Effect of Dietary Sesamin on Metabolic Fate of an Exogenous Linolelaidic Acid in Perfused Rat Liver

Nobuhiro Fukuda, Lei Zhang, Masaki Kodama, Masanobu Sakono, Takashi Ide, Kyosuke Yamamoto and Michihiro Sugano

1 Department of Biological Resource Sciences, Faculty of Agriculture, Miyazaki University, Miyazaki 889-2192, Japan
2 Laboratory of Nutrition Biochemistry, National Food Research Institute, Ministry of Agriculture, Fisheries and Forestry, Tsukuba 305-8642, Japan
3 Department of Internal Medicine, Saga Medical University School of Medicine, Saga 849-8501, Japan
4 Faculty of Human Life Sciences, Prefectural University of Kumamoto, Kumamoto 862-8502, Japan

(Received January 21, 1999)

Summary To estimate the relative significance of exogenous and endogenous fatty acid substrates in decreasing hepatic triacylglycerol secretion after sesamin feeding, livers from rats fed diets supplemented with and without sesamin (sesamin:episesamin, 1:1, w/w) were perfused in the presence and absence of an exogenous di-trans isomer of linoleic acid (linolelaidic acid, trans,trans-9,12-octadecadienoic acid). Both exogenous trans fatty acid and dietary sesamin, as compared with respective controls, resulted in a marked increase in hepatic ketogenesis; however, the β-hydroxybutyrate to acetoacetate ratio was elevated by exogenous fatty acid and decreased by dietary sesamin. On the other hand, hepatic secretions of triacylglycerol, phospholipid and cholesterol were markedly lowered in rats fed sesamin, especially when exogenous fatty acid substrate was provided. The relative significance of the exogenous fatty acid was observed in the dietary sesamin-induced decrease in hepatic secretion of triacylglycerol. These results suggest that increased fatty acid oxidation by dietary sesamin, as reflected by enhanced ketone body production, leads to decreased partition of fatty acid substrates to the esterification pathways, and this in turn reduces the synthesis and secretion of triacylglycerol. The altered metabolism of exogenous fatty acids in the liver was therefore a major determinant for the synthesis and secretion of triacylglycerol.

Key Words sesamin, linolelaidic acid, ketone body production, triacylglycerol secretion, perfused rat liver

*To whom correspondence should be addressed.
Sesamin is one of the sesame lignans occurring in sesame seeds and oils. Sesamin exhibits various physiological activities such as modification of lipid metabolism (1-8), availability of tocopherols (9-11), and protective effect against liver damage by alcohol (3). Among these, the hypolipidemic activity of dietary sesamin is the focus of attention since this lignan behaves like fibrate and the related drugs in its mode of action (12), which are widely used for the treatment of hypertriglyceridemic or mixed hyperlipidemic patients (13). In a recent communication, we have reported that dietary sesamin stimulates fatty acid oxidation in rats, and conversely reduces the synthesis and secretion of triacylglycerol by the liver. These reciprocal alterations are, in part, responsible for the reduction of serum lipids, especially triacylglycerol (1, 3). However, the relative contribution of endogenous and exogenous fatty acids to the sesamin-induced reduction of triacylglycerol secretion by the liver is unclear. An understanding of the relative contribution of these fatty acid sources to the formation of secretory triacylglycerol by the liver of sesamin-fed rat is important for an overall understanding of the mechanisms responsible for the hypotriglyceridemic action of dietary sesamin.

In this experiment, we therefore compared the metabolism of a geometrical isomer of linoleic acid (linolelaic acid, trans,trans-9,12-octadecadienoic acid) in the livers of rats fed diets supplemented with or without sesamin. We have previously reported the usefulness of this geometrical isomer of linoleic acid as an exogenous fatty acid tracer to compare the relative contribution of endogenous and exogenous fatty acid substrates in perfused rat liver experiments (14). In the present experiment, using exogenous trans fatty acid, we found that the hypotriglyceridemic action of dietary sesamin is partly due to alteration of exogenous free fatty acid relative to endogenous substrates in the hepatic metabolism. The mode of action of dietary sesamin on fatty acid oxidation in the liver, which is closely related to the decreased synthesis and secretion of triacylglycerol, was similar to that of fibrates, which are potent peroxisomal proliferators (15-18).

MATERIALS AND METHODS

Materials. Linolelaic acid (trans,trans-9,12-octadecadienoic acid) was purchased from Sigma Chemical, St. Louis, MO, USA. Bovine serum albumin Fraction V and β-hydroxybutyrate dehydrogenase were obtained from Boehringer Mannheim GmbH (Mannheim, Germany). Sesamin (sesamin : episesamin, 1 : 1, w/w, 99.5% purity) was supplied by Suntory (Osaka, Japan). All other chemicals used were of analytical grade.

Animals and diets. Five-wk-old male Wistar rats weighing an average 150 g (Kyudo, Kumamoto, Japan) were given a pellet stocked chow (Type CE-2, Clea Japan, Tokyo, Japan) and acclimated for 5 d in a temperature-controlled room (22-24°C, light on 07:00-19:00). The rats were then divided into two groups with equal body weights. The control group was fed diets containing the following ingredients, by weight%: vitamin-free casein (Oriental Yeast, Tokyo, Japan), 20.0;
corn oil, 5; mineral mixture (AIN-76\textsuperscript{TM}), 3.5; vitamin mixture (AIN-76\textsuperscript{TM}), 1; choline chloride, 0.15; cellulose, 4.0; and sucrose to 100. For the sesamin group, sesamin at the 0.2\% level was added at the expense of sucrose. The animals had free access to the diets and deionized water for 14 d. Food intake and body weight were recorded every other day.

Liver perfusion. On the day of experiments, livers from rats fed diets supplemented with and without sesamin were isolated under pentobarbital anesthesia at about 08:30 and perfused at the rate of 20 mL/min, with 120 mL of recirculating Krebs-Henseleit buffer (pH 7.4) containing 25 mM glucose, 1.5\% bovine serum albumin (w/v) and 25\% (w/v) washed bovine erythrocytes in the presence or absence of exogenous linolelaic acid substrate. When the livers were perfused in the presence of exogenous free fatty acid, 5 mL of 20 mM linolelaidic acid as a potassium salt (100 µmol) was added at the beginning of recirculation, and the same solution was continuously infused at the rate of 4.5 mL/h (90 µmol/h) by means of a mechanical infusion pump. Every 1 h, 20 mL of perfusate was removed for the measurement of lipids and ketone bodies, and the same volume of fresh perfusion medium was added at each removal. The liver perfusions of the control and sesamin groups were performed at the same time, and continued for a total of 4 h, as described in detail previously (18–21). The viability of each perfusion was evaluated as described previously (18–21). The experimental protocol was approved by the Ethics Committee for Animal Experiments of the Faculty of Agriculture, Miyazaki University.

Chemical analyses. The lipids of erythrocyte-free perfusate and post-perfused liver were extracted and purified according to the method of Folch et al (22). Triacylglycerol, cholesterol, and phospholipid contents in the lipid extract were measured chemically, and β-hydroxybutyrate and acetoacetate in a deproteinized sample of liver perfusate were measured enzymatically, as described elsewhere (18–21). Triacylglycerols in perfusate at the end of perfusion and post-perfused liver were separated by silica gel 60 G thin-layer chromatography with a solvent mixture of n-hexane : diethyl ether : glacial acetic acid, 80:20:1, v/v/v, transesterified with methanolic H\textsubscript{2}SO\textsubscript{4}, and analyzed by gas-liquid chromatography on a SILAR 10C column (23, 24). The free fatty acid composition of the perfusate at 1-h intervals was also analyzed after separation with thin-layer chromatography and gas-liquid chromatography as described above. The free fatty acid concentration was calculated using pentadecanoic acid as the internal calibration standard.

Statistical analysis. Data were analyzed by one-way analysis of variance, and the statistically significant difference of the means was considered at the level of $p<0.05$ using Duncan's multiple range test (25) or Student's $t$-test (26).

RESULTS

Hepatic uptake of exogenous fatty acid and lipid concentrations

The rats weighing an average of 165 g at the beginning of the experimental
Table 1. Effect of sesamin feeding on body weight, liver weight and post-perfused liver lipid concentrations.

|                | Final body weight (g) | Liver weight (g/100 g body weight) | Liver lipids (µmol/g) |
|----------------|-----------------------|------------------------------------|-----------------------|
|                |                       |                                    | Triacylglycerol | Cholesterol | Phospholipid |
| Without linolelaidic acid |                       |                                    |                |            |              |
| Control (4)    | 264 ± 7               | 4.7 ± 0.1a                         | 9.8 ± 1.0a     | 6.4 ± 0.3  | 41.6 ± 2.7a  |
| Sesamin (5)    | 269 ± 4               | 6.0 ± 0.2b                         | 12.6 ± 1.6a    | 5.7 ± 0.1  | 39.6 ± 1.3a  |
| With linolelaidic acid |                       |                                    |                |            |              |
| Control (5)    | 279 ± 6               | 5.2 ± 0.2a                         | 12.8 ± 2.0ab   | 5.9 ± 0.5  | 33.8 ± 0.5b  |
| Sesamin (4)    | 265 ± 8               | 5.8 ± 0.1b                         | 16.1 ± 0.9b    | 6.7 ± 0.5  | 40.5 ± 1.0a  |

Numbers in parentheses indicate the number of animals, and the results are expressed as mean ± SE. Values bearing a different letter within a column are significantly different at p < 0.05. Rats weighing about 165 g were fed the 20% casein diet with or without 0.2% sesamin for 14 d. The livers were then isolated and perfused in the presence or absence of exogenous linolelaidic acid substrate.

period consumed 18.5–19.5 g of diet per day and gained 99–114 g of body weight in 2 wk. There were no differences in these parameters between the control and sesamin groups, although the relative weights of post-perfused livers were significantly higher in the sesamin group than in the control group, irrespective of the presence or absence of exogenous trans fatty acid substrate (Table 1). No significant difference was noted in the concentration of post-perfused liver lipids between the control and sesamin-fed rats, except for phospholipid in the livers perfused in the presence of exogenous fatty acid; it was significantly higher in the sesamin group than in the control group. Hepatic uptakes of exogenous linolelaidic acid for 1, 2, 3 and 4 h (18–21), were comparable; 183 ± 3, 272 ± 4, 361 ± 3 and 453 ± 3 µmol/liver in the control group, and 185 ± 4, 273 ± 3, 363 ± 5 and 454 ± 5 µmol/liver in the sesamin group, respectively. This result therefore indicates that differences in ketogenesis and lipid secretion by the livers in the presence of exogenous fatty acid were due to the direct influence of the dietary lignan on intracellular fatty acid metabolism.

Ketone body production and lipid secretion

Table 2 shows ketone body production and the ratio of β-hydroxybutyrate to acetoacetate. The perfusion of control livers with an exogenous trans fatty acid as compared with those perfused without an added fatty acid substrate caused an approximately 3-fold increase in ketone body production. On the other hand, livers of the sesamin group produced significantly more ketone bodies from both endogenous and exogenous fatty acids than those of the corresponding control counterparts; an approximate 1.5-fold increase both in the absence and presence of exogenous fatty acids.
Table 2. Effect of sesamin feeding on cumulative production of ketone bodies and the ratio of $\beta$-hydroxybutyrate and acetoacetate in the perfused liver.

| Perfusion period (h) | 1          | 2          | 3          | 4          |
|----------------------|------------|------------|------------|------------|
| Control (4)          | 46.1 ± 2.1a| 87.5 ± 6.8a| 159 ± 18b  | 196 ± 20a  |
| Sesamin (5)          | 57.5 ± 4.8a| 138 ± 11b  | 228 ± 16b  | 311 ± 14b  |
| Control (5)          | 268 ± 19c  | 362 ± 14c  | 466 ± 22c  | 559 ± 26c  |
| Sesamin (4)          | 379 ± 13d  | 503 ± 28d  | 671 ± 12d  | 818 ± 18d  |

| $\beta$-Hydroxybutyrate/acetoacetate ratio |
|------------------------------------------|
| Without linolelaidic acid                |
| Control (4)                             | 0.42 ± 0.03ab | 0.47 ± 0.05a | 0.49 ± 0.06a | 0.59 ± 0.03a |
| Sesamin (5)                             | 0.35 ± 0.06a  | 0.40 ± 0.07a | 0.44 ± 0.08a | 0.59 ± 0.04a |
| With linolelaidic acid                   |
| Control (5)                             | 0.55 ± 0.05b  | 0.62 ± 0.08b | 0.74 ± 0.06b | 0.95 ± 0.07b |
| Sesamin (4)                             | 0.40 ± 0.07ab | 0.45 ± 0.04a | 0.51 ± 0.05a | 0.63 ± 0.06a |

Numbers in parentheses indicate the number of animals, and the results are expressed as mean ± SE. Values bearing a different letter within a column are significantly different at $p < 0.05$.

Effect of sesamin feeding on hepatic fatty acid metabolism. The ratio of $\beta$-hydroxybutyrate and acetoacetate, which is an indication of mitochondrial redox potential (18–21, 27), was significantly higher in livers perfused with linolelaidic acid than in those perfused without fatty acid, whereas the ratio observed in the livers of rats fed the sesamin diet was conversely lower than that in the livers of rats fed the control diet, especially when exogenous trans fatty acid was provided.

The cumulative secretions of triacylglycerol, phospholipid, and cholesterol by the livers are shown in Table 3. The infusion of exogenous trans fatty acid, compared without the infusion of fatty acid substrate, caused 1.6-, 1.5-, and 1.2-fold increases in the secretion of triacylglycerol, phospholipid, and cholesterol, respectively, indicating that exogenous trans fatty acid has a stimulatory effect on the synthesis and secretion of lipoproteins containing these lipids, which is consistent with previous observations (24). On the other hand, sesamin feeding resulted in a marked reduction in hepatic secretion of these lipid components: the extent of reduction in triacylglycerol (65%), phospholipid (43%) and cholesterol (16%) was statistically significant for all except cholesterol secretion at 3 and 4 h of perfusion. Thus, the effects of dietary sesamin appeared to be more marked on the secretion of glycerolipids than that of cholesterol.
Table 3. Effect of sesamin feeding on cumulative secretion of triacylglycerol, cholesterol and phospholipid in the perfused liver.

| Perfusion period (h) | 1     | 2     | 3     | 4     |
|----------------------|-------|-------|-------|-------|
| **Triacylglycerol secretion (μmol/liver)** |       |       |       |       |
| Without linoleic acid |       |       |       |       |
| Control (4)          | 8.6±1.7<sup>a</sup> | 12.1±1.6<sup>a</sup> | 17.3±2.0<sup>a</sup> | 22.3±2.3<sup>a</sup> |
| Sesamin (5)          | 6.9±1.1<sup>a</sup>  | 11.6±0.9<sup>a</sup> | 17.7±1.3<sup>a</sup> | 24.0±1.7<sup>a</sup> |
| With linoleic acid   |       |       |       |       |
| Control (5)          | 14.4±1.5<sup>b</sup> | 21.1±2.8<sup>b</sup> | 26.3±2.7<sup>b</sup> | 35.6±3.9<sup>b</sup> |
| Sesamin (4)          | 7.4±0.9<sup>c</sup>  | 8.3±0.3<sup>c</sup>  | 10.0±0.8<sup>c</sup> | 15.8±2.5<sup>c</sup> |

| **Cholesterol secretion (μmol/liver)** |       |       |       |       |
| Without linoleic acid |       |       |       |       |
| Control (4)          | 1.8±0.3<sup>a</sup> | 2.8±0.5<sup>a</sup> | 3.8±0.4<sup>a</sup> | 5.2±0.4<sup>a</sup> |
| Sesamin (5)          | 1.5±0.2<sup>a</sup> | 2.5±0.3<sup>a</sup> | 4.2±0.4<sup>a</sup> | 5.5±0.5<sup>a</sup> |
| With linoleic acid   |       |       |       |       |
| Control (5)          | 2.6±0.2<sup>b</sup> | 4.1±0.4<sup>b</sup> | 5.9±0.3<sup>b</sup> | 8.0±0.6<sup>b</sup> |
| Sesamin (4)          | 1.9±0.2<sup>a</sup> | 2.8±0.3<sup>a</sup> | 4.5±0.3<sup>a</sup> | 6.7±0.8<sup>a</sup> |

| **Phospholipid secretion (μmol/liver)** |       |       |       |       |
| Without linoleic acid |       |       |       |       |
| Control (4)          | 5.0±0.8<sup>a</sup> | 7.9±1.1<sup>a</sup> | 12.6±1.0<sup>a</sup> | 21.9±1.3<sup>a</sup> |
| Sesamin (5)          | 5.1±0.4<sup>a</sup> | 7.9±1.3<sup>a</sup> | 11.4±1.0<sup>a</sup> | 17.2±0.8<sup>a</sup> |
| With linoleic acid   |       |       |       |       |
| Control (5)          | 9.4±1.0<sup>b</sup> | 11.8±1.3<sup>b</sup> | 16.1±1.5<sup>b</sup> | 26.2±3.5<sup>b</sup> |
| Sesamin (4)          | 4.9±0.3<sup>a</sup> | 7.8±0.7<sup>a</sup> | 9.6±0.7<sup>a</sup>  | 16.5±1.4<sup>a</sup> |

Numbers in parentheses indicate the number of animals, and the results are expressed as mean±SE. Values bearing a different letter within a column are significantly different at p<0.05.

**Fatty acid composition of perfusate and liver triacylglycerol**

The fatty acid compositions of triacylglycerol in perfusate obtained at the end of perfusion and postperfused liver are shown in Table 4. The advantage of the use of di-trans fatty acid as a tracer of exogenous free fatty acid substrate in rat liver perfusion experiments was reported previously (14), although there are subtle differences in the metabolic fate of trans fatty acids as compared to cis-counterparts with respect to their oxidation and esterification (23, 24). In the livers perfused without an exogenous fatty acid substrate, the fatty acid compositions of perfusate and post-perfused liver triacylglycerol were roughly comparable between the groups. On the other hand, in control livers perfused in the presence of exogenous trans fatty acid, 23.4 and 4.8% of the perfusate and post-perfused liver triacylglycerol-fatty acids respectively, were replaced by this isomer. Thus, trans fatty acid was
Table 4. Fatty acid composition of triacylglycerol in perfusate and post-perfused liver.

| Fatty acids (weight%) | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 |
|----------------------|------|------|------|------|------|
|                       |      |      |      |      | cis,cis | trans,trans |
| Per fusate            |      |      |      |      |      |      |
| Without linolela idic acid |      |      |      |      |      |      |
| Control (4)           | 24.8±0.7a | 10.4±0.6a | 2.2±0.2 | 51.2±1.0a | 11.4±1.6 |      |
| Sesamin (5)           | 29.4±0.9b | 6.4±0.8b | 2.6±0.2 | 50.6±2.2a | 11.3±2.6 |      |
| With linolela idic acid |      |      |      |      |      |      |
| Control (5)           | 19.4±1.7c | 8.4±0.6c | 2.2±0.2 | 34.6±2.9b | 11.9±1.8 | 23.4±2.8 |
| Sesamin (4)           | 23.7±1.9ace | 4.0±0.4a | 2.8±0.3 | 44.6±3.3a | 12.0±2.5 | 13.0±1.1* |
| Post-perfused liver   |      |      |      |      |      |      |
| Without linolela idic acid |      |      |      |      |      |      |
| Control (4)           | 32.3±1.6 | 9.6±1.1a | 5.3±0.6 | 41.7±1.0 | 11.3±2.1a |      |
| Sesamin (5)           | 32.6±1.6 | 5.7±0.5bc | 4.9±0.5 | 44.8±0.4 | 11.7±2.4a |      |
| With linolela idic acid |      |      |      |      |      |      |
| Control (5)           | 32.1±2.1 | 8.4±1.4ab | 4.8±0.3 | 43.2±3.3 | 6.7±0.7b | 4.8±1.3 |
| Sesamin (4)           | 29.0±0.4 | 4.8±0.4a | 5.3±1.1 | 43.4±2.2 | 11.5±2.2a | 5.9±1.3 |

Numbers in parentheses indicate the number of animals, and the results are expressed as mean±SE. Values bearing a different letter within a column are significantly different at p<0.05. *Significantly different from control group at p<0.05.

Incorporated more into perfusate than post-perfused liver. This suggests that the fatty acid added exogenously during the perfusion periods was incorporated in hepatic triacylglycerol and secreted into perfusates actively as a component of triacylglycerol-fatty acids. On the other hand, neither the proportion of trans fatty acid nor endogenous fatty acids of post-perfused liver triacylglycerol were influenced by dietary sesamin, except for linoleic and palmitoleic acids; the percentage of the linoleic acid increased significantly, while that of the palmitoleic acid decreased more in the sesamin group than in the control group.

In the present study, the amounts of exogenous trans fatty acid and endogenous fatty acids in perfusate triacylglycerol were calculated, as described previously (14), to compare the extent of endogenous and exogenous fatty acid substrates contributing to the observed reduction in hepatic triacylglycerol secretion after sesamin feeding. Dietary sesamin caused a marked decrease in the concentration of exogenous linolela idic acid as well as that of endogenous fatty acids such as oleic, palmitic, linoleic, and palmitoleic acids in perfusate triacylglycerol-fatty acids (Table 5). However, the extent of reduction was more marked on the former exogenous trans fatty acid (75% reduction) than the latter endogenous fatty acids (56–59% reductions), except for palmitoleic acid (80% reduction).
Table 5. Calculated amount of endogenous\(^1\) and exogenous \textit{trans} fatty acids\(^2\) in perfusate triacylglycerol.

| Fatty acids | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 |
|-------------|------|------|------|------|------|
| \textit{cis,\textit{cis}} | | | | | |
| \textit{trans,\textit{trans}} | 20.5 ± 2.5 | 9.2 ± 1.5 | 2.4 ± 0.4 | 37.0 ± 4.8 | 13.0 ± 1.3 | 24.5 ± 4.2 |
| \textit{trans,\textit{trans}} | 11.2 ± 2.2* | 1.9 ± 0.4* | 1.3 ± 0.2 | 21.5 ± 4.2* | 5.3 ± 0.5* | 6.0 ± 0.7* |

Numbers in parentheses indicate the number of animals, and the results are expressed as mean ± SE. *Significantly different from control group at \(p<0.05\).

\(^1\)[\(\mu\text{mol of triacylglycerol secreted during }4\text{h} \times 3 \times \text{percentage of endogenous fatty acid in perfusate triacylglycerol/100}\)].

\(^2\)[\(\mu\text{mol of triacylglycerol secreted during }4\text{h} \times 3 \times \text{percentage of exogenous linolelaic acid in perfusate triacylglycerol/100}\)].

DISCUSSION

It has been reported that sesamin is a potent hypolipidemic dietary component (1–4, 7). However, the mechanism(s) responsible for the observed reduction of serum lipids, especially triacylglycerol, remains to be examined. In a previous rat liver perfusion experiment using an exogenous oleic acid substrate, sesamin feeding decreased the hepatic secretion rate of triacylglycerol (12). The present study, using linolelaic acid as an exogenous fatty acid source, showed again a reduction of hepatic triacylglycerol secretion in sesamin-fed rats. In addition, the reduction was concomitantly associated with the decreased proportion and concentration of exogenous \textit{trans} fatty acid in this lipid molecule. The concentrations of other major endogenous fatty acids in perfusate triacylglycerol were also significantly lowered in response to the reduction of triacylglycerol secretion. Therefore, the hypolipidemic action of dietary sesamin was presumably mediated through a reduction of hepatic synthesis and the secretion of triacylglycerol.

The extent of the reduction of exogenous \textit{trans} fatty acid in secreted triacylglycerol was markedly lower than that of endogenous fatty acids (Table 5), suggesting the relative significance of exogenous fatty acid in reducing the hepatic secretion of triacylglycerol. Fatty acids utilized for esterification, especially for the formation of triacylglycerol, are derived from serum free fatty acids, de novo fatty acid synthesis, and intrahepatic lipolytic process. The relative contribution of these fatty acid sources in the liver is variable under various physiological and nutritional conditions (18–21, 23, 24, 28). In a previous experiment, a marked increase in triacylglycerol secretion caused by emeriamine, an inhibitor of mitochondrial

\textit{J Nutr Sci Vitaminol}
Effect of Sesamin on Hepatic Fatty Acid Metabolism

carnitine palmitoyltransferase I (a rate-limiting enzyme for fatty acid oxidation), by the livers of fasting rats was concomitantly associated with a marked increase in the incorporation of exogenous trans fatty acid into the triacylglycerol molecule, in comparison with endogenous substrates (14). Conversely, the inhibition of fatty acid synthesis by 5-tetradecyloxy-2-furoic acid (TOFA) caused a marked decrease in triacylglycerol secretion, which was concurrently associated with the decreased incorporation of exogenous oleic acid substrate (20). However, the effect of other endogenous fatty acid substrates was less than that of exogenous fatty acid. A similar effect of exogenous free fatty acid on esterification pathways was observed in rat liver treated with another fatty acid synthesis inhibitor, 4-amino-5-ethyl-3-thiophenecarboxylic acid methyl ester hydrochloride (RO 22-0654) (21). These observations therefore indicate the relative significance of exogenous fatty acid substrates in comparison with that of the endogenous origins in modulating hepatic triacylglycerol secretion. Dietary sesamin appeared to influence the metabolism of exogenous fatty acids to a much greater extent than the endogenous counterparts.

Although the mechanism underlying the reduced triacylglycerol secretion by sesamin is not fully understood, the stimulated ketogenesis could account partly for that reduction, especially when exogenous trans fatty acid was provided since there were reciprocal responses in hepatic ketogenesis and triacylglycerol synthesis and secretion under various nutritional and physiological conditions (18–21, 23, 24, 28). In addition, the reduced secretion of triacylglycerol caused by dietary sesamin was inversely related to enhanced ketone body production by the liver, especially when exogenous oleic acid was provided (12). Thus, it is most likely that sesamin acts as a stimulator of hepatic ketogenesis and fatty acid oxidation. The increased conversion of endogenous and exogenous fatty acids into fatty acid oxidation pathways lead to the decreased incorporation of these substrates into esterification pathways, especially the synthesis and secretion of triacylglycerol.

Fatty acid β-oxidation in the liver proceeds in mitochondria and peroxisomes (29, 30). It was suggested that enhanced ketone body production by exogenous oleic acid is due to the increased transportation of fatty acids across the mitochondrial membrane through the stimulation of carnitine palmitoyltransferase activity (20, 21). In addition, elevation of the β-hydroxybutyrate to acetoacetate ratio suggested the increased production of NADH at the step of 3-hydroxyacyl CoA dehydrogenase following the stimulation of β-oxidation (30). Thus, the mitochondrial fatty acid β-oxidation pathway was the major site for the oxidation of endogenous and exogenous fatty acid substrates.

On the other hand, hypolipidemic drugs such as fibrate and its related drugs proliferate hepatic peroxisomes, another site of fatty acid oxidation (29–32), and thereby enhance fatty acid oxidation with a concomitant decrease in the ratio of β-hydroxybutyrate to acetoacetate (16, 18). With respect to the observed reduction in the ratio of β-hydroxybutyrate to acetoacetate, peroxisomes oxidize fatty acids to acetyl-CoA and medium-chain fatty acids. Medium-chain fatty acids are then diffused out of peroxisomes after converting to carnitine derivatives, transported

Vol 45, No 4, 1999
into mitochondria and oxidized there to acetyl-CoA (30). In this case, the enhanced peroxisomal fatty acid oxidation caused by fibric acid derivatives reduces the production of NADH via β-oxidation, as compared to the case of exogenous free fatty acid substrate in mitochondria described above. This implies a decrease in the ratio of β-hydroxybutyrate and acetoacetate and decreased redox potential in the mitochondria even during fatty acid oxidation, and thus, ketone body production is enhanced. Therefore, the reciprocal response in ketogenesis and the ratio of β-hydroxybutyrate to acetoacetate observed in peroxisomal proliferators suggests that the major site of hepatic fatty acid oxidation shifts from mitochondria to peroxisomes.

Sesamin caused an increase in relative liver weight accompanying the concomitant elevation of phospholipid (Table 2). Sesamin also represses polyunsaturated fatty acid desaturation processes in the liver (5, 6, 8). These characteristic responses caused by sesamin are very similar to those observed with fibric acid derivatives (33), although they are structurally unrelated each other. With regard to the dietary sesamin, we recently confirmed that this lignan is a peroxisomal proliferator and causes a marked increase in peroxisomal β-oxidation and, to a lesser extent, in mitochondrial β-oxidation (34). These observations therefore suggest that the sesamin-induced stimulation of ketone body production, with a concomitant decrease in the ratio of β-hydroxybutyrate to acetoacetate, is due to a marked increase in fatty acid oxidation in peroxisomes and, to a lesser extent, in mitochondria. This, in turn, leads to a decreased conversion of fatty acids for the synthesis and secretion of triacylglycerol-rich lipoproteins by the liver.

Exogenous trans fatty acid increased hepatic cholesterol secretion, while dietary sesamin tended to ameliorate the fatty acid-dependent increase (Table 3). Sesamin exhibits a hypocholesterolemic activity in experimental animals (1, 2) and humans (7) through decreased intestinal absorption and the inhibition of HMG-CoA reductase, a rate-limiting enzyme for cholesterol synthesis in the liver (2). The present study showed that the decreased hepatic secretion of cholesterol may additionally participate in the hypocholesterolemic effect of sesamin.

In conclusion, the decrease in the secretion of triacylglycerol-rich lipoproteins by the livers of sesamin-fed rats was a result of a selective increase in ketone body production. This reciprocal alteration was more relevant to the metabolism of exogenous fatty acid substrate than endogenous substrates, and was in part responsible for the observed reduction in the concentration of serum lipids, especially triacylglycerol in the rat.

REFERENCES

1) Sugano M, Inoue T, Koba K, Yoshida K, Hirose N, Shinmen Y, Akimoto K. 1990. Influence of sesame lignans on various lipid parameters in rats. *Agric Biol Chem* 54: 2669–2673.
2) Hirose Y, Inoue T, Nishihara K, Sugano M, Akimoto K, Shimizu S, Yamada H. 1991. *J Nutr Sci Vitaminol*
Effect of Sesamin on Hepatic Fatty Acid Metabolism

Inhibition of cholesterol absorption and synthesis in rats by sesamin. J Lipid Res 32: 629–638.

3) Akimoto K, Kitagawa Y, Akamatsu T, Hirose N, Sugano M, Shimizu S, Yamada H. 1993. Protective effects of sesamin against liver damage caused by alcohol or carbon tetrachloride in rodents. Ann Nutr Metab 37: 218–224.

4) Gu J-Y, Wakizono Y, Tsujita A, Lim B-O, Nonaka M, Yamada K, Sugano M. 1995. Effects of sesamin and α-tocopherol, individually or in combination, on the polyunsaturated fatty acid metabolism, chemical mediator production, and immunoglobulin levels in Sprague-Dawley rats. Biosci Biotechnol Biochem 59: 2198–2202.

5) Fujiyama-Fujiwara Y, Umeda-Sawada R, Kuzuyama M, Igarashi O. 1995. Effects of sesamin on the fatty acid composition of the liver of rats fed n-6 and n-3 fatty acid-rich diet. J Nutr Sci Vitaminol 41: 217–225.

6) Umeda-Sawada R, Takahashi N, Igarashi O. 1995. Interaction of sesamin and eicosapentaenoic acid against Δ5 desaturation and n-6/n-3 ratio of essential fatty acids in rat. Biosci Biotechnol Biochem 59: 2268–2273.

7) Hirata F, Fujita K, Ishikura Y, Hosoda K, Ishikawa T, Nakamura H. 1996. Hypocholesterolemic effect of sesame lignan in humans. Atherosclerosis 122: 135–136.

8) Umeda-Sawada R, Ogawa M, Igarashi O. 1998. The metabolism and n-6/n-3 ratio of essential fatty acids in rats: effect of dietary arachidonic acid and a mixture of sesame lignans (sesamin and episesamin). Lipoic 33: 567–572.

9) Yamashita K, Nohara Y, Katayama K, Namiki M. 1992. Sesame seed lignans and γ-tocopherol act synergistically to produce vitamin E activity in rats. J Nutr 122: 2440–2446.

10) Kamal-Eldin A, Pettersson D, Appelqvist L-A. 1995. Sesamin (a compound from sesame oil) increases tocopherol levels in rats fed ad libitum. Lipoic 30: 499–505.

11) Yamashita K, Iizuka Y, Imai T, Namiki M. 1995. Sesame seed and its lignans produce marked enhancement of vitamin E activity in rats fed a low α-tocopherol diet. Lipoic 30: 1019–1028.

12) Fukuda N, Miyagi C, Zhang L, Jayasooriya AP, Sakono M, Yamamoto K, Ide T, Sugano M. 1998. Reciprocal effects of dietary sesamin on ketogenesis and triacylglycerol secretion by the rat liver. J Nutr Sci Vitaminol 44: 715–722.

13) Sirtori CR, Manzoni C, Lovati MR. 1991. Mechanisms of lipid-lowering agents. Cardiology 78: 226–235.

14) Fukuda N, Fukui M, Kai Y, Jayasooriya AP, Sakono M, Maeda M, Ide T, Yamamoto K. 1998. Effect of emeriamine, an inhibitor of fatty acid oxidation, on metabolic fate of a geometrical isomer of linoleic acid in perfused rat liver. J Nutr Sci Vitaminol 44: 525–535.

15) Lazarow PB. 1977. Three hypolipidemic drugs increase hepatic palmitoyl-CoA oxidation in the rat. Science 197: 580–581.

16) Laker ME, Mayes PA. 1979. The immediate and long term effects of clofibrate on the metabolism of the perfused rat liver. Biochem Pharmacol 28: 2813–2827.

17) Henninger C, Clouet P, Danh HC, Pascal M, Bezard J. 1987. Effects of fenofibrate treatment to fatty acid oxidation in liver mitochondria of obese Zucker rats. Biochem Pharmacol 36: 3231–3236.

18) Yamamoto K, Fukuda N, Zhang L, Sakai T. 1996. Altered hepatic metabolism of fatty acids in rats fed a hypolipidemic drug, fenofibrate. Pharmacol Res 33: 337–342.

19) Fukuda N, Azain MJ, Ontko JA. 1982. Altered hepatic metabolism of free fatty acids underlying hypersecretion of very low density lipoproteins in the genetically obese

Vol 45, No 4, 1999
N FUKUDA et al

Zucker rat. *J Biol Chem* **257**: 14066–14072.

20) Fukuda N, Ontko JA. 1984. Interactions between fatty acid synthesis, oxidation, and esterification in the reduction of triglyceride-rich lipoproteins by the liver. *J Lipid Res* 25: 831–842.

21) Yamamoto M, Fukuda N, Triscari J, Sullivan AC, Ontko JA. 1985. Decreased hepatic production of very low density lipoproteins following activation of fatty acid oxidation by Ro 22-0654. *J Lipid Res* 26: 1196–1204.

22) Folch J, Lees M, Sloane-Stanley GH. 1957. A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem* 226: 497–509.

23) Fukuda N, Igari N, Etoh T, Hidaka T, Ikeda I, Sugano M. 1993. A comparison of the metabolism of cis,cis-, cis,trans/trans,cis- and trans,trans-9,12-octadecadienoic acids in rat liver. *Nutr Res* 13: 779–786.

24) Fukuda N, Etoh T, Wada K, Hidaka T, Yamamoto K, Ikeda I, Sugano M. 1995. Differential effects of geometrical isomers of octadecadienoic acids on ketogenesis and lipid secretion in the livers from rats fed a cholesterol-enriched diet. *Ann Nutr Metab* 39: 185–192.

25) Duncan DB. 1955. Multiple range and multiple F tests. *Biometrics* 11: 1–42.

26) Snedecor GW, Cochran WG. 1967. Statistical Method, 6th ed, p 258–338. Iowa State University Press, Ames, Iowa.

27) Wilson DF, Stubbs M, Veche RL, Ercinska M, Krebs HA. 1974. Equilibrium relations between the oxidation-reduction reactions and the adenine triphosphate synthesis in suspensions of isolated liver cells. *Biochem J* 140: 57–64.

28) Heimberg M, Goh EH, Klausner HA, Soler-Argilaga C, Weinstein I, Wilkox HG. 1978. Regulation of hepatic metabolism of free fatty acids: Interrelationships among secretion of very-low-density lipoproteins, ketogenesis, and cholesterogenesis. In: Disturbances in Lipid and Lipoprotein Metabolism (Dietsch JM, Gotto AM, Ontko JA, eds), p 251–267. Williams & Wilkins, Baltimore.

29) Osmundsen H, Bremer J, Pedersen J. 1991. Metabolic aspects of peroxisomal β-oxidation. *Biochim Biophys Acta* 1085: 141–158.

30) Guzman M, Geelen MJH. 1993. Regulation of fatty acid oxidation in mammalian liver. *Biochim Biophys Acta* 1167: 227–241.

31) Veldhoven VP, Declercq PE, Debeer LJ, Mannaepts GP. 1984. Effects of benfluorex and fenofibrate treatment on mitochondrial and peroxisomal marker enzymes in rat liver. *Biochem Pharmacol* 33: 1153–1155.

32) Mannaepts GP, Debeer LJ, Thomas J, De Schepper PJ. 1979. Mitochondrial and peroxisomal fatty acid oxidation in liver homogenates and isolated hepatocytes from control and clofibrate-treated rats. *J Biol Chem* 254: 4585–4595.

33) Sanchez RM, Vinals M, Alegret M, Vazquez M, Adzet T, Merlos M, Laguna JC. 1993. Fibrates modify rat hepatic fatty acid chain elongation and desaturation in vitro. *Biochem Pharmacol* 46: 1791–1796.

34) Ashakumary L, Rouyer I, Ide T, Fukuda N, Sugano M. 1998. Reciprocal responses to sesamin of the gene expression and activity of enzyme in fatty acid oxidation and synthesis in rat liver. The 52nd Annual Meeting of Nippon Eiyo Shokuryou Gakkai (J Jpn Nutr Food Sci) (Okinawa), Abstract: 90.