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Physiological effects of γ-linolenic acid and sesamin on hepatic fatty acid synthesis and oxidation☆

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Abstract

Interrelated effects of γ-linolenic acid (GLA) and sesamin, a sesame lignan, on hepatic fatty acid synthesis and oxidation were examined. Rats were fed experimental diets supplemented with 0 or 2 g/kg sesamin (1:1 mixture of sesamin and episesamin) and containing 100 g/kg of palm oil (saturated fat), safflower oil rich in linoleic acid, or oil of evening primrose origin containing 43% GLA (GLA oil) for 18 days. In rats fed sesamin-free diets, GLA oil, compared with other oils, increased the activity and mRNA levels of various enzymes involved in fatty acid oxidation, except for some instances. Sesamin greatly increased these parameters, and the enhancing effects of sesamin on peroxisomal fatty acid oxidation rate and acyl-CoA oxidase, enoyl-CoA hydratase and acyl-CoA thioesterase activities were more exaggerated in rats fed GLA oil than in the animals fed other oils. The combination of sesamin and GLA oil also synergistically increased the mRNA levels of some peroxisomal fatty acid oxidation enzymes and of several enzymes involved in fatty acid metabolism located in other cell organelles. In the groups fed sesamin-free diets, GLA oil, compared with other oils, markedly reduced the activity and mRNA levels of various lipogenic enzymes. Sesamin reduced all these parameters, except for malic enzyme, in rats fed palm and safflower oils, but the effects were attenuated in the animals fed GLA oil. These changes by sesamin and fat type accompanied profound alterations in serum lipid levels. This may be ascribable to the changes in apolipoprotein-B-containing lipoproteins.

Keywords: γ-Linolenic acid; Sesamin; Hepatic fatty acid oxidation; Hepatic fatty acid synthesis; Serum lipid levels

1. Introduction

Many studies have demonstrated that polyunsaturated fatty acids exert a profound influence on hepatic fatty acid metabolism. This may account for the physiological activities of dietary fats rich in polyunsaturated fatty acids affecting serum and tissue lipid levels. For example, fish oil and eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are n-3 polyunsaturated fatty acids (PUFAs) abundant in fish oil, up-regulate hepatic fatty acid oxidation, presumably through the activation of peroxisome proliferator-activated receptor α (PPARα) [1–6]. Perilla and linseed oils rich in α-linolenic acid, a member of the n-3 PUFAs, also stimulate the activity and gene expression levels of hepatic enzymes involved in fatty acid oxidation [1,7–10].

In contrast, fish oil [1–4], EPA and DHA [3–6] strongly reduce hepatic lipogenesis. Perilla and linseed oils rich in α-linolenic acid, compared with saturated fat (palm oil), also reduce the activities and mRNA levels of hepatic lipogenic enzymes [1,7,8,10]; however, the extent of the reduction was much smaller with perilla and linseed oils than with fish oil [1].

With regard to the physiological activity of n-6 PUFAs, our studies showed that safflower oil rich in linoleic acid, compared with palm oil, did not change hepatic activities or mRNA levels of enzymes involved in fatty acid oxidation [1,7–9]. We also found that fungal oil rich in dihomo-γ-linolenic acid or arachidonic acid, compared with corn oil rich in linoleic acid, has little effect on the activity or mRNA levels of enzymes involved in hepatic fatty acid oxidation [11]. Concerning the physiological activity of γ-linolenic acid (GLA) affecting hepatic fatty acid oxidation, Takada *et al.* [12] reported that fungal oil rich in GLA, compared with soybean oil, increased carnitine palmitoyltransferase activity and the peroxisomal β-oxidation rate in rat liver accompanying the reduction of body fat mass. Consistent with this, we observed that borage oil rich in GLA, compared with palm oil and a fat mixture containing similar amounts of n-6 PUFA in the form of linoleic acid but devoid of GLA, significantly increased the peroxisomal palmitoyl-CoA oxidation rate and the activities of acyl-CoA oxidase and carnitine palmitoyltransferase in the rat liver [10]. We [13] also demonstrated

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that borage oil rich in GLA, compared with safflower oil rich in linoleic acid, increased mRNA levels of hepatic enzymes involved in peroxisomal fatty acid oxidation and reduced body fat mass in rats.

With respect to the physiological effects of n-6 fatty acids affecting hepatic lipogenesis, oils rich in linoleic acid, compared with saturated fats, reduce the activity and mRNA levels of hepatic enzymes involved in fatty acid synthesis [1,7,8,10]. GLA in the form of borage oil was comparable to linoleic acid and α-linolenic acid in reducing hepatic lipogenesis [10]. Our recent study [11] indicated that fungal oil rich in either dihomo-γ-linolenic acid or arachidonic acid, compared with corn oil rich in linoleic acid, reduces the activity and mRNA levels of hepatic lipogenic enzymes; arachidonic acid was more competent in reducing lipogenesis than dihomo-γ-linolenic acid.

We previously demonstrated that sesame lignans are strong natural inducers of hepatic fatty acid oxidation [11,14–20]. Lignans also reduce hepatic lipogenesis [11,14–20]. Aside from the physiological activity of n-3 and n-6 PUFAs affecting hepatic fatty acid metabolism, we showed that fish oil [16,17], EPA and DHA in the form of ethyl esters [18] and fungal oil rich in either dihomo-γ-linolenic acid or arachidonic acid [11] strongly increased the physiological activity of the sesamin preparation composed of equal amounts of sesamin and episesamin to increase hepatic fatty acid oxidation. Linoleic acid, a member of the n-6 PUFAs, in the form of safflower oil or corn oil did not enhance the physiological activity of sesamin to increase fatty acid oxidation [11,16]. However, how the combination of sesamin and GLA affects hepatic fatty acid oxidation is not known.

In the present study, we studied the physiological activities of a diet containing both sesamin and oil rich in GLA affecting the hepatic fatty acid metabolism of rats.

2. Materials and methods

2.1. Animals and diets

Male Sprague–Dawley rats (4 weeks old) obtained from Charles River Japan (Kanagawa, Japan) were housed individually in animal cages in a room with controlled temperature (20°C–22°C), humidity (55%–65%), and lighting (lights on from 07:00 to 19:00 h) and fed commercial chow. After 7 days of acclimatization, rats were fed purified experimental diets supplemented with 0 or 2 g/kg sesamin (1:1 mixture of sesamin and episesamin; Takemoto Oil Co., Aichi) and containing 100 g/kg of palm oil, safflower oil or oil rich in γ-linolenic acid (GLA oil). GLA oil containing 42.6% GLA was prepared by selective hydrolysis of evening primrose oil containing 10.5% of GLA using lipase of *Candida cylindracea* origin, and the product was purified through usual oil refining processes (deacidification, deodorization and decoloration). Fatty acid compositions of dietary fats and the distribution of fatty acids among the mono-, di- and triacylglycerols are shown in Table 1. To analyze the fatty acid contents among different acylglycerols, palm, safflower and GLA oils were separated by thin-layer chromatography on silica gel G into respective lipid molecules using a solvent mixture of chloroform and acetone (95:5, v/v). Lipid bands were detected under UV (365 nm) after spraying 0.2% (w/v) 2′,7′-dichlorofluorescein in ethanol. Fatty acids in each fraction were methylated and quantified by gas–liquid chromatography using heptadecanoic acid as an internal standard. Lipid bands of 1,2- and 1,3-diacylglycerols were barely detectable in the palm and safflower oil samples. Therefore, the area on the thin-layer chromatogram corresponding to the position containing both of these two diacylglycerol species was scraped off to analyze fatty acids. More than 96% of the fatty acids were distributed in triacylglycerol for palm and safflower oils, while considerable amounts were recovered in 1,2- and 1,3-diacylglycerols for GLA oil. The basal composition of the purified experimental diets contained (g/kg): casein, 200; dietary fat, 100; corn starch, 150; cellulose, 20; mineral mixture [21], 35; vitamin mixture [21], 10; L-cystine, 3; choline bitartrate, 2.5 and sucrose to 1 kg. Sesamin was added to experimental diets in lieu of sucrose. The caloric contents were 4.23 and 4.22 kcal/g for sesamin-free and sesamin-containing diets, respectively. Experimental diets based on the AIN-93 diet [21] have been widely used among laboratories. In the present study, the compositions of mineral and vitamin mixtures were the same as those of AIN-93G-MX and AIN-93-VX [21], respectively. Corn starch is used as a major carbohydrate source in the AIN-93 diet (about 40% and 47% for AIN-93G and AIN-93M diets, respectively), and sucrose concentration is low (10% for both AIN-93G and -93M diets). However, information [22] suggested that hepatic lipogenesis is much lower with a diet high in starch than with a diet high in sucrose. Therefore, experimental diets high in starch are not suited for studies to investigate the physiological activity of food factors that reduce hepatic lipogenesis. Therefore, we employed experimental diets high in sucrose (about 48%) in this study.

Table 1
Fatty acid composition and distribution of dietary fats

	Dietary fats		
	Palm oil	Safflower oil	GLA oil
Fatty acid composition (wt%)			
10:0	0.2	0.0	0.0
12:0	0.7	0.0	0.0
16:0	44.0	5.2	4.1
18:0	1.2	1.2	1.3
18:1 (n-9)	39.3	9.5	5.6
18:2 (n-6)	14.7	84.1	46.4
18:3 (n-6)	0.0	0.0	42.6
Fatty acid distribution (wt%)			
Monoacylglycerol	0.8	0.7	1.6
1,2-Diacylglycerol	2.9[a]	1.3[a]	6.1
1,3-Diacylglycerol			12.7
Triacylglycerol	96.3	97.9	79.7

[a] Sum of the values recovered in 1,2- and 1,3-diacylglycerol fractions.

Animals had free access to the diets and water during the experimental period. This animal experiment was approved by the review board of animal ethics of Jumonji University (approval number of 1502; issued on June 16, 2015).

2.2. Enzyme assays

At the end of the experiment, rats were anesthetized by isoflurane and killed by bleeding from the abdominal aorta, after which the livers were quickly excised. Approximately 1.5 g of each liver was homogenized in 10 ml of 0.25 M sucrose containing 1 mM EDTA and 3 mM Tris–HCl (pH 7.2) and centrifuged at 200,000*g* for 30 min. The activity of enzymes involved in fatty acid synthesis was measured using the 200,000*g* supernatant of the liver homogenate, as detailed previously [4].

KCN-insensitive palmitoyl-CoA-dependent NAD reduction (peroxisomal palmitoyl-CoA oxidation) and the activity of enzymes involved in fatty acid oxidation, including acyl-CoA oxidase, carnitine acyltransferase, enoyl-CoA hydratase, 3-hydroxyacyl-CoA dehydrogenase and 3-ketoacyl-CoA thiolase, were measured spectrophotometrically in the whole liver homogenates as an enzyme source, as detailed previously [1,4,14]. Acyl-CoA oxidase activity was measured using palmitoyl-CoA as a substrate. Carnitine acyltransferase activity was measured using acetyl-, octanoyl- and palmitoyl-CoAs as substrates. Crotonyl-CoA was employed as a substrate in assaying the activity of enoyl-CoA hydratase. 3-Hydroxyacyl-CoA dehydrogenase and 3-keotacyl-CoA thiolase activities were measured using 3-hydroxybutyryl-CoA and acetoacetyl-CoA substrates, respectively. Acyl-CoA thioesterase activity was measured using octanoyl-CoA and palmitoyl-CoA substrates, and employing 200,000*g* supernatant and total homogenate as enzyme sources [23].

2.3. RNA analyses

Liver RNA was extracted, and mRNA abundance was analyzed by quantitative real-time polymerase chain reaction as previously described [24]. mRNA abundance was calculated as a ratio to the mRNA abundance of β-actin in each cDNA sample and expressed as a fold-change, assigning a value of 1 for rats fed a diet containing palm oil and devoid of sesamin.

2.4. Analyses of lipids and lignans

Liver lipids were extracted and purified, and triacylglycerol, phospholipid and cholesterol concentrations in the lipid extract were determined as described previously [25]. Triacylglycerol and phospholipids in the liver lipid extract were separated by thin-layer chromatography, and the fatty acid compositions were analyzed by gas-liquid chromatography (GLC) using a FAMEWAX column (30 m × 0.25 mm; Restek, Bellefonte, PA, USA). The serum high-density lipoprotein (HDL) fraction was prepared by precipitating apolipoprotein-B-containing lipoproteins using polyethylene glycol (average molecular weight of 6000; Sigma-Aldrich Co., Tokyo, Japan) [26]. Serum triacylglycerol, cholesterol and phospholipid concentrations, as well as cholesterol and phospholipid levels in the serum HDL fraction, were analyzed using commercial enzyme kits (Wako Pure Chemical, Osaka, Japan). Cholesterol and phospholipid concentrations in very low and low-density lipoprotein (VLDL + LDL) fractions were calculated by subtracting the values in the HDL fraction from the values in the unfractionated serum samples. Concentrations of sesamin and episesamin in the liver and serum were analyzed by high-performance liquid chromatography as detailed previously [27].

2.5. Statistical analysis

Microsoft Excel add-in software (Excel Statistics 2010; Social Survey Research Information Co., Tokyo, Japan) was used for statistical analyses. Data were expressed as