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Effect of Milk Whey and Its Fermentation Products by Lactic Acid Bacteria on Mitochondrial Lipid Peroxide and Hepatic Injury in Bile Duct-ligated Rats

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The study was carried out to assess whether bovine milk whey and its products fermented by lactic acid bacteria could ameliorate the lipid peroxidation of hepatic mitochondria associated with cholestatic liver injury due to bile duct ligation. Rats were maintained on one of five diets for 3 weeks before being operated upon and killed 3 weeks after bile duct ligation. The diets included one deficient in vitamin E (control diet) and others supplemented with either 5% milk whey or 5% milk whey fermented with *Bifidobacterium longum* (*B. longum*), *Lactobacillus acidophilus* (*L. acidophilus*), and *Streptococcus salivarius* subsp. *thermophillus* (*S. thermophillus*). Bile duct-ligated rats, compared with sham-operated rats, had higher organ weights (liver and spleen), higher serum alkaline phosphatase activity, higher serum bilirubin concentration, and higher content of hepatic mitochondrial lipid hydroperoxide. The rats fed on diets containing milk whey fermented with *B. longum* ameliorated the elevation of organ weights, enzyme activity, bilirubin concentration, and content of mitochondrial lipid hydroperoxide. Milk whey and milk whey fermented with *L. acidophilus* and *S. thermophillus* also suppressed the elevation of mitochondrial lipid hydroperoxide, but had no ameliorating effects on organ weights, enzyme activity, and bilirubin concentration. The elevation of serum lipid hydroperoxide was ameliorated in rats fed on diets containing milk whey and milk whey fermented with *B. longum* and *S. thermophillus*. The reduction in plasma α-tocopherol due to bile duct ligation was ameliorated in those rats fed on diets containing milk whey fermented with *B. longum* as well as by *S. themophillus*. These results suggest that a milk whey fermented with lactic acid bacteria exerts a beneficial effect on free radical-mediated hepatic injury.

Although the pathogenesis of hepatic injury during cholestasis is poorly understood, recent observations have suggested that an oxidant or free radical stress may play a role in cholestatic liver injury, since bile acids have been reported to enhance the release of oxygen free radicals from activated rat polymorphonulcear leukocytes,[1] inflammatory cells,[2] and human cholestatic liver lesions.[3] Although it has not been conclusively determined whether lipid peroxidation is involved in hepatic injury, mitochondrial lipid peroxidation in animal models of liver injury has been associated with a wide range of functional consequences that may impair cellular energy metabolism and viability.[4] In fact, Sokol *et al.*[5] have shown that free radical stress occurred after bile duct ligation in rats, and may result in mitochondrial lipid peroxidation. They have also shown that diets high in lipid and deficient in vitamin E may increase the free radical damage to hepatic mitochondria. Relatively little is known, however, on whether the diet can ameliorate mitochondrial hydroperoxidation and hepatic injury caused by cholestasis.

The dietary consumption of fermented milk has been reported to decrease serum cholesterol concentration[6] and to improve such disorders as intestinal diseases[7] and hepatic encephalopathy.[8] In many countries, therefore, there is the widespread belief that fermented milk products like yoghurt, butter milk, and cheese are beneficial to our health, although scientific evidence substantiating this belief is scarce.[9] Inconsistent results with respect to the effect of fermented milk on human health may partly be attributable to differences in experimental design and in the species and/or strains of lactic acid bacteria used for the production of fermented milk. In fact, we have shown that whey preparations prepared from cultured milk by 19 *Lactobacillus* (2 species) and 20 *Bifidobacterium* (5 species) strains affected differently the secretion and synthesis of bile acids in primary cultured rat hepatocytes and *in vivo*.[10]

The aim of this study is to assess directly whether bovine milk whey and its milk wheys fermented with three kinds of lactic acid bacteria are able to ameliorate the peroxidation of hepatic mitochondrial lipids and liver injury in bile duct-ligated rats.

Materials and Methods

Preparation of the cultured milk products. The fermented milk products were prepared by Snow Brand Milk Products Co. (Kawagoe, Japan). Briefly, a medium composed of 12% skim milk powder and 0.3% yeast extract (Asahi Brewery Co., Tokyo, Japan) in deionized water was sterilized at 115°C for 20 min, and aliquots were inoculated separately with *Bifidobacterium longum* (*B. longum*, SBT 2928 and SBT 2933R), *Lactobacillus acidophilus* (*L. acidophilus*, SBT 2062), and *Streptococcus salivarius* subsp. *thermophillus* (*S. thermophillus*, SBT 1035). After incubating at 37°C for 16 h, each medium was centrifuged and the whey was lyophilized. Bovine milk whey was also prepared by Snow Brand Milk Products Co. The fermented milk products contained 1.5–1.9 mg of vitamin C/100 g and 1.6–1.7 mg of vitamin B/100 g. The milk whey contained 6.9 mg of vitamin C/100 g and 1.5 mg of vitamin B/100 g.[12] Since defatted skim milk was used for the fermentation, the milk products seem to have contained a negligible amount of fat-soluble vitamins. In fact, tocopherols

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Table I. Composition of the Experimental Diets (g/100 g)

Ingredient	Dietary group		
	Control	Whey	Fermented whey
Milk whey[1]	—	5	—
Fermented milk whey[2]	—	—	5
Casein[3]	20	19.4	19.4
Safflower oil[4]	10	10	10
Vitamin mixture (without vitamin E)[5]	1.0	1.0	1.0
α-Tocopherol[5]	0.0005	0.0005	0.0005
Mineral mixture[3]	3.5	3.5	3.5
Choline bitartrate[6]	0.2	0.2	0.2
DL-Methionine[5]	0.3	0.3	0.3
Cellulose[7]	5	5	5
α-Corn starch[8]	15	15	15
Lactose[5]	3.5	—	1.1
Sucrose[9]	to 100	to 100	to 100

[1,2] The chemical and amino acid compositions (in wt%) were essentially similar among the milk whey and fermented milk wheys. They contained (in wt%): 0.38–0.42 of crude fat, 7.2–8.3 of protein, 12.1–13.2 of ash, and 71.1–71.8 of sugar. Their amino acid composition was as follows (in wt%): Asp, 8.2–9.2; Thr, 5.2–6.8; Ser, 5.8–6.8; Glu, 18.8–22.4; Pro, 7.2–8.2; Gly, 3.1–3.3; Ala, 4.3–5.2; Cys, 0.5–0.68; Val, 5.5–5.8; Met, 1.5–2.1; Ile, 5.4–5.6; Leu, 7.4–8.3; Try, 1.5–1.9; Phe, 3.5–4.1; Lys, 6.8–7.3; His, 3.0–3.3; Trp, 0.83–1.1; and Arg, 4.3–4.4. The milk whey contained approximately 70% lactose, the figure being approximately 40% in the fermented milk wheys.

[3] Oriental Yeast Co., Tokyo.

[4] Linol-Yusi, Tokyo.

[5] Nacalai Tesque, Kyoto.

[6] Wako Pure Chemical Industries, Osaka.

[7] Nitchiku Medical Industries, Kanagawa.

[8] Nihon Shokuhin Kakou Co., Aichi.

[9] Nishinihon Sugar Manufacturing Co., Fukuoka.

were not detected in the milk products after determination by high-performance liquid chromatography as described later.

Diet and animals. Male Sprague-Dawley rats, 4 weeks old, (Seiwa Experimental Animals Co., Fukuoka, Japan) were maintained on a commercial non-purified diet (NMF, Oriental Yeast Co., Tokyo, Japan) for 2 weeks before starting the experiment in a temperature-controlled room (22–25°C) with a 12 h-light/12 h-dark cycle. A control diet deficient in vitamin E (< 10 IU of all-ras-α-tocopherol acetate per kilogram of diet) was formulated according to the AIN 76^{TM} formula[11] as shown in Table I. To the control diet was added 5% of either milk whey or a fermented milk whey at the expense of sucrose. All the diets were adjusted to give the same content of protein and lactose. The rats were raised on these purified diets for 3 weeks before bile duct ligation.

Bile duct ligation was carried out on the rats under Nembutal anesthesia (5 mg/100 g of body weight) as described previously.[12] Briefly, the common bile duct was located through a midline abdominal incision and double-ligated near the liver with thread (Akiyama Medical Manufacturing Co., Tokyo, Japan). Two ml of sterile saline containing 5000 units of penicillin G was instilled into the peritoneum before the incision was closed. Sham surgery was identical to the ligation procedure, including locating and manipulating the common bile duct, except for ligation. The rats were maintained on the purified diets shown in Table I for another 3 weeks after surgery, the body weight and food intake being measured every other day. The rats were killed by withdrawing blood from the abdominal aorta under light diethyl ether anesthesia, and the liver and spleen were removed. A portion of the fresh liver was used for isolating mitochondria, and the rest was kept at −70°C.

Mitochondria, liver, and blood studies. Hepatic mitochondria were isolated as described previously.[13] Briefly, the liver was homogenized in 15% (w/v) of ice-cold 0.3 M sucrose supplied with 0.003 M EDTA to prevent lipid peroxidation during the subsequent extraction of lipids, and the homogenate was centrifuged at 600 × *g* for 12 min to remove the nucleic fraction. The nuclei-free fraction was then centrifuged at 8,000 × *g* for 10 min to separate the mitochondrial fraction, which was then purified by recentrifugation. Total lipids in the liver, mitochondria, and serum were extracted by the SDS procedure.[14] α-Tocopherol was determined by high-performance liquid chromatography (Waters 600E, Japan Millepore, Tokyo, Japan) according to the method described previously with a slight modification, using a Zorbax SIL column (4.6 mm × 25 cm; Rockland Technologies, U.S.A.) and a mobile phase mixture composed of *n*-hexane–dioxane–isopropanol (985 : 10 : 5, v/v).[15] Lipid hydroperoxide was estimated by measuring the triiodide ions released.[16] Since many substances which are not hydroperoxides may also oxidize iodide ions to triiodide ions,[16] the results are expressed as the amounts of triiodide ions produced. Serum was analyzed for alkaline phosphatase (EC 3.1.3.1) according to the method of Bretaudiere, Spillman *et al.*,[17] and total bilirubin by a commercially available kit (Bilirubin B II-Test, Wako Pure Chemical Industries, Osaka, Japan). Serum cholesterol and triglyceride were determined enzymatically by commercially available kits (Wako Pure Chemical Industries), and the mitochondrial total lipid content was determined gravimetrically.

Statistical analysis. All data are expressed as means ± SE, and statistical differences were determined by Duncan's multiple-range test[18] and Student's *t*-test.[19]

Results

Organs weights, activity of alkaline phosphatase, and concentration of bilirubin in the serum

The type of diet did not affect the food intake and body weight gain of the rats before bile duct ligation (data not shown). There were no differences in food intake and body weight gain after bile duct ligation between the sham and bile duct-ligated rats as shown in Table II. Relative weights of the liver and spleen, the activity of alkaline phosphatase and the concentration of bilirubin in the serum were greater in the bile duct-ligated rats fed on the control diet than in the sham-surgery rats. Among the bile duct-ligated groups, those fed on milk whey fermented with *B. longum* had lower weights for the liver and spleen, lower alkaline phosphatase activity and lower concentration of bilirubin in the serum compared with the rats fed on the control diet. The diets containing milk whey and milk wheys fermented with *L. acidophilus* and *S. thermophilus* did not ameliorate the elevation in organ weights, enzyme activity, or bilirubin concentration.

Hydroperoxide and α-tocopherol

As shown in Fig. 1, the bile duct-ligated rats fed on the control diet had a higher content of mitochondrial lipid hydroperoxide compared with the sham-surgery rats. Among the bile duct-ligated rats, the diets containing milk whey and milk wheys fermented with the three kinds of lactic acid bacteria resulted in a lower content of mitochondrial hydroperoxide when compared with the results for the control diet. Bile duct ligation did not affect the hepatic α-tocopherol content.

As shown in Fig. 2, the concentration of serum hydroperoxide was higher in the bile duct-ligated rats fed on the control diet than that in the sham-surgery rats. Among the bile duct-ligated rats, the diets containing milk whey and milk wheys fermented with *B. longum* and *S. thermophilus* resulted in a lower concentration of peroxide when compared with the results from the control diet. The

Table II. Relative Weights of the Liver and Spleen, Concentration of Total Bilirubin and Activity of Alkaline Phosphatase in the Serum of Sham-operated and Bile Duct-ligated Rats

Group		Food intake (g/day)	Body weight gain (g)	Liver weight (g/100 g of body weight)	Spleen weight	Alkaline phosphatase (U/liter)	Total bilirubin (mg/dl)
Sham	(6)	23.0±0.96	217.4±11.7	3.58±0.09	0.24±0.02	91.9±6.01	0.12±0.01
BDL[1]							
Control	(5)	22.1±0.31	202.1±14.1	6.10±0.44*[a]	0.55±0.05*[a]	202.0±21.69*[a]	6.46±1.31*[a]
Whey	(5)	22.1±1.58	215.0±26.0	5.94±0.56[ab]	0.49±0.07[ab]	181.0±45.00[a]	4.17±2.29[ab]
B. longum	(5)	22.4±1.07	206.3±14.8	4.45±0.52[b]	0.35±0.06[b]	74.0±12.2[b]	0.21±0.02[b]
L. acidophilus	(5)	21.6±0.44	222.8±7.6	5.36±0.45[ab]	0.41±0.05[ab]	179.0±26.11[a]	4.13±1.48[ab]
S. thermophilus	(5)	22.2±0.74	204.0±12.9	5.44±0.39[ab]	0.44±0.04[ab]	240.0±23.55[a]	7.18±1.77[a]

Figures in parentheses show the number of rats.
[1] BDL, bile duct ligation.
* The values for the BDL control are significantly different from those of the sham-operated animals at $p<0.05$.
[ab] Different superscript letters for the BDL group show significant differences at $p<0.05$.

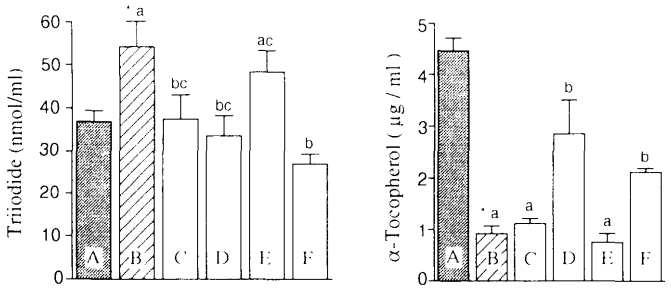

Fig. 1. Content of Mitochondrial Hydroperoxide and Liver α-Tocopherol.

Each bar shows the mean ±SE. A: Sham-operated rats. B, C, D, E, F: Bile duct-ligated rats fed on the control diet, milk whey, and milk wheys fermented with *B. longum*, *L. acidophilus*, and *S. thermophillus*, respectively. *The values for the bile duct-ligated rats fed on the control diet are significantly different from those for the sham-operated rats at $p<0.05$. [ab]Different superscript letters for the bile duct-ligated group show significant differences at $p<0.05$.

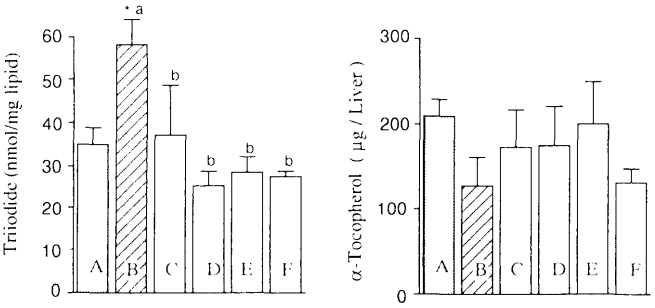

Fig. 2. Concentration of Serum Hydroperoxide and α-Tocopherol.

Each bar shows the mean ±SE. A: Sham-operated rats. B, C, D, E, F: Bile duct-ligated rats fed on the control diet, milk whey, and milk wheys fermented with *B. longum*, *L. acidophilus*, and *S. thermophillus*, respectively. *The values for the bile duct-ligated rats fed on the control diet are significantly different from those for the sham-operated rats at $p<0.05$. [abc]Different superscript letters for the bile duct-ligated group show significant differences at $p<0.05$.

concentration of serum α-tocopherol was lower in the bile duct-ligated rats fed on the control diet than in the sham-surgery rats. Among the bile duct-ligated groups, the diets containing milk wheys fermented with *B. longum* and *S. thermophilus* resulted in a higher concentration of plasma α-tocopherol compared with the results from the control diet.

Table III shows the relationship between the oxidation parameters (lipid hydroperoxide and α-tocopherol) and liver

Table III. Correlation between the Indices of Hepatic Injury, Lipid Peroxidation, and α-Tocopherol

Parameter	Group with bile duct ligation		Group with control diet	
	Bilirubin	Alkaline phosphatase	Bilirubin	Alkaline phosphatase
Mitochondrial hydroperoxide				
r	0.20	0.16	0.65	0.73
p	0.4	0.5	0.012	0.0029
Liver α-tocopherol				
r	−0.64	−0.60	−0.78	−0.69
p	0.0003	0.001	0.0009	0.0066
Serum hydroperoxide				
r	0.41	0.34	0.85	0.86
p	0.04	0.1	0.0004	0.0004
Serum α-tocopherol				
r	−0.66	−0.64	−0.85	−0.80
p	0.001	0.04	0.0005	0.0018

Correlation (r) and its significance (p) were calculated by a linear regression analysis, using data points for the rats with bile duct ligation ($n=25$) and for the rats fed on the control diet with or without bile duct ligation ($n=11$).

injury parameters (alkaline phosphatase and total bilirubin). The control diet-fed rats either with or without bile duct ligation show liver injury indices positively correlating with the lipid hydroperoxide level in mitochondria and serum, and negatively with α-tocopherol in the liver and serum. The bile duct-ligated rats fed on the control, milk whey and fermented milk whey diets show liver injury indices without significant correlation with the mitochondrial hydroperoxide level, although the bilirubin concentration was weakly correlated, but with positive correlation with the serum lipid hydroperoxide concentration. The level of α-tocopherol in the liver and serum was negatively correlated with the liver injury.

Concentration of serum lipids

As shown in Table IV, the bile duct-ligated rats fed on the control diet had a higher concentration of serum cholesterol compared with the sham-surgery rats. The milk whey and fermented milk whey diets did not ameliorate the elevation of serum cholesterol induced by bile duct ligation.

Table IV. Concentration of Serum Cholesterol and Triglyceride in the Sham-operated and Bile Duct-ligated Rats

Group	Cholesterol (mg/dl of plasma)	Triglyceride (mg/dl of plasma)
Sham (6)	160± 7.6	102±13.7
BDL[1]		
Control (5)	200±18.0*	99± 8.0[a]
Whey (5)	199±11.0	92±14.4[ab]
B. longum (5)	173± 9.6	64± 8.8[b]
L. acidophilus (5)	193±13.4	90± 5.7[ab]
S. thermophilus (5)	201±12.1	102±11.4[a]

Figures in parentheses shows the number of rats.
[1] BDL, bile duct ligation.
* The values for the BDL control are significantly different from those of the sham-operated animals at $p<0.05$.
[ab] Different superscript letters for the BDL group show significant differences at $p<0.05$.

Although there was no significant difference in the serum concentration of triglyceride between the sham-operated and bile duct-ligated rats fed on the control diet, the rats fed on the milk whey fermented with *B. longum* exhibited the lowest concentration of triglyceride.

Discussion

In the present experiment, rats were maintained on diets rich in linoleic acid and deficient in vitamin E in order to increase the free-radical mediated lipid peroxidation due to bile acids. Under these conditions, lipid hydroperoxides not only in the liver mitochondria but also in the serum were higher in the bile duct-ligated rats fed on the control diet than in the sham-operated rats fed on the same diet. In the rats fed on the control diet and in the sham-operated rats fed on the control diet with and without bile duct ligation, a positive correlation was observed between the hepatic injury indices (alkaline phosphatase activity and bilirubin concentration in the serum) and the level of hepatic mitochondrial lipid hydroperoxide, and a negative correlation between the hepatic injury indices and the level of hepatic α-tocopherol. These findings support the hypothesis that oxidant damage is in parallel to cholestatic injury to hepatocytes.[5,20]

With the bile duct-ligated groups fed on the control, milk whey and fermented milk whey diets, an inverse correlation was observed between the liver injury indices and α-tocopherol levels in the liver and serum. Such a significantly strong correlation was not apparent for lipid hydroperoxide in the bile duct-ligated groups, except for a weakly positive correlation between the serum hydroperoxide concentration and bilirubin concentration. These results suggest that the level of hepatic α-tocopherol reflects the protection against aggravated cholestatic liver injury. On the contary, Sokol *et al.*[20] have shown that a vitamin E-sufficient diet (50 IU/kg of diet) compared with a vitamin E-deficient diet (<10 IU/kg of diet) did not prevent elevation of the serum alkaline phosphatase activity and bilirubin concentration in cholestatic rats induced by bile duct ligation. Therefore, a preventive mechanism for the loss of hepatic α-tocopherol may be relevant to ameliorating the cholestatic injury to hepatocytes.

The present study shows that milk whey as well as fermented milk wheys were effective for suppressing the elevation of lipid hydroperoxide induced by bile duct ligation. Rats fed on milk whey and its fermentation product diets exhibited in a lower level of mitochondrial hydroperoxide compared with bile duct-ligated rats fed on the control diet. An elevation of serum hydroperoxide was also suppressed in the rats fed on milk whey and its fermentation products, except those rats fed on milk whey fermented with *L. acidophilus*. The reduction of these lipid hydroperoxide levels observed in the rats fed on milk whey and its fermentation products was not accompanied by any amelioration of hepatic injury, except those rats fed on the diet containing milk whey fermented with *B. longum*. In fact, the diet containing milk whey fermented with *B. longum* ameliorated the elevation of organ weights, alkaline phosphatase activity and bilirubin concentration. It is concluded from these results that simply reducing lipid peroxide formation seems not to be insufficient for protecting further liver injury in the bile duct-ligated rats. The reduced liver injury in rats fed on milk whey fermented with *B. longum* raises the possibility that this species may produce an active principle(s) different from the one(s) present in unfermented milk whey.

The present study shows that bile duct ligation resulted in an elevation of serum cholesterol concentration without exhibiting any significant change in the serum triglyceride. Rats fed on the diet containing milk whey fermented with *B. longum* tended to have a lower serum cholesterol concentration compared with the bile duct-ligated rats fed on the control diet, suggesting an ameliorating effect on the hepatic uptake of serum cholesterol associated with lipoproteins.

In summary, the present study indicates that bile duct-ligated rats are a suitable animal model for cholestatic liver injury. It remains to determine the active principle(s) present in the fermented products that protects against lipid peroxide formation and liver injury.

References

1) L. J. Dahm, J. A. Hewett, and R. A. Rothy, *Toxical. Appl. Pharmacal.*, **95**, 83–92 (1988).
2) J. Kountouras, B. H. Billing, and P. J. Scheuer, *Br. J. Exp. Pathol.*, **65**, 305–311 (1984).
3) P. J. Scheuer, in "Liver Biopsy Interpretation," Bailliere Tindall, London, 1980, pp. 36–59.
4) Y. A. Vladimirov, V. I. Olenev, T. B. Suslova, and Z. P. Cheremisina, *Ad. Lipid Res.*, **17**, 173–249 (1980).
5) R. J. Sokol, M. Devereaux, G. W. Mierau, K. M. Hambidge, and R. H. Shikes, *Gastroenterology*, **99**, 1061–1071 (1990).
6) D. R. Rao, C. B. Chawan, and S. R. Pulusani, *J. Food Sci.*, **46** 1339–1341 (1981).
7) C. Beck and H. Nechles, *Am. J. Gastroenterol.*, **35**, 522–530 (1961).
8) A. E. Read, C. F. McCarthy, K. W. Heaton, and J. Laidlaw, *Brit. Med. J.*, **1**, 1267–1269 (1966).
9) P. Rank, *Medical Hypothesis*, **20**, 317–338 (1986).
10) K. Imaizumi, K. Hirata, M. Zommara, M. Sugano, and Y. Suzuki, *J. Nutr. Sci. Vitaminol.*, **38**, 343–351 (1992).
11) Report from the American Institute of Nutrition Ad Hoc Committee on Standards for Nutritional Studies, *J. Nutr.*, **107**, 1340–1348 (1977).
12) G. R. Comeron and C. L. Oakley, *J. Pathol. Bacteripol.*, **35**, 769–798 (1932).
13) R. O. Recknagel and A. K. Ghoshal, *Exp. and Molec. Path.*, **5**, 413–426 (1966).
14) G. W. Burton, A. Weeb, and K. U. Ingold, *Lipids.*, **20**, 29–39 (1985).
15) K. Tanabe, M. Yamaoka, and A. Kato, *Yukagaku*, **30**, 116–118 (1981).

16) G. L. Cramer, J. F. Miller, R. B. Pendleton Jr., and W. E. M. Lands, *Anal. Biochem.*, **193**, 204 211 (1991).
17) J.-P. Bretaudiere and T. Spillman, in "Methods of Enzymatic Analysis," 3d Ed., ed. by H. U. Bergmeyer, Verlag Chemic Gmbh, D-6940 Weinheim, Germany, 1984, pp. 75–82.
18) D. B. Duncan, *Biometrics*, **11**, 1–42 (1955).
19) R. A. Fisher, in "Statistical Methods for Research Workers," 14th Ed., ed. by Oliver and Boyd, Edinburgh, Scotland, 1970, pp. 140–142.
20) R. J. Sokol, M. Devereaux, and A. R. Khandwala, *J. Lipid Res.*, **32**, 1349–1357 (1991).