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Circulating levels of endocannabinoids and oxylipins altered by dietary lipids in older women are likely associated with previously identified gene targets☆,☆☆

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ABSTRACT

Postmenopausal women (PMW) report marginal n−3 PUFA intakes and are at risk of chronic diseases associated with the skeletal, muscular, neuroendocrine, and cardiovascular systems. How n−3 PUFA affect the amounts of endocannabinoids (ECs) and oxylipins (OLs) of metabolic and physiologic importance in PMW is not clear. Based on our recent findings that dietary n−3 PUFA alter gene targets of the EC system and lower pro-inflammatory OL we proceeded to characterize these actions in blood of PMW. Our aim was to determine levels of the ECs, OLs, and global metabolites (GM) in white PMW (75 ± 7 y), randomized in a double-masked manner, from baseline to 6 mo after receiving a fish oil supplement of n−3 PUFA (720 mg 20:5n3 + 480 mg 22:6n3/d, n = 20) or placebo (1.8 g oleic acid/d, n = 20). ECs and OLs in serum were determined by UPLC-MS/MS and GM by GC–MS and LC-MS/MS. Plasma 20:5n3 and 22:6n3 levels increased in PMW given fish oil. EC n−6 acyl-ethanolamides, arachidonate-derived diols were decreased and 20:5n3 and 22:6n3 diols, epoxides, and alcohols were increased in PMW given fish oil. GM analysis revealed that n−3 PUFA supplementation increased renal steroid hormone and proteolytic metabolite levels in PMW. Herein, we confirm that gene targets of the EC system, previously found as modifiable by n−3 PUFA result in changes in the levels of ECs and OLs in PMW. This study shows phenotypic responses (in levels) to n−3 PUFA supplementation in PMW and increases of n−3 acyl-ethanolamide and n−3-derived OL of clinical considerations in aging.

Abbreviations: AEA, arachidonoyl ethanolamide, i.e. anandamide; 2-AG, 2-arachidonoylglycerol; DEA, docosatetraenoyl ethanolamide; DHA, docosahexaenoic acid; DHEA, docosahexaenoyl ethanolamide; DGLEA, dihomo-gamma-linolenoyl ethanolamide; ECs, endocannabinoids; ECS, endocannabinoid system; EPA, eicosapentaenoic acid; FAAH, fatty acid amide hydrolase; FAME, fatty acid methyl esters; LEA, linoleoyl ethanolamide; αLEA, alpha-linolenoyl ethanolamide; LTB_4, leukotriene B_4; NA-Gly, *N*-arachidonyl glycine; OEA, oleoyl ethanolamide; OLs, Oxylipins; PEA, palmitoyl ethanolamide; PGE_2, prostaglandin E_2; PMW, post-menopausal women; PUFA, polyunsaturated fatty acids; SEA, stearoyl ethanolamide; 5-HEPE, 5-hydroxy-6E,8Z,11Z,14Z,17Z-eicosapentaenoic acid; 5,6-DiHETrE, 5,6-dihydroxy-8Z,11Z,14Z-eicosatrienoic acid; 8(9)-EpETrE, 8(9)-epoxy-5Z,11Z,14Z-eicosatrienoic acid; 8,9-DiHETrE, 8,9-dihydroxy-5Z,11Z,14Z-eicosatrienoic acid; 11(12)-EpETrE, 11(12)-epoxy-5Z,8Z,14Z-eicosatrienoic acid; 11,12-DiHETrE, 11,12-dihydroxy-5Z,8Z,14Z-eicosatrienoic acid; 12-HEPE, 12-hydroxy-5Z,8Z,10E,14Z,17Z-eicosapentaenoic acid; 12-HETE, 12-hydroxy-5E,8Z,10Z,14Z-eicosatetraenoic acid; 14(15)-EpETrE, 14(15)-epoxy-5Z,8Z,11Z-eicosatrienoic acid; 14,15-DiHETE, 14,15-dihydroxy-5Z,8Z,11Z,17Z-eicosatetraenoic acid; 14,15-DiHETrE, 14,15-dihydroxy-5Z,8Z,11Z-eicosatrienoic acid; 15-HEPE, 15-hydroxy-5Z,8Z,11Z,13E,17Z-eicosapentaenoic acid; 16(17)-EpDPE, 16(17)-epoxy-4Z,7Z,10Z,13Z,19Z-docosapentaenoic acid; 17-HDoHE, 17-hydroxy-4Z,7Z,10Z,13Z,15E,19Z-docosahexaenoic acid; 17(18)-EpETE, 17(18)-epoxy-5Z,8Z,11Z,14Z-eicosatetraenoic acid; 17,18-DiHETE, 17,18-dihydroxy-5Z,8Z,11Z,14Z-eicosatetraenoic acid; 19,20-DiHDPA, 19,20-dihydroxy-4Z,7Z,10Z,13Z,16Z-docosapentaenoic acid.

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1. Introduction

Postmenopausal women (PMW) are at a risk of excessive bone loss, hypertension, and metabolic syndrome. Moreover, while n − 3 polyunsaturated fatty acids (PUFA) status generally increases with age [1], PMW typically have marginal intakes of n − 3 PUFA. Stark et al. [2] reported that short-term supplementation with n − 3 PUFA (20:5n3 EPA and 22:6n3 DHA) in PMW resulted in similarly higher EPA and DHA levels and lower linoleic acid levels that correspond to Inuit women but not the markedly lower level of arachidonic acid (AA) seen in these seal eating populations. A recent investigation revealed that aerobic exercise and n − 3 PUFA supplementation with fish oil had a synergistic action on reducing inflammation and improving bone mineral density associated with osteoporosis in PMW [3].

While the specific mechanisms for the observed health improvements of n − 3 PUFA are still being explored, hypotriglyceridemia [4], improved ratios of LDL/HDL [5], and reductions in coronary artery disease progression [6] and atherosclerosis incidence in PMW [7] are well documented. As n − 3 PUFA are precursors for many biologically active metabolites, including endocannabinoids (ECs) and oxylipins (OLs), a key to understanding their health promoting properties is to identify how dietary n − 3 PUFA influence the production of these metabolites.

Although the role of the endocannabinoid system (ECS) has yet to be characterized in PMW, this system affects food intake and energy metabolism in peripheral tissues, and ECS overstimulation contributes to obesity and loss of insulin sensitivity in muscle. Consequences of elevated AA-derived EC, include overstimulating of the ECS [8] leading to impaired glucose uptake in skeletal muscle [9], stimulating osteoclast proliferation [10], and generating pro-inflammatory cyclooxygenase (COX)-derived prostanoids [11]. Thus, the impact of dietary PUFA on physiology likely include mechanisms associated with alterations in lipid mediator tone. Recently, our laboratory reported that n − 3 PUFA alter expression of several genes associated with the ECS in myoblasts [12] and the mouse [13] to influence glucose uptake and fat accretion, respectively. These observations are a reason to investigate n − 3 PUFA effects on ECs and OLs in PMW.

The OLs are oxygenated fatty acids that are important regulators of physiology and health and include metabolites of all PUFA, whose balance can be altered by dietary lipid content [14]. The 20-carbon "eicosanoids" are OL derived from AA or EPA metabolized by a suite of enzymes including various cyclooxygenases, lipoxygenases, and cytochrome P450 (CYPs) hydroxylase and epoxygenases. The COX- and LOX-derived OLs consist of prostanoids, thromboxanes, and leukotrienes, and mid-chain alcohols including hydroxyeicosatetraenoic acids [15], while the CYPs yield the vasoactive and immunomodulatory omega-hydroxy and -epoxy fatty acids [16]. Several other OLs are derived as secondary products of these metabolites, further adding to the potential fates of PUFA. Pertinent to PMW, prostaglandin E_2 (PGE_2) has been found to be a potent stimulator of bone resorption and regarded as the primary prostaglandin affecting bone metabolism [17]. Leukotriene B_4 (LTB_4) has also been found to increase bone resorption and affect osteoclast cell division. PGE_2 and LTB_4 are both AA metabolites which can be altered by dietary n − 6/n − 3 PUFA balance [18], further elaborating the influence of dietary PUFA on biological processes where OLs are involved. A well-accepted effect in response to n − 3 PUFA supplementation is the enrichment of membrane phospholipids with the long-chain n − 3 PUFA, EPA and DHA. Given the relationship between dietary and tissue PUFA and the potential effects on circulating EC and OL levels, our research aim was to determine how dietary PUFA alter plasma levels of these metabolites in the elderly. However, little is known about the EC and OL serum levels in older adults and PMW. Therefore, the primary hypothesis for this research is that dietary PUFA determine blood and tissue concentrations of AA, while n − 3 PUFA EPA and DHA decrease AA to alter the types of EC and OL in blood. In the current study, postmenopausal subjects were given an n − 3 PUFA supplement or placebo for 6 mo after which serum and plasma was collected to measure EC, OL, and PUFA, respectively. We also examined the effect of the n − 3 PUFA supplement on global metabolites (GM) influenced by changes in ECs and systemic macronutrient metabolism [8,9]. The justification for this investigation with EPA and DHA is to determine the effects on the EC in PMW prior to studies on genes (cannabinoid receptors CB1 and CB2, GLUT 1, and insulin-R) related to these compounds which was shown in myoblast cultures and mice (protein expression of CB1, CB2, GLUT 4 and insulin-R) [12,13].

2. Materials and methods

2.1. Subjects, dietary supplements, and blood samples

Women with a mean age of 75 y (Table 1) were randomized in a double-masked manner to receive either 1.2 g EPA + DHA from fish oil (n = 20; 2 - 1 g capsules/d, 360 mg EPA and 240 mg DHA/capsule; Vital Nutrients, Middletown, CT) or olive oil placebo (n = 20; 2 - capsules/d, 1.8 g oleic acid/d, Vital Nutrients). Blood samples (serum and plasma) were collected at baseline and 6 mo into the intervention period, kept on ice during collection and stored at −80 °C, with sub-aliquots designated for the analysis of total lipid fatty acids (plasma; Watkins, University of Connecticut, Farmington, CT), EC and OL (serum; USDA-ARS-WHNRC, Davis, CA), and global metabolomics

Table 1
Baseline characteristics of study participant groups.

	Placebo (n = 20)	n − 3 PUFA (n = 20)	p value
Age (y)	74 ± 6	75 ± 6	0.77
Body mass index (kg/m^2)	26.3 ± 4.7	27.1 ± 6.0	0.59
PASE score	198 ± 96	269 ± 136	0.06
Dietary intake/d			
Calories	1694 ± 700	1519 ± 780	0.45
Fat (g)	57 ± 30	58 ± 34	0.87
Protein (g)	89 ± 112	60 ± 21	0.27
Calcium (mg)	626 ± 237	740 ± 369	0.25
Vitamin D (IU)	125 ± 81	119 ± 84	0.82
n − 3 PUFA (g)	0.65 ± 0.43	0.75 ± 0.53	0.54
Ethnicity %			0.32
White	97	92	
Hispanic	3	0	
Black	0	5	
Asian	0	3	
Education %			0.33
High school	15	15	
College	60	35	
Post graduate	25	40	
Marital Status %			0.90
Single	10	10	
Married	45	55	
Divorced	15	15	
Widowed	30	20	
Comorbidity %			
Coronary heart disease	10	10	1.00
Diabetes	0	0	
Hypertension	30	45	0.33
Depression	10	5	0.55
Smoker %	0	0	
Drinks alcohol %	70	70	1.00
Medications %			
Statins	56	38	0.29
Diuretic	28	23	0.62
Beta Blocker	31	25	0.69
ACE inhibitor	38	31	0.71
ARB	6	6	1.00
Calcium channel blocker	13	19	0.63
Aspirin	40	45	0.75
NSAID	10	0	0.155
Anti-acid	13	13	1.00

Values are means ± SD. Abbreviations: PASE, physical activity scale for the elderly; ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; NSAID, nonsteroidal anti-inflammatory drug.