Dietary proteins: Goodness and warnings for weight management

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High-protein diets (HPD)

- No definition of the maximal amount of dietary protein with no side effects.
- HPD are contraindicated in individuals with or at risk for chronic kidney diseases (Ko et al. Curr Opin Clin Nutr Metab Care 2017).

What is the balance between advantages of weight loss/metabolic health and the impact on the gut ecosystem?
Numerous bacterial metabolites

Undigested and not fully digested dietary and endogenous proteins

From Blachier et al. Clin Nutr 2018
Amino acid-derived bacterial metabolites and colon/rectum

Dietary and endogenous proteins
- Oligopeptides/Amino acids
  - Portal vein
  - Liver
  - Other tissues

Undigestible polysaccharides

Large intestine
- Residual proteins/peptides
  - Microbiota
  - Urease

Liver/Peripheral tissues
- Microbiota

Short-chain fatty acids
- Microbiota

Bacterial metabolites and co-metabolites
- Energy metabolism
  - Genotoxicity/Cytotoxicity
  - Epithelium renewal and barrier function

What experimental strategy did we used to test the impact of HPD on gut health?

The most simple: *in vitro* tests of the bacterial metabolites on the colonic epithelial cell.

A bit more complicated: to test in animal models the bacterial metabolites that are suspected to be active (intra-colonic instillation in anesthetized rodents).

Even more complicated: nutritional intervention in animal models and impact on the colorectal mucosa.

Very complicated: nutritional intervention in randomized double-blind clinical trial against placebo in volunteers and impact on the gut ecosystem.

Unbound bacterial metabolites

Bound bacterial metabolites

DIFFUSION

TRANSPORT

Bacterial metabolites

Intracellular metabolism

Co-metabolites

Bloodstream

Liver and peripheric organs

Kidney

Accumulation in urine

Luminal content

Impact on colonocyte metabolism and functions

Blachier et al. Clin Nutr 2018
Effects of amino acid-derived bacterial metabolites on colonocytes

*p-cresol* produced by the microbiota from tyrosine increases anion superoxide and DNA damage (Andriamihaja et al. Free Radic Biol Med 2015) → *p-cresol in excess is a genotoxic agent for colonocytes*
Effects of amino acid-derived bacterial metabolites on colonocytes

H$_2$S at low concentration $\rightarrow$ detoxified by oxidation in colonocytes allowing ATP synthesis. At higher concentrations $\rightarrow$ H$_2$S inhibits colonocyte respiration. **Intracolic instillation of NaHS** in excess increases the expression of inflammation-related genes in colonocytes (Beaumont et al. Free Radic Biol Med 2016) $\rightarrow$ H$_2$S oxidative substrate or « metabolic trouble maker » depending on its luminal concentration (reviewed in Curr Opin Clin Nutr Metab Care)
Effects of HP-diet on the colonic ecosystem in rodents

HPD given for 2 weeks to rats decreased in colonocytes the expression of genes related to
- cellular metabolism,
- NF-kappaB signaling,
- DNA repair,
- glutathione metabolism
- cellular adhesion;
while upregulating the genes related to
- cell proliferation
- chemical barrier function.

However, HPD displayed no genotoxic effect on colonocytes, and altered neither colonic epithelial renewal nor colonic barrier integrity. We proposed that the transcriptional regulation observed after HPD might result from an adaptive process (Beaumont et al. BMC Genomics 2017)
**My New Gut**: Human dietary intervention study with high-protein diets

- Randomized double-blind parallel-design trial with 38 overweight individuals
- Inclusion criteria: female and male participants, between 18 to 45 years, overweight (25<BMI<30), body stable within the last 3 months
- Exclusion criteria: gastrointestinal disease, antibiotic, prebiotic, or probiotic use, intolerance to the dietary supplements used, smoking
- 2 sources of proteins: animal source (milk protein isolate enriched in casein (92%)) and plant source (isolated soy protein) → different digestibility and different amino acid composition
Nutritional intervention

Nutritional intervention (3 weeks)

Normalisation period (2 weeks)

Randomized

Energy intake:
- habitual
- 15% of energy: proteins
- 50% carbohydrates
- 35% lipids

Normalisation period (2 weeks)

Energy intake:
- habitual
- 30% = 15% dietary protein + 15% casein
- 35% carbohydrates
- 35% lipids

Energy intake:
- habitual
- 30% = 15% dietary protein + 15% soy protein
- 35% carbohydrates
- 35% lipids

Energy intake:
- habitual
- 15% dietary protein
- 50% = 35% dietary carbohydrates + 15% maltodextrin
- 35% lipids

Feces, urine, blood

Metabolomics (1H-RMN)

Rectal biopsies

Gene expression (microarrays)

High-protein casein diet

High-protein soy diet

Normoproteic diet (Control)
Uremia (A) and fiber intake (B)

**Method:** blood urea measurement

Good compliance of participants

**Method:** 3-day food diaries

Similar intake of dietary fibers in the 3 groups

Metabolic parameters measured in the 3 groups each week during the 3-week trial

- Waist circumference: no effect
- Blood pressure: systolic blood pressure significantly lower in the soy protein group (isoflavones?)
- Cholesterol, LDL, HDL, triglycerides: no effect
- Glycemia, insulinemia: no effect
HPD (CAS, SOY) decreases fecal butyrate concentration and increases 2-methylbutyrate (ILEU).

HPD (CAS, SOY) increases urea, isobutyrate, phenylacetylglutamine, indoxylsulfate, CAS in addition increases p-cresol and p-cresylsulfate.
Fecal water cytotoxicity

- The effects of fecal water on the viability of human colonocytes HT-29 were not affected by the diets

- The most cytotoxic fecal water samples had the highest content in hydroxyphenyl acetate, bile acids, ethanol, and 5-aminovalerate

Hydroxyphenylacacetate and human colonocytes

HO-PAA appears genotoxic but not cytotoxic in *in vitro* experiments (Armand et al. Biochim Biophys Acta, in revision)
No significant effects of the nutritional intervention on the fecal and adherent (rectal biopsies) microbiota.

→ In our study, the availability of substrates is likely a central element for determining the urinary metabolite concentrations.
Transcriptional analysis in rectal biopsies

The **CASEIN diet** modifies the expression of gene related to:
- Extracellular matrix (increase)
- Cellular adhesion (increase)
- Mucus production (increase)

The **SOYA diet** modifies the expression of genes related to:
- Oxidative stress and detoxifying metabolism (increase and decrease)

Both diet (CAS et SOY) modifies the expression of genes related to:
- Cell proliferation and cell cycle (increase for 85% of genes)
- Cell apoptosis (increase for 83% of genes)
- Cytoskeleton formation (increase)

NB: No sign of inflammation in the rectal biopsies (proinflammatory cytokines in biopsies and fecal calprotectin and secretory immunoglobulin A)

Conclusion from this study

The HPD display an impact on the colorectal epithelium luminal environment that appears rather unfavourable (review in Blachier et al. Am J Pathol 2017).

The changes observed coincide with numerous (although of limited amplitude) modifications of expression of genes related to the homeostasis of the rectal mucosa (without sign of inflammation).
Although HPD appear efficient for weight loss, the effects of HPD on microbiota-derived metabolites and gene expression in the gut raise new questions on the impact of HPD on the large intestine mucosa homeostasis leading the authors to recommend some caution regarding the utilization of HPD, notably in a recurrent and/or long-term ways.

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