

Review

## Molecular basis of statin-associated myopathy

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### Abstract

Coronary artery disease (CAD) constitutes the most common cause of morbidity and mortality in developed countries. Statins effectively reduce low-density lipoprotein cholesterol, an important risk factor for CAD and related acute coronary syndromes. They are an extensively studied group of drugs with versatile properties. Overall, they are safe and effective drugs but their myotoxic potential cannot be overlooked. In this review we focus on the pathogenesis of statins' myopathic side effects. Statins can interfere with protein modification at multiple levels. They can affect protein prenylation, an important post-translational modification of membrane bound proteins. They can also adversely affect selenoprotein synthesis, or can interfere with the biosynthesis of dolichols, which are involved in the process of protein glycosylation. Statin-induced myopathy may be also associated with mitochondrial dysfunction. Statins remain the spearhead of our armamentarium in treating atherosclerotic disease. Consistent with their versatile properties it is anticipated to see in the future their indications to expand. Better understanding of the molecular mechanisms involved in statin-induced myopathy may help identify patient groups susceptible to statins' side effects, thereby increasing their safety.

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## 1. Introduction

Coronary artery disease (CAD) constitutes the most common cause of morbidity and mortality in developed countries. Elevated concentration of low-density lipoprotein cholesterol (LDL-C) is one of the most important risk factors for CAD, and effective reduction of its levels was shown to halt the progression of atherosclerosis and reduce CAD-associated morbidity and mortality. Statins are so far the most effective medication for serving the above purpose. They are competitive inhibitors of the 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase [1], an enzyme of the endoplasmic reticulum, which catalyzes the rate-limiting step in endogenous cholesterol synthesis [2] (Fig. 1). The therapeutic target organ of statins is the liver, as liver-derived cholesterol is the main source of hypercholesterolemia. In doing so, the intracellular levels of cholesterol in the hepatocytes fall, LDL-C receptors on their surface are upregulated, and consequently LDL-C hepatic uptake and catabolism increases.

Currently, statins have been proven effective in higher doses for aggressive lipid lowering therapy in patients with multiple comorbid conditions and complex dyslipidemias [3]. The patient populations indicated to receive long-term statins are expanding. Although statins are well tolerated by most patients, they can have various side effects with muscle-related complaints receiving the greatest attention. The risk of myopathy has been emphasized by the worldwide withdrawal of cerivastatin in August 2001 after the drug was associated with approximately 100 rhabdomyolysis-related deaths [4]. Purpose of this review is to summarize and provide a further insight into the molecular mechanisms of statin-induced myopathy. Better understanding of the molecular mechanisms involved in statin-induced myopathy may help identify patient groups susceptible to statins' side effects, thereby increasing their safety.

## 2. Definition and epidemiology of statin-associated myopathy

There has been significant inconsistency in the terminology used to describe muscle-related side effects. In an attempt to bridge these inconsistencies, the American College of Car-

diology/American Heart Association/National Heart, Lung, and Blood Institute (ACC/AHA/NHLBI) came up with the definitions presented in Table 1 [5,6].

The incidence of myopathy in randomized placebo-controlled clinical trials is low (Table 2). In these trials disease events are unlikely to have been missed as participants were followed closely [7]. However, some patient groups prone to statin-induced myopathy have either been excluded from these trials, such as those with elevated creatine kinase (CK) levels, or have been underrepresented, such as patients above 75 years and those with renal, and most notably, hepatic insufficiency [8]. In addition, in some studies, such as the most recent stroke prevention by aggressive reduction in cholesterol levels (SPARCL) study [9], subjects were allowed to take statins if indicated. This non-trial statin "drop-in" may have diluted the apparent risk [10]. Consequently, these trials may underestimate the incidence when statins are used in large unselected populations followed with less precision [4]. In addition, these trials provide limited data regarding higher doses of statins. On the other hand data from voluntary notifications to regulatory authorities of adverse events, although having the advantage of recording information from a very large pool, are plagued by under-reporting [7]. It is universally recognized that the occurrence of muscle-related symptoms less severe than rhabdomyolysis is under-reported, as complaints like minor muscle aches following exercise are frequently dismissed; the incidence of those may be at the range of 5% or even more. Therefore, it is difficult to estimate accurately the incidence of statin-induced myopathy.

## 3. Predisposing factors of statin-associated myopathy

Statin-associated myopathy is dose-dependent and has been associated with all statins. Certain predisposing conditions and precipitating factors have been described [4,11,12] (Table 3). Most of them promote myopathy by altering the metabolism and increasing the bioavailability of statins. In addition, many conditions and medications (e.g. strenuous exercise, alcohol, or treatment with steroids) may predispose patients to myopathy independently, producing an additive effect, and even precipitate myopathy when combined with statins. Statins may also unmask a latent, underlying

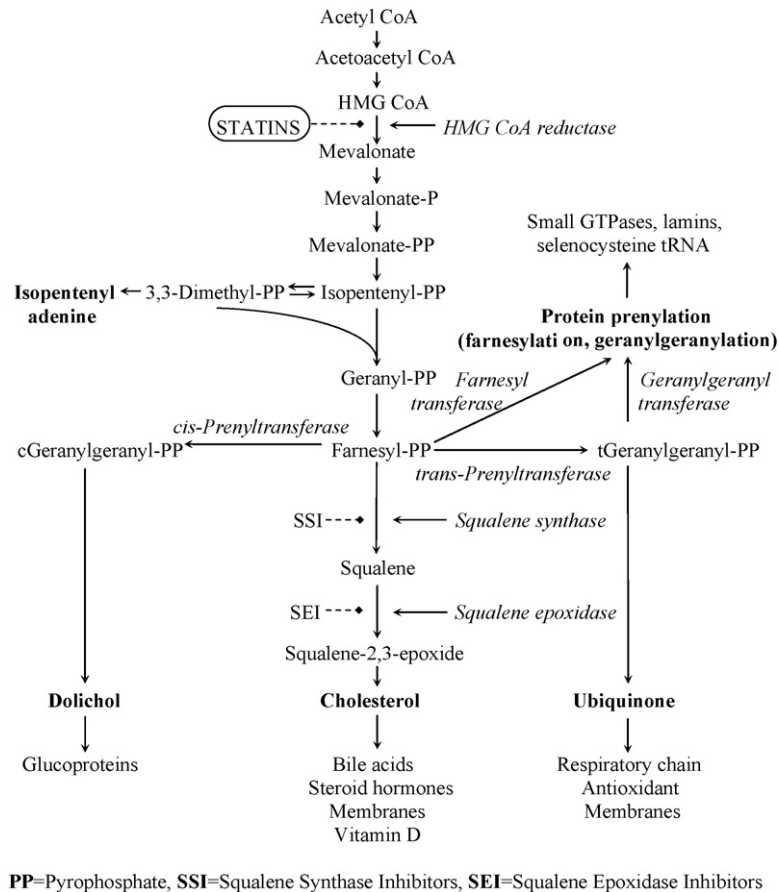


Fig. 1. The biosynthetic pathway of cholesterol and other co-metabolites.

neuromuscular disorder inherited or acquired, such as a mitochondrial myopathy or polymyalgia rheumatica, respectively.

#### 4. Pharmacokinetics of statins and myopathy

Many lines have been written about the association between the pharmacologic and pharmacokinetic characteristics of statins and their myotoxic potential (Table 3). Although the exact mechanisms of statins' myotoxic potential are largely unknown, a complex interplay among absorption, hepatic uptake, solubility, protein binding, drug elimination, and interaction with other drugs is likely to determine the systemic exposure to unbound active statins and consequently the risk of myopathy (Table 4).

The solubility of statins has received much attention [13], as in vivo [14,15] and in vitro [16,17] experiments suggested that hydrophilic statins like pravastatin rather than lipophilic ones like lovastatin or simvastatin are less likely to produce muscular effects (Table 4). Pravastatin demonstrates limited penetration into non-hepatic cells (e.g. muscle cells) because of low passive diffusion. However its hepatic extraction ratio is the lowest among other statins, resulting in expected mean plasma concentrations 10 times higher than those of lovastatin, simvastatin and fluvastatin. On the other

hand, lipophilicity confers more efficient hepatic extraction from the portal venous blood, but it is the same property that allows statins to permeate muscle cells more easily [18].

Atorvastatin, lovastatin, simvastatin and cerivastatin are metabolized by the cytochrome P450 isozyme 3A4 (Table 4). As this pathway is responsible for the metabolism and elimination of an extensive list of drugs (Table 5), it is expected that these statins interact more frequently with other medications. In fact, in approximately 60% of reported cases of rhabdomyolysis associated with simvastatin, lovastatin, or

Table 1  
The ACC/AHA/NHLBI [5] consensus definitions of muscle-related side effects of statins

Condition	Definition
Myopathy	A general term referring to any disease of muscles; myopathies can be acquired or inherited and can occur at birth or later in life
Myalgia	Muscle ache or weakness without CK elevation
Myositis	Muscle symptoms with increased CK levels
Rhabdomyolysis	Muscle symptoms with marked CK elevation (typically substantially greater than 10 times the upper limit of normal) and with creatinine elevation (usually with brown urine and urinary myoglobin)

CK: creatine kinase.

Table 2

Meta-analysis of randomized placebo-controlled clinical trials of statins regarding their myopathic side effects

Study	Type	Dose (mg)	Participants		Duration (years)	Rhabdomyolysis		Myopathy		Minor muscle pain		CK elevation	
			Statin	Placebo		Statin	Placebo	Statin	Placebo	Statin	Placebo	Statin	Placebo
Lovastatin													
AFCAPS/TexCAPS	Primary prevention	20–40	3,304	3,301	5.2	1	2	11	9	NR		21	21
CCAIT	CAD	36 av	165	166	2	NR		NR		NR	NR		
EXCEL	Both	20–80	6,582	1,663	0.9	0	0	5	0	512	125	17	7
ACAPS	Primary prevention	20–40	460	459	3	0	0	NR		NR		NR	
Pravastatin													
CARE	CAD	40	2,081	2,078	5 (median)	0	0	0	4	NR		12	7
WOSCOPS	CAD	40	3,302 (men)	3,293 (men)	4.9 (mean)	0	0	20	19	97	102	3	1
PLAC I	CAD	40	206	202	3	NR		0	0	NR		0	0
PLAC II	CAD	10–40	75	76	3	NR		NR		NR		NR	
REGRESS	CAD	40	323 (men)	330 (men)	2	0	0	1	0	NR		0	0
PREDICT	CAD	40	347	348	0.5	NR		NR		NR		NR	
LIPID	CAD	40	4,286	4,271	6 (median)	0	0	8	10	NR		NR	
LCAD	CAD	20–40 <sup>a</sup>	70	56	2	NR		NR		NR		NR	
KAPS	Primary prevention	40	224	223	3	NR		0	0	NR		NR	
PRINCE	Primary prevention <sup>b</sup>	40	666	673	24 weeks	NR		NR		NR		NR	
ALLHAT–LLT	Both	40	5,170	5,185	4.8 (median)	NR		NR		NR		NR	
PROSPER	Both	40	2,891	2,913	3.2	0	0	36	32	NR		0	0
FAST	Primary prevention	10	83	81	2	NR		NR		NR		NR	
Simvastatin													
4S	CAD	10–40	2,221	2,223	5.4 (median)	1	0	11	8	71	64	6	1
CIS	CAD	40	129	125	2.3 (mean)	NR		NR		NR		NR	
Wenke et al.	Heart transplantation	10 av	35	37	4	0	0	NR		NR		0	0
HPS	Both	40	10,269	10,267	5.3 (average)	5	3	49	50	3330	3359	1	2
MAAS	CAD	20	193	188	4	0	0	NR		NR		NR	
Fluvastatin													
LCAS	CAD	40 <sup>c</sup>	214	215	2.5	0	0	NR		NR		1	2
LiSA	CAD	80	187	178	1	0	0	0	0	NR		0	1
FLARE	CAD	80	409	427	0.8	0	0	7	3	NR		0	0
ALERT	Renal transplantation	40–80	1,045	1,049	5.1 (mean)	1	1	0	0	526	531	3	1
LIPS	CAD	80	844	833	3.9 (median)	0	0	NR		NR		0	3
Atorvastatin													
AVERT	CAD	80	164	177	1.5	0	0	0	0	0	0	0	0
MIRACL	CAD	80	1,538	1,548	0.3	0	0	0	0	NR		NR	
GREASE	CAD	24 av	800	800	3	0	0	0	0	NR		NR	
ASCOT–LLA	High-risk individuals	10	5,168	5,137	3.3 (median)	1	0	NR		NR		NR	
SPARCL	Secondary prevention	80	2,365	2,366	4.9	2	3	7	7	129	141		
Cerivastatin													
ENCORE I	CAD	0.4	114	119	0.5	NR		1	0	NR	0	2	0

Table 2 (Continued)

Study	Type	Dose (mg)	Participants		Duration (years)	Rhabdomyolysis		Myopathy		Minor muscle pain		CK elevation	
			Statin	Placebo		Statin	Placebo	Statin	Placebo	Statin	Placebo	Statin	Placebo
Rosuvastatin ASTEROID <sup>d</sup>	CAD	40	507		2	0		19		NR		0	
Incidence per 100,000 person years						4.04	4.11	70	65	2090	1977	24.24	21

AFCAPS/TexCAPS: Air Force/Texas coronary atherosclerosis prevention study; CCAIT: Canadian coronary atherosclerosis intervention trial; EXCEL: expanded clinical evaluation of lovastatin; ACAPS: asymptomatic carotid artery progression study; CARE: cholesterol and recurrent events trial; WOSCOPS: west of scotland coronary prevention study; PLAC I: pravastatin limitation of atherosclerosis in the coronary arteries; PLAC II: pravastatin lipids and atherosclerosis in the carotid arteries; REGRESS: regression growth evaluation statin study; PREDICT: prevention of restenosis by elisor after transluminal coronary angioplasty; LIPID: long-term intervention with pravastatin in ischaemic disease study; LCAD: lipid coronary artery disease study; KAPS: kuopio atherosclerosis prevention study; PRINCE: pravastatin inflammation/CRP evaluation; ALLHAT–LLT: the lipid-lowering trial component of the antihypertensive and lipid-lowering treatment to prevent heart attack trial; PROSPER: prospective study of pravastatin in the elderly at risk; FAST: Fukuoka atherosclerosis trial; 4S: Scandinavian simvastatin survival study; CIS: the multicenter coronary intervention study; HPS: heart protection study; MAAS: multicenter antiatheroma study; LCAS: lipoprotein coronary atherosclerosis study; LISA: lescol in severe atherosclerosis; FLARE: fluvastatin angiographic restenosis trial; ALERT: assessment of lescol in renal transplantation; LIPS: lescol intervention prevention study; AVERT: atorvastatin versus revascularization treatment; MIRACL: myocardial ischemia reduction with aggressive cholesterol lowering trial; GREASE: Greek atorvastatin and coronary heart disease evaluation study; ASCOT-LLA: Anglo-Scandinavian cardiac outcomes trial, lipid lowering arm; SPARCL: stroke prevention by aggressive reduction in cholesterol levels; ENCORE I: evaluation of nifedipine and cerivastatin on recovery of coronary endothelial function; ASTEROID: a study to evaluate the effect of rosuvastatin on intravascular ultrasound-derived coronary atheroma burden. NR: not reported, both: primary and secondary prevention, and av: average.

<sup>a</sup> Nicotinic acid and cholestyramine were also co-administered.

<sup>b</sup> Data from the primary prevention cohort presented. Data from the secondary prevention cohort was not placebo-controlled.

<sup>c</sup> Plus cholestyramine.

<sup>d</sup> Not a placebo-controlled clinical trial.

Table 3  
Predisposing factors of statin-induced myopathy

Predisposing factors
Increasing age
Female gender
Renal insufficiency
Hepatic dysfunction
Hypothyroidism
Polypharmacy
Perioperative period
Metabolic muscle diseases:
1. Carnitine palmitoyl transferase II deficiency
2. McArdle disease
3. Myoadenylate deaminase deficiency
Polymyalgia rheumatica

atorvastatin, there was an underlying interaction with a drug known to inhibit CYP 3A4 [7].

The combination of statins with a fibrate has received much attention since it is an attractive option to treat effectively complex hyperlipidemias (Table 5). An estimated 19% of statin-related rhabdomyolysis occurred in patients also taking fibrates, principally gemfibrozil [7]. Gemfibrozil inhibits the glucuronidation of statins [19], the pathway that mediates the removal of statins' hydroxyl acid metabolites [20]. Gemfibrozil undergoes extensive glucuronidation via the UGT1A1 and UGT1A3 pathways, the same as those employed by some statins. Fenofibrate, on the other hand, is metabolized by UGT1A9 and UGT2B7, a fact that suggests potentially less risk of interaction when used in combination therapy [10]. Bezafibrate and ciprofibrate also employ alternative pathways for their elimination [10,21]. It is possible that pharmacodynamic interactions between statins and fibrates also come into play as fibrates are considered myotoxic in their own right [21].

## 5. Endogenous biosynthetic pathway of cholesterol and other co-metabolites

Cholesterol is a very important molecule as it is an essential component of all cell membranes and the precursor molecule for steroid hormones, vitamin D and bile acids. The endogenous cholesterol biosynthesis occurs in the endoplasmic reticulum and cytosol and is shown in Fig. 1. Sequential condensation of three molecules of acetyl CoA by thiolase and HMG CoA synthase leads to the formation of HMG CoA. The next downstream reaction, HMG CoA reduction to mevalonate by HMG CoA reductase, represents the principal regulatory step in cholesterol synthesis. Very important intermediates of this pathway are geranyl pyrophosphate (G-PP) and farnesyl pyrophosphate (F-PP). These are derivatives of common 5-carbon building blocks, isopentenyl pyrophosphate and its isomer dimethylallyl pyrophosphate, called isoprene units [22]. Apart from the biosynthesis of cholesterol, F-PP and G-PP are involved in the post-translational

Table 4  
Pharmacokinetic properties of the currently approved (with the exception of cerivastatin) statins

Variable	Cerivastatin	Lovastatin	Simvastatin	Pravastatin	Fluvastatin	Atorvastatin	Rosuvastatin	Pitavastatin [53]
Elimination half-life (h)	2.1–3.1	2.9	2–3	1.3–2.8	0.5–2.3	15–30	19	11
Bioavailability (%)	60	5	5	18	19–29	12	20	>60%
Protein binding (%)	>99	>95	94–98	43–55	>99	80–90	88	96%
Solubility	Lipophilic	Lipophilic	Lipophilic	Hydrophilic	Intermediate <sup>a</sup>	Lipophilic	Hydrophilic	Lipophilic
Cytochrome 450 metabolism and isozyme	CYP 3A4 and CYP 2C8	CYP 3A4	CYP 3A4 and CYP 3A5	Hydrophilic <sub>b</sub>	CYP 2C9	CYP 3A4	Limited CYP 2C9	CYP2A9 and CYP3A4 <sup>c</sup>

Pitavastatin is currently available only in Japan. Cerivastatin was withdrawn in August 2001.

<sup>a</sup> Twice as hydrophilic as lovastatin but 40 times more lipophilic than pravastatin.

<sup>b</sup> Minimal CYP 3A4 metabolism. Primarily cleared by the kidneys.

<sup>c</sup> Minimal CYP3A4 metabolism. CYP2A9 is the principal elimination pathway.

Table 5  
Drug interactions with statins

Hypolipidemic drug	
Interactions on the CYP3A4 pathway (affect atorvastatin, lovastatin, simvastatin, and cerivastatin)	Fibrates <sup>a</sup> , nicotinic acid, macrolide antibiotics, azole antifungals, HIV protease inhibitors, mibefradil <sup>b</sup> , fluoxetine, nefazodone, verapamil, warfarin and grapefruit juice
Interactions on the CYP2C9 pathway (affect fluvastatin and rosuvastatin)	Warfarin, amiodarone, cimetidine, trimethoprim/sulfamethoxazole, fluoxetine, fluvoxamine, isoniazid, metronidazole, sulfapyrazone, ticlopidine, zafirlukast, itraconazole and ketoconazole

Co-administered medicines may serve as inhibitors or competing substrates of the respective elimination pathways of statins. Conversely, the clinician must be aware that many drugs, most notably barbiturates, phenytoin, carbamazepine, enhance elimination of statins.

<sup>a</sup> For statins other than cerivastatin combined with fibrates data from the US Food and Drug Administration (FDA) Adverse Events Reporting System (AERS) showed a 15-fold higher incidence of rhabdomyolysis when the fibrate was gemfibrozil than when it was fenofibrate [7].

<sup>b</sup> Withdrawn.

modification (i.e. prenylation) of various cellular proteins, as well as serve as precursors for the biosynthesis of important compounds, such as dolichol, and ubiquinone (Fig. 1).

### 5.1. Prenylated proteins

The post-translational prenylation of proteins occurs by the covalent addition of only two types of isoprenoids, F-PP and geranylgeranyl pyrophosphate (GG-PP), to cysteine residues at or near the C-terminus. Prenylated proteins, such as small GTPases and lamins constitute up to 2% of total cellular protein [22]. The lipophilic prenyl group enables these prenylated proteins to anchor to cell membranes, which in most cases is an essential requirement for their biologic function. Selenocysteine tRNA undergoes post-transcriptional prenylation, which is an important modification for its proper function.

#### 5.1.1. Biologic role of prenylated small GTPase family of proteins

Small GTPase proteins are prenylated proteins that cycle between an inactive guanosine diphosphate (GDP) – bound and active guanosine triphosphate (GTP) – bound state, and they have crucial roles in controlling multiple signaling pathways [23]. Upon tyrosine kinase receptor activation, the farnesylated membrane bound small GTPase protein Ras becomes activated by binding to GTP. Ras serves as a signal transduction intermediary by initiating a cascade of events culminating in positive regulation of cell growth.

Rab small GTPases are involved in organelle biogenesis and intracellular vesicular trafficking. More than 60 Rab small GTPase isoforms have been identified. Each has a specific intracellular localization and regulates a specific trafficking step. For example Rab1 is involved in the transportation of vesicles from the endoplasmic reticulum to the Golgi appa-

ratus and Rab8 carries newly synthesized transmembrane proteins from the Golgi apparatus to the plasma membrane [24]. Typically, they are doubly geranyl geranylated, an important modification for their exquisite localization, which is in turn required for their proper function [25,26].

### 5.1.2. *Biologic role of prenylated lamins*

Lamins are the main component of the intermediate filament lamina, which lines the inner nuclear membrane. Besides having a structural role, lamins have a role in chromatin organization as well, and there are several examples of lamin participation in gene expression [27]. Lamins B1 and B2 undergo farnesylation, whereas prelamin A, which is the precursor molecule of lamin A, undergoes prenylation-dependent processing.

### 5.1.3. *Biologic role of selenocysteine tRNA and selenoproteins*

Selenocysteine tRNA is isopentenylated at adenosine 37, (A37) a commonly modified position immediately 3' at the anticodon [28]. Selenocysteine tRNA decodes UGA, normally a stop codon, and inserts selenocysteine into nascent selenopeptides. The absence of tRNA isopentenylation was found to reduce the efficiency of the altered selenocysteine tRNA in decoding nonsense codons in bacteria and yeast [29]. Therefore, improper translational stop codon read-through may lead to premature termination of translation and production of truncated proteins.

## 5.2. *Dolichols*

Dolichols are derivatives of F-PP and isopentenylpyrophosphate. Dolichols are polyisoprenols with typically 16–22 isoprene units, whose single chain varies in length both within cells and between cell types and organisms. Dolichols mediate the N-linked glycosylation of nascent polypeptides by serving as carriers, as well as sites whereupon the core oligosaccharide unit for protein glycosylation is assembled. Glycosylation is an intricate modification that proteins undergo and is an integral component for proteins' proper biologic functioning.

## 5.3. *Ubiquinone*

Ubiquinone is composed of a hexameric quinone ring (Q) and a ten isoprenyl unit side chain, hence the name coenzyme Q10 (CoQ10). It acts as a mobile component of the respiratory chain in mitochondria that collects reducing equivalents from the more fixed flavoprotein complexes and passes them onto the cytochromes further downstream the respiratory chain.

## 6. Mechanisms of statin-induced myopathy

Statins, which share a HMG-like moiety, bind to HMG CoA reductase with three orders of magnitude greater affinity

than HMG CoA, effectively displacing the natural substrate [1,30]. Statin myotoxicity could be attributed to that effective inhibition of HMG CoA reductase by statins, which results in the reduced production of downstream intermediate metabolites. Indeed, in an in vitro model of statin-induced myotoxicity using neonatal rat skeletal myotubes, replacement of depleted mevalonate (i.e. the immediate product of HMG CoA reductase necessary for the synthesis of G-PP and F-PP; Fig. 1) restored in a concentration-dependent manner protein synthesis and reversed the changes induced by statins. In contrast, inhibition of squalene synthase, which catalyzes the first metabolic step in cholesterol synthesis uncommitted to isoprene synthesis (Fig. 1), did not induce myotoxicity in primary cultures of rat myotubes [31], in human rhabdomyosarcoma cell line and in human skeletal myotubes [32]. Similarly, inhibition of the next downstream step with squalene epoxidase inhibitors in L6 rat myocytes, and importantly at concentrations sufficient to inhibit completely cholesterol synthesis, did not have any adverse effect on cell viability [33]. These studies allow us to conclude that statin-induced myopathy is not due to decreased cholesterol synthesis, but due to the reduction of the availability of the isoprenoid co-metabolites (G-PP and F-PP). This in turn may adversely affect the prenylation of proteins and/or promote alternative modification (dysprenylation). The results of altered modification may be loss of function, gain of function, or altered function as a consequence of altered subcellular localization, or interaction with other proteins. Furthermore, reduced availability of isoprene units may adversely affect dolichol biosynthesis and consequently protein glycosylation. Ubiquinone biosynthesis may accordingly be impaired. The molecular and cellular effects of statins on the skeletal muscle cells are summarized in Table 6.

In vitro studies of the alterations in gene expression incurred by statins revealed that human skeletal muscle cells were substantially more sensitive than hepatocytes to statins regarding the inhibition of cholesterol biosynthesis [34]. This may explain the susceptibility of skeletal muscles to the toxic effects of statins at a genetic level, but only partly, as for example these drugs do not adversely affect the myocardium.

### 6.1. *Statins promote dysprenylation of proteins and selenocysteine tRNA*

#### 6.1.1. *Statin promote dysprenylation of small GTPases*

The Ras proteins of the Ras superfamily of small GTPases are involved in the functional maintenance and differentiation of myocytes, both of which are adversely affected by statins. Experiments on L6 myoblasts have shown that depletion of membrane bound Ras affected by statins was the inciting event for the induction of apoptosis [35]. More recent studies on isolated rat skeletal myofibers have highlighted the role of Rab small GTPases in statin-induced myopathy. Inactivation of Rabs effected by statins induced vacuolation of the myofibers, degeneration and swelling of organelles, and eventually apoptosis [36].

Table 6  
Molecular and cellular effects of statins on the skeletal muscle cells leading to myopathy

Molecule	Statin effect	Consequences at molecular level	Consequences at cellular level	Reference
Ras small GTPases	Dysprenylation	Impaired signal transduction	Deprivation of growth signals and impaired intracellular signaling	[23,35]
Rab small GTPases	Dysprenylation	Altered intracellular trafficking	Loss of physiologic cellular function and impaired translocation of receptors to the plasma membrane	[24–26,35,36]
Lamins	Dysprenylation	Nuclear fragility, heterochromatin disruption, impaired gene transcription	Susceptibility to mechanical stress and altered gene expression	[27,37–41,43]
Selenocysteine RNA	Dysprenylation	Premature termination of translation	Production of truncated and dysfunctional selenoproteins	[28,29,43]
Dolichols	Impaired post-translational <i>N</i> -glycosylation	Impaired expression of membrane receptors and alteration of structural proteins	Production of dysfunctional proteins and alteration or decrease of membrane receptors	[42]
Dystroglycans	Altered post-translational modification	Impaired extracellular matrix-cytoskeleton communication	Muscular dystrophy (?)	[43,44]
Coenzyme Q10	Decreased synthesis	Impaired oxidative phosphorylation	Impaired energy production	[45–49]

### 6.1.2. Statins promote dysprenylation of lamins

Lovastatin *in vitro* blocked the prenylation-dependent processing of lamin A [37] and prevented its assembly into the nuclear lamina [38]. However earlier experiments have shown that the concentrations of lovastatin required to inhibit prenylation of prelamin A are several magnitudes higher than those required to effectively inhibit endogenous cholesterol synthesis [39].

All the above observations lead to the assumption that statin-induced lamin dysprenylation may indeed underlie statin-associated myopathy, especially in settings where statins interact with other medicines and consequently can reach very high concentrations. Possible mechanisms may involve a fragile nucleus with defective nucleoskeleton unable to sustain the mechanical forces conducted during muscle contraction [40]. Defective nuclear mechanotransduction and impaired activation of transcription of genes involved in adaptive or protective pathways in response to mechanical stimulation may also be present [40]. Additionally, in congenital laminopathies severe heterochromatin disruption is observed along with nuclear dysfunction [41]. All these alterations facilitate apoptosis and perhaps to a greater or lesser extent participate in statin-induced myopathy.

### 6.1.3. Statins promote dysprenylation of selenocysteine tRNA and adversely affect selenoprotein synthesis

Warner et al employed *in vivo* lovastatin to illustrate the role of adenosine 37 isopentenyladenosine of selenocysteine tRNA in selenoprotein synthesis. Lovastatin reduced the amount of isopentenyladenosine containing selenocysteine tRNA by 50% in Chinese hamster ovary cells at a concentration of 10  $\mu$ M. This effect had a reflection on the ability of these cells to support selenoprotein synthesis. All cellular selenoproteins investigated with the exception of one were reduced, and some at an impressive degree. Truncated products resulting apparently from premature termination of

translation were produced that were subject to rapid degradation. In accordance with the above observations the same investigators showed that selenocysteine tRNA lacking A37 isopentenyladenosine was binding less efficiently to UGA stop codons [29]. The implications, however, of the above observations regarding statin-induced myopathy are appealing but not straightforward and require further investigation.

### 6.2. Statins promote inhibition of dolichol synthesis and *N*-linked glycosylation

The effect of statins on protein *N*-glycosylation has been highlighted *in vitro* on adipocytes by Siddals et al. [42]. The authors have shown that statins affected the *N*-glycosylation and consequently the processing of insulin-like growth factor receptors and insulin receptors. There may be a synergy between disruption of glycosylation culminating in decreased membrane expression of specific receptors and dysprenylation of downstream signaling intermediates, such as the Ras proteins [42]. Taken together, an impaired response to growth factors may underlie the statin-induced myopathy.

Most cell surface receptors however are not dependent on dolichol mediated *N*-glycosylation for correct processing. Other key cell surface proteins may be affected. A possible candidate would be dystroglycan, a cytoskeletal glycoprotein that undergoes extensive post-translational modification through the endoplasmic reticulum and Golgi apparatus [43]. Dystroglycan is cleaved into the extracellular matrix receptor  $\alpha$  dystroglycan and the transmembrane dystrophin binding protein  $\beta$  dystroglycan. It mediates a communication between extracellular matrix and muscle cytoskeleton, which may be defective when dystroglycan is hypoglycosylated. In fact, there is evidence to suggest that this defective communication may underlie the muscular dystrophy observed in congenital syndromes associated with mutated glycosyltransferases [44]. However, the direct effect of statins on dystroglycan processing has not been investigated.



### 6.3. Statin-induced mitochondrial myopathy: role of ubiquinone

There are several lines to suggest that statin-induced myopathy is associated with mitochondrial dysfunction. Theoretically, statins may inhibit the synthesis of CoQ10 in the mitochondria. Through this effect, statins may compromise the function of the mitochondrial respiratory chain, impair energy production in skeletal muscle cells ultimately inducing myopathy [45].

Although statins were found to reduce the serum CoQ10 levels [45–48], they showed no effect on CoQ10 levels within the skeletal muscle cells with the exception of high dose treatment with simvastatin [49]. Furthermore, a direct association between reduced levels of intramuscular CoQ10 and mitochondrial myopathy has not been conclusively shown [45]. Consequently, CoQ10 depletion does not appear to play an etiopathogenic role in statin-induced myopathy; more likely, it is a critical predisposing factor, especially in individuals in whom other CoQ10 depleting conditions co-exist [45]. Such conditions include old age, increased doses of statin treatment, increased statin bioavailability due to renal or hepatic dysfunction, hereditary metabolic syndromes, such as familial mitochondrial encephalomyopathy, and other comorbidities, such as cancer, heart failure, diabetes, familial hypercholesterolemia, and hypothyroidism.

### 7. Recommendations for prevention of statin-induced myopathy

The National Lipid Association Statin Safety Task Force recently reviewed the evidence regarding the safety of statins and edited practical clinical recommendations [50]. Clinicians should advise patients on statins about their side effects, drug interactions, risk factors and warning symptoms and signs of myopathy and rhabdomyolysis. Baseline CK measurements are not routinely recommended, although consideration should be given in patients at high risk for myopathy. No routine follow up CK measurements are recommended while on statin therapy, unless symptoms of myopathy develop [50].

When muscle symptoms develop, an attempt should be made to seek for other etiologies before deciding on the discontinuation of statins. Focal, rather than generalized muscle complaints argue against statin-induced myopathy. A CK measurement should also be obtained to assess the severity of muscle damage. It is the severity of clinical symptoms rather than the CK measurements alone that should prevail in clinical decision-making. This statement recognizes the fact that frequently muscle symptoms develop in the absence of CK elevation. Intolerable muscle complaints should prompt discontinuation of statins. When muscle complaints are tolerable a reduction in the dose of statins may be attempted first. Clinical follow up of the symptoms may serve as a guide for escalation or even discontinuation of the offending agent.

Any CK elevation above 10 times the upper limit of normal constitutes an indication for discontinuation of statins and should prompt inpatient management of an impending rhabdomyolysis [50]. Treatment is supportive and resolution of symptoms should be anticipated within a few days. Discontinuation of any interacting medicines, reinstitution of the statin at a lower dose or selection of a different statin and careful monitoring should be pursued once full recovery is achieved [51].

The etiopathogenic role of CoQ10 in statin-induced myopathy is not well established [46]. On the basis of the current evidence routine CoQ10 supplementation for all patients taking statins to prevent myotoxicity is not recommended. It is likely however that certain subgroups of patients susceptible to myopathy may benefit, and this fact may account for the inconsistency in the literature. After eliminating and treating, if possible, CoQ10 depleting conditions in such patients, it may be worth prescribing CoQ10 supplementation [45].

### 8. Future perspectives

Two thirds of the total body cholesterol is synthesized in the liver. Consequently, targeting the endogenous biosynthetic pathway produces effective reductions in LDL-C. Sparing the non-sterol pathways may eliminate the myotoxic potential on the one hand, but may deprive the lipid lowering therapy from the beneficial pleiotropic effects of statins. Squalene synthase inhibitors act on the first committed step of cholesterol synthesis (Fig. 1). They decrease LDL-C in a similar manner to statins. They are now entering clinical trials and the results are anticipated with great interest [52]. In addition, squalene epoxidase inhibitors and oxidosqualene cyclase inhibitors act further downstream in the pathway and are in various stages of development.

### 9. Conclusion

Statins remain the spearhead of our armamentarium in treating atherosclerotic disease as they effectively reduce LDL-C, an important risk factor for CAD and related acute coronary syndromes. Their pleiotropic effects render them invaluable in cardiovascular prevention. Overall, statins are safe and effective drugs but their myotoxic potential cannot be overlooked. Statins have been shown to affect protein synthesis and modification at multiple levels. Dysprenylation of signal transduction molecules and altered glycosylation of membrane proteins may deprive muscle fibers from growth signals. Their adverse effect on structural proteins, such as lamins, may render muscle fibers susceptible to mechanical stress. Statin-induced myopathy may also be associated with mitochondrial dysfunction. Further clinical and experimental investigations will provide insight into statin-induced myopathy, enhance the safety profile and expand the clinical use of statins.

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