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Influence of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors on mitochondrial respiration in rat liver during ischemia

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Abstract

Effects of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, pravastatin and simvastatin, on mitochondrial respiration in ischemic rat liver were examined. Either vehicle, pravastatin (2 or 4 mg/kg per day), or simvastatin (1 or 2 mg/kg per day) was orally administered for 3 weeks. Liver ischemia was induced by cessation of the systemic circulation for 60 min. Liver mitochondria were isolated and the respiration was determined by polarography using glutamate and succinate as substrates. In the vehicle-treated group, ischemia decreased QO_3, respiratory control index (RCI; QO_3/QO_4), and ADP/O ratio. Pretreatments with pravastatin and simvastatin enhanced the decreases in QO_3 measured with either glutamate or succinate, and in ADP/O ratio measured with succinate. Because of decreasing QO_4, HMG-CoA reductase inhibitors did not modify the changes in RCI due to ischemia. There were no significant differences in respiratory indices between pravastatin- and simvastatin-treated groups. In conclusion, HMG-CoA reductase inhibitors may enhance respiratory impairment of liver mitochondria under pathophysiological conditions, such as ischemia.

Key words: HMG-CoA (3-hydroxy-3-methylglutaryl conenzyme A) reductase inhibitor; Liver mitochondria; Ischemia

1. Introduction

A 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor is a very successful drug in the treatment of patients with hypercholesterolemia and hyperlipidemia. HMG-CoA reductase catalyzes the rate-limiting reaction in cholesterol biosynthesis (Rodwell et al., 1976). Inhibition of cholesterol biosynthesis in the liver increases cholesterol uptake from the blood to the liver, resulting in a reduction of its serum level. Pravastatin and simvastatin are competitive inhibitors of HMG-CoA reductase and prevent mevalonic acid formation. Mevalonic acid is a precursor not only in cholesterol biosynthesis but also in ubiquinone biosynthesis. Willis et al. (1990) have demonstrated that lovastatin, a HMG-CoA reductase inhibitor, decreases the level of ubiquinone in rat liver. The role of ubiquinone in mitochondrial electron transport system is well established in many organs (Ramasarma, 1968). Therefore, administration of HMG-CoA reductase inhibitors may influence mitochondrial oxidative phosphorylation in the liver, particularly under pathophysiological conditions. The present study was undertaken to examine the effects of pravastatin and simvastatin on respiration of mitochondria isolated from rat liver that was made ischemic.

2. Materials and methods

2.1. Animal preparations

52 male Sprague-Dawley rats weighing 160–220 g were randomly assigned to one of five treatment groups. The first ($n = 11$) and second ($n = 10$) groups received pravastatin at the dose of 2 and 4 mg/kg per day, respectively. The third ($n = 10$) and fourth ($n = 11$) groups received simvastatin at the dose of 1 and 2 mg/kg per day, respectively. The last group ($n = 10$) received vehicle (0.5% carboxymethyl cellulose sodium). Pravastatin and simvastatin suspended in 0.5% carboxymethyl cellulose solution were orally administered to the rats using a stomach tube once a day for 3

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weeks. The volume administered was fixed at 0.1 ml/100 g body weight. All animals had free access to standard rat chow and water and were removed from food for approximately 15 h before the experiments were started.

After 3 weeks of treatments, animals were anesthetized with sodium pentobarbital (50 mg/kg, i.p.)and ventilated with room air by using a respirator via a cannula inserted into the trachea. A silk thread was inserted into the thoracic cavity between the fourth and fifth ribs, and placed around the aorta. To interrupt the blood flow to the liver, the systemic circulation was ceased by ligating the aorta with the silk thread. After 60 min of aortic ligation, the abdomen was opened, and the liver was removed. We defined the liver obtained by this method as ischemic liver in the present study. An ischemic heart sample was also needed for another experiment. Cessation of the systemic circulation was carried out in about half of animals of each group. Sham operations were performed in the remaining animals by the same procedure except for the aortic ligation (non-ischemia).

2.2. Isolation of mitochondria

After 60 min of ischemia, the liver was removed and immediately placed in cold isolation buffer consisting of 250 mM sucrose, 5 mM EDTA 2K, 10 mM Tris and 0.5% bovine serum albumin (BSA) (pH 7.4). Basically, the procedure of Schneider and Hogeboom (1950) was modified for isolation of liver mitochondria. The liver was cut into small pieces, rinsed with the buffer, blotted dry and weighed. The liver tissue was finely minced and homogenized gently in 10 vols. of isolation buffer using a glass homogenizer with a teflon pestle. Homogenate was centrifuged at $600 \times g$ for 5 min to obtain the supernatant. The supernatant was then centrifuged at $10000 \times g$ for 10 min, and the pellet obtained was washed once. The final mitochondrial pellet was suspended in isolation buffer and used immedi-

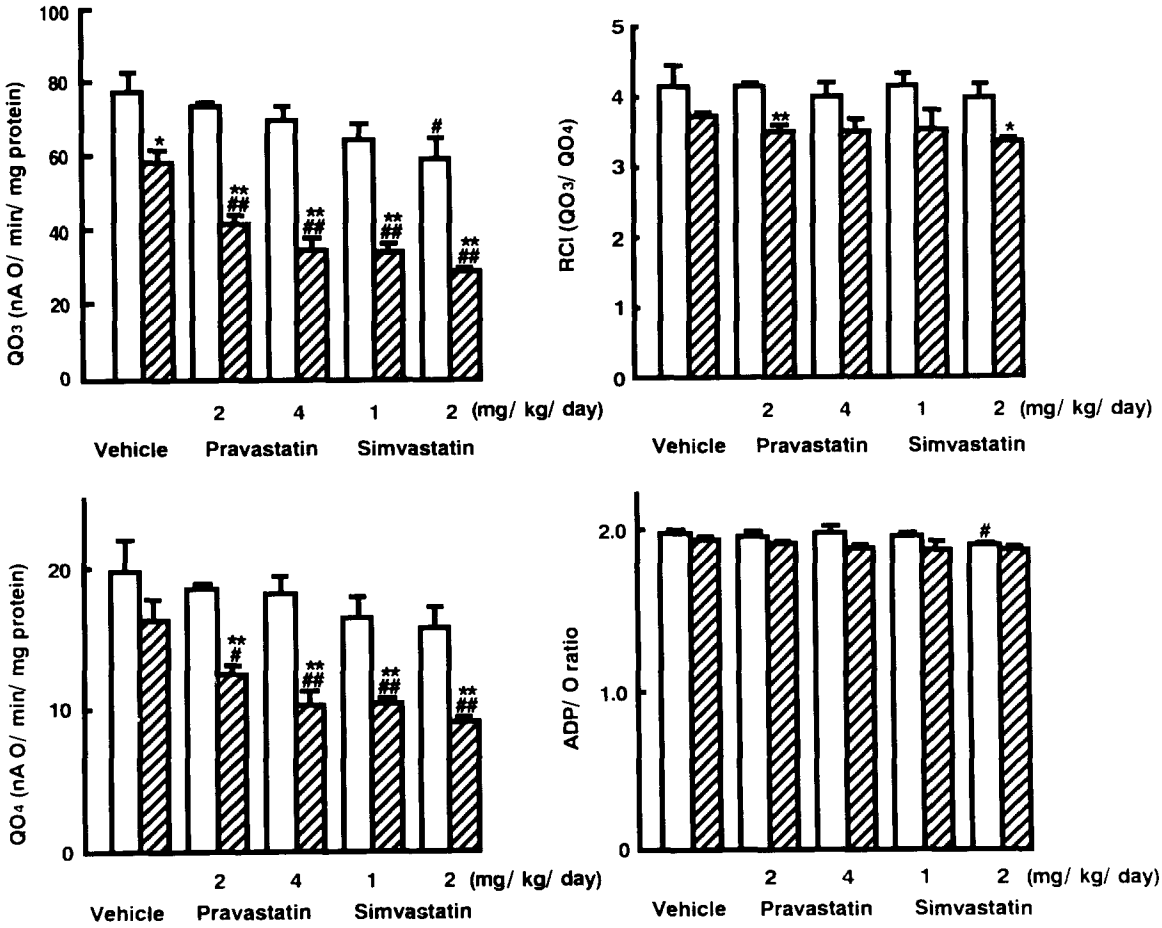

Fig. 1. Effects of pravastatin and simvastatin on QO_3, QO_4, RCI, and ADP/O ratio determined with glutamate as a substrate in rat liver mitochondria. Either vehicle, pravastatin (2 or 4 mg/kg per day), or simvastatin (1 or 2 mg/kg per day) was given orally for 3 weeks. The aorta was not ligated (non-ischemia; open column) or ligated for 60 min to cease the systemic circulation (ischemia; hatched column). Mitochondria were isolated by differential centrifugation. * $P < 0.05$; ** $P < 0.01$, vs. non-ischemia in each group. # $P < 0.05$; ## $P < 0.01$, vs. the respective values in vehicle-treated group.

ately for the experiment of mitochondrial respiration. Protein concentration was determined by the method of Lowry et al. (1951) using BSA (fraction V) as a standard. The mean yield of the mitochondria for all animals was 8.31 ± 0.19 mg protein/g tissue ($n = 52$), and there was no significant difference among the groups.

2.3. Assay of mitochondrial respiration

Mitochondrial respiration was measured polarographically at 25°C using an oxygen monitor (OHB-100, Otsuka Electronics Co., Osaka, Japan) equipped with an oxygen electrode modified by Hagihara (1961). Approximately 0.1–1 mg mitochondrial protein, 250 nmol of ADP, either 10 mM glutamate or 10 mM succinate with rotenone (8 μg/ml) as a substrate, and assay medium containing 3 mM K_2HPO_4, 1 mM EDTA 2K, 10 mM Tris and 250 mM sucrose (pH 7.4) were used for the assay. Mitochondrial protein was added into a cuvette containing assay medium equilibrated with air at 25°C, and then respiratory substrate was applied. After 2 min stabilization, state 3 respiration was initiated by the addition of ADP. Designations (QO_3 for ADP-stimulated and QO_4 for ADP-limitated respiration) and calculation of respiratory control index (RCI) and ADP/O ratios were carried out according to the method described by Chance and Williams (1955).

2.4. Statistical analysis

All values are means ± SEM. The significance of differences between groups were evaluated by two-way analysis of variance with repeated measures followed by the unpaired t test with the Bonferroni procedure. The P value less than 0.05 was considered statistically significant.

3. Results

3.1. Mitochondrial respiration determined with glutamate as a substrate

The values of QO_3, QO_4, RCI, and ADP/O ratio determined with glutamate as a substrate are illustrated in Fig. 1. In the vehicle-treated animals, all respiratory indices appeared to be decreased by 60 min

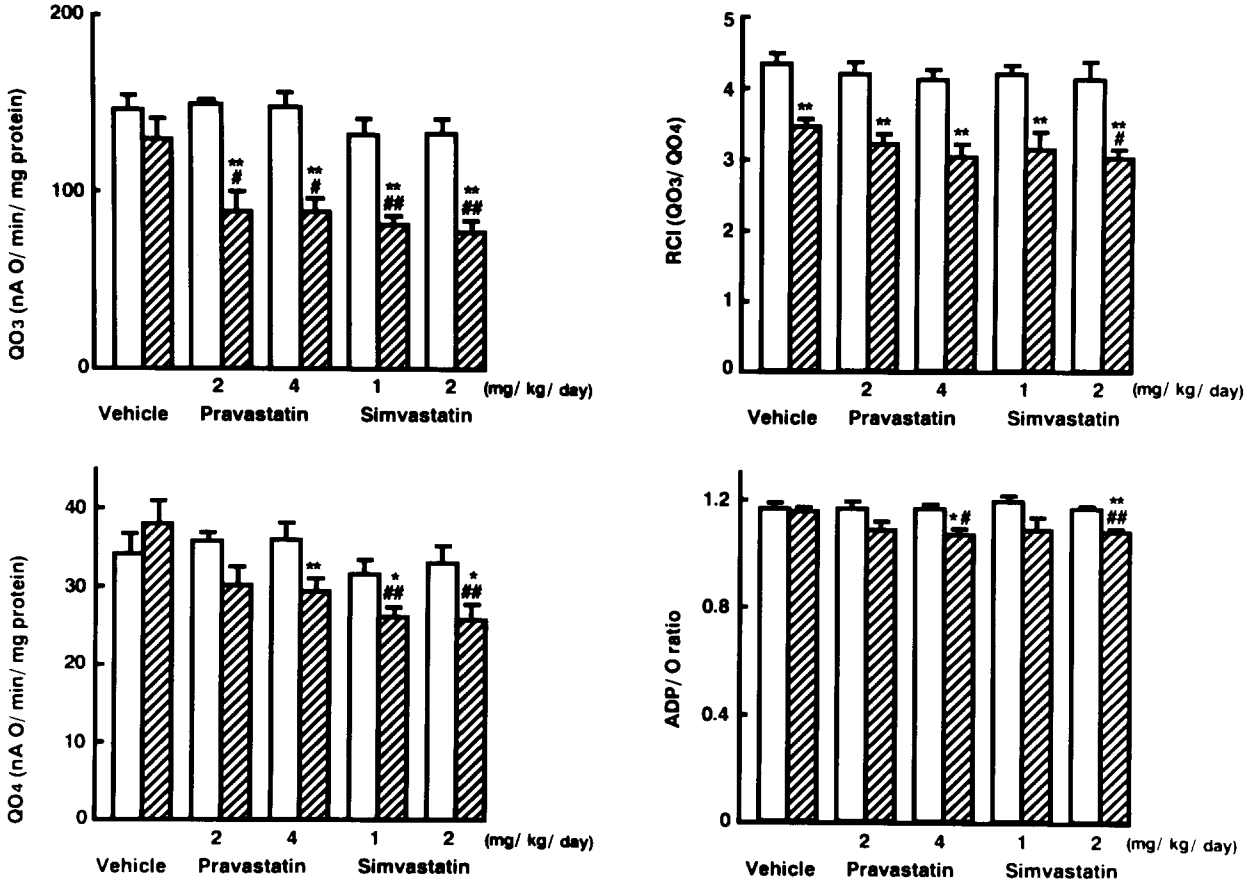

Fig. 2. Effects of pravastatin and simvastatin on QO_3, QO_4, RCI, and ADP/O ratio determined with succinate as a substrate in rat liver mitochondria. Experimental protocol and symbols are the same as those shown in Fig. 1. * $P < 0.05$; ** $P < 0.01$, vs. non-ischemia in each group. # $P < 0.05$; ## $P < 0.01$, vs. vehicle-treated ischemic group.

of ischemia. However, a significant change was observed in only QO_3. Pretreatment of the rat with pravastatin or simvastatin itself slightly decreased QO_3 and QO_4, and statistical significance was obtained in QO_3 of 2 mg/kg per day simvastatin-treated group. The values of RCI and ADP/O ratio were not modified by the pretreatment appreciably, except ADP/O ratio in 2 mg/kg per day simvastatin-treated group. The ADP/O ratio in that group significantly decreased as compared with that in the vehicle-treated group. Ischemia decreased QO_3 and QO_4 markedly and significantly in the pravastatin- and simvastatin-treated groups. The values of QO_3 and QO_4 in the pravastatin- and simvastatin-treated ischemic groups were significantly lower than those in the vehicle-treated ischemic group. Ischemia also decreased RCI and ADP/O ratio in the pravastatin- and simvastatin-treated groups. The differences in RCI in the 2 mg/kg per day pravastatin- and 2 mg/kg per day simvastatin-treated groups between non-ischemia and ischemia were significant. No significant difference in the respiratory indices was observed between 2 mg/kg per day pravastatin- and 1 mg/kg per day simvastatin-treated groups, and between 4 mg/kg per day pravastatin-and 2 mg/kg per day simvastatin-treated groups.

3.2. Mitochondrial respiration determined with succinate as a substrate

The respiratory indices determined with succinate as a substrate are shown in Fig. 2. In the vehicle-treated animals, QO_3 and RCI decreased during ischemia, whereas QO_4 appeared to increase. During ischemia, ADP/O ratio did not change. Pretreatment of the rat with pravastatin or simvastatin itself did not significantly altered all respiratory indices. In the pravastatin-and simvastatin-treated animals, ischemia significantly decreased QO_3 and RCI in all groups, QO_4 in 4 mg/kg per day pravastatin- and simvastatin-treated groups, and ADP/O ratio in 4 mg/kg per day pravastatin- and 2 mg/kg per day simvastatin-treated groups. The decrease in QO_3 caused by ischemia in pravastatin- and simvastatin-treated groups was more potent than that in the vehicle-treated group. The values of QO_4 in 1 and 2 mg/kg per day simvastatin-treated ischemic groups and RCI in 2 mg/kg per day simvastatin-treated ischemic group were significantly lower than the corresponding values in the vehicle-treated ischemic group. The ADP/O ratio in 4 mg/kg per day pravastatin- and 2 mg/kg per day simvastatin-treated ischemic groups was also significantly different as compared with that in the vehicle-treated ischemic group. There was no significant difference in the respiratory indices between pravastatin- and simvastatin-treated groups.

4. Discussion

Ubiquinone is essential for the production of energy through oxidative phosphorylation in many organs. HMG-CoA reductase inhibitors prevent ubiquinone biosynthesis as well as cholesterol biosynthesis, because mevalonate which is a product of HMG-CoA reductase reaction is a precursor in both cholesterol and ubiquinone biosynthesis. In fact, Willis et al. (1990) have reported that lovastatin decreases the tissue ubiquinone level in rat liver. The reduction of ubiquinone level in the liver mitochondria may impair its energy generating system, leading to the hepatic dysfunction. In the present study, almost all indices of mitochondrial respiration were not changed by administration of pravastatin and simvastatin for 3 weeks. When hepatic ischemia was induced for 60 min, however, the QO_3 and QO_4 values in the HMG-CoA reductase inhibitor-treated rats decreased more than those in the vehicle-treated rats. The decrease in ADP/O ratio in the 4 mg/kg per day pravastatin-treated and 2 mg/kg per day simvastatin-treated rats caused by ischemia was also greater than that in the vehicle-treated rats. These results indicate that pravastatin and simvastatin worsen ischemic impairment of the mitochondrial respiration in the liver. Although the decrease in QO_3 due to ischemia was enhanced by pretreatment with HMG-CoA reductase inhibitors, no significant effect of HMG-CoA reductase inhibitors on RCI (QO_3/QO_4) was obtained. The reason for this is that the decrease in QO_4 due to ischemia was also enhanced by pretreatment with HMG-CoA reductase inhibitors. Usually, ischemia should decrease QO_3, while it should increase QO_4 (Inoue and Tagawa, 1993). Increase in QO_4 does mean an uncoupling state in which an electron flows without energy generation. This is probably due to mitochondrial damage caused by ischemia (Inoue and Tagawa, 1993). Therefore, a decrease in QO_4 may mean a protection of mitochondria from ischemic damage. However, it is not likely that mitochondrial protection against 60 min of ischemia occurs even in the presence of pravastatin and simvastatin. If HMG-CoA reductase inhibitors decrease ubiquinone level in the mitochondrial respiratory chain, the rate of electron transfer could be slowed at the ubiquinone step. The slowing of electron transfer may cause the decrease in QO_4 value.

Influence of HMG-CoA reductase inhibitors at a high dosage on the decrease in ADP/O ratio during ischemia was clearly observed when succinate was used as a substrate. Electrons generated from glutamate oxidation flow into the respiratory chain at the nicotinamide-adenine dinucleotide (NAD) related enzyme step, whereas those from succinate oxidation at the ubiquinone step. If HMG-CoA reductase inhibitors would affect the mitochondrial respiration through re-

duction of ubiquinone level, the ADP/O ratio with succinate could change more markedly than those with glutamate. When an HMG-CoA reductase inhibitor attenuates the electron flow rate through ubiquinone step to 70%, for example, ATP generation decreases stoichiometrically from 2 to 1.4 mol/mol succinate (70% of initial value), whereas it decreases from 3 to 2.4 mol/mol glutamate (80% of initial value). Taking this consideration into account, the results obtained in the present study may indicate that the effect of HMG-CoA reductase inhibitors on mitochondrial respiration is due to a decrease in ubiquinone levels.

We adopted rather low dosages of the inhibitors (2 and 4 mg/kg per day of pravastatin and 1 and 2 mg/kg per day of simvastatin). Dosages of oral administration in other reports are 5–20 mg/kg per day for mice (Koga et al., 1990), 25 mg/kg per day for rats (Germershausen et al., 1989), and 50 mg/kg per day for rabbits and hamster (Watanabe et al., 1988; Bélichard et al., 1993). Because clinical dose of HMG-CoA reductase inhibitors is about 0.3–1 mg/kg per day, and because inhibitory effect of simvastatin on cholesterol synthesis is twice as potent as that of pravastatin (Tsujita et al., 1986), we chose the dosage used in the present study.

In conclusion, an HMG-CoA reductase inhibitor may result in the worsening of the hepatic mitochondrial respiration during ischemia, probably because of a decrease in ubiquinone level. For the control of plasma concentration of cholesterol in human, the widespread and long-term use of HMG-CoA reductase inhibitors may cause some hepatic dysfunctions, and a possibility must be investigated in future by appropriate clinical studies.

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References

Bélichard, P., D. Pruneau and A. Zhiri, 1993, Effect of a long-term treatment with lovastatin or fenofibrate on hepatic and cardiac ubiquinone levels in cardiomyopathic hamster, Biochim. Biophys. Acta 1169, 98.

Chance, B. and G.R. Williams, 1955, Respiratory enzymes in oxidative phosphorylation, J. Biol. Chem. 217, 409.

Germershausen, J.I., V.M. Hunt, R.G. Bostedor, P.J. Bailey, J.D. Karkas and A.W. Alberts, 1989, Tissue selectivity of the cholesterol lowering agents lovastatin, simvastatin and pravastatin in rats in vivo, Biochem. Biophys. Res. Commun. 158, 667.

Hagihara, B., 1961, Techniques for the application of polarography to mitochondrial respiration, Biochim. Biophys. Acta 46, 134.

Inoue, T. and K. Tagawa, 1993, Mitochondrial dysfunction in ischemic organs, in: Methods in Toxicology, Vol. 2. Mitochondrial Dysfunction, eds. L.H. Lash and D.P. Jones (Academic Press, San Diego, CA) p. 162.

Koga, T., Y. Shimada, M. Kuroda, Y. Tsujita, K. Hasegawa and M. Yamazaki, 1990, Tissue-selective inhibition of cholesterol synthesis in vivo by pravastatin sodium, a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor, Biochim. Biophys. Acta 1045, 115.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951, Protein measurement with the folin phenol reagent, J. Biol. Chem. 193, 265.

Ramasarma, T., 1968, Lipid quinones, Adv. Lipid Res. 6, 107.

Rodwell, V.W., J.L. Nordstrom and J.J. Mitschelen, 1976, Regulation of HMG-CoA reductase, Adv. Lipid Res. 14, 1.

Schneider, W.C. and G.H. Hogeboom, 1950, Intracellular distribution of enzymes. V. Further studies on the distribution of cytochrome c in rat liver homogenates, J. Biol. Chem. 183, 123.

Tsujita, Y., M. Kuroda, Y. Shimada, K. Tanzawa, M. Arai, I. Kaneko, M. Tanaka, H. Masuda, C. Tarumi, Y. Watanabe and S. Fujii, 1986, CS-514, a competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase: tissue-selective inhibition of sterol synthesis and hypolipidemic effect on various animal species, Biochim. Biophys. Acta 877, 50.

Watanabe, Y., T. Ito, M. Shiomi, Y. Tsujita, M. Kuroda, M. Arai, M. Fukami and A. Tamura, 1988, Preventive effect of pravastatin sodium, a potent inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase, on coronary atherosclerosis and xanthoma in WHHL rabbits, Biochim. Biophys. Acta 960, 294.

Willis, R.A., K. Folkers, J.L. Tucker, C.-Q. Ye, L.-J. Xia and H. Tamagawa, 1990, Lovastatin decreases coenzyme Q levels in rats, Proc. Natl. Acad. Sci. USA 87, 8928.