

Coenzyme Q10 Reduction with Statins: Another Pleiotropic Effect

Hiroshi Mabuchi^{1,*}, Atsusi Nohara¹, Junji Kobayashi¹, Masa-aki Kawashiri² and Akihiro Inazu³

¹Kanazawa University Graduate School of Medical Science, Department of Lipidology and ²Molecular Genetics of Cardiovascular Disorders; ³Kanazawa University Graduate School of Medical Science, Department of Laboratory Science

Abstract: Hypercholesterolemia is a major coronary risk factor, and extensive epidemiological data have shown that the higher the serum cholesterol level the higher the incidence of coronary heart disease (CHD). Over the past decade, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) have emerged as one of the most effective means of reducing risk for CHD. A number of clinical trials have demonstrated that statins are not only safe and well tolerated but also significantly decrease CHD morbidity and mortality in hyper- and normo-cholesterolemic patients in both primary and secondary prevention studies. These findings support “the lower the cholesterol, the better” concept.

HMG-CoA reductase converts HMG-CoA to mevalonate, and inhibition of this enzyme results in decreased synthesis of cholesterol and other products downstream of mevalonate. Sometimes clinical results from treatment with statins are not fully explained by reduction in serum cholesterol levels. These effects of the statins that go beyond the clinical effects brought about by cholesterol reduction are called “pleiotropic effects”. Many of these so-called pleiotropic effects have been shown to be secondary to inhibition of the synthesis of isoprenoid intermediates of the mevalonate pathway, and, thus, are completely independent of intracellular cholesterol biosynthesis. Although statins have been known to be safe, fatal cases of rhabdomyolysis in connection with this drug has raised major concerns about the possibility that certain pleiotropic effects of statins could also be harmful.

Mevalonate is a precursor of coenzyme Q10 (CoQ10) which is a central compound of the mitochondrial respiratory chain, and is a potent lipophilic antioxidant present in nearly all human tissues and plasma lipoproteins. Decreased content of CoQ10 found in the patient’s plasma could therefore increase its potential to oxidize. Supplementation of CoQ10 may restore tissue and plasma CoQ10 concentrations in patients treated with statins. Inhibitors of cholesterol biosynthesis beyond mevalonate may reduce cholesterol synthesis without inhibiting the biosynthesis of CoQ10 and dolichol. Here, we have reviewed CoQ10 metabolism in patients treated with statins, and the correlation with the possible side effects of statins.

Key Words: CoQ10, statin, mevalonate pathway, squalene synthase inhibitor, pleiotropic effect, rhabdomyolysis.

1. INTRODUCTION

Low-density-lipoprotein (LDL) hypercholesterolemia is a major coronary risk factor, and a large number of epidemiological data have shown that the higher the serum cholesterol level, the higher the incidence of coronary heart disease (CHD) [1]. Lowering LDL cholesterol with medications or life-habit changes reduces the morbidity and mortality of CHD. Of the LDL-cholesterol-lowering drugs, 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (statins) have been the most popular during the past decade. The long-term efficacy and safety of statins have been established in large multicenter trials regarding cholesterol lowering for preventing coronary events in both primary [2] and secondary prevention [3,4]. Statins account for the largest segment of the prescription-drug market in the United States and other countries [5].

HMG-CoA reductase converts HMG-CoA to mevalonate, with this catalysis constituting a rate-limiting step in the biosynthesis of cholesterol (Fig. 1). Feedback suppression of cholesterol synthesis in the liver by dietary cholesterol is mediated through changes in the activity of HMG-CoA reductase. These findings, obtained by 1970, supported

the concept that the inhibition of HMG-CoA reductase would be an effective means of lowering plasma cholesterol in humans. Endo *et al.* started researching HMG-CoA reductase inhibitors of microbial origin in 1971 [6]. They hoped that certain microorganisms would produce such inhibiting compounds as weapons in the fight against other microbes that required sterols or other isoprenoids for growth. Inhibition of HMG-CoA reductase would thus be lethal to these microbes. Approximately 6,000 microbial strains were tested for their ability to block cholesterol synthesis over a 2-year period. Finally they found a strong inhibitor of HMG-CoA reductase, and the compound was named compactin (ML-236B). The structural similarity between compactin and HMG-CoA clarified the structure-activity relationships in the inhibition of HMG-CoA reductase.

2. BENEFICIAL PLEIOTROPIC EFFECTS

Over the past decade, statins have emerged as one of the most effective types of drugs in reducing risk for CHD. Many large clinical trials have demonstrated that statins are not only safe and well tolerated but also significantly decrease CHD morbidity and mortality. These findings support the “the lower the cholesterol, the better” concept in the management of CHD [7-9].

Sometimes substantial reduction in coronary events can be observed in the very early phases of clinical trials, and significant reductions of coronary events were obtained

*Address correspondence to this author at the Department of Lipidology, Kanazawa University, University Takahara-machi 13-1, Kanazawa 920-8641, Japan; E-mail: mabuchi@med.kanazawa-u.ac.jp

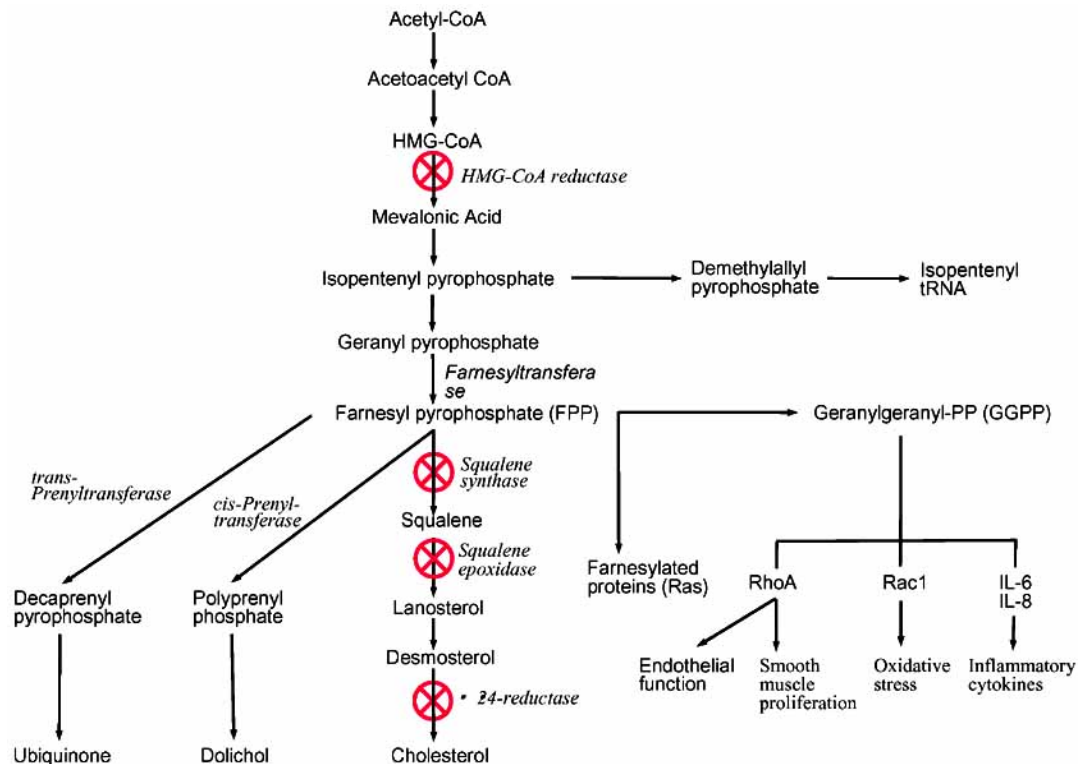


Fig. (1). Mevalonate pathway. Inhibition of HMG-CoA reductase by statins reduces the downstream products of mevalonate.

within several months of the trials [10]. These findings suggest that statin therapy favorably influences a number of diverse clinical events through both effects related to the lowering of LDL cholesterol levels and effects independent of the lowering of LDL cholesterol levels. Further evidence in support of the cholesterol-independent benefits of statin therapy was obtained from angiographic trials, and the beneficial effects of statins were attributed to plaque stabilization and remodeling [11-13]. In aggressive cholesterol-lowering trials statins were shown to be effective as early as 16 weeks after acute coronary ischemia, [10] which is too short a time for appreciable changes in vascular remodeling, and some other actions of statins, especially the improvement of endothelial function, may produce these early benefits. Many of these so-called pleiotropic effects have been shown to be secondary to the inhibition of the synthesis of isoprenoid intermediates of the mevalonate pathway, such as farnesylpyrophosphate (FPP) and geranylgeranylpyrophosphate (GGPP), and thus are completely independent of intracellular cholesterol biosynthesis (Fig. 1). These vascular biological pleiotropic effects have been extensively studied in *in vitro* and animal studies, and these effects are beyond the present review [11-13]. The mechanisms underlying most of the pleiotropic effects have been attributed to reduction of the products downstream of mevalonate, such as Rho, Ras, Rac, etc. Such research involving *in vitro* or animal studies, although interesting and important, should not obscure the primary effects of statins as effective LDL-cholesterol-lowering agents [14-16].

Although statins have been known to be safe, the withdrawal of cerivastatin [17] from the market because of fatal cases of rhabdomyolysis in connection with this compound

has raised major concerns about the possibility that certain pleiotropic effects of statins could be harmful. Most of the clinically favorable pleiotropic effects are the results of the reduction of one half of the mevalonate metabolic pathway, as with the inhibition of FPP and GGPP, while the reduction of the other half of the mevalonate pathway produces CoQ10 and dolichol deficiencies. These two compounds are essential for cell health in animals including humans, and a deficiency in these nonsteroidal isoprenoid substances may produce some adverse reactions to statins. Mevalonate is a precursor of CoQ10, which is a central compound of the mitochondrial respiratory chain and is a potent lipophilic antioxidant present in nearly all human tissues. Decreased levels of CoQ10 and α -tocopherol in a patient's plasma could therefore increase its potential for oxidation. Although the list of cellular effects of statins on the vascular wall continues to grow, it remains to be determined which, if any, of these effects accounts for the clinical benefits of statin therapy in cardiovascular disease [14-16].

3. BEYOND THE "ONE DRUG, ONE DISEASE" MODEL

Goldstein and Brown postulated the benefits that regulation of this system could have on malignancy and cardiovascular disease before clinical evidence lent its broad support [18]. Statins have greatly broadened the pleiotropic effects and clinical indications in the treatment of cardiovascular diseases, and basic and clinical studies have given statins further possible indications for other diseases. (A list of putative diseases indicated for statin therapy is shown in Table 1). Thus, clinical experiences with statins obtained in large clinical trials will transcend the limitations of the "one drug, one disease" model [19]. Cholesterol may have a dual role in

Table 1. Putative New Indications of Statin for Other Diseases

Disease category	Disease	Effects of statins	Reference
Infectious disease	Acute bacterial infection	reduce rate of sepsis	[69]
Heart disease (other than coronary heart disease)	Cardiomyopathy	improve cardiac function	[70]
	Heart transplant	reduce coronary vasculopathy	[71]
		decrease rejection episodes and improve 1-year survival	[72]
		improve 5-year survival	[73]
		better survival	[74]
		decrease risk of death and fatal rejection	[75]
	Heart failure	improve survival in patients with heart failure	[76]
Calcific aortic stenosis	no stop of the progression of calcific aortic stenosis	[77]	
Cancer	Cancer	meta-analysis	[78]
	Colorectal cancer	reduce risk of cancer	[79]
	Cancer	reduce cancer incidence	[80]
Neurological disease	Multiple sclerosis	decrease lesions	[81]
	Dementia	decrease the risk for developing dementia	[82]
		no changes by statin	[83]
		some clinical benefit	[84]
	no decreased risk of dementia	[85]	
Rheumatic disease	Systemic lupus erythematosus	reduce proteinuria	[86]
	Rheumatoid arthritis	reduce CRP	[86]
Bone disease	Bone fracture and osteoporosis	reduce bone fracture risk	[87]
		decrease the risk of osteoporotic bone fracture	[88]
		improvement	[89]
		no improvement of bone fracture and or bone density	[90]
Kidney disease	Contrast-induced nephropathy	reduction in nephropathy after PCI	[91]
	Renal transplant	no effect on graft loss	[92]
	Renal transplant	no lowering of acute rejection	[93]

(Table 1. Contd....)

Disease category	Disease	Effects of statins	Reference
	Chronic kidney disease	reduce proteinuria and progression of kidney disease	[94]
	Chronic kidney disease	prevent loss of kidney function	[95]
	Renal disease	arrest the progression of renal disease	[96]
	Chronic renal insufficiency	slow renal function loss	[97]
Hypertension	Hypertension and hypercholesterolemia	lower blood pressure	[98]
	Hypertensive type 2 diabetes	reduction of urinary albumin	[99]
	Hypertension	reduce proteinuria	[100]
Lung disease	Lung transplantation	clinical benefits after pulmonary transplant	[101]

the pathogenesis of Alzheimer's disease; increased concentrations of cellular cholesterol may promote neurodegeneration, while low cholesterol concentrations in the brain may stave off neurodegeneration. Two clinical trials are ongoing: the Cholesterol-Lowering Agent to Slow Progression of Alzheimer's Disease (CLASP) study, and the Lipitor's Effect in AD (LEAD) study [20]. Animal studies have suggested that statins increase bone's formation rate, volume, and density. Observational studies suggest an association between statins and reductions in bone fracture risk. However, large randomized controlled studies are needed to confirm this association [21]. Patients with chronic kidney disease administered statins show evidence of improved renal function [22]. Poynter *et al.* reported a reduction in the relative risk of colorectal cancer of 47% associated with statin therapy, after adjustment for a number of potential confounders and effect modifiers [23]. Many of these findings should currently be considered as hypothesis-generating, and prospective clinical studies would be required to demonstrate the beneficial effects of statins before clinical recommendations could be made.

4. SAFETY OF STATINS

Although statins are generally well tolerated and safe, the most worrisome adverse effects associated with statins are myopathy and an asymptomatic increase in hepatic transaminases, both of which occur infrequently [24]. The most severe form of myotoxicity, rhabdomyolysis, can occur with all statin drugs, either in monotherapy or in combination therapy, especially with fibrates [25, 26]. Fatal rhabdomyolysis has been reported to occur at an incidence of less than 1 per 1 million statin prescriptions, except with cerivastatin, which was associated with an incidence of over 3 deaths per 1 million prescriptions, leading to its withdrawal from the market in 2001 [17]. But the adverse effects are not limited to cerivastatin, and all currently marketed statins appear to have a similar potential for causing rhabdomyolysis. These adverse reactions are not limited to those patients sensitive to statins. Any patients may be sensitive to statins if they: are of

advanced age (>80 years); have a small body frame and/or frailty; have multisystem disease (e.g. chronic renal failure); are in a perioperative period; take multiple medications; consume large quantities of grapefruit juice; or abuse alcohol. Women are more susceptible than men [25].

Although the fundamental mechanisms of statin-associated myopathy and liver disease are unknown, it has been suggested that statins lead to inhibited synthesis of compounds arising from the synthetic pathway of cholesterol. In theory, this could lead to CoQ10 deficiency in muscle-cell mitochondria, disturbing normal cellular respiration and causing adverse effects including rhabdomyolysis [27].

5. MEVALONATE PATHWAY AND BIOSYNTHESIS OF COENZYME Q10

It may be estimated that with a normal diet, 60% of plasma CoQ10 is endogenous. Mevalonate, which is formed by HMG-CoA reductase, is essential for several isoprenoid compounds as well as cholesterol. The cholesterol biosynthesis pathway downstream from mevalonate includes a number of branches. The control of the synthesis of these different isoprenoid compounds is still far from being understood. The FPP and GGPP activate a number of cell-signaling proteins through the formation of covalent bonds (prenylation). Thus, statins stabilize plaque by favorably altering vascular biology, e.g. inhibiting proliferation and migration of endothelial cells and inhibiting inflammation and oxidative stress [28]. However, many *in vitro* studies used statin concentrations much higher than those we can achieve in humans using currently recommended doses, prompting skepticism of the *in vivo* relevance of the observed effects.

Mevalonate is a precursor of CoQ10, dolichol and heme through FPP (Fig. 1). In classical experiments using fibroblasts and smooth-muscle cells, radiolabeled mevalonate is converted into four end-products that are essential for cell growth: cholesterol, dolichol, ubiquinone, and isopentenyl transfer RNA [29]. Cholesterol and CoQ10 are thus synthesized through a common biosynthetic pathway involving

mevalonate [30]. It is reasonable to assume that anything that would interfere with the supply of mevalonate would greatly affect the rate and extent of CoQ10 biosynthesis.

In our primary research we observed no definite decrease in the serum levels of CoQ10, and we speculated that when HMG-CoA reductase is partially suppressed by compactin as well as LDL, cells must have some way of diverting the small amounts of synthesized mevalonate preferentially into the crucial nonsterol products (e.g. CoQ10) [31]. However, we suggested that when one uses drugs that decrease serum cholesterol levels by inhibiting HMG-CoA reductase, attention must be paid to the nonsteroid products of mevalonate. Even in the 1990s it was said that lovastatin does not inhibit HMG-CoA reductase completely, and biologically necessary amounts of mevalonate are available preferentially for the biosynthesis of CoQ10.

6. BIOLOGICAL FUNCTION OF COQ10

CoQ10 was discovered in 1957 by Crane *et al.* [32] as a component of beef heart mitochondria, and it is naturally found throughout the body, including in the heart, liver, and skeletal muscle. CoQ10 is well defined as a crucial component of the oxidative phosphorylation process in mitochondria, which converts the energy in carbohydrates and fatty acids into adenosine triphosphate (ATP) [33]. CoQ10 is clearly necessary for cellular ATP production and of particular importance in heart muscle function, given this tissue's extreme energy requirements.

CoQ10 is carried in the blood with LDL and serves to diminish the oxidation of LDL cholesterol in settings of oxidative stress. CoQ10 is known to be closely linked to vitamin E and serves to regenerate the reduced α -tocopherol of vitamin E as well as the reduced form of ascorbate [34]. Yamashita and Yamamoto [35] reported a method for the simultaneous determination of levels of ubiquinol-10 and ubiquinone-10 in human plasma. The ratio of ubiquinol to ubiquinone should be a good marker of oxidative stress. Oxidation of plasma lipoproteins is thought to represent a key step in the early development of atherosclerosis [36].

7. DISEASES ASSOCIATED WITH COQ10 DEFICIENCY

In humans, the only documented inborn error of CoQ10 biosynthesis is caused by a deficiency in mevalonate kinase (MK) [37]. Patients with this deficiency manifest mevalonic aciduria, which has a broad range of clinical symptoms, including psychomotor retardation, ataxia, cerebral atrophy, and myopathy. This study identified an inherited disorder in cholesterol and nonsterol isoprene biosynthesis in humans. The low rate of CoQ10 and dolichol synthesis in MK-deficient fibroblasts suggests that dietary CoQ10 may be masking a more profound deficiency in the tissues of these patients [38,39].

Mitochondrial encephalomyopathies present as a heterogeneous group of disorders characterized by morphological, biochemical, and genetic abnormalities of mitochondria. The first case of severe muscle CoQ10 deficiency associated with mitochondrial encephalopathy was described by Ogasawara

et al. [40]. Marked clinical improvement was reported in three of these patients after oral supplementation with CoQ10 (150 mg/day). CoQ10 is administered for an ever-widening range of disorders. The clinical aspects of CoQ10 treatment are reviewed by Littarru and Tiano [41].

There have been several other diseases reported due to CoQ10 deficiency. A deficiency of CoQ10 in myocardial tissue was first described by Folkers and co-workers in patients with various heart diseases, [42] and a relationship has been reported between the degree of CoQ10 deficiency in blood and myocardial tissue and the severity of heart disease [43]. In patients with atherosclerosis, where oxidative modification of LDL has been implicated as an important event in disease progression, [36] studies have reported decreased levels of reduced CoQ10 in plasma and LDL [44-47]. Reduced plasma concentrations of CoQ10 have been reported in conjunction with cancers such as breast cancer, myeloma, lymphoma, and lung cancer. Lower serum CoQ10 levels have been reported in association with neurodegenerative diseases such as Parkinson's disease, but normal concentrations of serum CoQ10 have been reported in cases of Alzheimer's disease and multiple sclerosis [48].

8. REDUCTIONS OF SERUM COQ10 BY STATINS IN HUMANS: UNBENEFICIAL PLEIOTROPIC EFFECTS OF STATINS

Most of the pleiotropic effects of statins are favorable for preventing and regressing atherosclerosis. In the earlier stages of the mevalonate pathway, other fields of the metabolic pathway had been illuminated through *in vivo* and *in vitro* studies. In our classical paper we reported the effects of compactin (a prototype of statin) on serum lipoprotein levels and CoQ10 concentrations in heterozygous patients with familial hypercholesterolemia, and we observed that LDL levels of CoQ10 decreased significantly, but serum CoQ10 levels did not change [31]. Thereafter, numerous papers reported the changes of serum CoQ10 levels enacted by statins (Table 2). Most of the clinical data show a definite reduction of CoQ10 in accordance with the LDL cholesterol-lowering power of statins. As CoQ10 is a lipid-soluble substance that is mainly carried by lipoproteins, the reduction in levels of LDL as a carrier of CoQ10 was followed by the reduction in LDL levels of CoQ10, and the CoQ10/LDL-cholesterol concentration did not change so greatly. Thus, the more potent statins usually show larger reductions of CoQ10 concentrations. In our recent paper we reported on a study describing serum ubiquinol-10 and ubiquinone-10 and the ratio of ubiquinol-10 to total CoQ10 in hypercholesterolemic patients treated with atorvastatin, and we observed definite reductions of serum CoQ10 levels in all patients (Fig. 2) [49]. Pravastatin treatment decreased plasma dolichol by 16%, while CoQ10 was decreased by 29%. The decrease in both substances is related to the dose as well as the potency of the statins [50].

9. COQ10 CHANGES IN ORGAN TISSUES IN ANIMALS AND HUMANS TREATED WITH STATINS

CoQ is synthesized in all cells *via* the mevalonate pathway, and the predominant CoQ homologue in rodents is

Table 2. Changes of CoQ10 Concentrations in Serum and Organ Tissues by Statins in Human Studies

Authors	Statins	Dosage	% Changes of CoQ10 concentration		Reference
		(mg/day)	Serum or plasma	Tissue	
Mabuchi H, <i>et al.</i>	Compactin	30-60	-7.9		[31]
Folkers K, <i>et al.</i>	Lovastatin	40	-18.8		[102]
Elmberger PG, <i>et al.</i>	Pravastatin	40	-33.1	Liver; no reduction	[50]
Watts GF, <i>et al.</i>	Simvastatin	10-80	-39.6		[103]
Ghirlanda G, <i>et al.</i>	Pravastatin	20	-51.2		[104]
	Simvastatin	20	-50.0		
Bargossi AM, <i>et al.</i>	Simvastatin	20	-27.7	Platelet -25.4%	[105]
Laaksonen R, <i>et al.</i>	Simvastatin	20-40	-25.2		[106]
	Lovastatin	20-40	-21.3		
Laaksonen R, <i>et al.</i>	Simvastatin	20	-31.2	Skeletal muscle +46.7%	[107]
Laaksonen R, <i>et al.</i>	Simvastatin	20	-27.3	Skeletal muscle +9.0%	[53]
De Pinieux G, <i>et al.</i>	Statins		decreased		[108]
Human JA, <i>et al.</i>	Simvastatin	10-20	-25.5		[109]
Palomaki A, <i>et al.</i>	Lovastatin	60	Ubiquinol/LDL -13		[110]
Mortensen SA, <i>et al.</i>	Pravastatin	10-40	-19.7		[111]
	Lovastatin	20-80	-28.8		
de Lorgeril M, <i>et al.</i>	Simvastatin	20	-19.4		[112]
Miyake Y, <i>et al.</i>	Simvastatin	5	-30.7		[113]
Bleske BE, <i>et al.</i>	Pravastatin	20	1.6		[114]
	Atorvastatin	10	-7.7		
Jula A, <i>et al.</i>	Simvastatin	20	-22.0		[115]
Passi S, <i>et al.</i>	Atorvastatin	20	-48.5	Lymphocyte -56.0%	[116]
	Pravastatin	40	-45.8	Lymphocyte -34.6%	
	Simvastatin	20	-32.0	Lymphocyte -47.0%	
Silver MA, <i>et al.</i>	Atorvastatin	20	variable		[117]
Rundek T, <i>et al.</i>	Atorvastatin	80	-50.8		[118]
Strey CH, <i>et al.</i>	Atorvastatin	40	-33.1		[119]
Mabuchi H, <i>et al.</i>	Atorvastatin	10	-42.9		[49]
Colquhoun DM, <i>et al.</i>	Simvastatin	20	-12.4		[120]

CoQ9, while in humans it is CoQ10 [51]. Effects of statins on CoQ10 concentrations in animal organ tissues are shown in Table 3. Statins block the endogenous biosynthesis of both cholesterol and CoQ10, and the decrease in both substances

is related to the dose as well as the potency of those drugs. Schaefer W.H. *et al.* [52] investigated the relationship between statin-induced myopathy and mitochondrial function. Mean CoQ9 levels in skeletal muscle slightly decreased, but

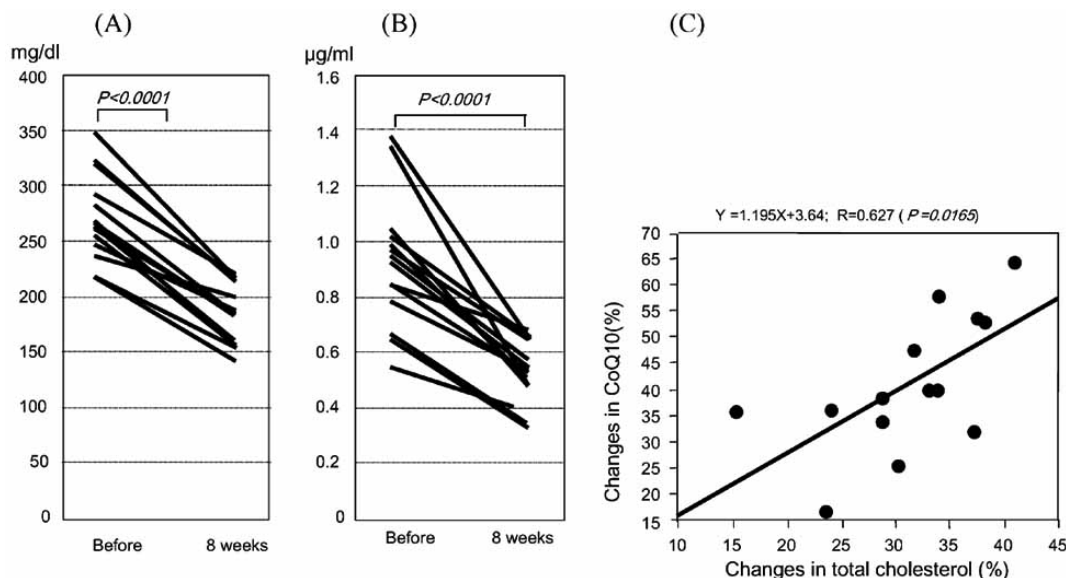


Fig. (2). Effects of atorvastatin on plasma total cholesterol (A) and CoQ10 (B) levels in hypercholesterolemic patients. (C) Correlations between percent changes of serum total cholesterol levels and percent reductions of serum CoQ10 levels before and after treatment with atorvastatin (Modified figures adapted with permission from J Atheroscler Thromb [49]).

not statistically significantly. CoQ9 levels did not correlate with circulating creatine kinase (CK) levels in cerivastatin-treated rats. This study suggests that neither mitochondrial injury nor a decrease in muscle CoQ9 levels is the primary cause of skeletal myopathy in cerivastatin-dosed rats. Although Laaksonen *et al.* indicated that the muscle CoQ10 concentration did not change in patients after treatment with simvastatin, they evaluated the CoQ10 concentration in the muscles of only about 20 patients, which seems to be too

small a number to evaluate the low frequency of statin-induced myotoxicities [53]. It was also reported that supplementation with CoQ10 ameliorated the myotoxicity in patients treated with lovastatin [54]. However, tissue levels of CoQ10 observed in cases of myopathy and liver dysfunction caused by statins are not definitely correlated with clinical features, and prophylactic supplementation of CoQ10 in the statin treatment has not been recommended yet.

Table 3. Changes of CoQ10 Concentrations in Organ Tissues in Animal Studies

Authors	Statins	Animal	Dosage/day	% Changes of CoQ9 or CoQ10 concentration			Reference
			(mg/Kg of body weight)	Heart	Skeletal muscle	Liver	
Willis RA, <i>et al.</i>	lovastatin	rat	400	-7.5%		-30.0%	[121]
Belichard P, <i>et al.</i>	lovastatin	hamster	50	-33%		-23%	[122]
Fukami M, <i>et al.</i>	simvastatin	rabbit	50	-25%	+4%	-24%	[123]
	pravastatin	rabbit	50	-10%	-4%	-12%	
Diebold BA, <i>et al.</i>	lovastatin	guinea pig	40	-31%			[124]
Loop RA, <i>et al.</i>	lovastatin	rat	67			-34%	[125]
Sato K, <i>et al.</i>	simvastatin	dog	2	decreased			[126]
	pravastatin	dog	4	no changes			
Morand OH, <i>et al.</i>	simvastatin	hamster	100	-11%		-45%	[127]
Nakahara K, <i>et al.</i>	simvastatin	rabbit	50	-23%	-22%	-20%	[128]
	pravastatin	rabbit	100	-14%	-18%	-15%	
Schaefer WH, <i>et al.</i>	cerivastatin	rat	1		-31%		[52]

10. SUPPLEMENTATION OF COQ10 IN PATIENTS TREATED WITH STATINS

Recognizing that serum CoQ10 concentrations are decreased in patients taking statins has led to the hypothesis that CoQ10 supplementation may be of benefit to these patients. A number of commercial sites that have recommend the concurrent use of CoQ10 and statins to prevent statin-induced adverse drug reactions lack strong scientific support. In all animal studies where supplemental CoQ was given to the animals prior to the introduction of statins, the depletion of CoQ in blood and tissue was completely prevented. Statin-induced CoQ10 deficiency is dose-related and the CoQ10 deficiency can be completely reversed by supplemental CoQ10. In addition, supplemental CoQ10 is safe and has no adverse effect on cholesterol lowering or on the anti-inflammatory effects of statins [53]. Although CoQ10 supplementation has been shown to increase CoQ10 concentrations in the blood of patients taking statins, there have been no published studies showing the effect of supplementation on tissue levels of CoQ10 (Table 4). In addition, any clinical benefits of CoQ10 supplementation have yet to be convincingly determined. There are no published clinical studies evaluating the efficacy of supplemental CoQ10 in reversing or preventing statin-induced adverse effects. A dosage of at least 100 mg/day has been recommended to significantly increase CoQ10 concentrations in the body. Although CoQ10 supplementation has been shown to reverse statin-induced decreases in plasma CoQ10 concentrations, there have not been any adequate studies on humans that have associated this with any pronounced clinical benefit in terms of either efficacy or safety. There are no efficacy data to support the routine use of CoQ10 for preventing statin-related adverse effects, and it is therefore not recommended for this purpose at this time [27,55].

11. SQUALENE SYNTHASE INHIBITOR AND SQUALENE EPOXIDASE INHIBITOR

As statins may prevent the synthesis of biologically important isoprenoids such as dolichol, ubiquinone, and isopen-tenyl tRNA, a more selective inhibition of cholesterol bio-

synthesis would be accomplished by blocking a step beyond the branches to the isoprenoids in the cholesterol synthesis pathway (Fig. 1). This idea prompted many major pharmaceutical companies in the 1990s to target selective cholesterol synthesis beyond FPP. The enzymes squalene synthase, squalene epoxidase, and oxidosqualene cyclase were identified as potential targets. Drugs that block the synthesis of cholesterol below the mevalonate level will not impair the biosynthesis of CoQ10.

In 1959 Blohm *et al.* reported that triparanol (MER-29) produced a cholesterol-lowering effect on rats, and that the effect was the result of an inhibition of cholesterol biosynthesis [56]. Triparanol inhibits cholesterol synthesis in the final step in the synthetic pathway, resulting in the accumulation of its immediate precursor, desmosterol, and other sterols, which produced side effects such as cataracts, ichthyosis, and hair loss [57]. Because of these side effects, triparanol was withdrawn from the market in the early 1960s.

The first enzyme committed in the sterol branch is squalene synthase, which can be specifically inhibited by squalene synthase inhibitor (squalenstatin). Squalene synthase inhibitors reduce cholesterol biosynthesis through inhibition of the conversion of FPP to squalene, sparing CoQ10 and dolichol, as well as farnesylated proteins and GGPP synthesis, so that squalene synthase inhibitors are less likely to cause adverse effects resulting from a lack of the intermediate metabolites of cholesterol biosynthesis [58]. One squalene synthase inhibitor, RPR107393, is an effective hypocholesterolemic agent in both rats and marmosets [59]. In marmosets, which have a lipoprotein profile closer to that of humans than rats, the reduction in plasma cholesterol was observed selectively in the LDL fraction without any change in the HDL fraction. The maximal reduction in plasma cholesterol observed with lovastatin was 31%, whereas RPR107393 demonstrated greater reduction in serum cholesterol (50%). Squalenstatin 1 also produced a 50% reduction in plasma cholesterol concentration in marmosets [60]. These results suggest that inhibitors of squalene synthase may be more effective agents than inhibitors of HMG-CoA reductase for the reduction of plasma cholesterol levels. The dual effects of squalene synthase inhibitor—inhibition of squalene synthase

Table 4. Supplementation of CoQ10 in Statin-Treated Humans and Animals

Authors	Statins	Humans or animals	CoQ10 dosage	% Changes of CoQ10 concentration		Reference
			(mg/day)	Serum	Tissue	
Willis RA, <i>et al.</i>	lovastatin	rat	15mg/Kg	315	Liver 920	[121]
					Heart 93	
Folkers K, <i>et al.</i>	lovastatin	human	100mg	127		[102]
Miyake Y, <i>et al.</i>	simvastatin	human	30mg	181		[113]
Bargossi AM, <i>et al.</i>	simvastatin	human	100mg	123	Platelet 153	[105]
Silver MA, <i>et al.</i>	atorvastatin	human	300mg	217		[117]
Palomaki A, <i>et al.</i>	lovastatin	human	180mg	417		[129]

and induction of HMG-CoA reductase—may result in the accumulation of metabolites of FPP, raising the possibility of toxicity with this treatment; however, these metabolites are readily excreted in urine, and rather biologically important CoQ10 and dolichol synthesis from FPP might be increased. This compound even increases CoQ biosynthesis three- to four-fold. Many other drugs based on squalene synthase inhibitors have been extensively developed in animal and clinical studies. At present we have several data concerning squalene synthase inhibitors used in human studies. At the 100 mg/day dose of TAK-475, mean LDL-C levels decreased significantly by 27.2% ($p < 0.001$), and no major safety issues were identified [61]. Therefore, it is suggested that squalene synthase inhibitors are ideal hypocholesterolemic agents because they do not inhibit the biosynthesis of CoQ, dolichol, or isopentenyl tRNA. This development signals a new direction for the pharmaceutical industry as it continues the search for more selective second-generation cholesterol-lowering drugs [62].

Squalene epoxidase (SE) is an attractive target for pharmacotherapeutic intervention, as it is the secondary rate-limiting enzyme, and blocking cholesterol synthesis at this step may result in accumulation of only squalene, which is known to be stable and non-toxic [63]. As most of the SE inhibitors have been developed as antifungal drugs and isolated from the fermentation broth of fungus like statins, they have received little attention as a target for hypocholesterolemic drugs. SE inhibitor NB-598 decreased serum cholesterol levels and increased serum squalene levels in a dose-dependent manner in dogs [64]. Another SE inhibitor, FR 194738, decreased plasma cholesterol levels by 40% and triglyceride levels by 80%. Induction of HMG-CoA reductase activity by FR 194738 was less than that displayed by simvastatin, which produced a concentration-dependent increase of HMG-CoA reductase activity [65]. These compounds are listed in Table 5 [66]. Though many inhibitors of these enzymes have been developed, until today no compound has been reported to have entered clinical trials except TAK-475 [67]. Therefore, the possibility of an additional

therapeutic effect of statins when administered with CoQ10 should be further evaluated. We also hope for the development of a new generation of cholesterol-reducing drugs that affect the biosynthesis of cholesterol beyond the FPP branch point of the mevalonate pathway and thus do not inhibit CoQ10 biosynthesis. Nishimoto *et al.* studied the myotoxic effects of T-91485, a metabolite of squalene synthase inhibitor, TAK-475, and statins in human skeletal myocytes and rhabdomyosarcoma cells. T-91485, atorvastatin, and simvastatin inhibited cholesterol biosynthesis concentration-dependently, although T-91485 inhibited cholesterol synthesis about five-fold less than atorvastatin and simvastatin. Atorvastatin and simvastatin decreased intracellular ATP content, whereas T-91485 did not decrease the intracellular ATP content, even at 100 μM Fig. (3). Myotoxicity was induced by atorvastatin and simvastatin, but not by T-91485 (Fig. 4). Furthermore, T-91485 attenuated the myotoxicity of atorvastatin. These findings suggest that squalene synthase inhibitors may decrease statin-induced myotoxicity in lipid-lowering therapy [68].

12. CONCLUSIONS

1. The long-term efficacy and safety of statins have been established in large multicenter trials on cholesterol-lowering for preventing coronary events in both primary and secondary prevention.

2. Many of the so-called pleiotropic effects have been shown to be secondary to inhibition of the synthesis of isoprenoid intermediates of the mevalonate pathway, such as FPP and GGPP and, thus, are completely independent of intracellular cholesterol biosynthesis.

3. Most of the clinically favorable pleiotropic effects of statins have been explained by the fact that reduction of half of the mevalonate metabolic pathway, and reduction of the other half of the mevalonate pathway produces deficiency in CoQ-10 and dolichol which are essential for cells and animals including humans, and deficiency of these nonsteroidal isoprenoid substances may result in some adverse reactions associated with statins.

Table 5. Inhibitors of Squalene Synthase and Squalene Epoxidase

Inhibitors	Compound	Animal	Dosage (mg/day)	% Changes of serum cholesterol levels	Reference
Squalene synthase inhibitors	Squalestatin 1	marmoset	10	-51	[60]
	ER-27856	rhesus monkey	10	-76	[130]
	YM-53601	hamster	50	-35	[131]
	TAK-475	mouse	110	-32	[132]
	YM-16638	rhesus monkey	30	-27	[133]
	RPR 107393	rat	30	-51	[59]
	YM-53546	hamster	50	-46	[134]
Squalene epoxidase inhibitor	NB-598	dog	10	-34	[64]
	FR194738	dog	32	-61(LDL-C)	[65]

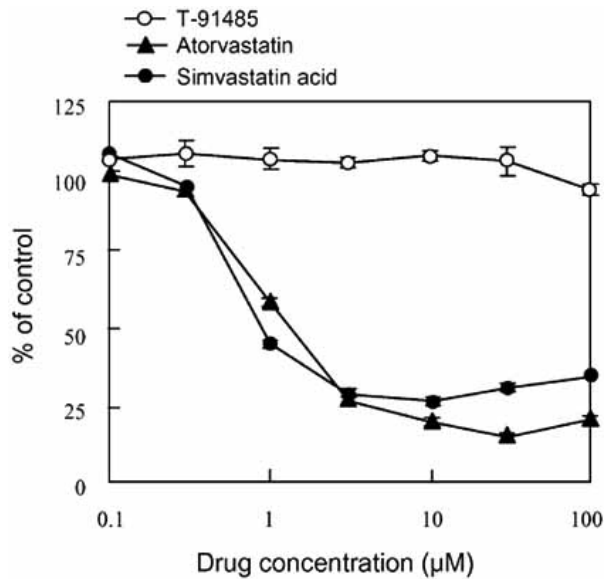


Fig. (3). Effects of T-91485, atorvastatin and simvastatin on intracellular ATP levels in differentiated rhabdomyosarcoma (RD) cells. Data represent the means \pm SEM (N=3). (Adapted with permission from *Biochem Pharmacol* [68]).

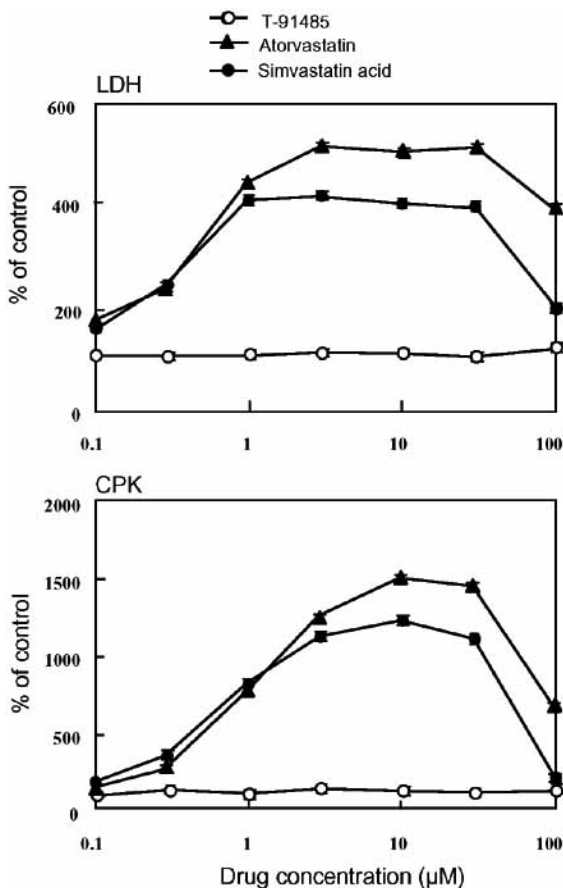


Fig. (4). Effects of T-91485, atorvastatin and simvastatin on medium LDH and CPK levels in differentiated rhabdomyosarcoma (RD) cells. Data represent the means \pm SEM (N=3). (Adapted with permission from *Biochem Pharmacol* [68]).

4. Most of the clinical data show a definite reduction in CoQ10 in accordance with the LDL-cholesterol lowering power of statins. The stronger statins usually show larger reductions of plasma CoQ10 concentrations.

5. Tissue levels of CoQ10 observed in myopathy and liver dysfunction caused by statins are not definitely correlated with clinical features, and prophylactic supplementation of CoQ10 in the statin treatment has not yet been recommended.

6. As the statins may prevent the synthesis of biologically important isoprenoids such as dolichol, CoQ10 and isopentenyl t-RNA, a more selective inhibition of the cholesterol biosynthesis would be accomplished by blocking a step beyond the branches to the isoprenoids in the cholesterol synthesis pathway. The enzymes squalene synthase, squalene epoxidase were identified as potential targets. Drugs that block the synthesis of cholesterol below the mevalonate level, will not impair the biosynthesis of CoQ10.

5. We hope for the development of a new generation of cholesterol-reducing drugs that affect the biosynthesis of cholesterol below the FPP branch point of the mevalonate pathway and thus will not inhibit CoQ10 biosynthesis.

REFERENCES

- [1] Stamler J, Wentworth D, Neaton JD. Is relationship between serum cholesterol and risk of premature death from coronary heart disease continuous and graded? Findings in 356,222 primary screenees of the Multiple Risk Factor Intervention Trial (MRFIT). *JAMA* 1986; 256: 2823-8.
- [2] Shepherd J, Cobbe SM, Ford I, *et al.* Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. West of Scotland Coronary Prevention Study Group. *N Engl J Med* 1995; 333: 1301-7.
- [3] Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). *Lancet* 1994; 344: 1383-9.
- [4] National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. *Circulation* 2002; 106: 3143-421.
- [5] Topol EJ. Intensive statin therapy -- a sea change in cardiovascular prevention. *N Engl J Med* 2004; 350: 1562-4.
- [6] Endo A. The discovery and development of HMG-CoA reductase inhibitors. *J Lipid Res* 1992; 33: 1569-82.
- [7] Downs JR, Clearfield M, Weis S, *et al.* Primary prevention of acute coronary events with lovastatin in men and women with average cholesterol levels: results of AFCAPS/TexCAPS. Air Force/Texas Coronary Atherosclerosis Prevention Study. *JAMA* 1998; 279: 1615-22.
- [8] Sacks FM, Pfeffer MA, Moye LA, *et al.* The effect of pravastatin on coronary events after myocardial infarction in patients with average cholesterol levels. Cholesterol and Recurrent Events Trial investigators. *N Engl J Med* 1996; 335: 1001-9.
- [9] LaRosa JC, Grundy SM, Waters DD, *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.
- [10] Schwartz GG, Olsson AG, Ezekowitz MD, *et al.* Myocardial Ischemia Reduction with Aggressive Cholesterol Lowering (MIRACL) Study Investigators. Effects of atorvastatin on early recurrent ischemic events in acute coronary syndromes: the MIRACL study: a randomized controlled trial. *JAMA* 2001; 285: 1711-8.

- [11] Massy ZA, Keane WF, Kasiske BL. Inhibition of the mevalonate pathway: benefits beyond cholesterol reduction? *Lancet* 1996; 347: 102-3.
- [12] Takemoto M, Liao JK. Pleiotropic effects of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors. *Arterioscler Thromb Vasc Biol* 2001; 21: 1712-9.
- [13] Liao JK. Beyond lipid lowering: the role of statins in vascular protection. *Int J Cardiol* 2002; 86: 5-18.
- [14] Gotto AM Jr, Farmer JA. Pleiotropic effects of statins: do they matter? *Curr Opin Lipidol* 2001; 12: 391-4.
- [15] Bonetti PO, Lerman LO, Napoli C, Lerman A. Statin effects beyond lipid lowering--are they clinically relevant? *Eur Heart J* 2003; 24: 225-48.
- [16] Davidson MH. Clinical significance of statin pleiotropic effects: hypotheses versus evidence. *Circulation* 2005; 111: 2280-1.
- [17] Furberg CD, Pitt B. Withdrawal of cerivastatin from the world market. *Curr Control Trials Cardiovasc Med* 2001; 2: 205-207.
- [18] Goldstein JL, Brown MS. Regulation of the mevalonate pathway. *Nature* 1990; 343: 425-30.
- [19] Hawk E, Viner JL. Statins and cancer--beyond the "one drug, one disease" model. *N Engl J Med* 2005; 352: 2238-9.
- [20] Kivipelto M, Solomon A, Winblad B. Statin therapy in Alzheimer's disease. *Lancet Neurol* 2005; 4: 521-2.
- [21] Coons JC. Hydroxymethylglutaryl-coenzyme A reductase inhibitors in osteoporosis management. *Ann Pharmacother* 2002; 36: 326-30.
- [22] Epstein M, Campese VM. Pleiotropic effects of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors on renal function. *Am J Kidney Dis* 2005; 45: 2-14.
- [23] Poynter JN, Gruber SB, Higgins PD, *et al.* Statins and the risk of colorectal cancer. *N Engl J Med* 2005; 352: 2184-92.
- [24] Bellosti S, Paoletti R, Corsini A. Safety of statins: focus on clinical pharmacokinetics and drug interactions. *Circulation* 2004; 109 (Suppl 1): III50-7.
- [25] Pasternak RC, Smith SC Jr, Bairey-Merz CN, Grundy SM, Cleeman JI, Lenfant C; American College of Cardiology; American Heart Association; National Heart, Lung and Blood Institute. ACC/AHA/NHLBI Clinical Advisory on the Use and Safety of Statins. *Circulation* 2002; 106: 1024-8.
- [26] Grundy SM. The issue of statin safety: where do we stand? *Circulation* 2005; 111: 3016-9.
- [27] Langsjoen PH, Langsjoen AM. The clinical use of HMG CoA-reductase inhibitors and the associated depletion of coenzyme Q10. A review of animal and human publications. *Biofactors* 2003; 18: 101-11.
- [28] Libby P, Aikawa M. Mechanisms of plaque stabilization with statins. *Am J Cardiol* 2003; 91: 4B-8B.
- [29] Brown MS, Goldstein JL. Multivalent feedback regulation of HMG CoA reductase, a control mechanism coordinating isoprenoid synthesis and cell growth. *J Lipid Res* 1980; 21: 505-17.
- [30] Faust JR, Goldstein JL, Brown MS. Synthesis of ubiquinone and cholesterol in human fibroblasts: regulation of a branched pathway. *Arch Biochem Biophys* 1979; 192: 86-99.
- [31] Mabuchi H, Haba T, Tatami R, *et al.* Effect of an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase on serum lipoproteins and ubiquinone-10-levels in patients with familial hypercholesterolemia. *N Engl J Med* 1981; 305: 478-82.
- [32] Crane FL, Hatefi Y, Lester RL, Widmer C. Isolation of a quinone from beef heart mitochondria. *Biochim Biophys Acta* 1957; 25: 220-1.
- [33] Crane FL. Biochemical functions of coenzyme Q10. *J Am Coll Nutr* 2001; 20: 591-8.
- [34] Ernster L, Forsmark-Andree P. Ubiquinol: an endogenous antioxidant in aerobic organisms. *Clin Investig* 1993; 71(8 Suppl): S60-5.
- [35] 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.
- [36] Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL. Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. *N Engl J Med* 1989; 320: 915-24.
- [37] Hoffmann G, Gibson KM, Brandt IK, Bader PI, Wappner RS, Sweetman L. Mevalonic aciduria -- an inborn error of cholesterol and nonsterol isoprene biosynthesis. *N Engl J Med* 1986; 314: 1610-4.
- [38] Hubner C, Hoffmann GF, Charpentier C, *et al.* Decreased plasma ubiquinone-10 concentration in patients with mevalonate kinase deficiency. *Pediatr Res* 1993; 34: 129-33.
- [39] DiMauro S, Schon EA. Mitochondrial respiratory-chain diseases. *N Engl J Med* 2003; 348: 2656-68.
- [40] Ogasahara S, Engel AG, Frens D, Mack D. Muscle coenzyme Q deficiency in familial mitochondrial encephalomyopathy. *Proc Natl Acad Sci USA* 1989; 86: 2379-82.
- [41] Littarru GP, Tiano L. Clinical aspects of coenzyme Q10: an update. *Curr Opin Clin Nutr Metab Care* 2005; 8: 641-6.
- [42] Folkers K, Littarru GP, Ho L, Runge TM, Havanonda S, Cooley D. Evidence for a deficiency of coenzyme Q10 in human heart disease. *Int Z Vitaminforsch* 1970; 40: 380-90.
- [43] Folkers K, Vadhanavikit S, Mortensen SA. Biochemical rationale and myocardial tissue data on the effective therapy of cardiomyopathy with coenzyme Q10. *Proc Natl Acad Sci USA* 1985; 82: 901-4.
- [44] Hanaki Y, Sugiyama S, Ozawa T, Ohno M. Ratio of low-density lipoprotein cholesterol to ubiquinone as a coronary risk factor. *N Engl J Med* 1991; 325: 814-5.
- [45] Hanaki Y, Sugiyama S, Ozawa T, Ohno M. Coenzyme Q10 and coronary artery disease. *Clin Investig* 1993; 71(8 Suppl): S112-5.
- [46] Kontush A, Reich A, Baum K, *et al.* Plasma ubiquinol-10 is decreased in patients with hyperlipidaemia. *Atherosclerosis* 1997; 129: 119-26.
- [47] Thomas SR, Witting PK, Stocker R. A role for reduced coenzyme Q in atherosclerosis? *Biofactors* 1999; 9: 207-24.
- [48] Hargreaves IP. Ubiquinone: cholesterol's reclusive cousin. *Ann Clin Biochem* 2003; 40: 207-18.
- [49] Mabuchi H, Higashikata T, Kawashiri M, *et al.* Reduction of serum ubiquinol-10 and ubiquinone-10 levels by atorvastatin in hypercholesterolemic patients. *J Atheroscler Thromb* 2005; 12: 111-9.
- [50] Elmberger PG, Kalen A, Lund E, *et al.* Effects of pravastatin and cholestyramine on products of the mevalonate pathway in familial hypercholesterolemia. *J Lipid Res* 1991; 32: 935-40.
- [51] Ernster L, Dallner G. Biochemical, physiological and medical aspects of ubiquinone function. *Biochim Biophys Acta* 1995; 1271: 195-204.
- [52] Schaefer WH, Lawrence JW, Loughlin AF, *et al.* Evaluation of ubiquinone concentration and mitochondrial function relative to cerivastatin-induced skeletal myopathy in rats. *Toxicol Appl Pharmacol* 2004; 194: 10-23.
- [53] Laaksonen R, Jokelainen K, Laakso J, *et al.* The effect of simvastatin treatment on natural antioxidants in low-density lipoproteins and high-energy phosphates and ubiquinone in skeletal muscle. *Am J Cardiol* 1996; 77: 851-4.
- [54] Thibault A, Samid D, Tompkins AC, *et al.* Phase I study of lovastatin, an inhibitor of the mevalonate pathway, in patients with cancer. *Clin Cancer Res* 1996; 2: 483-91.
- [55] Nawarskas JJ. HMG-CoA reductase inhibitors and coenzyme Q10. *Cardiol Rev* 2005; 13: 76-9.
- [56] Blohm TR, Mackenzie RD. Specific inhibition of cholesterol biosynthesis by a synthetic compound (MER-29). *Arch Biochem Biophys* 1959; 85: 245-9.
- [57] Laughlin RC, Carey TF. Cataracts in patients treated with triparanol. *JAMA* 1962; 181: 339-40.
- [58] Shen W, Garvey DS, Cohen J, Stein H, Rosenberg SH. Cyclopentenedi- and tricarboxylic acids as squalene synthase inhibitors: synthesis and evaluation. *Bioorg Med Chem Lett* 1998; 8: 891-6.
- [59] Amin D, Rutledge RZ, Needle SN, *et al.* RPR 107393, a potent squalene synthase inhibitor and orally effective cholesterol-lowering agent: comparison with inhibitors of HMG-CoA reductase. *J Pharmacol Exp Ther* 1997; 281: 746-52.
- [60] Baxter A, Fitzgerald BJ, Hutson JL, *et al.* Squalastatin 1, a potent inhibitor of squalene synthase, which lowers serum cholesterol *in vivo*. *J Biol Chem* 1992; 267: 11705-8.
- [61] Piper E, Price G, Chen Y. TAK-475, a squalene synthase inhibitor, improves lipid profiles in hyperlipidemic subjects. *American Heart Association Scientific Sessions* 2006. Abstract 1493.
- [62] Bliznakov EG, Wilkins DJ. Biochemical and clinical consequences of inhibiting coenzyme Q10 biosynthesis by lipid-lowering HMG-CoA reductase inhibitors (statins): a critical overview. *Adv Ther* 1998; 15: 218-228.
- [63] Chugh A, Ray A, Gupta JB. Squalene epoxidase as hypocholesterolemic drug target revisited. *Prog Lipid Res* 2003; 42: 37-50.

- [64] Horie M, Sawasaki Y, Fukuzumi H, *et al.* Hypolipidemic effects of NB-598 in dogs. *Atherosclerosis* 1991; 88: 183-92.
- [65] Sawada M, Washizuka K, Okumura H. Synthesis and biological activity of a novel squalene epoxidase inhibitor, FR194738. *Bioorg Med Chem Lett* 2004; 14: 633-7.
- [66] Watanabe S, Hirai H, Kambara T, *et al.* CJ-13,981 and CJ-13,982, new squalene synthase inhibitors. *J Antibiot (Tokyo)* 2001; 54: 1025-30.
- [67] Nishimoto T, Amano Y, Tozawa R, *et al.* Lipid-lowering properties of TAK-475, a squalene synthase inhibitor, *in vivo* and *in vitro*. *Br J Pharmacol* 2003; 139: 911-8.
- [68] Nishimoto T, Tozawa R, Amano Y, Wada T, Imura Y, Sugiyama Y. Comparing myotoxic effects of squalene synthase inhibitor, T-91485, and 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors in human myocytes. *Biochem Pharmacol* 2003; 66: 2133-9.
- [69] Almog Y, Shefer A, Novack V, *et al.* Prior statin therapy is associated with a decreased rate of severe sepsis. *Circulation* 2004; 110: 880-5.
- [70] Node K, Fujita M, Kitakaze M, *et al.* Short-term statin therapy improves cardiac function and symptoms in patients with idiopathic dilated cardiomyopathy. *Circulation* 2003; 108(7): 839-43.
- [71] Kobashigawa JA, Katznelson S, Laks H, *et al.* Effect of pravastatin on outcomes after cardiac transplantation. *N Engl J Med* 1995; 333: 621-7.
- [72] Mehra MR, Raval NY. Metaanalysis of statins and survival in de novo cardiac transplantation. *Transplant Proc* 2004; 36: 1539-41.
- [73] Stojanovic I, Vrtovec B, Radovancevic B, *et al.* Survival, graft atherosclerosis, and rejection incidence in heart transplant recipients treated with statins: 5-year follow-up. *J Heart Lung Transplant* 2005; 24: 1235-8.
- [74] Wenke K, Meiser B, Thiery J, Reichart B. Impact of simvastatin therapy after heart transplantation an 11-year prospective evaluation. *Herz* 2005; 30: 431-2.
- [75] Wu AH, Ballantyne CM, Short BC, *et al.* Statin use and risks of death or fatal rejection in the Heart Transplant Lipid Registry. *Am J Cardiol* 2005; 95: 367-72.
- [76] Fukuta H, Sane DC, Brucks S, Little WC. Statin therapy may be associated with lower mortality in patients with diastolic heart failure: a preliminary report. *Circulation* 2005; 112: 357-63.
- [77] Cowell SJ, Newby DE, Prescott RJ, *et al.* A randomized trial of intensive lipid-lowering therapy in calcific aortic stenosis. *N Engl J Med* 2005; 352: 2389-97.
- [78] Bjerre LM, LeLorier J. Do statins cause cancer? A meta-analysis of large randomized clinical trials. *Am J Med* 2001; 110: 716-23.
- [79] Poynter JN, Gruber SB, Higgins PD, *et al.* Statins and the risk of colorectal cancer. *N Engl J Med* 2005; 352: 2184-92.
- [80] Friis S, Poulsen AH, Johnsen SP, *et al.* Cancer risk among statin users: a population-based cohort study. *Int J Cancer* 2005; 114: 643-7.
- [81] Vollmer T, Key L, Durkalski V, *et al.* Oral simvastatin treatment in relapsing-remitting multiple sclerosis. *Lancet* 2004; 363: 1607-8.
- [82] Jick H, Zornberg GL, Jick SS, Seshadri S, Drachman DA. Statins and the risk of dementia. *Lancet* 2000; 356: 1627-31.
- [83] Li G, Higdon R, Kukull WA, *et al.* Statin therapy and risk of dementia in the elderly: a community-based prospective cohort study. *Neurology* 2004; 63: 1624-8.
- [84] Sparks DL, Sabbagh MN, Connor DJ, *et al.* Atorvastatin for the treatment of mild to moderate Alzheimer disease: preliminary results. *Arch Neurol* 2005; 62: 753-7.
- [85] Rea TD, Breitner JC, Psaty BM, *et al.* Statin use and the risk of incident dementia: the Cardiovascular Health Study. *Arch Neurol* 2005; 62: 1047-51.
- [86] Abud-Mendoza C, de la Fuente H, Cuevas-Orta E, Baranda L, Cruz-Rizo J, Gonzalez-Amaro R. Therapy with statins in patients with refractory rheumatic diseases: a preliminary study. *Lupus* 2003; 12: 607-11.
- [87] Meier CR, Schlienger RG, Kraenzlin ME, Schlegel B, Jick H. HMG-CoA reductase inhibitors and the risk of fractures. *JAMA* 2000; 283: 3205-10.
- [88] Chan KA, Andrade SE, Boles M, *et al.* Inhibitors of hydroxymethylglutaryl-coenzyme A reductase and risk of fracture among older women. *Lancet* 2000; 355: 2185-8.
- [89] Coons JC. Hydroxymethylglutaryl-coenzyme A reductase inhibitors in osteoporosis management. *Ann Pharmacother* 2002; 36: 326-30.
- [90] LaCroix AZ, Cauley JA, Pettinger M, *et al.* Statin use, clinical fracture, and bone density in postmenopausal women: results from the Women's Health Initiative Observational Study. *Ann Intern Med* 2003; 139: 97-104.
- [91] Khanal S, Attallah N, Smith DE, *et al.* Statin therapy reduces contrast-induced nephropathy: an analysis of contemporary percutaneous interventions. *Am J Med* 2005; 118: 843-9.
- [92] Fellstrom B, Holdaas H, Jardine AG, *et al.* Effect of fluvastatin on renal end points in the Assessment of Lescol in Renal Transplant (ALERT) trial. *Kidney Int* 2004; 66: 1549-55.
- [93] Lentine KL, Brennan DC. Statin use after renal transplantation: a systematic quality review of trial-based evidence. *Nephrol Dial Transplant* 2004; 19: 2378-86.
- [94] Bianchi S, Bigazzi R, Caiazza A, Campese VM. A controlled, prospective study of the effects of atorvastatin on proteinuria and progression of kidney disease. *Am J Kidney Dis* 2003; 41: 565-70.
- [95] Tonelli M, Sacks F, Pfeffer M, *et al.* Biomarkers of inflammation and progression of chronic kidney disease. *Kidney Int* 2005; 68: 237-45.
- [96] Vidt DG, Cressman MD, Harris S, Pears JS, Hutchinson HG. Rosuvastatin-induced arrest in progression of renal disease. *Cardiology* 2004; 102: 52-60.
- [97] Tonelli M, Moye L, Sacks FM, *et al.* Effect of pravastatin on loss of renal function in people with moderate chronic renal insufficiency and cardiovascular disease. *J Am Soc Nephrol* 2003; 14: 1605-13.
- [98] Abetel G, Poget PN, Bonnabry JP. Hypotensive effect of an inhibitor of cholesterol synthesis (fluvastatin). A pilot study. *Schweiz Med Wochenschr* 1998; 128: 272-7.
- [99] Tonolo G, Melis MG, Formato M, *et al.* Additive effects of Simvastatin beyond its effects on LDL cholesterol in hypertensive type 2 diabetic patients. *Eur J Clin Invest* 2000; 30: 980-7.
- [100] Lee TM, Su SF, Tsai CH. Effect of pravastatin on proteinuria in patients with well-controlled hypertension. *Hypertension* 2002; 40: 67-73.
- [101] Johnson BA, Iacono AT, Zeevi A, McCurry KR, Duncan SR. Statin use is associated with improved function and survival of lung allografts. *Am J Respir Crit Care Med* 2003; 167: 1271-8.
- [102] Folkers K, Langsjoen P, Willis R, *et al.* Lovastatin decreases coenzyme Q levels in humans. *Proc Natl Acad Sci USA* 1990; 87: 8931-4.
- [103] Watts GF, Castelluccio C, Rice-Evans C, Taub NA, Baum H, Quinn PJ. Plasma coenzyme Q (ubiquinone) concentrations in patients treated with simvastatin. *J Clin Pathol* 1993; 46: 1055-7.
- [104] Ghirlanda G, Oradei A, Manto A, *et al.* Evidence of plasma CoQ10-lowering effect by HMG-CoA reductase inhibitors: a double-blind, placebo-controlled study. *J Clin Pharmacol* 1993; 33: 226-9.
- [105] Bargossi AM, Battino M, Gaddi A, *et al.* Exogenous CoQ10 preserves plasma ubiquinone levels in patients treated with 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors. *Int J Clin Lab Res* 1994; 24: 171-6.
- [106] Laaksonen R, Ojala JP, Tikkanen MJ, Himberg JJ. Serum ubiquinone concentrations after short- and long-term treatment with HMG-CoA reductase inhibitors. *Eur J Clin Pharmacol* 1994; 46: 313-7.
- [107] Laaksonen R, Jokelainen K, Sahi T, Tikkanen MJ, Himberg JJ. Decreases in serum ubiquinone concentrations do not result in reduced levels in muscle tissue during short-term simvastatin treatment in humans. *Clin Pharmacol Ther* 1995; 57: 62-6.
- [108] De Piniex G, Chariot P, Ammi-Said M, *et al.* Lipid-lowering drugs and mitochondrial function: effects of HMG-CoA reductase inhibitors on serum ubiquinone and blood lactate/pyruvate ratio. *Br J Clin Pharmacol* 1996; 42: 333-7.
- [109] Human JA, Ubbink JB, Jerling JJ, *et al.* The effect of Simvastatin on the plasma antioxidant concentrations in patients with hypercholesterolaemia. *Clin Chim Acta* 1997; 263: 67-77.
- [110] Palomaki A, Malminiemi K, Metsa-Ketela T. Enhanced oxidizability of ubiquinol and alpha-tocopherol during lovastatin treatment. *FEBS Lett* 1997; 410: 254-8.

- [111] Mortensen SA, Leth A, Agner E, Rohde M. Dose-related decrease of serum coenzyme Q10 during treatment with HMG-CoA reductase inhibitors. *Mol Aspects Med* 1997; 18 Suppl: S137-44.
- [112] de Lorgeril M, Salen P, Bontemps L, Belichard P, Geysant A, Itti R. Effects of lipid-lowering drugs on left ventricular function and exercise tolerance in dyslipidemic coronary patients. *J Cardiovasc Pharmacol* 1999; 33: 473-8.
- [113] Miyake Y, Shouzu A, Nishikawa M, *et al.* Effect of treatment with 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors on serum coenzyme Q10 in diabetic patients. *Arzneimittelforschung* 1999; 49: 324-9.
- [114] Bleske BE, Willis RA, Anthony M, *et al.* The effect of pravastatin and atorvastatin on coenzyme Q10. *Am Heart J* 2001; 142: E2.
- [115] Jula A, Marniemi J, Huupponen R, Virtanen A, Rastas M, Ronne-*maa* T. Effects of diet and simvastatin on serum lipids, insulin, and antioxidants in hypercholesterolemic men: a randomized controlled trial. *JAMA* 2002; 287: 598-605.
- [116] 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.
- [117] Silver MA, Langsjoen PH, Szabo S, Patil H, Zelinger A. Effect of atorvastatin on left ventricular diastolic function and ability of coenzyme Q10 to reverse that dysfunction. *Am J Cardiol* 2004; 94: 1306-10.
- [118] 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.
- [119] Strey CH, Young JM, Molyneux SL, *et al.* Endothelium-*ameliorating* effects of statin therapy and coenzyme Q10 reductions in chronic heart failure. *Atherosclerosis* 2005; 179: 201-6.
- [120] Colquhoun DM, Jackson R, Walters M, *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.
- [121] Willis RA, Folkers K, Tucker JL, Ye CQ, Xia LJ, Tamagawa H. Lovastatin decreases coenzyme Q levels in rats. *Proc Natl Acad Sci USA* 1990; 87: 8928-30.
- [122] Belichard P, Pruneau D, Zhiri A. Effect of a long-term treatment with lovastatin or fenofibrate on hepatic and cardiac ubiquinone levels in cardiomyopathic hamster. *Biochim Biophys Acta* 1993; 1169: 98-102.
- [123] Fukami M, Maeda N, Fukushige J, *et al.* Effects of HMG-CoA reductase inhibitors on skeletal muscles of rabbits. *Res Exp Med (Berl)* 1993; 193: 263-73.
- [124] Diebold BA, Bhagavan NV, Guillory RJ. Influences of lovastatin administration on the respiratory burst of leukocytes and the phosphorylation potential of mitochondria in guinea pigs. *Biochim Biophys Acta* 1994; 1200: 100-8.
- [125] Loop RA, Anthony M, Willis RA, Folkers K. Effects of ethanol, lovastatin and coenzyme Q10 treatment on antioxidants and TBA reactive material in liver of rats. *Mol Aspects Med* 1994; 15 Suppl: s195-206.
- [126] Satoh K, Yamato A, Nakai T, Hoshi K, Ichihara K. Effects of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors on mitochondrial respiration in ischaemic dog hearts. *Br J Pharmacol* 1995; 116: 1894-8.
- [127] Morand OH, Aebi JD, Dehmlow H, *et al.* Ro 48-8.071, a new 2,3-oxidosqualene: lanosterol cyclase inhibitor lowering plasma cholesterol in hamsters, squirrel monkeys, and minipigs: comparison to simvastatin. *J Lipid Res* 1997; 38: 373-90.
- [128] Nakahara K, Kuriyama M, Sonoda Y, *et al.* Myopathy induced by HMG-CoA reductase inhibitors in rabbits: a pathological, electrophysiological, and biochemical study. *Toxicol Appl Pharmacol* 1998; 152: 99-106.
- [129] Palomaki A, Malminiemä K, Solakivi T, Malminiemä O. Ubiquinone supplementation during lovastatin treatment: effect on LDL oxidation *ex vivo*. *J Lipid Res* 1998; 39: 1430-7.
- [130] Hiyoshi H, Yanagimachi M, Ito M, *et al.* Squalene synthase inhibitors reduce plasma triglyceride through a low-density lipoprotein receptor-independent mechanism. *Eur J Pharmacol* 2001; 431: 345-52.
- [131] Ugawa T, Kakuta H, Moritani H, Inagaki O. Effect of YM-53601, a novel squalene synthase inhibitor, on the clearance rate of plasma LDL and VLDL in hamsters. *Br J Pharmacol* 2002; 137: 561-9.
- [132] Amano Y, Nishimoto T, Tozawa R, Ishikawa E, Imura Y, Sugiyama Y. Lipid-lowering effects of TAK-475, a squalene synthase inhibitor, in animal models of familial hypercholesterolemia. *Eur J Pharmacol* 2003; 466: 155-61.
- [133] Goto S, Shimokawa T, Ugawa T, *et al.* Species specificity in the blood cholesterol-lowering effect of YM-16638. *Br J Pharmacol* 1996; 118: 174-8.
- [134] Ishihara T, Kakuta H, Moritani H, Ugawa T, Yanagisawa I. Synthesis and biological evaluation of quinuclidine derivatives incorporating phenothiazine moieties as squalene synthase inhibitors. *Chem Pharm Bull (Tokyo)* 2004; 52: 1204-9.