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Soybean polar lipids differently impact adipose tissue inflammation and the endotoxin transporters LBP and sCD14 in flaxseed vs. palm oil-rich diets

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Abstract

Obesity and type 2 diabetes are nutritional pathologies, characterized by a subclinical inflammatory state. Endotoxins are now well recognized as an important factor implicated in the onset and maintain of this inflammatory state during fat digestion in high-fat diet. As a preventive strategy, lipid formulation could be optimized to limit these phenomena, notably regarding fatty acid profile and PL emulsifier content. Little is known about soybean polar lipid (SPL) consumption associated to oils rich in saturated FA vs. anti-inflammatory omega-3 FA such as α-linolenic acid on inflammation and metabolic endotoxemia. We then investigated in mice the effect of different synthetic diets enriched with two different oils, palm oil or flaxseed oil and containing or devoid of SPL on adipose tissue inflammation and endotoxin receptors. In both groups containing SPL, adipose tissue (WAT) increased compared with groups devoid of SPL and an induction of MCP-1 and LBP was observed in WAT. However, only the high-fat diet in which flaxseed oil was associated with SPL resulted in both higher WAT inflammation and higher circulating sCD14 in plasma. In conclusion, we have demonstrated that LPS transporters LBP and sCD14 and adipose tissue inflammation can be modulated by SPL in high fat diets differing in oil composition. Notably high-flaxseed oil diet exerts a beneficial metabolic impact, however blunted by PL addition. Our study suggests that nutritional strategies can be envisaged by optimizing dietary lipid sources in manufactured products, including fats/oils and polar lipid emulsifiers, in order to limit the inflammatory impact of palatable foods.

Keywords: LBP; CD14; Endotoxin; Lecithin; Flaxseed; Inflammation

1. Introduction

The occurrence of overweight is constantly increasing and is associated with an inflammatory state that contributes to the development of metabolic disorders such as obesity, type 2 diabetes and cardiovascular disease [1]. Our and other studies have shown that endotoxins (so-called lipopolysaccharides, LPS), components of Gram-negative bacteria wall, can contribute to the onset and maintenance of inflammation during obesity [2,3]. LPS transporter and soluble receptor in plasma, namely LBP and sCD14, have been suggested as clinical markers that reflect plasma exposure to endotoxins [4] and have been associated differently to obesity and inflammatory state [5]. More recently we have shown in humans that inflammation during the initial phase of weight gain was linked to the relative variations of LBP and sCD14 in plasma [6]. Several studies led by Moreno-Navarrete et al. [7] demonstrated that LBP is a pro-inflammatory soluble adipokine acting as a brake for adipogenesis and inflammation [8]. Soluble CD14 was described as LPS inhibitor at high concentrations by transferring LPS to plasma lipoproteins [9]; however, low concentrations of sCD14 were considered pro-inflammatory [10]. The white adipose tissue [11] secretes LBP, CD14 and actively participates to the development of inflammation. Indeed, in obese individuals, white adipose tissue expansion is associated with macrophage infiltration. A change in macrophage phenotype is also observed from M2, or "alternatively activated" non-inflammatory macrophages to M1, or "classically activated" pro-inflammatory macrophages [12,13]. M1 macrophages secrete cytokines, among which IL-6 and TNFα, and lead to a pro-inflammatory environment; contrary to M2 macrophages that predominate in a lean state and secrete anti-inflammatory cytokines [13,14].

We have shown previously that a diet enriched with rapeseed oil (rich in omega 3 FA α-linolenic acid, ALA) resulted in higher sCD14

Abbreviations: ALA, alpha-Linolenic acid; eWAT, epididymal white adipose tissue; FA, fatty acids; PL, polar lipids; SPL, soybean polar lipids; WAT, white adipose tissue; LAL, limulus amoebocyte lysate; SRB1, scavenger receptor class B member 1; LBP, lipopolysaccharide-binding protein; sCD14, soluble cluster of differentiation 14; MUC2, mucin 2.

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and lower LBP than a diet enriched with palm oil (rich in palmitic acid), which was associated for ALA-oil with lower inflammation in both plasma and adipose tissue despite higher plasma endotoxemia [2]. In this context, dietary lipids are composed mostly of triacylglycerols, which are the major components of fats and oils. However, other lipids present in lower amounts in the diet are polar lipids (PL). PL contribute to food texture and emulsion stability and are therefore incorporated into numerous manufactured food products. Soybean and egg lecithin are currently the major sources of PL. A recent review points out the beneficial effects of PL on lipid metabolism and inflammation [15]. Moreover, we have shown in rats that soybean PL used to emulsify flaxseed or sunflower oil increase FA absorption, gene expression of endotoxin transporters in the gut and postprandial endotoxemia [16,17]. However, none of these studies looked into the chronic effects on inflammation and metabolic endotoxemia of soybean PL consumption associated to oils rich in saturated FA vs. anti-inflammatory omega-3 FA such as α-linolenic acid.

The aim of this study was thus to compare the effect of the soybean PL (SPL) incorporation into high-fat diets differing in terms of fatty acid (FA) composition on adipose tissue inflammation and endotoxin receptors. Therefore we tested in mice synthetic diets enriched with two different oils, palm oil or flaxseed oil (a concentrated source of ALA), each with or without SPL.

2. Materials and methods

2.1. Animals and diets

Male C57BL/6 mice (6 week-old) were purchased from Janvier SA (Le Genest Saint-Isle, France) and kept in a temperature-controlled room (22 °C) with a 12 h light/12 h dark cycle with free access to food and tap water. After 1 week of chow diet, mice were randomly divided into four groups fed one of the four following diets for 8 weeks (Table 1): Palm (P), Flaxseed (F), Palm-soybean polar lipid (P-SPL) and Flaxseed-soybean polar lipid (F-SPL). There were 12 mice per group and, each group was divided into two cages of 6 mice each. Body weight was measured weekly and weights of distributed/remaining pellets were measured twice a week to calculate body weight gain and estimated food intake.

General composition and detailed oil composition of the diets are reported in Tables 1 and 2, respectively. The four diet formulations contained the same amount of lipids, proteins, and carbohydrates. P and P-SPL are the same diets used in our previous study about the impact of different emulsifiers, milk vs. soybean PL, on adiposity and inflammation [18]. Soybean lecithin was purchased from Atlantic Nature (Ploemeur, France), palm oil was provided by SIO (Bougival, France), rapeseed, sunflower, soybean and flaxseed oils were supplied by ITERG (Pessac, France). The different lipid blends were prepared at the labscale by direct fat mixing, stored under N_2 at −20 °C and sent to SAFE for diets preparation (Augy, France).

After 8 weeks of diet, mice were euthanized by intraperitoneal (IP) injection of sodium pentobarbital and blood was collected by cardiac puncture on heparin-containing tubes. Plasma was obtained by centrifugation (4600 rpm, 5 min, 4 °C) and stored at −80 °C. Liver and WAT (epididymal tissue) were collected, frozen in liquid nitrogen and stored at −80 °C.

Animal experiments were performed under the authorization N°CETIL 012014 and approved by the Animal Ethics Committee of INSA-Lyon and carried out in accordance with the EU Council Directive for the Care and Use of Laboratory Animals (no. 2010/63/EU).

Table 1
Formulation of the experimental diets

Ingredients (g/100 g)	P	P-SPL	F	F-SPL
Lipid mixture	20	20	20	20
Among which:				
Palm oil	17.8	17.8	-	-
Flaxseed oil	-	-	12	12.8
Soybean polar lipids (100% PL, no TAG):	-	1.2	-	1.2
Sunflower oil	0.8	1	-	-
High-oleic sunflower oil	-	-	7.0	6.0
Soybean oil	1.4	-	1.0	-
Corn starch	38.7	38.7	38.7	38.7
Casein	20	20	20	20
Saccharose	10	10	10	10
Cellulose	5	5	5	5
Mineral mix	5	5	5	5
Vitamin mix	1	1	1	1
Cystine	0.3	0.3	0.3	0.3

Table 2
Fatty acid profiles and polar lipid composition of the experimental diets

FA profile (g/100 g fat)	P	P-SPL	F	F-SPL
10:0	<0.1	<0.1	<0.1	<0.1
12:0	0.2	0.2	<0.1	<0.1
14:0	1.0	1.0	0.1	0.1
15:0	0.1	0.1	<0.1	<0.1
16:0	40.0	40.9	6.1	6.5
16:1	0.2	0.2	0.1	0.1
17:1	0.1	0.1	0.1	0.1
18:0	4.1	4.0	3.3	3.3
18:1	38.6	38.8	42.3	39.4
18:2 (n-6)	14.2	13.2	14.2	14.3
18:3 (n-3)	0.7	0.7	32.4	34.9
20:0	0.4	0.3	0.2	0.2
20:1	0.2	0.1	0.2	0.2
22:0	0.1	0.1	0.4	0.3
22:1	<0.1	<0.1	<0.1	<0.1
24:0	0.1	0.1	0.2	0.2
24:1	<0.1	<0.1	<0.1	<0.1
PL composition (g/100 g of diet)				
PE	0.03	0.16	0.03	0.15
PI	<0.01	0.11	<0.01	0.11
PC	0.01	0.23	0.01	0.22
SM	0.01	0.02	0.01	0.01
LysoPC	0.02	0.04	0.03	0.04
PS	0.01	0.06	0.01	0.06
Total PLs	0.09	0.62	0.09	0.60

2.2. Plasma analyses

Plasma concentrations of triacyglycerol (TAG) and cholesterol were measured with the Triglyceride PAP kit and, the Cholesterol RTU kit (BioMérieux, France), using culture plates (Corning) as previously described [19]. Plasma endotoxemia was determined by using the LAL assay kinetic chromogenic conditions (Biogenic, Pérols, France) [20]. Plasma concentrations of MCP-1, (Clinisciences, France), sCD14, LBP (Enzo Life Sciences) and insulin (Crystal Chem, USA) were assayed by ELISA kits according to the manufacturer's instructions. Plasma glucose levels were measured using a glucometer (Accu-Chek W, Roche, France).

2.3. Measurement of adipose cell size

The cell diameters and number from WAT were measured from analysis by Multisizer-IV (Beckman Coulter, Villepinte, France) as previously described [21].

2.4. Real-time PCR analysis

Total RNA were extracted from WAT, liver and duodenum with TRI Reagent (Ambion/Applied Biosystems). RNA concentration was measured with Nanodrop-ND1000 (Labtech). One microgram of RNA was used for target genes expression using RT-qPCR (quantitative PCR conditions available upon request, emmanuelle.meugnier@pop.univ-lyon1.fr). TATAbox binding protein (TBP) expression in duodenum and WAT; and 60S acidic ribosomal protein P0 (RPLP0) in liver were used as internal standard for normalization of target mRNA expression.

2.5. Immunohistochemistry and quantitative analysis of intestinal goblet cells

Paraffin sections were rehydrated and prepared as previously described [21]. The intestinal epithelium (colon) was analyzed by assessing the number of anti-mucin 2 (MUC2) positive cells (goblet cells) per crypt. The rabbit polyclonal MUC2 was used at 1/250 (Santa Cruz (H300); Santa Cruz Biotechnology, CA, USA).

2.6. Statistical analysis

All data are presented as means ± SEM and were analyzed by using GraphPad Prism software (GraphPad, version 6.01). Comparison were made between groups of normally distributed data by using a two-way ANOVA (SPL x Oil). When the interaction term was not significant, this analysis was followed by a Sidak-Holm post-hoc test. If the interaction term was significant an unpaired two-tailed Student's *t*-test was performed. Differences were considered significant at the $P<.05$ level.