REVIEW

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Pathophysiological mechanisms of statin-associated myopathies: possible role of the ubiquitin-proteasome system

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Background Statins are the cornerstone of pharmacotherapy for atherosclerotic cardiovascular disease. While these drugs are generally safe, treatment adherence is not optimal in a considerable proportion of patients because of the adverse effects on skeletal muscles in the forms of myopathy, myalgia, muscular pain, nocturnal muscle cramping, weakness, and rare rhabdomyolysis.

Methods For the purpose of this narrative review, we searched for the literature suggesting the involvement of the ubiquitin–proteasome system in the development of statin–induced myopathy.

Results Statins have been shown to up–regulate the expression of the muscle–specific ubiquitin–proteasome system as the major non–lysosomal intracellular protein degradation system. It has been postulated that statins may provoke instability in the myocyte cell membrane when subjected to eccentric exercise stress, triggering activation of intracellular proteolytic cascades and changes in protein degradation machinery. This is accompanied by the up–regulation of a series of genes implicated in protein catabolism, in addition to those of the ubiquitin–proteasome system.

Conclusions Based on the available literature, it seems that the involvement of ubiquitin–proteasome system is potentially implicated in the pathophysiology of statin–induced myopathy.

Keywords Statins; Myopathy; Ubiquitin-proteasome system

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The ubiquitin-proteasome system

The levels of intracellular proteins are the result of the balance between synthesis and degradation processes, both fundamental for the correct functioning of the cell. In particular, protein degradation is finely regulated taking place through two main routes. The first is represented by lysosomes, vesicular organelles containing acidic hydrolases with different specificities, responsible for the disposal of endogenous or exogenous proteins internalized by endocytosis and pinocytosis.[1,2] The other proteolytic pathway involves ubiquitin, a polypeptide of 76 amino acids, which acts as a marker for degradation, and a multienzymatic complex, the proteasome. The ubiquitin-proteasome system (UPS) does not require compartmentalization, and the catalytic complex acts ubiquitously (nucleus, cytoplasm, and in association with the endoplasmic reticulum), with ATP dependent and ATP independent mechanisms that involve numerous adjuvant molecules and that have been merged into the so-called 'signalosome'.[3]

The UPS acts on different classes of both short and long half-life proteins, and intervenes in the regulation of numerous cellular processes, being responsible for about 90% of extra-lysosomal degradation, such as cell cycle control by proteolysis of specific regulatory proteins, cell growth, and proliferation through the degradation of oncoproteins and proteins of the signal transduction pathway, DNA repair, regulation of transcription, regulation of immune and inflammatory responses, processing of the antigens presented in association with the major class I histocompatibility complex (MHC I), and degradation of mutated or damaged proteins (DRiPs).

Because the proteasome intervenes in processes of vital importance for the cell, its inhibition leads to cell death and its malfunctioning can be the basis of numerous pathological events.[4,5] The proteins that must be degraded by this system are covalently labelled with ubiquitin. The marking is intended to label the substrate to be degraded to the multicatalytic enzyme complex. The degradation involves also many different protein substrates and is based on a very ingenious and efficient recognition and marking mechanism.[4]

The ubiquitin attack on the substrate takes place through the formation of the isopeptide bond that is generated between the ubiquitin caboxide and the amino group of a Lys residue on the substrate to be degraded.

The first ubiquitin molecule can be covalently attached to the others, leading to the formation of ubiquitin chains (*Figure* 1). It has been observed that chains consisting of four or more ubiquitin units constitute the signal for proteasoma 26S. The ubiquitin attack is an ATP dependent process catalyzed by three enzymes: E1, E2, and E3, which also confer specificity to the process. The first reaction, catalyzed by E1, is responsible for the activation of ubiquitin: the C-terminal of Gly 76 of ubiquitin, activated in an ATP-dependent reaction, is linked to the Cys of the active site of the enzyme E1. The activated ubiquitin is transferred to E2, in whose active site there is a Cys residue. Through a transferase activity, E2 transfers ubiquitin to the E3 ligase that binds ubiquitin to Lys residues of the target protein, forming an isopeptide bond with the C-terminal of Gly 76. In some cases, E2 transfers ubiquitin directly to the target protein without the intervention of E3.[1–5]

The enzyme system E1, E2, and E3 acts cyclically, allowing the formation of a 'cluster' of ubiquitin molecules because of the progressive union of an ubiquitin monomer with the Lys 48 of the previous ubiquitin. The specificity for the substrate in the ubiquitination system is because of the combined action of the different E2 transferases and of the different E3 ligases, which in combination recognize the protein destined for degradation.

Degradation begins with the recognition of specific signals by the E2/E3 enzymes on the protein. Following signal recognition, the substrate is labelled with ubiquitin, thus directing it to the proteasome. The signals present on the protein that induce the ubiquitin attack are the N-terminal sequence, the phosphorylation of PEST sequences, the 'destruction box', and the loss of conformation.[1–5]

The presence of particular amino acids in the N-terminal of the protein is a universal signal. It has been shown that proteins with N-terminal residues loaded (Lys or Arg) or voluminous (Phe, Leu, Tyr, and Trp) are rapidly ubiquitinated and degraded; on the contrary, proteins with small N-terminal residues are quite stable.

The degradation signal requires the presence of a lysine close to the N-terminal residue to assemble the ubiquitin 'cluster'. The phosphorylation of specific sequences (Pro-Glu-Ser-Thr, PEST) or of 'destruction-box' (nine amino acids)

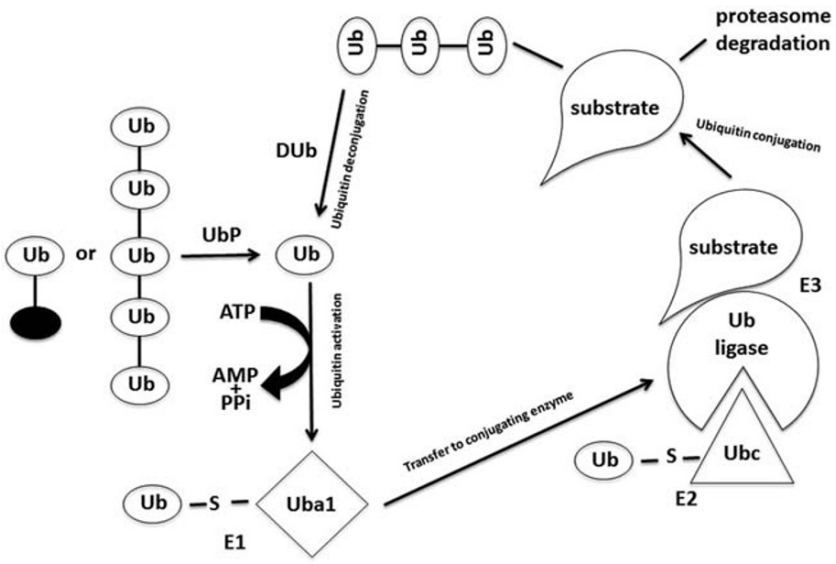

FIGURE 1 Protein degradation pathway by ubiquitin-proteasome system. Ub ubiquitin; UbP, ubiquitin protease; DUb, deubiquitinating enzyme.

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represents the signal that determines the labelling with ubiquitin of target proteins. This signal is used by some proteins that regulate the cell cycle (e.g. the cicline), by p53, and by the transcription factor inhibitor nuclear factor kB (NF-kB, IkB).[1–5]

Proteins damaged by oxidation, mutations, or cellular stress, such as molecules that lose their three-dimensional structure or are located in a wrong cellular compartment become excellent substrates for the ubiquitination process. The signals that activate this process are not yet perfectly clear but certainly involve the exposure of hydrophobic regions that are normally not exposed by proteins in their native structure. For example, proteins only partially or incorrectly synthesized in the endoplasmic reticulum are called 'Detective Ribosomal Products' (DRiPs); about 30% of the newly synthesized proteins—because they do not show up in their correct native conformation—are transferred by means of a specific transport from the endoplasmic reticulum to the cytoplasm and degraded by the UPS. In some cases, improperly shaped proteins are associated with assistance proteins (called chaperones) that mediate recognition with the ubiquitination enzymes.[1–5]

The proteasome is a multicatalytic enzymatic complex located in the cytoplasm and in the nucleus of eukaryotic cells. It constitutes the main system of protein degradation and possesses different types of proteolytic exopeptidasic and endopeptidasic activities. Because of its functionality, the enzyme requires ATP and the ubiquitin labelling of the substrate to be degraded. The complex is able to realize the compartmentalization of proteolysis because the region responsible for the proteolytic activity (proteolytic chamber) is confined within the complex structure called 'core' 20S that can be associated with different types of regulators (19S or 11S/PA28).

The compartmentalization is essential for the regulation of proteolysis and avoids the erroneous degradation of proteins that must remain intact and functional. Time and space control of degradation is regulated not only by ubiquitin labelling but also by changes in cellular localization of the proteasome. In fact, using specific localization signals, the proteasome can be addressed in different compartments of the cytoplasm or of the nucleus. The main activities carried out by the proteasome and its regulators are the recognition and binding of proteins linked to ubiquitin, the activity of assembling and disassembling proteins, and the ability to move proteins inside the proteolytic chamber, different peptidal activities used to degrade damaged and regulatory proteins. This proteolytic complex therefore plays a key role in the maintenance of protein homeostasis, in the cell cycle, in transcription, and in the immune response as it generates the peptides that bind the molecules of the MHC-I.[1–5]

Statin treatment and cardiovascular risk

It has been several decades since the awareness of the existence of a close association between high plasma levels of low-density lipoprotein (LDL) cholesterol and cardiovascular risk and the fact that its reduction is associated to a significant reduction in the risk to develop cardiovascular disease. In particular, the results of clinical studies conducted to test the efficacy of the inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (statins), indicate a direct relationship between the reduction of LDL-cholesterol levels and the reduction of cardiovascular events.[6] The results of the meta-analysis of Baigent *et al.*[6] clarified very well the proportionality of the relationship between LDL cholesterolemia and cardiovascular events, highlighting that, with each reduction of 1 mmol/L or 39 mg/dL of LDL levels, there is a 12% reduction in mortality, essentially linked to a reduction in mortality from coronary causes, and a 21% reduction in cardiovascular events. This reduction seems to be similar in all patients regardless of age, sex, and the presence of cardiovascular diseases and baseline cholesterol levels.

The more recent literature has shown that the cardiovascular risk reduction associated to lipid-lowering therapy is proportional to baseline cardiovascular risk of the patient and inversely to the achieved LDL values. In particular, a meta-analysis of individual participant data from randomized trials involving at least 1000 participants and at least 2 years treatment duration of more vs. less intensive statin regimens (five trials; 39 612 individuals; median follow-up 5.1 years) and of statin vs. control (21 trials; 129 526 individuals; median follow-up 4.8 years) was carried out. Across all 26 trials, all-cause mortality was reduced by 10% per 1·0 mmol/L LDL reduction (RR 0.90, 95% CI 0.87–0.93; $P < 0.0001$), largely reflecting significant reductions in deaths because of coronary heart disease (RR 0.80, 99% CI 0.74–0.87; $P < 0.0001$) and other cardiac causes (RR 0.89, 99% CI 0.81–0.98; $P = 0.002$), with no significant effect on deaths because of stroke (RR 0.96, 95% CI 0.84–1.09; $P = 0.5$) or other vascular causes (RR 0.98, 99% CI 0.81–1.18; $P = 0.8$).[7]

Based on these results, European and North American guidelines for cardiovascular disease prevention, recommend reaching an LDL-C level less than 115 mg/dL for subjects with low-to-moderate added cardiovascular risk, less than 100 mg/dL for subjects with high cardiovascular risk, and less than 70 mg/dL for subjects with very high cardiovascular disease risk.[8–10]

In spite of novel lipid-lowering therapies,[11,12] statins remain the most evidence-based and cost-effective approach to reduce LDL cholesterolemia and to reduce cardiovascular event risk. Besides, these drugs have pleiotropic cholesterol-independent activities that contribute to their cardioprotective action.[13–18]

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In spite of these benefits, the efficacy of statins is often limited by the relatively low tolerability of more intense treatments, leading to dramatically reduced persistence.[19] Muscle-related adverse events are among the most known and frequent causes of statin treatment interruption.

Statins' muscle-related adverse events

Statins are generally effective and safe drugs; however, the main side effects could limit their prescription, in particular as regard liver injury and skeletal muscle toxicity (i.e. myopathy, myalgia, muscular pain, nocturnal muscle cramping, weakness, and rare rhabdomyolysis).[20,21] In 2001, cerivastatin was withdrawn from the market worldwide because of 31 rhabdomyolisis-related deaths.[22]

Although the incidence of statin-induced myopathy is low in clinical trials, muscle symptoms are common in clinical practice. This is probably because of the fact that patients at high risk of statin-related adverse events linked to predisposing factors like drugs affecting statin metabolism or comorbidities, have usually been excluded in clinical trials.[23] The presence of a negative drucebo effects has also been suggested to explain a considerable part of statin-associated muscle symptoms in clinical practice.[24]

Epidemiological data assess that myalgia represents from 6% to 14% of all adverse events associated with statin use occurring in 5% to 7% of patients.[25–27] Myopathy occurs with an incidence of 195 cases per 100 000 patient-years (frequency <0.1% in patients on monotherapy with statins).[23] Lastly, in the post-marketing surveillance, data from the Food and Drug Administration's (FDA) adverse events reporting system (AERS) records that the incidence of rhabdomyolysis is approximately 0.70 per 100 000 patient-years with 0.15 deaths per 1 million prescriptions.[23,27]

Creatine kinase (CK) is commonly used as serum marker to define skeletal muscle damage and its severity. However, myopathy can occur without CK elevations.[28] Conversely, intense physical exercise increases CK levels but without chronic muscle pain.[29] For these reasons, CK is a non-sensitive biomarker of the induction of statin-induced myotoxicity. However, because of the lack of specific biomarkers, CK is currently used to characterize myotoxicity related to statin-therapy.

On the other hand, the terminology for describing muscle toxicity related to statin use is not yet harmonized. The American College of Cardiology/American Heart Association/National Lung Institute Clinical Advisory (ACC/AHA/NHLBI) defines myopathy as muscle pain, soreness, and frailty with or without abnormal serum CK and myalgia as muscle aches or weakness without CK elevation. Myositis has been described as muscle aches with CK enzyme level elevation (CK elevated but ≤10 times the ULN).[30,31] At the same time, according to National Lipid Association (NLA) myopathy includes the presence of previous symptoms plus a CK (>10 times the e limit of normal, ULN).[32]

With respect to rhabdomyolysis, the most severe myotoxic effects statin-related, the ACC characterizes this event with CK level 10 times the ULN, combined frequently with brown urine and urinary myoglobin.[30,31] NLA refers to rhabdomyolysis when CK level is over 10 000 IU/L plus an elevation in serum creatinine.[32]

Despite the previous definitions given by different medical societies, the FDA does not give a specific differentiation between myopathy, myalgia, and myositis which are defined as muscle pain, weakness, and an elevation of the plasma CK value (>10 times the ULN).[33] It refers to rhabdomyolysis when CK is 50 times the ULN (or greater than 10 000 IU/L) with acute renal failure because of myoglobin precipitation in the kidney tubules.[34]

The consequences of muscle symptoms as well as reduced drug compliance with possible discontinuation of therapy can lead to increased cardiovascular risk, impairment of the quality of life and limitation of physical activity.[27]

Variable factors may increase the risk of statin-related myopathy, both endogenous and exogenous.[25] The main endogenous factors are advanced age (75–80 years), gender (women are more predisposed), low body mass index, comorbidities (hypothyroidism, diabetes mellitus, liver disease, chronic renal failure, metabolic muscle disease, CYP450 polymorphism), vitamin D or carnitine palmityl transfer deficiency, and Asian ethnicity (Asians commonly require lower statin doses).[35–37]

Exogenous factors that have been associated with statin-related myopathy are: surgery, heavy alcohol consumption, vigorous physical exercise, drugs or foods that can interfere with the pharmacokinetics of statins.[35]

Related to that, statins with the exception of pravastatin (metabolized by sulfation), are subject to hepatic phase 1 metabolism mediated by CYP450 enzymes.[38] In particular, the CYP3A4 isoform is responsible of the biotransformation of atorvastatin, lovastatin and simvastatin; fluvastatin is metabolized via the CYP2C9 system while rosuvastatin is a CYP2C9 and CYP2C19 substrate.[38]

CYP3A4 inhibitors such as azole antifungals, anticoagulants (warfarin), immunosuppressants (cyclosporine), macrolide antibiotics, non-dihydropyridine calcium channel blockers (verapamil and diltiazem), HIV-protease inhibitors, antidepressants (nefazodone), amiodarone, excessive grapefruit juice or cranberry juice raise serum concentrations of statins increasing the risk of myopathy.[39,40]

At the same time, significant increases in the concentrations of fluvastatin and rosuvastatin have been observed following co-administration with fluconazole, amiodarone, cimetidine, fluvoxamine, trimethoprim-sulfamethoxazole and ticlopidine.[39,40]

Other drugs such as fibrates also increase the risk of myopathy by inhibiting the glucuronidation of statins.[41] While

fenofibrate is a mild inhibitor, concomitant use of a statin and gemfibrozil increases the incidence all statin-related myopathies and rhabomyolysis by 40%.[42] Gemfibrozil competes with statins for the hepatic microsomal enzymes uridine diphosphate glucuronosyl transferase (UGT)1A1 and inhibits human hepatic uptake transporter organic anion transporter 2 (OATP)2 increasing statins' plasma concentrations.[43,44]

Another factor which affects pharmacokinetics of statins is the inhibition of the multidrug-resistance protein (MRP)-2; it alters the egress of hydrophilic molecules that cause myopathy in the same way as lipophilic ones.[44]

There are multiple recommendations given by medical associations to prevent statins-related adverse events.

According to ACC/AHA, statin treatment should be particularly monitored in patients with the aforementioned risk factors. The cholesterol treatment guidelines advice to start moderate or high dose of any statin in elderly patients (>75 age), evaluating both clinical atherosclerotic cardiovascular disease (ASCVD) benefits and co-morbidities or drug interactions. For patients younger than 75 years tolerant to statins, it is not necessary to follow specific recommendations, except for pharmacokinetic factors.[30,45] Relating to CK levels, ACC/AHA envisage the measurement of its levels before starting statins treatment. If skeletal muscle symptoms appear during therapy, CK levels should be remeasured and reconciled with the parameters before treatment initiation. If CK levels exceed 10 times the baseline levels or statin myositis is suspected, statin treatment should be stopped immediately. If the CK level is less than five times ULN levels, patients should be monitored without discontinuation of therapy; if necessary statin switching or lower dosage could be evaluated until the normalization of the CK levels.[45] The European Society of Cardiology (ESC) and the European Atherosclerosis Society (EAS) recommend starting statin treatment in older people only if at least one other cardiovascular risk factor is present in addition to age. Based on CK levels, they recommend not to start treatment in those patients who have five-fold levels more at ULN.[46]

Instead, the NLA Muscle Safety Expert Panel proposed a questionnaire for patients treated with statins for monitoring symptoms.[32] If a patient has CK levels three times the ULN or above and intense myalgia, statin should be discontinued for 2–4 weeks and eventually start again at a lower dose in co-administration with another cholesterol lowering drug (colesevelam, ezetimibe).[32] Therefore, patients with a high CV risk should resume statin-therapy.

Conversely, the International Atherosclerosis Society (IAS) sustains that statin prescription to patients over 80 years of age should be reserved for those who have a cardiovascular risk of at least 15% upon the 10-year based on the Framingham risk algorithm.[47] Several algorithms for the diagnosis and management of myalgia have been created. They exclude confusing criteria of myalgia (e.g. hypothyroidism, extreme physical exercises, and vitamin D deficiency) and generally suggest to start with a low-dose or hydrophilic statin with or without ezetimibe.[47,48] Indeed, some recent studies showed that myalgia and myopathy are likely dose-related.

The Myocardial Ischemia Reduction with Aggressive Cholesterol Lowering (MIRACL) and the Pravastatin or Atorvastatin Evaluation and Infection Therapy-Thrombolysis in Myocardial Infarction 22 (PROVE IT-TIMI 22) trials compared three groups of patients, one treated with high a dose of atorvastatin, the second treated with placebo, and the third treated with low doses of drug. From the comparison of the patients results, the incidence of myopathy in the high-dose statin group is 0.6% higher than the other two groups.[49,50]

If statin treatment is not tolerated, an alternative option can be taken such as low-dosage drug, intermittent doses using long half-life statins like atorvastatin and rosuvastatin (14 and 19 h, respectively), switching to a different statin with another metabolism (e.g. from CYP450-dependent to CYP-450 independent) or solubility in monotherapy or with another hypolipidemic agent.[51,52] One last advice is to set up a therapy with nutraceuticals (e.g. rice fermented red, niacin) and CoQ10 supplementation even if data about that is still inconclusive or scarce.[53–57] However, if muscle symptoms persist even after discontinuation of statins, it is recommended to measure the CK levels and to pursue a muscle biopsy evaluating possible immunomediate necrosis that can be eventually treated with immunosuppressants.[58]

Role of ubiquitin proteasome in statin-induced myopathy

Statin-related myopathy risk factors have been identified and mechanisms proposed, but there is not yet a unified pathophysiological understanding. The mechanisms seem to be interdependent: an increased statin systemic exposure which increases skeletal muscle exposure leads to an intracellular skeletal myocyte susceptibility and perturbation of muscle function.[59] Studies suggest multiple hypotheses (Table 1). First of all, structural abnormalities appeared in the biopsy of skeletal muscle of statins-treated patients, even in asymptomatic subjects.[60] It has been postulated that statins alter energy metabolism by reducing beta-oxidation of fatty acids and by increasing the amount of intracellular lipids with vacuoles filled of lipids and fibre atrophy.[28] However, *in vitro* studies show that morphological alterations could be reduced with mevalonic acid pretreatment, assuming that this side effect is attributable to the inhibition of HMG-CoA.[61] Consequently, the depletion of intracellular cholesterol could lead to the instability of the myocyte cell membrane.

In addition to blocking cholesterol metabolism, statins also reduce products of the mevalonic pathway, thereby increasing the vulnerability of skeletal muscle cells as well as alteration of sterol metabolism.[28] Mevalonate is the precursor of

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Table 1 Proposed hypotheses on the pathogenesis of statin-induced myopathy.

Paper	Type of study	Mechanisms proposed
Guijarro *et al.*, 1998[62]	Cell line	Interfering with the isoprenylation of intracellular selenocysteine-GTP-binding proteins (i.e. Ras, Rac and Rho), which promote cell growth and attenuate the apoptosis of myofibers
Phillips *et al.*, 2002[28]	Human study (4 patients)	Altered energy metabolism by reducing beta-oxidation of fatty acids and by increasing the amount of intracellular lipids with vacuoles filled of lipids and fibre atrophy
Willoughby *et al.*, 2003[76]	Human study (9 patients)	Up-regulation of the UPS during eccentric exercise has been related to increased muscle injury, decreased muscle strength, and a decrease in myofibrillar protein
Sandri *et al.*, 2004[80]	Cell line	Blocking IGF-1 signalling promoting FoxO dephosphorylation, nuclear localization, and transcription of the atrogin-1 gene
Ludwig *et al.*, 2005[81]	Cell line	The depletion of intracellular cholesterol resulting from inhibition of HMG-CoA could lead to the instability of the myocyte cell membrane
Needham *et al.*, 2007[66]	Human study (8 patients)	Modulating the immune system by up-regulating the expression of MHC-I and the mediators of inflammation leads to muscle symptoms
Oh *et al.*, 2007[67]	Human study (133 statin-intolerant patients and 158 controls)	Genetic polymorphisms, in particular those of the coenzyme Q2 gene, involved in the biochemical activity of ubiquinone, and CYP450 related to drugs-metabolism
Hanai *et al.*, 2007[65]	Cell line	Increasing in atrophy-related genes (atrogenes) such as atrogin-1, results in enhanced degradation of skeletal muscle protein via the ubiquitin proteasome pathway
Marcoff *et al.*, 2007[64]	Systematic Review	Interfering with the isoprenylation of intracellular selenocysteine-GTP-binding proteins (i.e. Ras, Rac and Rho), which promote cell growth and attenuate the apoptosis of myofibers
Catapano *et al.*, 2012[44]	Systematic Review	Inhibition of the multidrug-resistance protein (MRP)-2; it alters the egress of hydrophilic molecules that cause myopathy
Bouitbir *et al.*, 2019[68]	Systematic Review	Statin-induced myopathy could be only the result of HMG-CoA reductase (HMGCR) gene inhibition.

prenylated isoprenoids including ubiquinone/coenzyme Q10 involved in electron transport in oxidative phosphorylation.[62] Even if low dosages of statins reduce serum levels of ubiquinone, intramuscular levels remain unaltered.[63] In particular, a study conducted in cultured rat vascular smooth muscle cells has shown that statins interfere in a dose-dependent manner with the isoprenylation of intracellular selenocysteine-GTP-binding proteins (i.e. Ras, Rac, and Rho), which promote cell growth and attenuate the apoptosis of myofibers.[62,64]

The atrophy of skeletal muscle cells could indicate the ability of statins to induce the expression of atrogin-1, which is responsible for the destruction of some muscle proteins. In other studies, statin-induced myopathy appeared to be immune-mediated.[65] In fact, in some patients, statins are able to modulate the immune system by upregulating the expression of MHC-I and some inflammatory mediators.[66] This is also supported by the fact that the muscle symptoms have been reduced in patients treated with immunosuppressants.

It has also been suggested that statin-related myopathic effects are related to genetic polymorphisms, in particular those of the coenzyme Q2 gene, involved in the biochemical activity of ubiquinone, and CYP450 related to drugs-metabolism.[67]

It is not definitively clear if the effect of statin treatment is a consequence of HMG-CoA reductase inhibition or another nonspecific drug effect. Fish knockdown of the HMG-CoA reductase (*HMGCR*) gene showed a muscle response similar to that seen in other studies, strongly questioning that statin-induced myopathy could be only the result of HMG-CoA reductase inhibition.[68]

Nevertheless, some studies have documented a controversial role of UPS inhibition in particular clinical conditions, such as Alzheimer disease.[69]

The impairment of the UPS has been indicated as one possible mechanism for muscle wasting and atrophy[70–75] as well as statin-induced muscle myopathy during exercise. Up-regulation of the UPS during eccentric exercise has been related to increased muscle injury, decreased muscle strength, and a decrease in myofibrillar protein[76] (*Figure* 2). Urso *et al*. investigated the role of ubiquitin proteasome pathway gene expression in skeletal muscle after exercise and statin use. In this study, individuals did eccentric exercises with one leg before and after placebo or high-dose atorvastatin (80 mg/d) treatment. The unexercised leg was used as a control. Among subjects taking statin treatment alone, only five genes were differentially expressed in comparison to the unexercised leg or the exercised leg of the placebo group, showing that statins do not impact significantly on skeletal muscle gene expression. On the other hand, eccentric exercise alone induced 80 genes that were differentially expressed compared with the non-exercised leg of the placebo group. Authors concluded that eccentric exercise along with statin treatment had the most significant effect on transcription factors and genes involved in the ubiquitin proteasome pathway when compared with eccentric exercise or statin use alone. This study showed that the statin-induced myopathy could be

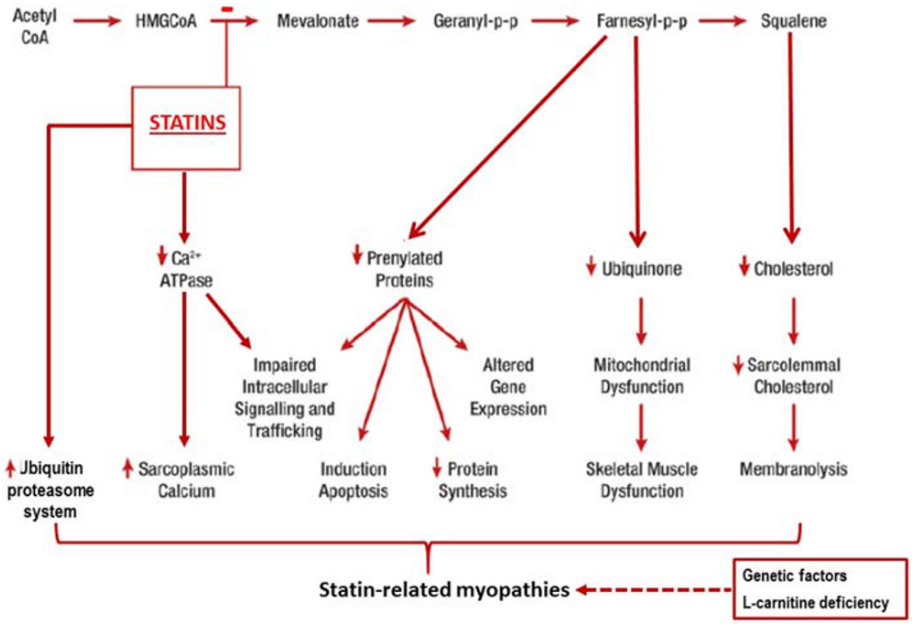

FIGURE 2 Potential mechanisms implicated in the pathophysiology of statin-associated myopathies.

explained by alterations of the expression of different genes because of the combination of statins and exercise.[77]

The relationship between statins and UPS has also been investigated in studies exploring the role of atrogin-1 that is a ubiquitin protein ligase significantly induced by any stimulus that promote muscle atrophy and that may be a key effector in muscle degradation during catabolic states.[78]

Authors hypothesized that statin myotoxicity could be similar to the atrophic response seen in other muscle-wasting diseases.[79] Increases in atrophy-related genes (atrogenes) such as *atrogin-1*, results in enhanced degradation of skeletal muscle protein via the ubiquitin proteasome pathway.[64]

Statin treatment blocks IGF-1 signalling promoting FoxO dephosphorylation, nuclear localization, and transcription of the atrogin-1 gene.[80] Authors demonstrated that the lack of geranylgeranyl induced by statin inhibition of HMGCoA reductase is responsible for at least part of this toxicity. They showed that geranylgeranyl precursors introduction in both muscle cell cultures and in zebrafish embryos treated with lovastatin blocks expression of atrogin-1 and avoids myotoxicity. On the contrary, blocking the enzymes that couple geranylgeranyl precursors onto intracellular proteins produces the same effects observed for lovastatin in terms of cell morphology and atrogin-1 expression. These results suggest that statins may exert their toxic effects in muscle by inhibiting the function of a geranylgeranyl-conjugated protein that directly or indirectly results in atrogin-1 expression.[81]

Recent data show that L-carnitine ((3R)-3-hydroxy-4-(trimethylazaniumyl)butanoate), an endogenous transporter of fatty acids into the mitochondrial matrix, biosynthesized within the human body from L-lysine and S-adenosyl-methionine, could partially counteract the UPS negative effects.[82] Carnitine plays a key role in importing acetyl-CoA into mitochondria. Excess mitochondrial acetyl-CoA is produced when an oversupply of fuel (predominantly glucose or fatty acids) enters the cell and mitochondrial oxidative capacity is saturated. The elevated concentration of acetyl-CoA may facilitate acetylation of lysine residues on mitochondrial enzymes, modulating their activity. Enzyme acetylation may inhibit fuel utilization when energy is in excess, favouring lipogenesis.[83] However, preclinical models suggest that L-carnitine supplementation could down-regulate UPS genes in skeletal muscle.[84] In particular, supplementation of carnitine markedly decreases the expression of MuRF1 and concentrations of ubiquitinated proteins in skeletal muscle of rats, indicating a diminished degradation of myofibrillar proteins by the UPS.[85] This effect could be mediated by activation of the IGF-1/PI3K/Akt signalling pathway which in turn might contribute to the observed down-regulation of MuRF1 and muscle protein ubiquitination.[85] L-carnitine supplementation seems to have a protective effect against soleus muscle atrophy caused by hind limb suspension and decreased E3 ligase messenger RNA expression, suggesting the possibility that L-carnitine protects against muscle atrophy, at least in part, through UPS inhibition.[86] These results could be of particular interest, because L-carnitine could exert some anti-inflammatory activity in humans, reducing the serum levels of some cytokines (Interleukin 6, tumour necrosis factor alpha, C-reactive protein) often increased in myopathies.[87] On the other side, some papers raise doubts about the usefulness of carnitine in some clinical conditions.[88,89]

The role of carnitine in cancer is also not well established. Recent findings have suggested that carnitine system could be involved in the metabolic flexibility of cancer cells.[90] On

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the contrary, the UPS down-regulation and autophagy activation trigger apoptosis in cancer cells.[91]

In addition, Vitamin D supplementation could exert some positive effects on prevention of muscle mass loss preventing muscle mass losses.[92,93] At the same time, essential aminoacids have also documented positive effects on preclinical models of statin-induced myopathy.[94,95] Other studies have shown that statin-induced creatine impairment could play a role in statin myopathy, suggesting creatine supplementation as a potential approach to prevent muscle-related adverse effects of statins and improve statin tolerance.[96]

Conclusions

In summary, it has been hypothesized that the introduction of statins into the myocyte cell membrane may provoke instability when subjected to eccentric exercise stress, provoking activation of intracellular proteolytic cascades and changes in protein degradative machinery. This is consistent with the up-regulation of a series of genes implicated in protein catabolism, in addition to those of the UPS. However, since there is a lack of *in vivo* studies, further research should be performed to elucidate the impact of statins on protein catabolism inducing myotoxicity.

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Conflict of interest

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