Original Article

Metabolism of omega-6 polyunsaturated fatty acids in women with dysmenorrhea

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Menstrual pain (dysmenorrhea) is one of the main complaints in clinics for women. The pain is often accompanied by other symptoms such as headache, nausea, constipation or diarrhea, urinary frequency, and vomiting which often leave the patients incapacitated for work or school for a few days. Dietary supplementation with polyunsaturated fatty acids (PUFA) has been shown to alleviate the menstrual pain. The purpose of the present study was to compare the effect of dietary supplementation with PUFA (sunflower seed oil, borage oil and fish oil concentrate) for three months on RBC membrane fatty acid composition in healthy and dysmenorrheica young women. Conversion of linoleic acid, via gamma-linolenic acid, to dihomo-gamma-linolenic acid (a precursor of anti-inflammatory prostaglandin E_1) in dysmenorrheic subjects as compared to the controls was slower whereas the level of arachidonic acid (a precursor of pro-inflammatory PGE_2) was not affected by the supplementation. Since there are no known side-effects associated with supplementation of these nutrients, management of dysmenorrhea through nutrition modulation should be an acceptable alternative to drug treatments.

Key Words: arachidonic acid, borage oil, fish oil, omega-3 fatty acids, prostaglandins, sunflower oil, supplementation

INTRODUCTION

Menstrual pain or dysmenorrhea is one of the main complaints in clinics for women. The pain is often accompanied by other symptoms such as headache, nausea, constipation or diarrhea, urinary frequency, and vomiting.[1-4] Over 50% of women report some type of pain during their monthly period. According to reports, the prevalence of the complaint can reach as high as 90%.[5] About 10% of patients have indicated that the menstrual pain has left them incapacitated for work or school for a few days.[6] It has been shown that patients with dysmenorrhea have higher levels of arachidonic acid (ARA, 20:4n–6)-derived eicosanoids, such as PGE_2 and $PGF_{2\alpha}$.[7,8] Thus, dysmenorrhea is typically treated with medicine such as inhibitors of the prostaglandin synthase. However, evidence show that chronic use of these drugs in treating inflammatory disorders is associated with significant adverse effects such as impaired kidney function and gastrointestinal bleeding.[9,10] As a result, many of this type of drugs have been removed from the market.[11]

Recently, both gamma-linoleic acid (GLA, 18:3n–6) and omega-3 polyunsaturated fatty acids (ω3-PUFAs), mainly eicosapentaenoic acid (EPA, 20:5n–3) and docosahexaenoic acid (DHA, 22:6n–3), have been shown to be effective in lowering the inflammatory symptoms.[12-14] In humans, GLA is rapidly elongated to dihomo-gamma-linolenic acid (DGLA, 20:3n–6), which can be converted into the anti-inflammatory PGE_1.[15] ω3–PUFAs are also known to suppress the formation of arachidonic acid and its pro-inflammatory eicosanoid derivatives (e.g., PGE_2)[16] (Figure 1). The purpose of the present study is, therefore, to compare the effect of supplementation with sunflower oil (SO, rich in linoleic acid, LA, 18:2n–6), borage oil (BO, rich in GLA) or fish oil (FO, rich in ω3-PUFAs), on fatty acid metabolism and subsequently the eicosanoid metabolism in women with dysmenorrhea.

The present study was a randomized, double-blinded trial. The patients were allocated into 3 groups and receive the SO, BO and FO capsules for 3 months. After 0, 1, 2, 3 month of treatment, blood samples were collected from the participants within 3 days of their period. The effect of treatment on erythrocyte membrane composition was examined.

SUBJECTS AND METHODS

The present study was conducted with the approval of the internal review board of the Da-Chien General Hospital. Thirty healthy young women and 30 subjects with history of primary dysmenorrhea were recruited for the study.

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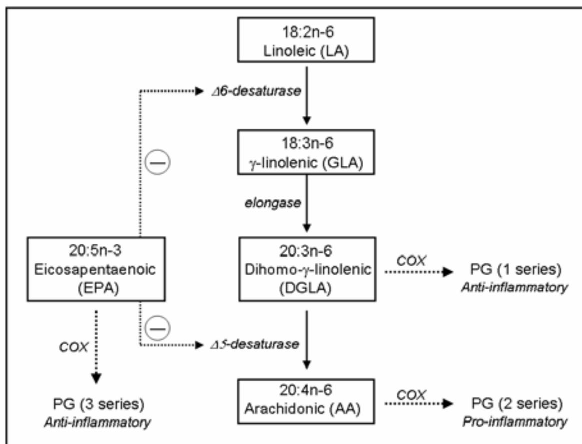

Figure 1. Metabolism of n-6 fatty acids and effect of n–3 fatty acid, (e.g., EPA). COX, cyclooxygenase; PG, prostaglandin; —, inhibition.

Table 1. Composition of major fatty acids in oil supplements (% by wt).

	18:2n–6	18:3n–6	20:5n–3	22:6n–3
SO	62.0	-	-	-
BO	38.1	24.4	-	-
FO	-	-	39.0	35.4

They were all well informed about the study, and gave informed consent. None of the subjects were taking hormonal contraceptives, or receiving long term drug therapy. The age, body weight, and height were matched between the healthy and experimental groups. They were randomly divided into 3 groups receiving supplementation per day of 6 oil capsules (~1g/capsule) containing sunflower oil (SO), borage oil (BO) or fish oil concentrate (FO, 2 capsules of fish oil concentrate and 4 capsules of sunflower oil) for 3 months. Each subject consumed about 3.8 g of additional polyunsaturated fatty acids (PUFA) daily from the supplementation (Table 1). Specifically, the SO group received 3.8 g of linoleic acid (LA, 18:2n–6), the BO group received 2.3 g of LA, and 1.5 g of GLA, and the FO group received 2.3 g of LA, 0.8 g of EPA and 0.7 g of DHA. Blood was collected from each subjects during the menstrual period (day 1-3), or after (day 8-12), a cycle prior to the supplementation, and from each subject during the period after supplementation for months one, two and three. Blood samples were kept cold until centrifugation (within 1 hour). Red blood cell (RBC) pellets were washed twice with saline solution, and stored in -20℃ until analysis.

Lipid analysis

Total RBC lipids were extracted following the method described by Dodge and Phillips (1967).[17] Total phospholipid fractions were separated from the neutral lipids by thin layer chromatography developed with a solvent mixture (hexane-diethyl ether-glacial acetic acid, 70:30:1, v/v/v). The phospholipid fraction was then methylated with 14% BF_3 in methanol.[18] The methyl ester of fatty acids (FAME) was then analysed by gas-liquid chromatography using an Agilent 6890 gas chromatograph equipped with flame ionization detector. The aliquot of FAME was injected onto a fused-silica capillary column (Omegawax, 30m x 0.32 mm ID, Supelco, Bellfonte, PA). The condition used for the study was as previously reported.[19] An internal standard (heptadecanoic acid, 17:0) and calibration standard (GLA-85) from Nu-Chek (Elysian, MN) were used for quantitation of fatty acids.

Statistics

Data (mean±SD) were analysed using SPSS (v. 11.5, Lead Technologies, Inc.) to examine predictors of change in specific fatty acids from baseline to 3 months. Significance of differences between two groups was compared using t-test. A *p-value of* $\leq$ 0.05 was considered significant.

RESULTS

Prior to oil supplementation, major fatty acid compositions of RBC membrane phospholipids in both healthy and dysmenorrhea subjects were compared. There were no significant differences between the two groups, and between the samples collected during the period or after the period (data not shown). The ratios of different fatty acids, such as arachidonic acid/linoleic acid (ARA/LA), and dihomo-gamma-linolenic acid/arachidonic acid (DGLA/ARA) were also similar between the two groups, and between two collection periods within the groups. After 3 months of supplementation, only 19 subjects in the control group (6 in SO, 7 in BO and 6 in FO group), and 23 subjects in the dysmenorrhea group (8 in SO, 8 in BO, and 7 in FO group) remained.

Supplementation with SO progressively increased the level of LA in both control and dysmenorrhea groups. They were increased by 5 and 13%, respectively in the control and the experimental group after 3 months of supplementation. The level of ARA in the healthy group compared to baseline increased by 22% one month after supplementation, but progressively decreased thereafter. After 3 months of supplementation, the level of ARA returned to the normal level. In the dysmenorrhea group, the level of ARA increased by only 13% after 1 month of supplementation, but fell back to normal level after 3 months of supplementation. Levels of other fatty acids in both control and dysmenorrhea groups were generally not affected by SO supplementation.

In the BO group, the level of LA in the dysmenorrhea group increased progressively, and had increased by 8% after 3 months of supplementation. However, no significant changes of LA level were observed in the control group. The level of DGLA in the control increased significantly ($p < 0.05$) by 62% after one month ofsupplementation, and maintained at that level throughout. In the dysmenorrhea group, the level of DGLA increased by only 16% after one month of supplementation, but fell to baseline level after 3 months of supplementation. The level of ARA in the control group increased by 7% after 3 months, but that in the dysmenorrhea group decreased by 7%.

In the FO group, after 3 months of supplementation, the level of LA had slightly increased, whereas that of DGLA had decreased in the dysmenorrhea group as compared to the control group. The level of ARA had increased slightly, by 0.8%, in the control group, but

decreased by 8% in the dysmenorrhea group after 3 months of supplementation. Levels of EPA and DHA in both the control and dysmenorrhea groups had increased significantly after supplementation with FO for one month and maintained at those levels thereafter. The levels of changes were similar between the two groups.

In order to examine the effect of different oil supplementations on omega-6 fatty acid metabolism, we have also calculated the fatty acid ratio, such as ARA/LA and DGLA/ARA (Figures 2 and 3).

Figure 2 shows the average change in ARA/LA ratios relative to baseline. In the control group, the ARA/LA ratios increased one month after supplementation, but decreased to baseline levels 3 months after. However, in the dysmenorrhea group, the ARA/LA ratio although slightly increased initially, decreased to below baseline levels after 3 months of supplementation.

The effect of oil supplementation on DGLA/ARA ratios are shown in Figure 3. There were no significant changes in both control and dysmenorrhea groups supplemented with either SO or FO. However, a significant increase in the DGLA/ARA ratio was observed in the control group after one month of BO supplementation. The DGLA/ARA ratio remained at the same level after 3 months of supplementation. There were no significant changes in the DGLA/ARA ratios in the dysmenorrhea group.

DISCUSSION

This study demonstrated the difference in metabolism of omega-6 fatty acids between the control and the dysmenorrhea groups. When PUFA was supplemented for one month, the level of ARA in the controls had rapidly increased. However, the levels of LA and DGLA were not elevated, suggesting that the metabolism of LA to ARA was rapid in the controls. On the other hand, the levels of LA in the dysmenorrhea group increased with the length of supplementation time, suggesting that the conversion of LA to ARA was slower in comparison with the controls. This was evident by a lower ARA/LA ratio as shown in Figure 2. Since the conversion of LA to ARA requires, in sequence, the action of enzymes such as Δ6-desaturases (conversion of LA to GLA), elongase (elongation of GLA to DGLA) and Δ5-desaturase (conversion of DGLA to ARA). Since there is a progressive accumulation of LA, but not GLA in the dysmenorrhea subjects supplemented with SO (rich in LA) suggesting slower Δ 6-desaturation. To examine whether the conversion of DGLA to ARA (Δ5-desaturation) was different between the control and dysmenorrhea groups, we compared the effect of oil supplementation on DGLA/ARA ratios. No significant differences were found between control and dysmenorrhea groups when supplemented with SO or FO, suggesting that the conversion of DGLA to ARA was not different between the two groups. However, supplementation with BO (rich in GLA) significantly increased the DGLA/ARA ratio in the control group, but not in the dysmenorrhea group. Since dietary supplementation of GLA is known to raise the levels of DGLA, and the ARA/DGLA ratio,[20,21] a slow increase in DGLA/ARA ratio in young women with dysmenorrhea suggest that the

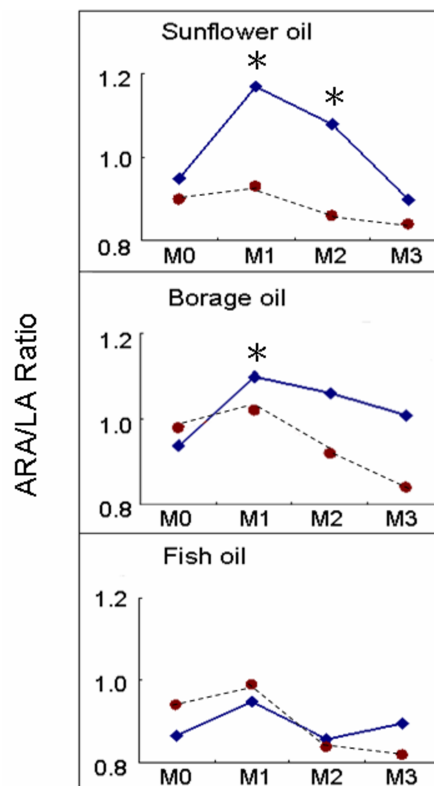

Figure 2. Comparison of mean ARA/LA ratios in RBC membrane PL of control (—◆—) and dysmenorrhea (···●···) subjects supplemented with SO, BO and FO for 0, 1, 2, and 3 months. Asterisk (*) indicates significant difference from the baseline levels.

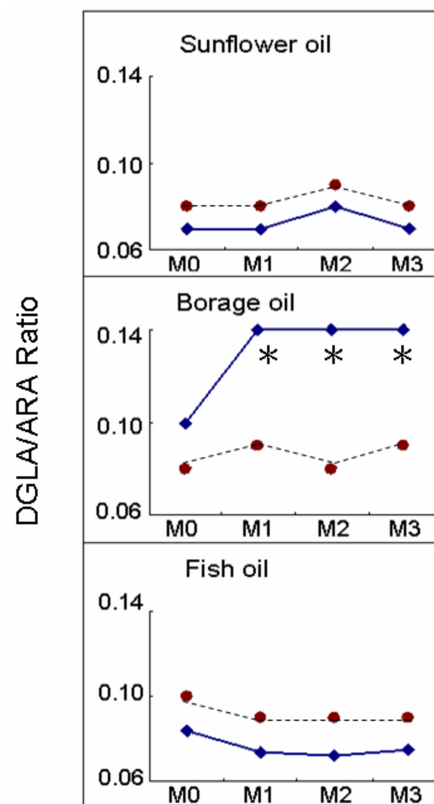

Figure 3. Comparison of mean DGLA/ARA ratios in RBC membrane PL of control (—◆—) and dysmenorrhea (···●···) subjects supplemented with SO, BO and FO for 0, 1, 2, and 3 months. Asterisk (*) indicates significant difference from the baseline levels.

elongation of GLA to DGLA was significantly faster in the control group than the dysmenorrhea group.

In conclusion, results from this study demonstrate that conversion of LA to DGLA, more specifically, LA to DGLA, in the dysmenorrhea group was slower than that in the control group. Since ARA is readily available in the human diet (e.g. in meat and eggs), the formation of pro-inflammatory eicosanoids, such as PGE_2, would not be affected. A slow formation of DGLA would on the other hand, impact on the formation of the anti-inflammatory eicosanoid, PGE_1. This would result in an imbalance toward inflammation, which might in part contribute to symptoms associated with dysmenorrhea.

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AUTHOR DISCLOSURES
Chao-Chih Wu, Mei-Yu Huang, Rakesh Kapoor, Chih-Hung Chen and Yung-Sheng Huang, no conflicts of interest.

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