Effects of perilla oil on plasma concentrations of cardioprotective (n-3) fatty acids and lipid profiles in mice

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Abstract

The aim of this study was to examine the effects of perilla oil as well as several vegetable oils, including flaxseed oil, canola oil, and rice bran oil on plasma levels of cardioprotective (n-3) polyunsaturated fatty acids in mice by feeding each vegetable oil for a period of eight weeks. Concentrations of docosapentaenoic acid (DHA) and eicosapentaenoic acid (EPA), fish-based (n-3) polyunsaturated fatty acids, showed an increase in the plasma of mice fed perilla and flaxseed oils compared to those of mice in the control group ($P < 0.05$), whereas rice bran and canola oils did not alter plasma DPA and EPA concentrations. Arachidonic acid concentration was increased by feeding rice bran oil ($P < 0.05$), but not canola, flaxseed, or perilla oil. In addition, oleic acid, linoleic acid, and docosahexaenoic acid concentrations were altered by feeding dietary rice bran, canola, perilla, and flaxseed oils. Findings of this study showed that perilla oil, similar to flaxseed oil, is cardioprotective and could be used as an alternative to fish oil or even flaxseed oil in animal models.

Key Words: Perilla oil, flaxseed oil, (n-3) polyunsaturated fatty acids, DPA, EPA

Introduction

Dietary choices, including lipids and sources of dietary fatty acids, play an important role in the etiology of cardiovascular disease (CVD). Animal studies have shown that diet can induce remarkable effects on blood pressure, cholesterol metabolism, and CVD.

Lipids supply a major source of energy to most organisms and play an important role as functional or structural constituents within cells [1-3]. Although lipids are essential components for physiological functions, excess dietary intake of lipids has been linked to increased obesity, hypertension, and CVD [4]. Accordingly, recent studies have recommended that consumption of excess dietary lipids such as fats, saturated fatty acids, and cholesterol be decreased in order to avoid subsequent disease onset (CVD, obesity, and hypertension) [5,6]. CVD is considered to be the number one cause of death in the United States, which may be due to consumption of a high fat diet [7]. In 1970, CVD was not a leading cause of death in Korea, whereas it has now become a leading cause of death due to increased Western dietary patterns. In addition, (n-3) polyunsaturated fatty acids, EPA and DHA, which are abundant in fish, have been reported to reduce the risk of CVD [7-11]. Further, recent studies have showed that (n-3) PUFAs may decrease the incidence of CVD and sudden death in patients with ischemic heart disease. An association of fish oil supplementation with beneficial effects on CVD mortality has been reported [12].

However, there are limitations associated with consumptions of fish due to pollution (waste-water) and decreased fish populations. Thus, vegetable oils rich in (n-3) PUFAs can be alternative sources of fish-based (n-3) fatty acids. In addition, administration of flaxseed oil in capsules has been shown to result in an increases in level of human plasma cardioprotective (n-3) PUFAs (EPA and DPA) [11]. Perilla oil, which contains a very high level of ALA (40-64%) similar to flaxseed oil, has been reported to shows anti-atherosclerosis properties in Japanish quail [13,14]. Dietary flaxseed meal exerts protective effects against hypertriglyceridemia and steatosis of the liver in an animal model of obesity [15].

The purpose of this study was to evaluate the effects of dietary perilla oil as well as several vegetable oils on plasma lipid profiles and cardioprotective (n-3) PUFA levels in mice.

Materials and Methods

Experimental animals, diet, and design

A total of 75 male ICR mice were purchased at eight weeks of age from Orient Bio (Sungnam, Korea). The mice were housed in plastic cages (five mice in each cage) and had free access to water and food. The room temperature was 21℃ with 50% humidity. The mice were acclimated for three weeks, during which they were fed an experimental basal diet. At 11 weeks of age, the 75 mice were randomly divided into experimental vegetable oil groups. Experimental diets consisted of a non-purified diet (Charles River mouse LabDiet) from Oriental Bio (Sungnam, Korea) and were composed of 20% protein, 62.3% carbohydrates, 4.5% fat, 4.7% fiber, 6% mineral mixture, and 2.5% vitamins. Olive oil was added to the control diet because it lacks α-linolenic acid (ALA), whereas select vegetable oils (rice bran, canola, flaxseed, or perilla oil) were added individually to the experimental diet groups (Table 1). In each experimental diet group, vegetable oil comprised 10% of the total mass. Sufficient water and diet were supplied for 60 days (*ad libitum*). Each cage of mice was provided daily with one of the experimental diets. The following day, any remaining food was collected before a new diet was added to each group. The amount of food remaining was weighed, and daily food intake was calculated by subtracting the amount of food remaining from that provided the previous day. Average body weights of mice at the end of the experiment ranged from 34-38 g. No significant differences in final body weight were observed among the five experimental groups. All procedures followed guidelines approved by appropriate committees and conformed to the institutional animal care regulations of Sahmyook University.

Analysis of blood profiles

After feeding for eight weeks, the mice were deprived of food overnight, anesthetized by inhaling ether, and killed by cervical dislocation. Treatment of experimental animals was carried out as follows: mice were fasted for 12 hours prior to sacrifice on the last day. Following slight anesthetizationed with ethyl ether, an abdominal incision was made, and blood was collected from the heart using a syringe. The collected blood was refrigerated for 1 hour at 4℃, followed by centrifugation at 3,000 rpm for 15 minutes at 5℃ for isolation of serum. The isolated serum was then placed in a 100 µl microtube, which was stored at -70℃ in a deep freezer prior to use. Plasma concentrations of lipoproteins, triacylglycerol, and cholesterol were quantified using commercial enzymatic kits (Asian Pharmacy).

Lipids were extracted from the plasma using chloroform-methanol, and plasma fatty acid concentrations were determined using an Aligent 6,890 GC/Mass Selective Detector (Agilent, U. S. A). Briefly, 5 ml of chloroform-methanol (1:1, v/v) and 0.5 ml of plasma were added to an 8 ml glass vial, which was mixed well, and centrifuged at $1,400 \times g$ for 5 minutes. The supernatant was transferred to a 20 ml-glass vial to which 200 µl of 100 µM heptacosanoic acid was added. 2 ml of chloroform and 1 ml of distilled water (DW) were added to the resulting solution, which was mixed well, and centrifuged at $1,400 \times g$ for 5 minutes. Next, the lower part of the solution, 2 ml of chloroform, and 1 ml of DW were added to an 8 ml glass vial, which was mixed, and centrifuged at $1,400 \times g$ for 5 minutes. The lower part of the solution was transferred to an 8 ml-glass vial, and organic solvents were removed with N_2 gas. Then, 2 ml of 1 M methanolic HCl was added to the resulting solution, which was incubated at 80℃ for 2 hours and allowed to stand at room temperature. 3 ml of hexane, then added to this solution, followed by mixing, and centrifugation at $1,400 \times g$ for 5 minutes. The supernatant was transferred to an 8 ml vial, followed by addition of organic solvents with N_2 gas and addition of 200 µl of hexane. The hexane phase containing the fatty acid sample was directly subjected to gas chromatography. The sample fatty acids were identified and quantified against a standard mixture of known fatty acids. Plasma fatty acid measurements were reported in µ M/L.

Statistical analysis

The mean and standard deviation were calculated from all collected data using the SPSS package (version 18.0) program. Comparison of the averages in each group was performed using one-way ANOVA, and analysis of significant differences in the averages between groups ($P < 0.05$) was performed using Duncan's multiple tests.

Results

Fatty acids in plasma

Fatty acid contents of plasma in mice are shown in Table 2. DPA and EPA concentrations in plasma of mice fed perilla (DPA: 44.71 ± 4.66 µM/L, EPA: 245.03 ± 62.04 µM/L) and flaxseed (DPA: 64.02 ± 21.99 µM/L, EPA: 217.44 ± 91.19 µM/L) oils showed an increased compared to those of mice in the control group (DPA: 12.20 ± 3.05 µM/L, EPA: 26.49 ± 8.76 µM/L) ($P < 0.05$), whereas rice bran and canola oils did not alter fatty acid concentrations. Plasma linoleic acid (LA), DHA, and oleic acid (OA) concentrations were not changed by the dietary

Table 1. Fatty acid composition of several vegetable oils (g/100 g)

Fatty Acids	Flaxseed oil	Perilla oil	Rice bran oil	Canola oil	Olive oil
Linoleic acid	17	14	37	15	8
α-Linolenic acid	58	63	1	9	0
Oleic acid	16	14	34	32	76
Palmitic acid	6	6	22	1	0
Stearic acid	3	2	2	0	16

Table 2. Plasma fatty acid concentrations in mice fed vegetable oils for eight weeks

(μM/L)	Control	Flaxseed	Perilla	Rice Brown	Canola	P-value
ALA	14.39 ± 3.60[1]	8.39 ± 4.15	9.59 ± 4.16	19.18 ± 12.63	13.19 ± 5.50	NS[2]
DPA	12.20 ± 3.05[a]	64.02 ± 21.99[b]	44.71 ± 4.66[b]	24.39 ± 6.10[a]	16.26 ± 6.34[a]	0.05[3]
EPA	26.49 ± 8.76[a]	217.44 ± 91.19[b]	245.03 ± 62.04[b]	28.70 ± 15.65[a]	47.46 ± 22.05[a]	0.05
DHA	274.35 ± 37.72	436.33 ± 185.14	284.64 ± 8.58	381.09 ± 150.92	268.84 ± 37.30	NS
OA	1,315.60 ± 386.97	1,486.99 ± 415.94	1,680.85 ± 401.49	1,297.08 ± 287.92	1,442.08 ± 287.92	NS
LA	888.09 ± 245.61	2,221.43 ± 709.44	2,033.33 ± 169.12	2,119.05 ± 447.98	1,519.05 ± 299.18	NS
AA	821.27 ± 177.80[ab]	654.60 ± 230.05[ab]	487.94 ± 10.05a	1,366.23 ± 693.14[c]	766.45 ± 348.95[ab]	0.05

[1] Mean ± SD
[2] NS, no statistically significant difference at $P < 0.05$ by ANOVA-test
[3] Significant at $P < 0.05$ by ANOVA-test
Values with different alphabet were significantly different among groups at * $P < 0.05$ by Duncan's multiple range test.
ALA, α-linolenic acid; DPA, Docosapentaenoic acid; EPA, eicosapentaenoic acid; DHA, docosahexanoic acid; OA, oleic acid; LA, linoleic acid; AA, arachidonic acid

Table 3. Effect of selective vegetable oils on plasma HDL-C, LDL-C, TG, and TC concentrations in mice fed vegetable oils for eight weeks

Lipo-protein (mg/dl)	Control	Flaxseed	Perilla	Canola	Rice Brown	P-value
TG	119.65 ± 24.29[1]bc	126.70 ± 20.51[c]	104.79 ± 12.15[ab]	97.30 ± 26.24[a]	85.71 ± 15.45[a]	0.05[2]
TC	108.07 ± 19.46	82.59 ± 21.97	91.71 ± 23.54	93.04 ± 24.19	99.34 ± 16.71	NS[3]
HDL-C	48.99 ± 9.76	41.08 ± 12.30	43.65 ± 16.54	41.86 ± 11.128	39.98 ± 8.01	NS
LDL-C	8.43 ± 2.08[b]	5.46 ± 1.97[a]	4.84 ± 1.26[a]	5.74 ± 1.41[a]	6.24 ± 1.61[a]	0.05

[1] Mean ± SD
[2] Significant at $P < 0.05$ by ANOVA-test
[3] NS, no statistically significant difference at $P < 0.05$ by ANOVA-test
Values with different alphabet were significantly different among groups at * $P < 0.05$ by Duncan's multiple range test.
TG, triacylglycerol; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol

vegetable oils. Plasma AA concentrations were increased by dietary rice bran oil (1,366.23 ± 693.14 μM/L), but not dietary canola (766.45 ± 348.95 μM/L), perilla (487.94 ± 10.05 μM/L), or flaxseed (654.60 ± 230.05 μM/L) oils ($P < 0.05$).

Effects of vegetable oils on plasma HDL-C, LDL-C, TG, and TC in mice

Lipid concentrations in mice fed vegetable oils are shown in Table 3. Total triacylglycerol (TG) concentrations in plasma were decreased by canola (97.30 ± 26.24 mg/dl) and rice bran (85.71 ± 15.45 mg/dl) oils but not by flaxseed (126.70 ± 20.51 mg/dl) or perilla (104.79 ± 12.15 mg/dl) oil. Plasma concentrations of total cholesterol (TC) and HDL-cholesterol (HDL-C) in mice were not altered in mice by the dietary oils. LDL-cholesterol (LDL-C) plasma levels were reduced by dietary flaxseed (5.46 ± 1.97 mg/dl), perilla (4.84 ± 1.26 mg/dl), canola (5.74 ± 1.41 mg/dl), and rice bran (6.24 ± 1.61 mg/dl) oils as compared to control diet ($P < 0.05$).

Discussion

In the present study, HDL-cholesterol levels in plasma of mice were not significantly altered by dietary vegetable oils. These data are somewhat contrary to previous reports in that we expected HDL-cholesterol levels to increase upon administration of vegetable oils high in (n-3) PUFAs. According to a previous

report, HDL-cholesterol levels in mice fed vegetable oils did not differ significantly from those of mice fed animal fat [16]. Meanwhile, association of ω-6-rich corn oil a lower plasma level of HDL-cholesterol, compared other vegetable oils, has been reported [16]. In addition, several previous studies have reported that ω-3 fatty acids increase HDL-cholesterol levels [17,18]. On the other hand, LDL-cholesterol levels in plasma of mice were reduced by dietary flaxseed, perilla, canola, and rice bran oils as compared to control (olive oil) diet. Our finding that these dietary vegetable oils induced a decreased in plasma LDL-cholesterol levels is similar to previous results [19,20]. Specifically, dietary unsaturated fatty acids have been shown to decrease plasma LDL-cholesterol levels as well as cardiovascular events [19,20]. Another study reported, a direct relationship between serum LDL-cholesterol and CVD, suggesting that reduction of plasma LDL-cholesterol through a non-drug means is most desirable [21]. In addition, LDL-cholesterol levels can be altered by dietary components, including minerals [22,23]. In contrast, other previous reports have failed to confirm the beneficial effects of (n-3) unsaturated fatty acids on cardiovascular disease since (n-3) polyunsaturated fatty acids (PUFAs) do not consistently cause a decrease LDL-cholesterol levels [20,24]. In addition, (n-3) PUFAs and phytosterols have been shown to act synergistically to reduce LDL-cholesterol and total cholesterol levels in hyperlipidemic men and women [25].

A previous study [26,27], reported that the effects of PUFAs on plasma lipids may vary according to the sources and ratio of PUFAs. In the present study, plasma triacylglycerol

concentrations were reduced by canola and rice bran oils but not by perilla or flaxseed oil. We expected that perilla and flaxseed oils rather than canola and rice bran oils would decrease plasma triacylglycerol concentrations since perilla and flaxseed oils are similar to fish oil in terms of (n-3) PUFAs. However, our expectations were not met. Previous studies, it was found that plasma triacylglycerol levels could be reduced by adminis- tration of marine (n-3) PUFAs [26-29]. Fish oils rich in (n-3) long-chain PUFAs have been reported to reduce circulating triacylglycerol and raise HDL-cholesterol levels [25,27,30]. Unexpectedly, findings of our study showed that plasma total cholesterol concentrations were not altered by dietary vegetable oils. Findings of a previous study [16], showed that the plasma total cholesterol concentration is dependent on the type of lipid rather than the lipid level. Whereas plasma triacylglycerol levels are almost invariably reduced by administration of marine (n-3) PUFAs, the effects of marine (n-3) PUFAs on plasma total cholesterol and LDL-cholesterol levels have been inconsistent [26,28,29]. On the other hand, besides vegetable oils, certain nuts (low in saturated fats and high in PUFA fats) have been reported to induce a significantly reduction of LDL-cholesterol, non-HDL- cholesterol, and total cholesterol concentrations [31]. In addition, LA, a (n-6) PUFA was proven to reduce cholesterol levels [32]. Reduction of serum cholesterol is a common dietary goal since cholesterol is a major component of atherogenic fatty plaques, which are associated with increased risk of cardiovascular disease [1]. Our findings demonstrate that dietary perilla and flaxseed oils induced an increase in the levels of plasma EPA and DPA but not LA, DHA, OA, or arachidonic acid (AA) levels in mice. In a previous trial [17], oral administration of EPA and ALA precursors, in the form of dietary supplementation (in capsules) of flaxseed oil for 26 weeks, resulted in increased EPA and DPA in human plasma. EPA and DPA, fish-based (n-3) PUFAs, are known to be cardioprotective, whereas ALA, a plant-based (n-3) PUFA, is known to be a precursor of EPA and DPA [1]. Earlier trials with ALA [32-36] yielded ambiguous results due to several factors, including duration of the experiment and experimental diet components, etc. In our study, dietary administration (not by tubing or capsules) of ALA in perilla oil resulted in increased plasma EPA and DPA in mice despite a shorter experimental duration. Our findings showing significant increases in EPA and DPA but not DHA in mice are similar to those of earlier reports [11,26,37], which found that ALA supplementation increases EPA and DPA in humans despite differences from our study in terms of experimental duration, subjects, and ALA sources. Previous studies [11] have suggested that significant increases in plasma EPA and DPA concentrations can be obtained by capsule administration of ALA (3 g/d). In addition to capsules, administration of ALA in foods, such as ALA-rich margarine, has been shown to increase serum EPA [38]. In addition, ALA-rich nuts, cereal, oil, and fortified breads display higher levels of EPA [32]. In all of these reports, ALA was introduced into the body through consumption of regular meals or snacks and not through capsules as in our study. However, disclosure of the process of EPA synthesis from ALA, the precursor of EPA, is not a trivial matter because several factors can modify the conversion of ALA to EPA, a longer-chain metabolite. For example, LA (n-6) in oil results in decreased conversion of ALA to EPA and DHA [39,40]. (n-3) PUFAs have been reported to compete with (n-6) PUFAs for metabolism [41,42]. In addition to LA (n-6), consumption of long-chain (n-3) PUFAs may reduce the conversion of ALA to EPA and DHA due to downregulation of desaturase enzymes [40]. A diet lacking long-chain (w-3) PUFAs is often characterized by upregulation of desaturase enzymes. On the other hand, foods such as cheese, which contains even small amounts of long-chain (w-3) PUFAs, have low levels of desaturase enzymes [43]. As expected, increases in EPA and DPA were not detected in the canola group, as canola oil contains little ALA, the precursor of EPA. In addition, due to a low level of ALA, the precursor of EPA in rice bran, rice bran oil did not induce an increase in plasma EPA and DPA levels. However, according to one report, that canola oil is considered to be an ALA source in the United States [32]. The focus of the present study was on the effects of dietary administration of rice bran, canola, and flaxseed oils as well as perilla oil, which is frequently used in Korea, on plasma cardioprotective effects. Rice is a primary daily food in Korea, whereas perilla oil is frequently used as a raw material in *bibimbab* and other traditional dishes. Accordingly, the fact that dietary administration of perilla resulted in an increase in plasma cardioprotective long-chain fatty acids is significant because perilla oil is a native product and frequently used as a traditional raw food material in Korea. Flaxseed oil is knowen to contains 58% ALA while canola oil contains a small amount [11,32]. Perilla oil is also known to contains up to 59% ALA (Table 1), along with small amounts of other kinds of PUFAs, including LA, which compete with ALA in synthesis of EPA from ALA. Therefore, large amounts of ALA in flaxseed and perilla oils likely induced an increase in level of plasma EPA and DHA in mice [10]. In addition, it is believed that canola and rice bran oils did not increase plasma EPA and DPA concentrations in mice because canola and rice bran oils contain small amounts of ALA, the precursor of EPA, and large amounts of LA, which inhibits conversion of ALA to EPA or DPA. Oils with large amounts of long-chain PUFAs have been proven to reduce the effects on myocardial infarction [44,45].

Although administration of ALA for a certain experimental period resulted in an increase in levels of plasma cardioprotective long-chain fatty acids, whether or not administration of ALA in capsule form to animals or humans over a long period can reduces CVD risk remains unknown. In addition, it is not clear whether or not ALA consumption in capsule form by humans for an extended period of time has beneficial effects on metabolism in addition to lowering the risk of CVD. Finally, it should be determined whether or not it is advisable for people to consume ALA sources during regular meal times or rather ALA in capsule

form at other times during the day.

In summary, perilla oil, a native product of Korea, currently used as a raw material for delicacies like *bibimbab*, increased cardioprotective DPA and EPA contents in the plasma of mice. Accordingly, it is possible that perilla oil can substitute for fish oil or imported flaxseed oil as an alternative cardioprotective vegetable oil. Finally, conduct of further studies will be necessary in order to elucidate the effects of perilla oil on plasma DHA and EPA concentrations in humans.

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