

Atorvastatin increases blood ratios of vitamin E/low-density lipoprotein cholesterol and coenzyme Q10/low-density lipoprotein cholesterol in hypercholesterolemic patients

Chin-San Liu^{a,b,1}, Chong-Kuei Lii^{c,1}, Li-Ling Chang^d, Chen-Ling Kuo^a, Wen-Ling Cheng^a, Shih-Li Su^e, Chia-Wen Tsai^{c,*}, Haw-Wen Chen^{c,*}

^aDepartment of Neurology and Vascular and Genomic Center, Changhua Christian Hospital, Changhua 500, Taiwan

^bGraduate Institute of Integrative Chinese and Western Medicine, China Medical University, Taichung 404, Taiwan

^cDepartment of Nutrition, China Medical University, Taichung 404, Taiwan

^dInstitute of Nutritional Science, Chung Shan Medical University, Taichung 402, Taiwan

^eDivision of Endocrinology and Metabolism, Department of Internal Medicine, Changhua Christian Hospital, Changhua 500, Taiwan

Received 28 October 2009; revised 7 January 2010; accepted 21 January 2010

Abstract

Statins are among the most widely used drugs in the management of hypercholesterolemia. In addition to inhibiting endogenous cholesterol synthesis, however, statins decrease coenzyme Q10 (CoQ10) synthesis. CoQ10 has been reported to have antioxidant properties, and administration of drugs that decrease CoQ10 synthesis might lead to increased oxidative stress *in vivo*. Our present study examined the hypothesis that atorvastatin increased oxidative stress in hypercholesterolemic patients due to its inhibition of CoQ10 synthesis. We investigated the effects of atorvastatin (10 mg/d) administration for 5 months on lowering hypercholesterolemia and blood antioxidant status. The study population included 19 hypercholesterolemic outpatients. Blood levels of lipid and antioxidant markers, consisting of vitamin C, vitamin E, CoQ10, and glutathione (GSH), and urinary levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG) were examined pre- and postadministration of atorvastatin. Atorvastatin administration resulted in a significant decrease in blood levels of total cholesterol, triglycerides, low-density lipoprotein (LDL) cholesterol, vitamin E, and CoQ10 ($P < .05$); however, a significant increase in the ratios of vitamin E/LDL cholesterol and CoQ10/LDL cholesterol was noted ($P < .05$). Atorvastatin had no significant effect on red blood cell (RBC) level of GSH and urinary 8-OHdG. The present study provides evidence that atorvastatin exerts a hypocholesterolemic effect, but on the basis of the urinary level of 8-OHdG and the **blood ratios of vitamin E/LDL cholesterol and CoQ10/LDL cholesterol, has no oxidative stress-inducing effect.**

© 2010 Published by Elsevier Inc.

Keywords:

Atorvastatin; CoQ10; Human; Hypercholesterolemia; 8-OHdG; Vitamin E

Abbreviations:

8-OHdG, 8-hydroxy-2'-deoxyguanosine; CK, creatine kinase; CoQ10, coenzyme Q10; GSH, reduced glutathione; HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A; HPLC, high-performance liquid chromatography; ox-LDLs, oxidized low-density lipoproteins; RBC, red blood cell; VLDL, very low-density lipoprotein.

1. Introduction

Hypercholesterolemia is a well-recognized risk factor for the development of cardiovascular disease [1], and cardiovascular disease is the leading cause of death throughout the Western world and the second most common cause of death worldwide [2]. Oxidized low-density lipoproteins

* Corresponding authors. Tel.: +886 4 2205 3366x7520; fax: +886 4 22062891.

E-mail addresses: cwtsai@mail.cmu.edu.tw (C.-W. Tsai), chenhw@mail.cmu.edu.tw (H.-W. Chen).

¹ Chin-San Liu and Chong-Kuei Lii contributed equally to this study.

(ox-LDLs) are known to play a crucial role in the progression of atherosclerosis [3]. Lipid-soluble antioxidants endowed in LDLs can prevent or minimize lipid peroxidation.

Coenzyme Q10 (CoQ10) is an antioxidant present in plasma and lipoproteins [4]. Despite its low concentration compared with other antioxidants, CoQ10 possesses a high antioxidant capacity [5], and its concentration in lipoproteins and plasma has been suggested as a clinically important index of oxidative stress [6]. Supplementation with CoQ10 inhibits *in vivo* lipoprotein oxidation and atherosclerosis in animals [7] and improves endothelial function in some subjects at risk of cardiovascular disease [8]. 8-Hydroxy-2'-deoxyguanosine (8-OHdG) is thought to come from repair of oxidized DNA and the DNA precursor pool [9,10], and it is considered to be one of the most reliable urinary biomarkers of DNA damage because it cannot be absorbed through the gut [11,12]. CoQ10 supplementation enhances *in vitro* DNA resistance toward H₂O₂-induced oxidative DNA damage in human lymphocytes [13] and inhibits *in vivo* DNA strand breaks in lymphocytes [14].

Statins are among the most widely used drugs in the management of hypercholesterolemia, and their mechanism of action is through inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in the biosynthesis of cholesterol. Several large clinical trials have shown the efficacy of statins to decrease coronary heart disease morbidity and mortality in hypercholesterolemic patients in both primary and secondary prevention [15,16]. A recent review showed that atorvastatin is associated with greater reductions in LDL cholesterol and total cholesterol than other statins administered at the same dose, with the exception of rosuvastatin [17]. In addition to lowering cholesterol, however, statins also decrease the synthesis of nonsterol derivatives of mevalonate, including CoQ10 [18].

The objective of the present study was to test the hypothesis that administration of atorvastatin exerted lipid-lowering effect and increased oxidative stress due to its inhibition of CoQ10 synthesis in hypercholesterolemic patients. The results of the present study will be helpful to suggest hypercholesterolemic patients about antioxidant supplementation when given statin therapy.

2. Methods and materials

2.1. Study subjects and sample collection

Nineteen subjects were recruited as outpatients from Changhua Christian Hospital in Changhua, Taiwan. Informed consent for study enrollment was obtained from each subject, and all human experimental procedures followed the ethical standards of Changhua Christian Hospital. The subjects were without diabetes (fasting blood glucose < 110 mg/dL), hypertension (systolic blood pressure < 135 mm Hg, and diastolic blood pressure < 85 mm Hg), vitamin supplementation use, and statin therapy experience. All

subjects had hypercholesterolemia with plasma concentrations of LDL cholesterol greater than 130 mg/dL. The smoking history and dietary habit of the subjects were obtained by questionnaire, and 2 subjects were smokers. Subjects were instructed not to change the lifestyle including dietary habit and smoking at the initiation of statin therapy. Atorvastatin (10 mg/d) was administered for 5 months to all subjects. Urine and blood samples were collected in the morning after the subjects had fasted overnight before and at the end of the 5-month atorvastatin therapy. A 6-mL urine sample was collected by centrifugation at 14 000g and 4°C for 20 minutes, divided into several aliquots, and stored at –80°C until analysis. A 10-mL quantity of whole blood was withdrawn from an antecubital vein, 0.5 mL of red blood cells (RBCs) was stored at –80°C until analysis, and the other blood was collected into a heparin-containing plastic tube. Plasma was collected by centrifugation at 1000g and 4°C for 10 minutes, divided into several aliquots, and stored at –80°C until analysis or freshly used for vitamin C analysis.

2.2. Red blood cell acid-soluble glutathione measurement

The reduced glutathione (GSH) content of RBC was determined by high-performance liquid chromatography (HPLC) as described by Reed et al [19] with some modification. Briefly, we prepared 10% RBC in phosphate-buffered saline (PBS). Alkylation of GSH was performed by adding 200 μ L of 100 mmol/L of iodoacetic acid into the 200 μ L of 10% RBC. After 15-minute reaction at dark, 400 μ L of 0.5 mol/L perchloric acid containing 2 mmol/L phenanthroline was added into each tube to precipitate protein. The acid-soluble GSH and oxidized glutathione in the cellular supernatant were prepared by centrifugation at 5500g for 10 minutes. The resultant pellets were used for total protein assay. Potassium bicarbonate (KHCO₃) was then added to neutralize the acidic solution. After 15 minutes of reaction in the dark, 440 μ L of 3% 2,4-dinitrofluorobenzene (in 100% ethanol) was added to each sample and then allowed to react at 48°C for 8 hours. This was followed by centrifugation, and GSH and oxidized glutathione were then analyzed at 365 nm with an HPLC system.

2.3. Urinary 8-OHdG assay

Fifty-microliter aliquots of urine were taken and analyzed for 8-OHdG by using a JaICA kit according to the manufacturer's instructions (JaICA, Nikken SEIL Corporation, Shizuoka, Japan).

2.4. Plasma CoQ10 measurement

The frozen samples of plasma were allowed to thaw at 4°C before analysis. The plasma level of CoQ10 was determined according to the methods described by Karpinska et al [20] and Li et al [21]. Briefly, plasma was pipetted into Eppendorf microcentrifuge tubes and deproteinized with methanol. Next, *n*-hexane was added. The mixture was mixed by vortexing for 5 minutes and

centrifuged at 2500g and 4°C for 15 minutes. Next, the clear *n*-hexane layer was transferred to another tube, and the *n*-hexane extraction procedure was repeated one more time. The plasma extracts were combined and evaporated to dryness under a stream of nitrogen gas. The dry residue was dissolved in mobile phase, filtered through a membrane (PVDF filter, 4 mm, 0.45 μm; Millipore, Bedford, Mass), and injected into the HPLC system. A microBondapak C-18 3.9 mm × 30-cm stainless steel column with a guard column 3 × 22 mm packed with microBondapak C-18 was used with a mobile phase of methanol-*n*-hexane 85:15 (vol/vol). The flow rate was 1 mL/min. The wavelength of the UV detector was 276 nm. To determine the amount of CoQ10 in plasma, a calibration curve was constructed by plotting the peak areas vs. the concentration. Linearity was achieved in the concentration range of 0.12 to 1.92 μg/mL.

2.5. Plasma lipoprotein measurement

Plasma levels of lipoproteins were measured from fasting blood samples. Such measurements, along with liver enzymes and the other laboratory measurements were made by using an automated biochemical analyzer.

2.6. Plasma vitamin E and vitamin C measurements

Plasma vitamin E and vitamin C concentrations were measured according to the method described in our previous study [22].

2.7. Statistical analyses

Data are expressed as means ± SD. All statistical procedures were performed with the SPSS statistical software package (SPSS, Chicago, IL). Multiple linear regression analysis was applied to assess the importance of various contributing factors to the blood ratios of vitamin E/LDL cholesterol and CoQ10/LDL cholesterol. The significance of the difference between mean values before and after treatment was determined by 1-way analysis of variance and paired *t* test; *P* values < .05 were taken to be statistically significant.

Table 1
Baseline demographic data of the hypercholesterolemic patients^a

Variables	Male (n = 11)	Female (n = 8)
Age, y	67 ± 12	61 ± 10
Height, cm	165 ± 6	156 ± 5
Weight, kg	68 ± 12	66 ± 12
BMI, kg/cm ²	24.9 ± 4.1	26.9 ± 4.3
Systolic blood pressure, mm Hg	134 ± 13	133 ± 12
Diastolic blood pressure, mm Hg	85 ± 12	85 ± 7
Smoking index, ^a pack year	7.3 ± 18.5	0
Smoking, %	18	0

Values are presented as means ± SD.

^a Smoking index, packs of cigarettes smoked per day × years of smoking.

Table 2

Multiple linear regression analysis of factors contributing to blood ratio of vitamin E/LDL cholesterol

Independent variables	β	SE	<i>t</i>	<i>P</i>
Age, y	-0.025	0.035	-0.727	.479
Sex	0.402	0.799	0.503	.623
Body mass index, kg/m ²	0.036	0.096	0.376	.713
Smoking ^a	0.011	0.027	0.411	.687

^a Smoking index, packs of cigarettes smoked per day × years of smoking.

3. Results

3.1. Baseline demographic data of subjects

The baseline demographic data of the 11 males and 8 females enrolled in this study are reported in Table 1.

3.2. Factors contributing to blood ratios of vitamin E/LDL cholesterol and CoQ10/LDL cholesterol

To identify the factors contributing to the changes in blood ratios of vitamin E/LDL cholesterol and CoQ10/LDL cholesterol, multiple linear regression analyses of factors contributing to blood ratios of vitamin E/LDL cholesterol and CoQ10/LDL cholesterol are presented in Tables 2 and 3. Age, sex, body mass index, and smoking did not contribute significantly to the blood ratios of vitamin E/LDL cholesterol and CoQ10/LDL cholesterol.

3.3. Effects of atorvastatin on blood and urinary measurements

To determine the lipid-lowering effect of atorvastatin and the possible side effect of atorvastatin treatment, the effects of atorvastatin on blood lipid, glucose, blood urea nitrogen, uric acid, myopathy indicator, liver function indicators, and oxidative damage indicator urinary 8-OHdG were investigated. The effects of atorvastatin on blood and urinary measurements are presented in Table 4. Blood levels of total cholesterol, triglycerides, and LDL cholesterol were significantly decreased by atorvastatin therapy (*P* < .05). However, atorvastatin had no effect on the blood level of high-density lipoprotein (HDL) cholesterol. Atorvastatin caused no adverse liver events on the basis of blood aspartate aminotransferase and alanine aminotransferase activities. Atorvastatin showed no effect on fasting blood glucose, uric acid, or creatinine levels or on blood creatine phosphokinase activity. The reliable urinary biomarker of DNA repair

Table 3

Multiple linear regression analysis of factors contributing to blood ratio of CoQ10/LDL cholesterol

Independent variables	β	SE	<i>t</i>	<i>P</i>
Age, y	0.0001	0.0001	0.789	.438
Sex	0.0001	0.001	0.601	.557
Body mass index, kg/m ²	0.0001	0.0001	-0.861	.403
Smoking ^a	0.0001	0.0001	0.777	.450

^a Smoking index, packs of cigarettes smoked per day × years of smoking.

Table 4
Effect of atorvastatin on blood and urinary measurements of subjects^a

Variable	Before dosing (n = 19)	After dosing (n = 19)	P ^a
Total cholesterol, mmol/L	6.16 ± 0.7	4.29 ± 0.59	.001*
Triglycerides, mmol/L	1.74 ± 0.62	1.21 ± 0.28	.001*
HDL cholesterol, mmol/L	1.47 ± 0.44	1.53 ± 0.44	.122
LDL cholesterol, mmol/L	3.93 ± 0.67	2.25 ± 0.54	.001*
Glucose (AC), mg/dL	99 ± 19	94 ± 7	.322
Blood urea nitrogen, mg/dL	17 ± 4	17 ± 6	.000
Uric acid, mg/dL	6 ± 1	6 ± 1	.722
Creatine phosphokinase, U/L	99 ± 43	116 ± 45	.087
Creatinine level, mg/dL	1.07 ± 0.26	1.11 ± 0.31	.340
Aspartate aminotransferase, IU/L	26 ± 11	26 ± 9	.625
Alanine aminotransferase, IU/L	28 ± 17	26 ± 11	.599
8-OHdG/creatinine level, ng/mg creatinine	11.30 ± 7.07	13.13 ± 9.13	.461

Values are presented as means ± SD. Glucose (AC) indicates fasting blood glucose.

^a One-way analysis of variance and paired *t* test.

* *P* < .05.

expressed as 8-OHdG/creatinine was not associated with atorvastatin therapy.

3.4. Effects of atorvastatin on blood antioxidants

To demonstrate the role of atorvastatin in the blood antioxidants status, blood concentrations of vitamin C, vitamin E, CoQ10, and glutathione were determined. The effects of atorvastatin on blood antioxidants are summarized in Table 5. Atorvastatin had no effect on blood water-soluble antioxidants such as vitamin C and glutathione. Blood lipid-soluble antioxidants such as vitamin E and CoQ10 were significantly decreased by atorvastatin (*P* < .05); however, atorvastatin showed no effect on these 2 parameters after correction for the total cholesterol level. In contrast, atorvastatin significantly increased the blood ratios of

Table 5
Effect of atorvastatin on blood antioxidants in subjects^a

Variable	Before dosing (n = 19)	After dosing (n = 19)	P ^a
Vitamin C, μmol/L	35.23 ± 11.93	37.5 ± 12.5	.429
RBC GSH, μmol/L	597 ± 111	524 ± 179	.085
RBC oxidized glutathione, μmol/L	58.8 ± 17.4	59.2 ± 26.4	.954
Vitamin E, μmol/L	26.47 ± 6.5	19.73 ± 7.89	.001*
CoQ10, μmol/L	0.42 ± 0.17	0.25 ± 0.07	.001*
Vitamin E/total cholesterol, μmol/L/mmol	4.27 ± 0.75	4.54 ± 1.29	.133
CoQ10/total cholesterol, μmol/L/mmol	0.068 ± 0.028	0.059 ± 0.014	.064
Vitamin E/LDL cholesterol, μmol/L/mmol	6.77 ± 1.43	8.98 ± 2.85	.001*
CoQ10/LDL cholesterol, μmol/L/mmol	0.106 ± 0.043	0.113 ± 0.027	.032*

Values are presented as means ± SD.

^a One-way analysis of variance and paired *t* test.

* *P* < .05.

vitamin E/LDL cholesterol and of CoQ10/LDL cholesterol (*P* < .05).

4. Discussion

In the present study, blood levels of total cholesterol, triglycerides, and LDL cholesterol were significantly decreased by atorvastatin therapy (*P* < .05). The total cholesterol- and LDL cholesterol-lowering effect of atorvastatin was consistent with that of Nozue et al [23]. The magnitude of decrease in blood LDL cholesterol is 39% in Nozue et al [23] and 43% in ours, both are significant. In addition to reducing cellular cholesterol and isoprenoid levels, certain statins are reported to produce large (22%–45%) reductions in triglyceride levels attributed to decreased hepatic secretion of very low-density lipoprotein (VLDL) cholesterol [24]. Statins have little effect on blood levels of HDL cholesterol. In the present study, atorvastatin resulted in a significant reduction in blood levels of triglycerides; however, it showed no effect on blood levels of HDL cholesterol. These results are consistent with those of previous studies.

The effect of statins on redox status is paradoxical. In addition to their lipid-lowering effect, statins exhibit pleiotropic actions including antiinflammation, antithrombosis, and antioxidation [25]. Oxidative stress is involved in atherosclerosis, and the antioxidative effect of statins is a concern. The GSH system, an important protective system against oxidative damage, may be affected by statins. In one study, plasma levels of GSH were significantly increased by 10 mg/d atorvastatin in hypercholesterolemic patients [26]. However, in a comparative dose-dependent study of atorvastatin, simvastatin, and pravastatin, erythrocyte GSH remained unchanged after statin treatment in hypercholesterolemic patients [27]. In the present study, 10 mg/d atorvastatin did not affect the RBC GSH level. Urinary 8-OHdG, a biomarker of oxidative stress, was significantly decreased by 10 mg/d atorvastatin in diabetic persons with hypercholesterolemia [28]; however, the same dosage of atorvastatin in the present study showed no significant effect on urinary 8-OHdG. The difference between these 2 studies may be the presence of diabetes.

In apoE^{-/-} mice, vitamin E may positively counteract atherosclerotic lesions, and the higher circulating levels of vitamin E are, the lower the atherosclerotic progression [29]. In hypercholesterolemic men treated with simvastatin (20 mg/d) for 12 weeks, serum vitamin E decreased by 16.2% (*P* < .001) and ubiquinol-10 by 22.0% (*P* < .001); however, glucose and vitamin C levels remained unchanged [30]. In men, approximately 30% of vitamin E is bound to HDL cholesterol and 60% to LDL cholesterol in plasma [31]. Thus, the decrease of serum vitamin E is expected to occur via lowering of LDL cholesterol by simvastatin. Simvastatin not only changes the level of circulating LDL cholesterol but also the composition of circulating LDL cholesterol [32]. The proportion of protein is increased; in contrast, the

proportions of free cholesterol and cholesterol esters are decreased. A more adequate expression of the circulating vitamin E level is that corrected by the cholesterol level [33].

One study showed that statin-treated patients with metabolic syndrome had higher plasma levels of vitamin E ($P = .02$) than did statin-free patients after correction for cholesterol levels [25]. Hypercholesterolemic patients treated with 10 mg/d atorvastatin showed a reduction of urinary isoprostanes after only 3 days (-18.8% ; $P < .01$); a stronger reduction of urinary isoprostanes was noted after 30 days (-37.1% ; $P < .01$) [34]. Meanwhile, an increase in plasma vitamin E ($+42\%$; $P < .01$) and a decrease in total cholesterol (-24.9% ; $P < .01$) were observed after 30 days. Hypercholesterolemic patients had enhanced oxidative stress and low levels of vitamin E compared with controls, and these defects were corrected after statin treatment [34]. These findings have several possible clinical implications. Oxidative stress is recognized to play a crucial role in atherosclerosis. First, the possible antiatherosclerotic property of statins is corroborated by the increase in the plasma antioxidant vitamin E. Second, it is not necessary for statin-treated patients to be supplemented with antioxidant vitamins because no further increase in oxidative stress resulted. This speculation is confirmed by a previous study showing no further decrease of isoprostanes in statin-treated patients given vitamin E concurrently [35].

Levels of lipid-soluble antioxidants such as vitamin E and CoQ10 were significantly lower in subjects receiving atorvastatin ($P < .05$). However, after correction for LDL cholesterol, the plasma levels of these two antioxidants were significantly greater in subjects receiving atorvastatin. These results are consistent with that of a previous study performed by Colquhoun et al [36]. In that study, simvastatin therapy of 20 mg/d significantly reduced plasma levels of vitamin E and CoQ10 in hypercholesterolemic subjects after 6 months. However, the plasma ratios of vitamin E/LDL cholesterol and CoQ10/LDL cholesterol increased significantly. Blood CoQ10 is mainly present in the plasma fraction (about 75%), where it is associated with lipoproteins; the remaining 25% is in cellular components [4].

It is reported that CoQ10 is secreted from the liver into VLDL [37]. VLDL undergoes remodeling during circulation in blood, and vitamin E and CoQ10 carried by VLDL are likely transferred to LDL. As suggested by the previous literature, atorvastatin did not cause any adverse effect on plasma levels of vitamin E and CoQ10. It might be inappropriate to express blood levels of lipid-soluble antioxidants in microgram per milliliter because they are carried by lipoproteins; however, their values should be corrected for blood lipoproteins. In the present study, blood level of water-soluble antioxidant vitamin C was not affected by atorvastatin treatment. Based on the criteria of optimal blood vitamin C range reported by Gey [38], the blood vitamin C nutritional status of our subjects is suboptimal and this may be due to the unhealthy dietary habits. However, blood vitamin E nutritional status is acceptable according to

the criteria proposed by Horwitt et al [39] and Thurnham et al [40], and plasma value of micromole of vitamin E per micromole of cholesterol greater than 2.25 is believed to be satisfactory. According to the level of blood antioxidants, the vitamin E intake of the subjects is appropriate and the vitamin C intake of the subjects needs to be improved.

Myopathy and asymptomatic but marked and persistent increases in liver transaminases are the most serious adverse effects of statin use [41]. In hypercholesterolemic patients, 10 mg/d atorvastatin for 8 weeks resulted in a significant increase in serum levels of aspartate aminotransferase and alanine aminotransferase, and several patients had serum concentrations higher than the upper normal limits [41]. However, 10 mg/d atorvastatin for 5 months did not cause abnormal liver enzyme elevation in hypercholesterolemic patients in the present study. This result indicates the safety of the used atorvastatin dosage to our subjects in the present study.

Myalgia is a disease related to statin therapy. However, no patients in the present study reported myalgia. Myopathy is a reversible adverse effect associated with all statins, and it is defined as a serum creatine kinase (CK) level more than 10 times the upper limit of normal with muscle symptoms [42]. The CK elevation has been reported in humans [43] and rabbits [44] receiving statins. Atorvastatin increased blood levels of CK of subjects in the present study; however, the effect was not significant. In the present study, fasting blood levels of glucose, urea nitrogen, uric acid, and creatinine were not affected by atorvastatin therapy.

In conclusion, treatment of hypercholesterolemic patients with 10 mg/d atorvastatin for 5 months is safe according to the effects on liver function, myopathy, urinary 8-OHdG, and blood levels of lipid-soluble antioxidants. The atorvastatin dose used in the present study is effective to lower blood levels of lipoproteins and **increase the blood ratios of vitamin E/LDL cholesterol and CoQ10/LDL cholesterol that indicate that administration of atorvastatin has no oxidative stress-inducing effect** and it is not necessary to give antioxidants supplementation to subjects receiving atorvastatin treatment. There are limitations of the study. The study subjects were from a single outpatient service in the Changhua Christian Hospital in Changhua, Taiwan, and thus limited by a small sample size.

Acknowledgment

This study was supported by grant CCH-93141 from Changhua Christian Hospital, Changhua, Taiwan.

References

- [1] Zulli A, Hare DL, Buxton BF, Black MJ. High dietary methionine plus cholesterol exacerbates atherosclerosis formation in the left main coronary artery of rabbits. *Atherosclerosis* 2004;176:83-9.
- [2] Braunwald E. Shattuck lecture—cardiovascular medicine at the turn of the millennium: triumphs, concerns, and opportunities. *N Engl J Med* 1997;337:1360-9.

- [3] Parthasarathy S, Steinberg D, Witztum JL. The role of oxidized low-density lipoproteins in the pathogenesis of atherosclerosis. *Annu Rev Med* 1992;43:219-25.
- [4] Tomasetti M, Alleva R, Solenghi MD, Littarru GP. Distribution of antioxidants among blood components and lipoproteins: significance of lipids/CoQ10 ratio as a possible marker of increased risk for atherosclerosis. *Biofactors* 1999;9:231-40.
- [5] Stocker R, Bowry VW, Frei B. Ubiquinol-10 protects human low density lipoprotein more efficiently against lipid peroxidation than does alpha-tocopherol. *Proc Natl Acad Sci U S A* 1991;88:1646-50.
- [6] Yamashita S, Yamamoto Y. Simultaneous detection of ubiquinol and ubiquinone in human plasma as a marker of oxidative stress. *Anal Biochem* 1997;250:66-73.
- [7] Witting PK, Pettersson K, Letters J, Stocker R. Anti-atherogenic effect of coenzyme Q10 in apolipoprotein E gene knockout mice. *Free Radic Biol Med* 2000;29:295-305.
- [8] Watts GF, Playford DA, Croft KD, Ward NC, Mori TA, Burke V. Coenzyme Q(10) improves endothelial dysfunction of the brachial artery in type II diabetes mellitus. *Diabetologia* 2002;45:420-6.
- [9] Cooke MS, Evans MD, Dove R, Rozalski R, Gackowski D, Siomek A, et al. DNA repair is responsible for the presence of oxidatively damaged DNA lesions in urine. *Mutat Res* 2005;574:58-66.
- [10] Dizdaroglu M, Jaruga P, Birincioglu M, Rodriguez H. Free radical-induced damage to DNA: mechanisms and measurement. *Free Radic Biol Med* 2002;32:1102-15.
- [11] Halliwell B, Whiteman M. Measuring reactive species and oxidative damage in vivo and in cell culture: how should you do it and what do the results mean? *Br J Pharmacol* 2004;142:231-55.
- [12] Gackowski D, Rozalski R, Roszkowski K, Jawien A, Foksinski M, Olinski R. 8-Oxo-7,8-dihydroguanine and 8-oxo-7,8-dihydro-2'-deoxyguanosine levels in human urine do not depend on diet. *Free Radic Res* 2001;35:825-32.
- [13] Tomasetti M, Littarru GP, Stocker R, Alleva R. Coenzyme Q10 enrichment decreases oxidative DNA damage in human lymphocytes. *Free Radic Biol Med* 1999;27:1027-32.
- [14] Niklowitz P, Sonnenschein A, Janetzky B, Andler W, Menke T. Enrichment of coenzyme Q10 in plasma and blood cells: defense against oxidative damage. *Int J Biol Sci* 2007;3:257-62.
- [15] Sever PS, Dahlof B, Poulter NR, Wedel H, Beevers G, Caulfield M, et al. ASCOT investigators. Prevention of coronary and stroke events with atorvastatin in hypertensive patients who have average or lower-than-average cholesterol concentrations, in the Anglo-Scandinavian Cardiac Outcomes Trial-Lipid Lowering Arm (ASCOT-LLA): a multicentre randomised controlled trial. *Lancet* 2003;361:1149-58.
- [16] Pedersen TR, Faergeman O, Kastelein JJ, Olsson AG, Tikkanen MJ, Holme I, et al. Incremental Decrease in End Points Through Aggressive Lipid Lowering (IDEAL) Study Group. High-dose atorvastatin vs usual-dose simvastatin for secondary prevention after myocardial infarction: the IDEAL study: a randomized controlled trial. *JAMA* 2005;294:2437-45.
- [17] Poli A. Atorvastatin: pharmacological characteristics and lipid-lowering effects. *Drugs* 2007;67:3-15.
- [18] Rundek T, Naini A, Sacco R, Coates K, DiMauro S. Atorvastatin decreases the coenzyme Q10 level in the blood of patients at risk for cardiovascular disease and stroke. *Arch Neurol* 2004;61:889-92.
- [19] Reed DJ, Babson JR, Beatty PW, Brodie AE, Ellis WW, Potter DW. High-performance liquid chromatography analysis of nanomole levels of glutathione, glutathione disulfide, and related thiols and disulfides. *Anal Biochem* 1980;106:55-62.
- [20] Karpinska J, Mikoluc B, Motkowski R, Piotrowska-Jastrzebska J. HPLC method for simultaneous determination of retinol, alpha-tocopherol and coenzyme Q10 in human plasma. *J Pharm Biomed Anal* 2006;42:232-6.
- [21] Li K, Shi Y, Chen S, Li W, Shang X, Huang Y. Determination of coenzyme Q10 in human seminal plasma by high-performance liquid chromatography and its clinical application. *Biomed Chromatogr* 2006;20:1082-6.
- [22] Liu CS, Lii CK, Ou CC, Tsai CH, Wei YH, Chen HW. Autoantibody against oxidized low-density lipoproteins may be enhanced by cigarette smoking. *Chem Biol Interact* 2000;127:125-37.
- [23] Nozue T, Michishita I, Ito Y, Hirano T. Effects of statin on small dense low-density lipoprotein cholesterol and remnant-like particle cholesterol in heterozygous familial hypercholesterolemia. *J Atheroscler Thromb* 2008;15:146-53.
- [24] Mason RP, Walter MF, Day CA, Jacob RF. Intermolecular differences of 3-hydroxy-3-methylglutaryl coenzyme a reductase inhibitors contribute to distinct pharmacologic and pleiotropic actions. *Am J Cardiol* 2005;96:11F-23F.
- [25] Cangemi R, Loffredo L, Carnevale R, Pignatelli P, Violi F. Statins enhance circulating vitamin E. *Int J Cardiol* 2008;123:172-4.
- [26] Save V, Patil N, Moulik N, Rajadhyaksha G. Effect of atorvastatin on type 2 diabetic dyslipidemia. *J Cardiovasc Pharmacol Ther* 2006;11:262-70.
- [27] Passi S, Stancato A, Aleo E, Dmitrieva A, Littarru GP. Statins lower plasma and lymphocyte ubiquinol/ubiquinone without affecting other antioxidants and PUFA. *Biofactors* 2003;18:113-24.
- [28] Endo K, Miyashita Y, Sasaki H, Ebisuno M, Ohira M, Saiki A, et al. Probuocol and atorvastatin decrease urinary 8-hydroxy-2'-deoxyguanosine in patients with diabetes and hypercholesterolemia. *J Atheroscler Thromb* 2006;13:68-75.
- [29] Pratico D, Tangirala RK, Rader DJ, Rokach J, FitzGerald GA. Vitamin E suppresses isoprostane generation in vivo and reduces atherosclerosis in ApoE-deficient mice. *Nat Med* 1998;4:1189-92.
- [30] Jula A, Marniemi J, Huupponen R, Virtanen A, Rastas M, Ronnema T. Effects of diet and simvastatin on serum lipids, insulin, and antioxidants in hypercholesterolemic men: a randomized controlled trial. *JAMA* 2002;287:598-605.
- [31] Behrens WA, Thompson JN, Madere R. Distribution of alpha-tocopherol in human plasma lipoproteins. *Am J Clin Nutr* 1982;35:691-6.
- [32] Kleinvelde HA, Demacker PN, De Haan AF, Stalenhoef AF. Decreased in vitro oxidizability of low-density lipoprotein in hypercholesterolemic patients treated with 3-hydroxy-3-methylglutaryl-CoA reductase inhibitors. *Eur J Clin Invest* 1993;23:289-95.
- [33] Traber MG, Jialal I. Measurement of lipid-soluble vitamins—further adjustment needed? *Lancet* 2000;355:2013-4.
- [34] Cangemi R, Loffredo L, Carnevale R, Perri L, Patrizi MP, Sanguigni V, et al. Early decrease of oxidative stress by atorvastatin in hypercholesterolemic patients: effect on circulating vitamin E. *Eur Heart J* 2008;29:54-62.
- [35] De Caterina R, Cipollone F, Filardo FP, Zimarino M, Bernini W, Lazzarini G, et al. Low-density lipoprotein level reduction by the 3-hydroxy-3-methylglutaryl coenzyme-A inhibitor simvastatin is accompanied by a related reduction of F2-isoprostane formation in hypercholesterolemic subjects: no further effect of vitamin E. *Circulation* 2002;106:2543-9.
- [36] Colquhoun DM, Jackson R, Walters M, Hicks BJ, Goldsmith J, Young P, et al. Effects of simvastatin on blood lipids, vitamin E, coenzyme Q10 levels and left ventricular function in humans. *Eur J Clin Invest* 2005;35:251-8.
- [37] Elmberger PG, Kalen A, Brunk UT, Dallner G. Discharge of newly-synthesized dolichol and ubiquinone with lipoproteins to rat liver perfusate and to the bile. *Lipids* 1989;24:919-30.
- [38] Gey KF. Cardiovascular disease and vitamins. Concurrent correction of 'suboptimal' plasma antioxidant levels may, as important part of "optimal" nutrition, help to prevent early stages of cardiovascular disease and cancer, respectively. *Bibl Nutr Dieta* 1995;52:75-91.
- [39] Horwitt MK, Harvey CC, Dahm Jr CH, Searcy MT. Relationship between tocopherol and serum lipid levels for determination of nutritional adequacy. *Ann N Y Acad Sci* 1972;203:223-36.
- [40] Thurnham DI, Davies JA, Crump BJ, Situnayake RD, Davis M. The use of different lipids to express serum tocopherol: lipid ratios for the measurement of vitamin E status. *Ann Clin Biochem* 1986;23:514-20.

- [41] Mabuchi H, Higashikata T, Kawashiri M, Katsuda S, Mizuno M, Nohara A, et al. Reduction of serum ubiquinol-10 and ubiquinone-10 levels by atorvastatin in hypercholesterolemic patients. *J Atheroscler Thromb* 2005;12:111-9.
- [42] LaRosa JC, Grundy SM, Waters DD, Shear C, Barter P, Fruchart JC, et al. Treating to New Targets (TNT) Investigators. Intensive lipid lowering with atorvastatin in patients with stable coronary disease. *N Engl J Med* 2005;352:1425-35.
- [43] Wortmann RL, Tipping RW, Levine JG, Melin JM. Frequency of myopathy in patients receiving lovastatin. *Am J Cardiol* 2005;95: 983-5.
- [44] Nakahara K, Kuriyama M, Sonoda Y, Yoshidome H, Nakagawa H, Fujiyama J, et al. Myopathy induced by HMG-CoA reductase inhibitors in rabbits: a pathological, electrophysiological, and biochemical study. *Toxicol Appl Pharmacol* 1998;152: 99-106.