Alleviation of Fatty Liver by α-Linolenic Acid

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Summary We compared the efficacy of alpha-linolenic acid (α-LNA, n-3) and linoleic acid (LA, n-6) on orotic acid (OA)-induced fatty liver in Sprague-Dawley rats. Rats were fed semi-synthetic diets containing either LA or α-LNA with or without 1% OA for 2 wk. OA supplementation lowered serum lipids in LA+OA groups. In addition to the decline of serum lipids in α-LNA groups compared to LA groups, a further decrease was found in α-LNA+OA groups compared to LA+OA groups. OA-containing diets significantly increased the liver weights and triacylglycerol (TG) accumulations compared with the OA-free diets. These results were attributed to the significant increases in the activities of phosphatidate phosphohydrolase (PAP), a rate-limiting enzyme of TG synthesis, and glucose-6-phosphate dehydrogenase, a fatty acid synthesis-related enzyme. However, the increase of PAP activity was significantly less in the α-LNA+OA group as compared with the LA+OA group. These results suggest that dietary α-LNA alleviates OA-induced hepatic TG accumulation through the attenuation of hepatic TG synthesis in rats.

Key Words orotic acid, fatty liver, α-linolenic acid, triacylglycerol, phosphatidate phosphohydrolase

Fatty liver is a disease characterized by the excessive accumulation of triacylglycerol (TG) in the liver and is induced by obesity, diabetes, or excessive consumption of alcohol (1, 2). Fatty liver may lead to the development of atherosclerosis, hypertension, and cirrhosis (2). Orotic acid (OA), an intermediate in pyrimidine nucleotide biosynthesis (3), is abundant in cow’s milk (4). It is well known that the administration of OA causes the accumulation of liver TG in rats (5, 6). Although the mechanism for this accumulation has not been fully clarified, it is believed that a disturbance in very low density lipoprotein synthesis and secretion may be one factor in hepatic TG accumulation (7, 8). We recently reported that enhanced TG synthesis mediated by changes in liver phosphatidate phosphohydrolase (PAP) activity might be involved in the development of fatty liver induced by OA administration (9).

Dietary polyunsaturated fatty acids (PUFAs), especially the n-3 subcategory, have been shown to positively affect atherogenic indices of the lipid metabolism in humans and other animals (10, 11). Epidemiological and clinical studies suggest that n-3 PUFAs might help to prevent coronary heart disease. Most antiatherogenic effects have been demonstrated with the fish oil derived n-3 PUFAs, such as alpha linolenic acid (α-LNA), which can be converted to the longer-chained EPA and DHA (14). Our previous results showed that, compared with dietary LA, dietary α-LNA, EPA, and DHA markedly lowered the concentration of hepatic and serum TG and reduced lipogenesis (15). However, there is no information about the effect of α-LNA on lipid metabolism in OA-induced fatty liver.

In the present study, we compared the effects of dietary n-3 α-LNA and n-6 LA on TG metabolism in OA-induced fatty liver.

MATERIALS AND METHODS

Animals and diets. Male Sprague-Dawley rats aged 4 wk were purchased from Seiwa Experimental Animals (Fukuoka, Japan) and housed individually in an air-conditioned room (24°C) with a 12-h light/dark cycle. After a 1-wk adaptation period, the rats were assigned to four groups (six rats each).

The basal diets were prepared according to recommendations of the AIN-76 and contained (in weight %): casein, 20; fat, 10; cornstarch, 15; vitamin mixture (AIN-76TM), 1; mineral mixture (AIN-76TM), 3.5; DL-Methionine, 0.3; choline bitartrate, 0.2; cellulose, 5; and sucrose, 45. The OA diets were prepared by the supplementation of 1.0% OA to the basal diet at the expense of sucrose. Dietary fats were designed to have a constant ratio (1:1:1) of polyunsaturated to monounsaturated to saturated fatty acids. Dietary fat in the LA diets was composed of a mixture of several vegetable oils (high oleic safflower oil, high linoleic safflower oil, and

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Table 1. Fatty acid composition of experimental diets.

|          | LA (18:2) | a-LNA (18:3) |
|----------|-----------|-------------|
| 14:0     | 0.9       | 0.7         |
| 16:0     | 31.1      | 29.2        |
| 16:1     | 0.1       | 0.1         |
| 18:0     | 3.0       | 3.0         |
| 18:1     | 32.3      | 33.3        |
| 18:2     | 31.9      | 11.5        |
| 18:3     | —         | 22.1        |

Table 2. Growth parameters of Sprague-Dawley rats after 2 wk of feeding.

|                 | LA       | LA+OA    | a-LNA    | a-LNA+OA |
|-----------------|----------|----------|----------|----------|
| Final body weight (g) | 225±5a   | 203±8b   | 233±4a   | 204±2b   |
| Food intake (g/d)     | 16.4±0.5 | 14.9±0.7 | 15.8±0.5 | 15.1±0.3 |
| Liver weight (g/100 g BW) | 4.56±0.1a | 6.37±0.1b | 4.03±0.0a | 5.58±0.2c |

Values are expressed as mean±standard error of six rats.
ab Different letters show significant difference at p<0.05.
Fig. 1. Effect of dietary fatty acids on the concentrations of hepatic lipids. Rats were fed semi-synthetic diets containing either LA or α-LNA with or without 1% OA for 2 wk. Values are expressed as mean±standard error of six rats. See Materials and Methods for composition of diets. abc Different letters show significant difference at p<0.05.

Fig. 2. Effect of dietary fatty acids on the concentrations of serum lipids. Rats were fed semi-synthetic diets containing either LA or α-LNA with or without 1% OA for 2 wk. Values are expressed as mean±standard error of six rats. See Materials and Methods for composition of diets. abc Different letters show significant difference at p<0.05.

Fig. 3. Effect of dietary fatty acids on the activities of hepatic glucose-6-phosphate dehydrogenase and malic enzyme in rats. Rats were fed semi-synthetic diets containing either LA or α-LNA with or without 1% OA for 2 wk. Values are expressed as mean±standard error of six rats. See Materials and Methods for composition of diets. abc Different letters show significant difference at p<0.05.
centrations of serum TG and cholesterol were markedly lowered in the OA-administrated groups compared to the LA groups. In addition to the decline of serum lipids in the α-LNA groups as compared to the LA groups, a further decrease was found in the α-LNA+OA groups as compared to the LA+OA groups. Serum phospholipid levels were significantly lowered by OA administration and dietary α-LNA.

We measured the activities of malic enzyme and G6PDH, which provide the NADPH required for fatty acid synthesis (Fig. 3). OA administration significantly increased G6PDH activity and tended to increase malic enzyme activity. Both LA and α-LNA suppress these fatty acid synthesis-related enzymes (32, 33), and our results showed that α-LNA could reduce the activities more than LA in non-OA-treated rats. Although the activities of malic enzyme and G6PDH tended to be lowered by dietary α-LNA, these activities were not significantly different between groups in OA-treated rats. Thus, it appears the effect of α-LNA on fatty acid synthesis is not as important as its alleviation of OA-induced fatty liver.

We measured the activities of the enzymes PAP and DGAT on the TG synthesis pathway (Fig. 4). OA-induced increases of the soluble and membrane-bound forms of Mg²⁺-dependent PAP activities were seen in the LA+OA group, but these activities were significantly suppressed in the α-LNA+OA group. Neither OA administration nor dietary fatty acids had a significant effect on DGAT activity. In previous studies, the effect of n-3 PUFAs on PAP and DGAT activities was unclear (34–36), although PAP is thought to be the key enzyme in the regulation of TG de novo synthesis (37). In this study, the liver TG concentration was highly and positively correlated with microsomal PAP activity (r=0.898). Therefore, the suppression of PAP activity seems to be one of the most important factors for the alleviation of OA-induced fatty liver by α-LNA. It is also possible that a synchronized reduction of fatty acid synthesis and TG synthesis is required for the alleviation of OA-induced fatty liver by n-3 PUFAs.

In conclusion, our findings revealed that OA-induced hepatic TG accumulation is associated with the enhancement of hepatic TG synthesis. Dietary α-LNA alleviates the OA-induced fatty liver by the attenuation of hepatic TG synthesis in rats.

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