Available online at www.sciencedirect.com

ScienceDirect

Journal of Nutritional Biochemistry 26 (2015) 585–595

Journal of Nutritional Biochemistry

RESEARCH ARTICLES

Intake of farmed Atlantic salmon fed soybean oil increases hepatic levels of arachidonic acid-derived oxylipins and ceramides in mice

Lisa Kolden Midtbø[a,b], Alison G. Borkowska[a,c,d], Annette Bernhard[a,b], Alexander Krokedal Rønnevik[a,b], Erik-Jan Lock[b], Michael L. Fitzgerald[c], Bente E. Torstensen[b], Bjørn Liaset[b], Trond Brattelid[b], Theresa L. Pedersen[d], John W. Newman[d,e,f], Karsten Kristiansen[a,*], Lise Madsen[a,b,**]

[a]Department of Biology, University of Copenhagen, Copenhagen, Denmark
[b]National Institute of Nutrition and Seafood Research, Bergen, Norway
[c]Massachusetts General Hospital, Center for Computational and Integrative Biology, Boston, MA, USA
[d]Obesity and Metabolism Research Unit, United States Department of Agriculture – Agricultural Research Service, Western Human Nutrition Research Center, CA, USA
[e]Department of Nutrition, University of California, Davis, USA
[f]West Coast Metabolomics Center, University of California, Davis, USA

Received 29 May 2014; received in revised form 18 November 2014; accepted 4 December 2014

Abstract

Introduction of vegetable ingredients in fish feed has affected the fatty acid composition in farmed Atlantic salmon (*Salmo salar L*). Here we investigated how changes in fish feed affected the metabolism of mice fed diets containing fillets from such farmed salmon. We demonstrate that replacement of fish oil with rapeseed oil or soybean oil in fish feed had distinct spillover effects in mice fed western diets containing the salmon. A reduced ratio of n-3/n-6 polyunsaturated fatty acids in the fish feed, reflected in the salmon, and hence also in the mice diets, led to a selectively increased abundance of arachidonic acid in the phospholipid pool in the livers of the mice. This was accompanied by increased levels of hepatic ceramides and arachidonic acid-derived pro-inflammatory mediators and a reduced abundance of oxylipins derived from eicosapentaenoic acid and docosahexaenoic acid. These changes were associated with increased whole body insulin resistance and hepatic steatosis. Our data suggest that an increased ratio between n-6 and n-3-derived oxylipins may underlie the observed marked metabolic differences between mice fed the different types of farmed salmon. These findings underpin the need for carefully considering the type of oil used for feed production in relation to salmon farming.

Keywords: EPA; DHA; Obesity; Diabetes; Aquaculture; Lipidomics

1. Introduction

The beneficial health effects of fatty fish such as salmon, herring and mackerel, have largely been attributed to their high content of marine n-3 PUFAs. Farmed Atlantic salmon has also been a rich source of these fatty acids, as high amounts of fish oil (FO) and fish meal have been used in the aquatic feed [1]. Such high use of marine ingredients in fish feed in the rapidly growing global aquaculture industry is not sustainable due to the stable harvest of wild fish stocks as 3–5 kg fresh fish were previously used to produce 1 kg farmed fish [2]. Hence, marine ingredients are increasingly replaced with vegetable ingredients in the fish feed used in commercial production [3]. This replacement alters fatty acid composition in the commercially available salmon fillets [4,5], and therefore, the potential spillover effects on consumers warrant further investigation. We have shown that replacement of FO with vegetable oils, soy bean oil (SO) in particular, in fish feed leads to a reduced n-3/n-6 PUFA ratio both in the salmon fillets and in red blood cells collected from mice consuming the salmon [6,7]. Of note, the reduced n-3/n-6 ratio in mice given the SO fed salmon was accompanied by increased insulin resistance and hepatic fat accumulation [7].

Whether there is a causal link between non-alcoholic fatty liver disease (NAFLD) and insulin resistance is a controversial question [8–10]. However, NAFLD and insulin resistance often co-occur, and both NAFLD and insulin resistance are strongly related to inflammation [11–13]. The role of inflammatory mediators, such as diacylglycerols (DAG) and ceramides, in modulating ectopic lipid accumulation involved in the development of insulin resistance is not fully elucidated, but ceramides are suggested to be key intermediates

* Correspondence to: K. Kristiansen, Department of Biology, University of Copenhagen, Ole Maaløes Vej 5, DK-2200 Copenhagen N, Denmark. Tel.: +45 3532 4443; fax: +45 3532 2128.
** Correspondence to: L. Madsen, National Institute of Nutrition and Seafood Research, P.O. Box 2029 Nordnes, N-5817 Bergen, Norway. Tel.: +47 4147 6177; fax: +47 5590 5299.
E-mail addresses: kk@bio.ku.dk (K. Kristiansen), lise.madsen@nifes.no (L. Madsen).

http://dx.doi.org/10.1016/j.jnutbio.2014.12.005

linking excess nutrients, specifically saturated fatty acids, and inflammatory cytokines, like tumor necrosis factor-α (TNF-α) to the induction of insulin resistance [14–16]. It is likely that insulin resistance and hepatic fat accumulation observed in mice given SO fed salmon compared with mice fed FO fed salmon [7], are related to the fatty acid composition in the salmon, and consequently, the mice feed. Of note, a low n-3/n-6 ratio has been associated with development of NAFLD [17] and n-3 PUFAs have been proposed as a therapeutic intervention in the treatment of NAFLD [18]. The n-3 PUFAs, EPA and DHA, are suggested to reduce accumulation of hepatic triacylglycerols (TAG) by increasing fatty acid oxidation and suppressing *de novo* lipogenesis and TAG formation [19–21]. Moreover, n-3 PUFAs might ameliorate NAFLD-progression and development of insulin resistance by their anti-inflammatory properties. The ability of n-3 PUFAs to inhibit diet-induced inflammation and insulin resistance is demonstrated to be, at least in part, mediated by their ability to activate the GPR120 receptor [22], but a number of additional mechanisms likely plays a role. EPA and DHA may inhibit arachidonic acid (AA)-dependent prostanoid production by incorporation into membrane phospholipids, reducing the amount of AA available as a substrate for cyclooxygenases. At the same time, the n-3 PUFAs serve as substrates for conversion to prostanoids of the 3- and 5-series that are considered less pro-inflammatory than AA-derived prostanoids [23], and n-3-epoxides which are reported to have anti-nociceptive [24], anti-angiogenic and anti-tumorogenic properties [25]. The n-3 PUFAs are also used as substrates for production of the anti-inflammatory resolvins and protectins [26]. Of note, resolvin E1 and protectin D1 have been demonstrated to mimic the insulin-sensitizing and anti-steatotic effects of n-3 PUFAs in animal studies [27]. Competition between n-3 PUFAs and AA for incorporation into phospholipids furthermore reduces substrate availability for syntheses of the two major endogenous endocannabinoids 2-arachidonoylglycerol (2-AG) and *N*-arachidonoylethanolamine (AEA) [28,29]. Given the importance of the cannabinoid receptor CB1 in development of diet-induced steatosis and insulin-resistance in mice [30,31], this represents an additional mechanism by which the levels of dietary n-3 and n-6 PUFAs can influence the development of hepatic steatosis and insulin resistance.

Since it is likely that the fatty acid composition is playing a key role in the metabolic effects we observed in mice fed salmon raised on different vegetable oil sources, We hypothesized that changes in hepatic neutral lipids, phospholipids and oxylipin, endocannabinoid and ceramide profiles, at least in part, contributed to hepatic lipid accumulation and the metabolic phenotypes. Accordingly, to investigate the molecular changes concurrent with and possibly involved in hepatic lipid accumulation, we evaluated changes in the liver lipidome. Specifically, we measured fatty acid profiles, lipid classes, phospholipids, oxylipins, endocannabinoid and ceramide profiles in combination with histological and molecular biological analysis of mice fed a Western diet (WD) containing either Atlantic salmon fed FO or Atlantic salmon where a high proportion of the FO was replaced with SO or rapeseed oil (RO).

2. Materials and methods

2.1. Ethical Statement

Animal handling and experiments were performed in accordance with the guidelines of the Norwegian Animal Research Authority (NARA) (Norwegian approval identification nr, FOTS Id:3196).

2.2. Experimental diets

Atlantic salmon were produced as described earlier [4]. All feed ingredients except the oil were the same and these are listed in [4]. The oil were either 100 % fish oil (FO) or 20% FO+80% rapeseed oil (RO), or 20% FO+80% soybean oil (SO). Fish fillets were subsequently freeze dried, and included in Western-diets (WD) as described [7]. Formulations of the mice diets were based on the standard Western diet (WD) D12079B Research diets, Inc. NJ, USA and prepared by Ssniff Spezialdiäten (Soest, Germany). We replaced 50% of the protein source in the standard WD with proteins from salmon fed FO, RO or SO. As references two groups of mice were fed a regular low fat diet (LF), D12450B Control Diet or a WD where 50% of the protein source was replaced with proteins from chicken breast filets. Chicken was chosen as a control since WD containing casein resulted in a lower fat digestibility compared with salmon fed FO [7]. Fat digestibility was equal for all mice fed the different WDs (Table S3).

2.3. Animals

Fifty male C57BL/6J BomTac mice were obtained from Taconic (Ejby, Denmark) at 8 weeks of age. The mice were maintained in a controlled environment with an artificial 12-h-light/dark cycle. To avoid thermal stress [32], mice were kept at thermoneutrality (30°C). Mice were allowed to acclimatize on a regular LF diet for 5 days. After acclimatization, the mice were housed individually and assigned by weight and body composition to the experimental diets. The mice had free excess to tap water and were fed the experimental diets described in Table S1 for 10 weeks ad libitum. Throughout the experiment, all mice were weighed once a week and feed intake was assessed every Monday, Wednesday and Friday. Lean mass and fat mass were determined prior to the feeding trial, and after 6 and 9 weeks by using nuclear magnetic resonance (NMR) (Minispec mq 7.5, NMR analyzer, Bruker, Germany). After a 10 weeks feeding period, mice were euthanized in fed state by cardiac puncture under Isoflurane anesthesia (Isoba-vet, Schering-Plough, Denmark). Liver, muscle and adipose tissue depots were quickly dissected out, weighed, snap-frozen in liquid nitrogen and stored at −80°C for further analysis. Blood was collected in EDTA-coated tubes, and plasma was prepared by centrifugation and stored in aliquots at −80°C for further use. Sampling was performed at room temperature (20°C).

2.4. Tissue and plasma analyses

Circulating insulin was measured in plasma using ELISA kit (EIA-3440, DRG International, INC., USA). Producer manuals were followed for measurements. Total RNA was isolated from mouse tissue using Trizol (Invitrogen). Reverse transcription was performed and cDNA was analyzed by qPCR using Light cycler 480 (Roche, Germany) as earlier described [33]. Fatty acid composition was measured in the neutral and phospholipid pools fractionated from liver samples as described earlier [6].

2.5. Histology

Liver was dissected out, fixed in 4% formaldehyde overnight, and stored in 0,1 M phosphate buffer before dehydration. Dehydration, paraffin embedding and sectioning were performed at Molecular Imaging Center (MIC) at the University of Bergen. Dehydration was performed by using Leica TP1020, Automatic Tissue Processor, 2 h in each container. The samples were cut into 5 μm-thick sections, and stained with Hematoxylin-Eosin for morphology investigations. For oil red O staining, samples were treated with a sucrose solution (20%) overnight before being stored in tissue tech at −80°C. Samples were cut into 10 μm-thick sections (CM 1950 Cryostat, Leica Biosystems, Germany), and stained with oil red O.

2.6. Targeted metabolomics

Liver Fatty Acids: Frozen liver samples were weighed (~20 mg) and placed in 5 μl anti-oxidant solution (0.2 mg/mL butylated hydroxytoluene (BHT)/EDTA) and 10 μL of a solution containing analytical surrogates were added to correct for extraction losses and ion suppression. Lipids were extracted from the liver subsamples via stepwise solvent extraction, using a modified protocol previously described [34]. After treatment with sodium methoxide/methanolic HCl, fatty acid methyl esters were analyzed by an Agilent 6890 Plus+equipped with 7683 Injector using a DB-225MS (30 m × 0.25 mm × 0.25 μm) column and detected by GC5973N mass selective detector. Conditions: 1 μL splitless injection, SIM/SCAN mode data collection; Column Flow: 1 mL/min for 5 min, +0.25 mL/min ramp to 3.5 mL/min, 4 mL/min for 28 min, 2 mL/min for 2 min; Oven Temperature: 65°C for 1 min, +35°C/min to 195°C, hold 0.5 min, +3°C/min to 235°C, +5°C/min to 240°C, hold 23.5 min, cool to 65°C in 2 min. SIM data was used for quantification against a 6-point calibration curve.

Liver Metabolite Acquisition: Non-esterified oxylipin, endocannabinoid and ceramides and their associated surrogates were analyzed by LC-MS/MS [35,36] from an aliquot of the stepwise extract described above. Additionally, esterified oxylipins within extracts were released by hydrolysis with 0.5 M sodium methoxide for 1 h at 60°C. Non-esterified oxylipins were isolated from total lipid extracts using a solid-phase extraction protocol with Oasis HLB columns (Waters, USA). Oxylipins were separated with a 16 min reverse phase gradient on a 2.1×150 mm, 1.7 μm Acquity BEH column at 60°C on a Waters Acquity UPLC. Analytes were ionized in negative mode by electrospray ionization (ESI) and data was acquired and collected in multi-reaction monitoring mode (MRM) with an AB Sciex 4000QTRAP tandem mass spectrometer. Endocannabinoids were separated on the same UPLC column and mobile phases but were ionized in positive mode by ESI in an independent 11 min run. Ceramides were separated on a BEH C8, 2.1×100 mm column and were ionized in positive mode by ESI with a 15 min run time, modified for UPLC from published methods [36].