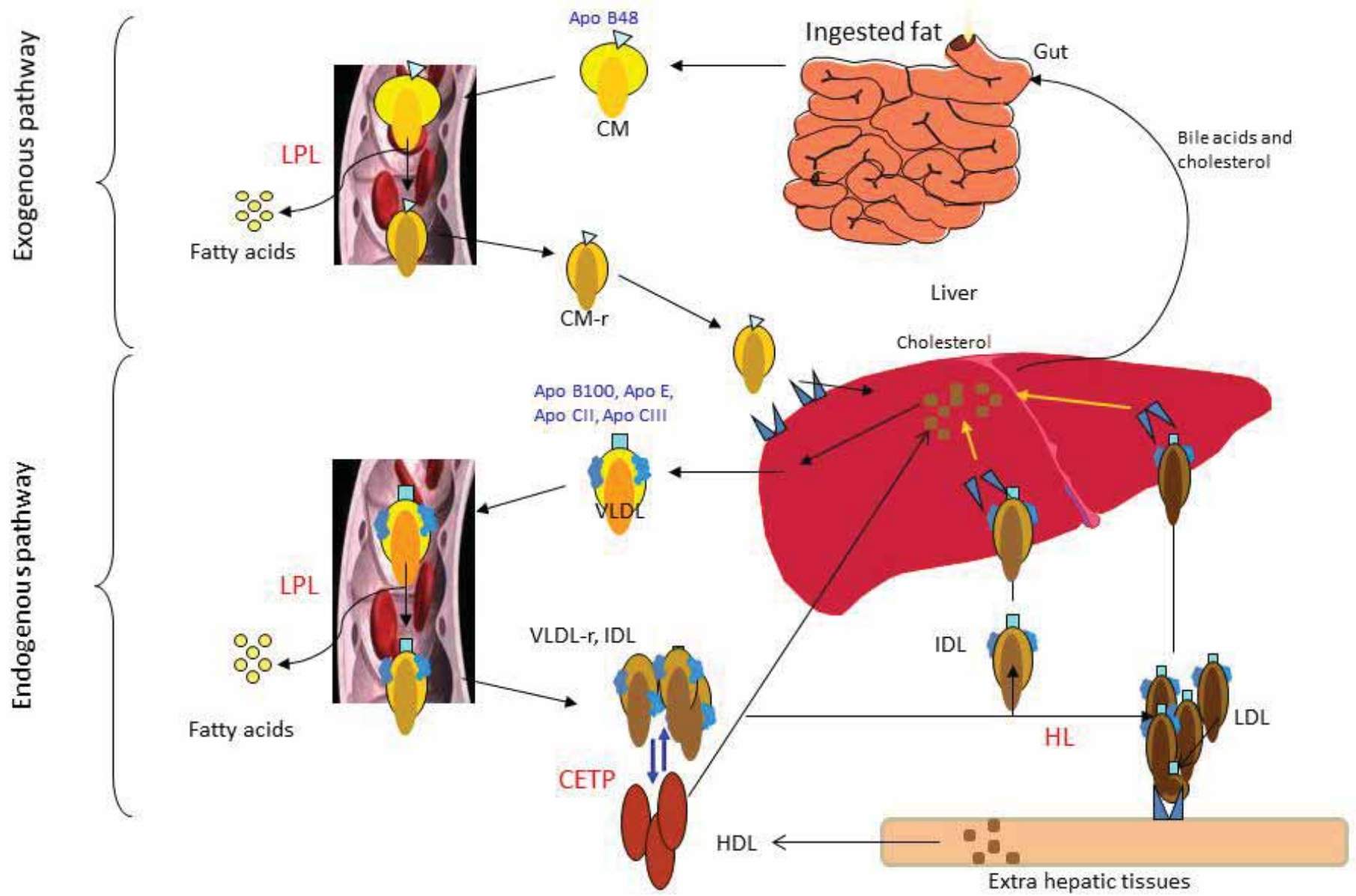
DIETARY FIBRE AND TYPE 2 DIABETES

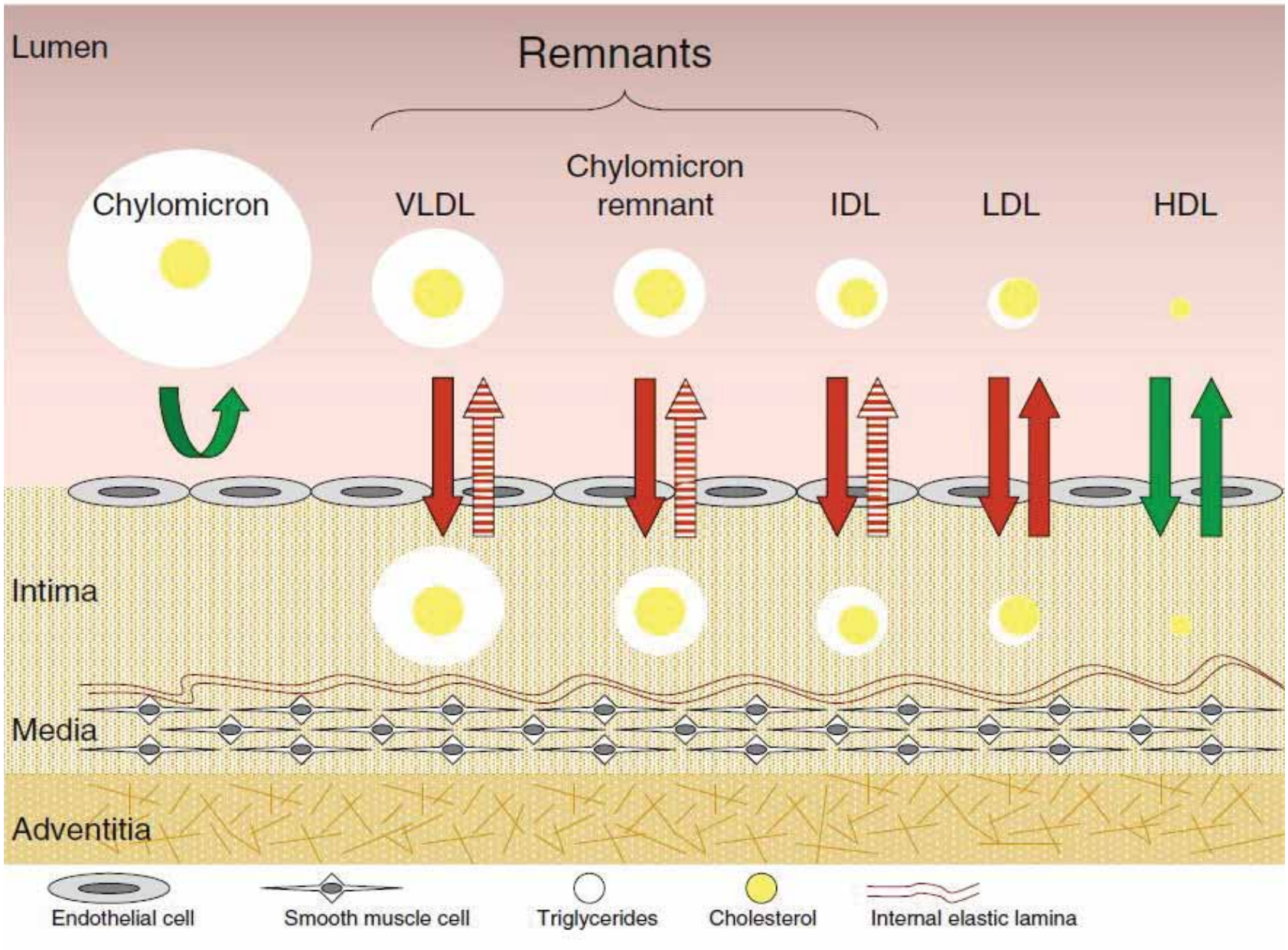


Yao et al. Eur J Epidemiol 2014


Insulin Resistance
High Blood Pressure
METABOLIC SYNDROME
High Triglyceride Levels
Low HDL Cholesterol

PATHWAYS INVOLVED IN DYSLIPIDAEMIA





Varbo et al., Pharmacology and Therapeutics (2014) 141: *358-367*

POSTPRANDIAL LIPAEMIA

- **Postprandial lipaemia: The increased level of plasma triglyceride following meal**
- **Non-fasting triglyceride between 2 and 10 mmol/l markedly increases the risk of cardiovascular disease**
- **Abdominally obese subjects exhibit greater postprandial triglyceride responses than lean controls despite normal fasting triglyceride**

Nordestgaard BG, Varbo A (2014). Lancet 384:626-635

Mekki N, et al. (1999) J Clin Endocrinol Metab 84:184-191

STUDY 1

Hypothesis:

A diet rich in arabinoxylan and resistant starch improve postprandial lipaemia and postprandial glucose regulation

Aim:

Investigate the effect of arabinoxylan and resistant starch on postprandial responses of circulating triglycerides, chylomicrons (apoB-48), FFA, glucose, insulin, glucagon, GLP-1 and GLP-2. Furthermore, fasting cholesterols were measured.

DESIGN

Healthy carbohydrate diet (HCD)

Western-style diet (WSD)

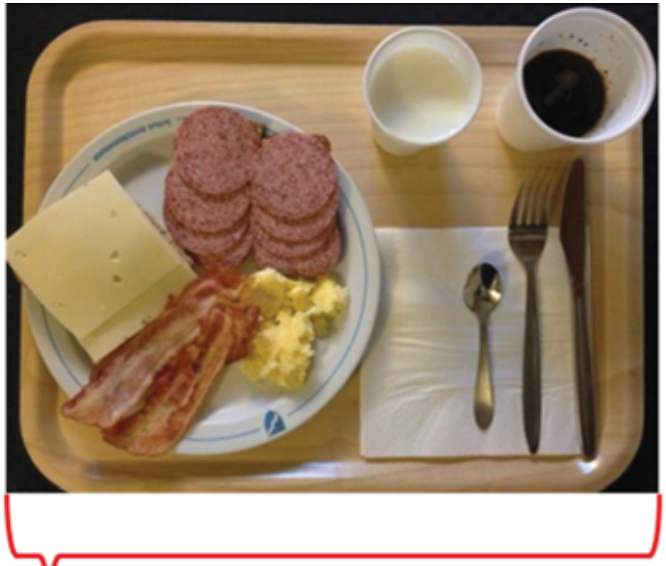
4 weeks washout

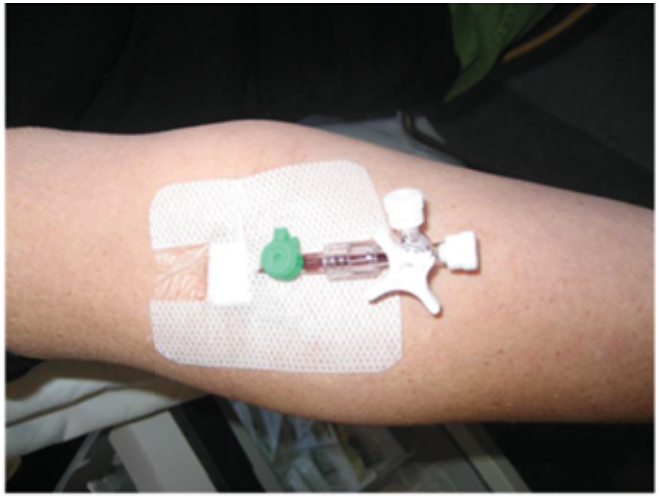
Total dietary fibre	63.9 g/dag	Total dietary fibre	17.7 g/dag

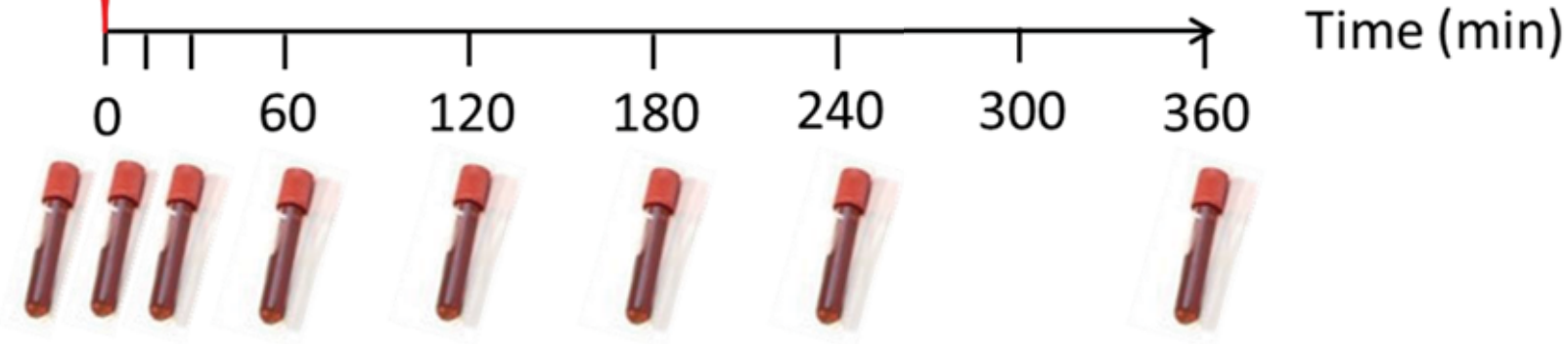
BASELINE CHARACTERISTICS

	Median (Range)
Gender (n)	5 F/14 M
Age (years)	60 (40-75)
BMI (kg/m^2)	30.6 (25.9-41.0)
Waist (cm)	106 (89-130)
Systolic blood pressure (mmHg)	140 (124-164)
Diastolic blood pressure (mmHg)	90 (84-105)
Fasting P-Glucose (mmol/l)	6.3 (5.4-6.9)
Total cholesterol (mmol/l)	5.4 (3.8-7.1)
HDL cholesterol (mmol/l)	1.3 (0.78-3.7)
LDL cholesterol (mmol/l)	3.3 (1.5-5.3)
Triglyceride (mmol/l)	1.5 (0.9-4.0)
Statin treatment (n)	7
Antihypertensive treatment (n)	7

STANDARD MIXED MEAL TEST

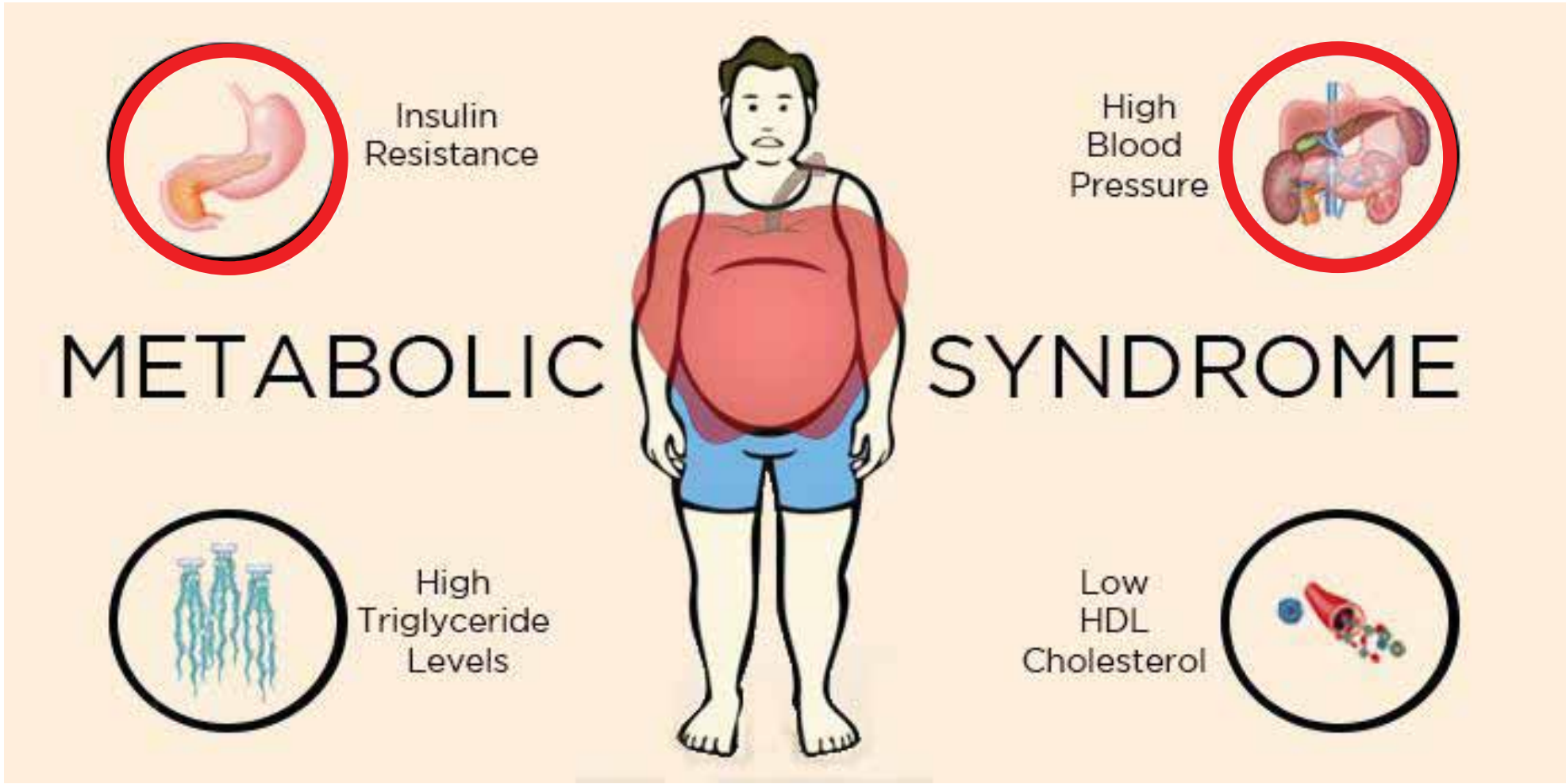






CONCLUSIONS FROM STUDY 1

- Postprandial lipaemia was not significantly reduced
- No other improved postprandial responses
- Significant interaction between statin and diet treatment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METABOLIC SYNDROME
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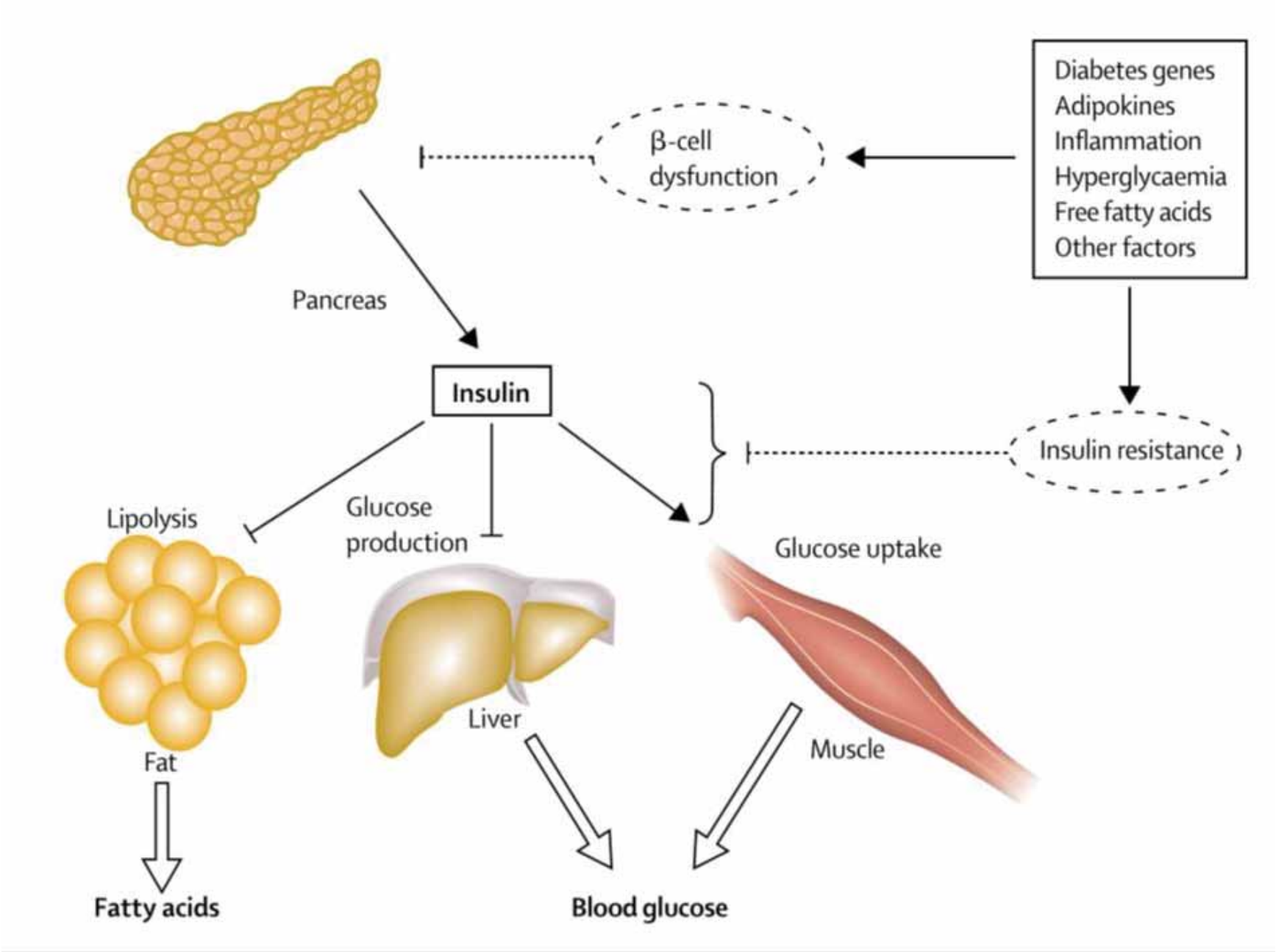
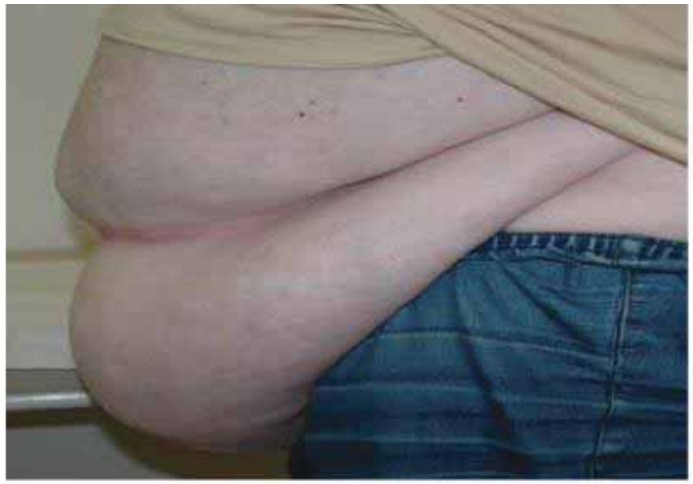
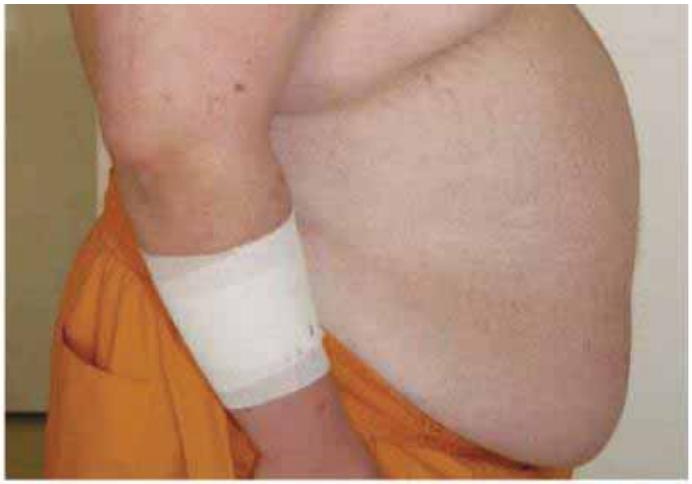
Low HDL Cholesterol

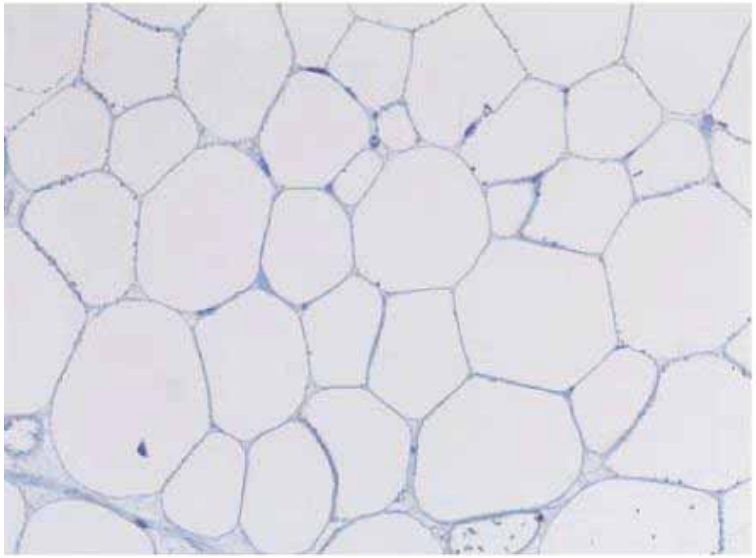


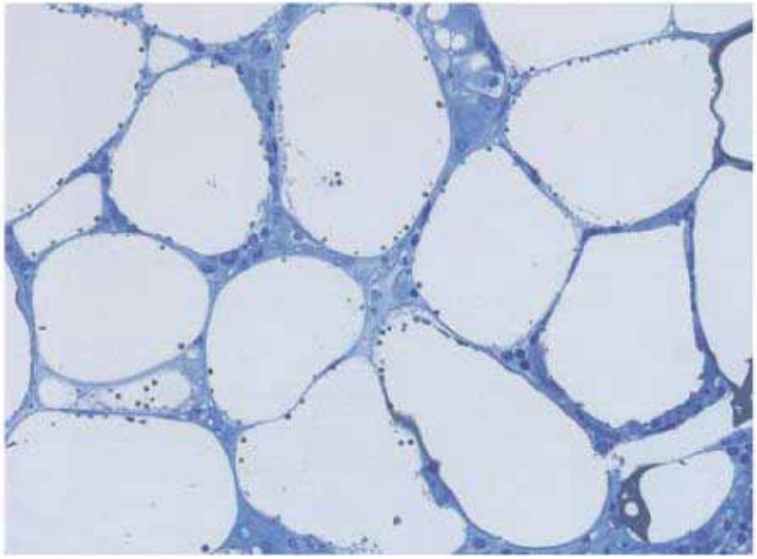
Stumvoll et al., The Lancet (2005) 365: *1333-1346*

INSULIN SENSITIVITY AND RESISTANCE



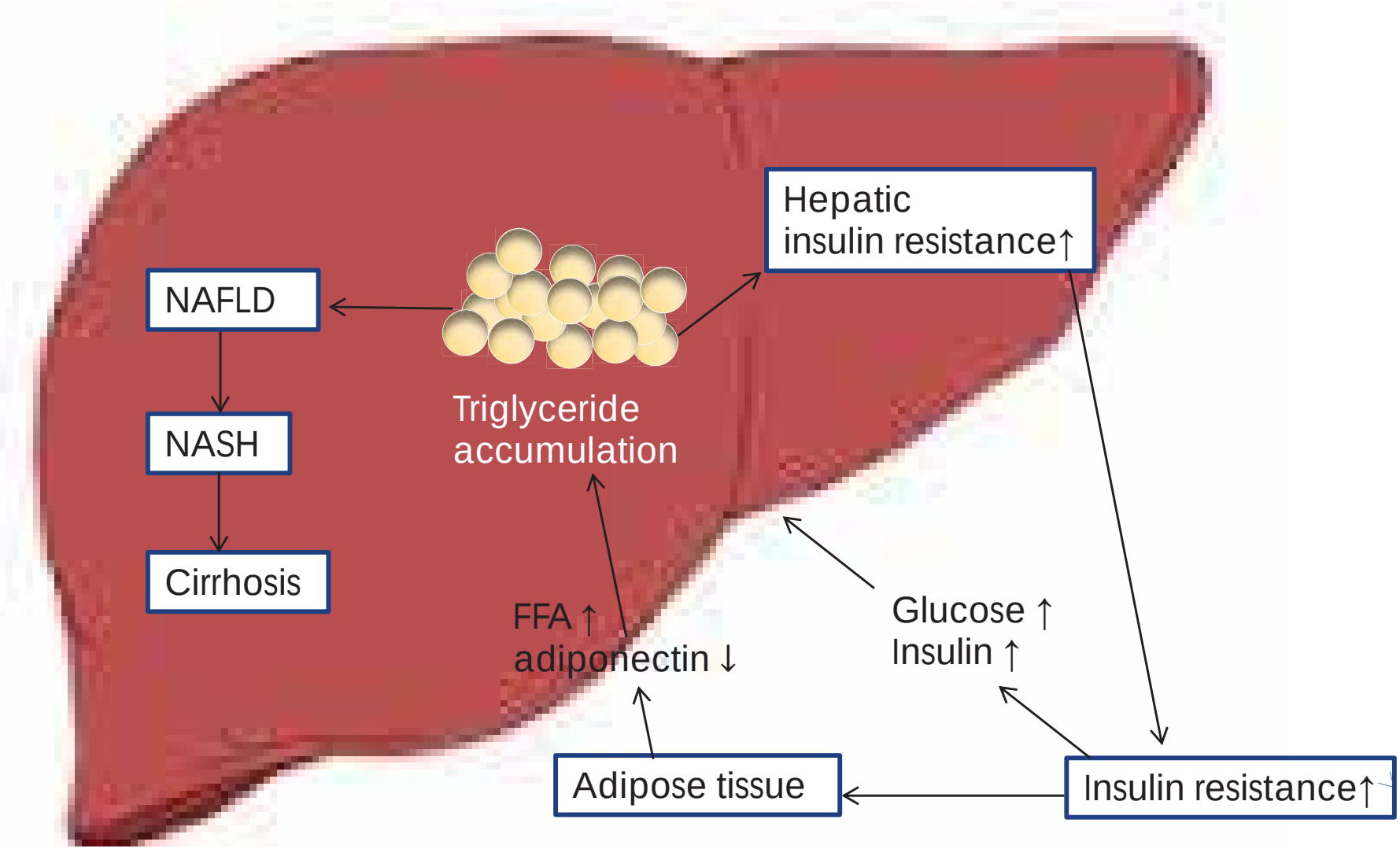






Klöting et al., American Journal of Physiology (2010) doi: 10.1152

HEPATIC STEATOSIS



STUDY 2

Hypothesis:

A diet rich in arabinoxylan and resistant starch decrease insulin resistance, intrahepatic lipid content, low-grade inflammation and blood pressure compared with a western-style diet

Aims:

Investigate whether a diet rich in arabinoxylan and resistant starch would improve insulin sensitivity, decrease intrahepatic lipid content and circulating low-grade inflammatory markers and blood pressure

DESIGN

Healthy carbohydrate diet (HCD)

4 weeks washout

Western-style diet (WSD)

CONCLUSIONS FROM STUDY 2

- Insulin resistance was not reduced by arabinoxylan and resistant starch
- Intrahepatic lipid content remained stable throughout the study
- Inflammatory markes were not significantly changed by the diets
- The blood pressure was not affected by the diets

PERSPECTIVES

- Longer intervention trials
- Measurement of the hormone PYY
- Weight loss vs. Weight stability
- Statin interaction with dietary fibre

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Department of Food Science and Technology,

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Mary E. Moore

Maria Marco

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KMC

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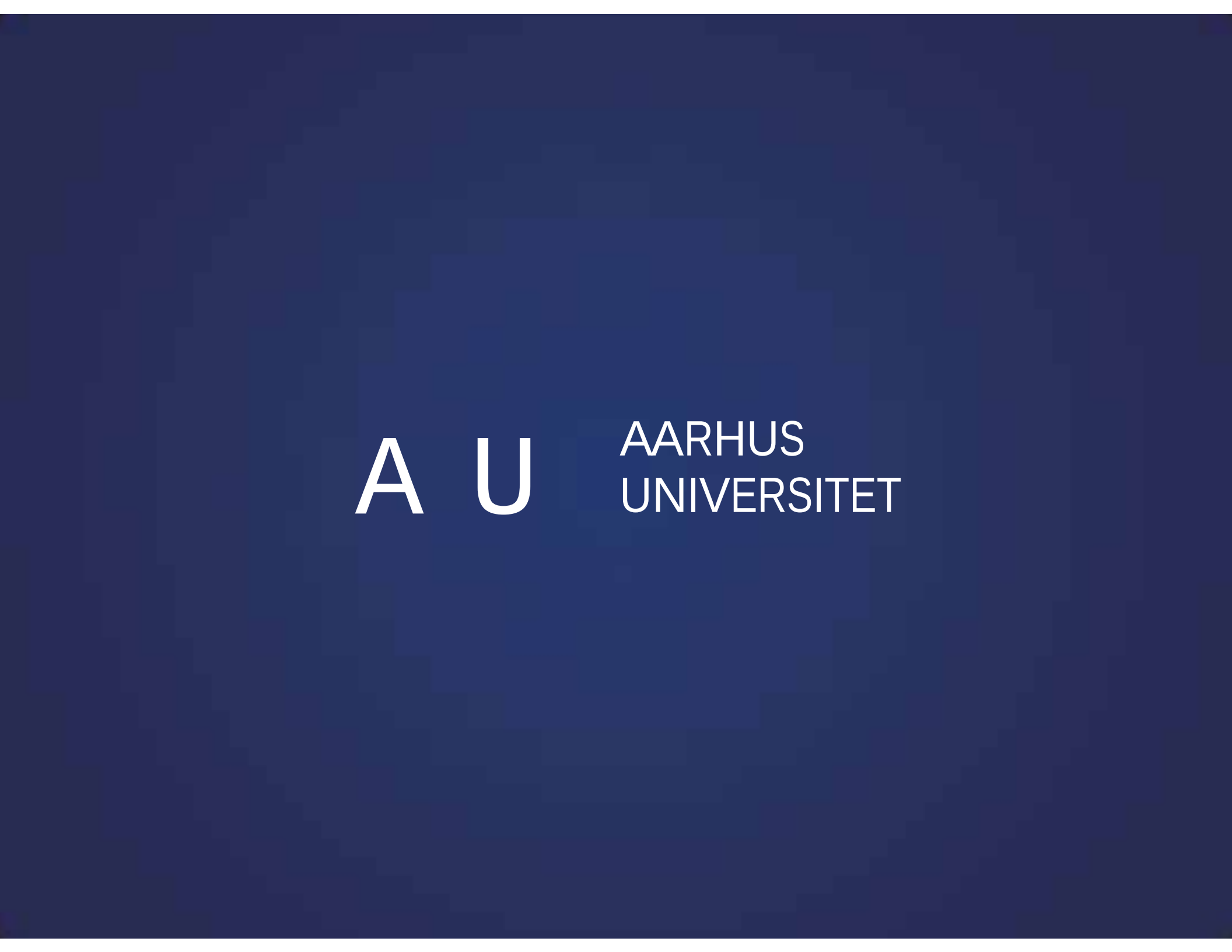
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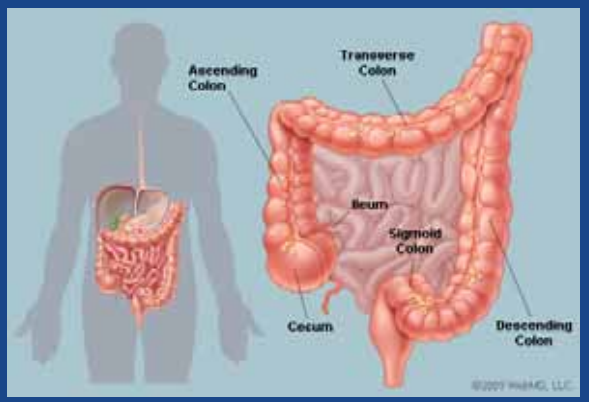
2 March 2016

ButCoIns – concluding remarks

Knud Erik Bach Knudsen
Department of Animal Science



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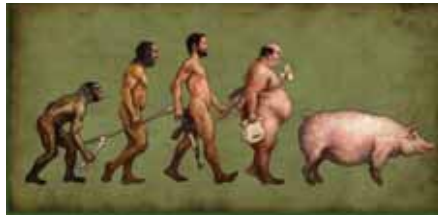
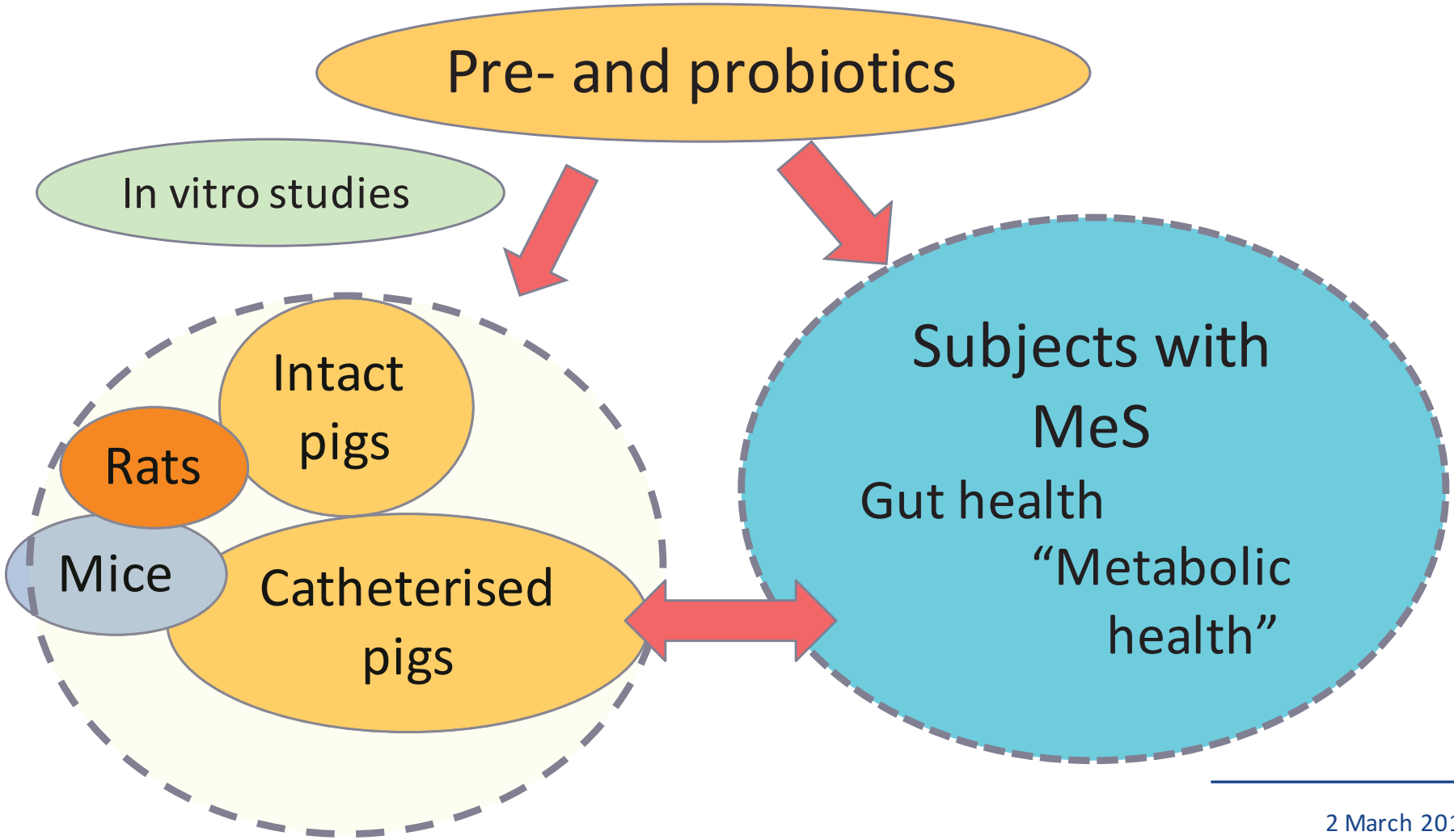


ButCoIns: Research elements



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Publications

Peer reviewed:

Ingerslev, A. K., P. K. Theil, M. S. Hedemann, H. N. Lærke, K. E. Bach Knudsen (2014). Resistant starch and arabinoxylan augment SCFA absorption, but affect postprandial glucose and insulin responses differently. *British Journal of Nutrition* 111, 1564-1576.

Nielsen T. S., H. N. Lærke, P. K. Theil, J. F. Sørensen, M. Saarinen, S. Forssten and K. E. Bach Knudsen (2014). Diets high in resistant starch and arabinoxylan modulate digestion processes and SCFA pool size in the large intestine and faecal microbial composition in pigs. *British Journal of Nutrition* **112**, 1837–1849.

Ingerslev, A.K., I. Karaman, M. Bağcıoğlu, A. Kohler, P. K. Theil, K. E. Bach Knudsen, M. S. Hedemann (2015). Whole grain consumption increases gastrointestinal content of sulfate-conjugated oxylipins in pigs – A multicompartmental metabolomics study. *Journal of Proteom Research* **14**, 3095-3110.

Nielsen, T. S., P. K. Theil, S. Purup, N. P. Nørskov, K. E. Bach Knudsen (2015). Effects of resistant starch and arabinoxylan on parameters related to the large intestinal and metabolic health in pigs fed fat-rich diets. *Journal of Agricultural and Food Chemistry* **63**, 10418-10430

Vangsøe, C. T., A. K. Ingerslev, P. K. Theil, M. S. Hedemann, H. N. Lærke, K. E. Bach Knudsen (2016). *In vitro* starch digestion kinetics of diets varying in resistant starch and arabinoxylan compared with *in vivo* portal appearance of glucose in pigs. *Food Research International* xxx, xxx-xxx (online) doi.org/10.1016/j.foodres.2016.02.005.

Nielsen, T. S., B. B. Jensen, P. K. Theil, S. Jackson, M. Saarinen, S. Forssten, S. Purup, K. E. Bach Knudsen (2016). Searching for synbiotics: Effects of enzymatically modified arabinoxylan and Butyrivibrio fibrisolvens on short-chain fatty acids in cecum content and plasma of rats. *Food & Function* **xx**, xxx-xxx (online) DOI: 10.1039/C6FO00114A.

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Publications

PhD thesis:

Ingerslev, A. K. (2015). The impact of short-chain fatty acids on metabolic responses - Studies in pigs fed diets with contrasting sources and levels of dietary fibres. PhD thesis Faculty of Science and Technology, Aarhus University.

Stine Hald. (2015). Effects of dietary fibres on gut microbiota, faecal short-chain fatty acids and intestinal inflammation in the metabolic syndrome. PhD thesis. Health, Aarhus University.

MSc thesis:

Nielsen, D. S. G. (2013). Colonic health in humans - in vitro effects of fermentation products on epithelial integrity parameters obtained by cellular- and transcriptome analysis. MSc thesis, Faculty of Science of Technology Aarhus University.

Dahl, R. C. (2013): Establishment of an adipocyte cell-based model to investigate the effect of short-chain fatty acids, especially butyrate, on insulin sensitivity and glucose homeostasis in fat tissue. MSc thesis, Faculty of Science of Technology Aarhus University.

BSc thesis:

Rydtoft, S. M. (2012). Effects of dietary fibre on glucose and insulin responses in pigs. BSc thesis, Faculty of Science of Technology Aarhus University

Vangsøe, C. T. (2014). In vitro starch digestion kinetics of diets varying in resistant starch and arabinoxylan compared with in vivo portal appearance of glucose in pigs. BSc thesis, Faculty of Science of Technology Aarhus University.

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