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Distinct effects of dietary flax compared to fish oil, soy protein compared to casein, and sex on the renal oxylipin profile in models of polycystic kidney disease☆

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ABSTRACT

Oxylipins are bioactive lipids derived from polyunsaturated fatty acids (PUFA) that are important regulators of kidney function and health. Targeted lipidomic analyses of renal oxylipins from four studies of rodent models of renal disease were performed to investigate the differential effects of dietary flax compared to fish oil, soy protein compared to casein, and sex. Across all studies, dietary fish oil was more effective than flax oil in reducing n-6 PUFA derived oxylipins and elevating eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) derived oxylipins, whereas dietary flax oil resulted in higher α-linolenic acid (ALA) oxylipins. Dietary soy protein compared to casein resulted in higher linoleic acid (LA) derived oxylipins. Kidneys from females had higher levels of arachidonic acid (AA) oxylipins, but similar or lower levels of oxylipins from other PUFA. Modulation of the oxylipin profile by diet and sex may help elucidate their effects on renal physiology and health.

1. Introduction

Oxylipins are oxygenated metabolites of polyunsaturated fatty acids (PUFA) formed by mono- or dioxygen-dependent reactions of cyclooxygenase (COX), lipoxygenase (LOX) and cytochrome P450 (CYP) enzymes [1]. Although eicosanoids formed from arachidonic acid (AA) are the most widely studied class of oxylipins, recent advances in lipidomics have revealed a large number of novel oxylipins formed from other PUFA with chain length varying from 18 (octadecanoids) to 22 carbons (docosanoids) [1–4]. These bioactive lipids play significant roles in many key physiological processes in kidney health and disease, including maintaining blood flow, hemodynamics, renin secretion, and glomerular filtration rate [5–7]. Oxylipins also are involved in inflammatory, fibrotic and proliferatory events in diseased kidneys [8,9].

Dietary oils influence the production of oxylipins in tissues, including the kidney. Increased dietary intake of n-3 PUFA has long been associated with an increase in beneficial n-3 prostaglandins (PG) and a reduction in n-6 PG [10], but the distinct effects of different n-3 PUFA [i.e. α-linolenic acid (ALA) vs. eicosapentaenoic acid (EPA)/docosahexaenoic acid (DHA)] on health [11–13] may be due to unique effects of their oxylipin metabolites. In kidney, few oxylipins have been studied, particularly in relation to dietary fish oil intake. In rats, a

Abbreviations: AA, arachidonic acid; ALA, α-linolenic acid; COX, cyclooxygenase; CYP, cytochrome P450; DGLA, dihomo-gamma-linolenic acid; DHA, docosahexaenoic acid; DiHDoHE, dihydroxy-docosahexaenoic acid; DiHDPE, dihydroxy-docosapentaenoic acid; DiHETrE, dihydroxy-eicosatrienoic acid; DiHOME, dihydroxy-octadecenoic acid; DiHOTrE, dihydroxy-octadecatrienoic acidEPA, eicosapentaenoic acid; EpOME, epoxy-octadecenoic acid; GLA, gamma-linolenic acid; HDoHE, hydroxy-docosahexaenoic acid; HEPE, hydroxy-eicosapentaenoic acid; HETE, hydroxy-eicosatetraenoic acid; HETrE, hydroxy-eicosatrienoic acid; HODE, hydroxy-octadecadienoic acid; HOTrE, hydroxy-octadecatrienoic acid; LA, linoleic acid; LOX, lipoxygenase; oxo-ETE, oxo-eicosatetraenoic acid; oxo-ODE, oxo-octadecadienoic acid; oxo-OTrE, oxo-octadecatrienoic acid; PG, prostaglandin; Rv, resolvin; TriHOME, trihydroxy-octadecenoic acid; Tx, thromboxane

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limited number of n-3 PUFA derived renal oxylipins were reported to be elevated and n-6 PUFA oxylipins were reduced with fish oil consumption in two recent studies [14,15]. With flax oil feeding, elevated levels of several oxylipins from ALA and EPA and lower levels of some n-6 oxylipins have been reported in kidneys from obese rats [16]. In a mouse model of renal disease, flax oil feeding lowered AA and linoleic acid (LA) oxylipin levels and elevated ALA, EPA and DHA oxylipins in renal tissues [17]. These findings are generally similar to human plasma oxylipin studies with fish [11,18,19] and flax oil [20,21], which have examined a much wider range of oxylipins. In the above studies, oxylipin alterations by dietary flax oil was associated with reduced renal disease [16,17], but fish oil induced oxylipin alterations did not consistently correlate with changes in disease [14,15]. However, neither the human plasma studies nor the rodent renal studies have compared the effects of fish to flax oil on the oxylipin profile directly in the same model. Additionally, a detailed profile of oxylipins in the kidney is lacking.

Much less is known about effects of dietary protein on oxylipins. We recently reported that soy protein compared to casein feeding reversed some of the disease associated alterations in n-6 PUFA derived oxylipins in rat renal tissues [14]. Peng et al. also demonstrated a reduction in AA derived levels of thromboxane B_2 (TxB_2), and 6-keto $PGF_{1\alpha}$ in rat renal tissues with soy protein feeding that also was associated with disease mitigation [22]. These results suggest that soy protein could alter renal oxylipins, but it is not clear whether this is an indirect effect due to its effects on disease. On the other hand, soy protein can reduce the activity of Δ6 desaturase, an enzyme that converts LA to longer chain PUFA, and raise the LA levels [23,24], and thus could potentially alter the formation of oxylipins from these fatty acids.

The effect of sex on oxylipins also is largely unknown, but there are some indications of differences between male and female oxylipin levels in the kidney. Female rats have higher renal levels of AA derived PGE_2, which may be due to lower levels of a PG-specific transporter responsible for PG clearance in rat renal tissues [25]. Higher levels of PGE_2 and TxB_2 in the urine of diseased female rats have also been reported [26]. Gender differences in enzymes that metabolize AA and AA oxylipins also may be responsible for these differences [27–31]. These limited results indicate that there are sex specific differences in renal oxylipins, but a comprehensive analysis of differences in the male and female oxylipin profile has not been performed in the kidney, or any other tissue.

We recently reported that there were few effects of dietary fish and flax compared to soy oil, soy protein compared to casein, and sex on disease progression in a rat and a mouse model of polycystic kidney disease [32]. We also examined these dietary and sex effects in 2 other studies with another model of this disease, but in these studies the disease progression was insufficient to examine dietary effects on disease. Since the dietary interventions and sex had only minor or no effects on disease in all four of these studies, the kidneys were examined herein to determine the effects of these diet interventions and sex on the comprehensive renal oxylipin profile. Findings across the different studies consistently revealed that fish and flax oil have distinct effects on the renal oxylipin profile, that soy protein increases LA derived oxylipins, and that AA derived oxylipins are uniquely higher in females.

2. Materials and methods

2.1. Animal models

Kidneys from four studies of rodent polycystic kidney diseases were used for the analyses. In the first study, weanling male PCK rats [33] purchased from a commercial breeder (Charles River, QC, Canada) were used. The second and third studies used Mx1Cre + $Pkd1^{flox/flox}$ (Pkd1) conditional knockout mice from our in-house colony, originally provided by Jing Zhou (Brigham and Women's Hospital and Harvard Medical School, Boston. MA, U.S.A) [34]. To induce disease, male and female Pkd1 mice in the second study were administered i.p. with 250 μg polyinosinic polycytidylic acid (pI: pC) for five consecutive days beginning at 5 weeks of age [hereafter called Pkd1 (5wk) mice], and in the third study were injected at 1 week of age [hereafter called Pkd1 (1wk) mice]. For the fourth study, $Pkd2^{ws25/ws25}$ and $Pkd2^{+/-}$breeders were obtained from Dr. Stefan Somlo (Yale University, New Haven, CT, USA) [35] and crossed to produce ($Pkd2^{ws25/-}$) mice with disease (hereafter called Pkd2 mice).

2.2. Diets

Diets were based on the American Institute of Nutrition (AIN) 93 G standard diet for laboratory rodents [36] and reported in detail in the previously published study on disease effects in PCK rats and Pkd2 mice [32]. All four studies had diets containing either soy oil, flax oil or fish oil with the only difference between these diets being that flax or fish oil replaced 80% of the soy oil in the standard soy oil diet (details in Supplemental Tables 1 and 2). Thus dietary oil effects were examined in all four studies. The studies with PCK rats, Pkd1 (5wk) mice and Pkd2 mice also had diets that replaced the standard protein source (casein) with soy protein, resulting in 6 different diets (Supplemental Table 1). In Pkd2 mice, however, diseased mice could only be identified upon termination and the number of mice in the protein groups was found to be too low in some subgroups to test protein effects on oxylipins, so protein effects were examined only in PCK rats and Pkd1 (5wk) mice. The three mouse studies also included both males and females, allowing the examination of sex effects in these studies. All female animals used were pre-menopausal for the duration of the studies. All diet ingredients were purchased from Dyets Inc. (Bethlehem, PA, USA). Oils contained 0.02% tert-butylhydroquinone (added by Dyets Inc) to prevent oxidation and diet ingredients were stored at 4 °C. Diet was freshly prepared twice per month and stored in sealed containers at −20 °C until feeding.

Animals were housed singly in a temperature and humidity controlled environment with a 12-h day/night cycle and were given free access to water and diet. The feeding period for each study was 12 weeks, 4–16 weeks of age), 16 weeks 6–22 weeks of age), 6 weeks 3–9 weeks of age) and 13 weeks 3–16 weeks of age), for the PCK rats, Pkd1 (5wk), Pkd1 (1wk) and Pkd2 mice, respectively. At the end of each study, animals were anesthetised with isoflurane. PCK rats were terminated by cardiac puncture and mice were terminated by decapitation. The right kidney was snap frozen in liquid nitrogen and stored at −80 °C until analysis. All animal procedures were approved by the University of Manitoba Animal Care Committee and adhered to the guidelines of the Canadian Council on Animal Care.

2.3. Oxylipin analysis

Lyophilized whole kidney tissues were homogenized in ice cold Tyrode's salt solution (pH 7.6) in a 1:28 weight:volume ratio. After homogenization, Triton X-100 was added to achieve a final concentration of 0.01%. Deuterated internal standards (10 ng each, Cayman Chemical, MI, USA) and 6.5 μL antioxidant cocktail [0.2 g/L BHT, 0.2 g/L EDTA, 2 g/L triphenylphosphine, and 2 g/L indomethacin in MeOH: EtOH:H_2O (2:1:1,by vol)] were added to 200 μL aliquots that were used for analysis. Samples were adjusted to pH < 3 and solid phase extraction was with Strata-X SPE columns (Phenomenex, CA, USA) preconditioned with methanol and pH 3 water. Samples were loaded onto the columns, rinsed with 10% methanol, and eluted with methanol. Evaporated samples were then resuspended in solvent for analysis by HPLC-MS/MS (API 4000, AB Sciex, Canada) as described [37] based on methods developed by Deems et al. [2]. Details of all oxylipins screened, the deuterated internal standards used and the detector response factors are listed in Supplemental Table 3. Detection and quantification limits were set at 3 and 5 levels above the background, respectively. Quantities of oxylipins were determined using the stable isotope dilution method [38] and expressed as pg/mg dry tissue.