Effects of two different high-fat diets on the gut microbiota of adult mice

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Introduction

Growing evidence supports the role of gut microbiota in the regulation of host energy homeostasis and its interaction with hyper-caloric diets to influence the development of metabolic disorders and obesity. However, little is known about how high-fat diets induce changes in the intestinal bacterial community and how this reshaped gut microbiota mediates the development of metabolic diseases. Recently, we reported that a soy oil-based high-fat diet can markedly affects cecal microbiota of weaning mice even over short periods of time (2). We suggested that the observed shifts of specific bacterial populations within the gut may represent an early consequence of increased dietary fat with potential implications for host disease. Here, we extend our previous research by investigating the effects of high-fat diets differing in their fatty acid composition on mouse physiology and gut ecology over a prolonged period of time.

Purpose

The aim of the present study was to characterize and compare alterations induced by two diets enriched with either soy oil (high in polyunsaturated fat) or coconut oil (high in saturated fat) with respect to gut microbiota and intestinal morphology, as well as growth, fat deposition and metabolic status of adult mice.

Methods

- Female C57BL/6 mice were fed either a low-fat, control diet (LFD; n=12), or a high-fat diet containing 30% soy oil (HFSD; n=12), or a high-fat diet containing 30% coconut oil (HFCD; n=12).
- Six mice from each group were sacrificed after 2 (W2) and 8 weeks (W8) of dietary exposure.
- Body weight and abdominal fat mass were measured.
- Total plasma cholesterol, triglycerides and haptoglobin were determined using commercial assays.
- Cecum tissues were subjected to histological examination (1).
- DNA was extracted from cecal content and used for PCR-DGGE analysis using universal 16S rRNA gene primers (2).
- Sequence analysis was performed on bands of interest for identification.
- DGGE results were checked by real-time quantitative PCR assays (3, this study).
- Volatile fatty acids (VFA) and lactate were determined by HPLC (4).
- Statistical analysis was performed using the PROC GLM of SAS version 9.2 software.

Results

HFSD and HFCD mice showed a higher daily weight gain both at W2 and W8, and an increased body fat storage at W8 as compared to LFD mice (**Figure 1**).

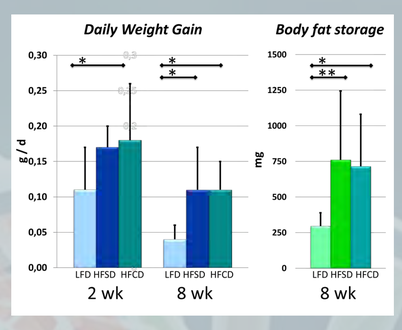

Figure 1. Daily weight gain and body fat storage of mice fed either LFD, HFSD or HFCD. Daily weight gain was calculated dividing the weight gain by the number of days of treatment. Values are means ± SD; * = P<0.05; ** = P<0.01

Plasma total cholesterol levels were higher in the HFSD and HFCD groups than LFD group at W2, but only in the HFCD group at W8 (**Figure 2**).

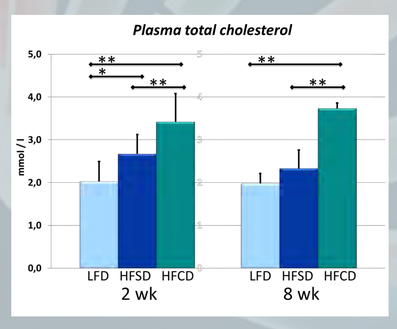

Figure 2. Plasma total cholesterol levels of mice fed either the LFD, HFSD or HFCD. Values are means ± SD; * = P<0.05; ** = P<0.01

Triglycerides were slightly increased (W2) only in HFCD (1.00 mmol/l) compared to LFD (0.61 mmol/l) and HFSD (0.64 mmol/l); P<0.05. No changes in acute phase proteins were observed, except for haptoglobin whose level was higher in HFCD vs. LFD at W2 (P<0.05).

At W8 the histological analysis of the cecum evidenced that HFSD mice showed an increment of lesions of the mucosa (P ≤ 0.01) (**Figure 3**) and inflammatory cell infiltration (P ≤ 0.05) (**Figure 4**) compared to LFD (**Figure 5**).

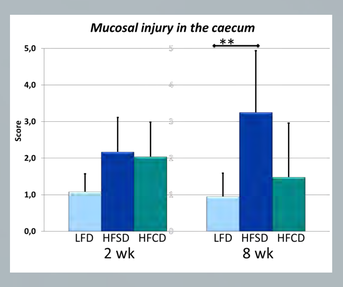

Figure 3. Mucosal injury in the cecum of mice fed either the LFD, HFSD or HFCD. A rating score between 0 (no change from normal tissue) and 5 (lesions involved most areas and all the layers of the intestinal section) was applied; * = P<0.05; ** = P<0.01

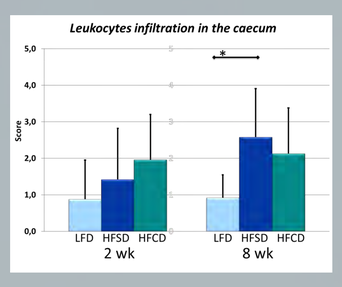

Figure 4. Leukocytes infiltration in the cecum of mice fed either the LFD, HFSD or HFCD. A rating score between 0 (no infiltration) and 5 (maximum infiltration rate) was applied; * = P<0.05; ** = P<0.01

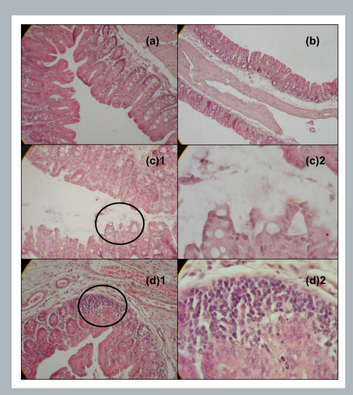

Figure 5. Histological sections of caecum stained with haematoxylin and eosin.
(a) Normal histological section at a 20x magnification
(b) Normal histological section at a 10x magnification
(c) Lesions of the mucosa at a 1) 20x and 2) 63x magnification
(d) Leukocytes infiltration at a 1) 20x and 2) 63x magnification

Results

UPGMA analysis of DGGE fingerprints showed that HFSD and HFCD mice samples clustered separately from the LFD control mice both at W2 and W8, indicating that the cecal bacterial community profile was significantly affected by the amount and type of dietary fat (**Figure 6**).

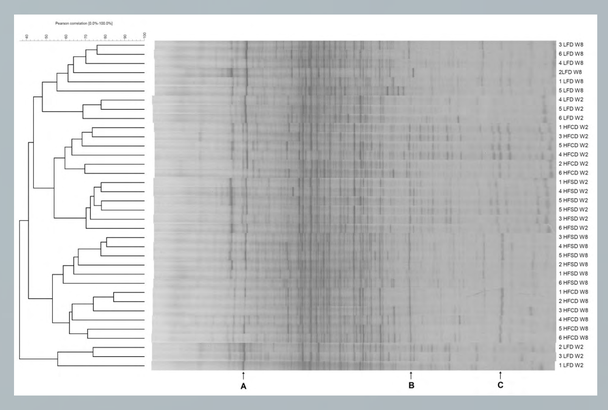

Figure 6. UPGMA analysis of PCR-DGGE profiles of mouse cecal microbiota obtained using universal 16S rRNA gene primers Hda1-GC/Hda2. Each of the LFD, HFSD and HFCD group consists of six mice numbered 1 to 6. Letters A, B and C indicate bands differentially present in specific dietary groups

The sequences obtained for these bands were compared to those available in the Ribosomal Database Project II database and the sequence similarities are presented in **Table 1**.

Band	Closest sequence (Accession no)	Score	Closest known species (Accession no)	Score
A			*Lactobacillus gasseri* (NR041920)	0.984
B	Uncultured *Lachnospiraceae* bacterium (CQ493042)	0.885	*Clostridium populeti* (X71858)	0.846
C	Uncultured *Coriobacteriaceae* bacterium (AY990782)	1.000	*Gordonibacter pamelaeae* (AF079507)	0.809

Table 1. Comparative sequence analysis of relevant bands excised from 16S rRNA gene PCR-DGGE gel

The intensity of the DGGE band corresponding to *L. gasseri* was lower in the HFSD and HFCD than in the LFD group, whereas the opposite trend was observed for the bands related to *G. pamelaeae* and *C. populeti* both at W2 and W8.

Quantitative real-time PCR confirmed a statistically significant 1-log decrease in *L. gasseri* numbers after HF feeding (**Figure 7**). The abundance of the uncultured *Coriobacteriaceae* bacterium was significantly higher in mice fed the HF diets than in controls, except for HFSD mice at W8 (**Figure 8**).

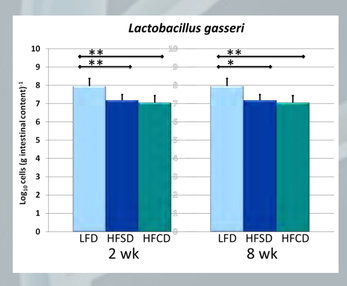

Figure 7. Quantitative real-time PCR for *Lactobacillus gasseri* levels in the DNA extracted from the cecum content of mice fed either the LFD, HFSD or HFCD. Values are means ± SD; * = P<0.05; ** = P<0.01

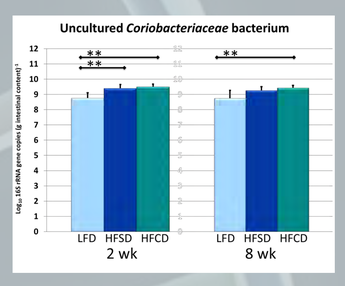

Figure 8. Quantitative real-time PCR for the uncultured *Coriobacteriaceae* bacterium levels in the DNA extracted from the cecum content of mice fed either the LFD, HFSD or HFCD. Values are means ± SD; * = P<0.05; ** = P<0.01

We are currently focusing on the development of a real-time PCR assay targeting the *C. populeti*-related phylotype.

No difference of volatile fatty acids and lactate levels between treatment and control groups was observed.

Literature cited

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Conclusions

These results corroborate and extend our previous findings on the impact of dietary fat on the gut microbial ecosystem and the physiology of the host. Our findings suggest that high-fat diets affect the composition of the cecal microbiota of mice by modulating the relative abundances of individual species/phylotypes rather than total community structure. The effect of HFCD on lipid metabolism persisted through time whereas a prolonged administration of HFSD seemed to result in metabolic adaptation to fat feeding. The soy oil-based diet appeared to trigger the onset of cecum inflammation inducing tissue alterations which were not observed with the HFCD. Further investigation is needed to gain insight into the relationship between gut bacterial community shifts and metabolic and physiological disturbances in mice.

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