Reproductive Sciences
2015, Vol. 22(9) 1083-1087

DOI: 10.1177/1933719114565030
rs.sagepub.com
SAGE

Serum Polyunsaturated Fatty Acids and Endometriosis

Margaret M. Hopeman, MD[1], Joan K. Riley, PhD[1], Antonina I. Frolova, MD, PhD[1], Hui Jiang, PhD[2], and Emily S. Jungheim, MD, MSCI[1]

Abstract

Polyunsaturated fatty acids (PUFAs) are fatty acids containing 2 or more double bonds, and they are classified by the location of the last double bond. Omega 3 (n-3) and omega 6 (n-6) PUFAs are obtained through food sources including fatty fish and seed/vegetable oils, respectively, and they are important to a number of physiologic processes including inflammation. Previous work demonstrates suppressive effects of n-3 PUFAs on endometriotic lesions in animal models and decreased risk of endometriosis among women with high n-3 PUFA intake. Thus, we sought to determine the relationship between circulating levels of PUFAs and endometriosis in women. To do this, we performed a cross-sectional study of serum PUFAs and clinical data from 205 women undergoing in vitro fertilization (IVF). Serum PUFAs were measured using liquid chromatography coupled to tandem mass spectroscopy and included n-3 PUFAs such as α-linolenic acid, eicosapentaenoic acid (EPA), and docosahexaenoic acid and n-6 PUFAs such as linoleic acid and arachidonic acid. Multivariable logistic regression was used to determine relationships between specific and total serum PUFAs and patient history of endometriosis. Women with high serum EPA levels were 82% less likely to have endometriosis compared to women with low EPA levels (odds ratio = 0.18, 95% confidence interval 0.04-0.78).

Keywords

eicosapentaenoic acid, endometriosis, omega-3 fatty acids, polyunsaturated fatty acids

Introduction

Endometriosis is common among reproductive-age women, particularly among women with infertility.[1] In addition to its association with infertility, endometriosis is also often associated with significant pain symptoms. Unfortunately, most medical options for managing endometriosis-associated pain also inhibit ovulation and are therefore unacceptable to women who are hoping to conceive.

A growing body of evidence from animal studies suggests endometriosis lesions may be suppressed by n-3 fatty acids.[2-6] Furthermore, data from the Nurses' Health Study demonstrate higher reported intake of n-3 fatty acids is associated with a decreased risk of endometriosis.[7] Despite this, associations between circulating levels of specific polyunsaturated fatty acids (PUFAs) and the diagnosis of endometriosis have been understudied in women.[8] This is important as the most efficient way to increase specific physiologic PUFA levels is through consumption of the specific fatty acid of interest.[9] Thus, we analyzed specimens and data from women with histories of surgically confirmed endometriosis and women without endometriosis to determine whether associations between specific PUFAs and endometriosis exist.

Materials and Methods

Study Participants and Samples

This cross-sectional study was approved by Washington University's Institutional Review Board. Fasting (overnight) serum specimens and data were obtained from women undergoing in vitro fertilization (IVF) and enrolled in the Women's and Infant's Health Specimen Consortium at Washington University (WIHSC), a prospective study of factors affecting reproductive and maternal–neonatal outcomes. Serum was extracted from whole blood and stored at -80°C for batched analysis. Women were included in this study if they consented

[1] Department of Obstetrics and Gynecology, Washington University School of Medicine, St Louis, MO, USA
[2] Department of Medicine, Diabetic Cardiovascular Disease Center, Washington University School of Medicine, St Louis, MO, USA

Corresponding Author:
Emily S. Jungheim, Department of Obstetrics and Gynecology, Washington University School of Medicine, 660 South Euclid Avenue, St Louis, MO 63110, USA.
Email: jungheime@wustl.edu

to use their serum and clinical data from the WIHSC study. Women were excluded if they did not undergo one of our standard controlled ovarian hyperstimulation (COH) protocols for IVF. Clinical data for each participant were obtained through the WIHSC database and included age, weight, height, infertility diagnosis, and surgical history.

Blood was collected from all women the morning of oocyte retrieval after COH. Briefly, 179 women underwent COH using a long gonadotropin-releasing hormone (GnRH) agonist protocol as described previously.[10] In all, 18 women underwent a short GnRH agonist (flare) protocol for COH and the remaining 8 underwent COH using a GnRH antagonist protocol. Women following the flare and GnRH antagonist protocols began gonadotropin injections (Follistim-Merck, Whitehouse Station, New Jersey, or Gonal-F-EMD Serono, Inc., Rockland, Massachusetts, and Menopur-Ferring Pharmaceuticals Inc., Parsippany, New Jersey) early in the follicular phase. Gonadotropin dosing was chosen based on age, antral follicle count, and serum anti-Mullerian hormone levels when available and was adjusted according to ovarian response. Women following the flare protocol took subcutaneous leuprolide acetate injections (Sandoz Inc, Princeton, New Jersey) along with their daily gonadotropin injections whereas women following the GnRH antagonist protocol initiated GnRH antagonist injections (Ganirelix-Merck, Whitehouse Station, or Cetrotide-EMD Serono, Inc) on day 6 of gonadotropin stimulation. Following either protocol, when 2 or more follicles were 18 to 20 mm in diameter and the serum estradiol level was at least 500 pg/mL, the patient was triggered with intramuscular human chorionic gonadotropin (hCG; Pregnyl-Merck, Whitehouse Station, New Jersey, or Novarel-Ferring, Parsippany, New Jersey). Oocyte retrieval was performed 36 hours after hCG administration.

Analysis of Serum PUFAs

Serum PUFAs were analyzed by liquid chromatography coupled to tandem mass spectroscopy (LC-MS/MS).[11] Briefly, serum PUFAs were extracted by a modified Bligh-Dyer protocol in the presence of d4-palmitic acid, an internal standard, and derivatized by dimethylaminopropylamine into amides to obtain high sensitivity in mass spectroscopy. Liquid chromatography was performed with a Shimadzu 10A HPLC system (Shimadzu, Columbia, Maryland) and a Phenomenex Luna C18 column (2.5 μm, 100 × 3 mm; Phenomenex, Torrance, California). Mass spectroscopy (MS) was performed with a TSQ Quantum Ultra triple quadrupole mass spectrometer (Thermo Fisher Scientific, Inc, Waltham, Massachusetts) operated in SRM mode under electrospray ionization (+). The identity of PUFA was determined by comparing retention time to a corresponding commercial standard. Data processing was conducted with Xcalibur 2.0.7 software (Thermo Fisher Scientific, Inc, Waltham, Massachusetts). The PUFA levels were reported as the peak area ratio of the analyte to the internal standard, based on the assumption that MS responses of analyte and internal standard were the same. Thus, reported levels do not have units. If the MS responses of the analyte and the internal standard differed, differences would in theory be eliminated when a specific PUFA species was compared across all patients. Coefficient of variation for each PUFA was calculated to evaluate the precision of measurement.

Statistical Analyses

Sample size was calculated using Epi Info 7.0 (Centers for Disease Control and Prevention, Atlanta, Georgia) and was based on the following assumptions: 0.05 type I error, 0.2 type II error, 50% prevalence of endometriosis in women with low n-3 fatty acid levels, and 25% prevalence of endometriosis in women with high n-3 fatty acid levels. Assumed prevalence in the 2 groups was based on the literature, suggesting 25% to 50% of women with infertility have endometriosis.[1] Given our assumptions, we determined specimens were needed from at least 116 women to address the question of whether or not differences in specific serum PUFAs existed between women with and without endometriosis. We identified 205 women in our WIHSC tissue databank who met inclusion and exclusion criteria. We analyzed specimens from all of these women to account for possible difficulty in specimen processing and to allow for investigation of possible dose-dependent associations between PUFA levels and endometriosis. All analyzed specimens yielded usable data.

Student's *t* test was used to determine whether the diagnosis of endometriosis was associated with total PUFA levels, total n-3 levels, total n-6 levels, and any of the specific serum PUFAs analyzed. The only differences noted were in serum eicosapentaenoic acid (EPA) levels which were noted to be lower in women with endometriosis. Serum EPA levels were categorized into quartiles and risk of endometriosis was compared in women with the highest EPA levels relative to women with the lowest levels using Fisher exact test. A logistic regression model was constructed including EPA quartiles and age as covariates with endometriosis as the outcome. All analyses were performed in SPSS version 22 (SPSS, Chicago, Illinois).

Results

Patient Characteristics

A total of 205 women met inclusion and exclusion criteria. Twenty-four women were classified as having endometriosis. All of these endometriosis diagnoses had been previously confirmed surgically. Among women classified as not having endometriosis, infertility diagnoses included male factor (n = 60), unexplained (n = 50), tubal occlusion (n = 37), ovulation disorder (n = 14), polycystic ovary syndrome (n = 10), diminished ovarian reserve (n = 9), and fibroids (n = 1). Of the 181 women, 24 classified as not having endometriosis had previously undergone laparoscopy for gynecologic pathology including hydrosalpinges or ovarian cysts. The remaining 157 women had no history of pelvic surgery, but all women underwent thorough pelvic evaluation with either a hysterosalpingogram or a saline-infused pelvic sonohysterogram.

Table 1. Characteristics of Women With Endometriosis Versus Those Without.

	Endometriosis (n = 24)	No Endometriosis (n = 181)	*P* Value	RR (95% CI)
Age, years	31.5 ± 2.6	33 ± 4.6	.03	–
Caucasian	22/24	153/181	.53	1.9 (0.47-7.6)
BMI, kg/m^2	26.6 ± 5.8	27.6 ± 7.0	.4	–
Previous pregnancy	4 (17%)	40 (22%)	.8	0.8 (0.3-1.9)
COH with long agonist protocol	22 (96%)	157 (88%)	.3	1.1 (1.0-1.2)

Abbreviations: RR, relative risk; CI, confidence interval; BMI, body mass index; COH, ovarian hyperstimulation.

Table 2. Serum FFAs in Women With and Without Endometriosis.

PUFA Species	Endometriosis, n = 24	No Endometriosis, n = 181	*P* Value
ALA (18:3)	0.52 ± 0.3	0.46 ± 0.2	.2
Linoleic acid (18:2)	6.5 ± 2.0	6.3 ± 2.0	.6
EPA (20:5)	$1.6 \times 10^{-2} \pm 0.006$	$2.0 \times 10^{-2} \pm 0.01$	.005
AA (20:4)	0.32 ± 0.09	0.36 ± 0.1	.1
DHA (22:6)	0.14 ± 0.06	0.15 ± 0.06	.7

Abbreviations: ALA, α-linolenic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid; DHA, docosahexaenoic acid.

Women with endometriosis were younger than those without endometriosis (31.5 vs 33 years, $P = .03$), but otherwise no demographic differences were noted between women with and without endometriosis (Table 1).

Associations Between Serum PUFAs and Endometriosis

No association was noted between total PUFAs, total n-3 PUFAs, or total n-6 PUFAs and endometriosis. The only specific serum PUFA associated with endometriosis was EPA. Women with a previous diagnosis of endometriosis had lower serum levels of EPA than women without the diagnosis ($1.56 \times 10^{-2} \pm 0.006$ vs $2.0 \times 10^{-2} \pm 0.011$, $P = .009$; Table 2). When analyzed by quartiles, women with the highest serum EPA levels were 82% less likely to have a diagnosis of endometriosis compared to women with the lowest serum EPA levels (95% confidence interval [CI] 0.03-0.71). This relationship persisted after adjusting for age (odds ratio = 0.15, 95% CI 0.03-0.72; Table 3). Total and specific n-3 to n-6 PUFA ratios were also investigated between women with and without endometriosis. No differences were noted, nor was any correlation noted between EPA levels and peak estradiol, amount of gonadotropin used, days of stimulation, or length of storage (results not shown).

As mentioned previously, 24 of the 181 women without endometriosis had previously undergone laparoscopy for gynecologic pathology including hydrosalpinges or ovarian cysts. Given that there was a possibility that some of the women with unexplained infertility could have had undiagnosed endometriosis, we stratified our analysis by the diagnosis of unexplained diagnosis. Consistent with our larger cohort, women with endometriosis had lower EPA levels than women with unexplained infertility ($1.6 \times 10^{-2} \pm 0.006$ vs $2.0 \times 10^{-2} \pm 0.01$, $P = .016$). This was also the case for women with endometriosis compared to women with other infertility diagnoses ($1.6 \times 10^{-2} \pm 0.006$ vs $2.0 \times 10^{-2} \pm 0.01$, $P = .01$).

Table 3. Relative Risk and Adjusted Odds Ratio (Adjusted for Age) of Endometriosis in Women Relative to Increasing Serum EPA Level.

	RR (95% CI)	aOR (95% CI)
EPA quartile 1 (n = 51)	–	–
EPA quartile 2 (n = 52)	0.47 (0.16-1.4)	0.43 (0.14-1.28)
EPA quartile 3 (n = 51)	0.40 (0.17-1.2)	0.38 (0.12-1.20)
EPA quartile 4 (n = 51)	0.18 (0.04-0.78)	0.15 (0.03-0.72)
Age	–	0.92 (0.83-1.03)

Abbreviations: EPA, eicosapentaenoic acid; RR, relative risk; CI, confidence interval aOR, adjusted odds ratio.

Discussion

This work demonstrates a negative association between serum EPA levels and the diagnosis of endometriosis among women undergoing IVF. In vitro studies and studies involving animal models have demonstrated suppressive effects of n-3 PUFAs and EPA specifically on endometriosis,[2-4,6] but to our knowledge, this is the first study to demonstrate an association between serum EPA levels and endometriosis in women.

N-3 Fatty acids measured in this study include α-linolenic acid (ALA), a precursor of EPA, and docosahexaenoic acid (DHA), a product of EPA. α-Linolenic acid is obtained primarily through vegetable oils (flaxseed, canola, and soy). However, conversion of ALA to EPA is poor. Although dietary DHA increases both physiologic EPA and DHA concentrations, the main source of EPA is from EPA-containing foods (fatty fish including salmon, sardines, and herring).[9] In 2010, Missmer et al showed a negative association between long-chain n-3 fatty acid consumption and the diagnosis of endometriosis in a cohort of nurses participating in the Nurses Health Study II.[7] Our data are consistent with Missmer's findings but perhaps more informative as we actually measured serum levels of specific PUFAs, whereas n-3 consumption was inferred in Missmer's study from nurses' self-reported composite intake of salad dressing, tuna, and dark fish on food frequency questionnaires.

Because we were able to investigate specific n-3 fatty acids, we believe our findings contribute additional insight into the physiologic mechanisms that may be involved in the pathogenesis of endometriosis. An association between dietary PUFA intake and decreased circulating inflammatory cytokines was first reported by Pischon and colleagues in 2003.[12] Inflammation is thought to play a key role in the pathogenesis of endometriosis.[13] Importantly, Tomio and colleagues recently demonstrated that EPA-derived metabolites, generated through the 12/15 lipoxygenase

pathway, suppress endometriotic lesions in mouse models.[5] In addition, 12/15-lipoxgenases may act as coactivators of peroxisome-proliferator activator receptor-γ (PPAR-γ).[14] This is consistent with work by Lebovic and colleagues showing that thiazolidinediones, PPAR-γ agonists, may prevent the development of endometriosis.[15-17]

Lipoxins, PUFA derivatives, also demonstrate anti-inflammatory and inflammation resolving properties.[18] Lipoxin A_4 has been shown to suppress inflammatory cytokine production in endometrium, and recently it has been demonstrated to suppress both the development and the progression of endometriosis in mouse models of endometriosis.[14,19-23] Although it is possible that increased consumption or circulating levels of EPA may push fatty acid substrates to the production of lipoxin A_4, further work investigating the metabolism of essential fatty acids to pro- and anti-inflammatory derivatives is needed.

Aside from our work, there is one study we are aware of that has investigated relationships between serum fatty acids and the diagnosis of endometriosis.[8] In this 2012 study, fasting serum fatty acids were analyzed in 138 women undergoing laparoscopy or laparotomy. Women were excluded from study if they had received anti-inflammatory drugs or if they had any history of ''endometritis, gastrointestinal or urological disease with pelvic pain, liver or endocrine neoplastic disorders, chronic inflammation disease in the pelvis, uterus, or ovaries.'' Based on surgical findings, 64 women were diagnosed with endometriosis and 74 women were diagnosed with a variety of gynecologic conditions including uterine fibroids and ovarian cysts. In contrast to our study, Khanaki et al did not find any differences in serum EPA levels between women with and without endometriosis. This discrepancy may be due, in part, to the fact that our study population and method of fatty acid analysis differed from that of Khanaki et al. Our study population consisted of women undergoing IVF, not women undergoing pelvic surgery. Although the presence of gynecologic pathology alone may drive the choice for operative intervention, oftentimes pain symptoms drive the choice for surgery over conservative management. In previous epidemiologic work, n-3 PUFA intake and pelvic pain were negatively correlated.[24] If pelvic pain was a motivating factor for Khanaki et al's study population to choose surgery, it is possible these women had low EPA intake overall which would dilute any associations to be made between endometriosis and EPA levels. Also, we used LC-MS/MS for serum PUFA assessment, whereas Khanaki et al used thin-layer chromatography to isolate total phospholipids and then gas chromatography to identify the fatty acids within this fraction (TLC-GC). Liquid chromatography coupled to tandem mass spectroscopy is a more sensitive technique and thus is better suited for measuring small concentrations of substances like EPA for comparison.[25,26]

There are several strengths to our study worth noting. The first is our patient population which consists of infertile women undergoing IVF. Many other studies investigating factors associated with endometriosis are at risk of type II error in that there may be women with endometriosis who are never diagnosed. Unlike studies of general populations of women, the participants in this study undergo a thorough workup for factors related to infertility including endometriosis. All women undergo an evaluation of their pelvic anatomy in the form of a hysterosalpingogram and/or laparoscopy as well as a number of pelvic ultrasounds. Given that there was a chance some of our unexplained cases of infertility could have in fact been due to endometriosis, we did a stratified analysis of EPA values in women with endometriosis compared to women with and without unexplained infertility. In both cases, we again found that women with endometriosis had lower serum EPA levels than women without.

Although Khanaki et al's article would have identified every case of endometriosis, we contend the relative homogeneity of our study population outside their infertility diagnosis limits confounding factors that may influence assessments of serum PUFAs. Like Khanaki et al, we were able to measure specific PUFAs, which is another strength of our study as it helps place our work into the growing body of literature regarding mechanisms involved in the pathophysiology of endometriosis.

Several limitations need to be kept in mind when considering our findings. First, EPA was measured in the serum and we have no knowledge of EPA consumption among our study cohort. On the other hand, it has been suggested that studies measuring fatty acid levels in blood are more accurate assessments of actual fatty acid consumption than dietary questionnaires as they can overcome misclassifications of assessments of dietary intake and assumptions regarding fatty acid content of food. Blood levels may also account for variations in absorption and metabolism of various fatty acids.[27] Another limitation to consider is that our study population was small and we only measured EPA levels 1 time. We have no way of knowing what chronic EPA levels were in these women. Finally, it is possible that women classified as not having endometriosis may have actually had undiagnosed endometriosis.

In conclusion, we found a decreased probability of endometriosis among women undergoing IVF with high serum EPA levels. As stated in the Introduction section, the most efficient way to increase circulating levels of a specific PUFA is through consumption of the specific fatty acid of interest.[9] Given this, our findings, and corroborating evidence from others, EPA supplementation and increased consumption of foods rich in n-3 fatty acids for women with symptoms related to endometriosis warrant further investigation including randomized controlled trials investigating EPA supplementation and symptoms related to endometriosis as well as additional epidemiologic studies of endometriosis with detailed information about long-term specific fatty acid exposures.

Acknowledgments

We would like to thank Dr Susan A. Lanzendorf and Janet Willand from Washington University's Infertility and Reproductive Medicine Center's clinical endocrinology lab as well as our nursing staff and members of the Women's and Infants Health Specimen Consortium at Washington University for their assistance in enrolling participants in this study and collecting and processing specimens.

Authors' Note

Presented in part at the 60th Annual Meeting of the Society for Gynecologic Investigation, Orlando, Florida, USA.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: National Institutes of Health: K12 HD063086 (ESJ), UL1 TR000448 (ESJ); Barnes-Jewish Hospital Foundation (ESJ); The Women's and Infants' Health Specimen Consortium was funded by the Children's Discovery Institute at Washington University.

References

1. ASRM. Endometriosis and infertility: a committee opinion. *Fertil Steril*. 2012;98(3):591-598.
2. Covens AL, Christopher P, Casper RF. The effect of dietary supplementation with fish oil fatty acids on surgically induced endometriosis in the rabbit. *Fertil Steril*. 1988;49(4):698-703.
3. Gazvani MR, Smith L, Haggarty P, Fowler PA, Templeton A. High omega-3: omega-6 fatty acid ratios in culture medium reduce endometrial-cell survival in combined endometrial gland and stromal cell cultures from women with and without endometriosis. *Fertil Steril*. 2001;76(4):717-722.
4. Netsu S, Konno R, Odagiri K, Soma M, Fujiwara H, Suzuki M. Oral eicosapentaenoic acid supplementation as possible therapy for endometriosis. *Fertil Steril*. 2008;90(4 suppl): 1496-1502.
5. Tomio K, Kawana K, Taguchi A, et al. Omega-3 polyunsaturated Fatty acids suppress the cystic lesion formation of peritoneal endometriosis in transgenic mouse models. *PloS One*. 2013; 8(9):e73085.
6. Herington JL, Glore DR, Lucas JA, Osteen KG, Bruner-Tran KL. Dietary fish oil supplementation inhibits formation of endometriosis-associated adhesions in a chimeric mouse model. *Fertil Steril*. 2013;99(2):543-550.
7. Missmer SA, Chavarro JE, Malspeis S, et al. A prospective study of dietary fat consumption and endometriosis risk. *Hum Reprod*. 2010;25(6):1528-1535.
8. Khanaki K, Nouri M, Ardekani AM, et al. Evaluation of the relationship between endometriosis and omega-3 and omega-6 polyunsaturated fatty acids. *Iran Biomed J*. 2012;16(1):38-43.
9. Arterburn LM, Hall EB, Oken H. Distribution, interconversion, and dose response of n-3 fatty acids in humans. *Am J Clin Nutr*. 2006;83(6 suppl):1467S-1476S.
10. Jungheim ES, Frolova AI, Jiang H, Riley JK. Relationship between serum polyunsaturated fatty acids and pregnancy in women undergoing in vitro fertilization. *J Clin Endocrinol Metab*. 2013;98(8):E1364-E1368.
11. Jiang X, Sidhu R, Porter FD, et al. A sensitive and specific LC-MS/MS method for rapid diagnosis of Niemann-Pick C1 disease from human plasma. *J Lipid Res*. 2011;52(7):1435-1445.
12. Pischon T, Hankinson SE, Hotamisligil GS, Rifai N, Willett WC, Rimm EB. Habitual dietary intake of n-3 and n-6 fatty acids in relation to inflammatory markers among US men and women. *Circulation*. 2003;108(2):155-160.
13. Weiss G, Goldsmith LT, Taylor RN, Bellet D, Taylor HS. Inflammation in reproductive disorders. *Reprod Sci*. 2009;16(2): 216-229.
14. Canny GO, Lessey BA. The role of lipoxin A4 in endometrial biology and endometriosis. *Mucosal Immunol*. 2013;6(3):439-450.
15. Lebovic DI, Kir M, Casey CL. Peroxisome proliferator-activated receptor-gamma induces regression of endometrial explants in a rat model of endometriosis. *Fertil Steril*. 2004;82(suppl 3):1008-1013.
16. Lebovic DI, Mwenda JM, Chai DC, et al. PPAR-gamma receptor ligand induces regression of endometrial explants in baboons: a prospective, randomized, placebo- and drug-controlled study. *Fertil Steril*. 2007;88(4 suppl):1108-1119.
17. Lebovic DI, Mwenda JM, Chai DC, Santi A, Xu X, D'Hooghe T. Peroxisome proliferator-activated receptor-(gamma) receptor ligand partially prevents the development of endometrial explants in baboons: a prospective, randomized, placebo-controlled study. *Endocrinol*. 2010;151(4):1846-1852.
18. Calder PC.Polyunsaturated fatty acids and inflammatory processes: New twists in an old tale. *Biochimie*. 2009;91(6):791-795.
19. Macdonald LJ, Boddy SC, Denison FC, Sales KJ, Jabbour HN. A role for lipoxin A(4) as an anti-inflammatory mediator in the human endometrium. *Reproduction*. 2011;142(2):345-352.
20. Wu R, Zhou W, Chen S, et al. Lipoxin A suppresses the development of endometriosis in an ALXR-dependent manner via the p38 mitogen-activated protein kinase pathway. *Br J Pharmacol*. 2014; 171(21):4927-4940.
21. Chen S, Wu RF, Su L, Zhou WD, Zhu MB, Chen QH. Lipoxin A4 regulates expression of the estrogen receptor and inhibits 17beta-estradiol induced p38 mitogen-activated protein kinase phosphorylation in human endometriotic stromal cells. *Fertil Steril*. 2014;102(1):264-271.
22. Chen QH, Zhou WD, Pu DM, Huang QS, Li T, Chen QX. 15-Epi-lipoxin A(4) inhibits the progression of endometriosis in a murine model. *Fertil Steril*. 2010;93(5):1440-1447.
23. Kumar R, Clerc AC, Gori I, et al. Lipoxin A(4) prevents the progression of de novo and established endometriosis in a mouse model by attenuating prostaglandin E(2) production and estrogen signaling. *PloS One*. 2014;9(2):e89742.
24. Deutch B. Menstrual pain in Danish women correlated with low n-3 polyunsaturated fatty acid intake. *Eur J Clin Nutr*. 1995; 49(7):508-516.
25. Sommer U, Herscovitz H, Welty FK, Costello CE. LC-MS-based method for the qualitative and quantitative analysis of complex lipid mixtures. *J Lipid Res*. 2006;47(4):804-814.
26. Vengurlekar SS, Heitkamp J, McCush F, Velagaleti PR, Brisson JH, Bramer SL. A sensitive LC-MS/MS assay for the determination of dextromethorphan and metabolites in human urine–application for drug interaction studies assessing potential CYP3A and CYP2D6 inhibition. *J Pharm Biomed Anal*. 2002;30(1):113-124.
27. Willett WC. *Nutritional Epidemiology*. New York, NY: Oxford University Press; 1990.