

# Effects of statins on mitochondrial pathways

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## Abstract

Statins are a family of drugs that are used for treating hyperlipidaemia with a recognized capacity to prevent cardiovascular disease events. They inhibit  $\beta$ -hydroxy  $\beta$ -methylglutaryl-coenzyme A reductase, i.e. the rate-limiting enzyme in mevalonate pathway, reduce endogenous cholesterol synthesis, and increase low-density lipoprotein clearance by promoting low-density lipoprotein receptor expression mainly in the hepatocytes. Statins have pleiotropic effects including stabilization of atherosclerotic plaques, immunomodulation, anti-inflammatory properties, improvement of endothelial function, antioxidant, and anti-thrombotic action. Despite all beneficial effects, statins may elicit adverse reactions such as myopathy. Studies have shown that mitochondria play an important role in statin-induced myopathies. In this review, we aim to report the mechanisms of action of statins on mitochondrial function. Results have shown that statins have several effects on mitochondria including reduction of coenzyme Q10 level, inhibition of respiratory chain complexes, induction of mitochondrial apoptosis, dysregulation of  $\text{Ca}^{2+}$  metabolism, and carnitine palmitoyltransferase-2 expression. The use of statins has been associated with the onset of additional pathological conditions like diabetes and dementia as a result of interference with mitochondrial pathways by various mechanisms, such as reduction in mitochondrial oxidative phosphorylation, increase in oxidative stress, decrease in uncoupling protein 3 concentration, and interference in amyloid- $\beta$  metabolism.

Overall, data reported in this review suggest that statins may have major effects on mitochondrial function, and some of their adverse effects might be mediated through mitochondrial pathways.

**Keywords** Statin; Myopathy; SAMS; Mitochondria; Apoptosis; Coenzyme Q10; Respiratory chain; Diabetes; Cognitive impairment

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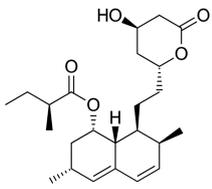
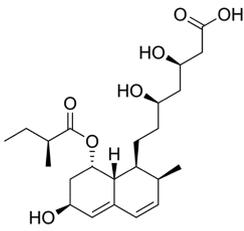
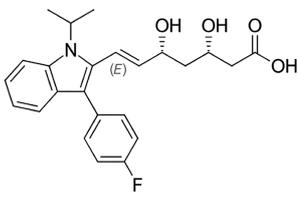
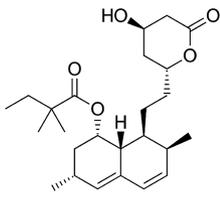
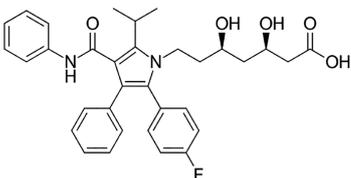
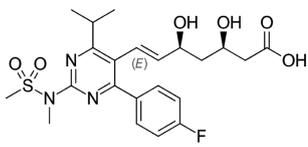
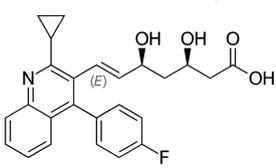
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## Introduction

Cardiovascular diseases (CVDs), such as myocardial infarction, angina, heart failure, and cerebrovascular accidents, contribute significantly to global mortality. World Health Organization estimated 17.9 million deaths in 2017 due to CVDs,

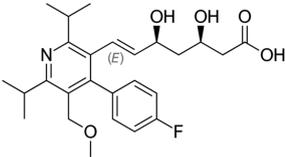
representing 31% of the whole global mortality.<sup>1</sup> The systematic analysis for the Global Burden of Disease Study estimates that CVD-associated mortality will raise to 23 million deaths per year by 2030.<sup>2</sup> Among the major risk factors, CVDs are associated with high plasma total cholesterol, low-density lipoprotein (LDL) cholesterol, and reduced high-density

**Table 1** Statins and their structures and classification

| Statin       | Generation | Structure  | Hydrophilicity/lipophilicity |
|--------------|------------|--|------------------------------|
| Lovastatin   | I          |     | Hydrophilic                  |
| Pravastatin  | I          |     | Lipophilic                   |
| Fluvastatin  | I          |    | Hydrophilic                  |
| Simvastatin  | II         |    | Hydrophilic                  |
| Atorvastatin | II         |  | Hydrophilic                  |
| Rosuvastatin | III        |  | Lipophilic                   |
| Pitavastatin | III        |  | Hydrophilic                  |

*(Continues)*

Table 1 (continued)

| Statin       | Generation | Structure  | Hydrophilicity/lipophilicity |
|--------------|------------|--|------------------------------|
| Cerivastatin | Withdrawn  |  | Lipophilic                   |

lipoprotein cholesterol. CVD risk is reduced by ~22% per each 1 mmol/L LDL cholesterol decrease.<sup>3</sup> There is overwhelming evidence showing an increasing clinical benefit when greater and earlier LDL cholesterol reductions are achieved.<sup>4</sup> Apo B-containing lipoproteins (like LDLs), which are the key sources of cholesterol, are responsible for initiation and progression of atherogenesis.<sup>5</sup> Therefore, one of the main goals of CVD prevention is LDL cholesterol level reduction.

$\beta$ -Hydroxy  $\beta$ -methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors, better known as statins, are among the most effective lipid-lowering agents.<sup>6</sup> Besides lifestyle approaches like diet and exercise interventions,<sup>7</sup> additional cholesterol-lowering therapies include nutraceuticals,<sup>8–10</sup> ezetimibe, proprotein convertase subtilisin/kexin type 9 inhibitors, lomitapide, and mipomersen.<sup>11,12</sup>

In 1976, Akira Endo isolated from *Penicillium citrinum*, a molecule (mevastatin) able to inhibit HMG-CoA reductase, a crucial enzyme in endogenous cholesterol biosynthesis.<sup>13</sup> Statins are reversible and competitive HMG-CoA reductase inhibitors and determine a reduction in serum and tissue levels of total cholesterol, LDL cholesterol, apo B, and triglycerides. Statins are prescribed in order to reduce plasma cholesterol levels both in primary and secondary prevention of CVD.

Because HMG-CoA is a key enzyme in the mevalonate pathway, its inhibition results in a reduced bioavailability of farnesyl pyrophosphate, geranylgeranyl pyrophosphate, heme A, coenzyme Q10 (CoQ10; also known as ubiquinone), and other metabolites that play essential roles in cellular physiology.<sup>14,15</sup> Moreover, cholesterol is not only a final product of this biochemical pathway but also itself an intermediate for many other compounds, such as corticosteroids, bile acids, and vitamin D.<sup>15</sup> This explains why statins pharmacodynamically exert pleiotropic effects,<sup>16–21</sup> which are responsible for both beneficial and adverse consequences during treatment. Apart from their cholesterol-lowering effect, statins are known to contribute to stabilization of atherosclerotic plaques,<sup>22</sup> improvement of endothelial dysfunction,<sup>23</sup> and immunomodulation and show anti-inflammatory,<sup>24</sup> antioxidant, and anti-thrombotic effects.<sup>25</sup> Lipid-dependent and lipid-independent effects of statins may vary according to the potency,<sup>6</sup> lipophilicity (Table 1), and pharmacokinetic properties<sup>14,26–28</sup> of different agents.

Statins have been proved to be safe drugs. However, they may elicit adverse reactions (muscle, metabolic, liver, and neurological side effects), being muscle adverse effects the most frequently reported.<sup>29</sup> These adverse reactions are commonly named as statin-associated symptoms (SAS).<sup>29</sup> It is important to underline that the causative relationship between statins and SAS is not always clearly demonstrable: some patients complaining of SAS can tolerate a low statin dose, and some non-specific side effects have been reported by patients treated with placebo.<sup>29</sup> Most of SAS appear to be dose dependent; half of the patients on high statin dosages stop the drug within 1 year because of SAS.<sup>30–32</sup> The risk of drug–drug interaction increases alongside with age and number of administered drugs.<sup>33</sup> Other risk factors for statin intolerance are frailty, surgery, infection, female sex, exertion, hypothyroidism, chronic kidney disease, and genetic predisposition.<sup>15,34</sup>

Statins may interfere with mitochondrial activity both via direct mechanisms, i.e. impairment of electron transport chain (ETC) complexes, and via indirect ones, i.e. as a consequence of mevalonate pathway metabolites depletion, e.g. CoQ10 and isoprenoids. Growing evidence is being provided showing that SAS are mostly due not to their direct cholesterol-lowering effect but rather to the induced impairment on mitochondrial function, as supported by histopathological, laboratory, and molecular findings in cell lines, rat models, and humans.<sup>34,35</sup> A better understanding of the pathogenic background of SAS will be fundamental for quickly identifying patients who are at major risk and acquire best results of treatment, so learning about their mechanisms, progression, and treatment is essential for better counteracting adverse reactions of statins responsible for therapy discontinuation.

## Aims and methods

The aim of this review was to provide an overview of achieved knowledge about mechanisms that underlie SAS, focusing on muscle effects, statin-induced diabetes, and cognitive impairment. In particular, we focused on statin-driven

molecular mechanisms interfering both directly and indirectly with mitochondrial function.

This review is based on evidence gathered performing a PubMed query by using 'statin AND mitochondria AND adverse reaction' as search terms. The search strategy was implemented by hand searching the references reported by the most relevant studies on this topic.

## Statin-associated muscle symptoms

Among all SAS, muscle side effects are the most frequently reported.<sup>29,36,37</sup> About 7–29% of patients complain of statin-associated muscle symptoms (SAMS).<sup>38</sup> Symptoms usually occur after 4–6 weeks of treatment initiation but sometimes after many years.<sup>39</sup> Weakness, pain, and muscle fatigue are responsible for 30–62% statin treatment discontinuation.<sup>40</sup> SAMS present as a wide range of clinical manifestations, such as myalgia, weakness, tendon pain, night muscle cramping, and rhabdomyolysis in most severe cases.<sup>41,42</sup> Weakness and pain usually involve large back muscles and proximal muscle groups symmetrically.<sup>43</sup> Underlying these manifestations is a statin-induced myopathy (SIM), whose pathophysiology has not been fully understood yet.<sup>44</sup> Further, the lack of reliable laboratory biomarkers for diagnosing myopathy complicates its clinical identification. The most used laboratory test is creatine kinase (CK) blood concentration, which however suffers from low sensibility and specificity.<sup>45</sup>

Up to now, there is no universally established terminology used to describe SIM. The National Lipid Association describes myopathy as muscle pain, soreness, weakness, and cramps together with an at least 10-fold increase in CK levels above the upper limit of normal (ULN).<sup>46</sup> European Atherosclerosis Society (EAS) defines myopathy as the presence of muscle symptoms accompanied by CK over 10-fold the ULN.<sup>38</sup> According to the American College of Cardiology/American Heart Association/National Heart, Lung, and Blood Institute, myopathy can be identified by either myalgia (without CK elevation), myositis (CK elevation within 10-fold the ULN), or blunt rhabdomyolysis (CK elevation over 10-fold the ULN, increase in creatinemia, and reddish or brown urine).<sup>47</sup> On the contrary, rhabdomyolysis is defined by the Food and Drug Administration (FDA) as a condition characterized by an increase in CK blood concentration over 50-fold the ULN associated with myoglobin-induced acute renal injury.<sup>48</sup> Conversely, according to EAS, CK over 40-fold the ULN associated with renal impairment and/or myoglobinuria configures the presence of rhabdomyolysis.<sup>38</sup> Importantly, albeit rhabdomyolysis is the most severe statin-associated side effect, it is a rare complication occurring in about 0.01% of treated patients.<sup>49</sup> Myoglobinuria, deriving from muscle necrosis and the release of myoglobin in the blood circulation,

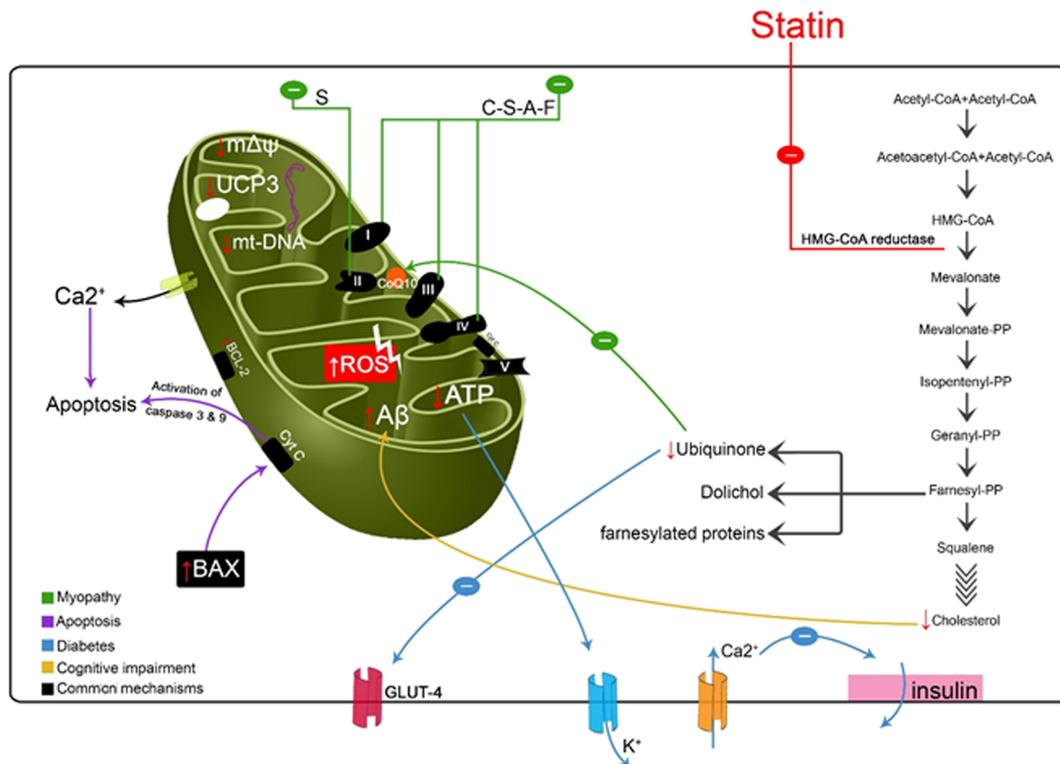
can cause mechanical obstruction and acute necrosis of the renal tubules and subsequently acute renal failure, a potentially life-threatening complication.<sup>50</sup>

Statin-associated muscle symptom definition refers to any muscle symptom related to statin treatment. In this frame, the EAS Consensus suggested three criteria for the diagnosis of SAMS: correspondence between CK levels increase and onset of statin treatment; decline of symptoms at treatment withdrawal; and symptoms recurrence at statin treatment rechallenging.<sup>38</sup> However, it must be underlined that SAMS might occur in patients who do not show increased CK blood concentrations.<sup>29</sup>

## Mitochondria

Mitochondria are organelles often referred to as the power station of eukaryotic cells, because several phases of cellular respiration take place there, such as the Krebs cycle, the oxidative phosphorylation, and fatty acid  $\beta$ -oxidation.<sup>51</sup> Oxidative phosphorylation is a key biochemical pathway in cellular respiration and is carried out by several protein complexes and molecules that are located at the inner mitochondrial membrane (IMM). It consists of two subsequent steps, the ETC and ATP synthesis. ATP synthesis consists of phosphorylation of ADP to ATP catalysed by ATP synthase, thanks to the proton motive force following the electrochemical gradient across the IMM. This electrochemical gradient is sustained by the proton translocation from mitochondrial matrix to intermembrane space (IMS) mediated by complexes I, III, and IV of the ETC.<sup>52</sup>

Complex I (NADH : ubiquinone oxidoreductase) is responsible for reduction of NADH yielded during glycolysis and Krebs cycle. While transferring electrons from NADH, thereby reducing ubiquinone to ubiquinol, it mediates transport of four protons ( $H^+$ ) from mitochondrial matrix to the IMS. Complex II (succinate : ubiquinone oxidoreductase) is part of the Krebs cycle. It catalyses succinate oxidation to fumarate while reducing ubiquinone to ubiquinol. Different to other complexes, complex II does not transport protons across the membrane. Complex III (cytochrome  $bc_1$ ) catalyses reoxidation of ubiquinol (moving freely along the IMM and yielded by complex I and II enzymatic activities) to ubiquinone, transferring electrons to the membrane-attached cytochrome  $c$  and four protons from matrix to IMS. Eventually, cytochrome  $c$  is again oxidized by complex IV (cytochrome  $c$  oxidase) that yields  $H_2O$  from transferring electrons to  $O_2$  and transfers four protons against gradient across the IMS.<sup>52</sup> The electron transport system (ETS) is thought to be an important source of reactive oxygen species (ROS). As a consequence of many redox reactions, mitochondria yield an important amount of ROS, which can act either as cell signalling molecules or as oxidative stress factors<sup>52–54</sup> (Figure 1).



**Figure 1** Effects of statins on mitochondrial function: (i) reduction in mitochondrial membrane potential; (ii) reduction in coenzyme Q10 (CoQ10) and GLUT-4 expression; (iii) increased reactive oxygen species (ROS) level and induction of intrinsic apoptosis; (iv) deregulation of Ca<sup>2+</sup> metabolism; (v) mitochondrial depletion; (vi) decrease in uncoupling protein 3 (UCP3) expression and reduction in β-oxidation efficiency; (vii) increase in amyloid-β (Aβ) concentration in mitochondria; (viii) direct inhibition in respiratory chain. A, atorvastatin; C, cerivastatin; F, fluvastatin; HMG-CoA, β-hydroxy β-methylglutaryl-coenzyme A; mtDNA, mitochondrial DNA; PP, pyrophosphate; S, simvastatin.

## Statin-associated muscle symptoms and mitochondrial dysfunction

Mitochondrial dysfunction is believed to play a pivotal role in the pathogenesis of SAMS, because skeletal muscles are avid energy consumers and closely depend on mitochondrial activity.<sup>55</sup> This is supported by histopathological findings, evidencing how SIM is characterized by lipid aggregation in mitochondria and ragged red fibres accumulating in subsarcolemmal region visible with Gömöri staining.<sup>56,57</sup> Abnormal high blood lactate/pyruvate ratio<sup>58</sup> and increased mean metabolic recovery time as assessed by <sup>31</sup>P magnetic resonance spectroscopy<sup>59</sup> in statin-treated patients when compared with untreated hypercholesterolaemic and healthy controls add evidence to this hypothesis. However, neither the LIFESTAT study nor 8 week treatment with simvastatin 80 mg/day on healthy subjects proved any difference in mitochondrial function after statin treatment.<sup>61,62</sup> According to the LIFESTAT study, 20 healthy middle-aged men were treated with 80 mg/day simvastatin (*n* = 10) or 40 mg/day pravastatin (*n* = 10). After 14 days, 50% of participants presented a different range of side effects from soreness, myalgia, to stomach ache, but no changes in mitochondrial

function with respect to respiratory chain capacity, mitochondrial density, or Q10 content, except a reduction in complex IV activity in both groups.<sup>61</sup> Hou et al. found the presence of mitochondrial dysfunction and abnormal respiratory chain enzyme activity in 24% and 10%, respectively, among 279 biopsies from patients with SIM. Hence, whether mitochondrial dysfunction may be considered a frequent pathogenic hallmark of SAMS is still debated.<sup>63</sup>

In the following sections, the proposed pathogenetic mechanisms of SAMS involving mitochondrial dysfunction are described (Table 2).

### Depletion of coenzyme Q10

Coenzyme Q10 (alias ubiquinone) is a hexameric quinone ring containing a 10-isoprenyl unit side chain; it is structurally similar to vitamin K and can be found in the hydrophobic layer of all cell membranes in mammals. Ubiquinone is an end product of the mevalonate pathway but can be supplied by diet being mostly present in some meats like beef, pork, and chicken<sup>64,65</sup> and by dietary supplements/nutraceuticals.<sup>66</sup> Because of its chemical structure, CoQ10 presents a high antioxidant activity. The highest tissue

**Table 2** Overview on statin mechanisms of mitochondrial toxicity

| Type of statin                | Mechanism  | Adverse effect                                      | Reference  |
|-------------------------------|--|---|--|
| Atorvastatin                  | Inhibition of complexes I, III, and IV               | Myopathy  | Salviati et al. <sup>68</sup>  |
|                               | Dysregulation of calcium homeostasis                 | Myopathy  | Liantonio et al. <sup>60</sup>   |
|                               | Reduction in CoQ10 concentration                     | Myopathy  | Saha and Whayne <sup>69</sup>  |
|                               | mtDNA depletion                                      | Myopathy  | Saha and Whayne <sup>69</sup>  |
|                               | PGC-1/Akt pathway disruption                         | Myopathy  | Mullen et al. <sup>83</sup>  |
|                               | ROS accumulation and oxidative stress                | Induced apoptosis                                   | Dirks and Jones <sup>104</sup>   |
|                               | Akt pathway disruption                               | Induced apoptosis                                   | Mikus et al. <sup>81</sup>   |
| Unknown                       | Impaired insulin secretion                           | Diabetes  | Shepherd et al., Sattar et al., and Sadighara et al. <sup>130,132,133</sup>                                    |
|                               | Unknown  | Cognitive impairment                                | Ott et al. and Swiger et al. <sup>153,154</sup>  |
| Fluvastatin                   | Inhibition of complexes I, III, and IV               | Myopathy  | Salviati et al. <sup>68</sup>  |
| Lovastatin                    | Dysregulation of calcium homeostasis                 | Myopathy  | Liantonio <sup>60</sup>  |
|                               | CPT-2 inhibition                                     | Myopathy  | Ghatak et al. <sup>123</sup>   |
| Pravastatin                   | Reduced activity of complex IV                       | Myopathy  | Sirvent et al. <sup>91</sup>   |
|                               | Impaired insulin secretion                           | Diabetes  | Shepherd et al. <sup>130</sup>   |
| Rosuvastatin                  | Inhibition of complex IV                             | Myopathy  | Phillips et al. <sup>57</sup>  |
| Simvastatin                   | Akt pathway disruption                               | Induced apoptosis                                   | Mikus et al. <sup>81</sup>   |
| Simvastatin                   | Impaired ETC function                                | Myopathy  | Salviati et al., Saha and Whayne, Vaughan et al., Kwak et al., and Sirvent et al. <sup>68,69,83,85,88,91</sup> |
|                               | Reduction in CoQ10 muscle concentration              | Myopathy  | Saha and Whayne and Sirvent et al. <sup>69,91</sup>  |
|                               | mtDNA depletion or reduced citrate synthase activity | Myopathy  | Saha and Whayne, Lamperti et al., Banach et al., Qu et al., and Schick et al. <sup>69,76–79</sup>              |
|                               | PGC-1/Akt pathway disruption                         | Myopathy  | Åberg et al., Stringer et al., and Singla et al. <sup>67,80,82</sup>   |
|                               | Dysregulation of calcium homeostasis                 | Myopathy  | De Pinieux et al., Murlasists and Radák, and Heringhaus et al. <sup>58,95,96</sup>                             |
|                               | Inhibition of complex IV                             | Myopathy  | Phillips et al. <sup>57</sup>  |
|                               | Calpain activation                                   | Induced apoptosis                                   | Hermann et al. <sup>103</sup>  |
|                               | ROS accumulation and oxidative stress                | Induced apoptosis                                   | Vaughan et al. <sup>85</sup>   |
|                               | Isoprenoid depletion                                 | Induced apoptosis                                   | Novelli and D'Apice and Guijarro et al. <sup>109,110</sup>   |
|                               | Akt pathway disruption                               | Induced apoptosis                                   | Stringer et al. and Mikus et al. <sup>80,81</sup>  |
| Peripheral insulin resistance | Diabetes   | Sadighara et al. and Moro et al. <sup>136,140</sup> |  |
| Unknown                       | Cognitive impairment                                 | Ott et al. <sup>153</sup>                           |  |
| Cerivastatin                  | Inhibition of complexes I, III, and IV               | Myopathy  | Salviati et al. <sup>68</sup>  |
|                               | Reduced activity of complex IV                       | Myopathy  | Brookes <sup>53</sup>  |
|                               | Isoprenoid depletion                                 | Induced apoptosis                                   | Hosseinzadeh et al. <sup>108</sup>   |

CoQ10, coenzyme Q10; CPT-2, carnitine palmitoyltransferase-2; ETC, electron transport chain; mtDNA, mitochondrial DNA; PGC-1, peroxisome proliferator-activated receptor gamma coactivator 1; ROS, reactive oxygen species.

concentrations of CoQ10 can be found in the kidney, liver, and heart.<sup>67</sup> As previously described, CoQ10 is an essential component of the ETC and therefore plays a pivotal role in cellular energy yield. Primary CoQ10 deficiency has been detected in several diseases comprising heart failure, nephrotic syndrome, and muscular and neurological disorders.<sup>68,69</sup> In addition, secondary CoQ10 deficiency may occur. In this regard, the inhibitory effect of statins on the mevalonate pathway (Figure 1) may reduce CoQ10 levels by 16–54%<sup>64</sup> and cause mitochondrial dysfunction, revealed by inhibition of mitochondrial ETC complexes,<sup>70,71</sup> disruption of mitochondrial membrane potential, decrease in mitochondrial DNA (mtDNA) copy number, interference with oxidative phosphorylation, mitochondrial swelling, and release of cytochrome c.<sup>71,72</sup> However, decrease in plasma CoQ10 level is mostly due to the reduction of circulating lipoproteins, because around 74% of CoQ10 is carried in the blood by apo B-containing lipoproteins.<sup>64</sup> Accordingly, no significant

difference in CoQ10 to total cholesterol ratio was found before and after statin treatment.

Scarce and contradictory data are available about the impact of statin treatment on skeletal muscle CoQ10 concentration.<sup>73</sup> In a randomized controlled trial, 8 weeks of treatment with simvastatin 80 mg/day but not the treatment with atorvastatin 40 mg/day or placebo was associated with a significant decrease in muscle ubiquinone concentration.<sup>72</sup> Analyses from a subgroup of patients showed that those with markedly reduced muscle CoQ10 had concurrent reduction in respiratory chain enzyme and citrate synthase activities.<sup>72</sup> However, conflicting data showing either increase or no change in muscle ubiquinone concentrations after both short-term and long-term statin treatments have been provided.<sup>74–76</sup>

Recent meta-analyses on the topic have led to controversial results about the effectiveness of CoQ10 supplementation in statin-treated patients in improving SIM and SAMS.<sup>77,78</sup>

### Mitochondrial depletion and oxidative stress

A significant reduction in mtDNA/nuclear DNA ratio was observed after 8 weeks of 80 mg/day simvastatin but not after 8 weeks of 40 mg/day atorvastatin.<sup>79</sup> The findings of this randomized controlled trial suggested a major impact of simvastatin on mitochondrial depletion due to its lipophilicity. Notably, changes in mtDNA/nuclear DNA ratio were irrespective of muscle symptoms but strongly associated with muscle CoQ10 concentration.<sup>79</sup> These results were replicated in a small cohort of patients experiencing SIM.<sup>80</sup>

Exercise training can enhance mitochondrial function by increasing oxygen uptake by ~10% and citrate synthase activity, a marker of skeletal muscle mitochondrial content, by 13% in overweight or obese patients after 12 week exercise training.<sup>81</sup> The addition of 40 mg/day simvastatin led to a lower increment of oxygen uptake during exercise training (1.5%) and a 4.5% reduction in citrate synthase activity.<sup>81</sup> Similar results (3.6% reduction in citrate synthase activity and cardiorespiratory fitness impairment) have been reported in a cohort of patients with type 2 diabetes mellitus (T2DM) treated with 40 mg/day simvastatin for 12 weeks.<sup>82</sup> Intriguingly, vitamin D supplementation resulted effective in preventing statin-related decrease in mitochondrial content and impairment in cardiorespiratory fitness.<sup>82</sup>

Statin-induced toxicity could be due to the reduced phosphorylation of protein kinase B (Akt) and subsequent disruption of its pathway, which plays an important role in the mitochondrial health.<sup>83,84</sup> The Akt pathway disruption is probably caused by decreased transcription and translation rates of peroxisome proliferator-activated receptor gamma coactivator (PGC)-1 $\alpha$  and PGC-1 $\beta$ . In fact, these transcriptional co-factors act as activators of the Akt pathway and are considered master inductors of mitochondrial biogenesis.<sup>85</sup> Different cell lines both *in vitro* and *in vivo* have shown to be differentially susceptible to the disruption of the Akt pathway. Primary mouse skeletal muscle myocytes, C2C12 myotubes,<sup>83</sup> human rhabdomyosarcoma cells,<sup>85</sup> and skeletal muscle cells from deltoid biopsies<sup>70</sup> were susceptible to statin treatment in terms of mitochondrial dysfunction, as assessed by several parameters, including PGC-1 $\alpha$  mRNA transcription and mitochondrial oxidative capacity. Interestingly, mitochondrial dysfunction could be rescued either by administration of ubiquinol,<sup>85</sup> exogenous antioxidant agents, or by PGC-1 $\alpha$  overexpression.<sup>70</sup> In similar studies done on various models such as murine liver HepG2 cells,<sup>83</sup> oxidative muscle fibres,<sup>86</sup> and human cardiac muscle fibres,<sup>70</sup> statins showed mitochondrial dysfunctions. The production of ROS and oxidative stress could be crucial hallmarks of statin-induced toxicity and have a role in regulating mitochondrial biogenesis.<sup>87,88</sup> In cells with high-efficient antioxidant systems, as cardiac myofibres, statin-induced ROS accumulation is limited and stimulates PGC-1 $\alpha$  activity, thus

promoting mitochondrial biogenesis and improving mitochondrial function. On the other hand, in cells with lower antioxidant capacity, such as fast glycolytic muscle fibres, a greater ROS increase could be responsible for the Akt pathway disruption.<sup>70,86</sup> The differential response in terms of mitochondrial biogenesis according to ROS production is known as mitochondrial hormesis and can explain why some tissues and cells, in particular fast muscle fibres, are more susceptible to statin toxicity.<sup>70</sup> It is notable that PGC-1 $\beta$  knockout mice showed mitochondrial dysfunction, increased ROS production, and induced apoptosis after oral administration of 5 mg/kg/day atorvastatin per 2 weeks both in glycolytic and oxidative muscle fibres.<sup>86</sup>

### Inhibition of electron transport chain complexes

Direct inhibition of ETC complexes has been proposed as a responsible mechanism for SIM.<sup>63,88–90</sup> *In vitro*, functional impairment of complexes I, III, and IV occurred in L6 rat skeletal muscle cells after a 24 hour exposure to 100  $\mu$ mol/L cerivastatin, simvastatin, fluvastatin, or atorvastatin. On the contrary, no functional impairment was reported after exposure up to 1 mmol/l pravastatin.<sup>71</sup> These findings were confirmed in human myocytes, onto which simvastatin acts mostly as an inhibitor of complex I.<sup>91</sup> However, assays on fresh and frozen muscle samples from rats treated with different statin regimens failed to identify abnormalities in ETC complexes activity.<sup>92,93</sup> A randomized controlled trial showed significant reduction in all ETC complexes activity in patients treated with simvastatin 80 mg/day for 8 weeks but not in those treated with atorvastatin 40 mg/day or placebo.<sup>72</sup> However, a systematic review of several studies on cell cultures, animal models, and humans showed highly contradicting results on this topic.<sup>34</sup>

In two patients affected by statin-induced rhabdomyolysis, skeletal muscle biopsies showed reductions in ubiquinone concentration and in complex IV activity.<sup>94</sup> A significant reduction by 60% of complex IV activity was identified in a patient presenting myoglobinuria after treatment with cerivastatin and gemfibrozil.<sup>56</sup>

Statins are known to interfere with beneficial exercise training adaptations, which mostly rely on improvements of the mitochondrial function,<sup>81,82,95</sup> and could be due to ETC complexes activity impairment.<sup>81</sup> Finally, it has been suggested that impaired ETC function could be found out in other tissues as well, though data on these aspects are still very scarce.<sup>96</sup>

### Dysregulation of Ca<sup>2+</sup> metabolism

As a consequence of mitochondrial dysfunction, studies on both animal and human models have identified an increased

Ca<sup>2+</sup> efflux from the sarcoplasmic reticulum, leading to abnormalities in excitation–contraction coupling in skeletal muscle cells.<sup>97</sup> Sirvent *et al.* hypothesized that ETC disruption (and, above all, complex I inhibition) due to statin treatment could be responsible both *in vitro* and *in vivo* for calcium metabolism deregulation.<sup>98</sup> Alterations in spontaneous Ca<sup>2+</sup> sparks (e.g. frequency and amplitude) in muscle cells indicate impaired Ca<sup>2+</sup> homeostasis.<sup>99</sup> Decreased frequency of spontaneous Ca<sup>2+</sup> sparks was found in patients treated with statin and complaining muscle symptoms but not in asymptomatic statin-treated patients when compared with healthy controls.<sup>98</sup> Increased Ca<sup>2+</sup> spark amplitude was found in all statin-treated patients when compared with controls<sup>98</sup> and in individuals treated with 80 mg/day simvastatin for 8 weeks displaying increased serum CK concentrations.<sup>62</sup> Statins have been demonstrated both *in vivo* and *in vitro* in animal and human models to raise cytosolic and sarcoplasmic Ca<sup>2+</sup> concentration: this increase does not seem to be attributable to abnormal sarcolemma permeability but rather to a primary Ca<sup>2+</sup> efflux from mitochondria mediated by mitochondrial permeability transition pore and Na<sup>+</sup>–Ca<sup>2+</sup> exchanger.<sup>99</sup> The latter secondarily leads to Ca<sup>2+</sup> release from the sarcoplasmic reticulum mediated by ryanodine receptors.<sup>60,70,96</sup> The abnormal opening of mitochondrial permeability transition pore and Na<sup>+</sup>–Ca<sup>2+</sup> exchanger may be caused by the decrease of mitochondrial membrane potential and depolarization of the IMM as a consequence of disrupted ETC activity.<sup>99</sup>

### Lactone toxicity

Statins are available in two different forms: hydroxy acid (active molecule) and the lactone (inactive prodrug). Interconversion through these two forms occurs spontaneously according to environmental pH or can be catalysed by enzymes, such as glucuronosyltransferases.<sup>100</sup> Lactone forms are more lipophilic and therefore more capable of crossing cell membranes and exerting their pharmacological activity.<sup>100</sup> Lactone forms have been described as more myotoxic both in rat and human cells *in vitro*.<sup>101,102</sup> Individuals affected by SIM showed higher circulating levels of lactone metabolites than controls.<sup>103</sup> Acute administration of lactone forms of atorvastatin, cerivastatin, and pitavastatin in C2C12 myoblasts elicited a fast decrease of oxygen consumption rate, indicating a net inhibitory effect on mitochondrial respiratory function.<sup>90</sup> In particular, lactone forms showed a higher capacity to impair ETC complex III activity.<sup>90</sup> Therefore, the conditions altering the hydroxy acid/lactone forms ratio, such as pH disturbances and pharmacokinetic interactions, might play a role in amplifying the muscle damage of statin treatment.

### Apoptosis

Statin-induced mitochondrial dysfunction has been correlated *in vitro* with an abnormal activation of the intrinsic apoptosis pathway, as a consequence of a decrease in the Bcl-2/Bax ratio. The statin-induced apoptosis occurs in different tissues and might be responsible for both beneficial pleiotropic and detrimental side effects of statin treatment.<sup>88,104</sup>

Several mechanisms have been proposed to explain the abnormal apoptosis in cells exposed to statins. *In vitro* studies on human primary skeletal muscle cells identified calpain, a calcium-dependent protease implicated in a variety of cellular processes such as signal transduction, cell proliferation, cell cycle progression, differentiation, and apoptosis,<sup>105</sup> as a plausible upstream effector for the statin-induced initiation of the intrinsic apoptotic pathway, which is only partially dependent on mevalonate depletion.<sup>106</sup> In fact, statin-induced calpain activation could be subsequent to increased Ca<sup>2+</sup> sarcoplasmic concentration, as discussed previously, and is responsible for Bax translocation to the outer mitochondrial membrane (OMM), leading to cytochrome *c* release into the cytosol and eventually to apoptosome assembly and activation of the caspases cascade.<sup>106</sup>

Accumulation of ROS as a consequence of ETC impairment could contribute to inducing mitochondria-mediated apoptosis.<sup>88,107</sup> Treatment with 10 mg/kg/day atorvastatin for 2 weeks suggests that statin-induced muscle cell apoptosis could be strictly dependent on the muscle fibre's metabolic phenotype. However, administration of quercetin, an antioxidant agent, resulted in apoptosis inhibition in fast glycolytic skeletal muscle fibres.<sup>107</sup> On the other hand, pretreatment with atorvastatin was able to protect human chondrocytes from high-glucose oxidative stress, by inducing expression of antioxidant enzymes, reducing ROS levels, and down-regulating the Bax/Bcl-2 ratio and caspase-3 activation.<sup>108</sup> Altogether, these findings imply that the differential cellular response to oxidative stress is an important determinant of tissue susceptibility to statins not only in terms of mitochondrial function but also in terms of apoptosis induction, according with the aforementioned concept of mitochondrial hormesis.<sup>70</sup>

Impairment of mitochondrial energy production affects isoprenoid biosynthesis. Statin treatment leads to depletion of isoprenoids compounds, such as farnesyl pyrophosphate and geranylgeranyl pyrophosphate, as a consequence of the blockade on the mevalonate pathway.<sup>14</sup> Protein farnesylation and geranylgeranylation are essential reactions for maintaining cellular homeostasis.<sup>109</sup> Depletion of isoprenoids may have a role in statin-related apoptosis induction, because HMG-CoA—but not squalene synthase inhibition—resulted in myotoxicity.<sup>104,110,111</sup> Isoprenoid depletion is likely to cause abnormal activation of  $\gamma$ -phospholipase C and PI3K signalling pathways,<sup>89,112,113</sup> thus leading to alteration in

calcium metabolism with the related detrimental consequences.<sup>104</sup> Finally, statins might alter the phosphorylation state of Akt by interfering with pathways involved in cell survival.<sup>83,84</sup> Despite a large amount of *in vitro* experiments, very few *in vivo* studies are currently available about the apoptotic effects of statins.

## Genetic background influences susceptibility to statin-associated symptoms

Polymorphisms on genes encoding for proteins involved in mitochondrial processes can modify the cell susceptibility to statin mitochondrial adverse effects. In fact, subclinical genetic mutations or polymorphisms have been associated with higher risk of SAS and SAMS.<sup>114</sup> Polymorphisms on gene *COQ2*, encoding for an enzyme involved in endogenous ubiquinone biosynthesis, have been associated to SAS and SAMS.<sup>115</sup> Similar findings have been reported for *ATP2B1*, encoding for a calcium transporting ATPase, and *DMPK*, encoding for a protein kinase with pathogenic implications in myotonic dystrophy.<sup>116</sup>

### *GATM* gene

The *GATM* gene encodes for the mitochondrial enzyme glycine amidinotransferase, which is the rate-limiting enzyme in creatine biosynthesis.<sup>117</sup> In a study carried out on lymphoblastoid cells of 480 statin-treated individuals, acute *in vitro* administration of simvastatin was associated with reduced gene expression levels and with reduced creatine synthesis in carriers of the rs9806699 single nucleotide polymorphism.<sup>118</sup> Moreover, the minor *GATM* allele, which is responsible for a reduced creatine yield, was associated with lower SIM rates but not with CK concentration in two different populations treated with 40 mg/day simvastatin for 6 weeks.<sup>118</sup> Although no causal mechanism can be inferred, it was speculated that reduced phosphocreatine storage impairs adenosine monophosphate-activated protein kinase signalling, in a way that could be protective against glucose and/or cholesterol deprivation.<sup>118,119</sup> However, these data were not replicated in subsequent studies.<sup>120,121</sup> Areas of uncertainty still exist on this topic.

### *Mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes syndrome*

Mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes syndrome is an archetypical model of mitochondrial genome inheritable disease. Because all mtDNA genes encode

for mitochondrial proteins, these pathologies are characterized by dysfunctions in organs and tissues with high-energy requirements.<sup>122</sup> Statin treatment has been shown to disclose several cases of previously asymptomatic mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes syndrome, possibly as a consequence of reduction in mitochondrial number and volume<sup>79,123</sup> or as a consequence of CoQ10 depletion,<sup>123,124</sup> as discussed previously.

### *Carnitine palmitoyltransferase-2 deficiency*

Carnitine palmitoyltransferase-2 (CPT-2) is an IMM enzyme that allows acyl-CoA to undergo  $\beta$ -oxidation in mitochondrial matrix. CPT-2 deficiency is the most common disorder of lipid metabolism and shows up with recurrent myalgia, rhabdomyolysis, and myoglobinuria that is precipitated by physical exercise, cold, infections, emotional stress, or fasting, usually during adulthood. Laboratory tests display high plasma acylcarnitine levels and acylcarnitine/carnitine ratio.<sup>125</sup> Such laboratory findings were found in a rabbit model treated with 30 mg/day lovastatin for 16 weeks, whereas the liver, heart, and skeletal muscle carnitine concentrations decreased after treatment. Interestingly, CPT-2 activity was significantly increased in the heart and liver but not in skeletal muscle.<sup>126</sup> In a case-control study, the frequency of the CPT-2 heterozygous mutation was 13-fold higher over the expected general population rate in patients affected by SIM. Statin treatment in those patients could be responsible for an impairment in CPT-2 activity, leading to a critical reduction of the enzyme overall activity and eliciting homozygous-like clinical manifestations.<sup>114</sup> These results corroborate the hypothesis that statin-induced mitochondrial dysfunction could also occur as impaired fatty acid oxidation, as suggested by histopathological findings.<sup>57</sup> The abnormally increased respiratory exchange ratio in patients who had experienced myositis<sup>127</sup> but not in those without SAMS<sup>128</sup> provides further evidence. Moreover, in high-fat-fed apoE knockout mice experiencing impaired exercise training adaptation after statin treatment, the administration of trimetazidine, a  $\beta$ -oxidation inhibitor, was successful in reversing statin-induced muscle injury,<sup>129</sup> suggesting that lipid metabolism disruption could be one of the several pathological mechanisms for SAMS.

## Statin-induced diabetes and mitochondrial function

In the Prospective Study of Pravastatin in the Elderly at Risk (PROSPER), the use of pravastatin (40 mg/day) in old patients was associated with an increased incidence of T2DM.<sup>130</sup> Similarly, in the Justification for the Use of Statins in Primary

Prevention: Intervention Trial Evaluating Rosuvastatin (JUPITER) trial, a significantly higher incidence of new-onset diabetes occurred in the rosuvastatin group when compared with the placebo group during the 2 years of statin administration.<sup>131</sup> A large meta-analysis of 13 trials including 91 140 patients reported a significant 9% increase in the risk of T2DM among patients treated with statins.<sup>132</sup> Because of these findings, FDA imposed to report the risk of new-onset diabetes on statin safety labels.

In an experimental setting, rats treated with 80 mg/kg/day lovastatin or 20 mg/kg/day atorvastatin for 2 weeks displayed increased glucose levels and decreased insulin secretion compared with controls.<sup>133</sup> Statin-treated rats also showed increased pancreatic cells ROS production, disrupted membrane potential, mitochondrial swelling, and reduced complex IV activity.<sup>133</sup> Administration of CoQ10 and L-carnitine separately or together was able to partially but significantly blunt these statin-induced abnormalities.<sup>133</sup>

We herein discuss the mitochondria-related mechanisms potentially explaining the increased risk of T2DM in statin-treated patients.

### Impaired insulin secretion

ATP levels are crucial in beta cells because they regulate the  $K_{ATP}$  channel closure that leads to cell depolarization and to insulin secretion. Mitochondrial dysfunction in beta cells could impair insulin secretion.<sup>134</sup> In a study on human pancreatic islets and in rat insulinoma INS-1 cells, atorvastatin—but not pravastatin—impaired both basal and glucose-induced insulin secretion.<sup>135</sup> This phenomenon is likely to be attributed to a significant decrease in ATP basal levels and ATP production rate, which is subsequent to a time and dose-dependent reduction in complexes I, III, and IV, and ATP synthase expression in atorvastatin-exposed cells.<sup>135</sup> Both mevalonate administration and *N*-acetylcysteine, an antioxidant agent, were able to rescue the impaired insulin secretion in statin-exposed cells.<sup>135</sup> In rat isolated pancreatic mitochondria, exposure to  $>75 \mu\text{M}$  atorvastatin significantly impaired complex II activity and reduced ATP levels.<sup>136</sup>

Nevertheless, experiments with patch clamping suggested that statins might impair insulin secretion by inhibiting L-type voltage-dependent  $\text{Ca}^{2+}$  channel as well, preventing the  $\text{Ca}^{2+}$  cytosolic concentration increase that is fundamental for insulin secretion.<sup>137</sup> The contrasting results obtained with atorvastatin and pravastatin account for their different diabetogenic potential.<sup>138</sup>

Atorvastatin exposure determined a significant increase in ROS levels both in human pancreatic islets and in INS-1 rat insulinoma cells<sup>135</sup> and in rat isolated pancreatic mitochondria<sup>136</sup> in a time and dose-dependent manner. Because pancreatic beta cells have weak antioxidant systems, oxidative stress may be crucial for beta-cell dysfunction, thus

increasing the risk of new-onset diabetes during statin treatment.<sup>136</sup>

Finally, CoQ10 concentration was reduced by atorvastatin administration as well, leading to ETC activity impairment.<sup>135</sup> As a consequence of ETC activity disruption, atorvastatin caused a decline in mitochondrial membrane potential, mitochondrial swelling, and cytochrome *c* release,<sup>136</sup> thus leading to intrinsic apoptosis induction in the pancreas.

### Peripheral insulin resistance

Statins could also induce peripheral insulin resistance via different pathways. Simvastatin determined a reduction in insulin-dependent glucose transporter GLUT4 expression in 3T3-L1 adipocytes, and this phenomenon could be rescued by CoQ10 administration.<sup>139</sup> The accumulation of non-esterified fatty acids in skeletal muscle cells is responsible for insulin resistance.<sup>140</sup> Mitochondrial uncoupling protein (UCP)-3 is a protein located in the IMM that acts as a fatty acid carrier, favouring their  $\beta$ -oxidation.<sup>141</sup> Patients affected by T2DM show lower expression of UCP3.<sup>142</sup> A case-control study pointed out a significantly reduced UCP3 expression in patients treated with simvastatin.<sup>143</sup> The complex relationship among statins, UCP3, and insulin resistance has not been fully clarified yet. Though no causative mechanisms can be inferred, it can be hypothesized that statins could play a role in determining skeletal muscle insulin resistance by down-regulating UCP3 expression.

## Cognitive impairment and dementia

Many studies have been published in the last two decades about the relationship between statin treatment and cognitive function. Like skeletal muscle, brain tissue has a high metabolic demand and hence suffers from mitochondrial vulnerability.<sup>144</sup>

A large retrospective study showed an increased risk for both Alzheimer's disease and vascular dementia in hypercholesterolaemic patients after a three-decade follow-up.<sup>145</sup>

Intriguingly, several case reports<sup>146,147</sup> and two randomized controlled trials<sup>148,149</sup> suggested a potential association between statin treatment and cognitive impairment, leading FDA to include in the warning section of all statin labels a statement about the risk for reversible memory loss or impairment. Contrariwise, large trials,<sup>130,150</sup> a Cochrane database systematic review,<sup>151</sup> a consensus statement by the Statin Cognitive Safety Task Force,<sup>152</sup> and more recent meta-analyses<sup>153,154</sup> failed to demonstrate any effect of statin treatment on cognitive function, while a large meta-analysis even showed a great risk reduction for dementia associated with statin treatment.<sup>155</sup> An extensive and

punctual review about the state of the art on this topic has been recently published.<sup>156</sup>

It is plausible that these contradicting results derive from different mechanisms that might be responsible for both cognitive protective effect of statins and treatment-related adverse cognitive effects.<sup>156</sup>

In terms of mitochondrial function, the pathological basis for these contradictory results is completely to be elucidated. It is conceivable that the mechanisms implicated in SAMS, such as CoQ10 depletion, ROS accumulation, and mitochondrial membrane depolarization, could account for cognitive impairment too. Accordingly, genetic disorders, dysthyroidisms, or the metabolic syndrome, which all underlie mitochondrial dysfunction, are well-known risk factors for statin-related cognitive adverse effects.<sup>15,157</sup> However, it must be underlined that simvastatin was able to protect a neuroblastoma cell line (i.e. SH-SY5Y) from amyloid- $\beta$  neurotoxicity by improving several aspects of mitochondrial function (e.g. cytochrome *c* release, ROS production, Bcl-2/Bax ratio, and membrane potential).<sup>158</sup> In addition, in a cellular model of mitochondrial dysfunction (i.e. cybrid), low-dose (1  $\mu$ M) simvastatin reduced hypoxia-inducible factor 1 $\alpha$  and  $\beta$ -site amyloid precursor protein cleaving enzyme, the latter molecules promoting amyloid- $\beta$  production. However, higher dose of simvastatin (10  $\mu$ M) increased hypoxia-inducible factor 1 $\alpha$  and  $\beta$ -site amyloid precursor protein cleaving enzyme expression both in the cybrid and control cell models,<sup>159</sup> thus suggesting that statin dose might differentially affect neuronal function irrespective of mitochondrial activity.

## Conclusions

Besides the well-known beneficial effects in primary and secondary prevention of CVD, statins may induce adverse effects, like myopathy. Mitochondrial dysfunction is likely to play an important role in the pathogenesis of these adverse reactions due to CoQ10 depletion, inhibition of ETC complexes, depletion of mevalonate pathway end products,

membrane depolarization and induction of intrinsic apoptosis, dysregulation of calcium metabolism, and fatty acid oxidation. Chronic statin treatment has been associated with increased risk for T2DM and cognitive impairment. The beneficial effects of statin treatment are not questionable; however, the widespread usage of these drugs and the considerable prevalence of side effects require the underlying pathological mechanisms to be carefully studied.

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## Conflict of interest

Dr M.B. has served on the speakers bureau of Abbott/Mylan, Abbott Vascular, Actavis, Akcea, Amgen, Biofarm, KRKA, MSD, Sanofi-Aventis, Servier, and Valeant and has served as a consultant to Abbott Vascular, Akcea, Amgen, Daichii Sankyo, Esperion, Lilly, MSD, Resverlogix, and Sanofi-Aventis; he also received grants from Sanofi and Valeant. S.v.H. has been a paid consultant for and/or received honoraria payments from Bayer, Boehringer Ingelheim, BRAHMS, Chugai, Grünenthal, Helsinn, Hexal, ovartis, Respicardia, Roche, Sorin, and Vifor; owns shares in Actimed. SvH; and reports research support from IMI and the German Center for Cardiovascular Research (DZHK).

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