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ORIGINAL CONTRIBUTION

Combined effect of sesamin and α-lipoic acid on hepatic fatty acid metabolism in rats

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Abstract
Purpose Dietary sesamin (1:1 mixture of sesamin and episesamin) decreases fatty acid synthesis but increases fatty acid oxidation in rat liver. Dietary α-lipoic acid lowers hepatic fatty acid synthesis. These changes can account for the serum lipid-lowering effect of sesamin and α-lipoic acid. It is expected that the combination of these compounds in the diet potentially ameliorates lipid metabolism more than the individual compounds. We therefore studied the combined effect of sesamin and α-lipoic acid on lipid metabolism in rats.
Methods Male Sprague–Dawley rats were fed diets supplemented with 0 or 2 g/kg sesamin and containing 0 or 2.5 g/kg α-lipoic acid for 22 days.
Results and conclusions Sesamin and α-lipoic acid decreased serum lipid concentrations and the combination of these compounds further decreased the parameters in an additive fashion. These compounds reduced the hepatic concentration of triacylglycerol, the lignan being less effective in decreasing this value. The combination failed to cause a stronger decrease in hepatic triacylglycerol concentration. The combination of sesamin and α-lipoic acid decreased the activity and mRNA levels of hepatic lipogenic enzymes in an additive fashion. Sesamin strongly increased the parameters of hepatic fatty acid oxidation enzymes. α-Lipoic acid antagonized the stimulating effect of sesamin of fatty acid oxidation through reductions in the activity of some fatty acid oxidation enzymes and carnitine concentration in the liver. This may account for the failure to observe strong reductions in hepatic triacylglycerol concentration in rats given a diet containing both sesamin and α-lipoic acid.

Keywords Sesamin · α-Lipoic acid · Hepatic lipogenesis · Hepatic fatty acid oxidation

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Introduction

Sesamin is one of the most abundant lignans in sesame seed and is epimerized during acid-clay bleaching in the oil-refining process to form episesamin [1]; therefore, sesamin preparations obtained as a by-product of the oil-refining process contain sesamin and episesamin at about an equivalent ratio. It has been demonstrated that this sesamin preparation exerts a serum lipid-lowering effect in experimental animals [2, 3] and humans [4]. We previously found that the sesamin preparation markedly and dose dependently increased the activity and gene expression of fatty acid oxidation enzymes in rat liver [2]. Also, the sesamin preparation decreased hepatic activity and mRNA levels of enzymes involved in fatty acid synthesis [3]. A later study showed that episesamin is more effective than sesamin in increasing the activity and gene expression of fatty acid oxidation enzymes [5].

α-Lipoic acid is widely distributed in plants and animals as a cofactor within mitochondrial enzymes [6]. Aside from its role in the mitochondrial metabolic pathway, α-lipoic acid, when supplemented in diets, induces various physiological activities in experimental animals. For instance, the hypolipidemic effect of α-lipoic acid has been reported by many investigators [6–9]. In relation to this, we previously demonstrated that α-lipoic acid markedly and dose

dependently decreased the activity and mRNA levels of lipogenic enzymes in rat liver [9].

It is apparent that sesamin and α-lipoic acid are dietary factors that greatly affect hepatic fatty acid metabolism and hence lower the serum lipid level. Moreover, both sesamin [10] and α-lipoic acid [11] act as potent antioxidants in vivo and hence attenuate oxidative stress. Therefore, it is expected that the combination of these compounds in the diet would potentially ameliorate disorders of lipid metabolism and reduce oxidative stress and hence would be effective in reducing the incidence of diseases such as atherosclerosis, diabetes, and cancer. In these contexts, here we studied the combined effect of sesamin and α-lipoic acid in affecting lipid metabolism in rats.

Materials and methods

Animals and diets

Male Sprague–Dawley rats obtained from Charles River Japan (Kanagawa, Japan) at 4 weeks of age were housed individually in animal cages in a room with controlled temperature (20–22 °C) and lighting (lights on from 07:00 to 19:00 h) and fed a commercial diet (Type NMF; Oriental Yeast Co., Tokyo, Japan). 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 0 or 2.5 g/kg of DL-α-lipoic acid (Tokyo Chemical Industrial Co., Tokyo, Japan) for 22 days. The basal composition of the purified experimental diets was (in g/kg): casein, 200; coconut oil, 90; corn oil, 10; corn starch, 150; cellulose, 20; mineral mixture [12], 35; vitamin mixture [12], 10; L-cystine, 3; choline bitartrate, 2.5; and sucrose to 1 kg. Sesamin and α-lipoic acid were added to experimental diets in lieu of sucrose. Animals had free access to the diets and water during the experimental period. This study was approved by the review board of animal ethics of our university, and we followed the university's guidelines in the care and use of laboratory animals.

Enzyme assays

The activity of lipogenic enzymes was measured spectrophotometrically using 20,000×g supernatant of the liver homogenate as an enzyme source [3, 9]. Activity levels of various hepatic fatty acid oxidation enzymes were measured spectrophotometrically using the whole liver homogenate as an enzyme source [2, 5]. The peroxisomal palmitoyl-CoA oxidation rate and acyl-CoA oxidase activities were measured using palmitoyl-CoA as a substrate. Carnitine acyltransferase activities were measured using acetyl-CoA, octanoy-CoA, and palmitoyl-CoA as substrates. We used crotonyl-CoA in assaying enoyl-CoA hydratase. 3-Hydroxyacyl-CoA dehydrogenase and 3-keotacyl-CoA thiolase activities were measured using acetoacetyl-CoA as a substrate. Acyl-CoA thioesterase activity was analyzed using octanoyl-CoA and palmitoyl-CoA as substrates [13].

RNA analysis

RNA in the liver was extracted, and mRNA abundance was analyzed by quantitative real-time PCR as detailed elsewhere [14, 15]. mRNA abundance was calculated as a ratio to the β-actin level in each cDNA sample and expressed as a fold change, assigning a value of 1 for rats fed a diet free of lignan and α-lipoic acid.

Analyses of serum and liver components

Serum triacylglycerol, cholesterol, phospholipid, and glucose concentrations were measured using commercial enzyme kits (Wako Pure Chemical, Osaka, Japan). The serum concentration of β-hydroxybutyrate was analyzed by HPLC [16]. Liver triacylglycerol [17], phospholipid [17], and cholesterol [18] concentrations were determined as described before. The hepatic concentration of carnitine was analyzed by the method of Pearson et al. [19]. Concentrations of malondialdehyde in serum and liver were analyzed by HPLC [20].

Statistical analysis

Microsoft Excel add-in software (Excel Statistics 2010; Social Survey Research Information Co., Tokyo, Japan) was used for statistical analysis. The data were analyzed by two-way ANOVA to establish the effect of sesamin and α-lipoic acid or any interaction between these two factors. When the interaction was significant, the data were reanalyzed by one-way ANOVA and Tukey's post hoc test. Differences were considered significant when $P < 0.05$.

Results

Animal growth and liver weight

The average daily food intake during the 0–22 day experimental period was about 7 % lower for diets containing α-lipoic acid than for diets free of this compound (Table 1). During the first half (0–11 days) of the feeding period, the reduction in food intake by α-lipoic acid was marked (about 13 % decrease); however,

Table 1 Effect of dietary sesamin and α-lipoic acid on growth parameters and liver weight

α-Lipoic acid (g/kg)	Sesamin (g/kg)				Two-way ANOVA (*P* value)		
	0		2		Sesamin	α-Lipoic acid	Sesamin × α-Lipoic acid
	0	2.5	0	2.5			
Food intake (*g/day*)							
0–11 days	21.5 ± 0.7	18.6 ± 0.4	21.2 ± 0.2	18.7 ± 0.7	NS	<0.001	NS
11–22 days	21.4 ± 0.6	21.4 ± 0.5	22.3 ± 0.2	20.9 ± 0.7	NS	NS	NS
0–22 days	21.3 ± 0.6	20.2 ± 0.4	21.8 ± 0.1	20.0 ± 0.6	NS	<0.01	NS
Body weight (g)	349 ± 8	319 ± 6	362 ± 5	327 ± 14	NS	<0.01	NS
Growth (g/22 days)	178 ± 7	147 ± 6	189 ± 5	157 ± 11	NS	<0.001	NS
Liver weight (g/100 g body weight)	4.89 ± 0.15	5.07 ± 0.11	5.68 ± 0.06	6.03 ± 0.16	<0.001	<0.05	NS

Values are the mean ± SEM ($n = 7$). NS, $P \geq 0.05$

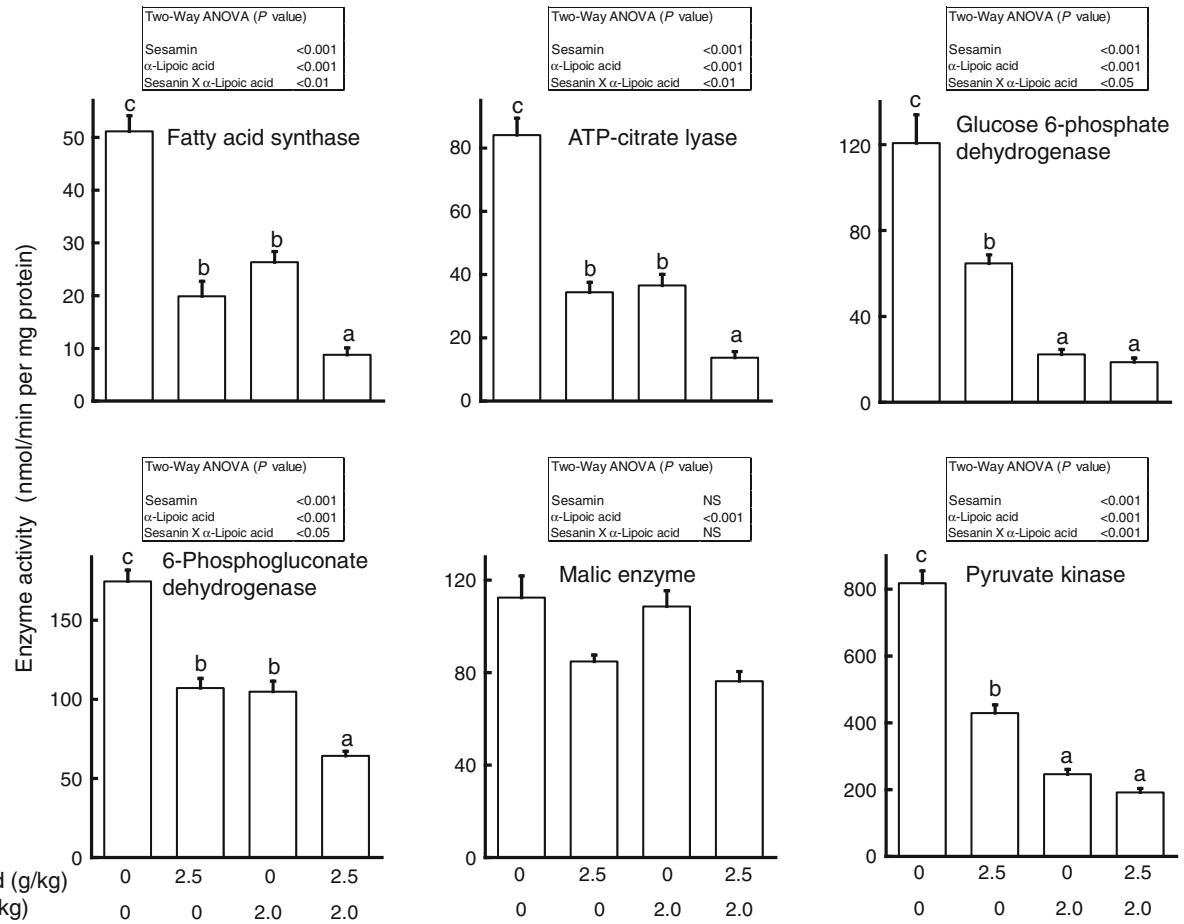

Fig. 1 Effect of dietary sesamin and α-lipoic acid on the activities of lipogenic enzymes in rat liver. Values are the means with their standard errors ($n = 7$). Two-way ANOVA revealed significant interactions between two dietary factors, that is, sesamin and α-lipoic acid, for the activities of various enzymes involved in hepatic lipogenesis except for malic enzyme; therefore, these values were reanalyzed by one-way ANOVA and Tukey's post hoc test. Means without a common letter differ, $P < 0.05$

α-lipoic acid-dependent reduction in food intake was not observed during the latter half of the experimental period (11–22 days). Body weight at the time of killing and growth were significantly lower in rats fed α-lipoic acid than in animals fed diets free of this compound. Sesamin did not affect food intake or growth. Both sesamin and α-lipoic acid significantly increased the liver weight of animals.

Effect of sesamin and α-lipoic acid on hepatic fatty acid synthesis

Both sesamin and α-lipoic acid significantly reduced the activities of lipogenic enzymes except for malic enzyme (Fig. 1). The diet containing either 2 g/kg sesamin or 2.5 g/kg α-lipoic acid was almost equally effective in reducing the activity levels of fatty acid synthase, ATP-citrate lyase, and 6-phosphogluconate dehydrogenase. Meanwhile, the reductions observed with glucose 6-phosphate dehydrogenase and pyruvate kinase were greater with a diet containing sesamin than with a diet containing α-lipoic acid. α-Lipoic acid but not sesamin significantly reduced the activity of malic enzyme. The combination of sesamin and α-lipoic acid effectively reduced the activity levels of fatty acid synthase, ATP-citrate lyase, and 6-phosphogluconate dehydrogenase in an additive fashion. The activity levels of glucose 6-phosphate dehydrogenase and pyruvate kinase were significantly lower in rats fed a diet containing both sesamin and α-lipoic acid than in animals fed a diet solely containing α-lipoic acid and a control diet; however, the values were comparable with those in animals fed a diet containing sesamin alone.

Figure 2 shows mRNA levels of proteins related to lipogenesis. There are two types of acetyl-CoA carboxylase, that is, alpha and beta. The alpha but not beta form appears to be involved in fatty acid synthesis in cytosol [21]. Mammalian tissues contain 3 distinct isoforms of malic enzyme (malic enzyme 1, 2, and 3). Malic enzyme 1 appears to be involved in the regulation of lipogenesis [22]. There are four isoforms of pyruvate kinase in mammals. L-Pyruvate kinase is an enzyme expressed in the liver [23]. Adiponutrin [24] is a protein presumed to be involved in the regulation of lipogenesis. We also analyzed mRNA expressions of enzymes involved in the desaturation of fatty acids, that is, stearoyl-CoA desaturase 1, and Δ^5- and Δ^6-desaturases.

Consistent with the observations made on enzyme activity, both sesamin and α-lipoic acid significantly reduced mRNA levels of acetyl-CoA carboxylase α, fatty acid synthase, ATP-citrate lyase, glucose 6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, and L-pyruvate kinase. The mRNA levels of these enzymes in rats fed diets solely containing either sesamin or α-lipoic acid were 40–67 % of those observed for a diet free of these compounds. The different effects of sesamin and α-lipoic acid observed on the activities of glucose 6-phosphate dehydrogenase and pyruvate kinase were not confirmed by mRNA levels of these enzymes. Combination

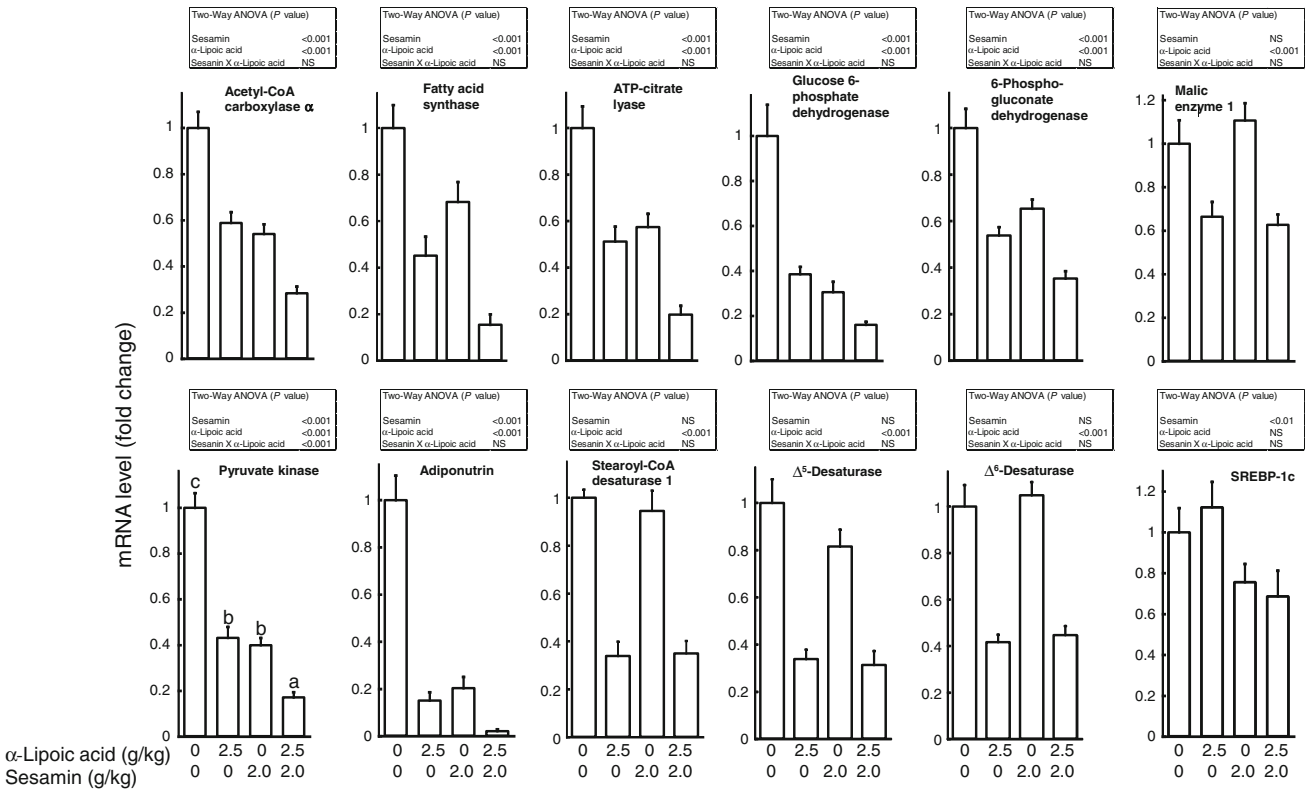

Fig. 2 Effect of dietary sesamin and α-lipoic acid on mRNA levels of lipogenic enzymes, adiponutrin, fatty acid desaturases and SREBP-1c in rat liver. Values are the means with their standard errors ($n = 7$). Two-way ANOVA revealed significant interactions between two dietary factors, that is, sesamin and α-lipoic acid, for mRNA levels of pyruvate kinase; therefore, these values were reanalyzed by one-way ANOVA and Tukey's post hoc test. Means without a common letter differ, $P < 0.05$

of sesamin and α-lipoic acid further decreased the mRNA levels of these enzymes. The values in rats fed a diet simultaneously containing sesamin and α-lipoic acid were 16–29 % of those observed with a diet free of these compounds. Both sesamin and α-lipoic acid also significantly lowered the mRNA expression of adiponutrin. The level in rats fed a diet solely containing either sesamin or α-lipoic acid was 15 % of that observed for the control diet. These values were very low for the diet containing both sesamin and α-lipoic acid (2.2 % of the value observed for the control diet). Consistent with the observations made on enzyme activity, α-lipoic acid but not sesamin lowered the mRNA expression of malic enzyme 1. The level observed for the diet containing sesamin and α-lipoic acid in combination was the same as the value observed for the diet solely containing α-lipoic acid. The responses to sesamin and α-lipoic acid of mRNA expression of stearoyl-CoA desaturase 1, and Δ^5- and Δ^6-desaturases resembled those observed with malic enzyme 1. Sterol regulatory element-binding protein-1c (SREBP-1c) is a transcription factor involved in the regulation of the gene expression of many lipogenic enzymes [25]. In spite of the fact that both sesamin and α-lipoic acid were effective in reducing the activity and mRNA levels of many lipogenic enzymes, sesamin but not lipoic acid significantly lowered mRNA levels of this transcription factor.

Effect of sesamin and α-lipoic acid on hepatic fatty acid oxidation

Consistent with the observations made in previous studies [2], sesamin significantly increased the activities of many enzymes involved in hepatic fatty acid oxidation (Table 2). In addition, we observed that the lignan increased the activities of acyl-CoA thioesterase. In contrast, α-lipoic acid did not increase the activities of enzymes involved in fatty acid oxidation except on one occasion for 3-hydroxyacyl-CoA dehydrogenase where α-lipoic acid significantly increased the enzyme activity in rats fed the sesamin-free diet. Instead, this compound significantly lowered the activities of some hepatic fatty acid oxidation enzymes. Accordingly, the activities of carnitine acyltransferase measured with palmitoyl-CoA substrate, enoyl-CoA hydratase, and 3-ketoacyl-CoA thiolase were significantly lower in rats fed α-lipoic acid-containing diets than in animals fed diets free of this compound. Also, α-lipoic acid significantly lowered the carnitine acyltransferase activity measured with octanoyl-CoA substrate in rats fed sesamin-free diets.

Consistent with the observations made on enzyme activities, sesamin increased mRNA levels of many enzymes involved in fatty acid oxidation. In addition, the lignan increased the mRNA expression of acyl-CoA thioesterase located in cytosol (acyl-CoA thioesterase 1) and mitochondria (acyl-CoA thioesterase 2). α-Lipoic acid lowered the activity of enzymes involved in hepatic fatty acid oxidation on some occasions. Among the various enzymes involved in fatty acid oxidation, α-lipoic acid-dependent reduction in mRNA levels was solely observed with carnitine palmitoyltransferase 1a not with other enzymes. Instead, α-lipoic acid added to a sesamin-free diet actually increased mRNA levels of carnitine palmitoyltransferase 1b, trifunctional enzyme subunit *β*, mitochondrial 3-ketoacyl-CoA thiolase, and acyl-CoA thioesterase 1 and 2; however, this compound added to a sesamin-containing diet did not affect the mRNA levels of various enzymes involved in fatty acid oxidation.

Effect of sesamin and α-lipoic acid on mRNA levels of proteins involved in the regulation of carnitine metabolism and fatty acid uptake in the liver

Consistent with a previous observation [26], sesamin greatly increased the carnitine concentration and mRNA level of carnitine transporter in the liver (Fig. 3). Interestingly, however, α-lipoic acid decreased the hepatic concentration of carnitine without affecting the mRNA level of carnitine transporter. Changes in the rate of hepatic carnitine synthesis appear to be one factor altering the concentration of carnitine in this tissue [27, 28]. We therefore analyzed mRNA levels of hepatic enzymes involved in carnitine biosynthesis (ε-trimethyllysine hydroxylase, γ-trimethylaminobutyraldehyde dehydrogenase, and γ-butyrobetaine hydroxylase 1) in the present study. α-Lipoic acid significantly decreased the mRNA level of all three enzymes. Sesamin slightly but significantly increased the mRNA level of γ-trimethylaminobutyraldehyde dehydrogenase but did not affect the value for the other two enzymes.

We previously found that sesamin increased hepatic mRNA levels of Cd36 located in the plasma membrane and involved in the cellular uptake of free fatty acid from the blood stream [26]. Cd36 gene is targeted by peroxisome proliferator-activated receptor (PPAR)γ2, and recent studies in mice showed that up-regulation of this gene is associated with a parallel increase in the mRNA expression of PPARγ2 in the liver [14, 29]. We therefore analyzed mRNA levels of three isoforms of PPAR in rat liver in the present study. As shown in Fig. 4, sesamin strongly increased the mRNA level of Cd36, but α-lipoic acid was ineffective in modulating the value. Sesamin did not affect mRNA levels of PPARα and PPARγ1 but doubled the value for PPARγ2. α-Lipoic acid did not influence the mRNA expression of PPARs.

Table 2 Effect of dietary sesamin and α-lipoic acid on the activity and mRNA levels of hepatic fatty acid oxidation enzyme

α-Lipoic acid (g/kg)	Sesamin (g/kg)				Two-way ANOVA (*P* value)		
	0		2		Sesamin	α-Lipoic acid	Sesamin × α-Lipoic acid
	0	2.5	0	2.5			
Enzymes and substrates	Enzyme activity (nmol/min per mg protein)						
Peroxisomal palmitoyl-CoA oxidation	2.01 ± 0.05	1.94 ± 0.15	7.93 ± 0.98	7.24 ± 0.67	<0.001	NS	NS
Acyl-CoA oxidase	1.49 ± 0.06	1.80 ± 0.08	7.92 ± 0.77	8.02 ± 0.63	<0.001	NS	NS
Carnitine acyltransferase							
Acetyl-CoA	0.38 ± 0.06	0.50 ± 0.08	23.8 ± 2.5	18.7 ± 1.3	<0.001	NS	NS
Octanoyl-CoA	2.67 ± 0.17[b]	1.30 ± 0.16[a]	9.69 ± 1.02[c]	8.01 ± 0.69[c]	<0.001	<0.001	<0.01
Palmitoyl-CoA	4.09 ± 0.31	1.66 ± 0.14	9.31 ± 0.83	6.06 ± 0.40	<0.001	<0.001	NS
Enoyl-CoA hydratase	4,640 ± 180	4,150 ± 130	10,800 ± 500	9,160 ± 320	<0.001	<0.01	NS
3-Hydroxyacyl-CoA dehydrogenase	635 ± 18[a]	678 ± 11[b]	1,610 ± 60[c]	1,500 ± 60[c]	<0.001	NS	<0.05
3-Ketoacyl-CoA thiolase	155 ± 6	116 ± 6	370 ± 18	303 ± 13	<0.001	<0.001	NS
Acyl-CoA thioesterase							
Octanoyl-CoA	2.58 ± 0.20	2.40 ± 0.27	16.4 ± 1.0	13.5 ± 0.9	<0.001	NS	NS
Palmitoyl-CoA	38.0 ± 2.0	38.2 ± 1.2	55.3 ± 1.8	55.3 ± 2.1	<0.001	NS	NS
Genes	mRNA level (fold change)						
Peroxisomal enzyme							
Carnitine octanoyltransferase	1.00 ± 0.09	0.89 ± 0.10	11.8 ± 1.2	9.48 ± 0.95	<0.001	NS	NS
Acyl-CoA oxidase 1	1.00 ± 0.03	1.08 ± 0.05	5.41 ± 0.65	4.86 ± 0.40	<0.001	NS	NS
Peroxisomal bifunctional enzyme	1.00 ± 0.05	1.02 ± 0.05	18.9 ± 3.0	16.4 ± 1.5	<0.001	NS	NS
Peroxisomal 3-ketoacyl-CoA thiolase A	1.00 ± 0.09[b]	0.63 ± 0.05[a]	6.49 ± 1.04[c]	7.37 ± 1.13[c]	<0.001	NS	<0.05
Mitochondrial enzyme							
Carnitine acetyltransferase	1.00 ± 0.16	1.37 ± 0.17	29.8 ± 6.2	21.77 ± 3.0	<0.001	NS	NS
Carnitine palmitoyltransferase 1a	1.00 ± 0.09	0.464 ± 0.076	1.67 ± 0.07	0.858 ± 0.112	<0.001	<0.001	NS
Carnitine palmitoyltransferase 1b	1.00 ± 0.13[a]	3.78 ± 0.34[b]	120 ± 42[c]	100 ± 25[c]	<0.001	<0.05	<0.01
Carnitine palmitoyltransferase 2	1.00 ± 0.05	1.13 ± 0.05	3.60 ± 0.25	3.33 ± 0.21	<0.001	NS	NS
Trifunctional enzyme subunit α	1.00 ± 0.07	1.27 ± 0.15	2.50 ± 0.24	2.30 ± 0.15	<0.001	NS	NS
Trifunctional enzyme subunit β	1.00 ± 0.04[a]	1.55 ± 0.10[b]	3.13 ± 0.25[c]	3.36 ± 0.13[c]	<0.001	<0.001	<0.01
Mitochondrial 3-ketoacyl-CoA thiolase	1.00 ± 0.07[a]	1.66 ± 0.09[b]	3.17 ± 0.16[c]	3.28 ± 0.20[c]	<0.001	<0.001	<0.01
Acyl-CoA thioesterase 2	1.00 ± 0.10[a]	3.57 ± 0.64[b]	8.34 ± 1.21[c]	11.5 ± 1.27[c]	<0.001	<0.001	<0.01
Cytosolic enzyme							
Acyl-CoA thioesterase 1	1.00 ± 0.06[a]	4.37 ± 0.57[b]	26.92 ± 3.71[c]	23.41 ± 1.67[c]	<0.001	<0.001	<0.001

Values are the mean ± SEM ($n = 7$). Means in a row with superscripts without a common letter are significantly different at $P < 0.05$. NS, $P \geq 0.05$

Effect of sesamin and α-lipoic acid on serum and liver lipids

Sesamin and α-lipoic acid significantly lowered serum concentrations of triacylglycerol, cholesterol, and phospholipid (Table 3). A diet containing 2 g/kg sesamin was comparable with a diet containing 2.5 g/kg α-lipoic acid in its potency to reduce serum lipid levels. Combination of α-lipoic acid and sesamin in the diet strongly reduced serum lipid concentrations. As expected, sesamin

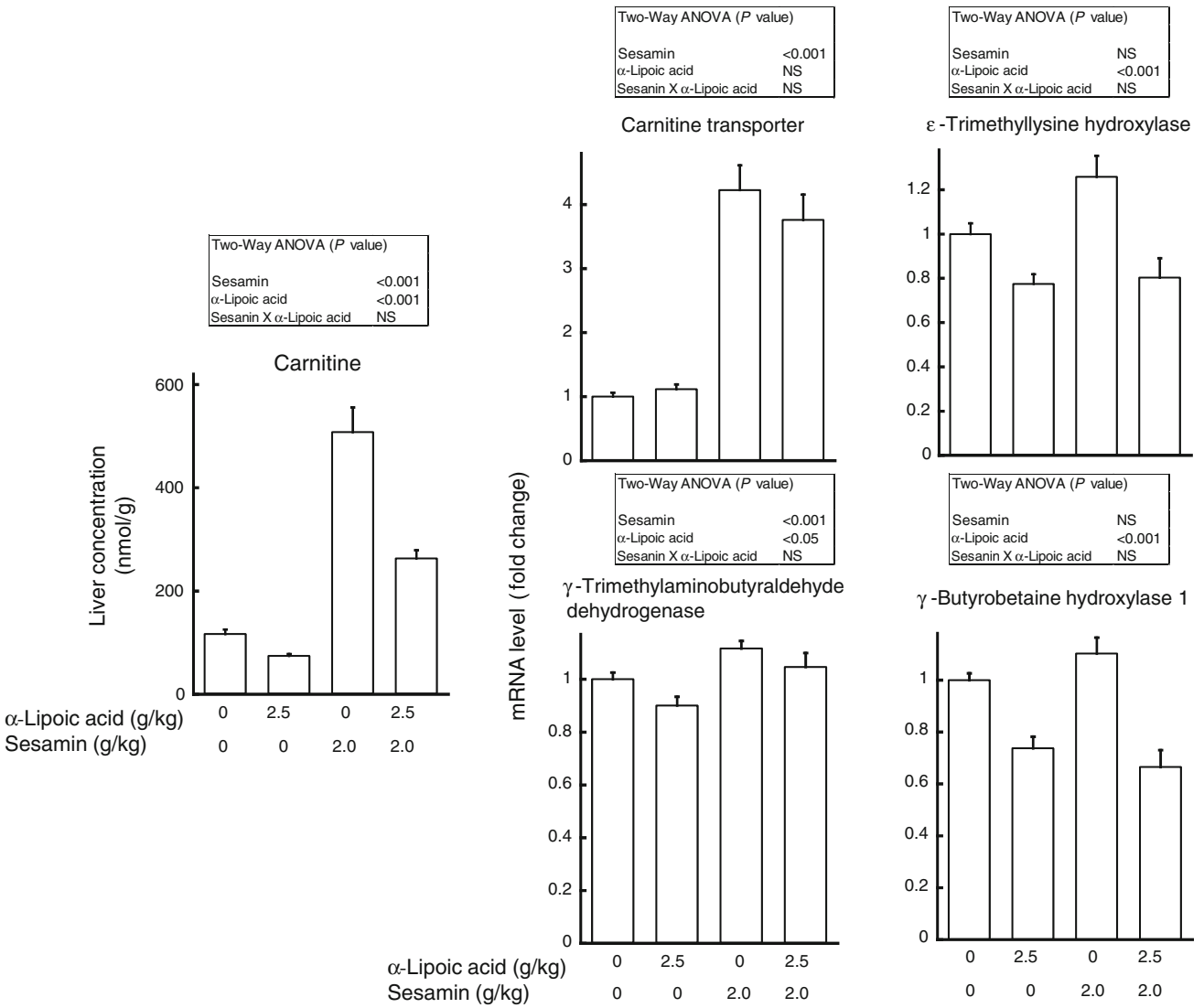

Fig. 3 Effect of dietary sesamin and α-lipoic acid on carnitine concentration, and mRNA levels of carnitine transporter and enzymes involved in carnitine biosynthesis in rat liver. Values are the means with their standard errors ($n = 7$)

significantly increased serum concentrations of β-hydroxy butyrate. In contrast, α-lipoic acid significantly reduced this parameter. α-Lipoic acid, but not sesamin, significantly decreased the serum concentration of glucose.

A diet containing α-lipoic acid but free of sesamin caused a strong 56 % decrease in the hepatic concentration of triacylglycerol. Also, a diet solely containing sesamin compared with a control diet decreased this parameter by 37 %; however, in contrast to serum, the combination of α-lipoic acid and sesamin did not cause an additional decrease in the hepatic concentration of triacylglycerol; that is, the hepatic concentration of triacylglycerol was the same between rats fed a diet containing sesamin alone and those fed a diet containing sesamin and α-lipoic acid in combination. Also, the value was much higher in rats fed a diet containing sesamin and α-lipoic acid in combination than in animals fed a diet containing α-lipoic acid alone. The experimental diet only containing either α-lipoic acid or sesamin compared with a control diet free of these compounds significantly lowered hepatic concentrations of cholesterol. Also, the hepatic concentration of cholesterol was lower in rats fed a diet containing α-lipoic acid and sesamin in combination than in animals fed a control diet free of these compounds; however, the values were comparable among the groups of rats fed diets solely containing α-lipoic acid or sesamin and a diet containing these compounds in combination. Both α-lipoic acid and sesamin increased the hepatic concentration of phospholipids, and the value was highest in rats fed a diet containing these compounds in combination.

It has been demonstrated that both sesamin [10] and α-lipoic acid [11] exert antioxidation activity in experimental animals. Consistent with previous observations, a

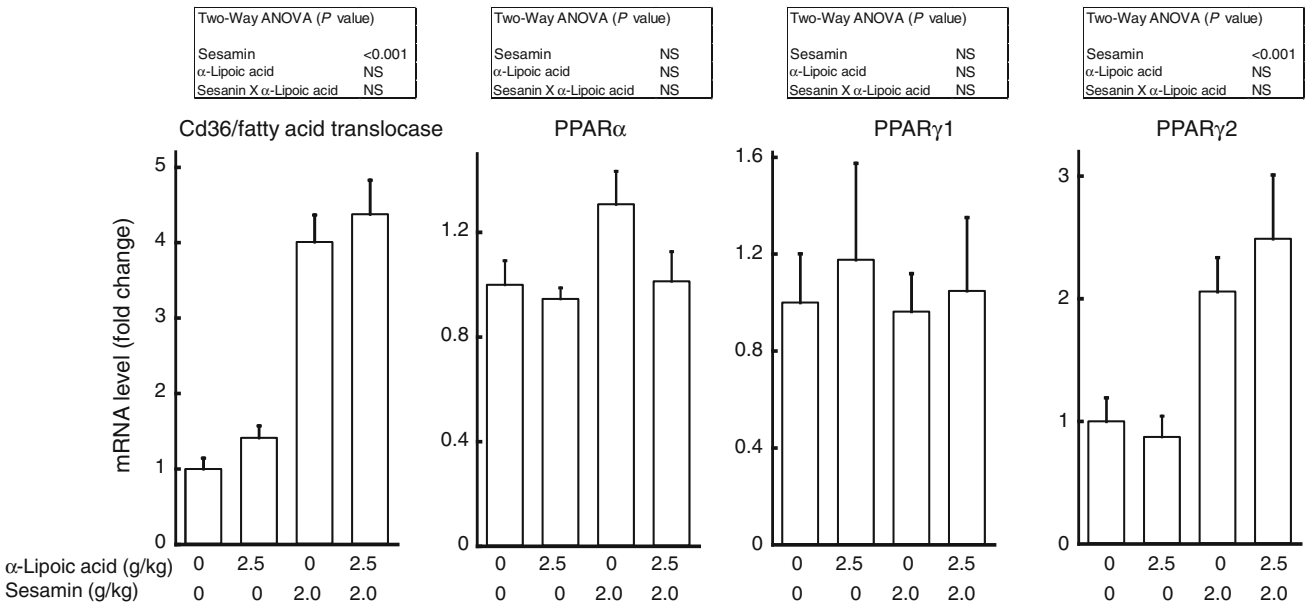

Fig. 4 Effect of dietary sesamin and α-lipoic acid on mRNA levels of Cd36/fatty acid translocase and three PPAR isoformes in rat liver. Values are the means with their standard errors ($n = 7$)

Table 3 Effect of dietary sesamin and α-lipoic acid on concentrations of serum components

α-Lipoic acid (g/kg)	Sesamin (g/kg)				Two-way ANOVA (*P* value)		
	0		2		Sesamin	α-Lipoic acid	Sesamin × α-Lipoic acid
	0	2.5	0	2.5			
Serum components							
Triacylglycerol (mmol/L)	3.29 ± 0.42	1.66 ± 0.15	1.85 ± 0.19	1.07 ± 0.11	<0.001	<0.001	NS
Cholesterol (mmol/L)	2.31 ± 0.13	2.11 ± 0.09	2.02 ± 0.13	1.51 ± 0.10	<0.01	<0.01	NS
Phospholipid (mmol/L)	2.80 ± 0.13	2.38 ± 0.14	2.44 ± 0.13	1.53 ± 0.09	<0.001	<0.001	NS
β-Hydroxybutyrate (mmol/L)	0.154 ± 0.013	0.124 ± 0.002	0.298 ± 0.015	0.227 ± 0.007	<0.001	<0.001	NS
Glucose (mmol/L)	9.69 ± 0.39	8.70 ± 0.32	9.02 ± 0.32	8.38 ± 0.42	NS	<0.05	NS
Malondialdehyde (μmol/L)	5.89 ± 0.69	2.58 ± 0.20	3.91 ± 0.20	1.99 ± 0.32	<0.01	<0.001	NS
Liver components							
Triacylglycerol (μmol/g)	80.1 ± 8.9^{c}	25.0 ± 3.0^{a}	50.3 ± 4.4^{b}	58.0 ± 3.3^{b}	NS	<0.001	<0.001
Cholesterol (μmol/g)	6.04 ± 0.25^{b}	4.94 ± 0.28^{a}	4.89 ± 0.23^{a}	4.99 ± 0.35^{a}	NS	NS	<0.05
Phospholipid (μmol/g)	32.0 ± 0.7	35.2 ± 0.4	38.6 ± 0.7	40.7 ± 0.7	<0.001	<0.001	NS
Malondialdehyde (nmol/g)	254 ± 14	198 ± 9	186 ± 13	179 ± 12	<0.01	<0.05	NS

Values are the mean ± SEM ($n = 7$). Means in a row with superscripts without a common letter differ, $P < 0.05$. NS, $P \geq 0.05$

diet only containing either sesamin or α-lipoic acid caused 34 and 56 % decreases, respectively, in the serum concentration of malondialdehyde. An additional strong decrease (66 %) in this parameter was observed with a diet simultaneously containing α-lipoic acid and sesamin. Sesamin and α-lipoic acid also caused 22 and 27 % decreases, respectively, in the liver concentration of malondialdehyde; however, a combination of these

compounds did not cause an additional decrease in this parameter (29 % decrease).

Discussion

Combined effect of sesamin and α-lipoic acid on hepatic lipogenesis

Many lipogenic enzymes are under the control of SREBP-1. This transcription factor also regulates the gene expression of enzymes involved in the desaturation of fatty acid (stearoyl-CoA desaturase 1, and Δ^5- and Δ^6-desaturases) [30, 31]. Previous studies showed that both sesamin [3] and α-lipoic acid [9] decreased the activity and mRNA levels of many enzymes involved in hepatic lipogenesis. We also demonstrated that down-regulation of SREBP-1 is the mechanism underlying the sesamin-dependent decrease in hepatic lipogenesis [3]. Consistent with these previous findings, sesamin and α-lipoic acid lowered the activity and mRNA levels of many hepatic lipogenic enzymes in the present study. The combination of these compounds also effectively decreased many of these parameters in an additive fashion; however, the responses to sesamin and α-lipoic acid of mRNA levels of malic enzyme 1 and of fatty acid desaturases were considerably different from those of other enzymes. It has been reported that the expressions of the genes of stearoyl-CoA desaturase 1 [30, 32], and Δ^5- and Δ^6-desaturases [31] as well as malic enzyme 1 [33] are dually regulated by SREBP-1 and PPARα. Sesamin appears to be an agonist of PPARα [2] and hence is expected to stimulate gene expressions of these enzymes and, at the same time, the lignan should conversely down-regulate their mRNA expression through SREBP-1-dependent mechanism [3]. These effects will cause only a moderate change in the mRNA expression of these genes, as actually observed in rats fed sesamin in the current study. As expected, α-lipoic acid, unlike sesamin, decreased mRNA expressions not only of the various lipogenic enzymes, including malic enzyme 1, but also fatty acid desaturases. The combination of this compound with sesamin results in lowering of the expression of malic enzyme 1 and fatty acid desaturases to the levels observable in rats fed a diet solely containing α-lipoic acid. This may not be due to the counteraction of PPAR action on these genes, because α-lipoic acid did not decrease mRNA levels of fatty acid oxidation enzymes, except for carnitine palmitoyltransferase 1a. Therefore, it was suggested that the combination of sesamin and α-lipoic acid potentiated down-regulation of the SREBP-1 signaling pathway and overcame the sesamin-mediated stimulation of PPAR action on various fatty acid desaturase genes and the malic enzyme 1 gene.

It has been well demonstrated that many enzymes involved in lipogenesis are under the control of SREBP-1 [25]. It is reasonable to consider that both sesamin and α-lipoic acid decreased hepatic lipogenesis through SREBP-1-dependent mechanism despite that sesamin, but not α-lipoic acid, reduced the mRNA level of SREBP-1c in the present study. In relation to this, we previously reported that 2 g/kg sesamin in the diet caused only marginal decreases in mRNA levels of SREBP-1 and protein levels of the precursor form of this transcription factor but caused strong reductions in protein levels of the mature form [3]. Therefore, it is possible that α-lipoic acid decreases the protein level of the mature form of SREBP-1 without affecting the mRNA level of this transcription factor and hence lowers hepatic lipogenesis. Information with respect to the effect of α-lipoic acid on the protein levels of immature and mature forms of SEEBP-1 has hitherto been lacking. This needs to be clarified in a future study. Insulin-induced gene (Insig)-1 and (Insig)-2 are proteins that control the proteolytic cleavage of immature SREBP to form nuclear mature SREBP [34, 35]. A recent study [36] showed that treatment with WY 14,643 and troglitazone, agonists of PPARα and PPARγ, respectively, increased mRNA levels of Insig-1 and Insig-2 in hepatoma Fao cells. This was accompanied by the reductions in mature SREBP-1 protein levels and lipogenesis. Sesamin appears to be a natural ligand to activate PPARα; therefore, it is possible that the lignan also decreases hepatic lipogenesis through the up-regulation of Insig-1 and 2.

Combined effect of sesamin and α-lipoic acid on hepatic fatty acid oxidation

In the present study, we confirmed previous findings that sesamin increased the hepatic activity and mRNA levels of fatty acid oxidation enzymes [2]. In addition, we found that sesamin increased the hepatic activity of acyl-CoA thioesterase and mRNA levels of acyl-CoA thioesterase 1 and 2 located in cytosol and mitochondria, respectively. There are many types of acyl-CoA thioesterase in mammals [37]. It has been reported that at least acyl-CoA thioesterase 1 and acyl-CoA thioesterase 2 genes are targeted by PPARα [37]; therefore, it is rational that sesamin activated PPARα and hence increased the mRNA levels of these acyl-CoA thioesterases and enzyme activity. There are three forms of carnitine palmitoyltransferase 1 in rats, that is, a, b, and c. Carnitine palmitoyltransferase 1a predominates in the liver while 1b and 1c are abundant in muscle and brain, respectively [38]. We confirmed previous findings [2] that sesamin increased the mRNA expression of carnitine palmitoyltransferase 1a. In addition, we found for the first time that sesamin caused a great (more than 100-fold) increase in the mRNA expression of carnitine

palmitoyltransferase 1b. It has been indicated that the expression level of 1b isoform in the liver is very low [38], and hence, it is generally considered that this isoform does not play a significant role in hepatic fatty acid oxidation; however, the present observation implies that 1b isoform may also play a significant role in oxidizing fatty acid in the liver, at least of rats fed sesamin. Sesamin appears to be a natural PPAR agonist. It is currently unknown whether PPAR agonists other than sesamin have the potential to cause a great increase in the mRNA expression of carnitine palmitoyltransferase 1b.

We observed in the present study that α-lipoic acid decreased the activity of some enzymes involved in hepatic fatty acid oxidation. In particular, α-lipoic acid-dependent reduction in the activity of carnitine acyltransferase was marked when palmitoyl-CoA was used as a substrate, the reduction being attenuated when using acetyl-CoA and octanoyl-CoA as substrates. In relation to this, we found in the present study that this compound significantly decreased mRNA levels of carnitine palmitoyltransferase 1a. α-Lipoic acid did not affect mRNA levels of other enzymes with carnitine acyltransferase activity (carnitine acetyltransferase, carnitine octanoyltransferase, and carnitine palmitoyltransferase 2) but actually increased the mRNA expression of carnitine palmitoyltransferase 1b in rats given sesamin-free diets. This implies that the reduction in the gene expression of carnitine palmitoyltransferase 1a is accountable for the α-lipoic acid-dependent decrease in the activity of carnitine acyltransferase observed in the present study. We also observed that α-lipoic acid significantly decreased the activity of enoyl-CoA hydratase and 3-ktoacyl-CoA thiolase; however, this compound did not decrease the mRNA levels of proteins having enoyl-CoA hydratase (peroxisomal bifunctional enzyme and trifunctional enzyme subunit α) and 3-ketoacyl-CoA thiolase activities (peroxisomal 3-ketoacyl-CoA thiolase A, trifunctional enzyme subunit β, and mitochondrial 3-ketoacyl-CoA thiolase); therefore, it is currently uncertain which enzyme proteins are responsible for the α-lipoic acid-dependent decrease in enoyl-CoA hydratase and 3-ketoacyl-CoA thiolase activities.

With respect to the physiological activity of sesamin and α-lipoic acid affecting hepatic fatty acid oxidation, we observed that sesamin strongly increased the hepatic concentration of carnitine, but α-lipoic acid reduced this parameter. The lignan-dependent increase in hepatic carnitine concentration is ascribable to the increase in mRNA expression of carnitine transporter. It has been indicated that the mRNA expression of carnitine transporter is under the control of PPARα [27, 28]; therefore, it is likely that sesamin activated PPARα and hence up-regulated mRNA expression of the transporter. Recent studies identified peroxisome proliferator response element (PPRE) in the promoter region of the genes not only of carnitine transporter [39], but also of enzymes involved in carnitine biosynthesis (γ-butyrobetaine hydroxylase 1 [40] and γ-trimethylaminobutyraldehyde dehydrogenase [41]) in mice; however, the effect of an agonist of PPARα (WY 14,643) on mRNA expression of enzymes involved in carnitine synthesis in mice appears rather moderate and inconsistent [27, 28]. Consistent with these previous observations, sesamin only moderately increased mRNA levels of γ-trimethylaminobutyraldehyde dehydrogenase but not of the other two enzymes involved in carnitine biosynthesis; therefore, more detailed examinations are required to clarify the involvement of PPARα in regulating carnitine biosynthesis. We found that an α-lipoic acid-dependent decrease in hepatic carnitine concentration accompanied the reduction in the mRNA expression of enzymes involved in carnitine biosynthesis, but not of carnitine transporter; therefore, it is likely that decreased carnitine biosynthesis is the mechanism of how α-lipoic acid decreases carnitine concentration in the liver. Our observations indicated that α-lipoic acid antagonized sesamin action to stimulate hepatic fatty acid oxidation through reductions in the activities of some enzymes involved in fatty acid oxidation and carnitine concentration in the liver. Consistent with this idea, two-way ANOVA showed that sesamin significantly increased the serum concentration of β-hydroxybutyrate, but α-lipoic acid decreased it.

Combined effect of sesamin and α-lipoic acid on serum and hepatic lipid levels

Alterations in hepatic fatty acid synthesis [42] and oxidation [43] modify the availability of fatty acids for the synthesis of triacylglycerol and, in turn, alter very low-density lipoprotein production by the liver; therefore, a change in the rate of these metabolic processes is crucial to determine serum lipid concentrations. In the current study, alterations by dietary α-lipoic acid and sesamin of hepatic fatty acid synthesis and oxidation were accompanied by large changes in serum lipid profiles. Large decreases in serum lipid levels were noted with a diet containing both sesamin and α-lipoic acid in combination; however, sesamin and α-lipoic acid did not necessarily cause changes in serum and hepatic lipid levels predictable from the alterations in hepatic fatty acid synthesis and oxidation. Both α-lipoic acid and sesamin lower the activities and mRNA levels of many enzymes involved in hepatic lipogenesis to similar levels. Also, sesamin but not α-lipoic acid increased hepatic fatty acid oxidation. In this case, it is reasonable that sesamin lowers serum and liver lipid levels more than α-lipoic acid; however, this was not the case in the present study. Serum triacylglycerol, cholesterol and

phospholipid levels, and hepatic triacylglycerol level were lower in rats fed diets solely containing either sesamin or α-lipoic acid than in animals fed a control diet; however, serum lipid levels were comparable between the former two groups. In addition, the hepatic triacylglycerol level was higher in animals fed sesamin than in animals fed α-lipoic acid. Moreover, the combination of sesamin and α-lipoic acid did not effectively lower hepatic triacylglycerol level. It is apparent that alterations in hepatic fatty acid oxidation and synthesis are not the only factors that control serum and hepatic lipid levels. In this context, we previously observed that various sesame lignans (sesamin, episesamin, and sesamolin) increased mRNA levels of Cd36 [26]. Consistent with this observation, in the present study, we observed that the sesamin preparation, composed of equivalent amounts of sesamin and episesamin, strongly increased mRNA expression of Cd36. It has been observed that increased hepatic Cd36 mRNA level is associated with the up-regulation of mRNA expression of PPARγ2 in mice [14, 29]. We found for the first time that up-regulation of the mRNA expression of Cd36 is associated with the increase in mRNA expression of PPARγ2 using rats as experimental animals in the present study. It is reasonable that the sesamin-dependent increase in the mRNA expression of Cd36 is a consequence of the up-regulation of the mRNA expression of this transcription factor. Although Cd36 levels in the liver are normally low in rodents, recent studies in mice [14, 29, 44] indicated that Cd36 plays a crucial role in the transport of fatty acid in hepatocytes and hence regulates hepatic triacylglycerol concentration. The sesamin-dependent increase in Cd36 expression may facilitate fatty acid uptake from the circulation and hence increase the availability of intracellular fatty acid for the hepatic synthesis of triacylglycerol despite the enhancement of fatty acid oxidation and reduction of lipogenesis. This would account for the inefficient capability of sesamin to lower the hepatic triacylglycerol concentration. We found in the present study that α-lipoic acid lowered the activities of some enzymes involved in hepatic fatty acid oxidation and the hepatic concentration of carnitine. α-Lipoic acid apparently antagonized the stimulating effect of sesamin of hepatic fatty acid oxidation. This may account for the ineffectiveness of the combination of sesamin and α-lipoic acid to lower the hepatic triacylglycerol concentration.

Consistent with the observations made in previous studies [2, 5, 9, 15], both sesamin and α-lipoic acid significantly increased liver weight, accompanying the increase in phospholipid concentration in this tissue. Sesamin appears to be a natural peroxisome proliferator and hence may increase liver weight and phospholipid level through the proliferation of peroxisomes and mitochondria. In fact, previous studies [45, 46] showed that xenobiotic peroxisome proliferators increased these parameters in rats. As α-lipoic acid did not increase hepatic fatty acid oxidation, this compound may increase liver weight and hepatic phospholipid level through the proliferation of cell organelles other than peroxisomes and mitochondria.

As expected, the combination of sesamin and α-lipoic acid decreased serum malondialdehyde level in an additive fashion in the present study, despite no particular treatment to load oxidative stress. Therefore, it is expected that the combined use of these compounds potentially prevents oxidative stress; however, an additive effect of these compounds to reduce hepatic malondialdehyde levels was not observed in the present study. There is still the possibility that the combination of sesamin and α-lipoic acid would effectively reduce hepatic malondialdehyde level more than an individual compound under conditions with excess oxidative load.

With respect to the bioavailability of α-lipoic acid, Peter and Borbe [47], using radiolabeled α-lipoic acid, reported that this compound was well absorbed in rats; that is, absorbability estimated from the time course of the changes in blood concentration of radioactivity after an single oral administration was 66 %, and the value estimated from the urinary excretion of radioactivity was 93 %. It has been reported that sesame lignans [15, 48, 49] and their metabolites [49] were detected in the serum, tissues and urine of rats fed these compounds, indicating that the lignans were absorbable and bioavailable; however, there has been no quantitative information on their absorbability.

It is generally observed that diets containing α-lipoic acid reduce the food intake of rats [9]. It is currently unknown whether this represents toxicity or the problem of the palatability [50] of this compound. A study suggested that α-lipoic acid decreased the activity of AMP-activated protein kinase in the hypothalamus and hence reduced food intake [51]. In the present study, reduction in food intake was observed in the early stage of the 22-day feeding period but was not confirmed in the later stage. It is possible that animals became accustomed to the unpleasant burning taste of α-lipoic acid [50] in the later stage of the experimental period.

In conclusion, sesamin and α-lipoic acid effectively decreased serum lipid concentrations and the combination of these compounds further decreased the parameters in an additive fashion. The diets solely containing either α-lipoic acid or sesamin compared to a diet free of these compounds reduced the hepatic triacylglycerol concentration; the lignan being less effective in decreasing this value. Combination of these compounds did not cause a strong decrease in the hepatic triacylglycerol concentration, and the value observed with a diet containing both compounds was the same as that observed with a diet containing sesamin alone. Combination of these compounds decreased the activity

and mRNA levels of hepatic lipogenic enzymes in an additive fashion. Sesamin but not α-lipoic acid strongly increased the activity and mRNA levels of hepatic fatty acid oxidation enzymes. α-Lipoic acid appeared to antagonize the stimulating effect of sesamin of hepatic fatty acid oxidation through the reduction in the activity and mRNA levels of fatty acid oxidation enzymes and carnitine concentration in the liver. This may account for the failure to observe strong reductions in hepatic triacylglycerol concentration in rats given a diet containing both sesamin and α-lipoic acid.

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Conflict of interest The authors declare no conflicts of interest.

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