Biochimica et Biophysica Acta 1841 (2014) 267–278

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbalip

Omega-3 phospholipids from fish suppress hepatic steatosis by integrated inhibition of biosynthetic pathways in dietary obese mice

Martin Rossmeisl [a], Dasa Medrikova [a,1], Evert M. van Schothorst [b], Jana Pavlisova [a], Ondrej Kuda [a], Michal Hensler [a], Kristina Bardova [a], Pavel Flachs [a], Barbora Stankova [c], Marek Vecka [c], Eva Tvrzicka [c], Ales Zak [c], Jaap Keijer [b], Jan Kopecky [a,*]

[a] Department of Adipose Tissue Biology, Institute of Physiology Academy of Sciences of the Czech Republic v.v.i., Prague, Czech Republic
[b] Human and Animal Physiology, Wageningen University, Wageningen, Netherlands
[c] 4th Department of Internal Medicine, 1st Faculty of Medicine of Charles University and the General Teaching Hospital, Prague, Czech Republic

ARTICLE INFO

Article history:
Received 3 June 2013
Received in revised form 14 November 2013
Accepted 21 November 2013
Available online 1 December 2013

Keywords:
Obesity
NAFLD
Marine phospholipids
PUFA
Lipogenesis
High-fat diet

ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) accompanies obesity and insulin resistance. Recent meta-analysis suggested omega-3 polyunsaturated fatty acids DHA and EPA to decrease liver fat in NAFLD patients. Anti-inflammatory, hypolipidemic, and insulin-sensitizing effects of DHA/EPA depend on their lipid form, with marine phospholipids showing better efficacy than fish oils. We characterized the mechanisms underlying beneficial effects of DHA/EPA phospholipids, alone or combined with an antidiabetic drug, on hepatosteatosis. C57BL/6N mice were fed for 7 weeks an obesogenic high-fat diet (cHF) or cHF-based interventions: (i) cHF supplemented with phosphatidylcholine-rich concentrate from herring (replacing 10% of dietary lipids; PC), (ii) cHF containing rosiglitazone (10 mg/kg diet; R), or (iii) PC + R. Metabolic analyses, hepatic gene expression and lipidome profiling were performed. Results showed that PC and PC + R prevented cHF-induced weight gain and glucose intolerance, while all interventions reduced abdominal fat and plasma triacylglycerols. PC and PC + R also lowered hepatic and plasma cholesterol and reduced hepatosteatosis. Microarray analysis revealed integrated down-regulation of hepatic lipogenic and cholesterol biosynthesis pathways by PC, while R-induced lipogenesis was fully counteracted in PC + R. Gene expression changes in PC and PC + R were associated with preferential enrichment of hepatic phosphatidylcholine and phosphatidylethanolamine fractions by DHA/EPA. The complex down-regulation of hepatic lipogenic and cholesterol biosynthesis genes and the antisteatotic effects were unique to DHA/EPA-containing phospholipids, since they were absent in mice fed soy-derived phosphatidylcholine. Thus, inhibition of lipid and cholesterol biosynthesis associated with potent antisteatotic effects in the liver in response to DHA/EPA-containing phospholipids support their use in NAFLD prevention and treatment.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD), a premorbid condition that can lead to fibrosis and cirrhosis, is frequently driven by obesity and insulin resistance [1]. Pharmacological interventions to treat obesity-associated diseases require multiple agents and are often associated with adverse side effects, as in the case of thiazolidinedione (TZD) antidiabetic drugs, thus lifestyle interventions remain an essential component of any treatment strategy. Marine fish oils, namely long-chain polyunsaturated fatty acids (FA) of *n*-3 series (omega-3), such as docosahexaenoic acid (DHA; 22:6n-3) and eicosapentaenoic acid (EPA; 20:5n-3), were shown to reduce the incidence and mortality of cardiovascular disease [2,3]. This beneficial effect was attributed to a reduction in plasma triacylglycerol (TG) levels as well as to the anti-inflammatory action of omega-3, both in rodents [4–6] and in humans [7,8]. Omega-3 supplementation potentiates health benefits of reduced calorie intake in humans [9] and obese mice [10]. While omega-3 improved insulin sensitivity in rodent models of metabolic syndrome [11], they could not revert insulin resistance in diabetic patients

Abbreviations: cHF, corn oil-based high-fat diet; Chow, standard low-fat diet; CLS, crown-like structures; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FA, fatty acids; FDR, false discovery rate; GEO, Gene Expression Omnibus; GSEA, Gene Set Enrichment Analysis; HDL-C, high-density lipoprotein cholesterol; HMW, high molecular weight; HOMA, homeostasis model assessment; LA, linoleic acid; NAFLD, non-alcoholic fatty liver disease; NEFA, non-esterified fatty acids; omega-3, long-chain *n*-3 polyunsaturated fatty acids of marine origin; omega-3 PL, omega-3 as marine phospholipids; omega-3 TG, omega-3 as triacylglycerols in fish oil; oPLS-DA, orthogonal partial least squares-discriminant analysis; PC, cHF supplemented with marine phospholipids; PCA, principal component analysis; PPAR, peroxisome proliferator-activated receptor; PC + R, cHF supplemented with marine phospholipids and rosiglitazone; qPCR, real-time quantitative RT-PCR; R, cHF supplemented with an antidiabetic drug rosiglitazone; SHP, small heterodimer partner; TC, total cholesterol; TG, triacylglycerol; TZD, thiazolidinedione; VIP, variable important to projection; VLDL-TG, very low-density lipoprotein triacylglycerol; WAT, white adipose tissue

* Corresponding author at: Department of Adipose Tissue Biology, Institute of Physiology AS CR, v.v.i., Videnska 1083, 142 20 Prague 4, Czech Republic. Tel.: +420 296442554; fax: +420 296442599.
E-mail address: kopecky@biomed.cas.cz (J. Kopecky).
[1] Present address: Joint Research Division Molecular Metabolic Control, German Cancer Research Center, Center for Molecular Biology, University of Heidelberg, Network Aging Research, University Hospital Heidelberg, Germany.

1388-1981/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbalip.2013.11.010

[12]. However, even these patients benefited from omega-3 supplementation through prevention of cardiovascular disease and improvement of dyslipidemia [13]. Moreover, omega-3 limited hepatosteatosis in rodents [5,6,10,11,14–16] and humans, as documented by a number of studies (e.g. [17,18]), as well as by a recent meta-analysis [1].

Metabolic effects of omega-3 are determined by: (i) modulation of expression by master transcriptional regulators, mainly by peroxisome proliferator-activated receptor α (PPARα; [19]) and sterol regulatory element-binding protein-1c [20], (ii) tissue production of eicosanoids and other lipid mediators [21], (iii) induction of adiponectin [22], and (iv) changes in the levels of endocannabinoids in metabolically relevant tissues [5,15,23]. White adipose tissue (WAT) and the liver represent the primary targets for omega-3 in dietary obese mice, as evidenced by induction of mitochondrial biogenesis and FA oxidation specifically in epididymal WAT [24] and by AMP-activated protein kinase dependent improvement of hepatic insulin sensitivity [11]. Hypolipidemic effects of omega-3 depend on complex metabolic and gene expression changes (see above), resulting in suppression of hepatic lipogenesis and increased FA oxidation [6].

Recent studies [5,6,15,16] document amelioration of hepatic steatosis in obese rodents in response to omega-3 administered as marine phospholipids (omega-3 PL), mostly as krill oil [6,15,16]. When compared with omega-3 contained in TG (omega-3 TG) in fish oils, omega-3 PL were more efficient in the reduction of steatosis [5,15] and improvement of metabolic profile [5]. Low-fat diet supplementation with krill oil resulted in decreased activities of gluconeogenic, lipogenic and cholesterol biosynthetic pathways, with enhanced mitochondrial oxidative activity, which was not observed after omega-3 TG supplementation [25]. However, a detailed analysis of pathways that are affected in response to omega-3 PL using a more clinically relevant setting, i.e. in the steatotic livers of mice with diet-induced obesity, is lacking. Moreover, although sea fish may contain up to one third of omega-3 as PL [26], and thus could serve as an abundant source of omega-3 PL, studies on the effects of fish-derived omega-3 PL are scarce [5].

We used our well-established mouse model of dietary obesity [4,5,10,11,22] to identify mechanisms involved in the antisteatotic action of omega-3 PL in the liver. By means of physiological assessment, gene expression profiling and lipidomic analysis, we examined the metabolic effects of phosphatidylcholine-rich omega-3 PL isolated from herring, administered either alone or in combination with a low dose of TZD rosiglitazone. Furthermore, in an attempt to distinguish the importance of FA composition and that of the PL moiety with regard to their contribution to the overall metabolic effect of omega-3 PL, we also compared herring-derived omega-3 PL with a phosphatidylcholine-rich concentrate isolated from soy, i.e. the type of PL molecule characterized by a completely different FA profile. We show here that besides preventing weight gain and dyslipidemia and preserving glucose homeostasis, omega-3 PL markedly reduced hepatic steatosis, which was associated with a profound suppression of hepatic biosynthetic processes, above all characterized by integrated inhibition of de novo FA synthesis and cholesterol biosynthesis. Omega-3 PL were even able to override the permissive effect (see refs. [4,27]) of TZD rosiglitazone on hepatic lipogenesis. Lastly, the strong down-regulation of the lipogenic and cholesterol biosynthesis pathways was specific for the livers of mice subjected to dietary intervention with omega-3-containing PL, since it was not observed in mice fed a high-fat diet supplemented with the phosphatidylcholine-rich PL concentrate isolated from soy. Furthermore, in mice fed soy-derived PL, the lack of effect on hepatic expression of enzymes involved in lipid metabolism was also associated with unchanged plasma lipid levels and hepatosteatosis.

2. Material and methods

2.1. Animals and dietary interventions

Male C57BL/6N mice (Charles River Laboratories, Sulzfeld, Germany) were fed a standard diet (Chow; extruded Ssniff R/M-H diet; Ssniff Spezialdieten GmbH, Soest, Germany) and were maintained on a 12-h light-dark cycle (light from 6:00 a.m.) at 22 °C. Three independent experiments labeled A, B and C were performed:

(A) Three-month-old mice were randomly assigned for 7 weeks to a corn oil-based high-fat diet (cHF; lipids ~35% wt/wt; see [4]) or to various cHF-based interventions (n = 8): (i) cHF with ~10% lipids replaced by herring-derived omega-3 PL concentrate rich in phosphatidylcholine (PC diet; produced by EPAX AS, Aalesund, Norway; 5 g DHA/EPA per kg diet); (ii) cHF supplemented with rosiglitazone (R diet; 10 mg/kg diet); and (iii) cHF with both omega-3 PL and rosiglitazone (PC + R diet). Chow-fed mice served as lean controls. See Supplemental Tables 1 and 3 for details regarding the macronutrient and FA composition of the diets, and Supplemental Table 2 regarding the PL composition of the phosphatidylcholine-rich omega-3 PL concentrate used to prepare the PC diet.

(B) Three-month-old male C57BL/6N mice were randomly assigned either the cHF diet or various cHF-based interventions (n = 6–8): (i) cHF with ~10% lipids replaced by herring-derived omega-3 PL of a similar composition as in the experiment A above (PC-M; produced by EPAX AS, Aalesund, Norway; 3.4 g DHA/EPA per kg diet – i.e. ~30% lower when compared with the phosphatidylcholine-rich PL concentrate used in the experiment A); and ii) cHF with ~10% lipids replaced by phosphatidylcholine-rich PL concentrate from soy (PC-S; L-α-Phosphatidylcholine, Cat. no. P3644, Sigma-Aldrich). Both supplemented diets were matched for the content of phosphatidylcholine (~14 g/kg diet), which represents the major PL fraction contained in both concentrates (see Supplemental Table 2 for details on the PL composition of the PL concentrates used in this study). Dietary interventions lasted for a period of 7 weeks.

(C) Three-month-old male C57BL/6N mice were randomly assigned either the cHF diet (see the experiment A above for details) or cHF-based intervention (PC; n = 7), in which ~10% of lipids in the cHF was replaced by herring-derived omega-3 PL of a similar composition as in the experiment A (5 g DHA/EPA per kg diet; see also Supplemental Table 2 for details on the PL composition of the omega-3 PL concentrate used in this study). Dietary intervention lasted for a period of 7 weeks. During the week 6 of the experiment, feces were collected over a 24-h period to estimate the loss of lipids in feces. At the end, mice were used for the measurement of hepatic secretion of TG in the form of VLDL (VLDL-TG).

Body weight of single-caged mice was monitored weekly, while a fresh ration was given every 2 days. Food intake of each mouse was assessed weekly during a 24-h period and recalculated per week. In the experiments A and B, mice were killed by cervical dislocation under diethylether anesthesia between 9:00 a.m. and 11:00 a.m. Dorsolumbar (subcutaneous) and epididymal (abdominal) WAT and the liver were snap-frozen in liquid N_2. Tissues and EDTA-plasma were stored at −70 °C. The experiments followed the guidelines for the use and care of laboratory animals of the Institute of Physiology.

2.2. Plasma metabolites, hormones and enzymes

Plasma lipids were assessed by using a clinical analyzer Hitachi 902 (Roche Diagnostics) and enzymatic kits [28]. Plasma HDL cholesterol (HDL-C) levels were assessed by the kit Biolatest HDL Cholesterol Direct Liquid from Erba Lachema (Brno, Czech Republic). Bile acids were assessed using a Mouse Total Bile Acids Kit (Crystal Chem, IL, USA). Plasma levels of insulin were determined using the Sensitive rat insulin RIA kit from Linco Research (St. Charles, MO, USA). The distribution of adiponectin multimeric complexes in plasma including its high molecular weight (HMW) form was determined by Western blotting [4]. The homeostasis model assessment (HOMA) was applied to quantify insulin