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Original Research

Increasing fat content from 20 to 45 wt% in a complex diet induces lower endotoxemia in parallel with an increased number of intestinal goblet cells in mice

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ABSTRACT

The impacts of high-fat diets (HFDs) on the onset of metabolic endotoxemia and low-grade inflammation are well established in rodent models. However, the dose-effect of dietary lipid intakes on these parameters is not known. We hypothesized that increasing dietary lipid amounts could be linked to parallel increases of endotoxemia, low-grade inflammation, and metabolic and intestinal alterations. Six-week-old male C57BL/6J mice were fed a low-fat diet (LFD, 2.6 wt% of lipids), a moderate HFD (mHFD, 22 wt% of lipids), or a very HFD (vHFD, 45 wt% of lipids) formulated mainly using chow ingredients and milk fat. After 12 weeks, white adipose tissues, liver, intestine, distal colon contents, and plasma were collected. Only vHFD mice significantly increased body weight and fat mass vs LFD mice. This was associated with increases of plasma concentrations of triglycerides, leptin and adiponectin, and liver lipids. No such differences were observed between LFD and mHFD mice. However, mHFD developed metabolic endotoxemia and inflammation, unlike vHFD mice. In turn, vHFD mice showed more goblet cells in all intestine segments vs both other groups and a decrease of *Bacteroides-Prevotella* in their microbiota vs LFD mice. Finally, mHFD mice colon exhibited a decrease in lactobacilli and in the levels of occludin phosphorylation. Altogether, using complex HFD, no

Abbreviations: ANOVA, analysis of variance; ELISA, enzyme-linked immunosorbent assay; HFD, high-fat diet; IL-6, interleukin-6; LBP, lipopolysaccharide-binding protein; LFD, low-fat diet; LPS, lipopolysaccharide; mHFD, moderate high-fat diet; MUC2, mucin 2; RT-PCR, real-time polymerase chain reaction; sCD14, soluble cluster of differentiation 14; TG, triglycerides; vHFD, very high-fat diet; WAT, white adipose tissue; ZO-1, zonula occludens 1.

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associations were observed between dietary lipid amounts and the magnitude of endotoxemia, inflammation, and physiological alterations developed. These results reveal the impact of the diet composition on intestinal goblet cells and mucus coat, bringing new insights about further consequences on HFD-induced metabolic disorders.

1. Introduction

To date, physiological alterations developed by mice fed high-fat diets (HFD) are well known and described. These include an impairment in glucose tolerance [1–7], an increase of plasma and liver lipids [2,4,6,8], and a decrease of plasma adiponectin concentrations [3,6,9]. Recently, interest has grown about the fact that HFDs are also associated with gut barrier alterations [10–12]. The gut barrier is a complex mucosal structure implicated in the host protection against luminal dangers. Its strength relies on (1) epithelial cells linked together by tight junctions and adhesion junctions, (2) goblet cells producing the protective mucus coat [13,14], (3) Paneth cells secreting antimicrobial peptides [15], and (4) commensal bacterial populations of gut microbiota [16,17]. Recent animal studies showed that HFDs modulate bacterial populations within the gut microbiota [1,2,4,5,18] and increase intestinal permeability [1,5,18], resulting in the increase of the translocation, from the gut lumen to the systemic circulation, of proinflammatory products of gram-negative bacteria of gut microbiota, the lipopolysaccharides (LPS), so-called endotoxins [2,19]. The onset of such a metabolic endotoxemia [1,2,8] may lead to the onset of low-grade inflammation [20]. In systemic circulation, LPS are bound by circulating LPS-binding proteins (LBPs) and then transferred to the circulating form of their receptor, the soluble cluster of differentiation 14 (sCD14) [21]. The complex LPS-LBP-CD14 initiates the secretion of proinflammatory cytokines, such as interleukin-6 (IL-6) [20], and so the low-grade inflammation.

Several studies have investigated the impact of HFDs on metabolic endotoxemia and low-grade inflammation in rodents. Cani et al [2,19] showed that mice fed a severe HFD (70% of the energy from fat) for 4 weeks developed a metabolic endotoxemia and a systemic inflammation in connection with metabolic and intestinal disorders. Consistent with these results, Serino et al [22] observed that, after 12 weeks of such an HFD, mice showed an elevated endotoxemia, an increased gut permeability, and changes in the composition of their gut microbiota. Finally, de La Serre et al [18] showed that rats fed a severe HFD had an increased endotoxemia associated with an increased intestinal permeability and substantial changes in the composition of their gut microbiota.

Despite the variety of dietary lipid intakes that can occur in human nutrition, there is virtually no information on the effect of dietary fat amount on the onset and the magnitude of metabolic endotoxemia: clearly, there is an effect of high-fat vs low-fat diets; but the dose-response relationship is not clear. Amar et al [23] reported that mice fed a diet with 72% of energy from fat had twice the level of endotoxemia compared to mice fed a diet with 35% of energy from fat. However, none of the endotoxemia related parameters, LBP and sCD14, were measured neither the metabolic and intestinal disorders associated with the increase of LPS plasma levels [23]. Therefore, available data are insufficient, to date, to assess whether dietary lipid amounts can influence the onset and the magnitude of metabolic endotoxemia, low-grade inflammation, and associated physiological disorders.

In this context, we hypothesized that increasing dietary lipid amounts could be linked to parallel increases of endotoxemia, low-grade inflammation, and metabolic and intestinal alterations. Thus, we investigated the effects of increasing the wt% of dietary fat from 2.5 to 20 to 45 on these parameters. In addition, the interaction with intestinal goblet cells and Paneth cells was determined, as little is known of their sensitivity to diets despite their importance in maintaining the homeostasis of gut microbiota. Here, we showed that, using complex diets based on chow diet ingredients and milk fat, there was no association between dietary lipid amounts and the magnitude of metabolic endotoxemia, low-grade inflammation, and physiological alterations developed in response to the diets. Furthermore, we brought new data on the impact of the diet composition on intestinal goblet cells and mucus gel in the frame of HFD-induced metabolic disorders.

2. Methods and materials

2.1. *Specific nonpyrogenic material*

With all measurements related to LPS, extreme care was taken to avoid contamination with exogenous LPS. Therefore, single-use nonpyrogenic supplies were used: PS Becton Dickinson tubes, Axygen tubes (VWR, Fontenay-sous-Bois, France), and pyrogen-free pipette tips (Biogenic, Perols, France).

2.2. *Animals, diets, and design of the study*

Male C57BL/6J mice (19-20 g, 6 weeks; Janvier SA, Saint Berthevin, France) were housed (5 per cage) in a controlled environment (24°C ± 1°C; 12-hour daylight cycle) with free access to food and water. After a week of acclimatization, they were randomly divided into 3 groups (n = 10): the low-fat diet group (LFD) fed a diet with 2.6 wt% of lipids (A04, diet used for maintenance of mice within the context of experimental protocol and containing barley, wheat, corn, soy meal, wheat bran, hydrolyzed fish protein, premixture of vitamins and minerals, dicalcium phosphate, and calcium carbonate), the moderate HFD group (mHFD) fed with a diet with 22 wt% of lipids, and the very HFD group (vHFD) fed with a diet with more than 45 wt% of lipids. These 3 fat amounts (2.6%, 22%, and 45%) were selected to cover a large range of fat intakes and were based on 3 commonly used fat loads in HFD studies with mice. Diets were prepared with the same base of ingredients by SAFE (Augy, France). Compositions and fatty acid profiles of diets are respectively detailed in Tables 1 and 2. Because of lower amounts