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Interrelated effects of dihomo-γ-linolenic and arachidonic acids, and sesamin on hepatic fatty acid synthesis and oxidation in rats

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Abstract

Interrelated effects of dihomo-γ-linolenic acid (DGLA) and arachidonic acid (ARA), and sesamin, a sesame lignan, on hepatic fatty acid synthesis and oxidation were examined in rats. Rats were fed experimental diets supplemented with 0 or 2 g/kg sesamin (1:1 mixture of sesamin and episesamin), containing 100 g/kg of maize oil or fungal oil rich in DGLA or ARA for 16 d. Among the groups fed sesamin-free diets, oils rich in DGLA or ARA, especially the latter, compared with maize oil strongly reduced the activity and mRNA levels of various lipogenic enzymes. Sesamin, irrespective of the type of fat, reduced the parameters of lipogenic enzymes except for malic enzyme. The type of dietary fat was rather irrelevant in affecting hepatic fatty acid oxidation among rats fed the sesamin-free diets. Sesamin increased the activities of enzymes involved in fatty acid oxidation in all groups of rats given different fats. The extent of the increase depended on the dietary fat type, and the values became much higher with a diet containing sesamin and oil rich in ARA in combination than with a diet containing lignan and maize oil. Analyses of mRNA levels revealed that the combination of sesamin and oil rich in ARA compared with the combination of lignan and maize oil markedly increased the gene expression of various peroxisomal fatty acid oxidation enzymes but not mitochondrial enzymes. The enhancement of sesamin action on hepatic fatty acid oxidation was also confirmed with oil rich in DGLA but to a lesser extent.

Key words: Arachidonic acid: Sesamin: Hepatic fatty acid synthesis: Hepatic fatty acid oxidation

PUFA exert a strong influence on hepatic fatty acid metabolism. Fish oil up-regulates hepatic fatty acid oxidation, presumably through the activation of PPARα[1,2]. EPA and DHA, which are *n*-3 PUFA abundant in fish oil, appear to be responsible for this[3–6]. Perilla oil rich in α-linolenic acid (ALA), a member of *n*-3 PUFA, also stimulates the activity and gene expression levels of hepatic enzymes involved in fatty acid oxidation in rats[1].

Fish oil[1–4,7], and EPA and DHA[3–7] also strongly reduce hepatic lipogenesis. Perilla oil rich in ALA compared with saturated fat (palm oil) also reduces the activities and mRNA levels of hepatic lipogenic enzymes[1]; however, the extent of the reduction was much smaller with perilla oil than with fish oil.

With regard to the physiological activity of *n*-6 PUFA, safflower oil rich in linoleic acid (LA) compared with saturated fat (palm oil) did not change hepatic activities and mRNA levels of enzymes involved in fatty acid oxidation[1]. On the other hand, this oil compared with palm oil decreased the parameters of hepatic lipogenesis[1]. Concerning the physiological activity of γ-linolenic acid (GLA), a member of *n*-6 PUFA, Takada *et al.*[8] showed that fungal oil rich in GLA compared with soyabean oil increased carnitine palmitoyltransferase activity and the peroxisomal β-oxidation rate in the rat liver accompanying the reduction in body fat mass. Consistent with this, we[9] observed that borage oil rich in GLA compared with palm oil rich in palmitic and oleic acids 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. We[10] also showed that borage oil rich in GLA compared with safflower oil increased mRNA levels of hepatic enzymes involved in peroxisomal fatty acid oxidation and reduced body fat mass in rats; however, GLA appeared comparable

Abbreviations: ALA, α-linolenic acid; ARA, arachidonic acid; cyp4a1, cytochrome P450 4a1; DGLA, dihomo-γ-linolenic acid; GLA, γ-linolenic acid; LA, linoleic acid; SREBP, sterol regulatory element binding protein.

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with LA and ALA in reducing hepatic lipogenesis[9]. Although these studies indicated that respective *n*-6 PUFA differ in how they affect hepatic fatty acid metabolism, animal studies that examined the physiological activity of *n*-6 PUFA other than LA and GLA are scarce because of their unavailability in nature as oils to feed experimental animals; however, recent developments in microbiology and biotechnology have made oils of fungal origin, rich in dihomo-γ-linolenic acid (DGLA)[11] and arachidonic acid (ARA)[12], available for use in animal[13,14] and human studies[15,16].

We previously demonstrated that sesame lignans are strong natural inducers of hepatic fatty acid oxidation[17–21]. Also, lignans have physiological activity in reducing hepatic lipogenesis[17–21]. Apart from the physiological activity of *n*-3 PUFA in affecting hepatic fatty acid synthesis and oxidation, we showed that fish oil[18], and EPA and DHA in the form of ethyl esters[19] strongly increased the physiological activity of a sesamin preparation composed of equal amounts of sesamin and episesamin to increase hepatic fatty acid oxidation. LA, a member of *n*-6 PUFA, in the form of safflower oil was not effective to enhance the physiological activity of sesamin to increase fatty acid oxidation[18]; however, how the combination of sesamin, and DGLA or ARA affects hepatic fatty acid oxidation is not known.

In the present study, we clarified the physiological activity of fungal oils rich in DGLA and ARA affecting hepatic fatty acid synthesis and oxidation. Moreover, despite the fact that these *n*-6 PUFA were rather ineffective in increasing hepatic fatty acid oxidation, we found that oils rich in DGLA and ARA strongly stimulated the physiological activity of sesamin to increase hepatic fatty acid oxidation, presumably through the up-regulation of the mRNA expression of peroxisomal fatty acid oxidation enzymes.

Materials and methods

Animals and diets

Male Sprague–Dawley rats (4 weeks old) obtained from Charles River Japan were housed individually in animal cages in a room with controlled temperature (20–22°C), humidity (55–65 %) and lighting (lights on from 07.00 to 19.00 hours), and fed commercial chow. After 7 d of acclimatisation, rats were fed purified experimental diets supplemented with 0 or 2 g/kg of sesamin (1:1 mixture of sesamin and episesamin), and containing 100 g/kg of maize oil or fungal oil rich in DGLA (DGLA oil) or ARA (ARA oil) for 16 d. The basal composition of the purified experimental diets contained: casein, 200 g/kg; dietary fat, 100 g/kg; maize starch, 150 g/kg; cellulose, 20 g/kg; mineral mixture[22], 35 g/kg; vitamin mixture[22], 10 g/kg; L-cystine, 3 g/kg; choline bitartrate, 2·5 g/kg and sucrose to 1 kg. Sesamin was added to the experimental diets in lieu of sucrose. Animals had free access to the diets and water during the experimental period. They were not deprived of food until before they were killed. Fatty acid compositions of the dietary fats are shown in Table 1. The present animal experiment was approved by the review board of animal ethics at our institute, and we followed the institute's guidelines for the care and use of laboratory animals.

Table 1. Fatty acid compositions of the dietary fats*

	Dietary fats		
	Maize oil	DGLA oil	ARA oil
16 : 0	10·0	17·9	9·2
18 : 0	1·9	7·2	6·7
22 : 0	0·2	2·9	3·4
24 : 0	0·1	7·4	8·2
18 : 1*n*-9	31·5	6·3	6·9
18 : 2*n*-6	55·0	5·7	9·3
18 : 3*n*-6	0·0	3·3	2·6
20 : 3*n*-6	0·0	45·3	3·1
20 : 4*n*-6	0·0	0·6	45·6
Others	1·2	3·4	5·0

DGLA, dihomo-γ-linolenic acid; ARA, arachidonic acid.
*Values are expressed as g fatty acids/100 g fatty acids.

Enzyme assays

At the end of the experiments, rats were anaesthetised using diethyl ether and killed by bleeding from the abdominal aorta, after which the liver was quickly excised. Approximately 1·5 g of each liver were homogenised 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 the enzymes involved in fatty acid synthesis was measured using the 200 000 ***g*** supernatant of the liver homogenate, as detailed previously[4].

Cyanide-insensitive palmitoyl-CoA-dependent NAD reduction (peroxisomal palmitoyl-CoA oxidation) and the activity of the 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 whole liver homogenates as an enzyme source, as detailed previously[1,4]. Acyl-CoA oxidase activity was measured using palmitoyl-CoA as a substrate. Carnitine acyltransferase activity was measured using both octanoyl-CoA and palmitoyl-CoA as substrates. Crotonyl-CoA, 3-hyroxybutyryl-CoA and acetoacetyl-CoA were employed as substrates in assaying the activities of enoyl-CoA hydratase, 3-hydroxyacyl-CoA dehydrogenase and 3-keotacyl-CoA thiolase, respectively.

RNA analyses

RNA in the liver was extracted, and mRNA abundance was analysed by quantitative real-time PCR, as detailed elsewhere[23]. The nucleotide sequences of primers and probes to analyse mRNA abundance of various genes were designed using Primer Express Software (Applied Biosystems), and those of sense and antisense primers, and probes were 5′-CATCTCCGCGCTGGAGTAC-3′, 5′-AGACAAGCACCGAGC-AAAGAC-3′ and 5′-CATGCCTGTCACCCTCATCGGAGA-3′ for 6-phosphogluconate dehydrogenase, 5′-GGCTTTGCAGGG-ATCTTCAAT-3′, 5′-CGGTCTGGAATCGAGACTTGAG-3′ and 5′-TGTGGCAATCCCCCCAGACG-3′ for carnitine/acylcarnitine

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translocase, 5′-CCAGCCAACGCCTTTGC-3′, 5′-TCCAGCACACCATACGACGTA-3′ and 5′-CCCACTCCAGAACCCAGACAACCA-3′ for cd36/fatty acid translocase, 5′-GCCGCCTCTATTGGGTTGAT-3′, 5′-TTCCGACCACCCCCATT-3′ and 5′-TCCACTCCATCTCCAGCATCGA-3′ for LDL receptor, and 5′-GCGCGGATGAGTTCACTTG-3′, 5′-TCATCCTGTCCATTGCACACA-3′ and 5′-TCCAGTGGCCGCTGTGTCTCCAG-3′ for VLDL receptor, respectively; those of other genes have been reported elsewhere[21]. mRNA abundance was calculated as the ratio to the mRNA abundance of β-actin in each complementary DNA sample and expressed as a percentage, assigning a value of 100 for rats fed a diet containing maize oil and devoid of sesamin.

Analyses of lipids and lignans

Liver lipids were extracted and purified, and TAG, phospholipid and cholesterol concentrations in the lipid extract were determined as described previously[24]. TAG and phospholipid in the liver lipid extract were separated by TLC and the fatty acid compositions were analysed by GLC using a FAMEWAX™ column (30 m × 0·25 mm; Restek). Serum TAG, cholesterol, phospholipid and NEFA concentrations were measured using commercial enzyme kits (Wako Pure Chemical). Concentrations of sesamin and episesamin in the liver and serum were analysed by HPLC as detailed previously[18].

Statistical analysis

Microsoft Excel add-in software (Excel Statistics 2010; Social Survey Research Information Company) was used for statistical analysis. Data are expressed as means with their standard errors. Levene's test was used to examine the constancy of the variance of the observations. If variances were heterogeneous, they were transformed logarithmically. These transformations were successful in rendering the variance of the observation constant, and hence the transformed values were used for subsequent statistical evaluations. The data for the two-way classification were analysed using two-way ANOVA. If no significant interaction existed, and a significant fat effect was detected, Tukey's *post hoc* test was conducted to detect differential effects of various fats differing in fatty acid composition. When the interaction was significant, the data were reanalysed with one-way ANOVA and the *post hoc* test to detect all the significant differences of means. The data for one-way classification were analysed using one-way ANOVA and the *post hoc* test. Differences were considered significant when $P<0·05$.

Results

Growth parameters and tissue weights

No significant effect of fat types and sesamin on food intake was seen among the groups (Table 2); however, some significant differences were seen in body weight at the time of killing and growth among the groups. Oils rich in DGLA and ARA compared with maize oil significantly decreased

Table 2. Effects of dietary sesamin and fat types on growth parameters and tissue weight
(Mean values with their standard errors, *n* 7)

Dietary sesamin (g/kg) …	Dietary fats (100 g/kg)										Two-way ANOVA (*P* value)			*Post hoc* analysis for fat effect (*P* value)		
	Maize oil				DGLA oil				ARA oil							
	0		2		0		2		0		2					
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Fat	Sesamin	Fat × sesamin	Maize oil *v.* DGLA oil	Maize oil *v.* ARA oil	DGLA oil *v.* ARA oil
Food intake (g/d)	19·0	0·5	19·8	0·5	20·6	0·3	19·5	0·7	20·4	0·6	19·3	0·5	NS*	NS*	NS*	–	–	–
Body weight (g)	268[a]	7	289[b]	6	283[a,b]	3	280[a,b]	5	288[a]	6	273[a,b]	5	NS*	NS*	< 0·01	–	–	–
Growth (g/16 d)	132[a]	6	152[c]	4	147[a,b,c]	3	142[a,b,c]	5	149[b,c]	6	134[a,b]	5	NS*	NS*	< 0·01	–	–	–
Tissue weight (g/100 g body weight)																		
Liver	5·00	0·14	6·38	0·18	4·74	0·11	6·00	0·13	4·81	0·11	5·69	0·08	< 0·01	< 0·01	NS*	< 0·05	< 0·01	NS*
Epididymal adipose tissue	1·14	0·07	0·980	0·069	1·01	0·03	0·920	0·068	0·862	0·040	0·802	0·086	< 0·01	NS*	NS*	NS*	< 0·01	NS*
Perirenal adipose tissue	1·29	0·07	0·933	0·068	1·10	0·08	0·897	0·089	0·896	0·105	0·822	0·069	< 0·05	< 0·01	NS*	NS*	< 0·01	NS*

DGLA, dihomo-γ-linolenic acid; ARA, arachidonic acid.
[a,b,c] Mean values with unlike superscript letters within a row were significantly different ($P<0·05$).
* $P\geq0·05$.

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the liver weight. Sesamin significantly increased this parameter. Dietary oil rich in ARA compared with maize oil significantly decreased the weights of epididymal and perirenal adipose tissues; however, DGLA oil compared with maize oil failed to do so. Dietary sesamin significantly decreased the weight of perirenal but not epididymal adipose tissue.

Activity and mRNA levels of enzymes involved in hepatic lipogenesis

Among the rats fed the sesamin-free diets, the activity levels of fatty acid synthase, ATP citrate lyase, glucose-6-phosphate dehydrogenase and pyruvate kinase were lower in rats fed oils rich in either DGLA or ARA than in those fed maize oil (Fig. 1). Oil rich in ARA compared with oil rich in DGLA more markedly reduced the activity levels of these enzymes. Oil rich in ARA compared with maize oil also significantly lowered the activity of 6-phosphogluconate dehydrogenase; however, oil rich in DGLA failed to do so. Sesamin, irrespective of the type of dietary fat, significantly reduced the activity of various lipogenic enzymes.

Fig. 2 shows mRNA levels of proteins related to hepatic lipogenesis. There are two types of acetyl-CoA carboxylase, i.e. α and β. The α but not β form appears to be involved in long-chain fatty acid synthesis in cytosols[25]. There are several isoforms of pyruvate kinase in mammals[26]. L-Pyruvate kinase is an enzyme expressed in the liver. Adiponutrin is a protein presumed to be involved in the regulation of lipogenesis[27]. Stearoyl-CoA desaturase 1 catalyses a rate-limiting step in the synthesis of unsaturated fatty acids. It has been observed that gene expression of this enzyme is coordinately regulated with those of various enzymes involved in lipogenesis[28]. Among the rats fed the sesamin-free diets, ARA oil compared with maize oil decreased the mRNA levels of various lipogenic enzymes and adiponutrin, and stearoyl-CoA desaturase 1. DGLA oil compared with maize oil also significantly reduced the mRNA expressions of acetyl-CoA carboxylase α, fatty acid synthase, ATP citrate lyase, glucose-6-phosphate dehydrogenase and stearoyl-CoA desaturase 1; however, the extent of the reduction was considerably attenuated compared with ARA oil. Oil rich in DGLA failed to reduce the mRNA levels of 6-phosphogluconate dehydrogenase, L-pyruvate kinase and adiponutrin. Sesamin, irrespective of the dietary fat type, significantly lowered the mRNA expression of acetyl-CoA carboxylase α, fatty acid synthase, ATP citrate lyase, 6-phosphogluconate dehydrogenase, pyruvate kinase and adiponutrin. The lignan decreased the mRNA levels of glucose-6-phosphate dehydrogenase in rats fed maize oil and DGLA oil, but failed to do so in animals fed ARA oil. In contrast to the cases observed in other genes related to lipogenesis, sesamin did not affect the gene expression of stearoyl-CoA desaturase 1 in rats given maize oil, but significantly increased this parameter in rats fed DGLA and ARA oils. Sesamin lowered the mRNA abundance of sterol regulatory element binding protein (SREBP)-1c involved in the regulation of the gene expression of lipogenic enzymes[7,29]; however, the type of dietary fat did not affect this parameter.

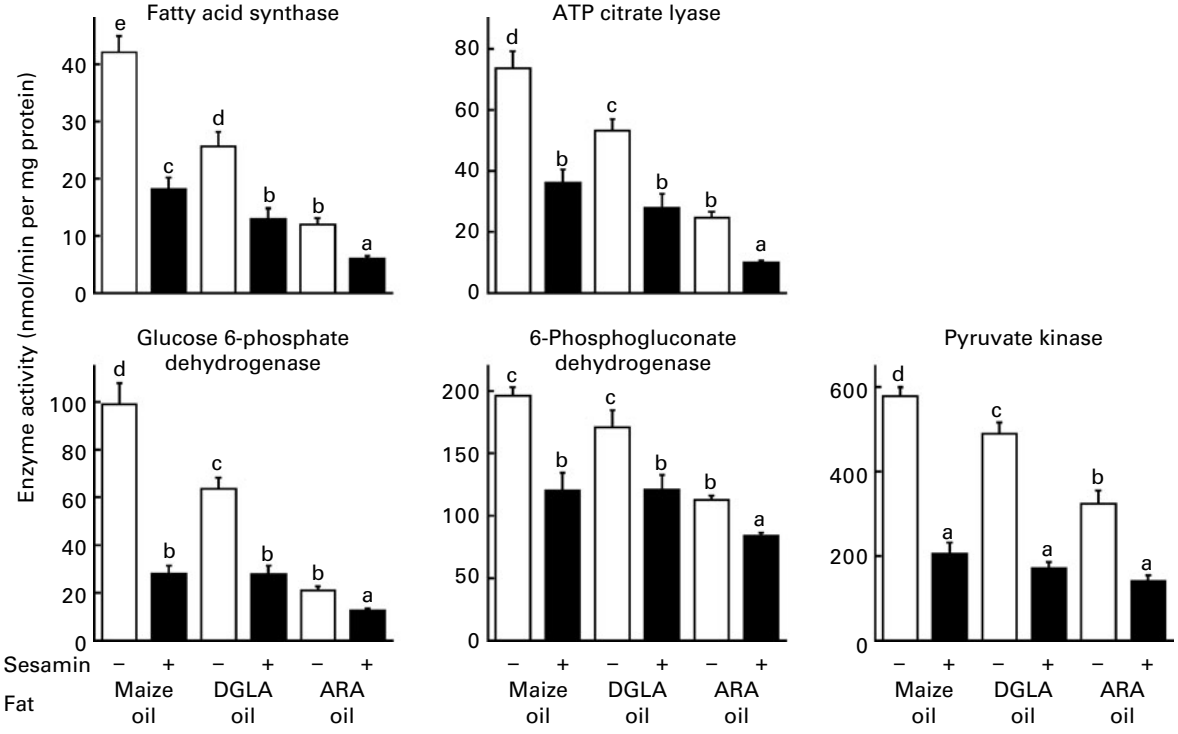

Fig. 1. Effect of dietary sesamin and fat types on the activity of lipogenic enzymes in the rat liver. Values are means, with their standard errors represented by vertical bars (n 7). Two-way ANOVA revealed significant interactions between two dietary factors, i.e. fat and sesamin, for the activities of various enzymes involved in hepatic lipogenesis; therefore, the values were reanalysed by one-way ANOVA and Tukey's *post hoc* test. [a,b,c,d,e] Mean values with unlike letters were significantly different ($P<0{\cdot}05$). DGLA, dihomo-γ-linolenic acid; ARA, arachidonic acid.

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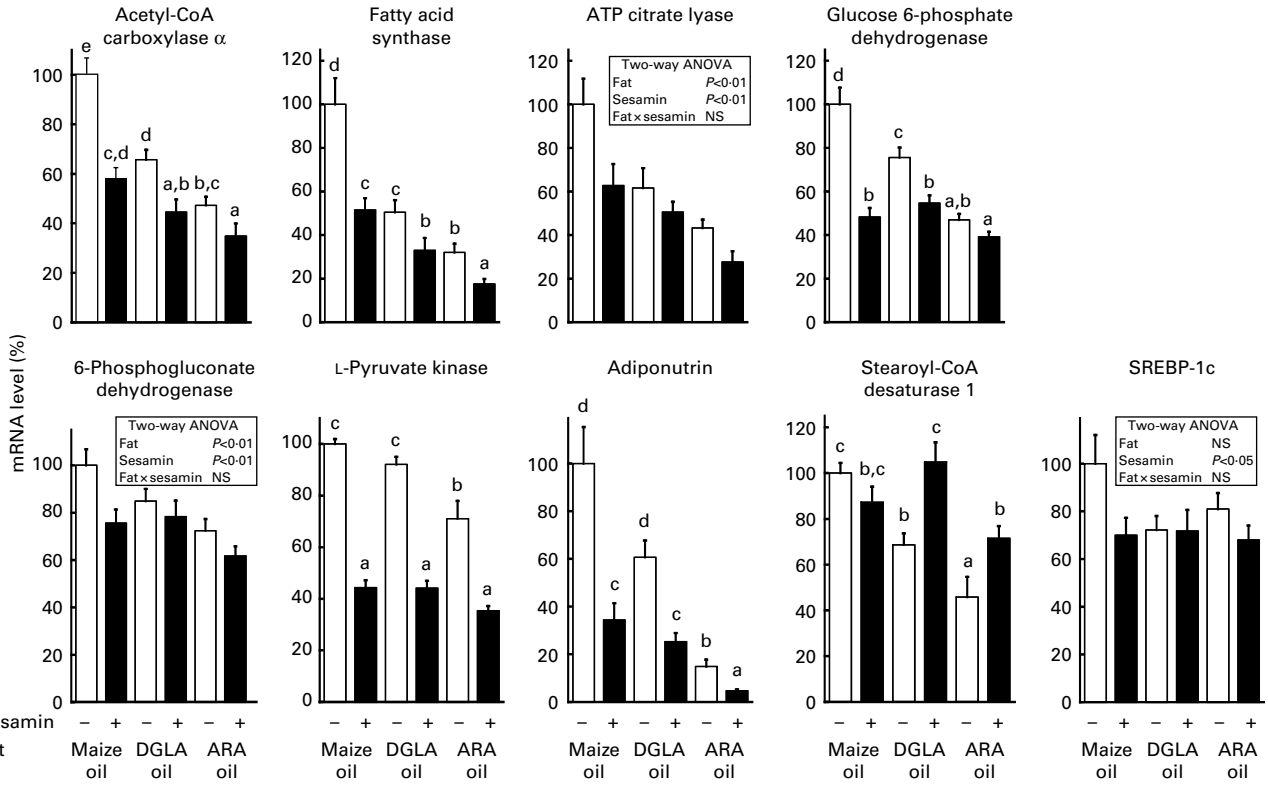

Fig. 2. Effect of dietary sesamin and fat types on the mRNA levels of proteins related to lipogenesis in the rat liver. Values are means, with their standard errors represented by vertical bars (*n* 7). Data were analysed by two-way ANOVA. *Post hoc* test for fat effect on the mRNA level of ATP citrate lyase revealed that the values were significantly lower in rats fed DGLA and ARA oils than in animals fed maize oil ($P<0·01$). Also, the value was lower in rats fed ARA oil than in animals fed DGLA oil ($P<0·05$). Two-way ANOVA revealed significant interactions between the two factors, fat and sesamin, for the mRNA levels of acetyl-CoA carboxylase α, fatty acid synthase, glucose 6-phosphate dehydrogenase, L-pyruvate kinase, adiponutrin and stearoyl-CoA desaturase 1; therefore, these values were reanalysed by one-way ANOVA and *post hoc* test. [a,b,c,d,e] Mean values with unlike letters were significantly different ($P<0·05$). NS, $P\geq0·05$. DGLA, dihomo-γ-linolenic acid; ARA, arachidonic acid.

Activity and mRNA levels of enzymes involved in hepatic fatty acid oxidation

Among the rats fed the sesamin-free diets, ARA oil compared with maize oil and DGLA oil caused a significant increase (1·3- and 1·4-fold compared with the former and latter, respectively) in the activity of acyl-CoA oxidase (Fig. 3). Also, the activity of carnitine acyltransferase measured with octanoyl-CoA as a substrate was higher in rats fed ARA oil than in those fed maize oil and DGLA oil (1·3- and 1·6-fold compared with the former and latter, respectively). In addition, the activity of 3-hydroxyacyl-CoA dehydrogenase was 1·6- to 1·7-fold higher in rats given oil rich in DGLA or ARA than in animals fed maize oil; however, oil rich in either DGLA or ARA compared with maize oil did not affect the activities of the other enzymes involved in fatty acid oxidation. Therefore, fungal oil rich in DGLA or ARA compared with maize oil may have moderate potency to increase the activity of some enzymes involved in hepatic fatty acid oxidation.

We confirmed the previous observations[17–21] that sesamin preparation composed of equal amounts of sesamin and episesamin strongly increased the activity levels of various enzymes involved in hepatic fatty acid oxidation in the present study. Moreover, the extent of the increase in many of these parameters apparently depended on dietary fat types. In spite of the fact that fat types were rather ineffective in altering the activities of the enzymes involved in hepatic fatty acid oxidation among the rats fed the sesamin-free diets, the activity levels were significantly higher in rats fed ARA oil than in those fed maize oil. The effects were most prominent in the peroxisomal palmitoyl-CoA oxidation rate and acyl-CoA oxidase activity. Actually, sesamin feeding caused 3·7- and 4·0-fold increases in rats fed maize oil, while it caused 7·4- and 7·9-fold increases in these parameters, respectively, in animals fed oil rich in ARA. Also, the activities of carnitine acyltransferase measured using octanoyl-CoA and palmitoyl-CoA substrates, enoyl-CoA hydratase, 3-hydroxyacyl-CoA dehydrogenase and 3-ketoacyl-CoA thiolase were significantly higher in rats fed oil rich in ARA than in those fed maize oil when the experimental diets contained sesamin. Although the extent of these changes was attenuated, oil rich in DGLA compared with maize oil also increased the enhancing effect of sesamin on the activity of the enzymes involved in hepatic fatty acid oxidation in many cases.

Fig. 4 and Table 3 show the mRNA levels of peroxisomal and mitochondrial enzymes involved in hepatic fatty acid oxidation, respectively. There are three forms of carnitine palmitoyltransferase 1 in rats, i.e. a, b and c. Carnitine palmitoyltransferase 1a predominates in the liver, while 1b and 1c are abundant in the muscle and brain, respectively. There are two isoforms

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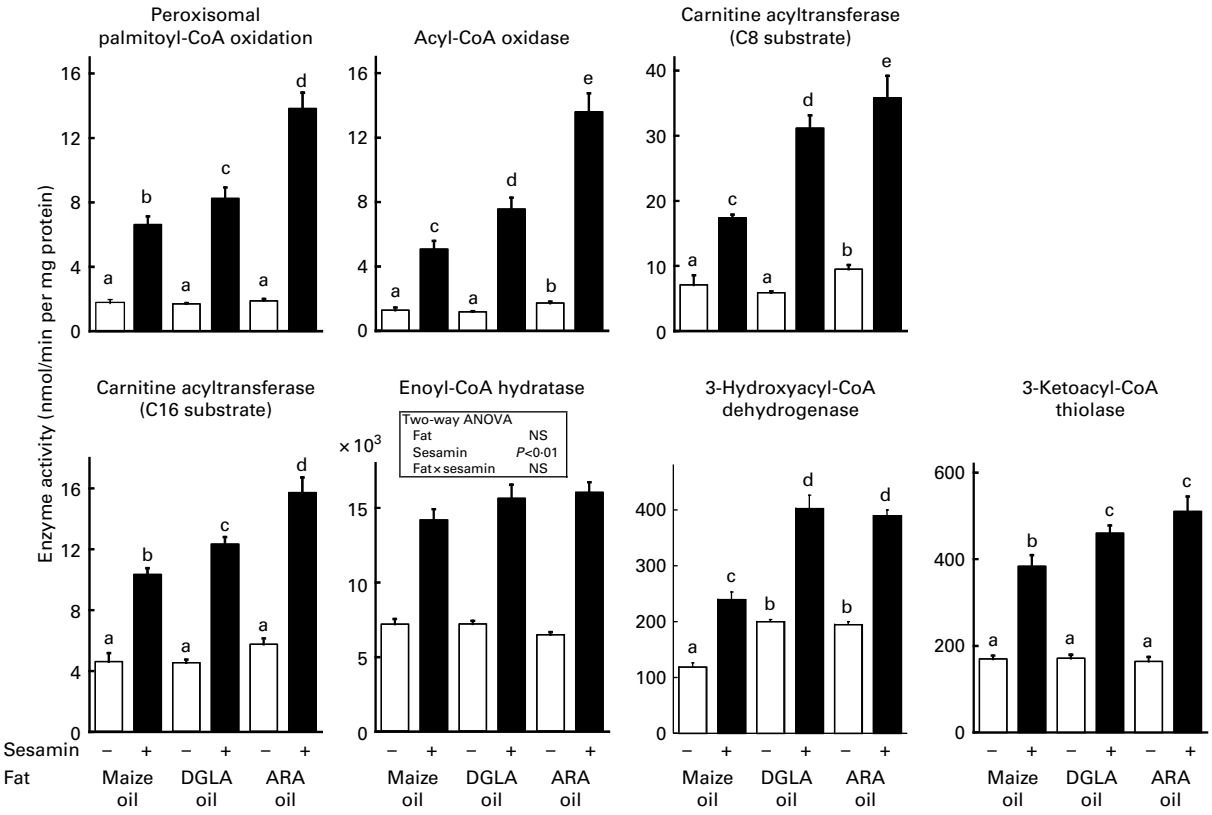

Fig. 3. Effect of dietary sesamin and fat types on the activity of enzymes involved in fatty acid oxidation in the rat liver. Values are means, with their standard errors represented by vertical bars (n 7). Two-way ANOVA revealed significant interactions between the two factors, fat and sesamin, for the activities of various enzymes except for enoyl-CoA hydratase; therefore, these values were reanalysed by one-way ANOVA and Tukey's *post hoc* test. [a,b,c,d,e] Mean values with unlike letters were significantly different ($P<0·05$). NS, $P\geq0·05$. DGLA, dihomo-γ-linolenic acid; ARA, arachidonic acid.

of acyl-CoA oxidase differing in substrate specificities in rat peroxisomes; acyl-CoA oxidase 1 oxidises long straight-chain fatty acids and eicosanoids, and acyl-CoA oxidase 2 is involved in the degradation of long-branched fatty acids and bile acid intermediates. Peroxisomal bifunctional enzyme exhibits enoyl-CoA hydratase and 3-hydroxyacyl-CoA dehydrogenase activities. Mitochondrial trifunctional protein composed of subunits α and β exhibits enoyl-CoA hydratase, 3-hydroxyacyl-CoA dehydrogenase and 3-ketoacyl-CoA thiolase activities. Table 3 also shows the value of microsomal cytochrome P450 4a1 (cyp4a1) involved in the ω-oxidation of fatty acids. Dietary sesamin strongly increased the mRNA levels of peroxisomal enzymes involved in hepatic fatty acid oxidation. With regard to the enzymes in the mitochondria, the sesamin-dependent increase in the mRNA expression of carnitine palmitoyltransferase 1a was moderate (less than 1·4-fold); however, stronger increases (more than 1·7-fold) were observed with the others. In addition, sesamin increased the mRNA expression of cyp4a1. Among the rats fed the sesamin-free diets, dietary fat types did not modify the mRNA levels of these proteins; however, sesamin-dependent increases in the mRNA expressions of various peroxisomal proteins were greater in rats fed ARA oil than maize oil. As a result, the mRNA levels of various peroxisomal enzymes became much higher in rats fed ARA oil and sesamin in combination than in those fed maize oil and sesamin in combination; however, dietary ARA did not enhance sesamin-dependent increases in the mRNA expressions of many mitochondrial fatty acid oxidation enzymes and microsomal cyp4a1 (Table 3). Although the effect was attenuated, DGLA oil also amplified sesamin-dependent increases in the mRNA expressions of peroxisomal carnitine octanoyltransferase, acyl-CoA oxidase 1 and bifunctional enzyme.

mRNA levels of Cd36 and LDL and VLDL receptors

Cd36/fatty acid translocase is located in the plasma membrane and involved in the transport of fatty acid, and plays a crucial role in regulating tissue lipid levels. We previously observed that sesamin greatly increased the mRNA expression of this protein[20]. LDL and VLDL receptors may also be involved in the regulation of tissue lipid levels. We therefore analysed the mRNA levels of these proteins in the present study (Fig. 5). Both fat and sesamin effects were observed by two-way ANOVA in the mRNA levels of Cd36. Accordingly, sesamin, irrespective of the dietary fat types, significantly increased this parameter. With regard to the fat effect, *post hoc* analysis indicated that the values were significantly higher in rats fed ARA oil than in those fed maize oil. Dietary fat types and sesamin were rather ineffective in altering the mRNA level of the LDL receptor despite that some significant differences in mean values were observed among the groups. Two-way ANOVA showed that sesamin significantly increased

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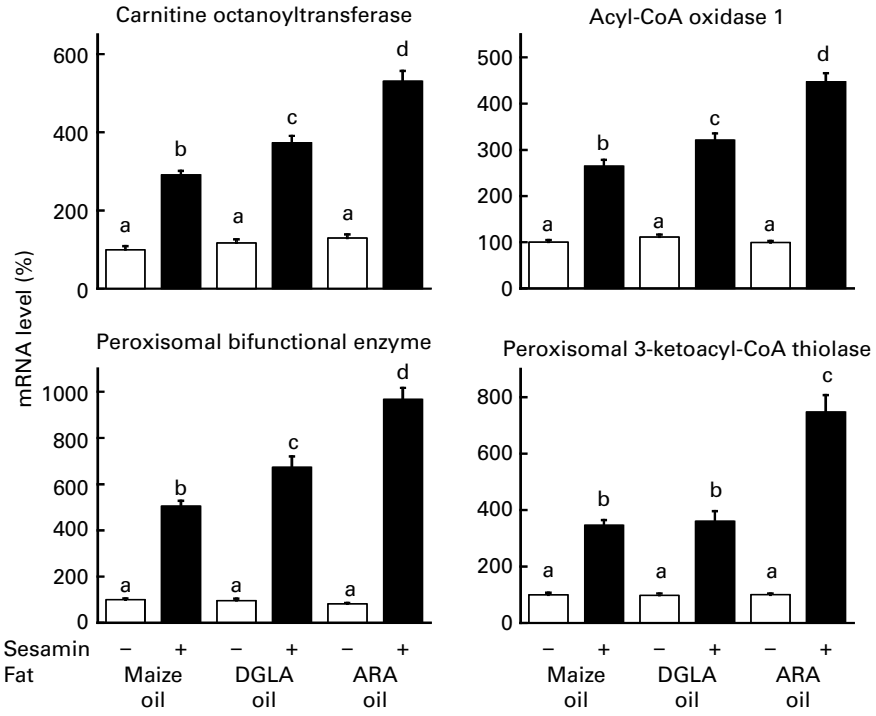

Fig. 4. Effect of dietary sesamin and fat types on the mRNA levels of peroxisomal enzymes involved in hepatic fatty acid oxidation. Values are means, with their standard errors represented by vertical bars (n 7). Two-way ANOVA revealed significant interactions between the two factors, fat and sesamin, for the mRNA levels of various peroxisomal enzymes; therefore, these values were reanalysed by one-way ANOVA and Tukey's *post hoc* test. [a,b,c,d]Mean values with unlike letters were significantly different ($P<0·05$). DGLA, dihomo-γ-linolenic acid; ARA, arachidonic acid.

the mRNA level of the VLDL receptor. Types of dietary fat also affected this parameter. The *post hoc* test revealed that the values were significantly higher in rats fed ARA oil than in those fed maize oil and DGLA oil.

Serum and liver concentrations of lipids and lignans

Oils rich in DGLA and ARA compared with maize oil significantly decreased the serum concentrations of TAG, cholesterol, phospholipid and NEFA (Table 4). The decreasing effects were stronger with ARA oil than with DGLA oil. Also, sesamin significantly decreased the serum concentrations of TAG, cholesterol and NEFA. Sesamin also decreased the serum phospholipid concentration in rats fed maize oil; however, sesamin-dependent changes in this parameter were not confirmed in rats given oils rich in DGLA and ARA.

Both oils rich in DGLA and ARA compared with maize oil significantly decreased the hepatic concentrations of TAG; DGLA and ARA oils were equally effective in decreasing this parameter. In contrast, sesamin significantly increased the hepatic TAG levels. Among the rats fed the sesamin-free diets, the hepatic concentration of cholesterol was significantly lower in rats fed DGLA oil than in the other groups. Sesamin significantly decreased this parameter in rats fed maize and ARA oils but not in animals fed DGLA oil. Sesamin significantly increased the hepatic concentration of phospholipid irrespective of the dietary fat source.

Lignans were detected in both the serum and liver among the rats fed the sesamin-containing diets but not in animals fed the sesamin-free diets. Although the sesamin preparation used in the present study contained both sesamin and episesamin in equal amounts, episesamin predominated in both the serum and liver. Total lignan and episesamin but not sesamin concentrations were significantly higher in rats fed maize oil than in the other groups.

Fatty acid concentrations in TAG and phospholipid in the liver

Fatty acid compositions of hepatic TAG and phospholipid were analysed by GLC, and the amounts of various fatty acids in each lipid molecule were calculated (Tables 5 and 6, respectively). Among the rats fed the sesamin-free diets, both dietary DGLA and ARA oils compared with maize oil decreased the hepatic concentrations of SFA (myristic, palmitic and stearic acids) and MUFA (palmitoleic and oleic acids) in TAG. The decreasing effects of these parameters aside from the value in myristic acid were stronger with ARA oil than with DGLA oil. Sesamin increased the hepatic concentrations of palmitic, stearic and oleic acids in TAG in rats fed the various oils. Dietary DGLA oil compared with maize oil increased not only the concentrations of DGLA, but also those of ARA and n-6 docosatetraenoic acid in TAG in both rats fed the sesamin-free and sesamin-containing diets. Also, ARA oil increased the values of ARA and n-6 docosatetraenoic acid in TAG.

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Table 3. Effect of dietary sesamin and fat types on the mRNA levels of mitochondrial fatty acid oxidation enzymes and microsomal cytochrome P450 4a1 (cyp4a1) in the liver*
(Mean values with their standard errors, *n* 7)

Dietary sesamin (g/kg) …	Dietary fats (100 g/kg)										Two-way ANOVA (*P* value)			*Post hoc* analysis for fat effect (*P* value)		
	Maize oil				DGLA oil				ARA oil							
	0		2		0		2		0		2					
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Fat	Sesamin	Fat × sesamin	Maize oil *v.* DGLA oil	Maize oil *v.* ARA oil	DGLA oil *v.* ARA oil
Mitochondrial enzymes																		
Carnitine palmitoyltransferase 1a	100	9	115	7	90·2	5·5	114	4	81·3	5·1	110	4	NS†	< 0·01	NS†	–	–	–
Carnitine/acylcarnitine translocase	100	5	176	12	110	6	201	13	108	4	193	12	NS†	< 0·01	NS†	–	–	–
Carnitine palmitoyltransferase 2	100	6	196	3	97·3	4	194	8	124	4	221	14	< 0·01	< 0·01	NS†	NS†	< 0·01	< 0·01
Trifunctional protein subunit α	100	5	201	10	100	7	234	7	122	2	223	17	NS†	< 0·01	NS†	–	–	–
Trifunctional protein subunit β	100	8	207	10	106	6	245	6	107	6	220	12	< 0·05	< 0·01	NS†	< 0·05	NS†	NS†
3-Ketoacyl-CoA thiolase	100	4	185	9	90·6	7·3	198	11	110	3	218	13	< 0·05	< 0·01	NS†	NS†	< 0·05	NS†
Microsomal enzyme																		
cyp4a1	100	10	563	45	106	10	630	35	103	3	581	35	NS†	< 0·01	NS†	–	–	–

DGLA, dihomo-γ-linolenic acid; ARA, arachidonic acid.
* Values are expressed as percentages, assigning a value of 100 for rats fed a diet containing maize oil and devoid of sesamin.
† $P \geq 0{\cdot}05$.

The increasing effect of DGLA and ARA oils on *n*-6 docosatetraenoic acid was attenuated in rats fed sesamin.

In the phospholipid fraction, dietary DGLA oil compared with maize oil significantly increased the concentration of DGLA, ARA and *n*-6 docosatetraenoic acid in rats fed the sesamin-free or sesamin-containing diets. DGLA oil also increased the hepatic concentration of *n*-6 docosapentaenoic acid in phospholipid in rats fed the sesamin-free diets, but the change observed in rats fed the sesamin-containing diets was not significant. Dietary ARA oil compared with maize oil increased the concentrations of ARA and *n*-6 docosatetraenoic acid in the phospholipid fraction.

Discussion

Effects of dihomo-γ-linolenic acid, arachidonic acid and sesamin on hepatic fatty acid synthesis

It has been well demonstrated that various PUFA exert profound effects on hepatic fatty acid synthesis and oxidation in experimental animals[1–10]; however, the physiological activities of DGLA and ARA, members of *n*-6 PUFA, affecting hepatic fatty acid metabolism in experimental animals have not yet been well clarified.

Some studies using a monolayer culture of rat hepatocytes have indicated that ARA is effective in reducing lipogenesis. Stabile *et al.*[30] showed that insulin and glucose caused a several-fold increase in the activity and mRNA levels of glucose-6-phosphate dehydrogenase in rat hepatocytes. LA and ARA added to the culture medium attenuated the increase. ARA was more effective than LA in inhibiting the insulin–glucose action. Armstrong *et al.*[31] also reported that ARA dose-dependently suppressed the insulin-dependent increase in the mRNA expression of fatty acid synthase, but gondoic acid (20 : 1*n*-9) actually stimulated the mRNA expression in rat hepatocytes. These studies indicated that ARA is stronger than LA and monoenoic acid in reducing lipogenesis in rat hepatocytes; however, studies examining the physiological activity of ARA to affect hepatic lipogenesis in experimental animals have been lacking, except for a report by Berger *et al.*[14] who examined the effect of fungal oil rich in ARA on hepatic gene expression using DNA microarray in mice. The oil was added to the experimental diet at 11 g/kg (this diet provided 5 g/kg ARA). Microarray analysis showed that the oil caused significant decreases in the hepatic mRNA levels of ATP citrate lyase and fatty acid synthase; however, confirmation of this finding by measuring the mRNA levels using real-time PCR or by the analysis of the activity or protein levels of lipogenic enzymes was not performed in their study. No study is available that examined the physiological activity of DGLA in affecting hepatic lipogenesis.

The present study unequivocally demonstrated that oils rich in DGLA and ARA, especially the latter, compared with maize oil rich in linoleic acid profoundly decreased the activity and mRNA levels of enzymes involved in lipogenesis in the rat liver. The present study therefore supports the ideas from studies using rat hepatocytes[30,31] that ARA has greater activity than LA in reducing hepatic lipogenesis. In addition, for the first time, we provided

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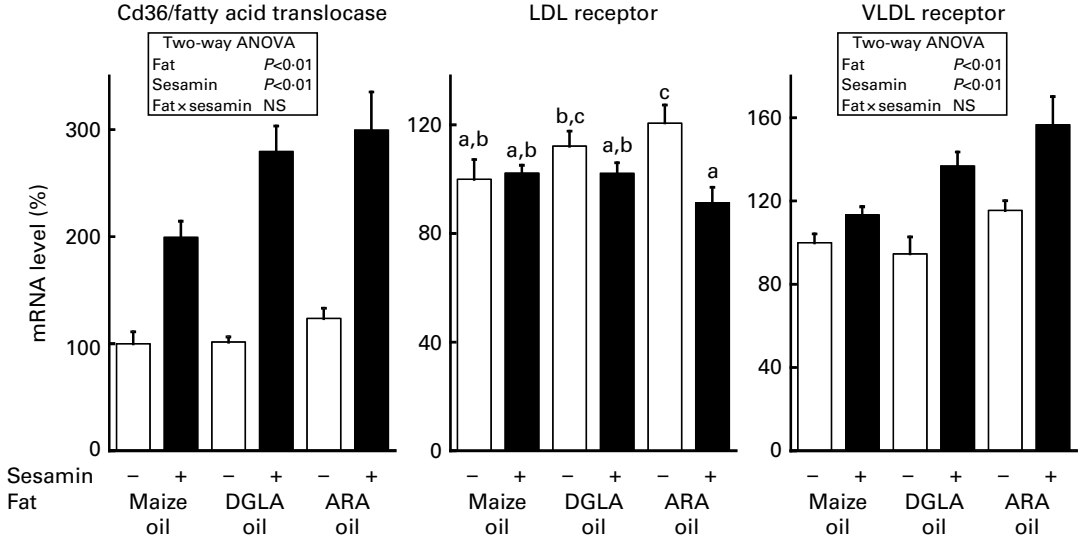

Fig. 5. Effect of dietary sesamin and fat types on the mRNA levels of Cd36, and LDL and VLDL receptors. Values are means, with their standard errors represented by vertical bars (n 7). Two-way ANOVA revealed significant interactions between the two factors, fat and sesamin, for the mRNA levels of the LDL receptor; therefore, the values for the LDL receptor were reanalysed by one-way ANOVA and Tukey's *post hoc* test. [a,b,c] Mean values with unlike letters were significantly different ($P<0·05$). NS, $P\geq0·05$. DGLA, dihomo-γ-linolenic acid; ARA, arachidonic acid.

evidence that DGLA also effectively reduces the activity and mRNA levels of hepatic lipogenic enzyme more than LA. Using the dietary level (46 g/kg ARA), which is higher than the level employed by Berger *et al.*[14] for a DNA microarray study, we observed in the present study that ARA reduced the mRNA expressions of many genes involved in lipogenesis in addition to fatty acid synthase and ATP citrate lyase. In a separate experiment, we tested the physiological activity of diets containing lower dietary levels of ARA affecting hepatic lipogenesis (TIde *et al.*, unpublished results). The results indicated that 9 g/kg appears to be the lower limit of the dietary level of ARA to observe apparent effects on hepatic lipogenesis under our experimental conditions.

In addition to oil rich in ARA, oil rich in DGLA decreased various parameters for lipogenesis. As DGLA is converted to ARA by Δ5 desaturase, it is possible that the metabolite (ARA) rather than DGLA *perse* reduced hepatic fatty acid synthesis. In fact, oil rich in DGLA compared with maize oil increased not only DGLA but also ARA concentrations in liver TAG and phospholipid.

We previously demonstrated that various sesame lignans, including the sesamin preparation containing equivalent amounts of sesamin and episesamin employed in the present study, decreased the activity and mRNA levels of hepatic lipogenic enzymes[17–21]. This was confirmed in the present study irrespective of the dietary fat sources.

It has been demonstrated that SREBP-1c is a transcription factor responsible for the regulation of the gene expression of hepatic lipogenic enzymes[7,29]; however, no clear-cut relationship between the indices for lipogenesis and SREBP-1c mRNA levels was observed in the present study. The amounts of the mature active form of SREBP-1c are regulated not only by its gene expression levels but also by changes in the proteolytic process of the immature form of the transcription factor to convert it to the mature form[7,29]. In addition, it has been reported that the activity of SREBP to stimulate the gene expression of its targets is modulated by covalent modifications of the transcription factor[32]; therefore, it is still possible that DGLA, ARA and sesamin modulated these processes and hence decreased the activity and mRNA levels of hepatic lipogenic enzymes.

Stearoyl-CoA desaturase 1 gene is a target to be activated by SREBP-1c, and it has been demonstrated that the gene expression of this enzyme is coordinately regulated with those of other enzymes involved in lipogenesis[28]. Consistent with these observations, ARA and DGLA compared with maize oil not only decreased the gene expressions of various lipogenic enzymes but also that of stearoyl-CoA desaturase 1 when diets were free of sesamin. Interestingly, however, the lignan rather up-regulated the expression of this gene in rats fed DGLA and ARA oils and did not modify the value in animals given maize oil. In relation to this, hepatic concentrations of oleic acid in TAG were characteristically modified both by dietary fat types and sesamin, although the changes did not necessarily parallel those of stearoyl-CoA desaturase 1 mRNA levels. The reason why sesamin caused the response of stearoyl-CoA desaturase 1 mRNA level to be different from those of other genes related to lipogenesis is not clear. It has at least been indicated that not only SREBP-1 but also various other transcription factors are involved in the regulation of the expression of stearoyl-CoA desaturase 1 gene[28].

Effects of dihomo-γ-linolenic acid, arachidonic acid and sesamin on hepatic fatty acid oxidation

Information on the physiological activity of DGLA and ARA affecting hepatic fatty acid oxidation has hitherto been lacking. The present study showed that these n-6 PUFA, unlike

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Table 4. Effect of dietary sesamin and fat types on the serum and liver concentrations of lipids and lignans
(Mean values with their standard errors, *n* 7)

Dietary sesamin (g/kg) …	Dietary fats (100 g/kg)												Two-way ANOVA (*P* value)			*Post hoc* analysis for fat effect (*P* value)		
	Maize oil				DGLA oil				ARA oil									
	0		2		0		2		0		2							
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Fat	Sesamin	Fat × sesamin	Maize oil *v.* DGLA oil	Maize oil *v.* ARA oil	DGLA oil *v.* ARA oil
Serum lipids (mmol/l)																		
TAG	1·80	0·12	1·37	0·12	0·936	0·079	0·854	0·067	0·790	0·043	0·533	0·027	<0·01	<0·01	NS*	<0·01	<0·01	<0·05
Cholesterol	2·49	0·09	1·97	0·09	1·87	0·13	1·79	0·08	1·46	0·08	1·12	0·05	<0·01	<0·01	NS*	<0·01	<0·01	<0·01
Phospholipid	2·83[d]	0·08	2·40[c]	0·12	1·82[b]	0·07	2·05[b]	0·10	1·38[a]	0·05	1·28[a]	0·04	<0·01	NS*	<0·01	–	–	–
NEFA	0·843	0·051	0·781	0·08	0·787	0·080	0·537	0·042	0·476	0·024	0·363	0·021	<0·01	<0·01	NS*	<0·05	<0·01	<0·01
Serum lignans (μmol/l)																		
Sesamin	–		0·076	0·012	–		0·052	0·011	–		0·103	0·028	–	–	–	–	–	–
Episesamin	–		0·973[b]	0·107	–		0·695[a]	0·056	–		0·497[a]	0·048	–	–	–	–	–	–
Total	–		1·05[b]	0·120	–		0·747[a]	0·058	–		0·599[a]	0·058	–	–	–	–	–	–
Liver lipids (μmol/l)																		
TAG	33·0	3·4	38·9	3·5	12·2	0·7	26·7	3·6	10·5	1·4	15·8	2·1	<0·01	<0·01	NS*	<0·01	<0·01	NS*
Cholesterol	5·26[c]	0·15	4·30[b]	0·10	4·04[a,b]	0·05	3·76[a]	0·10	5·05[c]	0·09	3·79[a]	0·08	<0·01	<0·01	<0·01	–	–	–
Phospholipid	40·7	1·5	50·2	1·1	42·2	0·9	52·7	0·9	41·1	0·4	53·5	0·8	NS*	<0·01	NS*	–	–	–
Liver lignans (nmol/g)																		
Sesamin	–		4·01	0·77	–		2·67	0·52	–		2·46	0·41	–	–	–	–	–	–
Episesamin	–		12·5[b]	1·2	–		9·33[a]	1·04	–		7·21[a]	0·67	–	–	–	–	–	–
Total	–		16·5[b]	1·8	–		12·0[a]	1·5	–		9·67[a]	1·08	–	–	–	–	–	–

DGLA, dihomo-γ-linolenic acid; ARA, arachidonic acid.
[a,b,c,d] Mean values with unlike superscript letters within a row were significantly different ($P<0·05$).
* $P\geq0·05$.

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Table 5. Effect of dietary sesamin and fat types on the hepatic concentration of fatty acids in TAG
(Mean values with their standard errors, *n* 7)

	Dietary fats (100 g/kg)																	
	Maize oil				DGLA oil				ARA oil				Two-way ANOVA (*P* value)				*Post hoc* analysis for fat effect (*P* value)		
Dietary sesamin (g/kg) . . .	0		2		0		2		0		2								
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Fat	Sesamin	Fat × sesamin	Maize oil *v.* DGLA oil	Maize oil *v.* ARA oil	DGLA oil *v.* ARA oil
Fatty acids (μmol/g liver)																		
14 : 0	1·3	0·2	0·9	0·1	0·5	0·1	0·5	0·1	0·4	0·1	0·4	0·1	<0·01	NS*	NS*	<0·01	<0·01	NS*
16 : 0	31·0	4·1	41·9	3·0	13·3	0·9	28·7	4·2	8·5	1·9	15·5	2·1	<0·01	<0·01	NS*	<0·01	<0·01	<0·05
18 : 0	1·8	0·2	2·6	0·3	1·3	0·0	2·0	0·2	1·1	0·2	1·2	0·1	<0·01	<0·01	NS*	<0·01	<0·01	<0·05
16 : 1*n*-7	5·8[d]	1·3	3·9[d]	0·4	2·0[b,c]	0·2	3·5[c,d]	0·8	1·0[a]	0·4	1·4[b]	0·2	<0·01	NS*	<0·05	–	–	–
18 : 1*n*-9	31·9	3·7	53·9	3·1	8·5	0·7	33·3	5·8	5·6	1·1	14·8	2·6	<0·01	<0·01	NS*	<0·01	<0·01	<0·01
18 : 2*n*-6	14·5[c]	0·8	22·9[d]	2·0	1·7[a]	0·1	3·6[b]	0·2	3·7[b]	0·5	4·6[b]	0·6	<0·01	<0·01	<0·01	–	–	–
18 : 3*n*-6	0·1	0·0	0·4	0·1	0·2	0·0	0·3	0·1	0·2	0·0	0·4	0·1	NS*	<0·01	NS*	–	–	–
20 : 3*n*-6	0·1	0·0	0·3	0·1	2·9	0·2	3·6	0·6	0·5	0·1	0·6	0·1	<0·01	NS*	NS*	<0·01	NS*	<0·01
20 : 4*n*-6	0·4	0·0	1·1	0·2	3·9	0·1	3·7	0·7	7·2	0·5	7·0	0·9	<0·01	NS*	NS*	<0·01	<0·01	<0·01
22 : 4*n*-6	0·1[a]	0·0	0·3[a]	0·1	1·9[c]	0·1	1·0[b]	0·2	3·5[d]	0·3	1·5[c]	0·2	<0·01	<0·01	<0·01	–	–	–
22 : 5*n*-6	0·0[a]	0·0	0·1[b,c]	0·0	0·2[d]	0·0	0·1[a,b]	0·0	0·2[d]	0·0	0·2[c,d]	0·0	<0·01	NS*	<0·01	–	–	–

DGLA, dihomo-γ-linolenic acid; ARA, arachidonic acid.
[a,b,c,d] Mean values with unlike superscript letters within a row were significantly different ($P<0·05$).
* $P \geq 0·05$.

n-3 PUFA such as ALA, EPA and DHA[1–6], are rather irrelevant in affecting hepatic fatty acid oxidation.

Aside from the physiological activity of *n*-3 PUFA in increasing hepatic fatty acid oxidation, we previously demonstrated that the combination of sesamin and fish oil rich in EPA and DHA increased the activity of many enzymes involved in hepatic fatty acid oxidation in a synergistic manner[18]. The combination of sesamin, and purified EPA and DHA in the form of ethyl esters caused similar responses to the activities of hepatic fatty acid oxidation enzymes[19]. Analysis of the mRNA levels of various hepatic enzymes involved in fatty acid oxidation strongly indicated that the up-regulation of the gene expression of peroxisomal enzymes is responsible for this effect[18,19]; therefore, it was suggested that EPA and DHA not only increase hepatic fatty acid oxidation but also act as co-activators of sesamin to enhance peroxisomal fatty acid oxidation. The present study showed that the activity of many hepatic fatty acid oxidation enzymes was much higher in rats fed sesamin and ARA oil in combination than in animals fed sesamin and maize oil in combination; therefore, it is apparent that ARA has physiological activity to increase the potency of sesamin to stimulate hepatic fatty acid oxidation. Moreover, the marked increases by the combination of sesamin and ARA in mRNA expression were confirmed in enzymes involved in hepatic fatty acid oxidation located in peroxisomes but not in the mitochondria; therefore, in spite of the fact that ARA is dissimilar to EPA and DHA in how it affects hepatic fatty acid oxidation, this *n*-6 PUFA is quite similar to *n*-3 PUFA in how it enhances the sesamin-dependent increase in the activity and mRNA expression of enzymes involved in hepatic fatty acid oxidation. Although the responses were attenuated, DGLA also appears to have activity to amplify sesamin-dependent increases in hepatic fatty acid oxidation. It is again possible that the metabolite (ARA) rather than DGLA itself is responsible for this effect.

The mechanism by which ARA as well as EPA and DHA enhances the potency of sesamin to increase hepatic fatty acid oxidation is not clear at present. PPARα plays a central role in regulating hepatic fatty acid oxidation. Studies have indicated that activation of PPARα by peroxisome proliferators results in an increase in the gene expression of many peroxisomal and mitochondrial fatty acid oxidation enzymes[33,34]. In spite of these observations, ARA, EPA and DHA potentate sesamin-dependent increases in the mRNA levels of peroxisomal fatty acid oxidation enzymes, but not mitochondrial enzymes; therefore, the PPARα-dependent mechanism alone cannot account for the marked increase in hepatic fatty acid oxidation caused by the combination of sesamin and these PUFA.

Effects of dihomo-γ-linolenic acid, arachidonic acid and sesamin on serum and hepatic lipid levels

Alterations in hepatic fatty acid synthesis[35] and oxidation[36] modify the availability of fatty acids for the synthesis of TAG, and in turn alter VLDL production by the liver; therefore, a change in the rate of these metabolic processes is crucial in determining serum lipid concentrations. In the present study, alterations by dietary fats and sesamin of hepatic fatty acid synthesis and oxidation were accompanied by parallel changes in

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Table 6. Effect of dietary sesamin and fat types on the hepatic concentration of fatty acids in phospholipids
(Mean values with their standard errors, *n* 7)

	Dietary fats (100 g/kg)																	
	Maize oil				DGLA oil				ARA oil				Two-way ANOVA (*P* value)			*Post hoc* analysis for fat effect (*P* value)		
Dietary sesamin (g/kg) …	0		2		0		2		0		2							
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Fat	Sesamin	Fat× sesamin	Maize oil *v.* DGLA oil	Maize oil *v.* ARA oil	DGLA oil *v.* ARA oil
Fatty acids (μmol/g liver)																		
14 : 0	0·2[c]	0·0	0·2[b]	0·0	0·2[c]	0·0	0·1[a]	0·0	0·2[c]	0·0	0·1[b]	0·0	<0·05	<0·01	<0·01	–	–	–
16 : 0	15·8	0·6	21·6	0·7	17·8	0·5	22·4	0·8	18·2	0·5	25·0	0·5	<0·01	<0·01	NS*	NS*	<0·01	<0·05
18 : 0	18·0	0·7	24·5	0·5	20·9	0·7	25·6	0·8	18·3	0·3	24·8	0·6	<0·01	<0·01	NS*	<0·01	NS*	<0·05
16 : 1*n*-7	0·7[b]	0·1	0·5[a]	0·0	0·8[b]	0·0	0·7[b]	0·1	0·5[a]	0·1	0·8[b]	0·1	<0·05	NS*	<0·01	–	–	–
18 : 1*n*-9	5·6[c]	0·2	5·8[c]	0·2	4·9[b]	0·2	6·4[d]	0·2	4·2[a]	0·1	6·0[c,d]	0·2	<0·01	<0·01	<0·01	–	–	–
18 : 2*n*-6	10·0	0·5	10·4	0·6	1·4	0·1	2·3	0·1	2·1	0·1	4·2	0·2	<0·01	<0·01	NS*	<0·01	<0·01	<0·01
18 : 3*n*-6	0·1	0·0	0·1	0·0	0·1	0·0	0·2	0·0	0·2	0·0	0·1	0·0	NS*	NS*	NS*	–	–	–
20 : 3*n*-6	0·6[a]	0·1	1·3[b]	0·1	6·0[c]	0·3	7·6[d]	0·3	0·7[a]	0·0	0·9[a,b]	0·1	<0·01	<0·01	NS*	–	–	–
20 : 4*n*-6	23·7	1·2	29·6	1·0	25·6	0·7	34·3	0·7	29·9	0·3	38·6	0·7	<0·01	<0·01	NS*	<0·01	<0·01	<0·01
22 : 4*n*-6	0·5[a]	0·0	0·5[a]	0·0	2·5[c]	0·1	1·8[b]	0·2	4·4[d]	0·2	2·5[c]	0·1	<0·01	<0·01	<0·01	–	–	–
22 : 5*n*-6	1·7[b]	0·2	1·0[a]	0·2	2·6[c]	0·2	1·2[a]	0·1	1·7[b]	0·1	1·0[a]	0·0	<0·01	<0·01	<0·05	–	–	–
22 : 5*n*-3	0·2	0·0	0·4	0·0	0·3	0·0	0·5	0·0	0·4	0·0	0·6	0·0	<0·01	<0·01	NS*	<0·05	<0·01	<0·01
22 : 6*n*-3	4·2[c]	0·1	4·4[c]	0·2	1·3[a]	0·1	2·5[b]	0·2	1·4[a]	0·1	2·5[b]	0·1	<0·01	<0·01	<0·01	–	–	–

DGLA, dihomo-γ-linolenic acid; ARA, arachidonic acid.
[a,b,c,d]Mean values with unlike superscript letters within a row were significantly different ($P<0·05$).
* $P\geq 0·05$.

serum lipid profiles. Noticeably, the large increases in the activity and mRNA levels of hepatic fatty acid oxidation enzymes together with the decreases in these parameters for lipogenesis caused by a diet containing sesamin and ARA oil in combination accompanied great decreases in serum lipid levels. However, in spite of the fact that sesamin increased hepatic fatty acid oxidation and decreased fatty acid synthesis in rats fed various types of fats, lignan rather increased hepatic TAG concentration. In relation to this, we previously found that sesamin increased the mRNA expression of Cd36[20]. This was confirmed in the present study irrespective of the dietary fat sources. It has been considered that Cd36 is crucial to facilitate fatty acid translocation in adipocytes, the heart and skeletal muscle[37]. Although Cd36 levels in the liver are normally low in rodents, recent studies in mice[23,38,39] have indicated that Cd36 plays a crucial role in the transport of fatty acids in hepatocytes and hence regulates hepatic TAG concentration. It is possible that the sesamin-dependent increase in Cd36 expression facilitated fatty acid uptake from the circulation and hence increased the availability of intracellular fatty acids for the hepatic synthesis of TAG despite the enhancement of fatty acid oxidation and reduction of lipogenesis. In this case, it is possible that adipose tissue supplied the bulk of circulating fatty acids to be taken up by the liver through enhanced lipolysis in this tissue. Consistent with this notion, sesamin significantly decreased at least the weight of perirenal adipose tissue. Although we previously observed[20] that sesamin was rather ineffective in modulating the expression of various genes involved in lipid metabolism in epididymal adipose tissue, we did not examine the effect of sesamin on lipolytic activity and the expression of genes regulating lipolysis in adipose tissue in previous studies. These need to be clarified to test this hypothesis. However, the sesamin-dependent decrease in NEFA concentration in the serum may not support the notion that this lignan increases lipolysis in adipose tissue. The LDL receptor is abundantly expressed in the liver and may play a key role in regulating lipid levels in the liver and serum[40]; however, sesamin and dietary fat types failed to have a clear-cut effect on the mRNA expression levels of this protein. Instead, we observed that sesamin increased the mRNA expression of the VLDL receptor. It has been reported that VLDL receptor mRNA levels are high in the heart, muscle, adipose tissue and brain, but are relatively low in the liver[41]; however, several studies[42,43] have indicated that alterations in the mRNA expression of the VLDL receptor accompanied parallel changes in the liver TAG level; therefore, it is possible that the VLDL receptor may also be involved in sesamin-dependent changes in lipid levels in the liver. ARA oil compared with maize oil significantly increased the mRNA levels of Cd36 and the VLDL receptor; therefore, these proteins may also play a role in dietary fat-dependent changes in serum and liver lipid levels.

Despite that DGLA and ARA oils compared with maize oil strongly lowered the hepatic concentration of TAG, sesamin increased this parameter, particularly in rats given these fungal oils, and the increase was rather

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moderate in rats fed maize oil. However, the values in rats given diets containing sesamin and DGLA or ARA oil in combination were still lower than those observed in rats fed sesamin-free and -supplemented diets containing maize oil as a fat source. In a previous study[18], we observed that hepatic TAG concentration was much lower in rats fed fish oil rich in EPA and DHA (14·0 μmol/g) than in animals fed safflower oil rich in LA (45·4 μmol/g) and palm oil rich in palmitic and oleic acids (81·5 μmol/g). The combination with sesamin in the diet strongly lowered this parameter in rats given palm oil (26·2 μmol/g). A moderate decrease was observed with safflower oil (34·8 μmol/g), but the lignan actually increased this parameter (33·1 μmol/g) in rats given fish oil. Although more detailed examinations are still required, these observations indicated that sesamin increases the hepatic concentration of TAG only under nutritional conditions where the level in the liver of this lipid molecule is low, and the increased level may not exceed the 'normal value'. However, there is still a concern that the sesamin-dependent increase in TAG is accompanied by progressive inflammation of the liver (hepatitis); therefore, histological examination of liver tissue is required to clarify this point in a future study.

In conclusion, we found that fungal oils rich in DGLA and ARA compared with maize oil rich in LA, especially the latter, markedly decreased hepatic activity and mRNA levels of hepatic lipogenic enzymes. Oils rich in DGLA and ARA were rather ineffective in affecting hepatic fatty acid oxidation; however, a diet containing a sesamin preparation containing both sesamin and episesamin, and oil rich in ARA in combination markedly increased hepatic fatty acid oxidation much more than a diet containing sesamin and maize oil in combination. Examinations of the activity and mRNA levels of various hepatic fatty acid oxidation enzymes indicated that up-regulation of the gene expression of peroxisomal enzymes is principally involved in this phenomenon. Although the response was much attenuated, a diet simultaneously containing sesamin and DGLA affected hepatic fatty acid oxidation in a similar way.

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