ELSEVIER

Journal of Nutritional Biochemistry 13 (2002) 200–208

THE JOURNAL OF Nutritional Biochemistry

Antioxidative effects of lovastatin in cultured human endothelial cells

Qi Zhou[a], Fred A. Kummerow[a,b]

[a]*Harlan E. Moore Heart Research Foundation, 503 S. Sixth St., Champaign IL, USA, 61820*
[b]*Burnsides Research Laboratory, University of Illinois, 1208 West Pennsylvania Avenue, Urbana, IL, USA 61801*

Received 27 June 2001; received in revised form 25 September 2001; accepted 5 October 2001

Abstract

The effects of lovastatin on glutathione peroxidase activity, hydrogen peroxide consumption, [^{3}H]cholesterol uptake and [^{14}C]acetate incorporation were investigated in cultured human endothelial cells. Treatment of endothelial cells with lovastatin in a medium without serum for 4 hr significantly increased both glutathione peroxidase activity and hydrogen peroxide consumption. This treatment also significantly inhibited cholesterol synthesis and cholesterol esterification. However, lovastatin stimulated cholesterol uptake by the cells. These alterations produced by lovastatin continued up to 24 hr. When serum was present in the culture medium, only decreased cholesterol synthesis and esterification were detected. We suggest that the *in vitro* antioxidative ability of lovastatin resulted, in part at least, from [1] its activating effect on glutathione peroxidase, [2] its stimulative effect on the ability of endothelial cell to scavenge H_2O_2, and [3] its hypolipidemic effect.

Keywords: Lovastatin; cholesterol; H_2O_2 consumption; Glutathione peroxidase; Endothelial cells

1. Introduction

Our recent studies showed that concentrations of oxidized products of cholesterol (oxysterols) and lipid peroxides in the plasma of cardiac catheterized patients with established stenosis of their coronary arteries were significantly higher than those in age- and sex-matched controls [1,2]. It has been reported that the concentrations of lipid oxidation products conformed with the severity of stenosis, whereas the plasma cholesterol concentration did not conform with the stenosis severity [3,4]. These results indicate that oxidation products played an important role in the initiation and progression of atherosclerosis [1–4]. Studies from other laboratories also showed that low density lipoprotein (LDL) must be oxidatively modified to oxidized LDL (ox-LDL) in order to trigger the atherogenic pathological events, such as macrophage cholesterol accumulation, foam cell formation, cytotoxicity, thrombosis and inflammation, leading finally to atherosclerosis [5–7]. An increase in ox-LDL, found in patients with coronary artery disease [8,9] and in patients subjected to heart transplants [10], has been credited with an important role in the initiation and progression of atherosclerosis [3,4]. The increase in ox-LDL correlated not only with the extent of coronary artery stenosis but also with the development of the stenosis [10]. Imai et al [11] demonstrated that aortas of rabbits fed concentrates of impure cholesterol (that had been oxidized in an unsealed drum for several years) exhibited diffuse fibrous lesions in the intima. Bovine aortic endothelial cells cultured with LDL plus cholesterol oxidase (an enzyme catalyzing the oxidation of cholesterol to cholest-4-en-3-one in the cell) were significantly damaged, compared to the cells cultured only with LDL [12]. Scanning and transmission electron microscopy showed damaged aortic endothelial cells in rabbits within 24 hr after cholesterol oxidation products were given intravenously or by gastric gavage [13,14]. Both *in vivo* and *in vitro* studies showed that ox-LDL induced damage of cellular membrane, caused loss of cell viability [15], inhibited wound-healing response of vascular endothelial cells [16], produced an increase in cytosolic Ca^{++} [17], activated endothelial recruitment of leukocytes, enhanced macrophage cytokine production and stimulated smooth muscle cell proliferation [18].

A strong role of ox-LDL in triggering atherosclerosis points to the importance of inhibiting oxidation to prevent the initiation of atherosclerosis [19–21]. LDL can be oxidized by release of reactive oxygen species (ROS) from activated leukocytes, platelets and monocytes both *in vivo*

* Corresponding author. Tel.: +1-217-333-1806; fax: +1-217-333-7370.

E-mail address: fkummero@uiuc.edu (Q. Zhou)

0955-2863/02/$ – see front matter
PII: S0955-2863(01)00213-3

and *in vitro* [22,23]. Cholesterol is also directly oxidized by either free radical autoxidation reaction or enzymatic oxidation [24]. Previous studies showed that oxidation in the plasma is challenged by a host of defense mechanisms [25] including ceruloplasmin, fibrinogen, transferrin and albumin [26] and that hypocholesterolemic therapy significantly inhibited both *in vivo* and *in vitro* oxidation of LDL [27–29]. We suggest that the antioxidative effect of hypocholesterolemic therapy be related to its hypolipidemic effect [29], to its inhibiting effect on free radical generation [27], and to its scavenging effect on free radical. [7] The current study is to provide further evidence for our suggestion.

Evidence from clinical and laboratory studies indicate that endothelial injury is involved in a loss of endothelial integrity and endothelial permeability properties [22] and in the early events leading to atherosclerotic lesion formation [30,31]. The endothelium is not only constantly exposed to ox-LDL, to free radicals and to other harmful materials in the blood, but it also is in direct contact with lovastatin in the circulation. We therefore chose the endothelial cell as an experimental model to study the antioxidative effect of lovastatin.

2. Materials and methods

2.1. Materials

[1,2,6,7-^{3}H]cholesterol (3.1 TBq/mmol, 84 Ci/mmol) and [1,2-^{14}C]acetate (1.9 GBq/mmol, 51.8 mCi/mmol) were obtained from NEN Research Products (Boston, MA USA). Fetal bovine serum (FBS), lovastatin (mevinolin), nutrient mixture F-12 ham (F12K), modified eagle's medium nutrient mixture F-12 ham (MF12K), bovine serum albumin (BSA), glutathione reductase, hydrogen peroxide (H_2O_2) and other reagents were purchased from Sigma Chemical Co. (St. Louis, MO USA).

The concentrations of [^{3}H]cholesterol, [^{14}C]acetate, and H_2O_2 were chosen based on our previous experiments [32, 33]. Lovastatin (4 mg) was dissolved in 0.1 ml of absolute ethanol. Stock solution was stored at –20°C. Final concentration of ethanol in culture medium was 0.05%. BSA was dissolved in 0.01 M phosphate buffered saline (PBS) at a concentration of 5% and the BSA solution was sterilized.

2.2. Cell culture

Endothelial cells (ECs) from human umbilical veins were obtained from ATCC (Rockville, MA USA) and used at passages from 15–21. Cells were cultured in F12K supplemented with 10% FBS (F12K-10%FBS) in a 5% CO_2 incubator at 37°C. The confluent ECs were seeded into 6-well plates, 60 x 15 mm dishes and 75 cm^2 flasks (Corning Medical and Scientific Co., East Walpol, MA USA). After the cells grew to about 80% confluence, the cells were cultured for 24 hr with lovastatin at levels of 5, 1, 0.1 and 0.01 μM in either MF12K or MF12K containing 5% FBS (MF12K-5%FBS). Control incubations were carried out in medium (either MF12K or MF12K-5%FBS) containing 0.05% ethanol.

2.3. Measurement of glutathione peroxidase ($GSHP_x$) activity

The cells in flasks were treated with or without lovastatin for designed periods, washed with PBS, trypsinized briefly, harvested by centrifugation, resuspended in 3.5 ml of PBS and sonicated. Aliquots were taken for protein measurement. The remaining 3.4 ml of the sample was centrifuged at 8000 x g for 5 min in order to obtain clean supernatant. GSHPx activity was assayed with a spectrophotometer at 366 nm by addition of 0.05 M PBS (pH 7.0), 1 mM EDTA, 0.36 U/ml glutathione reductase, 0.125 mM NADPH, 0.25 mM GSH, and 0.6 mM sodium azide into 1 ml of supernatant [34]. GSHPx activity was calculated using a millimolar extinction coefficient of 3.2 for NADPH at 366 nm.

2.4. Measurement of H_2O_2 consumption

After the cells in the 6-well plates were cultured in the media containing lovastatin for 4 and 24 hr, the monolayer was washed twice with Hanks' balanced salt solution (HBSS), then 2 ml of HBSS containing 0.2 mM H_2O_2 was added to the monolayer. At time point 1, 5, 10, 15 and 20 min after the H_2O_2 addition, 0.1 ml of the HBSS was collected and the remaining H_2O_2 in HBSS was measured with a spectrophotometer at 560 nm according to the method of Jiang, *et al* [35]. The concentration of H_2O_2 was calculated by comparison with a standard curve generated with known amounts of H_2O_2. The protein content in the monolayers was assayed.

2.5. Measurement of cholesterol uptake, synthesis and esterification

[^{3}H]cholesterol in ethanol was first dispersed in FBS or BSA at a concentration of 9 μCi of [^{3}H]cholesterol in 0.2 ml of FBS or BSA. The mixture was shaken constantly at 37°C for 2 hr and then transferred into culture medium with or without lovastatin. ECs in dishes were first cultured with lovastatin for 1 hr, media were then replaced by freshly prepared media containing 9 μCi of [^{3}H]cholesterol or 9 μCi of [^{14}C]acetate. After the cells were kept for another 3 or 23 hr in the incubator at 37°C, the media were aspirated and the cells were gently rinsed thrice with PBS, trypsinized, collected by centrifugation, resuspended in 1 ml of methanol and sonicated twice for 30 sec on ice. Aliquots were taken for protein assay (Bio-Rad, Richmond, CA USA). Lipids in the sonicated cells were extracted with 15 ml of chloroform/methanol (2:1, by vol.) overnight at 4°C. The lipid extracts were separated by TLC (Silica Gel G plates) using hexane : diethyl ether : acetic acid (80:20:1, by vol.). The cholesterol and cholesterol ester bands were collected and radioactivities were counted. The nonspecific

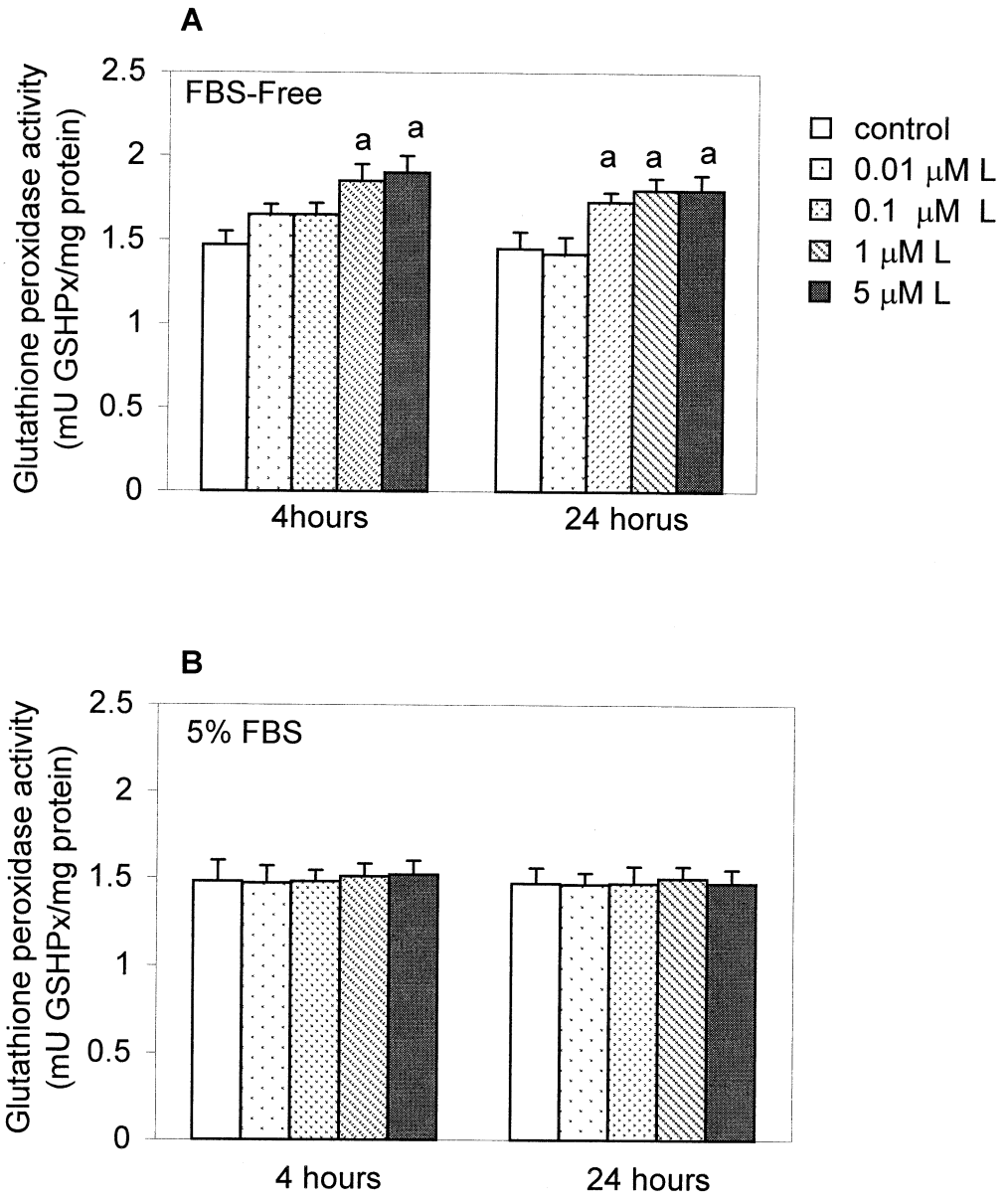

Fig. 1. The effect of lovastatin (L) on glutathione peroxidase activity in endothelial cells in the presence or absence of fetal bovine serum (FBS) in the culture medium. Results are expressed as mean±SE of duplicate for each independent determination in six experiments. Mean values at the same incubation periods under the same experiment condition with a letter are significantly different at a level of $P < 0.05$, compared with control group.

activities obtained from some silica gel on blank spaces were subtracted from sample radioactivity.

2.6. Statistical analysis

Data were subjected to ANOVA and a Student-Newmankeuls method. Differences with $p < 0.05$ were considered significant. All data are presented as mean±SE.

3. Results

We observed that GSHPx activity was significantly enhanced by lovastatin at a concentration higher than 0.1 μM in the absence of FBS after 4 hr of the treatment (Figure 1A). In the presence of FBS, however, no alteration of GSHPx activity was detected by lovastatin (Figure 1B).

The rate of degradation of H_2O_2 present in the culture medium was measured in this study. We found that the amount of H_2O_2 left in the wells decreased significantly after 4 hr of exposure of the cells to lovastatin in the medium without FBS (Figure 2A), indicating that more H_2O_2 was consumed by the lovastatin-treated cells. This decrease was continuous up to 24 hr. No increase in consumption of H_2O_2, however, was detected in the cells cultured in the medium containing FBS (Figure 2B).

The incorporation of [^{14}C]acetate into cholesterol was

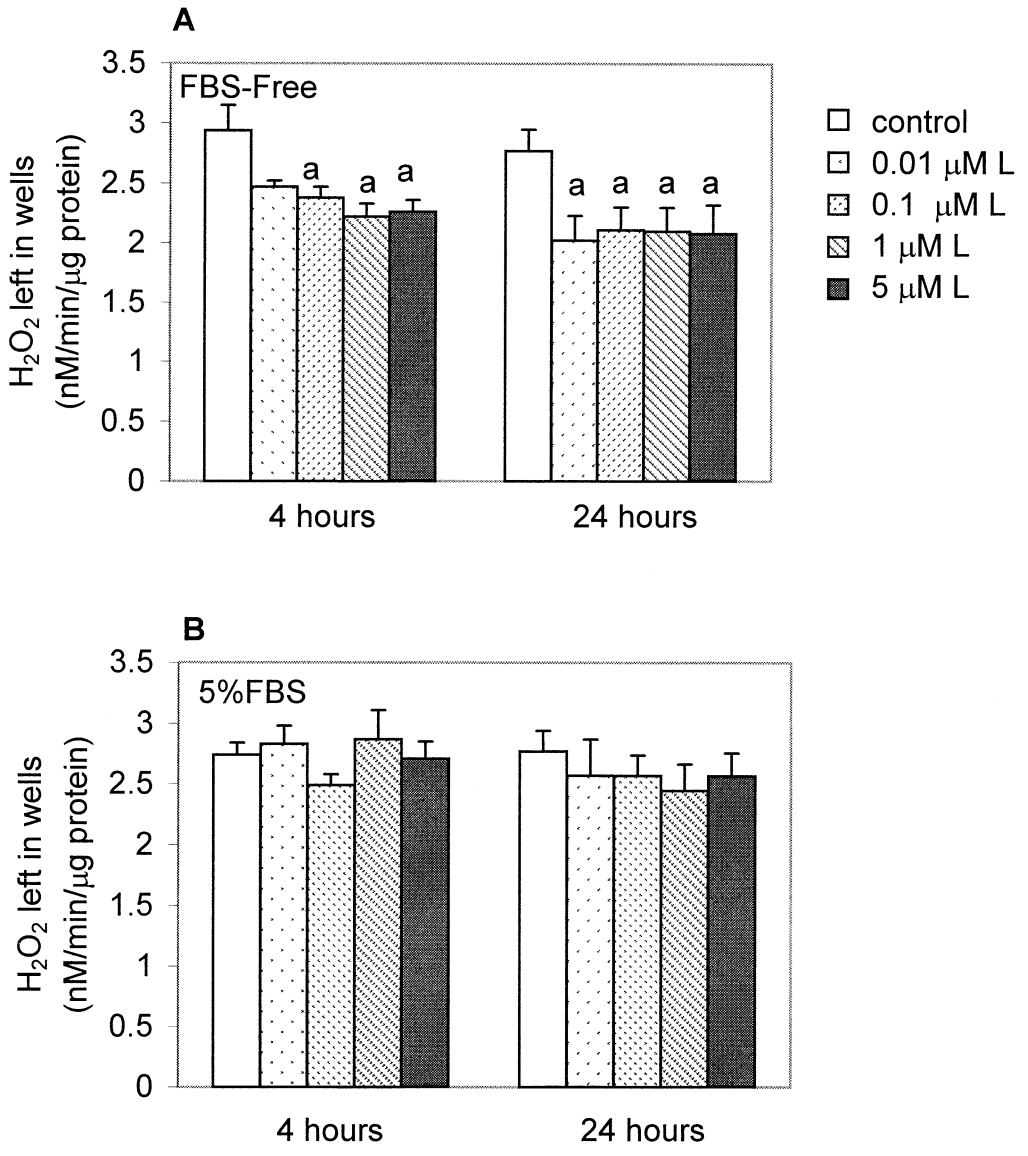

Fig. 2. The effect of lovastatin (L) on H_2O_2 consumption by endothelial cells in the presence or absence of fetal bovine serum (FBS) in the culture medium. Results are expressed as mean±SE of duplicate for each independent determination in six experiments. Mean values at the same incubation periods under the same experiment condition with a letter are significantly different at a level of $P < 0.05$, compared with control group.

significantly inhibited after 4 hr of treatment of the cells with lovastatin at all concentrations in the absence of FBS in the culture medium (Figure 3A). This inhibiting effect of lovastatin lasted up to 24 hr. In the presence of FBS, lovastatin at concentrations of l μM or higher significantly inhibited [^{14}C]acetate incorporation after 4 hr of treatment and at all concentrations after 24 hr (Figure 3B).

The effects of lovastatin on cholesterol uptake are shown in Figure 4. With 5 μM lovastatin added into the medium without FBS, the uptake of [^{3}H]cholesterol significantly increased after 4 hr of culture and the increased uptake continued for up to 24 hr (Figure 4A). After FBS was added into the medium, no significant increase in cholesterol uptake was detected (Figure 4B). In the absence of FBS, lovastatin at all concentrations significantly decreased the percentage of cellular [^{3}H]cholesterol incorporated into cholesterol ester for up to 24 hr (Figure 5A). In the presence of FBS, lovastatin at concentrations of 1 μM or higher significantly reduced the percentage of cellular [^{3}H]cholesterol incorporated into cholesterol ester only after 24 hr (Figure 5B). These data indicated that cholesterol esterification was inhibited by lovastatin, especially in the absence of FBS.

4. Discussion

In this study, we investigated the effects of lovastatin on GSHPx activity, H_2O_2 consumption and cholesterol synthesis and uptake in cultured human endothelial cells. We found that lovastatin increased intracellular GSHPx activity. We believe that the antioxidant effect of statins is related to

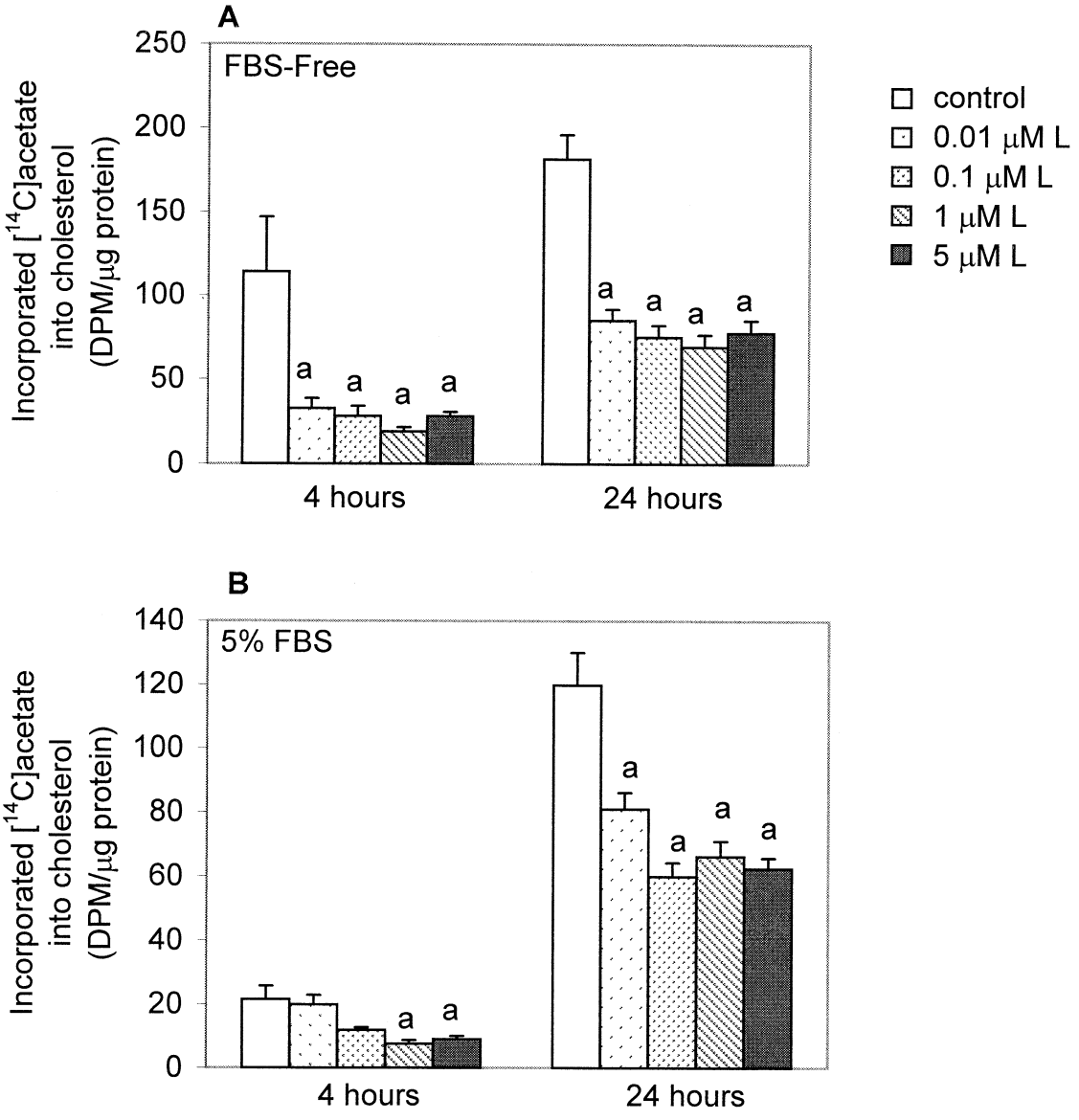

Fig. 3. The effect of lovastatin (L) on [^{14}C]acetate incorporation into cholesterol in the presence or absence of fetal bovine serum (FBS) in the culture medium. Results are expressed as mean±SE of six separate experiments. Mean values at the same incubation periods under the same experiment condition with a letter are significantly different at a level of $P < 0.05$, compared with control group.

its ability to reduce free radical formation. A pervious study showed that when patients with ischemic heart disease were treated with lovastatin, the superoxide anion generating capacity of neutrophil granulocytes decreased markedly while the activity of GSHPx increased significantly [36]. When LDL was directly incubated with statins, direct antioxidative influence of statins themselves on LDL was also observed [27,37]. Indeed, simvastatin has decreased superoxide formation in human monocyte-derived macrophages [37], and lovastatin not only preserved the endogenous antioxidant enzyme, superoxide dismutase (SOD) in hyperlipidemic rabbits but also increased the SOD activity in phorbol 12-myristate 13-acetate-activated leukocytes [27]. Addition of exogenous mevalonic acid to statin-treated macrophages, however, restored their ability for superoxide production and for oxidative modification of LDL [37]. Since the lovastatin molecule *per se* does not contain an antioxidant moiety, lovastatin might inhibit free radical generation from leukocytes and other tissues by inhibiting the isoprenoid reaction during the activation of NADPH oxidase [27].

Cellular GSHPx activity was enhanced only in the cells treated with lovastatin in absence of serum, but no change was observed in the presence of serum. ECs have been shown to have an oxidizing effect and can modify LDL *in vitro* [38]. Serum which contain several antioxidants such as vitamin C, vitamin E, albumin, ceruloplasmin and other antioxidants could compensate for the oxidative effect of ECs. However, in the absence of serum, there is an urgent need for antioxidant to prevent the exposure of cells to

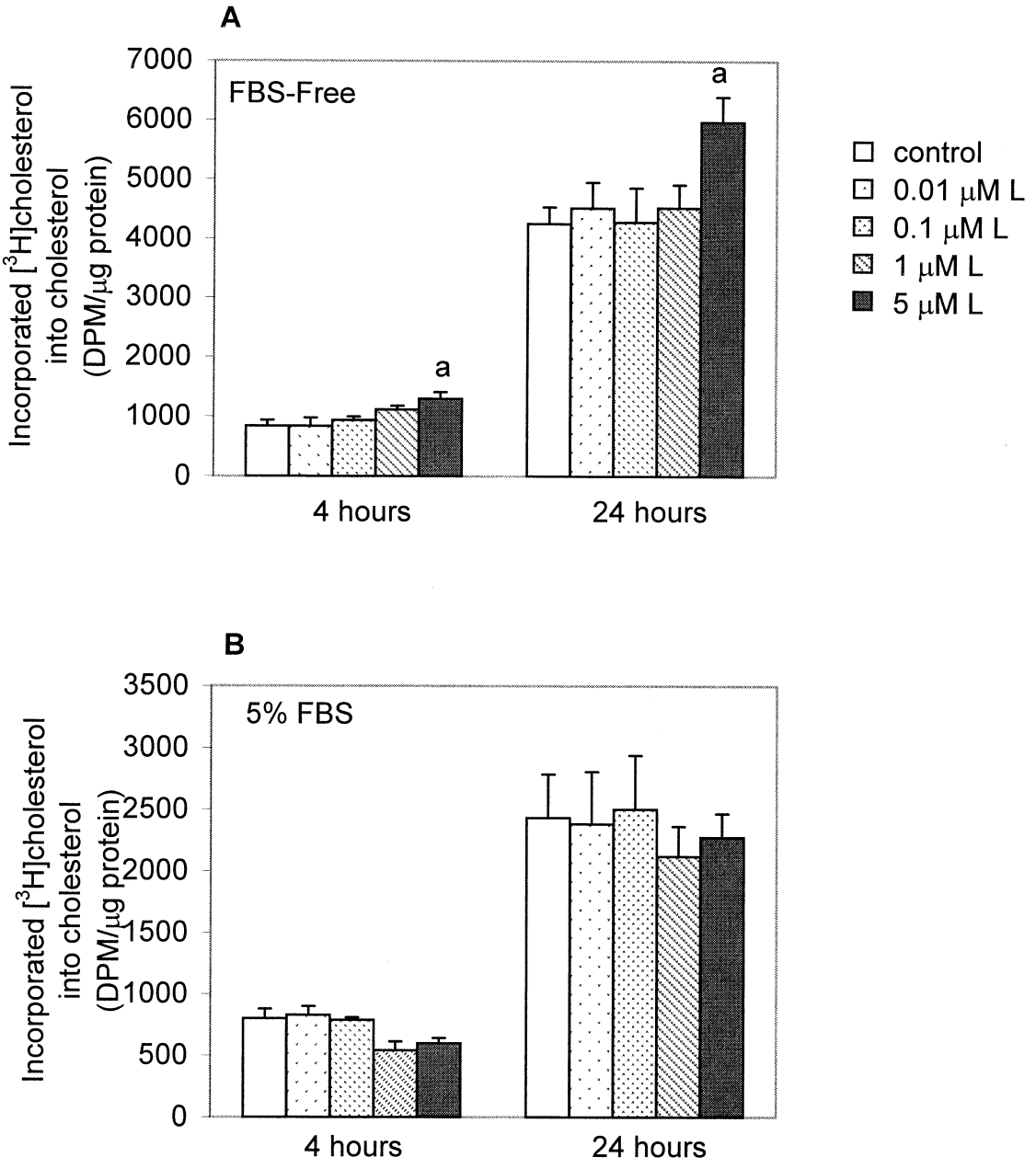

Fig. 4. The effect of lovastatin (L) on [^{3}H]cholesterol uptake by the cultured cells in the presence or absence of fetal bovine serum (FBS) in the culture medium. Results are expressed as mean±SE of six separate experiments. Mean values at the same incubation periods under the same experiment condition with a letter are significantly different at a level of $P < 0.05$, compared with control group.

oxidative stress. Lovastatin was capable of enhancing the GSHPx activity in ECs incubated without serum. We suspect that hypercholesterolemic patients who need cholesterol lowing therapy are challenged by a high level of oxidative stress. Under these conditions lovastatin is capable of enhancing GSHPx activity in addition to its lowing effect on plasma cholesterol.

We also found that lovastatin stimulated cell consumption of H_2O_2. In other words, lovastatin increased H_2O_2 scavenging by ECs. ROS (e.g., superoxide anion, hydroxyl radical and H_2O_2) in the blood can be released by activated leukocytes, platelets and monocytes [22,39]. A high concentration of H_2O_2 has been found to be harmful because H_2O_2 can induce oxidation of protein sulfhydryls, can activate poly(ADP-ribose)poly-merase and can change membrane fluidity by peroxidation of membrane lipid [40–42]. Consequently the increased H_2O_2 scavenging by ECs could reduce the oxidative effect of H_2O_2 in the blood. It is well known that serum contains several antioxidant molecules that have an important effect on antioxidation. As demonstrated in our experiments in the presence of serum in culture medium, an increased consumption of H_2O_2 by the cells could not be obtained after the treatment of the cells with lovastatin. The reason, we suggest, could be that serum

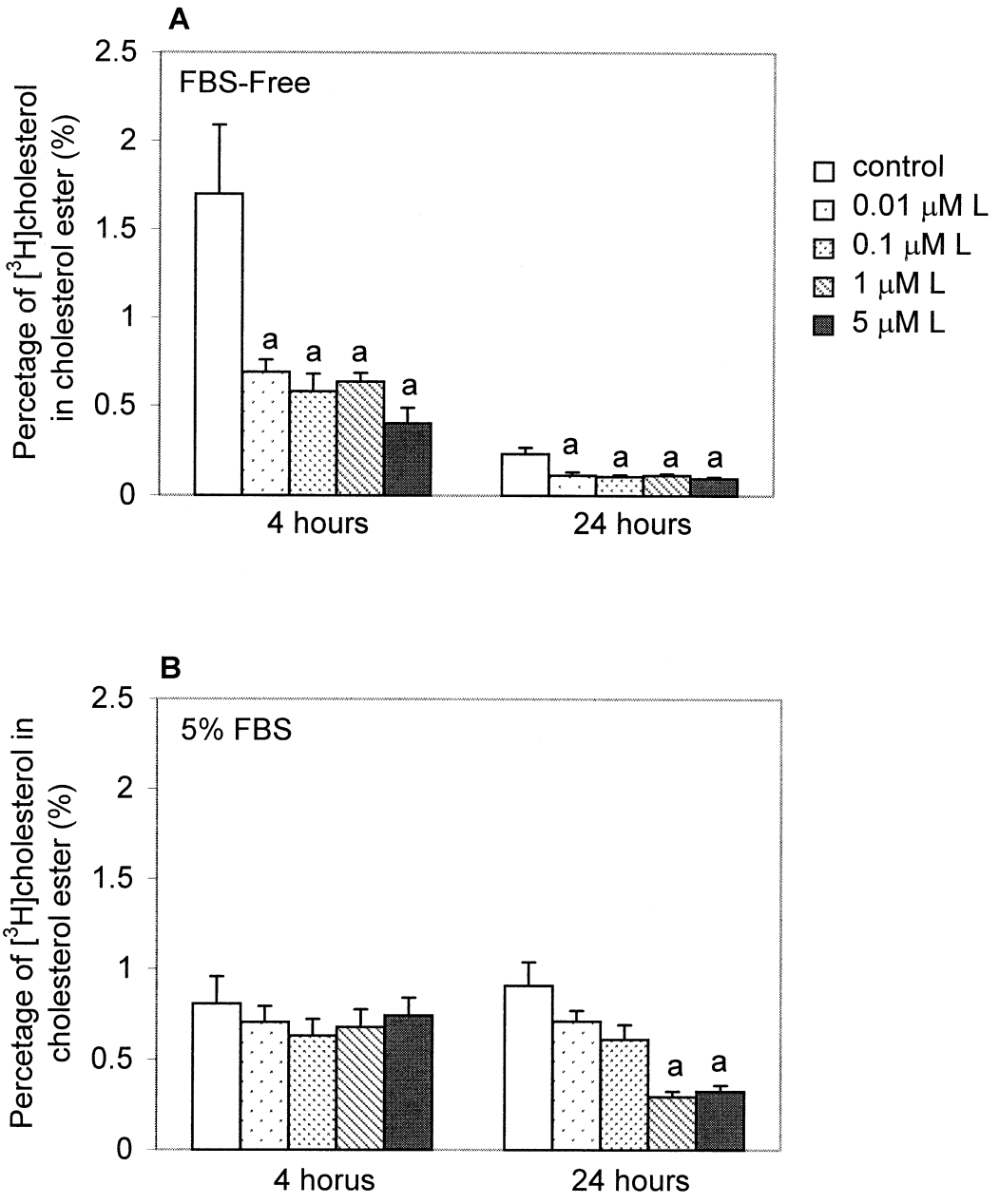

Fig. 5. The alternations of the percentage of [^{3}H]cholesterol in cholesterol ester by lovastatin (L) in the presence or absence of fetal bovine serum (FBS) in the culture medium. Results are expressed as mean±SE of six separate experiments. Mean values at the same incubation periods under the same experiment condition with a letter are significantly different at a level of $P < 0.05$, compared with control group.

has stronger antioxidative effect than lovastatin does under normal conditions.

An inhibition of cholesterol synthesis by lovastatin was confirmed by this study. Reduction in the cholesterol content lowered the amount of substrate available for oxidation. So hypolipidemic effect of statins was thought to play a role in antioxidation [29]. Inhibition of cholesterol synthesis by lovastatin was observed in previous studies with various cell lines by measuring [^{14}C]acetate incorporation [43,44] and mRNA levels of HMG-GoA reductase [45,46]. In the presence of serum the cellular [^{3}H]cholesterol uptake was stimulated by lovastatin. In the absence of serum, no change in the cellular uptake of [^{3}H]cholesterol was observed. We believe that cholesterol present in serum could compete with [^{3}H]cholesterol for transfer into the cells, resulting in undetectable change of [^{3}H]cholesterol uptake by the cells treated with lovastatin.

In brief, our data suggest that the antioxidant effect of lovastatin may result, at least in part, from the lovastatin's activating effect on GSHPx and its stimulatory effect on scavenging H_2O_2 by ECs. The decreased cholesterol synthesis induced by lovastatin may be another factor related to its *in vitro* antioxidant effect.

Acknowledgment

We acknowledge the support of the Wallace Research Foundation. The authors thank Dr. M. Mahfouz for assistance in manuscript preparation.

References

[1] Q. Zhou, E. Wasowicz, B. Handler, L. Fleischer, F.A. Kummerow, An excess concentration of oxysterols in the plasma is cytotoxic to cultured endothelial cells, Atherosclerosis 149 (2000) 191–197.
[2] F.A. Kummerow, R.M. Olinescu, L. Fleischer, B. Handler, S.V. Shinkareva, The relationship of oxidized lipids to coronary artery stenosis, Atherosclerosis 149 (2000) 181–190.
[3] E. Maggi, G. Finardi, M. Poli, P. Bollati, M. Filipponi, P.L. Stefano, G. Paolini, A. Grossi, P. Clot, E, Albano, and et al, Specificity of autoantibodies against oxidized LDL as an additional marker for atherosclerotic risk, Coron. Artery. Dis. 4 (1993) 1119–1122.
[4] C.P. Sparrow, T.W. Doebber, J. Olszewski, M.S. Wu, J. Ventre, K.A. Stevens, Y.S. Chao, Low density lipoprotein is protected from oxidation and the progression of atherosclerosis is slowed in cholesterol-fed rabbits by the antioxidant N,N′-diphenyl-phenylenediamine, J. Clin. Invest. 89 (1992) 1885–18915.
[5] M. Navab, J.A. Berliner, A.D. Watson, S.Y. Hama, M.C. Territo, A.J. Lusis, The yin and yang of oxidation in the development of the fatty streak, Arterioscler. Thromb. Vasc. Biol. 16 (1996) 831–842.
[6] J.W. Heinecke, Oxidants and antioxidants in the pathogenesis of atherosclerosis: implications for the oxidized low density lipoprotein hypothesis, Atherosclerosis 141 (1998) 1–15.
[7] M. Aviran, M. Rosenblat, C.L. Bisgaier, R.S. Newton, Atorvastatin and gemfibrozil metabolites, but not the parent drugs, are potent antioxidants against lipoprotein oxidation, Atherosclerosis 138 (1998) 271–280.
[8] I.B. Kovacs, M. Jahangiri, G.M. Rees, P. Gorog, Elevated plasma lipid hydroperoxides in patients with coronary artery disease, Am. Heart. J. 34 (1997) 572–576.
[9] L. Mosca, M. Rubenfire, T. Tarshis, A. Tsai, T. Pearson, Clinical predictors of oxidized low-density lipoprotein in patients with coronary artery disease, Am. J. Cardiol. 80 (1997) 825–830.
[10] P. Holvoet, J.-M. Stassen, J.V. Cleemput, D. Collen, J. Vanhaecke, Oxidized low density lipoproteins in patients with transplant-associated coronary artery disease, Arterioscler. Thromb. Vasc. Biol. 18 (1998) 100–107.
[11] H. Imai, N.T. Werthessen, B. Taylor, K.T. Lee, Angiotoxicity and arteriosclerosis due to contaminants of USP-grade cholesterol, Arch. Pathol. Lab. Med. 100 (1976) 565–572.
[12] A.W. Bernheimer, W.G. Robinson, R. Linder, D. Mullins, Y.K. Yip, N.S. Cooper, I. Seidman, T. Uwajima, Toxicity of enzymically-oxidized low-density lipoprotein, Biochem. Biophys. Res. Commun. 148 (1987) 260–266.
[13] C.B. Taylor, S.K. Peng, Cytotoxicity and atherogenicity of oxidized cholesterol, J. Am. Oil. Chem. Soc. 62 (1985) 633.
[14] S.K. Peng, P. Tham, C.B. Taylor, B. Mikkelson, Cytotoxicity of oxidation derivatives on cultured aortic smooth muscle cells and their effect on cholesterol biosynthesis, Am. J. Clin. Nutr. 32 (1979) 1033–1042.
[15] N. Ide, B.H. Lau, Garlic compounds protect vascular endothelial cells from oxidized low density lipoprotein-induced injury, J. Pharm. Pharmacol. 49 (1997) 908–911.
[16] G. Murugesan, G.M. Chisolm, P.L. Fox, Oxidized low density lipoprotein inhibits the migration of aortic endothelial cells in vitro, J. Cell. Biol. 120 (1993) 1011–1019.
[17] A. Negre-Salvayre, G. Fitoussi, V. Reaud, M.T. Pieraggi, J.C. Thiers, R. Salvayre, A delayed and sustained rise of cytosolic calcium is elicited by oxidized LDL in cultured bovine aortic endothelial cells, FEBS Lett. 299 (1992) 60–65.
[18] J. Nilsson, Lipoproteins and inflammation in atherosclerosis, Fibrinolysis Proteolysis 11 (suppl.1) (1997) 129–132.
[19] M.L. Bierenbaum, R.P. Reichstein, H.N. Bhagavan, T.R. Watkins, Relationship between serum lipid peroxidation products in hypercholesterolaemic subjects and vitamin E status, Biochem Mol Biol Int 28 (1992) 57–66.
[20] A.L. Stewart-Lee, L.A. Forster, J. Nourooz-Zadeh, G.A.A. Ferns, E.E. Anggard, Vitamin E protects against impairment of endothelium-mediated relaxations in cholesterol-fed rabbits, Arterioscler. Thromb. Vasc. Biol. 14 (1994) 494–499.
[21] A. Polette, D. Blache, Effect of vitamin E on acute iron load-potentated aggregation, serotonin, calcium uptake and thromboxane biosynthesis in rat platelets, Atherosclerosis 96 (1992) 171–179.
[22] B. Hennig, C.K. Chow, Lipid peroxidation and endothelial cell injury: implications in atherosclerosis, Free. Radic. Biol. Med. 4 (1988) 99–106.
[23] B. Halliwell, Oxidants and human disease: some new concepts, FASEB J. 1 (1987) 358–364.
[24] L.L. Smith, The oxidation of cholesterol, in: S. Peng, R.J. Morin (Eds.), Biological effects of cholesterol oxides, CRC Press, Florida, 1992. pp. 7–31.
[25] D.D. Wayner, G.W. Burton, K.U. Ingold, L.R. Barclay, S.J. Locke, The relative contributions of vitamin E, urate, ascorbate and proteins to the total peroxyl radical-rapping antioxidant activity of human blood plasma, Biochim Biophys Acta 924 (1987) 408–419.
[26] J.M. Gutteridge, Antioxidant properties of the proteins ceruloplasmin, albumin and transferrin. A study of their activity in serum and synovial fluid from patients with rheumatoid arthritis, Biochim. Biophys. Acta. 869 (1986) 119–127.
[27] L. Chen, W.H. Haught, B. Yang, T.G.P. Saldeen, S. Parathasarathy, J.L. Mehta, Preservation of endogenous antioxidant activity and inhibition of lipid peroxidation as common mechanisms of antiatherosclerotic effects of vitamin E, lovastatin and amlodipine, J. Am. Coll. Cardiol. 30 (1997) 569–575.
[28] R.B. Singh, N.K. Singh, S.S. Rastogi, G.S. Wander, M. Aslam, Z. Onouchi, F.A. Kummerow, S. Nangia, Antioxidant effects of lovastatin and vitamin E on experimental atherosclerosis in rabbits, Cardiol. Drugs Therapy 11 (1997) 575–580.
[29] R. Hoffman, G.J. Brook, M. Aviram, Hypolipidemic drugs reduce lipoprotein susceptibility to undergo lipid peroxidation: in vitro and ex vivo studies, Atherosclerosis 93 (1992) 105–113.
[30] H. Plachta, E. Bartnikowaka, A. Obara, Lipid peroxides in blood from patients with atherosclerosis of coronary and peripheral arteries, Clin Chim Acta 211 (1992) 101–112.
[31] C. Smith, M.J. Mitchinson, O.I. Aruoma, B. Halliwell, Stimulation of lipid peroxidation and hydroxyl-radical generation by the contents of human atherosclerotic lesions, Biochem. J. 286 (1992) 901–905.
[32] Q. Zhou, F.A. Kummerow, Alterations of Ca^{++} uptake and lipid content in cultured human arterial smooth muscle cells treated with 26-hydroxycholesterol, Artery 21 (1994) 182–192.
[33] Q. Zhou, R.M. Olinescu, F.A. Kummerow, Influence of low magnesium concentrations in the medium on the antioxidant system in cultured human arterial endothelial cells, Magnes. Res. 12 (1999) 19–29.
[34] N. Suttorp, W. Toepfer, L. Roka, Antioxidant defense mechanisms of endothelial cells: glutathione redox cycle versus catalase, Am. J. Physiol. 251 (1986) C671–C680.
[35] Z.-Y. Jiang, J.V. Hunt, S.P. Wolff, Ferrous ion oxidation in the presence of xylenol orange for detection of lipid hydroperoxide in low density lipoprotein, Anal. Biochem. 202 (1992) 384–389.
[36] L. Czopf, R. Halmosi, G. kesmarky, T. Habon, K. Toth, I. Jurisckay, E. Roth, G. Mozsik, Lovastatin and nitrate therapy induced changes in hemorheological parameters and in free radical mediated processes in patients, Perfusion 12 (1999) 50–61.

[37] L.M. Giroux, J. Davignon, M. Naruszewicz, Simvastatin inhibits the oxidation of low-density lipoproteins by activated human monocyte-derived macrophages, Biochim. Biophys. Acta. 1165 (1993) 335–338.

[38] U.P. Steinbrecher, S. Parthasarathy, D.S. Leake, J.L. witztum, D. Steinberg, Modification of low density lipoprotein by endothelial cells involves lipid peroxidation and degradation of low density lipoprotein phospholipids, Proc. Natl. Acad. Sci. USA 81 (1984) 3883–3887.

[39] R.C. Kukreja, M.L. Hess, Biochemistry of oxygen and nitrogen metabolites, in: R.C. Kukreja, M.L. Hess (Eds.), Free Radicals, cardiovascular dysfunction and protection strategies, R.G. Landes Company, Austin, 1994. pp. 3–8.

[40] J.B. Kirkland, Lipid peroxidation, protein thiol oxidation and DNA damage in hydrogen peroxide-induced injury to endothelial cells: Role of activation of poly(ADP-ribose) polymerase, Biochim. Biophys. Acta. 1092 (1991) 319–325.

[41] G. Bellomo, F. Mirabelli, P. Richelmi, S. Orrenius, Critical role of sulfhydryl group(s) in ATP-dependant Ca^{2+} sequestration by the plasma membrane fraction from rat liver, FEBS Lett. 163 (1983) 136–139.

[42] E.R. Block, J.M. Patel, K.J. Angelides, N.P. Sheridan, L.C. Garg, Hyperoxia reduces plasma membrane fluidity: A mechanism for endothelial cells dysfunction, J. Appl. Physiol. 60 (1986) 826–835.

[43] T. Koga, Y. Shimada, M. Kuroda, Y. Tsujita, K. Hasegawa, M. Yamazaki, Tissue-selective inhibition of cholesterol synthesis in vivo by pravastatin sodium, a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor, Biochim. Biophys. Acta. 1045 (1990) 115–120.

[44] S.T. Mosley, S.S. Kalinowski, B.L. Schafer, R.D. Tanaka, Tissue-selective acute effects of inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A reductase on cholesterol biosynthesis in lens, J. Lipid. Res. 30 (1989) 1411–1420.

[45] A.K. van Vliet, G.C.F. van Thiel, R.H. Huisman, H. Moshage, S.H. Yap, L.H. Cohen, Different effects of 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors on sterol synthesis in various human cell types, Biochim. Biophys. Acta. 1254 (1995) 105–111.

[46] J.W. Choi, E.N. Lundquist, D.M. Peffley, Inhibition of protein synthesis in baby-hamster kidney cells blocks oxysterol-mediated suppression of 3-hydroxy-3-methylglutaryl-CoA reductase messenger RNA at a post-transcriptional levels, Biochem. J. 296 (1993) 859–866.