Comparative Effects of Two Forms of $\gamma$-Oryzanol in Different Sterol Compositions on Hyperlipidemia Induced by Cholesterol Diet in Rats

Sadao NAKAYAMA, Atsufumi MANABE, Junichi SUZUKI, Koji SAKAMOTO and Tetsuya INAGAKI*

Department of Pharmacology, School of Medicine, Showa University, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142, Japan
*Zeria Pharmaceutical Co., Ltd., 10-11 Nihonbashikobuna-cho, Chu-o-ku, Tokyo 103, Japan

Accepted February 20, 1987

Abstract—Hypolipidemic effects of the usual $\gamma$-oryzanol ($\gamma$-OZ) and a new $\gamma$-OZ (N-$\gamma$-OZ) with a different sterol composition from $\gamma$-OZ were investigated on the hyperlipidemia induced by ingestion of a high cholesterol diet (HCD) containing 1% cholesterol for 12 days in male Sprague-Dawley rats. Treatment with $\gamma$-OZ for 6 days significantly inhibited the increase in serum total cholesterol (TC) and phospholipids (PL) induced by HCD, while the treatment with $\gamma$-OZ for 12 days did not inhibit the increase of TC and PL. Treatment with N-$\gamma$-OZ at 100 or 1000 mg/kg for 6 days slightly inhibited the increase of TC by HCD. The decrease of TC in high density lipoprotein (HDL-TC) was markedly inhibited by treatment with N-$\gamma$-OZ for 12 days, but N-$\gamma$-OZ for 6 days and $\gamma$-OZ for 6 and 12 days did not inhibit the decrease of HDL-TC. Treatment with N-$\gamma$-OZ for 12 days significantly inhibited the increase of PL and free cholesterol (FC) by HCD. $\gamma$-OZ at 1000 mg/kg for 12 days also inhibited the increase of FC. N-$\gamma$-OZ significantly reduced the atherogenic index using TC and HDL-TC by affecting the HDL-TC increase. $\gamma$-OZ at 1000 mg/kg for 6 days reduced the atherogenic index using TC and HDL-TC by the inhibition of TC increase. The atherogenic index using PL and HDL-PL was only reduced by the treatment with N-$\gamma$-OZ at 1000 mg/g for 12 days. The increase of triglyceride (TG) by HCD was inhibited by the treatment of N-$\gamma$-OZ for 6 days (all doses) and 12 days (500, 1000 mg/kg), and $\gamma$-OZ at 500 mg/kg for 6 and 12 days also inhibited the increase of TG by HCD. $\gamma$-OZ and N-$\gamma$-OZ had no effects on liver lipid contents. The hypolipidemic effect of N-$\gamma$-OZ was slightly more potent than that of $\gamma$-OZ. The inhibition of decrease in HDL-TC and increase in FC by HCD in the treatment with N-$\gamma$-OZ was more potent than that of $\gamma$-OZ, and these effects of N-$\gamma$-OZ may be related to the acceleration of HDL function in the serum.

$\gamma$-Oryzanol ($\gamma$-OZ) is used in the treatments of menopausal disorders and autonomic nerve imbalance and for the removal of stress (1, 2). The hypolipidemic effect of $\gamma$-OZ has been studied experimentally and clinically (3-7). Kuzuya et al. (3) has reported that $\gamma$-OZ inhibits an increase of total cholesterol (TC) and a formation of serum lipid peroxide induced by the feeding of a 0.1% cholesterol diet to rats. In the examination of lipid metabolism in high cholesterol diet (1% cholesterol in normal chow diet containing 0, 0.5 and 2% $\gamma$-OZ) -fed rats, $\gamma$-OZ showed an increase of TC in high density lipoprotein (HDL-TC), and it inhibited increases of esterified cholesterol (EC) and triglyceride (TG) in serum, TC in liver and acyl Co A cholesterol acyl transferase activity
in aorta by the feeding of the high cholesterol diet for 4 to 13 weeks (4, 5). It has also been reported that the treatment of \( \gamma \)-OZ for 12 to 16 weeks in the hyperlipidemia of humans showed decreases of TC and TG and an increase of HDL-TC (6, 7).

\( \gamma \)-OZ is a ferulic acid (4-hydroxy-3-methoxycinnamic acid) ester of sterols (cycloartenol, 24-methylene cycloartenol, cyclobroanol and other sterols). New \( \gamma \)-OZ (N-\( \gamma \)-OZ), which has a different sterol composition than \( \gamma \)-OZ, used in the present study also is a ferulic acid ester of sterols.

In the present paper, the effect of N-\( \gamma \)-OZ on the hyperlipidemia induced by a high cholesterol diet (HCD) in rats was compared with that of \( \gamma \)-OZ.

**Materials and Methods**

Male Sprague-Dawley strain rats, 5 weeks of age and weighing about 100 g, were used. Control rats were fed commercial food pellets (Nippon Clea Co., Ltd., Tokyo). Rats were made hyperlipidemia by HCD. The composition of HCD is as follows: 1% cholesterol, 1% cholic acid, 20% casein, 50% sucrose, 12% coconut hardened oil, 4% cellulose, 4% minerals (29.29% CaCO\(_3\), 0.43% CaHPO\(_4\)
\( \bullet \) 2H\(_2\)O, 34.31% KH\(_2\)PO\(_4\), 25.06% NaCl, 0.623% Fe(C\(_6\)H\(_5\)O\(_7\))
\( \bullet \) 6H\(_2\)O, 0.0025% (NH\(_4\))\(_6\)Mo\(_7\)O\(_{24}\)
\( \bullet \) 4H\(_2\)O, 0.516% CuSO\(_4\)
\( \bullet \) 5H\(_2\)O, 0.12% MnSO\(_4\)
\( \bullet \) H\(_2\)O, 0.020% ZnCl\(_2\), 9.98% MgSO\(_4\)
\( \bullet \) 7H\(_2\)O, 0.0005% KI), 0.5% vitamins (5.0 mg thiamine hydrochloride, 5.0 mg riboflavin, 25.0 mg nicotinic acid, 20.0 mg calcium pantothenate, 2.5 mg pyridoxine hydrochloride, 0.5 mg menadione, 0.1 mg biotin, 0.2 mg folic acid, 0.02 mg vitamin B\(_12\), 100.0 mg inositol, 50.0 mg vitamin C, 4000 I.U. vitamin A, 2000 I.U. vitamin D, 150 I.U. vitamin E and choline chloride, 1500 mg/kg of diet) and 7.5% white fishmeal.

\( \gamma \)-OZ and N-\( \gamma \)-OZ were obtained from Zeria Pharmaceutical Co. Ltd., (Tokyo). The sterol composition of \( \gamma \)-OZ is as follows: 35% cycloartenol, 45% 24-methylene cycloartenol, 10% cyclobroanol and 10% other sterols. The sterol compositions of N-\( \gamma \)-OZ is as follows: 60% cycloartenol, 30% 24-methylene cycloartenol and 10% cyclobroanol.

\( \gamma \)-OZ and N-\( \gamma \)-OZ were suspended in a vehicle (0.06% carboxy methylcellulose, 0.004% propylparaben, 0.2% polyvinyl-alcohol, 99.73% saline) and administered by gavage at doses of 100, 500 and 1000 mg/kg/4 ml volume once a day for 12 days with HCD feeding. Six rats in each group were sacrificed at the end of the 7th and 13th day. Serum was obtained by centrifuging the blood at 2500 r.p.m. for 15 min. Liver lipids were extracted by the method of Bragdon (8).

TC in serum and liver and HDL-TC in serum were determined by a colorimetry method (Cholesterol-B Test) (9). Phospholipids (PL) and HDL-PL in serum were determined enzymatically (Phospholipid-B Test) (10). PL in the liver was determined by the method of Wako (Phospholipids Test) (11). Free cholesterol (FC) in the serum and TG in the serum and liver were determined enzymatically (Free cholesterol-C Test, Tri-glyceride-G Test) (12, 13). Activities of transaminase (GOT, GPT) in serum were determined by the colorimetry method (Transaminase-C Test) (14). Total protein (TP) and albumin in serum were determined by the Buret method (15) and the bromocresol green method (A/G Test) (16). HDL-TC and HDL-PL in serum were separated by the dextran sulfate-magnesium precipitation method (17). These assays were performed using commercial kits (Wako Pure Chemical Ind. Ltd., Tokyo). TP in the liver was determined by the method of Lowry et al. using bovine serum albumin as a standard (18). In the histological examination, a part of each liver sample was fixed with 10% formalin solution, and it was sectioned and embedded in paraffin. Each section was stained with hematoxylineosin and was examined under light microscopy.

**Results**

**Body weight and liver weight:** Changes in the body weight and liver weight are shown in Fig. 1. Control animals gained weight steadily throughout the experimental period. The animals fed HCD for 12 days showed a significant inhibition of body weight gain. Treatment with N-\( \gamma \)-OZ at 1000 mg/kg daily for 6 days apparently increased the body weight in comparison to the HCD fed group. N-\( \gamma \)-OZ at 500 and 1000 mg/kg for 12 days
Fig. 1. Effects of r-ΟΖ and N-r-ΟΖ on body and liver weight of hyperlipidemia induced by a high cholesterol diet (HCD) in rats. Each column represents the mean±S.D. of 5 to 6 rats. △: duration of treatment for 6 days, □: duration of treatment for 12 days, Cont.: fed a normal diet, Cholesterol diet: fed a 1% cholesterol diet (HCD). △: Significantly different from the control group (P<0.01), △ and △△: Significantly different from the cholesterol diet group (P<0.05 and P<0.01).

The liver weight increased at 6 and 12 days after the feeding of HCD. Treatment with r-ΟΖ at 500 mg/kg for 6 days inhibited the increase of liver weight by HCD. Treatment with N-r-ΟΖ at 500 and 1000 mg/kg and r-ΟΖ at 100 and 1000 mg/kg for 12 days increased the liver weight as compared to the weight of the HCD fed group.

Lipid contents in the serum: The contents of TC in the serum were significantly increased by the feeding of HCD for 6 and 12 days (Fig. 2). Treatment of N-r-ΟΖ for 6 days and r-ΟΖ for 6 and 12 days did not significantly inhibit the increase of TC contents, but a marked inhibition was caused by the treatment of r-ΟΖ for 12 days. Treatment with r-ΟΖ at 100 mg/kg for 12 days slightly, but not significantly, inhibited an increase of TC content by the feeding of HCD. r-ΟΖ at 500 and 1000 mg/kg for 12 days had no effect on the TC content.

Treatment of N-r-ΟΖ and r-ΟΖ for 6 and 12 days did not inhibit the decrease of HDL-TC by HCD. N-r-ΟΖ treatment for 12 days significantly inhibited the decrease of HDL-TC contents caused by the feeding of HCD.

Changes of the PL and HDL-PL in serum are shown in Fig. 3. PL content in the serum was markedly increased by the feeding of HCD as compared to the control. The increase in PL contents was significantly inhibited by the 6 day-treatment with N-r-ΟΖ (100 mg/kg) and r-ΟΖ (500, 1000 mg/kg) and 12 day-treatment with N-r-ΟΖ (100 to 1000 mg/kg), but 12 day-treatment with r-ΟΖ had no effect on the increase in PL.

Treatment of N-r-ΟΖ and r-ΟΖ for 6 and 12 days did not inhibit the decrease of HDL-PL by HCD (Fig. 3).

Changes of the atherogenic index using HDL-TC and HDL-PL (TC/HDL-TC, PL/HDL-PL) are shown in Fig. 4. The atherogenic index can be increased by the increments of TC and PL and
the decreases of HDL-TC and HDL-PL in the HCD feeding. Treatment with N-γ-OZ at 100 mg/kg for 6 days and 1000 mg/kg for 12 days showed a slight, but not significant,
Effects of γ-OZ and N-γ-OZ on the atherogenic index using HDL-TC and HDL-PL of hyperlipidemia induced by HCD in rats. A: Significantly different from the control group (P<0.001), AA and AAA: Significantly different from the cholesterol diet group (P<0.05, P<0.01 and P<0.001). See explanation in Fig. 1.

decrease of TC and a significant increase of HDL-TC, resulting in a reduction of the atherogenic index (TC—HDL-TC/HDL-TC). Treatment with N-γ-OZ at 500 mg/kg for 12 days also showed a reduction of the atherogenic index using HDL-TC by the increase of HDL-TC, but at doses of 100 mg/kg for 12 days and 500 and 1000 mg/kg for 6 days, N-γ-OZ did not cause a reduction of the atherogenic index because these doses of N-γ-OZ did not inhibit the increase of TC content by HCD feeding. The increases in PL contents were significantly inhibited at doses of N-γ-OZ and γ-OZ such as those shown in Fig. 3, but the reduction of the atherogenic index using HDL-PL was only found with the treatment with N-γ-OZ at 1000 mg/kg for 12 days.

The contents of FC in the serum were increased by the feeding of HCD for 6 and 12 days (Fig. 5). The increase in FC contents was not inhibited by the treatment with N-γ-OZ and γ-OZ for 6 days. Treatment with N-γ-OZ at all the doses used and γ-OZ at 1000 mg/kg for 12 days significantly inhibited the increase of FC by HCD feeding.

Changes of TG contents in serum are shown in Fig. 5. The increase in the TG contents induced by HCD feeding was markedly inhibited by the treatment of N-γ-OZ at all the doses used for 6 days and at 500 and 1000 mg/kg for 12 days, but the inhibition by γ-OZ was only observed with the treatment of 500 mg/kg for 6 days.

Serum TP content was increased by the feeding of HCD. N-γ-OZ and γ-OZ treatment for 6 and 12 days did not change the TP contents in the HCD fed-rats (Fig. 6). Serum albumin contents did not change by the feeding of HCD. The contents of serum albumin was increased with N-γ-OZ treatment at all doses for 12 days compared to the control and HCD fed groups (Fig. 6).

Transaminase levels in the serum: The levels of GOT in the serum were significantly increased by the feeding of HCD for 6 and 12 days, while a significant increase of GPT levels was found by the feeding of HCD for 6 days only. Treatment with N-γ-OZ for 6 and 12 days did not cause significant inhibition on the increase of GOT and GPT levels by HCD. At 500 mg/kg γ-OZ for 6 and 12 days inhibited the increase of GOT level by HCD; furthermore, at 500 mg/kg for 6
Fig. 5. Effects of γ-OZ and N-γ-OZ on FC and TG in the serum of hyperlipidemia induced by HCD in rats. FC: serum-free cholesterol, TG: serum triglyceride. △: Significantly different from the control group (P<0.001), ▲, ▲▲ and ▲▲▲: Significantly different from the cholesterol diet group (P<0.05, P<0.01 and P<0.001). See explanation in Fig. 1.

Fig. 6. Effects of γ-OZ and N-γ-OZ on TP and albumin in the serum of hyperlipidemia induced by HCD in rats. TP: serum total protein. ▲ and ▲▲: Significantly different from the control group (P<0.05 and P<0.001), ▲ and ▲▲: Significantly different from the cholesterol diet group (P<0.05 and P<0.001). See explanation in Fig. 1.

days, it caused a significant decrease in the GPT level. Treatment of γ-OZ at 1000 mg/kg for 12 days showed a significant increase of the GPT level (Fig. 7).

Lipid and TP contents in liver: Increases in TC and TG contents and decrease in PL.
Effects of r-OZ on Hyperlipidemia

Fig. 7. Effects of r-OZ and N-r-OZ on transaminase (GOT, GPT) in serum of hyperlipidemia induced by HCD in rats. A and AA: Significantly different from the control group (P<0.01 and P<0.001), A: Significantly different from the cholesterol diet group (P<0.05).

content in liver were observed in all of the HCD fed rats. TC, TG and PL content in the liver of rats fed a normal diet for 12 days were 10.6 mg/g, 0.66 mg/g and 42.0 mg/g, respectively. The feeding of HCD for 12 days increased TC content to 45.8 mg/g, TG content to 10.13 mg/g and PL content to 38.1 mg/g. Treatments with N-r-OZ and r-OZ had no effect on the contents of TC, TG and PL in the liver (data not shown).

The feeding of HCD and the treatments with N-r-OZ and r-OZ for 6 and 12 days did not cause noticeable changes of TP contents in the liver (data not shown).

Histological observations: Diffuse fatty degeneration was found in the liver of HCD-fed rats. The steatosis by HCD was not improved by the treatments of N-r-OZ and r-OZ (photo not shown).

Discussion

It is interesting that the inclusion of phytosterols as a component of the chemical structure in the r-OZ may be related to the effect of this drug on hyperlipidemia.

In the present study, the treatment with r-OZ for 6 days inhibited the increase of serum TC content by HCD feeding. This result of r-OZ is similar to the report of Kuzuya et al. (3). Shinomiya et al. (4) reported that the content of EC was reduced by the admixed-treatment of r-OZ in the cholesterol diet on the cholesterol-induced hyperlipidemia in rats. Our results is at variance with the observation of Shinomiya et al. It may be related to the difference of the treatment routes in both studies. Adsorption in the treatment of r-OZ by gavage may be lower than that by the treatment by admixture; therefore, treatment of r-OZ for 12 days did not inhibit the increase of TC content by HCD. Effect of r-OZ on hyperlipidemia induced by HCD was dose-independent, and it also may be related to the absorption of r-OZ. N-r-OZ showed slight, but not significant, decrease of TC content. The reduced effect of N-r-OZ on the TC content in hyperlipidemic rats was found to be weaker than that of r-OZ. These results suggest that the inhibition of r-OZ on TC absorption in the intestine and the acceleration of serum TC excretion is more potent than that of N-r-OZ; it may be due to the different sterol compositions of r-OZ and N-r-OZ.

The contents of HDL-TC and HDL-PL in the serum were significantly decreased by the feeding of HCD in the experimental animals (19–21). A few reports (3–5) have
shown that \( r \)-OZ does not inhibit the decrease of HDL-TC by the feeding of HCD. The reduction of serum TC levels by the treatment with \( r \)-OZ was in parallel with the decrease of serum HDL-TC; therefore, the atherogenic index was not improved (3). In the present study, the atherogenic index using HDL-TC significantly reduced in the treatment with N-\( r \)-OZ at 100 mg/kg for 6 days and at 500 and 1000 mg/kg for 12 days since there was a slight decrease in TC and an increase in HDL-TC. Only the treatment with \( r \)-OZ at 100 mg/kg for 6 days showed a reduction of atherogenic index using HDL-TC. N-\( r \)-OZ at 100 mg/kg for 6 days at all doses used for 12 days and \( r \)-OZ at 500 and 1000 mg/kg for 12 days inhibited the increase of PL content by HCD, while the decrease of HDL-PL content was not inhibited. In the above results, the reduction of atherogenic index using HDL-PL was only shown by the treatment of N-\( r \)-OZ at 1000 mg/kg for 12 days. The reduction of atherogenic index is significantly noticeable under the conditions where there are both increases in HDL contents and decreases of TC and PL (22). The effects of N-\( r \)-OZ and \( r \)-OZ on the atherogenic index were related to the changes in HDL, TC and PL contents.

The inhibitions by N-\( r \)-OZ of the decrease in HDL-TC and the increases in FC and TG were found to be greater than of \( r \)-OZ. The decrease of HDL-PL content by HCD was not inhibited by the treatment of N-\( r \)-OZ. It was indicated that the increase of HDL-TC by N-\( r \)-OZ does not necessarily mean an increase of HDL levels. The increase of HDL-TC content by N-\( r \)-OZ suggests the increase in the transformation of FC into EC rather than the acceleration of HDL synthesis in the liver since the decrease of serum FC is in parallel with the increase of HDL-TC content. These results suggest that the hypolipidemic effect of N-\( r \)-OZ on the hyperlipidemia induced by HCD may be more potent than that of \( r \)-OZ.

The GPT levels were markedly decreased by the treatment with \( r \)-OZ at 500 mg/kg for 6 days, while these levels were significantly increased by \( r \)-OZ at 1000 mg/kg for 12 days. The changes of GOT and GPT levels by feeding of HCD and treatments with N-\( r \)-OZ and \( r \)-OZ were not related to the increases of liver weight and lipid levels and observations on dissected specimens, therefore, the mechanism of \( r \)-OZ on the changes of GPT levels are still not clarified.

N-\( r \)-OZ and \( r \)-OZ had no effect on the lipid contents and fatty degeneration in the liver. Shinomiya et al. (4, 5) reported a decrease of liver EC and TG in addition to the decrease in serum TC levels by admixed treatment of \( r \)-OZ for 4 to 13 weeks. In our study, the reason for the negative effects of N-\( r \)-OZ and \( r \)-OZ on the liver lipids seemed to be the short duration of these treatments and the difference of treatment routes in both studies.

From the results in the present study, it was suggested that the hypolipidemic mechanisms of \( r \)-OZ in HCD-induced hyperlipidemia were mainly inhibition of cholesterol absorption in the intestine and acceleration of cholesterol excretion from the serum, while that of N-\( r \)-OZ were an improvement of lipid metabolism such as transformation of FC into EC and decrease of TG in the serum.

With respect to the sterol compositions, the inclusion of cycloartenol in N-\( r \)-OZ is greater than in \( r \)-OZ. The hypolipidemic effect and HDL-TC increase by N-\( r \)-OZ were more potent than that of \( r \)-OZ, and it may be due to the concentration of cycloartenol as an inclusive ratio of sterols. From the results showing the increase of HDL-TC and decrease of FC in the serum, it was suggested that the HDL function was accelerated by the treatment of N-\( r \)-OZ.

References
1 Murase, Y. and Ishima, H.: Clinical studies of oral administration of \( r \)-oryzanol on climatic complaints and its syndrome. Obstet. Gynecol. Pract. 12, 147–149 (1963) (in Japanese)
2 Sasagawa, T., Kimura, A., Nee-Chouhou, Sano, M. and Kojima, H.: Clinical studies on \( r \)-oryzanol in treatment of gastro-entero neuritis. Mainly subjecting the autonomic instability with gastrointestinal symptoms. Basic Pharmacol. Ther. 4, 588–591 (1976) (in Japanese)
3 Kuzuya, F., Yoshimine, N., Kato, S., Fujita, K. and Uchigome, H.: Effects of \( r \)-oryzanol on experimental hyperlipidemia in rats. Geriat. Med. 18, 519–524 (1980) (in Japanese)
4 Shinomiya, M., Morisaki, N., Matsuoka, N.,...
Effects of γ-OZ on Hyperlipidemia

Izumi, S., Saito, Y., Kumagai, A., Mitani, K. and Morita, S.: Effects of γ-oryzanol on lipid metabolism in rats fed high-cholesterol diet. Tohoku J. Exp. Med. 141, 191–197 (1983)

5 Shinomiya, M., Morisaki, N., Fujiyama, Y., Shirai, K., Saito, Y., Kumagai, A., Matsuoka, N., Murano, S., Mitani, K. and Morita, S.: Effect of γ-oryzanol on the lipid metabolism in high cholesterol diet-administered rats. J. Japan. Atheroscler. Soc. 10, 1069–1075 (1983) (Abs. in English)

6 Osawa, A. and Kanō, Y.: Hypolipidemic effects of γ-oryzanol. J. Japan. Atheroscler. Soc. 10, 1077–1079 (1983) (Abs. in English)

7 Saito, Y., Kumagai, A., Morisaki, N. and Matsuoka, N.: Effects of γ-oryzanol on lipid metabolism. Basic Pharmacol. Ther. 8, 2839–2842 (1980) (in Japanese)

8 Braden, J.H.: Method for determination of total serum lipids. In Lipids and Steroid Hormones in Clinical Medicine, Edited by Sunderman, F.W., p. 9–14, Lippicott, Philadelphia and Montreal (1960)

9 Zlatkis, A. and Zak, B.: Study of a new cholesterol reagent. Anal. Biochem. 29, 143–148 (1968)

10 Takayama, M., Itoh, S., Nagasaki, T. and Tanimizu, I.: A new enzymatic method for determination of serum choline-containing phospholipids. Clin. Chem. Acta 79, 93–98 (1977)

11 Yamanishi, K., Ohashi, M. and Saito, M.: Direct colorimetry for determination of serum phosphorus. J. Clin. Pathol. 17, Supp., p. 92 (1969) (in Japanese)

12 Allain, C.C., Poon, L.S., Chan, C.S.G., Richmond, W. and Fu, P.C.: Enzymatic determination of total serum cholesterol. Clin. Chem. 20, 470–475 (1974)

13 Bucolo, G. and David, H.: Quantitative determination of serum triglycerides by the use of enzymes. Clin. Chem. 19, 476–482 (1973)

14 Ohkawa, J., Miyoshi, Y., Matsuura, S. and Misaki, H.: A new colorimetric method for the determination of transaminase activity using pyruvate oxidase. Japan. J. Clin. Pathol. 26, Supp., p. 70 (1978) (in Japanese)

15 Weichselbaum, T.E.: An accurate and rapid method for the determination of proteins in small amounts of blood and plasma. Am. J. Clin. Pathol. 16, 40–49 (1946)

16 Doumas, B.T., Watson, W.A. and Biggs, H.G.: Albumin standards and the measurement of serum albumin with bromocresol green. Clin. Chem. Acta 31, 87–86 (1971)

17 Finley, P.R., Schifman, R.B., Williams, J. and Lichti, D.A.: Cholesterol in high density lipoprotein; Use of Mg²⁺/dextran sulfate in its enzymatic measurement. Clin. Chem. 24, 931–933 (1978)

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

19 Tammi, M., Ronnemaa, T. and Viikari, J.: Rapid increase of glycosaminoglycans in the aorta of hypercholesterolemic rats; a negative correlation with plasma HDL concentration. Acta Physiol. Scand. 105, 188–194 (1979)

20 Ohnishi, H., Ito, C., Suzuki, K., Niho, T., Imazumi, Y., Yamazaki, Y., Morishita, S., Shimura, M. and Ito, R.: Effects of trapidil on various experimental hyperlipemias. Folia Pharmacol. Japon. 76, 469–477 (1980) (Abs. in English)

21 Nakayama, S., Sakashita, M., Nishimura, T. and Sakamoto, K.: Variation of lipids in rats fed a cholesterol diet. Folia Pharmacol. Japon. 78, 91–107 (1981) (Abs. in English)

22 Nakayama, S., Negishi, K. and Sakamoto, K.: Effects of soysterol, pantethine and dl-α-tocopheryl nicotinate on hyperlipemia in rats. Folia Pharmacol. Japon. 78, 191–202 (1981) (Abs. in English)