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Suppression of hepatic fatty acid synthase by feeding α-linolenic acid rich perilla oil lowers plasma triacylglycerol level in rats

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Abstract

This study was performed to determine the effects of dietary perilla oil, a n-3 α-linolenic acid (ALA) source, on hepatic lipogenesis as a possible mechanism of lowering triacylglycerol (TG) levels. Male Sprague-Dawley rats were trained for a 3-hour feeding protocol and fed one of five semipurified diets as follows: 1% (w/w) corn oil control diet, or one of four diets supplemented with 10% each of beef tallow, corn oil, perilla oil, and fish oil. Two separate experiments were performed to compare the effects of feeding periods, 4 weeks and 4 days. Hepatic and plasma TG levels were decreased in rats fed perilla oil and fish oil diets, compared with corn oil and beef tallow diets. The activities of hepatic lipogenic enzymes such as fatty acid synthase (FAS), glucose-6-phosphate dehydrogenase, and malic enzyme were suppressed in the fish oil, perilla oil, and corn oil–fed groups, and the effect was the most significant in the fish oil–fed group. Also, the activities of glycolytic enzymes, glucokinase, and L-pyruvate kinase showed the similar trend as that of lipogenic enzymes. The activity of FAS, the key regulatory enzyme in lipogenesis, was positively correlated with hepatic and plasma TG levels and reduced significantly in the perilla oil–fed group compared with corn oil–fed group. In addition, the FAS activity was negatively correlated with the hepatic microsomal content of EPA and DHA. In conclusion, suppression of FAS plays a significant role in the hypolipidemic effects observed in rats fed ALA rich perilla oil and these effects were associated with the increase of hepatic microsomal EPA and DHA contents.

Keywords: α-Linolenic acid; Fatty acid synthase; Glycolysis; Lipogenesis; Perilla oil; Triacylglycerol

1. Introduction

Recent studies have shown that elevated triacylglycerol (TG) concentrations, especially in the postprandial state, may be associated with the increased risk of coronary heart disease [1–3]. It has been established that fish oil suppresses plasma TG concentrations in both postprandial and fasting states in human [1,4] and experimental animals [5,6]. However, as fish oil is not used widely as a common cooking oil because of its unpleasant smell, we cannot actually apply its hypolipidemic capacity directly to our daily eating habits. The effect of fish oil has been ascribed to the long chain n-3 polyunsaturated fatty acid (PUFA) such as eicosapentaenoic acid (EPA; 20:5 n-3) and docosahexaenoic acid (DHA; 22:6 n-3) [6–8].

Considering that α-linolenic acid (ALA) can be a precursor of these very long–chain fatty acids, it is plausible that dietary ALA exerts similar physiological effect as in the case of very long–chain fatty acids in fish oil. The previous results of ALA have not always been consistent according to the experimental conditions such as given oil status, amount, or feeding period [9–12]. Our previous report [13] indicated that dietary perilla oil rich in ALA was effective in reducing postprandial lipid level and it was related to the increase of EPA and DHA in hepatic membrane fractions.

The exact mechanism by which PUFA lowers blood lipids, particularly TG, is not completely understood. Several studies demonstrated that PUFA exerted hypolipidemic effect by coordinately suppressing hepatic lipid synthesis and secretion while inducing hepatic and skeletal muscle fatty acid oxidation [14–16]. Suppression of hepatic lipogenesis is accompanied by inhibiting hepatic synthesis of lipogenic and glycolytic enzyme proteins (fatty acid synthase [FAS], acetyl-CoA carboxylase, malic enzyme [ME], glucokinase [GK], and L-pyruvate kinase [PK]) [17–19]. Contrary to the numerous studies on PUFA comparing with fat-free or saturated fatty acids, there have been limited

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studies on the comparison of various PUFA explaining the hypotriglyceridemic effect of n-3 and n-6 PUFA.

Thus, we compared the activities of hepatic lipogenic and glycolytic enzymes and FAS gene expression in perilla oil–fed rats with other dietary fats. The relationships to the fatty acid composition of hepatic membrane lipid were also investigated. To achieve an experimental design similar to our real eating habits and de novo lipogenesis high, we estimated 10% (w/w) as moderate intake of daily dietary fat and compared effects of commonly used dietary fats.

2. Methods and materials

2.1. Animals and diets

The Animal Experiment Ethics Committee of Seoul National University approved the protocol of animal preparation. Male Sprague-Dawley rats, supplied by Laboratory Animal Center of Seoul National University, were individually caged with free access of water during the entire experimental periods. Two separate experiments were conducted to compare the effects of dietary fat feeding periods. In the first feeding trial, weanling rats were assigned to one of five groups, with 10 rats in each group, and were fed experimental diets for 4 weeks. To facilitate control of diet intake, all animals were adapted to a meal-eating regimen in which one meal allowed access to food for a 3-hour period (9 AM to 12 noon). Experimental diets were prepared by mixing fat-free basal diet with 10% (100 g/kg diet) each of beef tallow (BT), corn oil (CO), perilla oil (PO), fish oil (FO), and 1% (10 g/kg diet) corn oil (1C) to provide minimal essential fatty acids as a control. The fat-free basal diet contained the following percentage of ingredients according to weight: casein 20%, sugar 34.85%, corn starch 34.85%, α-cellulose 5%, AIN mineral mixture 4%, AIN vitamin mixture 1%, and DL-methionine 0.3%. The percentages of macronutrients in the diets (% of total energy) were as follows. Control diet (1C) consisted of 22% protein, 76% carbohydrate, and 2% fat. The composition of four experimental diets was 18% protein, 62% carbohydrate, and 20% fat. The amount of fat-free basal diet was kept constant by matching intakes of each day. In the second part of this study, rats were fed the basal diet supplemented with 5% CO until they weighed about 140 g and divided into five groups with 10 rats in each group. For 7 days before the experimental period of fat supplementation, all rats were adapted to the 3-hour feeding regimen, receiving 1C diet to have similar fatty acid status by exhausting the stored fatty acids. After this preliminary feeding, rats were fed one of five experimental diets described in the first model for 4 days. The fatty acid compositions of dietary fats are shown in Table 1. To prevent autoxidation, α-tocopherol was supplemented in PO (0.015%) and FO (0.019%), based on peroxidizability index [20]. In addition, meals were offered daily and diets were stored in the freezer and replenished with N_2 gas to minimize the peroxidation of lipids.

Table 1
Fatty acid composition of dietary fats

Fatty acid	Beef tallow	Corn oil	Perilla oil	Fish oil
C 14:0	3.96	—	—	3.58
C 15:0	—	—	—	1.36
C 16:0	28.36	11.85	6.80	25.66
C 16:1	2.79	—	—	5.50
C 18:0	20.87	—	2.07	6.65
C 18:1	44.02	28.94	15.23	13.83
C 18:2(n-6)	—	57.29	13.67	1.62
C 18:3(n-3)	—	0.93	61.49	1.08
C 20:0	—	0.54	—	—
C 20:1	—	0.45	0.48	1.52
C 20:2(n-6)	—	—	0.25	—
C 20:5(n-3)	—	—	—	6.47
C 22:6(n-3)	—	—	—	32.72
ΣSFA	53.19	12.39	8.87	37.25
ΣMUFA	46.81	29.39	15.71	20.85
Σn-6	—	57.29	13.92	1.62
Σn-3	—	0.93	61.49	40.27
n-6/n-3	—	61.60	0.23	0.04

Values are expressed as percentage of total fatty acids.
ΣSFA = sum of saturated fatty acids; ΣMUFA = sum of monounsaturated fatty acids; Σn-6 = sum of n-6 fatty acids; Σn-3 = sum of n-3 fatty acids; n-6/n-3 = ratio of n-6 to n-3 fatty acids.

2.2. Preparation of samples

Animals were killed by decapitation 90–120 minutes after completion of the final meal. An aliquot of each liver was quickly removed and snap frozen in liquid $N_2$and kept at −70°C for mRNA isolation and hepatic TG concentration assay. About each 5 g of liver was homogenized in 5 vol of ice-cold homogenizing buffer solution (154 mmol/L KCl, 50 mmol/L Tris-HCl, and 1 mmol/L EDTA buffer, pH 7.4) and centrifuged at 10,000 × g for 20 minutes, followed by recentrifugation of the supernatant at 105,000 × g for 60 minutes. The supernatant was considered as the cytosol, and the pellet the microsome. The aliquots of cytosol fraction were stored at −70°C until assays were performed. The entire fractionation procedure was conducted at 0–4°C. Blood was collected in heparinized tube and plasma was obtained by centrifugation at 3000 rpm.

2.3. Lipid analyses

Microsomal total phospholipids were isolated by TLC with hexane/diethylether/formic acid (80:20:20, by vol). After visualizing by spraying with 2′, 7′-dichlorofluorescein and marking under UV light (366 nm), spots were scraped off and collected into glass tubes for fatty acid analysis. The procedures of fatty acid analysis and TG content assay were described our previous report [13] in detail.

2.4. Enzyme assays

All the enzymatic activities were measured in hepatic cytosolic fraction. FAS was determined from the rate of

malonyl-CoA–dependent NADPH oxidation by the method of Linn [21]. The activities of glucose-6-phosphate dehydrogenase (G6PD) and ME activity was measured according to the method of Löhr and Waller [22], and Ochoa [23]. The glycolytic enzyme, PK and GK was measured by the method of Bücher and Pfleiderer [24], and Pilkis [25], respectively. Soluble protein contents were measured according to the method of Scopes [26], and the modified method of Lowry et al. [27].

2.5. Isolation of total RNA and Northern-blot hybridization of FAS

Total RNA was extracted from the liver by acid guanidinium thiocyanate-phenol-chloroform (AGPC) method [28]. Nucleic acid concentration was estimated spectrophotometrically by absorption at 260 nm. Northern blot analysis was performed as described by Sambrook et al. [29]. The plasmid containing cDNA insert for rat FAS was a gift from Dr. Steven Clarke (University of Texas). The cDNA probes for FAS and glyceraldehyde phosphate dehydrogenase (GAPDH) were radiolabeled with [α-^{32}P]-dCTP using random primer labeling kit (Promega, Madison, WI). Quantification of Northern blots was achieved by scanning microdensitometer (PDS, Perkin-Elmer, Norwalk, CT). Relative FAS mRNA levels were normalized to their respective GAPDH signals by quantitative densitometric scanning of autoradiograms.

2.6. Statistical analysis

All statistical analyses were carried out using analysis of variance and Duncan's multiple range test. A value of $P < 0.05$ was selected as a limit of statistical significance. The statistical program used was the SAS software package (SAS Institute, Cary, NC).

3. Results

3.1. Food intake and body weights

There were no significant differences in diet intake, final body weight, or weight increase rate among 10% fat-feeding experimental groups. Diet intakes were not different within a group because rats were individually housed and adapted to a 3-hour feeding regimen.

3.2. TG levels and fatty acid composition of hepatic membrane

The postprandial plasma and liver TG was shown in Table 2. As we reported earlier [13], the content of plasma and hepatic TG in the FO and PO groups were significantly lower than other groups in both the 4-week and 4-day feeding models. On the contrary, CO, which is rich in n-6 linoleic acid (LA), did not result in any significant decrease in plasma and hepatic TG levels. The proportions of the fatty acids in the microsomal phospholipids are shown in Table 3. The fatty acid composition reflected dietary fatty acids and their metabolic conversion. The significant fatty acids in rats fed BT were palmitic and stearic acids. High contents of LA and arachidonic acid (AA, C20:4, n-6) were detected in the CO group, contrasting to the higher EPA and DHA contents in the PO and FO groups.

Table 2
Effect of dietary fats on hepatic and plasma triglyceride levels

Dietary treatment	Plasma triglyceride (mg/dL plasma)		Hepatic triglyceride (mg/g liver)
	4-Week feeding	4-Day feeding	4-Week feeding
1C	74.31 ± 3.37^{cd}	64.96 ± 4.16^{c}	4.64 ± 0.25^{a}
BT	122.78 ± 8.75^{b}	95.69 ± 3.28^{b}	4.56 ± 0.21^{a}
CO	144.95 ± 10.63^{a}	139.46 ± 6.33^{a}	4.52 ± 0.24^{a}
PO	90.02 ± 6.85^{c}	106.09 ± 6.76^{b}	3.38 ± 0.43^{b}
FO	62.92 ± 6.59^{d}	42.96 ± 2.82^{d}	2.74 ± 0.56^{b}

Data expressed as mean ± SEM (n = 9). Values with the different superscript letters, in a column are significantly different at $P < 0.05$ by Duncan's multiple range test. Sprague-Dawley rats were fed each of the experimental diets for 4 weeks and 4 days by a 3-hour feeding protocol. 1C = 1% corn oil diet; BT = 10% beef tallow diet; CO = 10% corn oil diet; PO = 10% perilla oil diet; FO = 10% fish oil diet.

3.3. Hepatic lipogenic enzyme activity and FAS gene expression

The activity of FAS, the overall rate-limiting enzyme for fatty acid synthesis, is affected by dietary fats (Fig. 1). FAS activity of the BT group was comparable to that of 1C group, but the activities were reduced in polyunsaturated fat-fed groups. Among the polyunsaturated fat groups, the reduction of the activity was the greatest in the rats fed FO. PO was more effective than CO in reducing the activity. The differences among each three groups were statistically significant. The activities of G6PD and ME, two regulatory enzymes in supplying NADPH requirement, showed similar tendencies (Fig. 2). The activities were reduced in polyunsaturated fat–fed groups like FAS. The difference between PO and FO was not significant in 4 days of feeding. The distinct suppression of these two enzyme activities in FO appeared only in longer periods, whereas the differences among the polyunsaturated fats were evident in case of FAS. The relative contents of FAS mRNA as an estimate of gene expression are shown in Fig. 3. The mRNA content was reduced in rats fed 10% fat diets, compared to 1C fed rats. In the 4-week feeding experiment, FO supplementation resulted in the lowest FAS mRNA content, which was reduced to 25% of that in the 1C group. The FAS mRNA content of PO was intermediate level between FO and CO, although this was not significant. However, the difference between FO and CO was significant. In the 4-day feeding experiment, the overall trend was similar to that of the

Table 3
Effect of dietary fats on fatty acid composition in hepatic microsomal phospholipid

Fatty acid	Dietary treatment				
	1C	BT	CO	PO	FO
4-Week feeding					
C14:0	0.26 ± 0.04^{a}	0.29 ± 0.04^{a}	0.18 ± 0.02^{b}	0.15 ± 0.02^{b}	0.10 ± 0.02^{c}
C16:0	16.92 ± 0.53^{bc}	17.92 ± 0.8^{ab}	14.52 ± 0.45^{c}	14.20 ± 0.43^{c}	19.50 ± 1.3^{a}
C16:1	1.78 ± 0.04^{a}	1.30 ± 0.09^{b}	0.58 ± 0.06^{d}	0.83 ± 0.08^{c}	1.55 ± 0.07^{ab}
C18:0	21.54 ± 0.3^{b}	23.60 ± 0.5^{a}	21.00 ± 0.75^{b}	21.08 ± 0.38^{b}	16.71 ± 0.42^{c}
C18:1	8.98 ± 0.29^{a}	8.67 ± 0.27^{a}	4.84 ± 0.25^{d}	5.86 ± 0.37^{c}	7.13 ± 0.4^{b}
C18:2	10.58 ± 0.27^{c}	8.54 ± 0.21^{d}	15.78 ± 0.59^{a}	12.24 ± 0.57^{b}	2.20 ± 0.15^{c}
C18:3	1.42 ± 0.26^{b}	0.59 ± 0.19^{c}	1.53 ± 0.21^{b}	7.63 ± 0.52^{a}	0.92 ± 0.17^{bc}
C20:2	5.65 ± 0.38^{b}	7.50 ± 0.42^{a}	3.20 ± 0.42^{c}	2.29 ± 0.47^{c}	2.91 ± 0.25^{c}
C20:4	28.94 ± 0.54^{b}	27.30 ± 0.47^{b}	34.60 ± 0.6^{a}	12.80 ± 0.38^{d}	19.00 ± 0.52^{c}
C20:5	0.33 ± 0.03^{c}	0.36 ± 0.05^{c}	0.20 ± 0.03^{c}	14.57 ± 0.31^{a}	7.52 ± 0.27^{b}
C22:6	3.52 ± 0.30^{c}	3.94 ± 0.15^{c}	3.09 ± 0.28^{c}	8.35 ± 0.4^{b}	22.46 ± 0.84^{a}
ΣSFA	38.72 ± 0.87^{ab}	41.81 ± 1.34^{a}	35.70 ± 1.22^{b}	35.43 ± 0.83^{b}	36.31 ± 1.74^{b}
Σn-6	45.17 ± 1.19^{b}	43.34 ± 1.10^{b}	53.58 ± 1.01^{a}	27.33 ± 1.42^{c}	24.11 ± 0.92^{c}
Σn-3	5.27 ± 0.59^{b}	4.89 ± 0.39^{b}	4.82 ± 0.52^{b}	30.55 ± 1.23^{a}	30.90 ± 1.28^{a}
4-Day feeding					
C14:0	1.45 ± 0.06^{a}	1.33 ± 0.14^{a}	0.75 ± 0.07^{b}	0.71 ± 0.06^{b}	0.68 ± 0.04^{b}
C16:0	19.54 ± 0.65^{a}	18.87 ± 0.51^{ab}	17.40 ± 0.83^{b}	14.06 ± 0.57^{c}	15.87 ± 0.71^{c}
C16:1	2.71 ± 0.23^{a}	3.02 ± 0.31^{a}	1.00 ± 0.09^{b}	1.06 ± 0.12^{b}	1.09 ± 0.06^{b}
C18:0	22.60 ± 0.45^{a}	23.64 ± 0.43^{a}	21.31 ± 0.58^{a}	22.64 ± 0.41^{a}	19.68 ± 0.34^{b}
C18:1	10.47 ± 0.21^{b}	13.71 ± 0.58^{a}	8.22 ± 0.39^{c}	6.38 ± 0.19^{d}	6.05 ± 0.13^{d}
C18:2	9.12 ± 0.25^{c}	8.40 ± 0.27^{e}	12.10 ± 0.68^{a}	10.26 ± 0.55^{b}	2.62 ± 0.14^{d}
C18:3	1.02 ± 0.09^{bc}	0.84 ± 0.06^{c}	1.25 ± 0.04^{b}	6.73 ± 0.36^{a}	0.88 ± 0.06^{c}
C20:2	7.83 ± 0.45^{a}	5.42 ± 0.51^{bc}	4.65 ± 0.38^{c}	4.58 ± 0.47^{c}	6.51 ± 0.27^{ab}
C20:4	22.30 ± 0.58^{b}	20.24 ± 0.75^{c}	30.10 ± 1.54^{a}	17.73 ± 0.71^{d}	18.90 ± 0.49^{cd}
C20:5	0.05 ± 0.02^{c}	0.27 ± 0.04^{c}	0.14 ± 0.09^{c}	8.90 ± 0.42^{a}	5.02 ± 0.37^{b}
C22:6	2.91 ± 0.10^{d}	4.26 ± 0.19^{c}	3.08 ± 0.09^{cd}	6.95 ± 0.47^{b}	22.70 ± 0.56^{a}
ΣSFA	43.59 ± 1.16^{a}	43.84 ± 1.08^{a}	39.46 ± 1.48^{b}	37.41 ± 1.04^{bc}	36.23 ± 1.09^{c}
Σn-6	39.25 ± 1.28^{b}	34.06 ± 1.53^{c}	46.85 ± 2.6^{a}	32.57 ± 1.73^{c}	28.03 ± 0.9^{d}
Σn-3	3.98 ± 0.21^{d}	5.37 ± 0.29^{c}	4.47 ± 0.22^{cd}	22.58 ± 1.25^{b}	28.60 ± 0.99^{a}

Data expressed as percentage of total fatty acids and mean ± SEM (n = 9). Values with different superscript letters in a line are significantly different at $P < 0.05$ by Duncan's multiple range test. Sprague-Dawley rats were fed each of the experimental diets for 4 weeks and 4 days by a 3-hour feeding protocol. For abbreviations see Tables 1 and 2.

4-week feeding. However, the extent of suppression was small and there were no significant differences among the 10% fat diets except for FO group.

3.4. Hepatic glycolytic enzyme activity

Major glycolytic enzymes, PK and GK, activities are shown in Table 4. Both enzymes showed similar tendencies in both feeding trials. The suppression of the activities was the greatest in the rats fed FO. PO was more effective than CO in 4-day feeding, but the difference disappeared as the feeding period was extended to 4 weeks. BT did not result in any significant reduction in the activities.

4. Discussion

Our results showed that 1C control diet produced the lowest plasma TG level, compared with hypercaloric10% fat diets except for fish oil diet. Numerous studies have shown that PUFA reduced the plasma TG level in human and experimental animals [4–8,30–31]. Our previous study confirmed that PUFA, especially n-3 PUFA sources such as FO and PO, were effective to reduce plasma and hepatic TG levels [13]. Unlike FO with its unpleasant smell, PO can easily be applied to usual eating habits because it is a common cooking oil used in various ways such as for flavoring, salad dressings, and for pan-frying in Korea.

Some researchers suggested that the hypotriglyceridemic effect is due to the decreased synthesis and secretion of hepatic VLDL-TG [14,16,32]. Considering that the liver is the major site of synthesizing fatty acids and TG, suppression of hepatic lipogenic enzymes was supposed to be a crucial factor in our study. De novo lipogenesis is influenced by dietary macronutrient content [33]. Massive overconsumption of carbohydrate can increase lipogenesis, whereas consuming diets high in fat result in extremely low lipogenesis. Maximum inhibition of hepatic lipogenic enzyme occurs when the diet contains approximately 20% of its calories as PUFA, but as little as 5% of energy as PUFA is sufficient to inhibit lipogenic enzyme [34]. In addition, researches on the effect of fat level have used conventionally 20% (w/w) fat-fed rats versus 5% fat-fed rats, which are

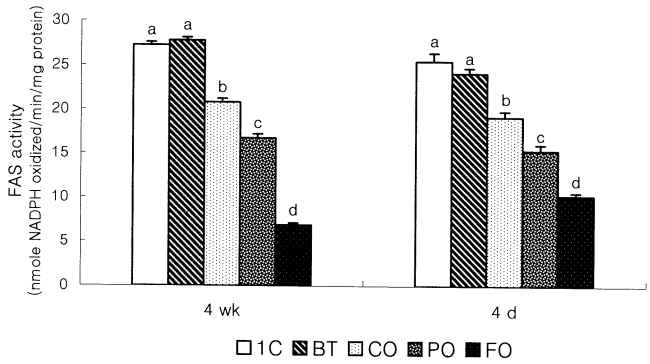

Fig. 1. Effects of dietary fats on the rat hepatic fatty acid synthase (FAS) activities. Rats were fed each of the experimental diets for 4 weeks and for 4 days by a 3-hour feeding protocol. 1C = 1% corn oil diet; BT = 10% beef tallow diet; CO = 10% corn oil diet; PO = 10% perilla oil diet; FO = 10% fish oil diet. Values are mean ± SEM (n = 8–9). Values with different superscript letters (a–e) are significantly different at $P < 0.05$ by Duncan's multiple range test.

comparable to high fat (~40% of total energy from fat) and low fat (~10% of total energy from fat) [35,36]. Thus, we used 1C diet with minimum essential fatty acids as a control, and selected 10% (w/w, estimated 20% calories), as the moderate fat intake level to compare the inhibitory effect of dietary fats on lipogenesis. Experimental groups fed 10% fat consumed the same amount of calories, carbohydrate, and other nutrients, so all the results can be considered as a sole effect of different dietary fats.

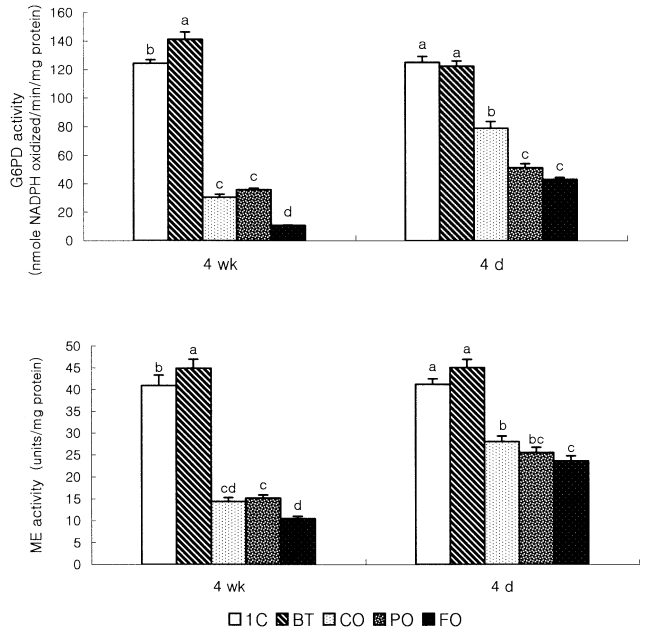

Fig. 2. Effects of dietary fats on the rat hepatic glucose-6-phosphate dehydrogenase (G6PD) and malic enzyme (ME) activities. Rats were fed each of the experimental diets for 4 weeks and for 4 days by a 3-hour feeding protocol. 1C = 1% corn oil diet; BT = 10% beef tallow diet; CO = 10% corn oil diet; PO = 10% perilla oil diet; FO = 10% fish oil diet. Values are mean ± SEM (n = 8–9). Values with different superscript letters (a–d) are significantly different at $P < 0.05$ by Duncan's multiple range test.

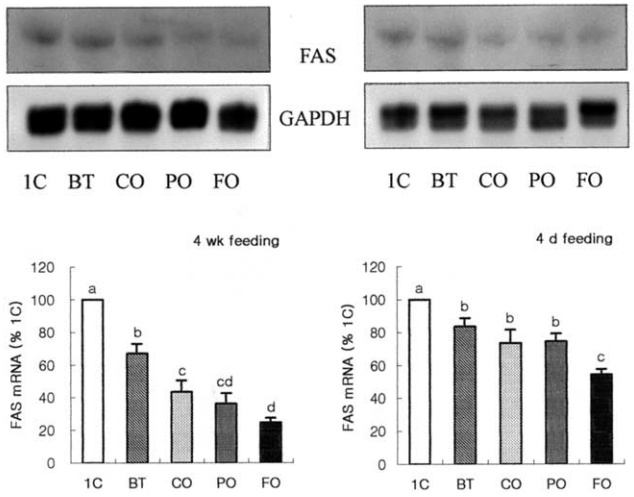

Fig. 3. Effects of dietary fats on the rat hepatic fatty acid synthase (FAS) mRNA expression. Rats were fed each of the experimental diets for 4 weeks and for 4 days by a 3-hour feeding protocol. 1C = 1% corn oil diet; BT = 10% beef tallow diet; CO = 10% corn oil diet; PO = 10% perilla oil diet; FO = 10% fish oil diet. Values are mean ± SEM (n = 4). RNA samples (20 μg) were denatured and separated on a 1.3% agarose gel containing formaldehyde, then transferred to a nylon membrane and fixed with UV crosslinking. Fatty acid synthase (FAS) and GAPDH mRNA levels were analyzed by hydridization with radiolabeled cDNA probes. Relative FAS mRNA levels were normalized to their respective GAPDH levels and are presented as percentage of 1C. Values not sharing a common superscripts (a–c) are significantly different at $P < 0.05$ by Duncan's multiple range test.

FAS plays a central role in lipogenesis by catalyzing the synthesis of saturated long chain fatty acids from acetyl-CoA, malonyl-CoA, and NADPH. Considering the absence of reducing FAS activity in rats fed BT rich in saturated fatty acids and oleic acid, the suppressive effect was absolutely due to the specific action of PUFA. The inhibitory potency of PUFA seems to vary, depending upon the degree of unsaturation and chain length, as shown in other studies [37,38]. As repeatedly observed in previous studies [17,19,37,38], it is obvious that fish oil rich in EPA and DHA suppressed the hepatic activity of lipogenic enzyme effectively. Polyenoic long-chain n-3 PUFA (DHA, EPA) are the most potent fatty acids in reducing hepatic lipogenic enzyme activities and fatty acid synthesis [17,19,34]. Although there were some studies that failed to show any significant differences between LA and ALA [39,40], our study showed that FAS activity in rats fed PO rich in ALA was lower than that of rats fed CO rich in LA. This is consistent with the results of several studies [34,41]. Dietary PO, containing neither EPA nor DHA, resulted in high content of EPA and DHA in hepatic membrane with low content of AA as we have reported earlier [13]. This result was probably due to the suppressed biosynthesis of AA from LA by the presence of α-LNA in PO, because LA and ALA compete for Δ^6-desaturase to elongate and desaturate further. Even though the products of Δ^6-desaturase are 2- to 4-fold more potent suppressors than LA and ALA itself

Table 4
Effect of dietary fats on pyruvate kinase and glucokinase activity in rat liver

Dietary treatment	Pyruvate kinase (nmol NADP reduced/min/mg protein)		Glucokinase (nmol NADP reduced/min/mg protein)	
	4-Week feeding	4-Day feeding	4-Week feeding	4-Day feeding
1C	938.6 ± 50.9[a]	915.2 ± 29.4[a]	21.01 ± 1.24[b]	15.77 ± 0.89[b]
BT	879.7 ± 34.1[a]	810.9 ± 33.2[a]	24.44 ± 1.42[a]	17.56 ± 0.47[a]
CO	759.5 ± 48.7[b]	881.4 ± 29.7[a]	13.35 ± 0.90[c]	11.93 ± 0.59[c]
PO	660.2 ± 24.3[b]	701.4 ± 57.1[b]	13.05 ± 0.68[c]	9.54 ± 0.41[d]
FO	456.7 ± 10.4[c]	530.3 ± 25.4[c]	9.60 ± 0.41[d]	8.59 ± 0.30[d]

Data expressed as mean ± SEM ($n = 9$). Values with the different superscript letters in a column are significantly different at $P < 0.05$ by Duncan's multiple range test. Sprague-Dawley rats were fed each of the experimental diets for 4 weeks and 4 days by a 3-hour feeding protocol. 1C = 1% corn oil diet; BT = 10% beef tallow diet; CO = 10% corn oil diet; PO = 10% perilla oil diet; FO = 10% fish oil diet.

[34,42,43], the resulting high concentration of EPA and DHA in the PO-fed group could suppress FAS activity more effectively than AA in the CO-fed group. Statistical analyses in our study also revealed that FAS activity was negatively correlated with EPA and DHA content of hepatic membrane fraction ($r = -0.541$ for EPA, $r = -0.846$ for DHA in 4-week feeding, $P < 0.01$; $r = -0.639$ for EPA, $r = -0.789$ for DHA in 4-day feeding, $P < 0.05$). Therefore, feeding PO and FO diets rich in n-3 PUFA (ALA, EPA, DHA) resulted in lowering FAS activity compared to feeding a CO diet rich in LA.

The result of FAS mRNA content confirms the strong inhibitory capability of FO on gene expression [16,17,37]. Unlike the rapid response of FO after even a single meal [17], we found that CO and PO showed the inhibitory effect after extended periods of feeding. In our study, a positive correlation was found between FAS specific activity and relative amount of FAS mRNA ($r = 0.757$, $P < 0.0005$ in 4-week feeding; $r = 0.620$, $P < 0.0005$ in 4-day feeding), which supports that enzyme induction is exerted partly through the process of transcription, leading to a increase of enzyme synthesis rate [18,19,43]. On the other hand, it was also observed that PO had stronger inhibitory effect on FAS activity compared with CO, despite no difference of FAS mRNA contents between two groups. Thus, it is worthwhile to consider the effect of fatty acids on FAS degradation rate as a possible factor to cause this discrepancy. Further studies are required to clarify this possibility because the effect of dietary fat on enzyme degradation rate has not been reported yet.

Glycolysis, supplying acetyl-CoA as a substrate for fatty acid synthesis from surplus glucose, seems to be related with lipogenesis. The activities of two key regulatory enzymes, PK and GK, were found to be positively correlated with that of FAS ($r = 0.856$ for PK, $r = 0.840$ for GK in 4-week feeding; $r = 0.911$for PK, $r = 0.910$ for GK in 4-day feeding; $P = 0.0001$). Our results confirmed the inhibitory capability of polyunsaturated fatty acids, especially FO, on glycolytic enzymes [17,44]. Considering that glucose-6-phosphate is thought to be a signal metabolite to trigger the transcriptional response of lipogenic enzyme [45,46], the suppression of glucose metabolism by inhibition of glycolytic enzyme was probably related with the low FAS activity in polyunsaturated fatty acids. In contrast to the lower GK and PK activities of the PO group in the 4-day feeding period, a significant difference was not observed between PO and CO groups as the feeding period was extended to 4 weeks. There have been inconsistent results of comparing glycolytic enzyme activities between LA and ALA [39,40]. It might be ascribed to the multiple experimental factors such as feeding periods, fat intake levels, and oil status.

The activities of G6PD and ME were also significantly reduced in polyunsaturated fat–fed groups, with the greatest in FO group. Positive correlations between NADPH supplying enzymes and FAS were observed ($r = 0.874$ for G6PDH, $r = 0.821$ for ME in 4-week feeding; $r = 0.854$ for G6PDH, $r = 0.792$ for ME for 4-day feeding; $P = 0.0001$), which suggests that the activities of NADPH providing enzymes correlate positively with the rate of fatty acid synthesis. However, each of these enzymes is usually near equilibrium with respect to substrate and products in liver [47]. Thus, the rate of NADPH production is thought to be dependent upon the fatty acid synthesis rate. In addition, for rats of all dietary groups, the hepatic lipogenic enzyme activities and hepatic TG were directly correlated ($r = 0.588$, $P < 0.0005$ for FAS; $r = 0.483$, $P < 0.01$ for G6PD; $r = 0.378$, $P < 0.05$ for ME), and plasma TG concentration was positively correlated with FAS activity ($r = 0.442$, $P < 0.05$) in the long-term feeding experiment. These are consistent with the report that the activities of hepatic lipogenic enzymes were reflected by the concentration of TG in liver and plasma [48,49]. Thus, this study elucidate that the type of dietary fat can probably affect the rate of hepatic TG synthesis via coordinate changes of lipogenic enzymes and, as a result, the plasma TG concentration can be lowered.

In summary, suppression of FAS plays a significant role in the hypolipidemic effects observed in rats fed ALA-rich perilla oil, and these effects were associated with the increase of hepatic microsomal EPA and DHA contents.

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