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Perinatal antibiotic treatment of sows affects intestinal barrier and immune system in offspring

European project INTERPLAY – WP6 task 6.1

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INRA SCIENCE & IMPACT

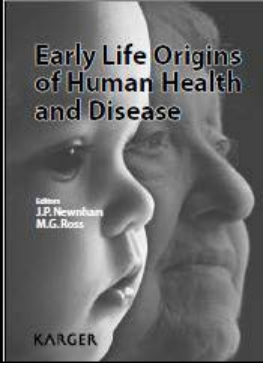
DNc Alimentation & Adaptations Digestives Nerveuses et Comportementales

SEVENTH FRAMEWORK PROGRAMME

INTERPLAY

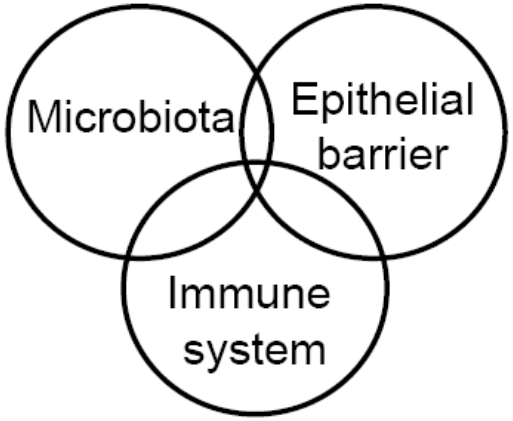

Background

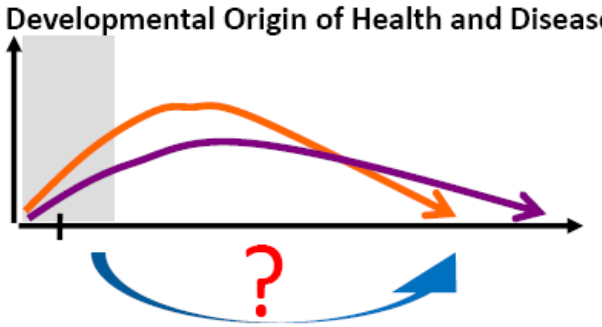
- Association between early life nutrition and health later in life has emerged = perinatal programming
- Neonatal period = key period in acquisition of gut microbiota and education of immune system, especially towards LPS
- Disturbances of microbiota colonization -> alter mucosal and systemic immunity leading to asthma, allergy, obesity…



Hypothesis

Post-natal intestinal development





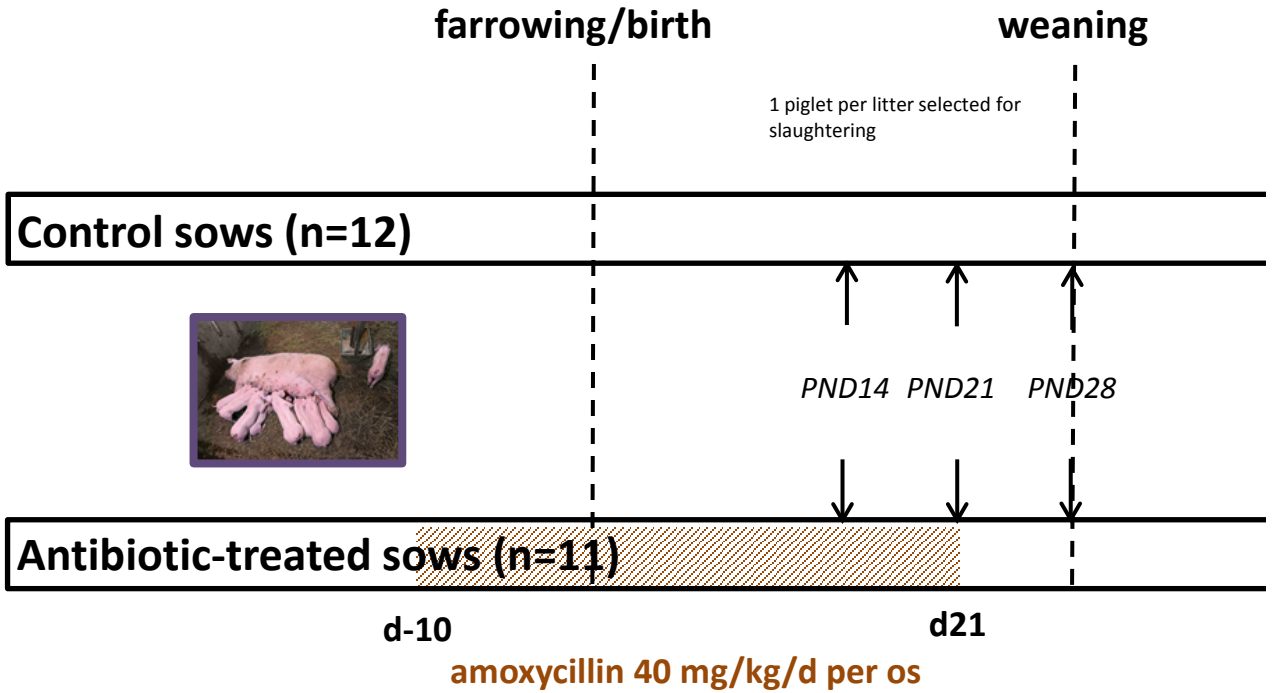
- Altering microbiota implantation during early life would change the post-natal development of enterocyte functions and local immune cell sensitivity to bacterial products

- This will have consequences on their responses to a High Fat (HF) diet later in life

Objectives

- Use of peripartum antibiotic treatment to alter piglet microbiota acquisition
- Investigate the post-natal development of the intestinal barrier function and transcriptome as well as GALT education towards LPS in piglets born to ATB sows
- Analyze their gut adaptation to a HF diet later in life

Experimental protocol



	LF	HF
Energy (kcal/g)	410	455
% of energy		
protein	17	14
carbohydrate	79	64
fat	4	22

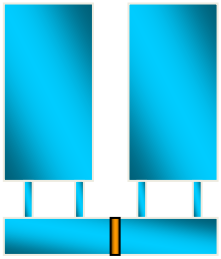
Maternal and offspring microbiota

Changes in microbiota diversity in collaboration with O. Perrez-Gutteriez, J. Zhang, H. Smidt (WUR, Netherlands)

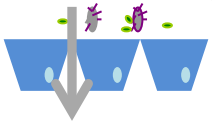
- Peripartum antibiotic treatment modified sow faecal microbiota profile

- Maternal antibiotic treatment reduced piglet ileal microbiota diversity

Ileal barrier function during the post-natal period

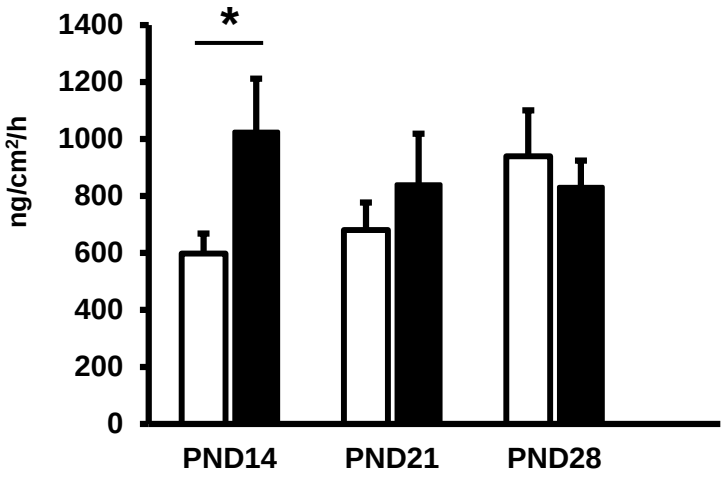


Ileum mounted in Ussing chambers

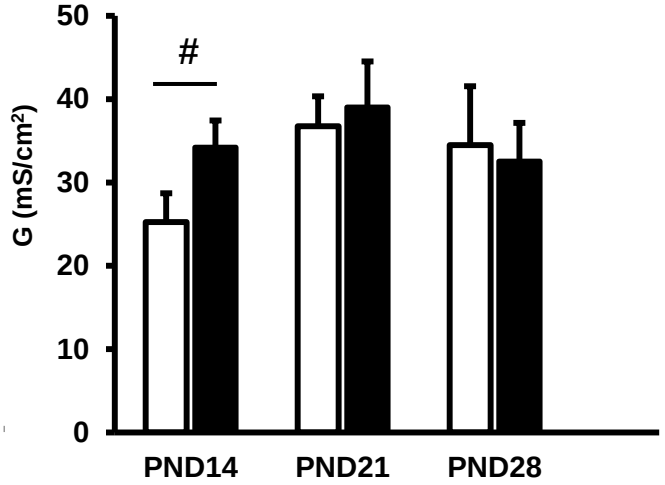


CTRL
ATB
* P<0.05
# P=0.07

Paracellular permeability (FD4 flux)



Tissue conductance

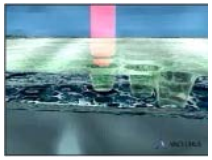


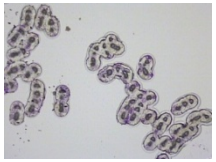
=> The maternal antibiotic treatment was associated with early ileal barrier function defaults, as in rodents *(Fåk et al 2008)*

Transcriptomic analysis of laser-captured enterocytes from 21 day piglets

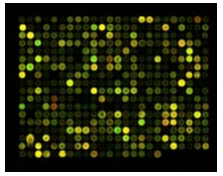
Laser capture microdissection

Enterocytes of 21 day piglets





RNA extraction

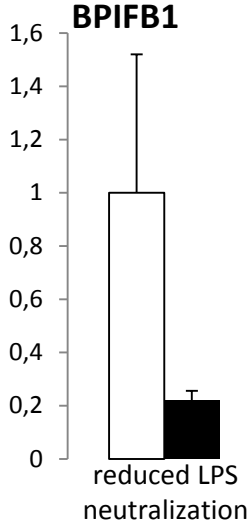


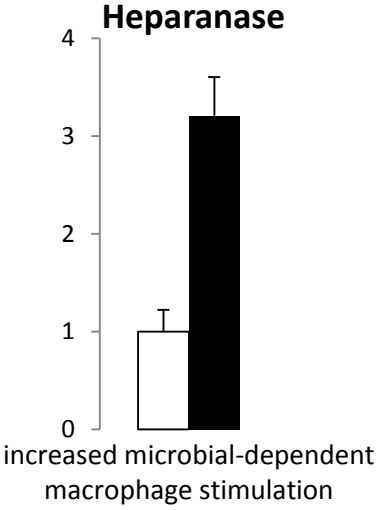
Expression analysis using a 60K porcine microarray

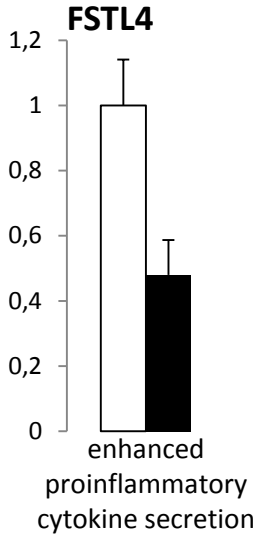
32 (29 annotated) differentially expressed genes in ATB *vs* CTRL (p<0.001) of which :

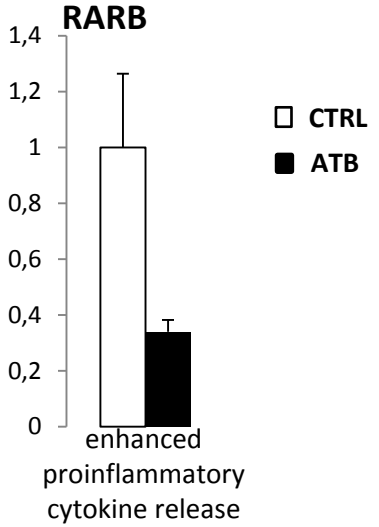
- 11 dow-regulated genes (< 0.5)
- 16 up-regulated genes (> 2)

} *protein catabolism, **immune response**, energetic metabolism*





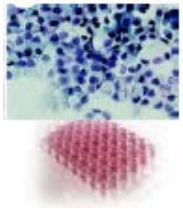




=> Changes on enterocyte transcriptome could orientate local macrophages towards a pro-inflammatory profile in ATB group

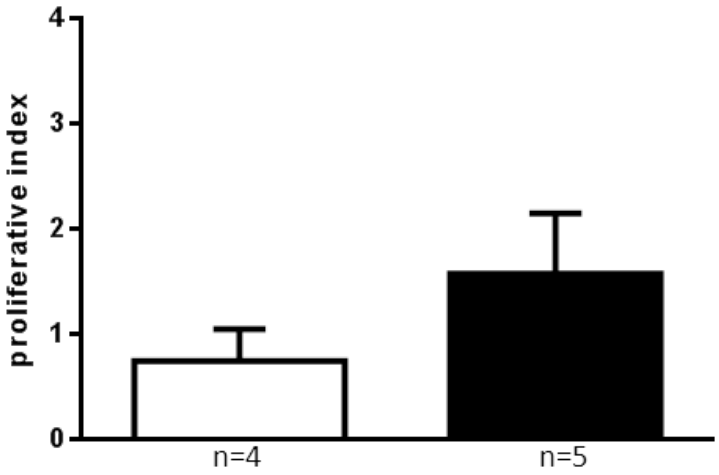
Education of the local immune system of 21 day piglets

Isolation and purification of lamina propria mononuclear cells (LPMC) from PND21 piglets
-> culture with LPS for 72hrs



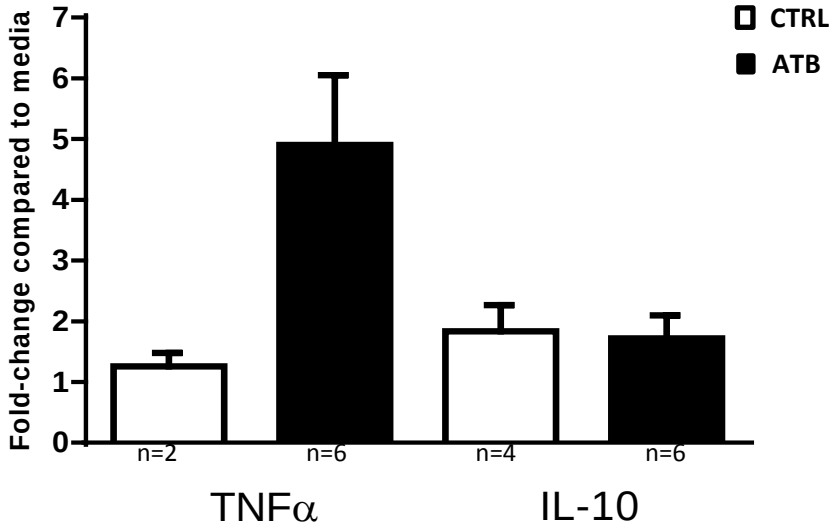
Proliferative index

Incorporation of ^{3}H-thymidine



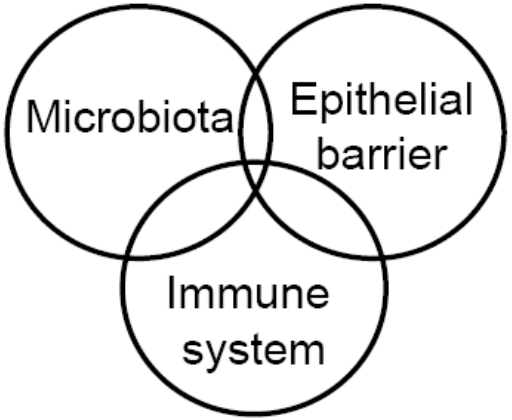
Cytokine secretion

TNFα and IL-10 ELISA assays



=> LPMC were still tolerant to LPS in both groups
=> Cytokine responses seemed to be oriented towards a pro-inflammatory profile in LPS-challenged LPMC of ATB piglets

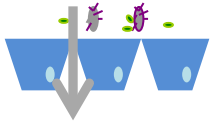
Peripartum antibiotic treatment affects the molecular cross-talk between commensal bacteria, epithelial barrier and immune cells in the early period of life.



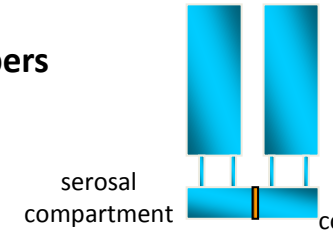
Is the adaptation to a HF diet later in life modified?

Ileal barrier function in adults

Adult pigs deriving from CTRL or ATB sows given either a LF or a HF diet for 4 weeks

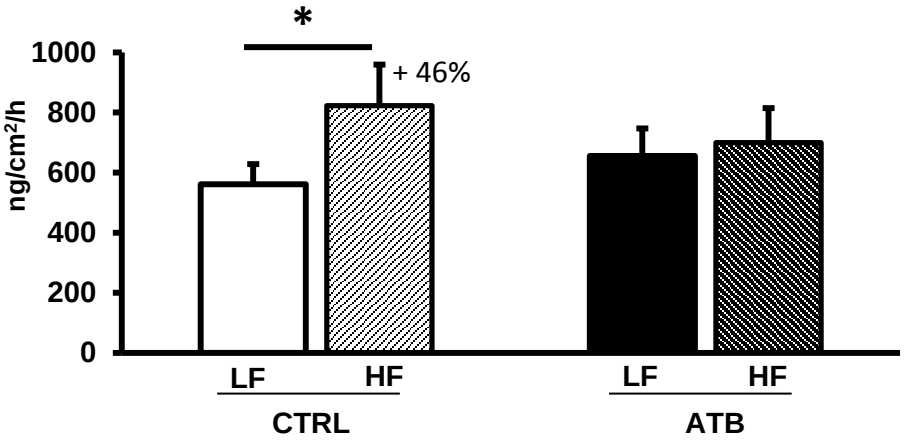


Ileum mounted in Ussing chambers

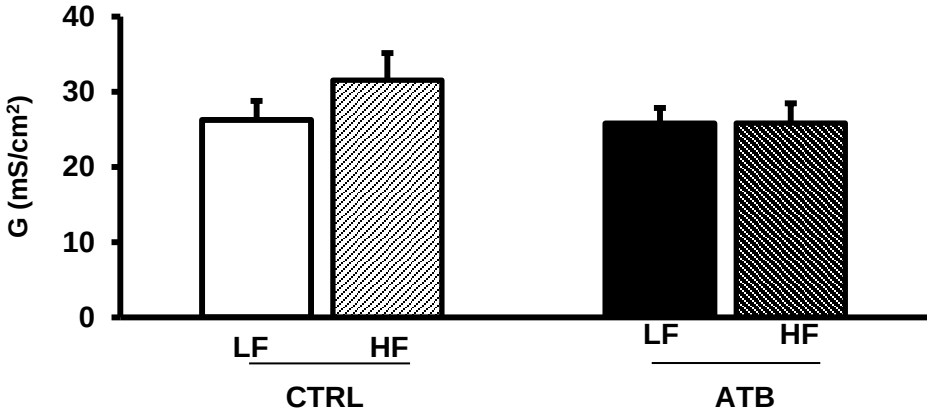


* P<0.05

Paracellular permeability (FD4 flux)



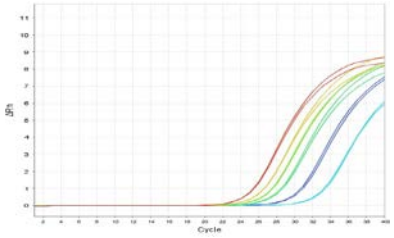
Tissue conductance



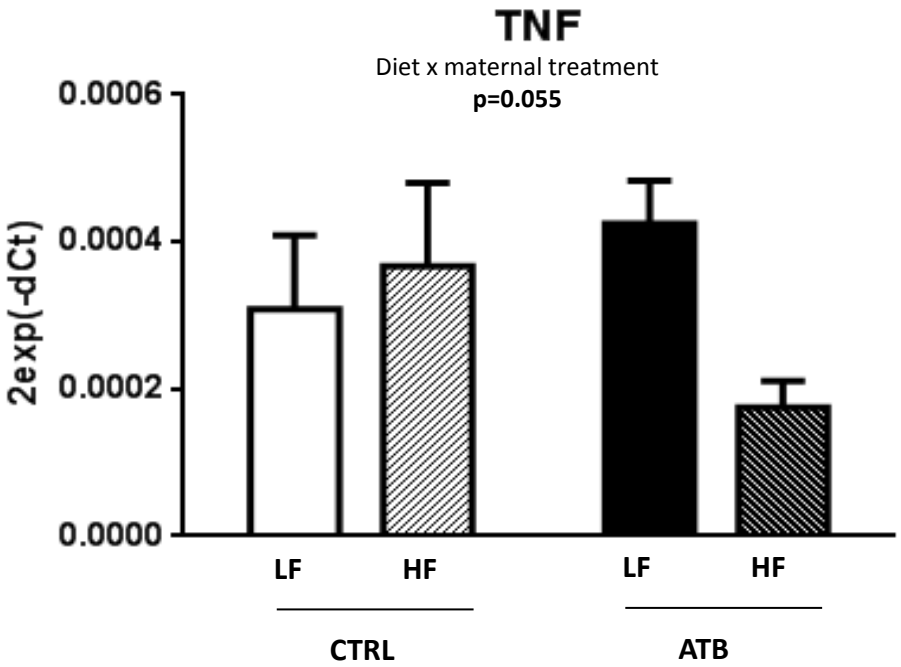
=> CTRL pigs displayed increased ileal permeability under a HF diet

=> Such an increase was not observed in ATB pigs *(Cani et al 2008)*

Gene expression in adult ileum



qPCR on ileum extracted RNA



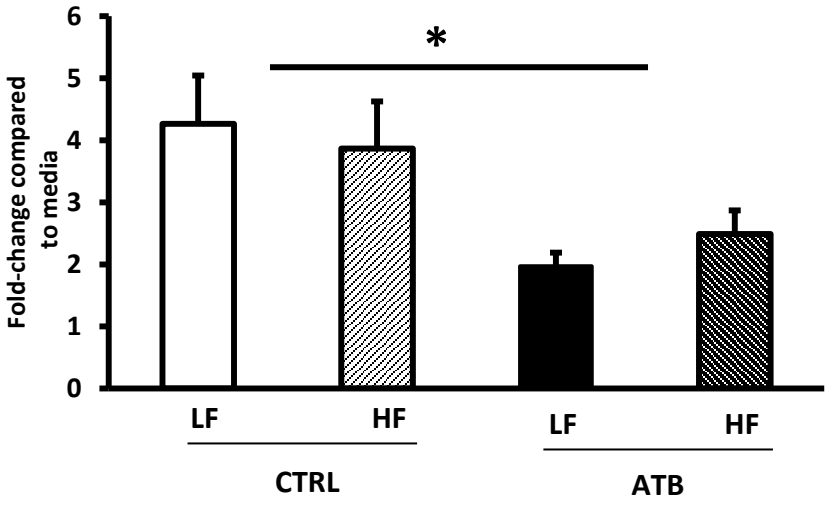
=> TNFα gene expression tended to decrease in ATB-HF

TNFα secretion by ileal biopsies

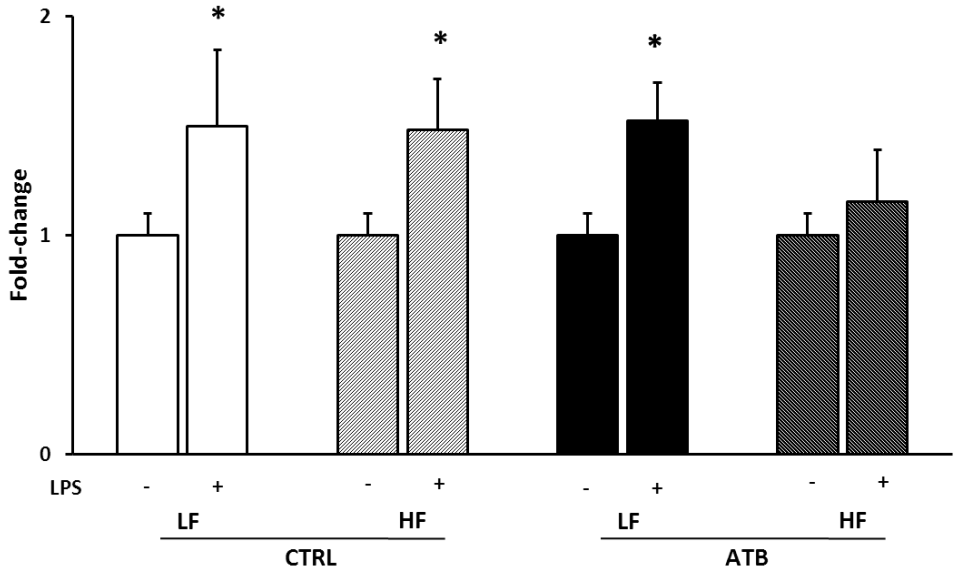
Ileal biopsies cultivated for 20hrs with media, PWM or 100 µg/mL LPS
TNFα secretion in supernatant (ELISA)

* P<0.05

TNFα secretion in response to PWM



TNFα secretion in response to LPS



=> TNFα secretion was blunted in ATB pigs
=> The sensitivity to LPS was altered in ATB-HF pigs

Summary and conclusion

Maternal antibiotic treatment

during the neonatal period:

-modified piglet ileal microbiota;
-increased ileal permeability;
-altered cross-talk between microbiota, enterocytes and immune cells (LPMC response to LPS) towards a pro-inflammatory profile;

later in life:

-did not modify ileal barrier function in LF-fed animals but prevented the HF diet-induced increase in permeability;
-blunted the TNFα response of ileal biopsies to inflammatory stimuli, especially in ATB-HF pigs (corroborating gene expression).

The gut response to a HF diet is dependent upon early-life microbiota colonization

Acknowledgments

Alimentation & Adaptations Digestives Nerveuses et Comportementales

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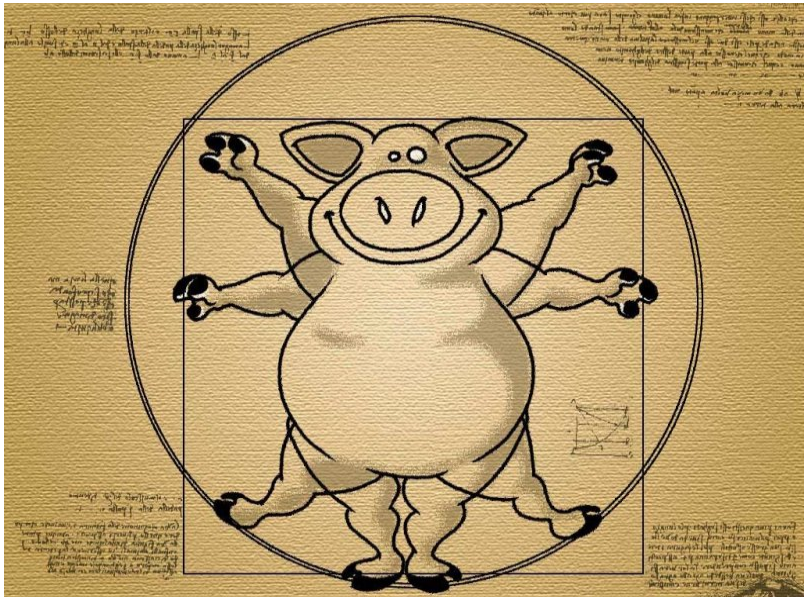

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