Succinate Dehydrogenase and Synthetic Pathways of Glucose 6-Phosphate Are also the Markers of the Toxicity of Orally Administered Secondary Autoxidation Products of Linoleic Acid in Rat Liver

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Summary In order to find the markers of the toxicity of the autoxidized lipids in the liver, rats were given a lethal amount of secondary autoxidation products of linoleic acid (400 mg/rat/day for 3 days) and then changes in the hepatic metabolic functions were analyzed. A decrease in acetyl-CoA level to half caused by the depletion of CoASH was reported in an associated paper (J. Nutr. Sci. Vitaminol., 35, 11-23, 1989). Citrate, isocitrate, and 2-oxoglutarate also decreased to half the level of those of the control group. Reduction in isocitrate dehydrogenase activity was only 25%, while NADH2 and ATP levels remained unchanged. Thus, the reduction in the citrate cycle activity was due to the decrease in acetyl-CoA. The activity of mitochondrial succinate dehydrogenase was decreased to 1/5. Other appreciable changes were depletion of glucose 6-phosphate and fructose 6-phosphate, accumulation of glucose 1-phosphate, reductions in hexokinase, phosphofructokinase, glucose-6-phosphatase, phosphoglucomutase, and phosphoglucuronate dehydrogenase activities, and decrease in the NADPH2 level. It was considered that these changes were caused by the depletion of glucose 6-phosphate whose synthetic pathways were abnormal. Therefore, the markers of the hepatotoxicity of secondary products were the changes in the CoASH level and the activities of succinate dehydrogenase and synthetic pathways for glucose 6-phosphate.

Key Words autoxidation products, lipid peroxide, toxicity by oral intake, marker of hepatotoxicity, glucose 6-phosphate, succinate dehydrogenase
Oral intake of autoxidation products of unsaturated fatty acid such as linoleic acid is deleterious for an animal body. When 400 mg/rat/day of secondary autoxidation products was orally administered for 3 days, the rats diet at 30-40 h after the third dose (1). It is important to know the markers in vivo of the toxicity of secondary products. Secondary products administered orally are incorporated into the body, accumulate in the liver unchanged in their forms (2, 3), and severely injure the hepatic functions (4-7). It is, therefore, considered that the markers are the changes in the hepatic metabolic function. Previously (1), changes in the hepatic lipid metabolism was analyzed at 24 h after the third dose of 400 mg/rat/day of secondary products. The levels of CoASH, acetyl-CoA, and long-chain acyl-CoA were decreased to 1/9, 1/2, and 1/4, respectively, and hereby it was concluded that one of the markers was the depletion of hepatic CoASH. The other markers in the liver must be also made clear.

The triple dose of secondary products injured the digestive tract, made absorption of nutrients difficult, and then gave the rats a stress such as starvation (1, 8). Therefore, when attempting to clarify a mechanism of the disturbance in metabolic functions, two factors must be taken into consideration the starvation and the toxicity of secondary products. In the present study, two controls were used, normal and pair-feeding groups. The normal rats were given saline solution 3 times and the pair-feeding rats were fed on limited amounts of diets to put them into a state of starvation. The changes in the activities of hepatic citrate cycle, glycolysis, glyconeogenesis, glycogenolysis, and pentose-phosphate cycle, and the levels of ATP, NADH$_2$, and NADPH$_2$ in the secondary products-dosed group were measured as compared to those in control groups. Reductions in the activities of succinate dehydrogenase and synthetic systems of glucose 6-phosphate were found.

**MATERIALS AND METHODS**

*Secondary autoxidation products of linoleic acid.* Linoleic acid was purchased from Tokyo Kasei Kogyo Co., Ltd., and autoxidized at 37°C for 7 days. Secondary product fraction was obtained from the autoxidized linoleic acid by silica gel column and thin-layer chromatographies, and was characterized as previously shown in detail (1).

*Animals and diet.* Male Wistar rats, 5 weeks old and each weighing about 110 g (KY, SPF: Shizuoka Laboratory Animal Center), were housed in a room in which the light cycle (a light and dark of 12 h each), temperature, and humidity were controlled. A 1-week period was allowed for acclimatization before the animals were used. The diet was prepared daily and its peroxide value was maintained at less than 0.5 meq/kg. The detailed composition of the diets has been described previously (4). Briefly, it consisted of 30% sucrose, 25% casein, 24% corn starch, 15% soybean oil, 4% McCollum’s salt mixture, 1% cellulose powder, and 1% vitamin mixture. The rats were divided at random into three groups of 8 rats each and their foods were withheld for 4 h before treatments. One group was intragastric-
cally given 400 mg/rat/day of secondary products for 3 days. The other two groups were given 400 mg/rat/day of saline solution for 3 days and one of them was fed on a limited amount of diet to use it as a pair-feeding group. After the administrations, the food (except for the pair-feeding group) and water were supplied ad libitum. In order to have the body weights of pair-feeding rats coincide with those of the secondary product group, the diet amount of the pair-feeding rats was reduced to half at the first day, and later the foods were withheld. The animals were sacrificed at 72 h after the start of experiments.

Treatment of the rat liver. The liver was perfused for 10 s with a saline solution using a cannula and then treated immediately according to the freeze-clamped method of Williamson et al. (9). The frozen liver was pulverized and the liver powder was subjected to the following analyses within 24 h.

Levels of metabolites. The liver powder (3 g) was transferred to a tube containing 12 ml of cold 9% (w/v) perchloric acid, and gently homogenized. The homogenate was centrifuged at 30,000×g for 10 min at 4°C. The supernatant was adjusted with 40% KOH to a pH of around 6, allowed to stand for 30 min, and centrifuged to remove the KClO₄ precipitate. Florisil (0.1 g/ml) was added to the supernatant and shaken vigorously. After removing the florisil by centrifugation, clear supernatant was used for spectrophotometric analyses of metabolites: oxaloacetate, malate, fumarate, 2-oxoglutarate, isocitrate, and citrate (10), adenine nucleotides, NADP and NAD, UDP-glucose (11), glycogen, glucose (12), glucose 1-phosphate, glucose 6-phosphate, fructose 6-phosphate, fructose 1,6-bisphosphate, triose 3-phosphate, phosphoenolpyruvate, pyruvate (13), and lactate (14). The other 1 g of liver powder was submitted to alkaline-extraction with 10 ml of 0.5 N KOH in ethanol for assaying of NADPH₂ and NADH₂ levels (11). The metabolite levels were expressed as the totals per whole liver, because the liver weights varied with the treatment group as shown previously (1) (saline group, 9.8±0.6 g; pair-feeding group, 5.8±0.3 g; and secondary product group, 7.2±0.4 g).

Preparation of subcellular fractions. The liver powder (1.5 g) was homogenized with 10 volumes of a 1.15% KCl solution. The homogenate was centrifuged at 700×g for 10 min and the supernatant was further centrifuged at 5,000×g for 20 min. The pellet was suspended in the original volume of the KCl solution and centrifuged again at the same g. The pellet was suspended in 150 mM KCl solution and used as a mitochondrial fraction. The supernatant from 5,000×g was recentrifuged at 24,000×g for 10 min and the supernatant was further centrifuged at 57,000×g for 60 min. The pellet was referred to as microsomal fraction and suspended in 150 mM KCl solution. The supernatant of 57,000×g was recentrifuged at 105,000×g for 60 min and the supernatant was used as a cytosol fraction. The protein contents in these fractions were determined by the method of Lowry et al. (15).

Measurement of enzyme activities. The activities of citrate cycle enzymes, isocitrate dehydrogenase (16), succinate dehydrogenase (17), fumarate hydratase (16), and malate dehydrogenase [EC 1.1.1.37] (16) were spectrophotometri-
cally determined using the mitochondrial fraction. The activities of NADPH$_2$-supplementary enzymes, glucose-6-phosphate dehydrogenase (18), phosphogluconate dehydrogenase (18), malate dehydrogenase [EC 1.1.1.40] (19), and isocitrate dehydrogenase (16) were measured using the cytosol fraction. The activities of phosphoglucomutase (16), hexokinase (16), phosphofructokinase (20), and lactate dehydrogenase (16) were also measured using the cytosol fraction. The activities of microsomal glucose-6-phosphatase and cytosolic fructose-1,6-bisphosphatase were determined by the measurement of the production of inorganic phosphate (21).

**Statistic analysis.** When F-test for homogeneity of variance showed that variances were heterogeneous, Student's t-test was employed to determine the statistical significance. The variability of the data is presented as means ± SE and a 0.05 probability level was chosen.

**RESULTS**

**The activity of hepatic citrate cycle**

Figure 1 shows the metabolite levels of the citrate cycle in the secondary product-triply dosed group and its pair-feeding group when the metabolite levels in the saline group were taken as 100. The pair-feeding rats were not given the diet for more than 2 days and hereby they were in a state of starvation. The levels of acetyl-CoA, which is one of the substrates of citrate cycle, have been determined previously (1). The acetyl-CoA level in the pair-feeding group was half that in the saline group, while citrate and isocitrate recovered to the same levels in the saline group but the other metabolites decreased to the half levels. In the secondary product group, all metabolite levels were significantly low as compared to those in the saline group. When they were compared to those in the pair-feeding group, the acetyl-CoA level was half (1), and the citrate, isocitrate, and 2-oxoglutarate levels were also half, and fumarate, malate, and oxaloacetate levels were at the same level or higher.

Table 1 shows the enzyme activities of the mitochondrial citrate cycle. The enzyme activities in the pair-feeding group remained unchanged as compared to those in the saline group. In the secondary product group, the activity of isocitrate dehydrogenase, which is a rate-limiting enzyme in the citrate cycle, was slightly reduced by 25%, but the activity of succinate dehydrogenase was markedly decreased by 80% as compared to that in the pair-feeding group. Fumarate hydratase and malate dehydrogenase activities remained unchanged. These results showed that the reduction in citrate cycle activity in the secondary product group was due to the decrease in acetyl-CoA level, but the reduction in succinate dehydrogenase activity was not due to this.

**Levels of adenosine nucleotides in the liver**

The reduction in citrate cycle activity is believed to decrease a production of ATP through an oxidative phosphorylation system. Figure 2 shows the levels of
Fig. 1. Comparisons of metabolite levels of the hepatic citrate cycle in the secondary product group and its pair-feeding group with those in the saline group. The levels of metabolites in the saline group were taken as 100 and the levels in the secondary product group (▲) and its pair-feeding group (●) were expressed as percentage. Letters a and b show the significant difference from the saline group and pair-feeding group, respectively (8 rats in each group). The levels of acetyl-CoA were cited from the results in the previous paper (1).

Table 1. Enzyme activities of the mitochondrial citrate cycle.

| Group of rats1 | Saline      | Pair-feeding | Secondary products (nmol/min/mg protein) |
|---------------|-------------|--------------|------------------------------------------|
| Isocitrate dehydrogenase | 36.0 ± 3.0  | 34.9 ± 2.2   | 25.9 ± 1.7a,b                            |
| Succinate dehydrogenase     | 131 ± 10   | 108 ± 12     | 22 ± 13a,b                               |
| Fumarate hydratase          | 213 ± 15   | 197 ± 21     | 167 ± 7a                                 |
| Malate dehydrogenase        | 90 ± 6     | 96 ± 3       | 101 ± 5                                  |

1 Eight rats in each group. a Significant difference from the saline group. b Significant difference from the pair-feeding group.

hepatic adenosine nucleotides. The ATP levels in the secondary product group and its pair-feeding group were lower by 60–70% than that in the saline group and the ADP levels were lower by 35%. However, no differences in ATP and ADP levels
between the groups of secondary products and pair-feeding were detected. Thus, the extent of the decrease in the hepatic ATP level by the triple dose of secondary products was not greater but was in fact similar to that by the starvation.

**Levels of NAD(H) \(_2\) and NADP(H) \(_2\) in the liver**

\(\text{NADH}_2\) mediates between the citrate cycle and the production of ATP. A decrease in the \(\text{NADH}_2\) level in the pair-feeding group was detected (Fig. 3), but the ratios of \(\text{NADH}_2/\text{NAD}\) were similar among the secondary product (0.0925), pair-feeding (0.0754), and saline groups (0.0806). No appreciable change in the levels of \(\text{NADH}_2\) was detected between the secondary product group and its pair-feeding group.

The level of another biological reductant, \(\text{NADPH}_2\), was also determined in the liver. Its level in the secondary product group was markedly lower than the levels in both controls, and the \(\text{NADP}\) level was higher. The ratios of \(\text{NADPH}_2/\text{NADP}\) in the secondary product, pair-feeding, and saline groups were 0.395, 2.73, and 3.38, respectively. In the rat body, most \(\text{NADPH}_2\) was supplied with a pentose phosphate cycle and others were supplied with a malic enzyme and a cytosolic isocitrate dehydrogenase. The phosphogluconate dehydrogenase activity of pentose phosphate cycle in the secondary product group was reduced by 30\%, and the activities of malic enzyme and isocitrate dehydrogenase remained unchanged as compared to those in the pair-feeding group (Table 2). Thus, the marked changes in the \(\text{NADPH}_2\) and \(\text{NADP}\) levels were produced by the triple dose of secondary products, and a part of the changes was caused by the reduction in the pentose phosphate cycle activity.
Fig. 3. Levels of NAD (H<sub>2</sub>) and NADP (H<sub>2</sub>) in the liver. Letters a and b show the significant difference from the saline group and pair-feeding group, respectively (8 rats in each group).

Table 2. Enzyme activities of the NADPH<sub>2</sub>-supplementary system in the cytosol.

| Group of rats<sup>1</sup> | Saline | Pair-feeding | Secondary products (nmol/min/mg protein) |
|--------------------------|--------|--------------|-----------------------------------------|
| Glucose-6-phosphate dehydrogenase | 57.5 ± 9.8 | 28.2 ± 3.8<sup>a</sup> | 22.6 ± 1.1<sup>a</sup> |
| Phosphogluconate dehydrogenase | 54.8 ± 3.4 | 33.7 ± 2.6<sup>a</sup> | 24.1 ± 1.4<sup>a,b</sup> |
| Malate dehydrogenase<sup>2</sup> | 13.4 ± 0.8 | — | 12.6 ± 1.2 |
| Isocitrate dehydrogenase | 51.6 ± 4.2 | — | 47.3 ± 6.0 |

<sup>1</sup> Eight rats in each group. <sup>2</sup> A cytosolic NADP-dependent malic enzyme. <sup>a</sup> Significant difference from the saline group. <sup>b</sup> Significant difference from the pair-feeding group.

Activity in the hepatic glucose metabolism

Pentose phosphate cycle is linked by glucose 6-phosphate to glucose metabolism. Figure 4 shows the metabolite levels of glycogenolysis, and glycolysis and gluconeogenesis in the secondary product group compared with those in its pair-feeding group when the levels in the saline group were taken as 100. Glycogen is one of the energy sources in the rat liver. It is believed that glycogen is exhausted when starvation is continued, and then the glucose 6-phosphate level is decreased. In the pair-feeding rats, the glycogen was exhausted to 7% of that in the saline group, but glucose 6-phosphate remained almost unchanged. On the contrary, in the secondary...
Fig. 4. Comparisons of metabolite levels of the hepatic glycogenolysis and glycolysis in the secondary product group and its pair-feeding group with those in the saline group. The levels of metabolites in the saline group were taken as 100 and the levels in the secondary product group (▲) and its pair-feeding group (●) were expressed as percentage. Letters a and b show the significant difference from the saline group and the pair-feeding group, respectively (8 rats in each group). Abbreviations: UDP-G, UDP-glucose; G-1-P, glucose 1-phosphate; G-6-P, glucose 6-phosphate; F-6-P, fructose 6-phosphate; F-1,6-P, fructose 1,6-bisphosphate; T-3-P, triose 3-phosphate; P-pyruvate, phosphoenolpyruvate.

product group, glycogen was also depleted, and glucose 6-phosphate and fructose 6-phosphate levels were 1/2 and 1/3 of those in its pair-feeding group, respectively.

Glucose 6-phosphate is supplied by glycogenolysis via glucose 1-phosphate, whose accumulation was detected in the secondary product group. Glucose 6-phosphate is also supplied by glycolysis from glucose. The glucose level in the secondary product group was significantly higher than that in its pair-feeding group. Table 3 shows the enzyme activities of these pathways. Phosphoglucomutase activity, which changes glucose 1-phosphate to glucose 6-phosphate, was significantly low in the secondary product group as compared to that in the saline group. Its activity was also lower by 15% than that in the pair-feeding group, but not significantly. Hexokinase activity which produced glucose 6-phosphate from glucose in the secondary product group was 1/3 of that in its pair-feeding group.

The activities of glucose-6-phosphatase and phosphofructokinase, whose substrates are glucose 6-phosphate, in the secondary product group were 1/2 and 1/5 of those in the pair-feeding group, respectively. On the contrary, the activities of fructose bisphosphatase and lactate dehydrogenase of glyconeogenesis which did

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Table 3. Enzyme activities of the hepatic glucose metabolism.

| Group of rats | Saline (nmol/min/mg protein) | Pair-feeding (nmol/min/mg protein) | Secondary products (nmol/min/mg protein) |
|---------------|-----------------------------|-----------------------------------|----------------------------------------|
| Phosphoglucomutase | 78.9 ± 7.9 | 40.2 ± 2.6<sup>a</sup> | 34.6 ± 2.6<sup>a</sup> |
| Hexokinase    | 19.3 ± 1.1 | 13.3 ± 1.8<sup>a</sup> | 4.0 ± 0.6<sup>a,b</sup> |
| Glucose-6-phosphatase | 10.0 ± 0.7 | 12.9 ± 0.6<sup>a</sup> | 7.5 ± 0.4<sup>a,b</sup> |
| Phosphofructokinase | 20.7 ± 4.6 | 20.2 ± 1.5 | 4.2 ± 0.7<sup>a,b</sup> |
| Fructose bisphosphatase | 1.94 ± 0.11 | 2.16 ± 0.14 | 2.21 ± 0.17 |
| Lactate dehydrogenase | 1,218 ± 95 | — | 1,130 ± 83 |

<sup>1</sup> Eight rats in each group. <sup>a</sup> Significant difference from the saline group. <sup>b</sup> Significant difference from the pair-feeding group.

not directly link to glucose 6-phosphate, were normal as compared to those in both controls. The levels of metabolites of glyconeogenesis, fructose 1,6-bisphosphate, triose 3-phosphate, phosphoenolpyruvate, and pyruvate were also normal. Therefore, it is considered that the disturbance in the glucose metabolism was caused by the depletion of glucose 6-phosphate, which was induced by the abnormalities in its synthetic pathways.

DISCUSSION

In order to find the markers in vivo of the toxicity of autoxidized lipids orally administered, a lethal amount of secondary autoxidation products of linoleic acid was given to rats and then changes in the activity of the hepatic carbohydrate metabolism were measured as compared to those in the pair-feeding group.

The level of acetyl-CoA was half, which was caused by the depletion of CoASH as shown previously (1). Citrate level is closely related to the acetyl-CoA level. In the secondary product group, the levels of citrate, isocitrate, and 2-oxoglutarate were reduced to the same extent as the acetyl-CoA level (Fig. 1). Therefore, the decreases in these metabolites may be due to the reduction in the acetyl-CoA level. On the other hand, oxaloacetate is supplied by glycolysis and glyconeogenesis. Glyconeogenesis in the secondary product group was normal, and thus the other metabolite levels remained unchanged (Fig. 4 and Table 3). Subsequently, the decrease in citrate cycle activity was slight (Table 1) and the NADH<sub>2</sub> and ATP levels were not affected (Figs. 2 and 3). If the decrease in citrate cycle activity had disturbed the metabolism, a significant accumulation of acetyl-CoA and decreases in NADH<sub>2</sub> and ATP levels should have been detected. It is considered that the change in the hepatic citrate cycle activity was not the marker for the toxicity of secondary products.

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The reduction in succinate dehydrogenase activity was remarkable (Table 1). Succinate dehydrogenase is one of the enzymes in the citrate cycle, but the reduction in its activity may be another problem. Previously (6), when a single dose of 400 mg of secondary products was administered to rats, the decreases in the activities of isocitrate dehydrogenase and succinate dehydrogenase were 30 and 35%, respectively, as compared to those in the saline group. Here, by the triple dose of secondary products, the reduction in isocitrate dehydrogenase activity was also 25%, but that in succinate dehydrogenase activity was 80% (Table 1). It is considered that mitochondrial succinate dehydrogenase was specifically inactivated by the administration of secondary products. The specific inactivation of succinate dehydrogenase has been reported by Yoshioka et al. (22). They described that the autoxidized lipids destroyed mitochondrial membrane and then the membrane-dependent succinate dehydrogenase leaked from the membrane. On the other hand, our recent study showed that the administrations of autoxidation products of linoleic acid produced no appreciable changes in the fatty acid compositions of membrane phospholipids (7). There is another possibility concerning the inactivation mechanism of succinate dehydrogenase.

Other appreciable changes were detected in the glucose 6-phosphate, fructose 6-phosphate, glucose 1-phosphate (Fig. 4) and NADPH₂ (Fig. 3) levels, and the phosphogluconate dehydrogenase (Table 2), phosphoglucomutase, hexokinase, glucose-6-phosphatase and phosphofructokinase (Table 3) activities. It is considered that these disturbances were caused by the depletion of glucose 6-phosphate. The activity of glucose-6-phosphatase whose substrate was glucose 6-phosphate was reduced. As described previously (6), the depletion of glucose 6-phosphate decreased fructose 6-phosphate level, and later reduced the phosphofructokinase activity. Pentose phosphate cycle also uses glucose 6-phosphate, and the production of NADPH₂ was decreased following the reduction in pentose phosphate cycle activity. Moreover, the dose of secondary products stimulates glutathione peroxidase (1), which consumes NADPH₂ as a reductant. These changes may facilitate exhaustion of NADPH₂.

In the rat, glucose 6-phosphate is supplied mainly from glucose 1-phosphate and glucose. The former is catalyzed by phosphoglucomutase and the latter by hexokinase. The accumulation of glucose 1-phosphate was significant in the secondary product group (Fig. 4). The magnitude of reduction in phosphoglucomutase activity was negligible as compared to that in the starved rats (Table 3), but the inactivation of phosphoglucomutase has been supported by two other pieces of evidence. The single dose of secondary products significantly reduced the phosphoglucomutase activity by 25% as compared to that in the saline group (6), and endogeneous lipid peroxidation caused specific inactivation of hepatic phosphoglucomutase (23). Therefore, it seems likely that the reduction in the phosphoglucomutase activity in the secondary product group was at least in part due to the hepatotoxicity of secondary products. Glucose level was 60% higher than that in its pair-feeding group (Fig. 4), and hexokinase activity was significantly lowered by
70% (Table 3). It is concluded that the depletion of glucose 6-phosphate in the secondary product group was caused by the abnormality of phosphoglucomutase and the inactivation of hexokinase.

On the basis of the present and previous results (1), four toxic effects of the orally administered secondary products were found. One of them was the stress such as starvation caused by the injury of digestive tracts as described in detail previously (1). The other three effects were the depletion of CoASH, inactivation of mitochondrial succinate dehydrogenase, and disturbance of synthetic pathways of glucose 6-phosphate in the liver. We consider that such hepatotoxicity is caused by the aldehydic components of secondary products (24, 25). For example, 9-oxononanoic acid (one of the components) is incorporated into the liver unchanged in the form (2), and may attack the enzyme proteins and SH compounds such as CoASH (26). Benedetti et al. also predicted that aldehydic components, 4-hydroxy nonenals and 4,5-dihydroxydecenal, were toxic in vivo (27). However, other information on the toxicity of autoxidation products of methyl linoleate was reported by Tovar and Kaneda (28). They described that hydroperoxy alkenals were the most toxic components. In order to clear the toxic components, secondary autoxidation products should be fractionated and administered orally to rats, and then the changes in the above targets should be measured. Thereafter, the toxic components will be identified. In our laboratory, this study is in progress.

REFERENCES

1) Kanazawa, K., Ashida, H., Mizuno, M., and Natake, M. (1989): Depletion of hepatic coenzyme A derivatives is one of the markers of the toxicity of orally administered secondary autoxidation products of linoleic acid in rat. J. Nutr. Sci. Vitaminol., 35, 11–23.

2) Kanazawa, K., and Natake, M. (1986): Identification of 9-oxononanoic acid and hexanal in liver of rat orally administered with secondary autoxidation products of linoleic acid. Agric. Biol. Chem., 50, 115–120.

3) Kanazawa, K., Kanazawa, E., and Natake, M. (1985): Uptake of secondary autoxidation products of linoleic acid by the rat. Lipids, 20, 412–419.

4) Kanazawa, K., Ashida, H., Minamoto, S., and Natake, M. (1986): The effect of orally administered secondary autoxidation products of linoleic acid on the activity of detoxifying enzymes in the rat liver. Biochim. Biophys. Acta, 879, 36–43.

5) Ashida, H., Kanazawa, K., and Natake, M. (1987): Decrease of the NADPH level in rat liver on oral administration of secondary autoxidation products of linoleic acid. Agric. Biol. Chem., 51, 2951–2957.

6) Ashida, H., Kanazawa, K., Minamoto, S., Danno, G., and Natake, M. (1987): Effect of orally administered secondary autoxidation products of linoleic acid on carbohydrate metabolism in rat liver. Arch. Biochem. Biophys., 259, 114–123.

7) Ashida, H., Kanazawa, K., and Natake, M. (1988): Comparison of the effects of orally administered linoleic acid, and its hydroperoxides and secondary autoxidation products on hepatic lipid metabolism in rats. Agric. Biol. Chem., 52, 2007–2014.

8) Kanazawa, K., Ashida, H., Minamoto, S., Danno, G., and Natake, M. (1988): The
effects of orally administered linoleic acid and its autoxidation products on intestinal mucosa in rat. J. Nutr. Sci. Vitaminol., 34, 363–373.

9) Williamson, D. H., Lund, P., and Krebs, H. A. (1967): The redox state of free nicotinamide-adenine dinucleotide in the cytoplasm and mitochondria of rat liver. Biochem. J., 103, 514–527.

10) Williamson, J. R., and Corkey, B. E. (1969): Assays of intermediates of the citric acid cycle and related compounds by fluorometric enzyme methods, in Methods in Enzymology, ed. by Lowenstein, J. M., Vol. XIII, Academic Press, New York, pp. 434–513.

11) Jaworek, D., and Welsch, J.; Klingenberg, M.; and Keppler, D., and Decker, K. (1985): Adenosine 5'-triphosphate (UV-method with phosphoglycerate kinase) and adenosine 5'-diphosphate and adenosine 5'-monophosphate (UV method); Nicotinamide-adenine dinucleotides, and dinucleotide phosphates (NAD, NADP, NADH, NADPH) (endpoint UV-methods); and Uridine 5'-diphosphoglucose and uridine 5'-diphosphogalactose, in Methods of Enzymatic Analysis, ed. by Bergmeyer, H. U., Bergmeyer, J., and Grassl, M., Vol. VII, Verlag Chemie, Weinheim, pp. 340–346 and 365–370; pp. 251–271; and pp. 524–530, respectively.

12) Keppler, D., and Decker, K.; and Kunst, A., Draeger, B., and Ziegenhorn, J. (1984): Glycogen, and d-Glucose, in Methods of Enzymatic Analysis, ed. by Bergmeyer, H. U., Bergmeyer, J., and Grassl, M., Vol. VI, Verlag Chemie, Weinheim, pp. 11–18; and pp. 163–172, respectively.

13) Michal, G., and Lamprecht, W., and Heinz, F. (1984): d-Glucose 1-phosphate; d-Glucose 6-phosphate and d-Fructose 6-phosphate; and d-Fructose 1,6-bisphosphate, dihydroxyacetone phosphate and d-glyceraldehyde 3-phosphate; and d-Glycerate 2-phosphate and phosphoenolpyruvate, and Pyruvate, in Methods of Enzymatic Analysis, ed. by Bergmeyer, H. U., Bergmeyer, J., and Grassl, M., Vol. VI, Verlag Chemie, Weinheim, pp. 185–191, 191–198, and 342–350; and pp. 555–561, and 570–577, respectively.

14) Barker, S. B., and Summerson, W. H. (1941): The colorimetric determination of lactic acid in biological material, J. Biol. Chem., 138, 535–554.

15) Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951): Protein measurement with the Folin phenol reagent. J. Biol. Chem., 193, 265–275.

16) Bergmeyer, H. U., Grassl, M., and Walter, H.-E. (1983): Biochemical reagents for general use; Fumarase; Hexokinase; Isocitrate dehydrogenase; l-(+)-Lactate dehydrogenase; Malate dehydrogenase; and Phosphoglucomutase, in Methods of Enzymatic Analysis, ed. by Bergmeyer, H. U., Bergmeyer, J., and Grassl, M., Vol. II, Verlag Chemie, Weinheim, pp. 180–191; 222–223; 230–231; 232–233; 246–247; and 277–278, respectively.

17) Veeger, C., Dervartanian, D. V., and Zeylemaker, W. P. (1969): Succinate dehydrogenase, in Methods in Enzymology, ed. by Lowenstein, J. M., Vol. XIII, Academic Press, New York, pp. 81–90.

18) Glock, G. E., and McLean, P. (1953): Further studies on the properties and assay of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase of rat liver, Biochim. J., 55, 400–408.

19) Ochoa, S. (1955): “Malic” · Enzyme, in Methods in Enzymology, Colowick, S. P., and Kaplan, N. O., Vol. I, Academic Press, New York, pp. 739–753.

20) Massey, T., and Deal, W. C., Jr. (1975): Phosphofructokinases from porcine liver and kidney and from other mammalian tissues, in Methods in Enzymology, ed. by Wood, J. Nutr. Sci. Vitaminol.
W. A., Vol. XLII, Academic Press, New York, pp. 99–110.

21) Swanson, M. A.; and McGilvery, R. W. (1955): Glucose-6-phosphatase from liver; and Fructose-1,6-diphosphatase from liver, in Methods in Enzymology, ed. by Colowick, S. P., and Kaplan, N. O., Vol. II, Academic Press, New York, pp. 541–543; and pp. 543–546, respectively.

22) Yoshioka, M., Tachibana, K., and Kaneda, T. (1974): Studies on the toxicity of the autoxidized oils. IV. Impairment of metabolic functions induced by autoxidized methyl linoleate, Yukagaku (in Japanese), 23, 327–331.

23) Kanazawa, K., Ashida, H., Inoue, N., and Natake, M. (1988): Endogenous lipid peroxidation causes specific inactivation of hepatic phosphoglucomutase, in Medical, Biochemical and Chemical Aspects of Free Radicals, Elsevier Science Publ., B.V., Amsterdam, in press.

24) Minamoto, S., Kanazawa, K., Ashida, H., Danno, G., and Natake, M. (1985): The induction of lipid peroxidation in rat liver by oral intake of 9-oxononanoic acid contained in autoxidized linoleic acid. Agric. Biol. Chem., 49, 2747–2751.

25) Minamoto, S., Kanazawa, K., Ashida, H., and Natake, M. (1988): Effect of orally administered 9-oxononanoic acid on lipogenesis in rat liver. Biochim. Biophys. Acta, 958, 199–204.

26) Esterbauer, H., Ertl, A., and Scholz, N. (1976): The reaction of cysteine with \( \alpha,\beta \)-unsaturated aldehydes. Tetrahedron, 32, 285–289.

27) Benedetti, A., Comporti, M., Fulceri, R., and Esterbauer, H. (1984): Cytotoxic aldehydes originating from the peroxidation of liver microsomal lipids, identification of 4,5-dihydroxydecalenal. Biochim. Biophys. Acta, 792, 172–181.

28) Tovar, G. L. R., and Kaneda, T. (1977): Studies on the toxicity of the autoxidized oils. VI. Comparative toxicity of secondary oxidation products in autoxidized methyl linoleate. Yukagaku (in Japanese), 26, 169–172.