Impact of Dietary Protein on Polyunsaturated Fatty Acid Desaturation in Rats Fed Diets Rich in \( \alpha \)-Linolenic Acid

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Delta 6-desaturase activity and fatty acid composition of liver microsomes were measured periodically in rats fed diets high in \( \alpha \)-linolenic acid (perilla oil) containing either casein (CAS) or soybean protein (SOY) as a protein source. Delta 6-desaturase activity measured by using linoleic acid as a substrate (18:2n-6→18:3n-6) and the linoleic acid desaturation index as estimated by the (20:3+20:4)/18:2 ratio in microsomal phospholipids were significantly higher in rats fed the CAS diet than in those fed the SOY diet at days 4 and thereafter. The proportion of eicosapentaenoic acid (20:5n-3) was also significantly higher in the CAS group than in the SOY group. These results confirmed a differential impact of the dietary protein type on the metabolism of polyunsaturated fatty acids even when rats were fed fat rich in \( \alpha \)-linolenic acid.

The desaturation processes leading from linoleic acid (18:2n-6) and \( \alpha \)-linolenic acid (18:3n-3) to the corresponding highly polyunsaturated fatty acids are of physiological and pathological significance in respect to membrane function and eicosanoid production as well.1,2 Imbalanced eicosanoid synthesis may cause inflammation, thrombosis, atherosclerosis, and immunosuppression.1,2 Various dietary factors influence the desaturation of linoleic acid.3,4 Dietary protein, especially casein (CAS) and soybean protein (SOY), differently influences not only the plasma cholesterol concentration,4–6 but also desaturation of linoleic acid to arachidonic acid.7–10 However, the protein effects have been studied on the metabolism of n-6 polyunsaturated fatty acids (PUFAs), and little attention has been paid to the metabolic consequences of n-3 fatty acids. In this communication, the impact of dietary CAS or SOY on n-6 and n-3 PUFA metabolism was studied in rats fed a diet containing perilla oil, which is rich in \( \alpha \)-linolenic acid.

Materials and Methods

Animals and diets. Male Sprague-Dawley rats (4 weeks old), obtained from Seiwa Experimental Animals, Yoshitomi, were acclimatized for 4 days on a commercial non-purified diet (NMF, Oriental Yeast Co., Tokyo) in a room with controlled temperature (20–22°C) and a 12-h light/dark cycle. They were divided into two groups and fed experimental diets ad libitum for 0, 1, 2, 4, 7, and 14 days. The diets were prepared according to the formula recommended by the American Institute of Nutrition11 and contained in g/100 g diet: protein 20, fat 5, vitamin mixture 1.0 (AIN-76), mineral mixture 3.5 (AIN-76), choline bitartrate 0.2, DL-methionine 0.3, cellulose 5, cornstarch 15, and sucrose to 100. Either casein (Wako Pure Chemicals, Osaka) or soybean protein isolate (Fujipro R, Fuji Oil Co., Osaka) served as the protein source. Perilla oil (Ohta Oil Co., Okazaki) was used as a fat source, and its fatty acid composition is shown in Table I. Vitamin and mineral mixtures were purchased from Oriental Yeast Co., Tokyo. The rats were killed by decapitation without anaesthesia. The liver was then immediately excised. Blood serum was used for lipid and fatty acid analyses.

Microsomal desaturase assay. The Δ6-desaturase activity in liver microsomes was assayed using [1-14C]linoleic acid and [1-14C]α-linolenic acid (59 and 52 mCi/mmole, respectively, New England Nuclear, Boston, MA) by the method of Svensson.12 Under the experimental conditions we used, the desaturation reaction was linear with microsomal protein concentration and incubation time. Microsomal protein was measured by the method of Lowry et al.13

Lipid analyses. Lipids were extracted by the method of Folch et al.14 Liver microsomal phosphatidylcholine (PC) and phosphatidylethanolamine (PE) and serum cholesterol ester were separated by thin-layer chromatography,15–17 and their fatty acid compositions were analysed by gas-liquid chromatography on a Silar 10C column.18 Serum lipids were measured enzymatically by using commercial kits (Cholesterol C-Test, Triglyceride G-Test, and Phospholipid C-Test, Wako Pure Chemicals, Osaka). Liver lipids were measured as described previously.9

Statistics. Data were analyzed by one-way analysis of variance followed by inspection of all differences by Student’s t-test.

Results

Delta 6-Desaturase activity of liver microsomes

The course of the change in Δ6-desaturase activity of liver microsomes is shown in Fig. 1. When the activity was measured using [1-14C]linoleic acid as a substrate, Δ6-desaturase activity (18:2n-6→18:3n-6) became higher in rats fed the CAS diet than in those fed the SOY diet at

<table>
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Table I. Fatty Acids Composition of Dietary Fat and Commercial Non-purified Diet

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Fig. 1. Course of the Changes in \( \delta 6 \)-Desaturase Activity in the Liver Microsomes of Rats Given Different Proteins.

Each point represents the mean \( \pm SE \) for 4 rats. *Significantly different from the casein group at \( p<0.01 \). Difference in pooled values of \( \delta 6 \)-desaturase activity (18:2n-6 \rightarrow 18:3n-6) from days 4 to 14 was significant at \( p<0.05 \).

Fig. 2. Course of the Changes in the Proportion of Linoleic Acid (2A) and Arachidonic Acid (2B), and Linoleic Acid Desaturation Index in Liver Microsomal Phosphatidylincholine (2C) and Serum Cholesterol Ester (2D) of Rats Given Different Proteins.

Each point represents the mean \( \pm SE \) for 4 rats. **Significantly different from the casein group at \( p<0.05 \) and \( p<0.01 \), respectively. Difference in pooled values of the proportion of linoleic acid in phosphatidylincholine, and linoleic acid desaturation index in phosphatidylincholine and cholesterol ester from days 4 to 14 was significant at \( p<0.01 \).

days 4 and thereafter (the difference in the pooled values from days 4 to 14 was significant at \( p<0.05 \)) (Fig. 1A). No such a difference could be seen in the enzyme activity when \([1-^{14}C]2\)-linolenic acid was used as a substrate (18:3n-3 \rightarrow 18:4n-3) (Fig. 1B).

Fig. 3. Course of the Changes in the Proportion of Eicosapentaenoic acid in Liver Microsomal Phosphatidylincholine (3A) and Phosphatidylethanolamine (3B), and the Proportion of Docosapentaenoic Acid (3C) and \( \delta 4 \)-Desaturation Index (3D) in Liver Microsomal Phosphatidylincholine of Rats Given Different Proteins.

Each point represents the mean \( \pm SE \) for 4 rats. *Significantly different from the casein group at \( p<0.05 \). Difference in pooled values of the proportion of eicosapentaenoic acid in phosphatidylincholine, and the proportion of docosapentaenoic acid and \( \delta 4 \)-desaturation index in phosphatidylethanolamine from days 4 to 14 was significant at \( p<0.05 \), \( p<0.01 \), and \( p<0.01 \), respectively.

**Fatty acid composition of liver microsomes**

The course of the changes in the proportion of linoleic acid and arachidonic acid in the liver microsomal PC and serum cholesterol ester is shown in Fig. 2. The proportion of linoleic acid in PC was significantly lower at days 4 and thereafter in the CAS group than in the SOY group (Fig. 2A), while that of arachidonic acid was essentially comparable in these two groups (Fig. 2B). Therefore, the ratio of (20:3n-6 + 20:4n-6)/18:2n-6, the linoleic acid desaturation index, was significantly higher in the CAS group than in the SOY group after 7 days (Fig. 2C). Thus, the changes observed in fatty acid compositions of microsomal PC were consistent with those in the desaturation of linoleic acid to \( \gamma \)-linolenic acid by \( \delta 6 \)-desaturase. No significant protein-dependent change was observed in the n-6 PUFA composition of liver microsomal PE throughout the experimental period (data not shown).

The changes in the proportions of n-3 PUFA, eicosapentaenoic acid (EPA) and docosapentaenoic acid (DPA), are shown in Fig. 3. The proportion of EPA in the liver microsomal PC and PE was increased by feeding perilla oil, and it tended to be higher in the CAS group than in the SOY group (the difference in the pooled values from days 4 to 14 was significant at \( p<0.05 \) in PC but not in PE) (Fig. 3A and 3B). In contrast, the proportion of DPA in PE was lower in the CAS group. Since there was no difference in the proportion of docosahexaenoic acid (22:6n-3), the ratio of 22:6n-3/22:5n-3, the \( \delta 4 \)-desaturation index, was significantly higher in rats fed the CAS diet as compared with those fed the SOY diet (Fig. 3C and 3D).
Fatty acid composition of serum cholesterol ester

The serum cholesterol ester fraction responded to dietary protein more evidently than liver microsomal phospholipids. The proportion of linoleic acid was significantly lower, while that of arachidonic acid was slightly higher in the CAS group than in the SOY group at days 4 and thereafter (mean values from 4 to 14 days were 17.1 ± 0.9 and 25.4 ± 0.4%, p < 0.01 and 22.9 ± 0.5 and 21.1 ± 0.7%, p < 0.05 for the CAS and SOY groups, respectively) and hence, the linoleic acid desaturation index was obviously higher in rats fed CAS than in those fed SOY (Fig. 2D). The proportion of EPA was also higher in the CAS group than in the SOY group (mean values from 4 to 14 days, 24.5 ± 0.8 and 17.9 ± 0.8%, p < 0.01).

Serum and liver lipids

There was a trend toward a decreasing concentration of serum cholesterol in rats fed a SOY diet as compared with those fed a CAS diet, but the difference was not statistically significant (74.6 ± 10.9 and 63.4 ± 3.5 mg/100 ml at days 14). No difference also was observed in the concentration of serum triglyceride and phospholipid between the two groups (data not shown).

In contrast, there was a significant dietary protein-dependent difference in the concentration of liver cholesterol and triglyceride (cholesterol 4.14 ± 0.30 and 2.29 ± 0.11 mg/g, and triglyceride 60.0 ± 13.4 and 11.2 ± 2.5 mg/g for the CAS and SOY groups at days 14, respectively). This was due to a periodic increase in the concentration in the CAS group, while it remained unchanged in the SOY group. The liver phospholipid levels were comparable in the two groups.

Discussion

We have previously shown a dietary protein-dependent difference in the metabolism of linoleic acid in rats fed diets rich in linoleic acid, the most common dietary n-6 PUFA.8,19,20 In this study, the protein effect was confirmed on desaturation of linoleic acid and the fatty acid profile of liver microsomal PC even in rats fed diets rich in ω-linolenic acid, n-3 PUFA. Thus, dietary CAS, in relation to SOY, caused a decrease in the proportion of linoleic acid, but not arachidonic acid. In addition, the proportion of EPA also increased after feeding CAS, indicating a common regulatory mechanism for desaturation of both n-6 and n-3 PUFAs. These effects could be detected within 4 d after starting a perilla oil diet.

However, when we compared the response of the fatty acid compositions after changing from a commercial non-purified diet to the experimental diets, there was a contrasting difference depending on the source of dietary fat. The proportion of arachidonic acid increased when dietary fat was rich in linoleic acid,20 while it decreased when dietary fat rich in ω-linolenic acid was fed (Fig. 2B). The decrease in arachidonic acid and the increase in EPA after starting perilla oil suggest a metabolic interference between n-6 and n-3 fatty acids.21–23 However, the effect of dietary protein on the fatty acid profile still persisted, indicating an inherent nature of the protein effect.

In accordance with the preceding experiments,8,19,20 both Δ6-desaturase activity (18:2n-6→18:3n-6) and the linoleic acid desaturation index of liver microsomes were higher in rats fed the CAS diet than in those fed the SOY diet. Lindholm and Eklund10 suggested that CAS in relation to SOY stimulates the release of arachidonic acid from membrane phospholipids and its subsequent metabolism. Under these situations, desaturation of linoleic acid will be accelerated to an extent that its hepatic pool becomes depleted.10 In fact, the protein-dependent change in the linoleic acid desaturation index was mainly attributable to the change in linoleic acid rather than arachidonic acid. Although the metabolic consequence of these changes in the PUFA profile is not clear, the production of eicosanoids such as prostacyclin and thromboxane A2 tended to be higher in rats fed casein than in those fed soy protein.8 The protein-dependent difference in linoleic acid desaturation can at least partly be attributed to the difference in the amino acid composition of the proteins.24

The proportion of EPA was higher in the CAS group than in the SOY group, but there was no difference in Δ6-desaturation of ω-linolenic acid (18:3n-6→18:4n-3) between the two groups, suggesting an influence of dietary protein on the Δ5-desaturation (20:4n-3→20:5n-3). In addition, the ratio of docosahexaenoic acid (DHA) to docosapentaenoic acid (DPA) in liver microsomal PE was higher in the CAS group than in the SOY group. This effect could be regarded as a stimulatory effect of CAS on the Δ4-desaturation of DPA to DHA as compared with SOY. The Δ5-desaturation reaction also seems to proceed effectively since essentially no intermediate fatty acids between 18:4n-3 and 20:5n-3 were detected.

In conclusion, CAS promoted the desaturation of linoleic acid compared with SOY even in rats fed a diet rich in ω-linolenic acid. The change in the Δ6-desaturase activity reflected that in the n-6 PUFA composition of liver microsomal phospholipid. These effects were observed shortly after feeding different proteins, within 4 days. In contrast, the effects of dietary protein on the metabolism of n-3 PUFAs were diverse.

References

15. H. Takamura, H. Narita, J. H. Park, K. Tanaka, T. Matsuura, and