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Mitochondrial Oxidative Stress and Calcium-Dependent Permeability Transition are Key Players in the Mechanisms of Statins-Associated Side Effects

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Estela N.B. Busanello, Ana C. Marques,
Estela Lorza-Gil, Helena C.F. de Oliveira and
Anibal E. Vercesi

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Abstract

Statins are cholesterol-lowering medicines utilized worldwide and are associated with reduced risk of cardiovascular mortality and events. However, 0.5–10% of patients suffer from adverse effects especially on skeletal muscle. Recently, new onset of diabetes has been reported in subjects on statin therapy. Pro- and anti-oxidant effects of statins have been reported, thus fostering a debate. Previously reported data provide evidence that statins induce alterations in intracellular calcium homeostasis and mitochondrial dysfunctions that can be counteracted by antioxidants (e.g., CoQ10, creatine, and L-carnitine). Therefore, we have proposed that statin-induced inhibition of mitochondrial respiration leads to oxidative stress that opens a calcium-dependent permeability transition pore, an event that may lead to cell death. In addition, mitochondrial oxidative stress caused by statin treatment may be a signal for cellular antioxidant system responses such as catalase upregulation, possibly explaining the alleged statins' antioxidant properties. Muscle mitochondrial dysfunction induced by statin treatment may be associated with the peripheral insulin resistance and may explain statins-induced new onset of diabetes. Together, the data presented in this review suggest that the statins' detrimental effects can be prevented by co-administration of antioxidants.

Keywords: statins adverse effects, statins pleiotropic effects, reactive oxygen species (ROS), mitochondrial permeability transition, antioxidants

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1. Introduction

Familial hypercholesterolemia (FH) is an autosomal dominant disorder characterized by the presence of very high levels of low-density lipoprotein cholesterol (LDLc) in the blood stream

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since birth. This cholesterol disorder was first described in the 1960s, and the existence of a mutated LDL receptor (LDLr) in FH patients was later discovered by Brown and Goldstein [1]. They observed that FH fibroblasts did not specifically bind and internalize LDL when compared with normal fibroblasts; that finding was the beginning of decades of work and discoveries concerning cholesterol metabolism regulation that led the pair to Nobel Prize award in 1985. Although the homozygous mutants for LDLr have an early cardiac death in the first or second decade of life, heterozygous FH patients usually do not present any early severe symptoms. The lack of diagnosis and treatment may have severe consequences considering the lifetime exposure to high LDLc concentrations. Increased LDLc levels are a well-established independent risk factor for cardiovascular diseases [2], and lowering LDL serum levels remains the primary treatment target in hypercholesterolemia [3, 4] that is undertaken in order to prevent and reduce cardiovascular and coronary heart diseases [5, 6].

Cholesterol is synthesized from acetyl-CoA by a 30-step pathway, in which 3-hydroxy-3-methylglutaryl coenzyme-A (HMG-CoA) reductase is the rate-limiting enzyme, converting HMG-CoA into mevalonate. However, besides being involved in cholesterol synthesis, mevalonate is also a precursor for isoprenoids farnesyl diphosphate. Geranyl- (GPP), farnesyl- (FPP) and geranylgeranyl-pyrophosphate (GGPP) are precursors of sterols, dolichols, CoQ10, isoprenoids, and carotenoids. These important metabolites are involved in membrane structures, protein glycosylation and prenylation, electron transport in mitochondrial respiratory chain, and scavenging of ROS [7].

The first cholesterol-lowering agent, citrinin, was discovered in the 1970s. It was derived from fungal cultures, but this product was discontinued due to its hepatotoxicity [8, 9]. After this, another fungal-derived compound called compactin was purified and tested in rats; however, it failed to reduce plasma cholesterol because it had the rebound effect of inducing HMG-CoA reductase activity a few hours after administration [10]. At the end of the 1970s, a very potent compound chemically similar to compactin was synthesized based on independent studies from Endo and Alberts [11, 12], and after several trials, this potent compound, lovastatin, was approved and commercially available in 1986 [13]. Presently, there are seven natural (fungal-derived) or synthetic statins that are commercially available; this group consists of three hydrophilic (pravastatin, rosuvastatin, and pitavastatin) and four lipophilic (lovastatin, simvastatin, fluvastatin, and atorvastatin) [14–16]. Cerivastatin was approved by the Food and Drug Administration in 1998, but it was removed from the market in 2001 after reports of fatal rhabdomyolysis [17].

Statins are one of the most successful drugs for reducing cardiovascular diseases. High-intensity statins treatment is associated with the greatest reduction in mortality [18]. In addition to lowering plasma cholesterol, various studies have reported that statins have pleiotropic effects such as antioxidant, anti-inflammatory, and anti-tumorigenesis. Regarding statins redox effects, some groups have demonstrated protective roles of these compounds against cell oxidative damage [19, 20], whereas others have reinforced their toxic effects [21, 22]. Despite these discrepancies in these results over the last decade, accumulated data have indicated that alterations in mitochondrial energy-linked functions such as respiration, oxidative phosphorylation, redox state,

Ca²⁺-dependent permeability transition underlie statins toxicity. The impact on cell or tissue pathophysiology will depend on the intensity of statins' effects on mitochondria. In this chapter, we review the literature data on the statins effects on mitochondrial functions and consequent toxic tissue events.

1.1. Mitochondrial energy-linked functions and reactive oxygen generation

Mitochondria participation in the process of statin toxicity adds to the numerous roles of these organelles in cell pathophysiology [23, 24]. Considering that statin-mediated mitochondrial dysfunctions include many aspects of mitochondrial physiology such as inhibition of respiration, depletion of ubiquinone, redox imbalance, opening of the mitochondrial permeability transition pore (PTP) and disruption of energy conservation, we next outline some of these mitochondrial properties in the following sections.

During the last several decades, mitochondria have emerged as the center of attention in processes of cell signaling, cell injury, and cell death [25, 26]. According to the concept of coupling between respiration and oxidative phosphorylation through a transmembrane proton electrochemical potential that was introduced by Peter Mitchell [27], it is not difficult to understand that any condition that interferes with the ability to sustain the inner membrane proton potential leads to mitochondrial dysfunction [28]. In addition, the continuous oxygen reduction by the mitochondrial electron transport chain to build up the transmembrane proton gradient also generates a well-regulated amount of superoxide [23, 29]. Therefore, mitochondria have developed a complex antioxidant defense system composed of Mn-superoxide dismutase that converts the superoxide radical generated during respiration into hydrogen peroxide (H₂O₂). H₂O₂ is then reduced to water by glutathione and thioredoxin peroxidase or catalase [30]. Oxidized glutathione (GSSG) and thioredoxin (TSST) generated by peroxidases are converted to their reduced forms by glutathione and thioredoxin reductases, using NADPH as reducing power. NADH then reduces NADP⁺, in a reaction catalyzed by NADP transhydrogenase that is present in the inner mitochondrial membrane [31–33]. Therefore mitochondria redox state is tightly regulated and connected with whole cell redox balance [34–36]. Furthermore, it is now generally accepted that superoxide as well as other forms of ROS can function as a signal for either adaptation or maladaptation to stress conditions [35]. In this regard, mitochondrial ROS generation leads to a nonlinear dose-response relationship called mitohormesis. In mitohormesis, high reactive oxygen concentrations exert devastating and irreversible effects on cell function and structures, whereas low concentrations may be associated with protective effects due to activation of cellular defense mechanisms [37, 38]. In fact, at progressively increasing physiological levels, ROS may successively regulate cellular processes such as proliferation and differentiation, activate adaptive programs such as transcriptional upregulation of antioxidant genes, and at higher levels, ROS may be a signal for senescence and regulated cell death [35]. In addition to the physiological processes, it seems that mitochondrial oxidative stress is responsible for the development and progression of a series of diseases such as cancer, diabetes, inflammatory diseases, hypertension, neurodegenerative and ischemia-related diseases, and aging [39–46]. Statin toxicity may also include the participation of mitochondrial generated ROS [47–49].

1.2. Mitochondrial Ca^{2+} transport and mitochondrial membrane permeability transition (MPT)

Ca^{2+} modulates several metabolic pathways through transient changes in its free concentrations in different cell compartments [50, 51]. In order to fulfill these physiological roles, Ca^{2+} movements across cell membranes are driven directly or indirectly by ATP hydrolysis. Therefore, defects in processes that supply cellular ATP may lead to deregulation in Ca^{2+} signaling that may compromise cell functioning, redox balance, and mitochondrial membrane permeability transition (MPT) [51, 52]. In this review, we briefly describe how mitochondrial Ca^{2+} load promotes MPT [53].

MPT is characterized by the opening of a high conductance, nonspecific proteinaceous pore, the PTP. It was first described by Hunter and collaborators [54] and then demonstrated by Vercesi's group to be dependent on redox imbalance promoted either by thiol oxidants or oxidative stress [55]. Matrix Ca^{2+} participates in at least two steps in the process of PTP opening: (a) stimulates superoxide generation by mitochondria and (b) binds to membrane sites exposing specific buried thiols to the oxidants (**Figure 1**) [55]. Accordingly, Ca^{2+} binding to cardiolipin alters mitochondria inner membrane lipid organization characterized by increased lipid packing and domain formation. As a consequence, the electron transfer along the respiratory complexes is impaired favoring superoxide generation [56].

Robust data has provided evidence that PTP opening is a main step in the mitochondrial pathway leading to cell death either by apoptosis or necrosis [57, 58], and is a major cause of cell death under a variety of pathophysiological conditions, including ischemia/reperfusion injury, traumatic brain injury, neurodegenerative diseases, metabolic diseases, muscular dystrophy, and drug toxicity [59–67].

Since mitochondrial Ca^{2+} overload stimulates superoxide generation and MPT, the mechanisms of Ca^{2+} transport by mitochondria will be outlined next. The inner mitochondrial membrane possesses three different carriers for Ca^{2+} influx and efflux [68]. A mitochondrial calcium uniporter (MCU) located in the inner membrane mediates the influx of Ca^{2+} down its electrochemical gradient without coupling Ca^{2+} transport to the flux of another ion. This mechanism was discovered in the 1960s [69, 70], but the molecular nature of the channel was only recently identified [71, 72]. Ca^{2+} release from mitochondria occurs via $\text{Ca}^{2+}/3\text{Na}^{+}$ or a $\text{Ca}^{2+}/2\text{H}^{+}$ exchangers [73–75] depending on the tissue [68, 76].

The high loads of matrix Ca^{2+} that stimulate ROS production in mitochondria [55] appear to be associated with either dysregulation of cellular Ca^{2+} homeostasis or regulated release from endo(sarco)plasmic reticulum [77–79] (**Figure 1**). Under both conditions, the opening of the PTP can occur allowing for the movements of molecules up to 1.5 kDa. The entry of solutes and water to the matrix causes large amplitude mitochondrial swelling. These conditions disrupt both the electrochemical proton potential and oxidative phosphorylation [23, 55]. When PTP opens in a large number of mitochondria, cell death occurs by necrosis due to the lack of ATP, and when PTP is limited to a small number of mitochondria, apoptosis is triggered by the release of cytochrome c [80]. Anti-apoptotic proteins (members of Bcl-2 family) or cyclosporine A inhibits the opening of PTP [81, 82]. Evidence has been provided that high intracellular Ca^{2+} levels and ROS have additive effects in the process of PTP opening [23, 53, 55, 83–88].

It is well recognized that mitochondrial Ca^{2+} is essential for PTP opening [54, 55, 89, 90], whereas oxidative modifications of inner membrane protein thiols, oxidative stress, presence

Figure 1. Statins triggers mitochondrial oxidative stress and calcium-dependent permeability transition. Statins diminishes the respiratory capacity at the level of complexes I, II and III of the respiratory chain, increasing superoxide generation ($O_2^{\cdot-}$). The Fe-S clusters present in these respiratory complexes are vulnerable to superoxide attack, thus inhibiting their activity and diminishing their resistance to Ca^{2+} induced MPT. Superoxide is dismutated in hydrogen peroxide (H_2O_2). When not metabolized by mitochondrial antioxidant systems, H_2O_2 can induce (directly or indirectly) membrane protein sulfhydryl-disulfide transitions, a process involved in PTP opening. Statins also impair cellular Ca^{2+} homeostasis, inducing Ca^{2+} release from the ER via IP₃R and increasing cytosolic Ca^{2+} levels. Thus, mitochondria uptake the excessive cytosolic Ca^{2+} via VDAC and MCU channels, leading to its accumulation in mitochondrial matrix. Ca^{2+} binds to membrane sites exposing specific buried thiols to the oxidants and also impairs mitochondrial respiration, increasing $O_2^{\cdot-}$ formation. The association of ROS and mitochondrial Ca^{2+} overload, PTP may open and trigger cell death. In addition, a decrease in the levels of CoQ10 that acts as an electron carrier and antioxidant also occurs due to inhibition of the mevalonate pathway by statins. The antioxidants CoQ10, L-carnitine and creatine prevent PTP opening induced by statins.

of inorganic phosphate [53, 55, 83, 85, 91], and Bcl-2 family proteins [81, 82] participate in PTP modulation. The close location of mitochondria and the endoplasmic reticulum (ER) [75] permits mitochondria to take up large amounts of Ca^{2+} that are released from the ER. This process seems to be controlled via a redox-regulated cross talk between mitochondria and ER that is mediated by NADPH oxidases [36]. Such redox interactions may link PTP opening to the induction of Ca^{2+} signals specifically for cell death [26]. Considering the understanding on how Ca^{2+} and ROS act synergistically in the mechanism of PTP opening, it should be emphasized that mitochondria are more susceptible to MPT when their antioxidant systems are exhausted, especially due to an oxidized state of NADPH and GSH [55]. Accordingly, mitochondria isolated from mice deficient in nicotinamide nucleotide transhydrogenase (NNT), which cannot sustain NADPH in the reduced state, present defective antioxidant capacity and increased susceptibility to MPT [92, 93]. Thus, MPT can be induced by pro-oxidants and prevented or even reversed by antioxidants [85, 86, 94, 95].

2. Statins pleiotropic effects

Statins are among the most commonly prescribed medicines worldwide. They are safe and well-tolerated and seem to present a range of cholesterol-independent protective actions called pleiotropic effects. Indeed, several studies claim that statins act as antioxidants [19, 96], anti-inflammatory agents [97], and can increase stability of the atherosclerotic plaque [98], improve endothelial function [99], and induce cancer cell death [100].

2.1. Antioxidant responses triggered by statins

Extensive literature reports have indicated that antioxidant effects can be attributed to statins. It has been postulated that statins decrease systemic or local oxidative stress and this appears to confer additional vascular protection. The first possible mechanism for this protective effect could be secondary to statins' main target effect, which is to decrease the concentration of the oxidizable substrate, LDLc. This decrease may lead to a reduction in oxidized-LDL, which constitutes a very early step involved in atherosclerosis development [101–103].

Another antioxidant mechanism frequently attributed to statins is the upregulation of cellular antioxidant defenses. For instance, atorvastatin treatment decreased the expression of essential NAD(P)H oxidase subunits and upregulated catalase expression in cultured rat vascular smooth muscle cells and in the vasculature of spontaneous hypertensive rats (SHR) [104]. Simvastatin treatment restored endothelial function in SHR by increasing superoxide dismutase and glutathione peroxidase activities [105].

Other studies have demonstrated a protective effect by statins against oxidative damage of biomolecules. In whole blood leukocytes of non-treated dyslipidemic diabetic type 2 patients, simvastatin treatment [19] protected against DNA oxidative damage. Similarly, rosuvastatin inhibited lipid peroxidation and attenuated the oxidative damage to DNA in treated rat liver [106]. Rosuvastatin-treated HL-60 cells exhibited a glutathione-dependent protective mechanism against DNA oxidation [107]. In addition, simvastatin or fluvastatin administration prevented lipid peroxidation, superoxide generation, cytokine production, and neutrophil accumulation in a rat colitis model [108].

With respect to statins' effects on specific mitochondrial redox homeostasis, literature reports are more controversial. It was shown that atorvastatin and simvastatin reduced oxidative stress triggered by Ca^{2+} and prevented MPT and cytochrome c release in rat liver mitochondria [96]. On the other hand, results from our group and others suggest that statins, when administered to mitochondria, muscle biopsies, or *in vivo* exert pro-oxidant activities (this will be discussed in more detail in the next section) [47, 49, 109]. Thus, our hypothesis for the alleged statin antioxidant effects is based on the mitohormesis concept [37, 38]: mild mitochondrial oxidative stress caused by statins may function as a signal that leads to a cellular adaptive response such as increasing the expression and activity of cellular antioxidant systems in order to overcome this stress.

2.2. Statins and cancer

Statins have been proposed as adjuvant in cancer therapy since the 1990s and, until then, several mechanisms have been proposed for this specific function depending on the type of cancer and

statins lipophilicity [100, 110–112]. In this regard, literature reports suggest that the mevalonate pathway inhibition is associated with anti-proliferative, pro-apoptotic, and anti-metastatic statins effects [113]. In addition, statins may impair cell membrane function, due to the lowering of cholesterol levels and inhibition of the tumor cell cycle, and may lead to cell death by distinct pathways, including the mitochondrial pathway (for more details, see Ref. [114] and other reviews).

Prostate cancer is one of the most commonly diagnosed cancer in men and is a significant cause of male morbidity and mortality [115]. Literature reports have shown that statins protect against prostate cancer in human patients [116, 117], and some of these effects may be attributed to a decreased isoprenoid synthesis due to mevalonate pathway inhibition. As a consequence, Ras proteins that regulate signaling pathways of cell proliferation, angiogenesis, and metastasis are not able to be isoprenylated, thus reducing their function and triggering apoptosis [118]. Statins also stimulate the mitochondrial apoptosis pathway [119, 120] via an increase in pro- and decrease in anti-apoptotic Bcl-2 proteins [121], activation of caspases 3, 7, 8, and 9 [122–124], and decrease in the formation of lipid rafts, membrane microdomains involved in several regulatory functions, including cell survival [125, 126]. In addition, statins have a dose-dependent effect on cell death. For instance, simvastatin at concentrations below 10 μM induced PC3 prostate cancer cells apoptosis [21] via a mechanism sensitive to mevalonate but not to cyclosporin A (CysA), an MPT inhibitor. On the other hand, necrosis is stimulated by higher doses of simvastatin ($\geq 60 \mu\text{M}$) and is preceded by an increase in free cytosolic Ca^{2+} concentration and PTP opening, sensitive to CysA, but not to mevalonate [21]. Both MPT and necrosis induced by simvastatin (60 μM) are sensitive to L-carnitine (antioxidant) and piracetam (membrane stabilizer) in an additive manner. When combined, these compounds act at lower doses than when each compound is used separately [22]. These data provide evidence that statin toxicity to tumor cells is not only the result of HMG-CoA reductase inhibition but also is mediated by the increase in free cytosolic Ca^{2+} concentration, stimulation of ROS generation, and PTP opening [21, 22]. Although many studies show that statins which are efficient in inducing tumor cell death claim their potential use as adjuvant therapy, there are no robust data that non-tumor cells are less affected by statins' toxic effects than tumor cells. Therefore, it is still premature to conclude that statins are anti-tumorigenic agent.

3. Statins adverse effects

After decades of statins' use, some side effects have been consistently described in a minority of patients, particularly regarding muscle function. Adverse effects other than muscle symptoms such as headache, digestive problems, liver enzymes abnormalities, and neurological dysfunction may occur in some patients [127, 128]. The side effects are often the decisive factor for the noncompliance to statins treatment [129, 130] and its discontinuation usually makes the side effect symptoms disappear [131].

The precise mechanisms involved in statins toxicity and the reasons why only a few subjects are affected remain unclear. Several groups, including ours, have proposed that mitochondria are the main players in statin-induced toxicity.

3.1. Mitochondrial dysfunction caused by statins treatment

Mitochondrial redox imbalance is associated with aging, degenerative disorders, and drug-induced toxicity [26, 132]. Several reports concerning statin *in vitro* effects on isolated tissues or mitochondria from experimental models demonstrated that statins promote inhibition of mitochondrial respiration, mitochondrial oxidative stress, and cell death [47, 49, 109, 133]. It has been previously shown that lipophilic (cerivastatin, fluvastatin, atorvastatin, and simvastatin) and hydrophilic (pravastatin) statins-induced mitochondrial membrane potential decrease in rat skeletal muscle cell line (L6) [133]. The four lipophilic statins also induced mitochondrial swelling, cytochrome c release, and DNA fragmentation in these L6 cells. Mitochondrial β -oxidation enzymes activities were strongly impaired by all lipophilic statins, but in the case of pravastatin, it occurred only at high concentrations. In isolated rat skeletal muscle mitochondria, glutamate-supported state 3 respiration and respiratory control ratios were decreased by all lipophilic statins, but not by pravastatin [133]. According to the authors, this mitochondrial dysfunction caused by lipophilic statins in skeletal muscle might partially explain the muscle symptoms presented by some patients. Abdoli and coworkers demonstrated in isolated rat liver mitochondria that atorvastatin, simvastatin, and lovastatin increased ROS formation followed by lipid peroxidation, inner mitochondrial membrane depolarization, and a decreased GSH/GSSG ratio [47].

More recently, mitochondrial redox imbalance [67, 134] was observed in a genetic human familial hypercholesterolemia mouse model, the LDL receptor knockout mouse (*LDLr^{-/-}*) [135]. Mitochondria isolated from several tissues of these mice (liver, heart, and brain) and intact spleen mononuclear cells presented higher ROS production and higher susceptibility to MPT. In addition, these mitochondria showed lower capacity to sustain reduced NADPH [67, 134], which is the most important reducing power involved in reconstituting mitochondrial antioxidant systems [132]. As a consequence, H_2O_2 accumulates and PTP opens [67, 134]. Since cholesterol synthesis consumes a large amount of NADPH, we have proposed that the increased steroidogenesis observed in these mice would be partially responsible for the lower mitochondrial content of NADPH and Krebs cycle intermediates observed in their liver mitochondria [67, 134]. Therefore, we hypothesized that inhibition of cholesterol synthesis by statins treatment could prevent the decrease in NADPH oxidation in *LDLr^{-/-}* mice mitochondria. Unexpectedly, liver mitochondria from wild type and *LDLr^{-/-}* mice treated with lovastatin presented a higher susceptibility to PTP opening, and *in vitro* experiments revealed a drug dose- and class-dependence of this effect [109]. Statin induced PTP opening was shown to be Ca^{2+} -dependent and associated with oxidation of protein thiol groups. Thus, statins induced a direct oxidative damage in mitochondrial proteins [109].

3.2. Ca^{2+} and statins toxicity

It has been proposed by our group and others that statins impair cellular Ca^{2+} homeostasis, leading to mitochondrial dysfunction. Increased cytosolic Ca^{2+} levels were observed after simvastatin treatment of myoblasts culture [136], rat skeletal muscle [137], and human skeletal muscle fibers, and this was followed by mitochondrial Ca^{2+} accumulation [138]. Indeed, Hattori and coworkers [139] proposed that statins induced Ca^{2+} release from the endoplasmic reticulum to the cytosol in human CD19+ primary lymphocytes. As a consequence of high Ca^{2+} levels in the cytosol, Ca^{2+} enters the mitochondria and induces MPT as demonstrated by our group in PC3 cells after simvastatin treatment [21, 22].

3.3. Statins effects on respiratory chain complexes

It is well known that enzymes containing 4Fe-4S clusters are particularly vulnerable to damage by superoxide or peroxynitrite radicals [140–145]. Complexes I and II present six and one of these 4Fe-4S clusters, respectively, thus showing a high superoxide-sensitivity. Some studies have demonstrated that superoxide generation inhibits respiration at complex I and II levels as a result of 4Fe-4S clusters damage. These alterations diminish resistance to Ca^{2+} -induced MPT and induce necrotic cell death [65, 145]. As mentioned before, our group demonstrated that mitochondrial dysfunction caused by simvastatin incubation in permeabilized skeletal muscle was L-carnitine and CoQ10 sensitive [49]. L-carnitine did not protect against CoQ10 depletion, indicating that both CoQ10 and L-carnitine are protecting mitochondrial respiration due to its ROS scavenging properties. Since L-carnitine also binds Fe^{2+} [146], it is feasible that this antioxidant molecule interacts with 4Fe-4S clusters in complexes I and II of the respiratory chain, protecting these sites against superoxide attack. Simvastatin lowered the ADP-stimulated respiration supported by substrates of complexes I and II in primary human skeletal myotubes and increased susceptibility to MPT, mitochondrial oxidative stress, and apoptosis [48]. These results are in agreement with a decrease in complex I activity in muscle of patients undergoing statin treatment [147].

Another study performed in myoblasts culture (C2C12) incubated with several statins (atorvastatin, cerivastatin, fluvastatin, lovastatin, pitavastatin, pravastatin, rosuvastatin, and simvastatin) showed that the respiratory capacity is reduced not only at the levels of respiratory chain complexes I and II but also in complex III [148]. In this case, it was suggested that statins in the lactone form binds to Q_o site of complex III, inhibiting its activity. Similarly, complex III activity of muscle from patients presenting myopathies induced by statins was also reduced [148]. On the other hand, statins do not seem to affect the complex IV-supported respiration [49, 148].

4. Muscle sensitivity to statins

It is well known that about 10% of patients undergoing statin treatment develop mild myopathic symptoms such as weakness, muscle pain, exercise intolerance, and other symptoms that are usually with normal or minimally elevated creatine kinase (CK) serum levels [149, 150]. Moreover, myositis, defined as muscle symptoms associated with increased CK, is usually present [151, 152]. Rhabdomyolysis, the most severe adverse effect of statins, is a very rare condition affecting 1.6/100,000 patients-years. It may result in acute renal failure and disseminated intravascular coagulation, leading to death. This condition is frequently related to drug interactions and occurs with CK levels 10-fold higher than the normal limit and elevated levels of creatinine [153, 154]. Increased intracellular lipid stores, cytochrome oxidase-negative myofibers, ragged red fibers, and subsarcolemmal accumulation of mitochondria were found in patients with muscle symptoms during statin therapy [155, 156]. Schick and colleagues also observed reduced mitochondrial DNA levels in patients treated with simvastatin [157]. Muscle-associated statin toxicity seems to be more severe with increasing lipophilicity, whereas more hydrophilic statins exert only mild or no toxicity [133, 153]. The myotoxic effect is attributed to their ability to penetrate and accumulate in cell membranes and alter their structural conformation [158–160].

On the other hand, high statin sensitivity may also be related to genetic factors; for instance, the activity of specific liver transporters may be impaired, thus reducing statins hepatic uptake and increasing its plasma concentrations that may potentially affect muscles [161–163].

Skeletal muscles are highly heterogeneous and present distinct fiber types classified as I or II and their respective subtype spectrum as determined by the myosin heavy chain isoforms. Type I and II fibers present relatively distinct metabolic, contractile, and motor properties in addition to antioxidant defense capacity. Thus, type I fibers appear red due to high myoglobin content, extensive mitochondrial content, and oxidative capacity, whereas type II fibers have relatively low myoglobin and mitochondrial content that depends mostly on glycolytic activity [164, 165]. In this regard, our group observed that respiratory rates were inhibited in the presence of Ca^{2+} in permeabilized plantaris muscle (predominantly type II fibers) in *LDLr*^{-/-} mice chronically treated with pravastatin and catalase activity increased. In contrast, no alterations were observed in soleus muscle (predominantly type I fibers) [166]. Similarly, previous studies reported a distinct sensitivity of different muscle fiber types to lovastatin [162]. After 10 days of lovastatin administration, rat gastrocnemius muscles showed organelle degeneration, microvacuolization, and 20–50% necrosis, whereas soleus muscle was spared, suggesting that type II fibers are more vulnerable to lovastatin-induced myopathy [167]. In line with this finding, Westwood and colleagues characterized time-dependent muscle necrosis triggered by simvastatin or cerivastatin in rats after 10 days of treatment. The authors demonstrated that glycolytic fibers were more prone to necrosis than oxidative fibers, which in turn were consistently spared even when myotoxicity was severe. Since these fibers present distinct metabolism and MPT may precede necrosis, it is conceivable that mitochondria exert a central role in this process. In fact, it was observed that the first subcellular alterations were found in mitochondria of type II fibers, characterized by vacuolization as well as myeloid and vesicular body accumulation in sarcolemma areas [168]. Later, the same group performed a similar study using rosuvastatin in rats. Although a much higher statin dose was required to achieve muscle necrosis in comparison to the earlier study, the same pattern of muscle damage was observed and the soleus muscle remained unaffected [169]. Specific soleus-insensitivity to statin toxicity has also been demonstrated by other groups. Schaefer and coworkers demonstrated necrosis and inflammation in muscles with predominance of type II fibers in rats after 15 days of cerivastatin administration. Sarcomere disruption and altered mitochondria was also found in degenerated fibers, while these alterations were not found in type I fibers [170]. Similarly, cerivastatin-induced degeneration was evident in several muscles but not in the soleus muscle of female rats after the same treatment time (15 days). After 15 days of treadmill exercises, the severity of muscle damage had increased, but the soleus remained unaltered. Degenerated mitochondria were also observed with no changes in contractile elements such as endoplasmic reticulum and other subcellular compartments [171]. Although the role of mitochondria in myotoxicity in type II fibers is well established, there is no consensus as to whether this involvement precedes myofiber degeneration, thus justifying further studies to clarify this matter [170, 171]. In addition, MPT is associated with apoptosis or necrosis in several diseases [172] and is probably an important statin-induced event in muscle necrosis.

5. Statins toxicity to liver

Although rare, the main liver injury studies have reported statins toxicity alone [173–176] or in combination with other drugs with variable patterns of injury [177–181]. Some cases exhibited autoimmune features [180, 182, 183] and a range of latencies to onset [184] and progression was also observed [182, 185]. Liver adverse symptoms are unspecific and most patients remain asymptomatic [186]. A 3-fold increase in serum aspartate (AST) and alanine (ALT) aminotransferases activities have been described in less than 1% of patients receiving starting and intermediate statins doses [187–191] and this alteration may be accompanied by bilirubin elevation [192]. Two factors are frequently related to the hepatotoxic effects of statins: (a) the lipophilicity of these medicines and (b) alterations in cytochrome P450 system [193–195]. Accordingly, lipophilic statins (atorvastatin and simvastatin) are associated with more than 130 cases of liver injury, and a few cases progress to liver transplantation and death [173, 174, 178]. Rare cases of portal inflammation or fibrosis and mild necrosis were also described in patients undergoing lovastatin treatment [196] or atorvastatin treatment [197]. On the other hand, hydrophilic statins are minimally metabolized by the cytochrome P450 pathway [193–195] and are generally less toxic [109, 198]. A multicenter report also showed that pravastatin was well-tolerated in patients with compensated chronic liver disease [199]. Our group also attributes statin-induced liver toxicity to mitochondrial dysfunction associated with oxidative stress and MPT [193].

6. Statins and new onset of diabetes

Recent studies suggest that chronic use of statins is associated with risk of developing type 2 diabetes [200–202]. Meta-analyses of large-scale statin trials support the concept of the diabetogenic effect of statins, but the precise mechanisms have not yet been identified [203, 204]. We have recently revealed diabetes-related mechanisms induced by statin treatment in a familial hypercholesterolemia animal model, the *LDLr^{-/-}*. We demonstrated that pravastatin-treated *LDLr^{-/-}* mice exhibit marked reductions of insulinemia and of glucose-stimulated insulin secretion by isolated pancreatic islets. These effects were associated with increased oxidative stress and apoptosis [205] and were counteracted by co-treatment with CoQ10 (Lorza-Gil et al., unpublished data). Therefore, we have proposed that pancreatic toxic effects of pravastatin could be caused by statin inhibition of CoQ10 biosynthesis. On the other hand, we and others have hypothesized that insulin signaling in their target tissues (such as muscle) could also be impaired by chronic statin treatment. However, studies relating statins therapy and insulin sensitivity are controversial [206–208]. A meta-analysis by Baker and colleagues shows that while pravastatin improved insulin sensitivity, atorvastatin, simvastatin, and rosuvastatin worsened it [209]. Experimental studies suggest that atorvastatin leads to reduced expression of GLUT4 in adipocytes *in vivo* and *in vitro* [210] and that simvastatin decreases IGF-1 signaling (pAKT, pERK) in muscle cells [211]. Kain et al. [212] showed that myotubes treated with simvastatin and atorvastatin presented impaired insulin signaling pathway and glucose uptake. We have evidence that long-term pravastatin treatment of hypercholesterolemic mice also induces

marked insulin resistance and increased muscle protein degradation (Lorza-Gil et al., unpublished data). Therefore, toxic effects on insulin secreting cells in conjunction with impaired muscle insulin signaling may explain the new onset of diabetes reported in statin-treated subjects.

7. Antioxidant supplement and statins toxicity

The cholesterol biosynthesis pathway generates several products including CoQ10 [213]. CoQ10 is an essential component of the electron transport chain where it acts as an electron carrier [214]. Ubiquinol, the reduced form of ubiquinone, when associated with proteins in the inner mitochondrial membrane, has an important function as a lipophilic antioxidant [215, 216]. CoQ10 also has additional functions such as regeneration of reduced intra- and extracellular forms of ascorbic acid and tocopherol (vitamin E) [217, 218], participation in redox processes associated with PTP opening [219], and regulation of muscle uncoupling proteins [220]. It is also known that the reduced form of ubiquinone occurs in all cellular membranes [221–223] as well as in serum lipoproteins and DNA, protecting them from oxidative damage [224]. CoQ10 content is larger in tissues such as cardiac and skeletal muscles that have high energy demand [223]. Therefore, decreased synthesis of ubiquinone may result in two harmful conditions: (a) insufficient rates of mitochondrial ATP synthesis [225] and (b) decreased mitochondrial antioxidant capacity [49].

Some studies have proposed that statin-induced mitotoxicity may be mediated by diminished CoQ10 content with consequent impairment of mitochondrial respiration [111, 226–234]. On the other hand, our group has provided evidence that under our experimental conditions, the reduction of mitochondrial respiration associated with CoQ10 depletion was mainly due to its free radical scavenging action rather than its electron carrier function. Indeed, it has been demonstrated that incubation of permeabilized rat soleus muscle with simvastatin inhibited both ADP and FCCP-stimulated oxygen consumption supported by complex I or II substrates. Additionally, ubiquinone content was diminished by 40% and the H_2O_2 content was significantly increased. Under these conditions, all of the following compounds, including mevalonate, CoQ10, or L-carnitine protected against H_2O_2 generation but only mevalonate prevented CoQ10 depletion. Thus, independent of CoQ10 levels, L-carnitine prevented the toxic effects of simvastatin. This allows for the conclusion that L-carnitine antioxidant action prevailed in the protection against simvastatin-induced respiratory inhibition [49]. Therefore, it can be concluded that CoQ10 also acted as a free radical scavenger in this mechanism. Accordingly, Kettawan and coworkers previously demonstrated that a decrease in ubiquinone levels in serum, liver, and heart in mice undergoing simvastatin treatment increased lipoperoxidation. Simvastatin also reduced NADPH-CoQ reductase activity, whereas the co-administration of CoQ10 and simvastatin to mice diminished these deleterious effects [235]. Another study revealed that simvastatin reduced mitochondrial CoQ10 levels associated with DNA oxidative damage and reduced ATP synthesis followed by cell death in hepatocytes (HepG2). All of these alterations were reversed by CoQ10 supplementation [236]. Furthermore, it was recently shown that CoQ10 supplementation improved respiratory control in liver mitochondria isolated from rats treated with high

doses of atorvastatin and/or a cholesterol-rich diet [237]. Despite all data correlating CoQ10 depletion with statin toxicity, the efficacy of ubiquinone supplementation in patients with side effects is still under debate [231, 238–240].

Creatine is a guanidine compound synthesized endogenously [241] and widely and safely used as supplement by athletes to increase their performance [242]. The role of creatine on the maintenance of ATP/ADP ratio by activating CK is very well known, but it also exerts other actions. Creatine participates on a protein complex involved in MPT regulation [55, 243, 244] and was firstly mentioned as antioxidant in 1998 [245]. A few years later, Lawler and coworkers showed that this compound was capable of scavenging radicals such as superoxide and peroxynitrite [246]. In our recent work, we showed that diet supplementation with creatine protected *LDLr*^{-/-} mice against pravastatin sensitization to Ca²⁺-induced MPT [166].

L-carnitine stimulates β -oxidation by increasing carnitine palmitoyltransferase 1A mRNA expression. This action prevents mitochondrial oxidative stress induced by free fatty acids, increasing mitochondrial function [22, 247]. Another property of L-carnitine is to bind Fe²⁺ [248] that participates in the mitochondrial oxidative stress involved in MPT [249]. Thus, it is feasible to propose that L-carnitine protects complexes I and II of the respiratory chain against superoxide attack by interacting with 4Fe-4S clusters in these sites. In a previous work performed in PC3 prostate cancer, we showed that L-carnitine and piracetam (a membrane stabilizer) prevented MPT and necrosis induced by simvastatin (60 μ M) [22].

Taken together, these experimental results suggest that ROS generation and mitochondrial oxidative stress play an important role on statins toxicity.

8. Conclusions

Cardiovascular benefits of statins therapy are undoubted and appear to be present across diverse demographic and clinical groups. However, the side effects may affect a minority of patients. In this review, we addressed the cellular and molecular mechanisms related to statin side effects. Mitochondrial oxidative stress seems to be the main cause of toxicity in statin sensitive tissues (**Figure 1**). The levels and consequences of mitochondrial oxidative stress seem to be more deleterious in skeletal muscle. This effect is secondary to: (a) inhibition of electrons flow at the levels of respiratory complexes I, II, and III, and (b) decrease in the levels of CoQ10 due to inhibition of the mevalonate pathway. In association with mitochondrial Ca²⁺ overload due to increased cytosolic free Ca²⁺ concentrations, the PTP may open and trigger cell death. *In vitro* experiments provide evidence that this can be blocked in a concerted manner by L-carnitine plus the membrane stabilizer piracetam. Experiments performed with muscle biopsies taken from hypercholesterolemic mice, chronically treated with pravastatin, show that either CoQ10 or creatine can protect against statin-induced mitochondrial muscle toxicity both *in vitro* and *in vivo*. Statin treatment may also result in pro- or antioxidant actions depending on statin class (lipophilicity), dose, and patient's background. We suggest that mitochondrial oxidative stress caused by statin treatment may be a signal for cellular antioxidant system response (such as

catalase upregulation) possibly explaining the alleged statin antioxidant properties. Together, the experimental evidence presented in this review suggests that statins' detrimental effects could be prevented by antioxidants administration such as CoQ10, L-carnitine, and creatine.

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Conflict of interest statement

The authors declare no conflicts of interest.

Abbreviations

ALT	Alanine transaminase
AST	Aspartate transaminase
CK	Creatine kinase
CoQ10	Coenzyme Q10
Cys A	Cyclosporin A
ER	Endoplasmic reticulum
FCCP	Carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone
FH	Familial hypercholesterolemia
FPP	Farnesyl-pyrophosphate
GGPP	Geranylgeranyl-pyrophosphate
GPP	Geranyl-pyrophosphate
GSH	Glutathione
GSSG	Glutathione oxidized
HMG-CoA	3-Hydroxy-3-methylglutaryl coenzyme-A
IMM	Inner mitochondria membrane

IP ₃ R	Inositol 1,4,5-trisphosphate receptor
MCU	Mitochondrial calcium uniporter
MPT	Mitochondrial permeability transition
NNT	Nicotinamide nucleotide transhydrogenase
OMM	Outer mitochondria membrane
PTP	Permeability transition pore
ROS	Reactive oxygen species
SHR	Spontaneous hypertensive rats
TSST	Thioredoxin
VDAC	Voltage-dependent anion-selective channel

Author details

Estela N.B. Busanello¹, Ana C. Marques¹, Estela Lorza-Gil², Helena C.F. de Oliveira² and Anibal E. Vercesi^{1*}

*Address all correspondence to: anibal@unicamp.br

1 Faculty of Medical Sciences, Department of Clinical Pathology, UNICAMP, SP, Brazil

2 Institute of Biology, Department of Structural and Functional Biology, UNICAMP, SP, Brazil

References

- [1] Brown MS, Goldstein JL. Receptor-mediated endocytosis: Insights from the lipoprotein receptor system. *Proceedings of the National Academy of Sciences of the United States of America*. 1974;**71**:788-792
- [2] Vogt A. The genetics of familial hypercholesterolemia and emerging therapies. *The Application of Clinical Genetics*. 2015;**8**:27-36. DOI: 10.2147/TACG.S44315
- [3] Shepherd J, Cobbe SM, Ford I, Isles CG, Lorimer AR, MacFarlane PW, McKillop JH, Packard CJ. Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. West of Scotland Coronary Prevention Study Group. *The New England Journal of Medicine*. 1995;**333**:1301-1307. DOI: 10.1056/NEJM199511163332001
- [4] Collins R, Armitage J, Parish S, Sleight P, Peto R, HPSC Group. MRC/BHF Heart Protection Study of cholesterol-lowering with simvastatin in 5963 people with diabetes: A randomised placebo-controlled trial. *Lancet*. 2003;**361**:2005-2016. DOI: 10.1016/S0140-6736(03)13636-7

- [5] Gazzerо P, Proto MC, Gangemi G, Malfitano AM, Ciaglia E, Pisanti S, Santoro A, Laezza C, Bifulco M. Pharmacological actions of statins: A critical appraisal in the management of cancer. *Pharmacological Reviews*. 2012;**64**:102-146. DOI: 10.1124/pr.111.004994
- [6] Vaughan CJ, Gotto AM, Basson CT. The evolving role of statins in the management of atherosclerosis. *Journal of the American College of Cardiology*. 2000;**35**:1-10. DOI: 10.1016/S0735-1097(99)00525-2
- [7] Goldstein JL, Brown MS. Regulation of the mevalonate pathway. *Nature*. 1990;**343**:425-430. DOI: 10.1038/343425a0
- [8] Endo A, Kuroda M. Citrinin, an inhibitor of cholesterol synthesis. *The Journal of Antibiotics*. 1976;**29**:841-843. DOI: 10.7164/antibiotics.29.841
- [9] Tanzawa K, Kuroda M, Endo A. Time-dependent, irreversible inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A reductase by the antibiotic citrinin. *Biochimica et Biophysica Acta*. 1977;**488**:97-101
- [10] Endo A, Tsujita Y, Kuroda M, Tanzawa K. Effects of ML-236B on cholesterol metabolism in mice and rats: Lack of hypocholesterolemic activity in normal animals. *Biochimica et Biophysica Acta*. 1979;**575**:266-276
- [11] Endo AJ. Monacolin K. A new hypercholesterolemic agent produced by a monascus species. *Antibiotics*. 1979;**32**:852-854. DOI: 10.7164/antibiotics.32.852
- [12] Alberts AW, Chen J, Kuron G, Hunt V, Huff J, Hoffman C, Rothrock J, Lopez M, Joshua H, Harris E, Patchett A, Monaghan R, Currie S, Stapley E, Albers-Schonberg G, Hensens O, Hirshfield J, Hoogsteen K, Liesch J, Springer J. Mevinolin: A highly potent competitive inhibitor of hydroxymethylglutaryl-coenzyme A reductase and a cholesterol-lowering agent. *Proceedings of the National Academy of Sciences of the United States of America*. 1980;**77**:3957-3961
- [13] Endo A. A historical perspective on the discovery of statins. *Proceedings of the Japan Academy. Series B, Physical and Biological Sciences*. 2010;**86**:484-493. DOI: 10.2183/pjab.86.484
- [14] Endo A. The discovery and development of HMG-CoA reductase inhibitors. *Journal of Lipid Research*. 1992;**33**:1569-1582
- [15] Tobert JA. Lovastatin and beyond: The history of the HMG-CoA reductase inhibitors. *Nature Reviews. Drug Discovery*. 2003;**2**:517-526. DOI: 10.1038/nrd1112: 10.1038/nrd1112
- [16] Saito Y. Pitavastatin: An overview. *Atherosclerosis. Supplements*. 2011;**12**:271-276. DOI: 10.1016/S1567-5688(11)70886-8
- [17] Psaty BM, Furberg CD, Ray WA, Weiss NS. Potential for conflict of interest in the evaluation of suspected adverse drug reactions: Use of cerivastatin and risk of rhabdomyolysis. *Journal of the American Medical Association*. 2004;**292**:2622-2263. DOI: 10.1001/jama.292.21.2622

- [18] Wise J. High intensity statins are associated with greatest reduction in mortality. *British Medical Journal*. 2016;**355**:1-1. DOI: 10.1136/bmj.i6048
- [19] Manfredini V, Biancini GB, Vanzin CS, Dal Vesco AM, Cipriani F, Biasi L, Treméa R, Deon M, Peralba MC, Wajner M, Vargas CR. Simvastatin treatment prevents oxidative damage to DNA in whole blood leukocytes of dyslipidemic type 2 diabetic patients. *Cell Biochemistry and Function*. 2010;**28**:360-366. DOI: 10.1002/cbf.1654
- [20] Li J, Sun YM, Wang LF, Li ZQ, Pan W, Cao HY. Comparison of effects of simvastatin versus atorvastatin on oxidative stress in patients with coronary heart disease. *Clinical Cardiology*. 2010;**33**:222-227. DOI: 10.1002/clc.20724
- [21] Oliveira KA, Zecchin KG, Alberici LC, Castilho RF, Vercesi AE. Simvastatin inducing PC3 prostate cancer cell necrosis mediated by calcineurin and mitochondrial dysfunction. *Journal of Bioenergetics and Biomembranes*. 2008;**40**:307-314. DOI: 10.1007/s10863-008-9155-9
- [22] Costa RA, Fernandes MP, de Souza-Pinto NC, Vercesi AE. Protective effects of l-carnitine and piracetam against mitochondrial permeability transition and PC3 cell necrosis induced by simvastatin. *European Journal of Pharmacology*. 2013;**701**:82-86. DOI: 10.1016/j.ejphar.2013.01.001
- [23] Vercesi AE, Kowaltowski AJ, Oliveira HC, Castilho RF. Mitochondrial Ca²⁺ transport, permeability transition and oxidative stress in cell death: Implications in cardiotoxicity, neurodegeneration and dyslipidemias. *Frontiers in Bioscience*. 2006;**11**:2554-2564. DOI: 10.2741/1990
- [24] Kowaltowski AJ. Alternative mitochondrial functions in cell physiopathology: Beyond ATP production. *Brazilian Journal of Medical and Biological Research*. 2000;**33**:241-250. DOI: 10.1590/S0100-879X2000000200014
- [25] Vercesi AE, Castilho RF, Kowaltowski AJ, Oliveira HC. Mitochondrial energy metabolism and redox state in dyslipidemias. *IUBMB Life*. 2007;**59**:263-268. DOI: 10.1080/15216540601178091
- [26] Figueira TR, Barros MH, Camargo AA, Castilho RF, Ferreira JC, Kowaltowski AJ, Sluse FE, Souza-Pinto NC, Vercesi AE. Mitochondria as a source of reactive oxygen and nitrogen species: From molecular mechanisms to human health. *Antioxidants & Redox Signaling*. 2013;**18**:2029-2074. DOI: 10.1089/ars.2012.4729
- [27] Mitchell P. Coupling of phosphorylation to electron and hydrogen transfer by a chemiosmotic type of mechanism. *Nature*. 1961;**191**:144-148
- [28] Korshunov SS, Skulachev VP, Starkov AA. High protonic potential actuates a mechanism of production of reactive oxygen species in mitochondria. *FEBS Letters*. 1997;**416**:15-18. DOI: 10.1016/S0014-5793(97)01159-9
- [29] Turrens JF. Mitochondrial formation of reactive oxygen species. *The Journal of Physiology*. 2003;**552**:335-344. DOI: 10.1113/jphysiol.2003.049478

- [30] Radi R, Turrens JF, Chang LY, Bush KM, Crapo JD, Freeman BA. Detection of catalase in rat heart mitochondria. *The Journal of Biological Chemistry*. 1991;**266**:22028-22034
- [31] Yin F, Sancheti H, Cadenas E. Silencing of nicotinamide nucleotide transhydrogenase impairs cellular redox homeostasis and energy metabolism in PC12 cell. *Biochimica et Biophysica Acta*. 2012;**1817**:401-409. DOI: 10.1016/j.bbabo.2011.12.004
- [32] Rydström J. Mitochondrial NADPH, transhydrogenase and disease. *Biochimica et Biophysica Acta*. 2006;**1757**:721-726. DOI: 10.1016/j.bbabo.2006.03.010
- [33] Jo SH, Son MK, Koh HJ, Lee SM, Song IH, Kim YO, Lee YS, Jeong KS, Kim WB, Park JW, Song BJ, Huh TL, Huhe TL. Control of mitochondrial redox balance and cellular defense against oxidative damage by mitochondrial NADP⁺-dependent isocitrate dehydrogenase. *The Journal of Biological Chemistry*. 2001;**276**:16168-16176. DOI: 10.1074/jbc.M010120200
- [34] Toledo JC, Augusto O. Connecting the chemical and biological properties of nitric oxide. *Chemical Research in Toxicology*. 2012;**25**:975-989. DOI: 10.1021/tx300042g
- [35] Hamanaka RB, Chandel NS. Mitochondrial reactive oxygen species regulate cellular signaling and dictate biological outcomes. *Trends in Biochemical Sciences*. 2010;**35**:505-513. DOI: 10.1016/j.tibs.2010.04.002
- [36] Dikalov S. Cross talk between mitochondria and NADPH oxidases. *Free Radical Biology & Medicine*. 2011;**51**:1289-1301. DOI: 10.1016/j.freeradbiomed.2011.06.033
- [37] Ristow M. Unraveling the truth about antioxidants: Mitohormesis explains ROS-induced health benefits. *Nature Medicine*. 2014;**20**:709-711. DOI: 10.1038/nm.3624
- [38] Yun J, Finkel T. Mitohormesis. *Cell Metabolism*. 2014;**19**:757-766. DOI: 10.1016/j.cmet.2014.01.011
- [39] Husain K, Hernandez W, Ansari RA, Ferder L. Inflammation, oxidative stress and renin angiotensin system in atherosclerosis. *World Journal of Biological Chemistry*. 2015;**6**:209-217. DOI: 10.4331/wjbc.v6.i3.209
- [40] Liu J, Wang Z. Increased oxidative stress as a selective anticancer therapy. *Oxidative Medicine and Cellular Longevity*. 2015;**2015**:294-303. DOI: 10.1155/2015/294303
- [41] Santilli F, D'Ardes D, Davì G. Oxidative stress in chronic vascular disease: From prediction to prevention. *Vascular Pharmacology*. 2015;**74**:23-37. DOI: 10.1016/j.vph.2015.09.003
- [42] Bhat AH, Dar KB, Anees S, Zargar MA, Masood A, Sofi MA, Ganie SA. Oxidative stress, mitochondrial dysfunction and neurodegenerative diseases: A mechanistic insight. *Biomedicine & Pharmacotherapy*. 2015;**74**:101-110. DOI: 10.1016/j.biopha.2015.07.025
- [43] Ferrari RS, Andrade CF. Oxidative stress and lung ischemia-reperfusion injury. *Oxidative Medicine and Cellular Longevity*. 2015;**2015**:590-987. DOI: 10.1155/2015/590987
- [44] Jones DP. Redox theory of aging. *Redox Biology*. 2015;**5**:71-79. DOI: 10.1016/j.redox.2015.03.004

- [45] Sinha N, Dabla PK. Oxidative stress and antioxidants in hypertension-a current review. *Current Hypertension Reviews*. 2015;**11**:132-142. DOI: 10.2174/1573402111666150529130922
- [46] Maiese K. New insights for oxidative stress and diabetes mellitus. *Oxidative Medicine and Cellular Longevity*. 2015;**2015**:875-961. DOI: 10.1155/2015/875961
- [47] Abdoli N, Heidari R, Azarmi Y, Eghbal MA. Mechanisms of the statins cytotoxicity in freshly isolated rat hepatocytes. *Journal of Biochemical and Molecular Toxicology*. 2013;**27**:287-294. DOI: 10.1002/jbt.21485
- [48] Kwak HB, Thalacker-Mercer A, Anderson EJ, Lin CT, Kane DA, Lee NS, Cortright RN, Bamman MM, Neufer PD. Simvastatin impairs ADP-stimulated respiration and increases mitochondrial oxidative stress in primary human skeletal myotubes. *Free Radical Biology & Medicine*. 2012;**52**:198-207. DOI: 10.1016/j.freeradbiomed.2011.10.449
- [49] La Guardia PG, Alberici LC, Ravagnani FG, Catharino RR, Vercesi AE. Protection of rat skeletal muscle fibers by either L-carnitine or coenzyme Q10 against statins toxicity mediated by mitochondrial reactive oxygen generation. *Frontiers in Physiology*. 2013;**4**:103. DOI: 10.3389/fphys.2013.00103
- [50] Berridge MJ, Bootman MD, Roderick HL. Calcium signalling: Dynamics, homeostasis and remodelling. *Nature Reviews. Molecular Cell Biology*. 2003;**4**:517-529. DOI: 10.1038/nrm1155
- [51] Lehninger AL, Vercesi A, Bababunmi EA. Regulation of Ca²⁺ release from mitochondria by the oxidation-reduction state of pyridine nucleotides. *Proceedings of the National Academy of Sciences of the United States of America*. 1978;**75**:1690-1694
- [52] Bhosale G, Sharpe JA, Sundier SY, Duchen MR. Calcium signaling as a mediator of cell energy demand and a trigger to cell death. *Annals of the New York Academy of Sciences*. 2015;**1350**:107-116. DOI: 10.1111/nyas.12885
- [53] Vercesi AE, Kowaltowski AJ, Grijalba MT, Meinicke AR, Castilho RF. The role of reactive oxygen species in mitochondrial permeability transition. *Bioscience Reports*. 1997;**17**:43-52. DOI: 10.1023/A:1027335217774
- [54] Hunter DR, Haworth RA, Southard JH. Relationship between configuration, function, and permeability in calcium-treated mitochondria. *The Journal of Biological Chemistry*. 1976;**251**:5069-5077
- [55] Kowaltowski AJ, Castilho RF, Vercesi AE. Mitochondrial permeability transition and oxidative stress. *FEBS Letters*. 2001;**495**:12-15. DOI: 10.1016/S0014-5793(01)02316-X
- [56] Grijalba MT, Vercesi AE, Schreier S. Ca²⁺-induced increased lipid packing and domain formation in submitochondrial particles. A possible early step in the mechanism of Ca²⁺-stimulated generation of reactive oxygen species by the respiratory chain. *Biochemistry*. 1999;**38**:13279-13287. DOI: 10.1021/bi9828674
- [57] Javadov S, Kuznetsov A. Mitochondrial permeability transition and cell death: The role of cyclophilin d. *Frontiers in Physiology*. 2013;**4**:76. DOI: 10.3389/fphys.2013.00076

- [58] Lemasters JJ, Nieminen AL, Qian T, Trost LC, Elmore SP, Nishimura Y, Crowe RA, Cascio WE, Bradham CA, Brenner DA, Herman B. The mitochondrial permeability transition in cell death: A common mechanism in necrosis, apoptosis and autophagy. *Biochimica et Biophysica Acta*. 1998;**1366**:177-196. DOI: 10.1016/S0005-2728(98)00112-1
- [59] Bernardi P, Krauskopf A, Basso E, Petronilli V, Blachly-Dyson E, Blalchy-Dyson E, Di Lisa F, Forte MA. The mitochondrial permeability transition from in vitro artifact to disease target. *The FEBS Journal*. 2006;**273**:2077-2099. DOI: 10.1111/j.1742-4658.2006.05213.x
- [60] Halestrap AP, Pasdois P. The role of the mitochondrial permeability transition pore in heart disease. *Biochimica et Biophysica Acta*. 2009;**1787**:1402-1415. DOI: 10.1016/j.bbabbio.2008.12.017
- [61] Kroemer G, Galluzzi L, Brenner C. Mitochondrial membrane permeabilization in cell death. *Physiological Reviews*. 2007;**87**:99-163. DOI: 10.1152/physrev.00013.2006:
- [62] Mbye LH, Singh IN, Carrico KM, Saatman KE, Hall ED. Comparative neuroprotective effects of cyclosporin A and NIM811, a nonimmunosuppressive cyclosporin A analog, following traumatic brain injury. *Journal of Cerebral Blood Flow and Metabolism*. 2009;**29**:87-97. DOI: 10.1038/jcbfm.2008.93
- [63] Russmann S, Kullak-Ublick GA, Grattagliano I. Current concepts of mechanisms in drug-induced hepatotoxicity. *Current Medicinal Chemistry*. 2009;**16**:3041-3053. DOI: 10.2174/092986709788803097
- [64] Starkov AA, Chinopoulos C, Fiskum G. Mitochondrial calcium and oxidative stress as mediators of ischemic brain injury. *Cell Calcium*. 2004;**36**:257-264. DOI: 10.1016/j.ceca.2004.02.012
- [65] Vaseva AV, Marchenko ND, Ji K, Tsirka SE, Holzmann S, Moll UM. p53 opens the mitochondrial permeability transition pore to trigger necrosis. *Cell*. 2012;**149**:1536-1548. DOI: 10.1016/j.cell.2012.05.014
- [66] Alberici LC, Oliveira HC, Patrício PR, Kowaltowski AJ, Vercesi AE. Hyperlipidemic mice present enhanced catabolism and higher mitochondrial ATP-sensitive K⁺ channel activity. *Gastroenterology*. 2006;**131**:1228-1234. DOI: 10.1053/j.gastro.2006.07.021
- [67] Oliveira HC, Cosso RG, Alberici LC, Maciel EN, Salerno AG, Dorigheo GG, Velho JA, de Faria EC, Vercesi AE. Oxidative stress in atherosclerosis-prone mouse is due to low antioxidant capacity of mitochondria. *The FASEB Journal*. 2005;**19**:278-280. DOI: 10.1096/fj.04-2095fje
- [68] Gunter TE, Sheu SS. Characteristics and possible functions of mitochondrial Ca²⁺ transport mechanisms. *Biochimica et Biophysica Acta*. 2009;**1787**:1291-1308. DOI: 10.1016/j.bbabbio.2008.12.011
- [69] Deluca HF, Engstrom GW. Calcium uptake by rat kidney mitochondria. *Proceedings of the National Academy of Sciences of the United States of America*. 1961;**47**:1744-1750

- [70] Vasington FD, Murphy JV. Ca ion uptake by rat kidney mitochondria and its dependence on respiration and phosphorylation. *The Journal of Biological Chemistry*. 1962; **237**:2670-2677
- [71] Baughman JM, Perocchi F, Girgis HS, Plovanich M, Belcher-Timme CA, Sancak Y, Bao XR, Strittmatter L, Goldberger O, Bogorad RL, Koteliensky V, Mootha VK. Integrative genomics identifies MCU as an essential component of the mitochondrial calcium uniporter. *Nature*. 2011;**476**:341-345. DOI: 10.1038/nature10234
- [72] De Stefani D, Raffaello A, Teardo E, Szabo I, Rizzuto R. A forty-kilodalton protein of the inner membrane is the mitochondrial calcium uniporter. *Nature*. 2011;**476**:336-340. DOI: 10.1038/nature10230
- [73] Palty R, Silverman WF, Hershfinkel M, Caporale T, Sensi SL, Parnis J, Nolte C, Fishman D, Shoshan-Barmatz V, Herrmann S, Khananshvil D, Sekler I. NCLX is an essential component of mitochondrial Na⁺/Ca²⁺ exchange. *Proceedings of the National Academy of Sciences of the United States of America*. 2010;**107**:436-441. DOI: 10.1073/pnas.0908099107
- [74] Kang TM, Hilgemann DW. Multiple transport modes of the cardiac Na⁺/Ca²⁺ exchanger. *Nature*. 2004;**427**:544-548. DOI: 10.1038/nature02271
- [75] Hajnóczky G, Csordás G. Calcium signalling: Fishing out molecules of mitochondrial calcium transport. *Current Biology*. 2010;**20**:888-891. DOI: 10.1016/j.cub.2010.09.035
- [76] Docampo R, Vercesi AE. Ca²⁺ transport by coupled *Trypanosoma cruzi* mitochondria in situ. *The Journal of Biological Chemistry*. 1989;**264**:108-111
- [77] Rizzuto R, Brini M, Murgia M, Pozzan T. Microdomains with high Ca²⁺ close to IP₃-sensitive channels that are sensed by neighboring mitochondria. *Science*. 1993;**262**:744-747. DOI: 10.1126/science.8235595
- [78] Pozzan T, Rizzuto R, Volpe P, Meldolesi J. Molecular and cellular physiology of intracellular calcium stores. *Physiological Reviews*. 1994;**74**:595-636
- [79] Rizzuto R, Pinton P, Carrington W, Fay FS, Fogarty KE, Lifshitz LM, Tuft RA, Pozzan T. Close contacts with the endoplasmic reticulum as determinants of mitochondrial Ca²⁺ responses. *Science*. 1998;**280**:1763-1766. DOI: 10.1126/science.280.5370.1763
- [80] Bonfils C, Bec N, Larroque C, Del Rio M, Gongora C, Pugnière M, Martineau P. Cyclophilin A as negative regulator of apoptosis by sequestering cytochrome c. *Biochemical and Biophysical Research Communications*. 2010;**393**:325-330. DOI: 10.1016/j.bbrc.2010.01.135
- [81] Kowaltowski AJ, Vercesi AE, Fiskum G. Bcl-2 prevents mitochondrial permeability transition and cytochrome c release via maintenance of reduced pyridine nucleotides. *Cell Death and Differentiation* 2000;**7**:903-910. DOI: 10.1038/sj.cdd.4400722
- [82] Murphy AN, Bredesen DE, Cortopassi G, Wang E, Fiskum G. Bcl-2 potentiates the maximal calcium uptake capacity of neural cell mitochondria. *Proceedings of the National Academy of Sciences of the United States of America*. 1996;**93**:9893-9898

- [83] Castilho RF, Kowaltowski AJ, Meinicke AR, Bechara EJ, Vercesi AE. Permeabilization of the inner mitochondrial membrane by Ca^{2+} ions is stimulated by t-butyl hydroperoxide and mediated by reactive oxygen species generated by mitochondria. *Free Radical Biology & Medicine*. 1995;**18**:479-486. DOI: 10.1016/0891-5849(94)00166-H
- [84] Castilho RF, Kowaltowski AJ, Vercesi AE. The irreversibility of inner mitochondrial membrane permeabilization by Ca^{2+} plus prooxidants is determined by the extent of membrane protein thiol cross-linking. *Journal of Bioenergetics and Biomembranes*. 1996;**28**:523-529
- [85] Fagian MM, Pereira-da-Silva L, Martins IS, Vercesi AE. Membrane protein thiol cross-linking associated with the permeabilization of the inner mitochondrial membrane by Ca^{2+} plus prooxidants. *The Journal of Biological Chemistry*. 1990;**265**:19955-19960
- [86] Kowaltowski AJ, Netto LE, Vercesi AE. The thiol-specific antioxidant enzyme prevents mitochondrial permeability transition. Evidence for the participation of reactive oxygen species in this mechanism. *The Journal of Biological Chemistry*. 1998;**273**:12766-12769. DOI: 10.1074/jbc.273.21.12766
- [87] Kowaltowski AJ, de Souza-Pinto NC, Castilho RF, Vercesi AE. Mitochondria and reactive oxygen species. *Free Radical Biology & Medicine*. 2009;**47**:333-343. DOI: 10.1016/j.freeradbiomed.2009.05.004
- [88] Vercesi AE, Pereira-da-Silva L. NADP redox state and mitochondrial Ca^{2+} efflux: A controversial issue. *Brazilian Journal of Medical and Biological Research*. 1984;**17**:353-356
- [89] Maciel EN, Kowaltowski AJ, Schwalm FD, Rodrigues JM, Souza DO, Vercesi AE, Wajner M, Castilho RF. NADP redox state and mitochondrial Ca^{2+} efflux: A controversial issue. *Journal of Neurochemistry*. 2004;**90**:1025-1035. DOI: 10.1111/j.1471-4159.2004.02565.x
- [90] Kowaltowski AJ, Castilho RF. Ca^{2+} acting at the external side of the inner mitochondrial membrane can stimulate mitochondrial permeability transition induced by phenylarsine oxide. *Biochimica et Biophysica Acta*. 1997;**1322**:221-229. DOI: 10.1016/S0005-2728(97)00078-9
- [91] Kowaltowski AJ, Castilho RF, Vercesi AE. Opening of the mitochondrial permeability transition pore by uncoupling or inorganic phosphate in the presence of Ca^{2+} is dependent on mitochondrial-generated reactive oxygen species. *FEBS Letters*. 1996;**378**:150-152. DOI: 10.1016/0014-5793(95)01449-7
- [92] Ronchi JA, Figueira TR, Ravagnani FG, Oliveira HC, Vercesi AE, Castilho RF. A spontaneous mutation in the nicotinamide nucleotide transhydrogenase gene of C57BL/6J mice results in mitochondrial redox abnormalities. *Free Radical Biology & Medicine*. 2013;**63**:446-456. DOI: 10.1016/j.freeradbiomed.2013.05.049
- [93] Ronchi JA, Francisco A, Passos LA, Figueira TR, Castilho RF. The contribution of nicotinamide nucleotide transhydrogenase to peroxide detoxification is dependent on the respiratory state and counterbalanced by other sources of NADPH in liver mitochondria. *The Journal of Biological Chemistry*. 2016;**291**:20173-20187. DOI: 10.1074/jbc.M116.730473

- [94] Valle VG, Fagian MM, Parentoni LS, Meinicke AR, Vercesi AE. The participation of reactive oxygen species and protein thiols in the mechanism of mitochondrial inner membrane permeabilization by calcium plus prooxidants. *Archives of Biochemistry and Biophysics*. 1993;**307**:1-7. DOI: 10.1006/abbi.1993.1551
- [95] Castilho RF, Kowaltowski AJ, Meinicke AR, Vercesi AE. Oxidative damage of mitochondria induced by Fe(II)citrate or t-butyl hydroperoxide in the presence of Ca²⁺: Effect of coenzyme Q redox state. *Free Radical Biology & Medicine*. 1995;**18**:55-59
- [96] Parihar A, Parihar MS, Zenebe WJ, Ghafourifar P. Statins lower calcium-induced oxidative stress in isolated mitochondria. *Human & Experimental Toxicology*. 2012;**31**:355-363. DOI: 10.1177/0960327111429141
- [97] Futterman LG, Lemberg L. Statin pleiotropy: Fact or fiction? *American Journal of Critical Care*. 2004;**13**:244-249
- [98] Du R, Zhao XQ, Cai J, Cui B, Wu HM, Ye P. *Journal of Clinical Lipidology*. 2016;**10**:587-593. DOI: 10.1016/j.jacl.2016.01.004
- [99] Zhou Q, Liao JK. Changes in carotid plaque tissue composition in subjects who continued and discontinued statin therapy. *Circulation Journal*. 2010;**74**:818-826. DOI: 10.1253/circj.CJ-10-0110
- [100] Demierre MF, Higgins PD, Gruber SB, Hawk E, Lippman SM. Statins and cancer prevention. *Nature Reviews. Cancer*. 2005;**5**:930-942. DOI: 10.1038/nrc1751
- [101] Steinberg D. The LDL modification hypothesis of atherogenesis: An update. *Journal of Lipid Research*. 2009;**50**(Suppl):S376-S381. DOI: 10.1194/jlr
- [102] Morel DW, DiCorleto PE, Chisolm GM. Endothelial and smooth muscle cells alter low density lipoprotein in vitro by free radical oxidation. *Arteriosclerosis*. 1984;**4**:357-364. DOI: 10.1161/01.ATV.4.4.357
- [103] Lamb DJ, Wilkins GM, Leake DS. The oxidative modification of low density lipoprotein by human lymphocytes. *Atherosclerosis*. 1992;**92**:187-192. DOI: 10.1016/0021-9150(92)90277-N
- [104] Wassmann S, Laufs U, Müller K, Konkol C, Ahlbory K, Bäumer AT, Linz W, Böhm M, Nickenig G. Cellular antioxidant effects of atorvastatin in vitro and in vivo. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2002;**22**:300-305
- [105] Carneado J, Alvarez de Sotomayor M, Perez-Guerrero C, Jimenez L, Herrera MD, Pamies E, Martin-Sanz MD, Stiefel P, Miranda M, Bravo L, Marhuenda E. Simvastatin improves endothelial function in spontaneously hypertensive rats through a superoxide dismutase mediated antioxidant effect. *Journal of Hypertension*. 2002;**20**:429-437
- [106] Ajith TA, Riji T, Anu V. In vitro anti-oxidant and DNA protective effects of the novel 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor rosuvastatin. *Clinical and Experimental Pharmacology and Physiology*. 2008;**35**:625-629. DOI: 10.1111/j.1440-1681.2007.04853.x

- [107] Schupp N, Schmid U, Heidland A, Stopper H. Rosuvastatin protects against oxidative stress and DNA damage in vitro via upregulation of glutathione synthesis. *Atherosclerosis*. 2008;**199**:278-287. DOI: 10.1016/j.atherosclerosis.2007.11.016
- [108] Jahovic N, Gedik N, Ercan F, Sirvanci S, Yüksel M, Sener G, Alican I. Effects of statins on experimental colitis in normocholesterolemic rats. *Scandinavian Journal of Gastroenterology*. 2006;**41**:954-962. DOI: 10.1080/00365520600554444
- [109] Velho JA, Okanobo H, Degasperis GR, Matsumoto MY, Alberici LC, Cosso RG, Oliveira HC, Vercesi AE. Statins induce calcium-dependent mitochondrial permeability transition. *Toxicology*. 2006;**219**:124-132. DOI: 10.1016/j.tox.2005.11.007
- [110] Menter DG, Ramsauer VP, Harirforoosh S, Chakraborty K, Yang P, Hsi L, Newman RA, Krishnan K. Differential effects of pravastatin and simvastatin on the growth of tumor cells from different organ sites. *PLoS One*. 2011;**6**:28813. DOI: 10.1371/journal.pone.0028813
- [111] Thibault A, Samid D, Tompkins AC, Figg WD, Cooper MR, Hohl RJ, Trepel J, Liang B, Patronas N, Venzon DJ, Reed E, Myers CE. Phase I study of lovastatin, an inhibitor of the mevalonate pathway, in patients with cancer. *Clinical Cancer Research*. 1996;**2**:483-491
- [112] Larner J, Jane J, Laws E, Packer R, Myers C, Shaffrey M. A phase I-II trial of lovastatin for anaplastic astrocytoma and glioblastoma multiforme. *American Journal of Clinical Oncology*. 1998;**21**:579-583
- [113] Hindler K, Cleeland CS, Rivera E, Collard CD. The role of statins in cancer therapy. *The Oncologist*. 2006;**11**:306-315. DOI: 10.1634/theoncologist.11-3-306
- [114] Matuszewicz L, Meissner J, Toporkiewicz M, Sikorski AF. The effect of statins on cancer cells—review. *Tumour Biology*. 2015;**36**:4889-4904. DOI: 10.1007/s13277-015-3551-7
- [115] Ke Zhou C, Check DP, Lortet-Tieulent J, Laversanne M, Jemal A, Ferlay J, Bray F, Cook MB, Devesa SS. Prostate cancer incidence in 43 populations worldwide: An analysis of time trends overall and by age group. *International Journal of Cancer*. 2015;**6**:1388-1400. DOI: 10.1002/ijc.29894
- [116] Marcella SW, David A, Ohman-Strickland PA, Carson J, Rhoads GG. Statin use and fatal prostate cancer: A matched case-control study. *Cancer*. 2012;**118**:4046-4052. DOI: 10.1002/cncr.26720
- [117] Mondul AM, Weinstein SJ, Virtamo J, Albanes D. Serum total and HDL cholesterol and risk of prostate cancer. *Cancer Causes & Control* 2011;**22**:1545-1552. DOI: 10.1007/s10552-011-9831-7
- [118] Konstantinopoulos PA, Karamouzis MV, Papavassiliou AG. Post-translational modifications and regulation of the RAS superfamily of GTPases as anticancer targets. *Nature Reviews. Drug Discovery*. 2007;**6**:541-555. DOI: 10.1038/nrd2221
- [119] Park YH, Seo SY, Lee E, Ku JH, Kim HH, Kwak C. Simvastatin induces apoptosis in castrate resistant prostate cancer cells by deregulating nuclear factor- κ B pathway. *The Journal of Urology*. 2013;**189**:1547-1552. DOI: 10.1016/j.juro.2012.10.030

- [120] Ghosh PM, Ghosh-Choudhury N, Moyer ML, Mott GE, Thomas CA, Foster BA, Greenberg NM, Kreisberg JL. Role of RhoA activation in the growth and morphology of a murine prostate tumor cell line. *Oncogene*. 1999;**18**:4120-4130. DOI: 10.1038/sj.onc.1202792
- [121] Peng X, Li W, Yuan L, Mehta RG, Kopelovich L, McCormick DL. Inhibition of proliferation and induction of autophagy by atorvastatin in PC3 prostate cancer cells correlate with downregulation of Bcl2 and upregulation of miR-182 and p21. *PLoS One*. 2013;**8**:e70442. DOI: 10.1371/journal.pone.0070442
- [122] Marcelli M, Cunningham GR, Haidacher SJ, Padayatty SJ, Sturgis L, Kagan C, Denner L. Caspase-7 is activated during lovastatin-induced apoptosis of the prostate cancer cell line LNCaP. *Cancer Research*. 1998;**58**:76-83
- [123] Goc A, Kochuparambil ST, Al-Husein B, Al-Azayzih A, Mohammad S, Somanath PR. Simultaneous modulation of the intrinsic and extrinsic pathways by simvastatin in mediating prostate cancer cell apoptosis. *BMC Cancer*. 2012;**12**:409. DOI: 10.1186/1471-2407-12-409
- [124] Hoque A, Chen H, Xu XC. Statin induces apoptosis and cell growth arrest in prostate cancer cells. *Cancer Epidemiology, Biomarkers & Prevention*. 2008;**17**:88-94. DOI: 10.1158/1055-9965.EPI-07-0531
- [125] Li YC, Park MJ, Ye SK, Kim CW, Kim YN. Elevated levels of cholesterol-rich lipid rafts in cancer cells are correlated with apoptosis sensitivity induced by cholesterol-depleting agents. *The American Journal of Pathology*. 2006;**168**:1107-1118. quiz 1404-1105. DOI: 10.2353/ajpath.2006.050959
- [126] Zhuang L, Kim J, Adam RM, Solomon KR, Freeman MR. Cholesterol targeting alters lipid raft composition and cell survival in prostate cancer cells and xenografts. *The Journal of Clinical Investigation*. 2005;**115**:959-968. DOI: 10.1172/JCI19935
- [127] Hu M, Cheung BM, Tomlinson B. Safety of statins: An update. *Therapeutic Advances in Drug Safety*. 2012;**3**:133-144. DOI: 10.1177/2042098612439884
- [128] Evans M, Rees A. The myotoxicity of statins. *Current Opinion in Lipidology*. 2002;**13**:415-420
- [129] Andrade SE, Walker AM, Gottlieb LK, Hollenberg NK, Testa MA, Saperia GM, Platt R. Discontinuation of antihyperlipidemic drugs—do rates reported in clinical trials reflect rates in primary care settings? *The New England Journal of Medicine*. 1995;**332**:1125-1131. DOI: 10.1056/NEJM199504273321703
- [130] Sung JC, Nichol MB, Venturini F, Bailey KL, McCombs JS, Cody M. Factors affecting patient compliance with antihyperlipidemic medications in an HMO population. *The American Journal of Managed Care*. 1998;**4**:1421-1430
- [131] Stojaković N, Igić R. Simvastatin-Induced nocturnal leg pain disappears with pravastatin substitution. *Srpski Arhiv za Celokupno Lekarstvo*. 2013;**141**:387-389. DOI: 10.2298/SARH1306387S
- [132] Kowaltowski AJ, Vercesi AE. Mitochondrial damage induced by conditions of oxidative stress. *Free Radical Biology and Medicine*. 1999;**26**:463-471. DOI: S0891-5849(98)00216-0

- [133] Kaufmann P, Török M, Zahno A, Waldhauser KM, Brecht K, Krähenbühl S. Toxicity of statins on rat skeletal muscle mitochondria. *Cellular and Molecular Life Sciences*. 2006;**63**:2415-2425. DOI: 10.1007/s00018-006-6235-z
- [134] Paim BA, Velho JA, Castilho RF, Oliveira HC, Vercesi AE. Oxidative stress in hypercholesterolemic LDL (low-density lipoprotein) receptor knockout mice is associated with low content of mitochondrial NADP-linked substrates and is partially reversed by citrate replacement. *Free Radical Biology & Medicine*. 2008;**44**:444-451. DOI: 10.1016/j.freeradbiomed.2007.10.005
- [135] Ishibashi S, Brown MS, Goldstein JL, Gerard RD, Hammer RE, Herz J. Hypercholesterolemia in low density lipoprotein receptor knockout mice and its reversal by adenovirus-mediated gene delivery. *The Journal of Clinical Investigation*. 1993;**92**:883-893. DOI: 10.1172/JCI116663
- [136] Nakahara K, Yada T, Kuriyama M, Osame M. Cytosolic Ca²⁺ increase and cell damage in L6 rat myoblasts by HMG-CoA reductase inhibitors. *Biochemical and Biophysical Research Communications*. 1994;**202**:1579-1585. DOI: 10.1006/bbrc.1994.2112
- [137] Pierno S, De Luca A, Liantonio A, Camerino C, Conte Camerino D. Effects of HMG-CoA reductase inhibitors on excitation-contraction coupling of rat skeletal muscle. *European Journal of Pharmacology*. 1999;**364**:43-48. DOI: 10.1016/S0014-2999(98)00817-6
- [138] Sirvent P, Mercier J, Vassort G, Lacampagne A. Simvastatin triggers mitochondria-induced Ca²⁺ signaling alteration in skeletal muscle. *Biochemical and Biophysical Research Communications*. 2005;**329**:1067-1075. DOI: 10.1016/j.bbrc.2005.02.070
- [139] Hattori T, Saito K, Takemura M, Ito H, Ohta H, Wada H, Sei Y, Kawamura M, Seishima M. Statin-induced Ca(2+) release was increased in B lymphocytes in patients who showed elevated serum creatine kinase during statin treatment. *Journal of Atherosclerosis and Thrombosis*. 2009;**16**:870-877. DOI: 10.5551/jat.2048
- [140] Flint DH, Tuminello JF, Emptage MH. The inactivation of Fe-S cluster containing hydro-lyases by superoxide. *The Journal of Biological Chemistry*. 1993;**268**:22369-22376
- [141] Radi R, Rodriguez M, Castro L, Telleri R. Inhibition of mitochondrial electron transport by peroxynitrite. *Archives of Biochemistry and Biophysics*. 1994;**308**:89-95. DOI: 10.1006/abbi.1994.1013
- [142] Fridovich I. Superoxide radical and superoxide dismutases. *Annual Review of Biochemistry*. 1995;**64**:97-112. DOI: 10.1146/annurev.bi.64.070195.000525
- [143] Bouton C, Raveau M, Drapier JC. Modulation of iron regulatory protein functions. Further insights into the role of nitrogen- and oxygen-derived reactive species. *The Journal of Biological Chemistry*. 1996;**271**:2300-2306
- [144] Demicheli V, Quijano C, Alvarez B, Radi R. Inactivation and nitration of human superoxide dismutase (SOD) by fluxes of nitric oxide and superoxide. *Free Radical Biology & Medicine*. 2007;**42**:1359-1368. DOI: 10.1016/j.freeradbiomed.2007.01.034

- [145] Panov A, Dikalov S, Shalbuyeva N, Taylor G, Sherer T, Greenamyre JT. Rotenone model of Parkinson disease: Multiple brain mitochondria dysfunctions after short term systemic rotenone intoxication. *The Journal of Biological Chemistry*. 2005;**280**:42026-42035. DOI: 10.1074/jbc.M508628200
- [146] Gülçin I. Antioxidant and antiradical activities of L-carnitine. *Life Sciences*. 2006;**78**:803-811. DOI: 10.1016/j.lfs.2005.05.103
- [147] Sirvent P, Fabre O, Bordenave S, Hillaire-Buys D, Raynaud De Mauverger E, Lacampagne A, Mercier J. Muscle mitochondrial metabolism and calcium signaling impairment in patients treated with statins. *Toxicology and Applied Pharmacology*. 2012;**259**:263-268. DOI: 10.1016/j.taap.2012.01.008
- [148] Schirris TJ, Renkema GH, Ritschel T, Voermans NC, Bilos A, van Engelen BG, Brandt U, Koopman WJ, Beyrath JD, Rodenburg RJ, Willems PH, Smeitink JA, Russel FG. Statin-induced myopathy is associated with mitochondrial complex III inhibition. *Cell Metabolism*. 2015;**22**:399-407. DOI: 10.1016/j.cmet.2015.08.002
- [149] Bruckert E, Hayem G, Dejager S, Yau C, Bégaud B. Mild to moderate muscular symptoms with high-dosage statin therapy in hyperlipidemic patients—the PRIMO study. *Cardiovascular Drugs and Therapy*. 2005;**19**:403-414. DOI: 10.1007/s10557-005-5686-z
- [150] Stroes ES, Thompson PD, Corsini A, Vladutiu GD, Raal FJ, Ray KK, Roden M, Stein E, Tokgözoğlu L, Nordestgaard BG, Bruckert E, De Backer G, Krauss RM, Laufs U, Santos RD, Hegele RA, Hovingh GK, Leiter LA, Mach F, März W, Newman CB, Wiklund O, Jacobson TA, Catapano AL, Chapman MJ, Ginsberg HN. Statin-associated muscle symptoms: Impact on statin therapy-European atherosclerosis society consensus panel statement on assessment, aetiology and management. EASC panel. *European Heart Journal*. 2015;**36**:1012-1022. DOI: 10.1093/eurheartj/ehv043
- [151] Trøseid M, Henriksen OA, Lindal S. Statin-associated myopathy with normal creatine kinase levels. Case report from a Norwegian family. *Acta Pathologica, Microbiologica et Immunologica Scandinavica*. 2005;**113**:635-637. DOI: 10.1111/j.1600-0463.2005.apm_270.x
- [152] Draeger A, Monastyrskaya K, Mohaupt M, Hoppeler H, Savolainen H, Allemann C, Babiychuk EB. Statin therapy induces ultrastructural damage in skeletal muscle in patients without myalgia. *The Journal of Pathology*. 2006;**210**:94-102. DOI: 10.1002/path.2018
- [153] Taha DA, De Moor CH, Barrett DA, Gershkovich P. Translational insight into statin-induced muscle toxicity: From cell culture to clinical studies. *Translational Research*. 2014;**164**:85-109. DOI: 10.1016/j.trsl.2014.01.013
- [154] Tomaszewski M, Stępień KM, Tomaszewska J, Czuczwar SJ. Statin-induced myopathies. *Pharmacological Reports*. 2011;**63**:859-866
- [155] Phillips PS, Haas RH, Bannykh S, Hathaway S, Gray NL, Kimura BJ, Vladutiu GD, England JD. Statin-associated myopathy with normal creatine kinase levels. SMCR Center. *Annals of Internal Medicine*. 2002;**137**:581-585. DOI: 10.7326/0003-4819-137-7-200210010-00009

- [156] Gambelli S, Dotti MT, Malandrini A, Mondelli M, Stromillo ML, Gaudiano C, Federico A. Mitochondrial alterations in muscle biopsies of patients on statin therapy. *Journal of Submicroscopic Cytology and Pathology*. 2004;**36**:85-89
- [157] Schick BA, Laaksonen R, Frohlich JJ, Päivä H, Lehtimäki T, Humphries KH, Côté HC. Decreased skeletal muscle mitochondrial DNA in patients treated with high-dose simvastatin. *Clinical Pharmacology and Therapeutics*. 2007;**81**:650-653. DOI: 10.1038/sj.clpt.6100124
- [158] Masters BA, Palmoski MJ, Flint OP, Gregg RE, Wang-Iverson D, Durham SK. In vitro myotoxicity of the 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, pravastatin, lovastatin, and simvastatin, using neonatal rat skeletal myocytes. *Toxicology and Applied Pharmacology*. 1995;**131**:163-174. DOI: 10.1006/taap.1995.1058
- [159] Pierno S, Didonna MP, Cippone V, De Luca A, Pisoni M, Frigeri A, Nicchia GP, Svelto M, Chiesa G, Sirtori C, Scanziani E, Rizzo C, De Vito D, Conte Camerino D. Effects of chronic treatment with statins and fenofibrate on rat skeletal muscle: A biochemical, histological and electrophysiological study. *British Journal of Pharmacology*. 2006;**149**:909-919. DOI: 10.1038/sj.bjp.0706917
- [160] Chatzizisis YS, Koskinas KC, Misirli G, Vaklavas C, Hatzitolios A, Giannoglou GD. Risk factors and drug interactions predisposing to statin-induced myopathy: Implications for risk assessment, prevention and treatment. *Drug Safety*. 2010;**33**:171-187. DOI: 10.2165/11319380-000000000-00000
- [161] Generaux GT, Bonomo FM, Johnson M, Doan KM. Impact of SLCO1B1 (OATP1B1) and ABCG2 (BCRP) genetic polymorphisms and inhibition on LDL-C lowering and myopathy of statins. *Xenobiotica*. 2011;**41**:639-651. DOI: 10.3109/00498254.2011.562566
- [162] Voora D, Shah SH, Spasojevic I, Ali S, Reed CR, Salisbury BA, Ginsburg GS. The SLCO1B1*5 genetic variant is associated with statin-induced side effects. *Journal of the American College of Cardiology*. 2009;**54**:1609-1616. DOI: 10.1016/j.jacc.2009.04.053
- [163] Donnelly LA, Doney AS, Tavendale R, Lang CC, Pearson ER, Colhoun HM, McCarthy MI, Hattersley AT, Morris AD, Palmer CN. Common nonsynonymous substitutions in SLCO1B1 predispose to statin intolerance in routinely treated individuals with type 2 diabetes: A go-DARTS study. *Clinical Pharmacology and Therapeutics*. 2011;**89**:210-216. DOI: 10.1038/clpt.2010.255
- [164] Cornachione AS, Benedini-Elias PC, Polizello JC, Carvalho LC, Mattiello-Sverzut AC. Characterization of fiber types in different muscles of the hindlimb in female weanling and adult Wistar rats. *Acta Histochemica et Cytochemica*. 2011;**44**:43-50. DOI: 10.1267/ahc.10031
- [165] Ji LL, Fu R, Mitchell EW. Glutathione and antioxidant enzymes in skeletal muscle: Effects of fiber type and exercise intensity. *Journal of Applied Physiology*. 1992;**73**:1854-1859
- [166] Busanello ENB, Marques AC, Lander N, de Oliveira DN, Catharino RR, Oliveira HCF, Vercesi AE. Pravastatin chronic treatment sensitizes hypercholesterolemic mice muscle

- to mitochondrial permeability transition: Protection by creatine or coenzyme Q10. *Frontiers in Pharmacology*. 2017;**8**:185. DOI: 10.3389/fphar.2017.00185
- [167] Waclawik AJ, Lindal S, Engel AG. Experimental lovastatin myopathy. *Journal of Neuropathology and Experimental Neurology*. 1993;**52**:542-549
- [168] Westwood FR, Bigley A, Randall K, Marsden AM, Scott RC. Statin-induced muscle necrosis in the rat: Distribution, development, and fibre selectivity. *Toxicologic Pathology*. 2005;**33**:246-257. DOI: 10.1080/01926230590908213
- [169] Westwood FR, Scott RC, Marsden AM, Bigley A, Randall K. Rosuvastatin: Characterization of induced myopathy in the rat. *Toxicologic Pathology*. 2008;**36**:345-352. DOI: 10.1177/0192623307311412
- [170] Schaefer WH, Lawrence JW, Loughlin AF, Stoffregen DA, Mixson LA, Dean DC, Raab CE, NX Y, Lankas GR, Frederick CB. Evaluation of ubiquinone concentration and mitochondrial function relative to cerivastatin-induced skeletal myopathy in rats. *Toxicology and Applied Pharmacology*. 2004;**194**:10-23. DOI: 10.1016/j.taap.2003.08.013
- [171] Seachrist JL, Loi CM, Evans MG, Criswell KA, Rothwell CE. Roles of exercise and pharmacokinetics in cerivastatin-induced skeletal muscle toxicity. *Toxicological Sciences*. 2005;**88**:551-561
- [172] Rasola A, Sciacovelli M, Pantic B, Bernardi P. Signal transduction to the permeability transition pore. *FEBS Letters*. 2010;**584**:1989-1996. DOI: 10.1016/j.febslet.2010.02.022
- [173] Björnsson E, Jacobsen EI, Kalaitzakis E. Hepatotoxicity associated with statins: Reports of idiosyncratic liver injury post-marketing. *Journal of Hepatology*. 2012;**56**:374-380. DOI: 10.1016/j.jhep.2011.07.023
- [174] Perdices EV, Medina-Cáliz I, Hernando S, Ortega A, Martín-Ocaña F, Navarro JM, Peláez G, Castiella A, Hallal H, Romero-Gómez M, González-Jiménez A, Robles-Díaz M, Lucena MI, Andrade RJ. Hepatotoxicity associated with statin use: Analysis of the cases included in the Spanish Hepatotoxicity Registry. *Revista Española de Enfermedades Digestivas*. 2014;**106**:246-254
- [175] Carrascosa MF, Salcines-Caviedes JR, Lucena MI, Andrade RJ. Acute liver failure following atorvastatin dose escalation: is there a threshold dose for idiosyncratic hepatotoxicity? *Journal of Hepatology*. 2015;**62**:751-752. DOI: 10.1016/j.jhep.2014.11.019
- [176] Medina-Caliz I, Robles-Díaz M, García-Muñoz B, Stephens C, Ortega-Alonso A, García-Cortés M, González-Jiménez A, Sanabria-Cabrera JA, Moreno I, Fernández MC, Romero-Gómez M, Navarro JM, Barriocanal AM, Montane E, Hallal H, Blanco S, Soriano G, Roman EM, Gómez-Domínguez E, Castiella A, Zapata EM, Jiménez-Pérez M, Moreno JM, Aldea-Perona A, Hernández-Guerra M, Prieto M, Zoubek ME, Kaplowitz N, Lucena MI, Andrade RJ. Definition and risk factors for chronicity following acute idiosyncratic drug-induced liver injury. SD registry. *Journal of Hepatology*. 2016;**65**:532-542. DOI: 10.1016/j.jhep.2016.05.003

- [177] Goldberg AC, Sapre A, Liu J, Capece R, Mitchel YB, Group ES. Efficacy and safety of ezetimibe coadministered with simvastatin in patients with primary hypercholesterolemia: a randomized, double-blind, placebo-controlled trial. *Mayo Clinic Proceedings*. 2004;**79**:620-629. DOI: 10.1016/S0025-6196(11)62283-0
- [178] Björnsson ES. Hepatotoxicity of statins and other lipid-lowering agents. *Liver International*. 2017;**37**:173-178. DOI: 10.1111/liv.13308
- [179] Andrade RJ, Lucena MI, Fernández MC, Pelaez G, Pachkoria K, García-Ruiz E, García-Muñoz B, González-Grande R, Pizarro A, Durán JA, Jiménez M, Rodrigo L, Romero-Gomez M, Navarro JM, Planas R, Costa J, Borrás A, Soler A, Salmerón J, Martín-Vivaldi R. SGfSoD-IL disease. Drug-induced liver injury: An analysis of 461 incidences submitted to the Spanish registry over a 10-year period. *Gastroenterology*. 2005;**129**:512-521. DOI: 10.1016/j.gastro.2005.05.006
- [180] van Heyningen C. Drug-induced acute autoimmune hepatitis during combination therapy with atorvastatin and ezetimibe. *Annals of Clinical Biochemistry* 2005;**42**:402-404. DOI: 10.1258/0004563054890105
- [181] Chalasani N, Fontana RJ, Bonkovsky HL, Watkins PB, Davern T, Serrano J, Yang H, Rochon J. DILIN. Causes, clinical features, and outcomes from a prospective study of drug-induced liver injury in the United States. *Gastroenterology*. 2008;**135**:1924-1934. 1934.e1921-1924. DOI: 10.1053/j.gastro.2008.09.011
- [182] Russo MW, Scobey M, Bonkovsky HL. Drug-induced liver injury associated with statins. *Seminars in Liver Disease*. 2009;**29**:412-422. DOI: 10.1055/s-0029-1240010
- [183] Lucena MI, Kaplowitz N, Hallal H, Castiella A, García-Bengoechea M, Otazua P, Berenguer M, Fernandez MC, Planas R, Andrade RJ. Recurrent drug-induced liver injury (DILI) with different drugs in the Spanish Registry: The dilemma of the relationship to autoimmune hepatitis. *Journal of Hepatology*. 2011;**55**:820-827. DOI: 10.1016/j.jhep.2010.12.041
- [184] Reuben A, Koch DG, Lee WM, ALFS Group. Drug-induced acute liver failure: results of a U.S. multicenter, prospective study. *Hepatology*. 2010;**52**:2065-2076. DOI: 10.1002/hep.23937
- [185] Russo MW, Hoofnagle JH, Gu J, Fontana RJ, Barnhart H, Kleiner DE, Chalasani N, Bonkovsky HL. Spectrum of statin hepatotoxicity: Experience of the drug-induced liver injury network. *Hepatology*. 2014;**60**:679-686. DOI: 10.1002/hep.27157
- [186] Tzefos M, Olin JL. 3-hydroxyl-3-methylglutaryl coenzyme A reductase inhibitor use in chronic liver disease: A therapeutic controversy. *Journal of Clinical Lipidology*. 2011;**5**:450-459. DOI: 10.1016/j.jacl.2011.06.013
- [187] Tolman KG. Defining patient risks from expanded preventive therapies. *The American Journal of Cardiology*. 2000;**85**:15-19. DOI: 10.1016/S0002-9149(00)00946-2
- [188] Chalasani N. Statins and hepatotoxicity: focus on patients with fatty liver. *Hepatology*. 2005;**41**:690-695. DOI: 10.1002/hep.20671

- [189] Cohen DE, Anania FA, Chalasani N. NLASSTFLE panel. An assessment of statin safety by hepatologists. *The American Journal of Cardiology*. 2006;**97**:77C-81C. DOI: 10.1016/j.amjcard.2005.12.014
- [190] McKenney JM. An assessment of statin safety. *The American Journal of Managed Care*. 2006;**12**:S310-S317
- [191] Aguirre L, Hijona E, Macarulla MT, Gracia A, Larrechi I, Bujanda L, Hijona L, Portillo MP. Several statins increase body and liver fat accumulation in a model of metabolic syndrome. *Journal of Physiology and Pharmacology*. 2013;**64**:281-288
- [192] Herrick C, Bahrainy S, Gill EA. Statins and the liver. *Endocrinology and Metabolism Clinics of North America*. 2016;**45**:117-128. DOI: 10.1016/j.ecl.2015.09.008
- [193] Bhardwaj SS, Chalasani N. Lipid-lowering agents that cause drug-induced hepatotoxicity. *Clinics in Liver Disease*. 2007;**11**:597-613, vii. DOI: 10.1016/j.cld.2007.06.010
- [194] Parra JL, Reddy KR. Hepatotoxicity of hypolipidemic drugs. *Clinics in Liver Disease*. 2003;**7**:415-433
- [195] Souk K, Al-Badri M, Azar ST. The safety and benefit of statins in liver cirrhosis: A review. *Experimental and Clinical Endocrinology & Diabetes*. 2015;**123**:577-580. DOI: 10.1055/s-0035-1564093
- [196] MacDonald JS, Gerson RJ, Kornbrust DJ, Kloss MW, Prahalada S, Berry PH, Alberts AW, Bokelman DL. Preclinical evaluation of lovastatin. *The American Journal of Cardiology*. 1988;**62**:16J-27J
- [197] Nakad A, Bataille L, Hamoir V, Sempoux C, Horsmans Y. Atorvastatin-induced acute hepatitis with absence of cross-toxicity with simvastatin. *Lancet*. 1999;**353**:1763-1764. DOI: 10.1016/S0140-6736(99)00569-3
- [198] Argo CK, Loria P, Caldwell SH, Lonardo A. Statins in liver disease: A molehill, an iceberg, or neither? *Hepatology*. 2008;**48**:662-669. DOI: 10.1002/hep.22402
- [199] Lewis JH, Mortensen ME, Zweig S, Fusco MJ, Medoff JR, Belder R. PiCLDS investigators. Efficacy and safety of high-dose pravastatin in hypercholesterolemic patients with well-compensated chronic liver disease: Results of a prospective, randomized, double-blind, placebo-controlled, multicenter trial. *Hepatology*. 2007;**46**:1453-1463. DOI: 10.1002/hep.21848
- [200] Sattar NA, Ginsberg H, Ray K, Chapman MJ, Arca M, Aversa M, Betteridge DJ, Bhatnagar D, Biliannou E, Carmena R, Ceška R, Corsini A, Erbel R, Flynn PD, Garcia-Moll X, Gumprecht J, Ishibashi S, Jambart S, Kastelein JJ, Maher V, da Silva PM, Masana L, Odawara M, Pedersen TR, Rotella CM, Salti I, Teramoto T, Tokgozoglu L, Toth PP, Valensi P, Vergès B. The use of statins in people at risk of developing diabetes mellitus: evidence and guidance for clinical practice. *Atherosclerosis*. 2014;**15**(Suppl):1-15. DOI: 10.1016/j.atherosclerosissup.2014.04.001
- [201] Cederberg H, Stančáková A, Yaluri N, Modi S, Kuusisto J, Laakso M. Increased risk of diabetes with statin treatment is associated with impaired insulin sensitivity and

- insulin secretion: a 6 year follow-up study of the METSIM cohort. *Diabetologia*. 2015; **58**:1109-1117. DOI: 10.1007/s00125-015-3528-5
- [202] Betteridge DJ, Carmena R. The diabetogenic action of statins—mechanisms and clinical implications. *Nature Reviews. Endocrinology*. 2016;**12**:99-110. DOI: 10.1038/nrendo.2015.194
- [203] Chan DC, Pang J, Watts GF. Pathogenesis and management of the diabetogenic effect of statins: A role for adiponectin and coenzyme Q10? *Current Atherosclerosis Reports*. 2015;**17**:472. DOI: 10.1007/s11883-014-0472-7
- [204] Thakker D, Nair S, Pagada A, Jamdade V, Malik A. Statin use and the risk of developing diabetes: A network meta-analysis. *Pharmacoepidemiology and Drug Safety*. 2016; **25**:1131-1149. DOI: 10.1002/pds.4020
- [205] Lorza-Gil E, Salerno AG, Wanschel AC, Vettorazzi JF, Ferreira MS, Rentz T, Catharino RR, Oliveira HC. Chronic use of pravastatin reduces insulin exocytosis and increases β -cell death in hypercholesterolemic mice. *Toxicology*. 2016;**344-346**:42-52. DOI: 10.1016/j.tox.2015.12.007
- [206] Koh KK, Quon MJ, Han SH, Lee Y, Kim SJ, Park JB, Shin EK. Differential metabolic effects of pravastatin and simvastatin in hypercholesterolemic patients. *Atherosclerosis*. 2009;**204**:483-490. DOI: 10.1016/j.atherosclerosis.2008.09.021
- [207] Muscogiuri G, Sarno G, Gastaldelli A, Savastano S, Ascione A, Colao A, Orio F. The good and bad effects of statins on insulin sensitivity and secretion. *Endocrine Research*. 2014;**39**:137-143. DOI: 10.3109/07435800.2014.952018
- [208] Benes LB, Bassi NS, Davidson MH. The risk of hepatotoxicity, new onset diabetes and rhabdomyolysis in the era of high-intensity statin therapy: Does statin type matter? *Progress in Cardiovascular Diseases*. 2016;**59**:145-152. DOI: 10.1016/j.pcad.2016.08.001
- [209] Baker WL, Talati R, White CM, Coleman CI. Differing effect of statins on insulin sensitivity in non-diabetics: a systematic review and meta-analysis. *Diabetes Research and Clinical Practice*. 2010;**87**:98-107. DOI: 10.1016/j.diabres.2009.10.008
- [210] Nakata M, Nagasaka S, Kusaka I, Matsuoka H, Ishibashi S, Yada T. Effects of statins on the adipocyte maturation and expression of glucose transporter 4 (SLC2A4): Implications in glycaemic control. *Diabetologia*. 2006;**49**:1881-1892. DOI: 10.1007/s00125-006-0269-5
- [211] Ogura T, Tanaka Y, Nakata T, Namikawa T, Kataoka H, Ohtsubo YJ. Simvastatin reduces insulin-like growth factor-1 signaling in differentiating C2C12 mouse myoblast cells in an HMG-CoA reductase inhibition-independent manner. *Toxicological Sciences*. 2007;**32**:57-67. DOI: 10.2131/jts.32.57
- [212] Kain V, Kapadia B, Misra P, Saxena U. Simvastatin may induce insulin resistance through a novel fatty acid mediated cholesterol independent mechanism. *Scientific Reports*. 2015;**5**:13823. DOI: 10.1038/srep13823
- [213] Löw P, Andersson M, Edlund C, Dallner G. Effects of mevinolin treatment on tissue dolichol and ubiquinone levels in the rat. *Biochimica et Biophysica Acta*. 1992;**1165**:102-109

- [214] Ernster L, Dallner G. Biochemical, physiological and medical aspects of ubiquinone function. *Biochimica et Biophysica Acta*. 1995;**1271**:195-204
- [215] James AM, Smith RA, Murphy MP. Antioxidant and prooxidant properties of mitochondrial coenzyme Q. *Archives of Biochemistry and Biophysics*. 2004;**423**:47-56
- [216] Crane FL, Navas P. The diversity of coenzyme Q function. *Molecular Aspects of Medicine*. 1997;**18**(Suppl):S1-S6
- [217] Arroyo A, Navarro F, Gómez-Díaz C, Crane FL, Alcaín FJ, Navas P, Villalba JM. Interactions between ascorbyl free radical and coenzyme Q at the plasma membrane. *Journal of Bioenergetics and Biomembranes*. 2000;**32**:199-210
- [218] Constantinescu A, Maguire JJ, Packer L. Interactions between ubiquinones and vitamins in membranes and cells. *Molecular Aspects of Medicine*. 1994;**15**(Suppl):s57-s65
- [219] Kowaltowski AJ, Castilho RF, Vercesi AE. Ca(2+)-induced mitochondrial membrane permeabilization: Role of coenzyme Q redox state. *The American Journal of Physiology*. 1995;**269**:C141-C147
- [220] Jarmuszkiewicz W, Navet R, Alberici LC, Douette P, Sluse-Goffart CM, Sluse FE, Vercesi AE. Redox state of endogenous coenzyme q modulates the inhibition of linoleic acid-induced uncoupling by guanosine triphosphate in isolated skeletal muscle mitochondria. *Journal of Bioenergetics and Biomembranes*. 2004;**36**:493-502. DOI: 10.1023/B:JOB B.0000047331.25248.7a
- [221] Marcoff L, Thompson PD. The role of coenzyme Q10 in statin-associated myopathy: A systematic review. *Journal of the American College of Cardiology*. 2007;**49**:2231-2237. DOI: 10.1016/j.jacc.2007.02.049
- [222] Miles L, Miles MV, Tang PH, Horn PS, Wong BL, DeGrauw TJ, Morehart PJ, Bove KE. Muscle coenzyme Q: A potential test for mitochondrial activity and redox status. *Pediatric Neurology*. 2005;**32**:318-324. DOI: 10.1016/j.pediatrneurol.2005.01.009
- [223] Turunen M, Olsson J, Dallner G. Metabolism and function of coenzyme Q. *Biochimica et Biophysica Acta*. 2004;**1660**:171-199. DOI: 10.1016/j.bbamem.2003.11.012
- [224] Littarru GP, Tiano L. Bioenergetic and antioxidant properties of coenzyme Q10: Recent developments. *Molecular Biotechnology*. 2007;**37**:31-37
- [225] Laaksonen R, Jokelainen K, Sahi T, Tikkanen MJ, Himberg JJ. Decreases in serum ubiquinone concentrations do not result in reduced levels in muscle tissue during short-term simvastatin treatment in humans. *Clinical Pharmacology and Therapeutics*. 1995;**57**:62-66. DOI: 10.1016/0009-9236(95)90266-X
- [226] Ghirlanda G, Oradei A, Manto A, Lippa S, Uccioli L, Caputo S, Greco AV, Littarru GP. Evidence of plasma CoQ10-lowering effect by HMG-CoA reductase inhibitors: A double-blind, placebo-controlled study. *Journal of Clinical Pharmacology*. 1993;**33**:226-229. DOI: 10.1002/j.1552-4604.1993.tb03948.x
- [227] Miyake Y, Shouzu A, Nishikawa M, Yonemoto T, Shimizu H, Omoto S, Hayakawa T, Inada M. Effect of treatment with 3-hydroxy-3-methylglutaryl coenzyme A reductase

- inhibitors on serum coenzyme Q10 in diabetic patients. *Arzneimittel-Forschung*. 1999;**49**:324-329. DOI: 10.1055/s-0031-1300422
- [228] Rundek T, Naini A, Sacco R, Coates K, DiMauro S. Atorvastatin decreases the coenzyme Q10 level in the blood of patients at risk for cardiovascular disease and stroke. *Archives of Neurology*. 2004;**61**:889-892. DOI: 10.1001/archneur.61.6.889
- [229] Päivä H, Thelen KM, Van Coster R, Smet J, De Paepe B, Mattila KM, Laakso J, Lehtimäki T, von Bergmann K, Lütjohann D, Laaksonen R. High-dose statins and skeletal muscle metabolism in humans: A randomized, controlled trial. *Clinical Pharmacology and Therapeutics* 2005;**78**:60-68. DOI: 10.1016/j.clpt.2005.03.006
- [230] Littarru GP, Langsjoen P. Coenzyme Q10 and statins: Biochemical and clinical implications. *Mitochondrion*. 2007;**7**(Suppl):S168-S174. DOI: 10.1016/j.mito.2007.03.002
- [231] Mabuchi H, Nohara A, Kobayashi J, Kawashiri MA, Katsuda S, Inazu A, Koizumi J, HLR Group. Effects of CoQ10 supplementation on plasma lipoprotein lipid, CoQ10 and liver and muscle enzyme levels in hypercholesterolemic patients treated with atorvastatin: A randomized double-blind study. *Atherosclerosis*. 2007;**195**:e182-e189. DOI: 10.1016/j.atherosclerosis.2007.06.010
- [232] Young JM, Molyneux SL, Reinheimer AM, Florkowski CM, Frampton CM, Scott RS, George PM. Relationship between plasma coenzyme Q10, asymmetric dimethylarginine and arterial stiffness in patients with phenotypic or genotypic familial hypercholesterolemia on long-term statin therapy. *Atherosclerosis*. 2011;**218**:188-193. DOI: 10.1016/j.atherosclerosis.2011.04.034
- [233] Bookstaver DA, Burkhalter NA, Hatzigeorgiou C. Effect of coenzyme Q10 supplementation on statin-induced myalgias. *The American Journal of Cardiology*. 2012;**110**:526-529. DOI: 10.1016/j.amjcard.2012.04.026
- [234] Vaughan RA, Garcia-Smith R, Bisoffi M, Conn CA, Trujillo KA. Ubiquinol rescues simvastatin-suppression of mitochondrial content, function and metabolism: implications for statin-induced rhabdomyolysis. *European Journal of Pharmacology*. 2013;**711**:1-9. DOI: 10.1016/j.ejphar.2013.04.009
- [235] Kettawan A, Takahashi T, Kongkachuichai R, Charoenkiatkul S, Kishi T, Okamoto T. Protective effects of coenzyme q(10) on decreased oxidative stress resistance induced by simvastatin. *Journal of Clinical Biochemistry and Nutrition*. 2007;**40**:194-202. DOI: 10.3164/jcbn.40.194
- [236] Tavintharan S, Ong CN, Jeyaseelan K, Sivakumar M, Lim SC, Sum CF. Reduced mitochondrial coenzyme Q10 levels in HepG2 cells treated with high-dose simvastatin: A possible role in statin-induced hepatotoxicity? *Toxicology and Applied Pharmacology*. 2007;**223**:173-179. DOI: 10.1016/j.taap.2007.05.013
- [237] Jiménez-Santos MA, Juárez-Rojop IE, Tovilla-Zárate CA, Espinosa-García MT, Juárez-Oropeza MA, Ramón-Frías T, Bermúdez-Ocaña DY, Díaz-Zagoya JC. Coenzyme Q10 supplementation improves metabolic parameters, liver function and mitochondrial respiration in rats with high doses of atorvastatin and a cholesterol-rich diet. *Lipids in Health and Disease*. 2014;**13**:22. DOI: 10.1186/1476-511X-13-22

- [238] Caso G, Kelly P, McNurlan MA, Lawson WE. Effect of coenzyme q10 on myopathic symptoms in patients treated with statins. *The American Journal of Cardiology*. 2007;**99**:1409-1412. DOI: 10.1016/j.amjcard.2006.12.063
- [239] Young JM, Florkowski CM, Molyneux SL, McEwan RG, Frampton CM, George PM, Scott RS. Effect of coenzyme Q(10) supplementation on simvastatin-induced myalgia. *The American Journal of Cardiology*. 2007;**100**:1400-1403. DOI: 10.1016/j.amjcard.2007.06.030
- [240] Levy HB, Kohlhaas HK. Considerations for supplementing with coenzyme Q10 during statin therapy. *The Annals of Pharmacotherapy*. 2006;**40**:290-294. DOI: 10.1345/aph.1G409
- [241] Wyss M, Kaddurah-Daouk R. Creatine and creatinine metabolism. *Physics Review*. 2000;**80**:1107-1213
- [242] Volek JS, Duncan ND, Mazzetti SA, Staron RS, Putukian M, Gómez AL, Pearson DR, Fink WJ, Kraemer WJ. Performance and muscle fiber adaptations to creatine supplementation and heavy resistance training. *Medicine and Science in Sports and Exercise*. 1999;**31**:1147-1156
- [243] Dolder M, Walzel B, Speer O, Schlattner U, Wallimann T. Inhibition of the mitochondrial permeability transition by creatine kinase substrates. Requirement for micro-compartmentation. *The Journal of Biological Chemistry*. 2003;**278**:17760-17766. DOI: 10.1074/jbc.M208705200
- [244] Meyer LE, Machado LB, Santiago AP, da-Silva WS, De Felice FG, Holub O, Oliveira MF, Galina A. Mitochondrial creatine kinase activity prevents reactive oxygen species generation: Antioxidant role of mitochondrial kinase-dependent ADP re-cycling activity. *Journal of Biological Chemistry*. 2006;**281**:37361-37371. DOI: 10.1074/jbc.M604123200
- [245] Matthews RT, Yang L, Jenkins BG, Ferrante RJ, Rosen BR, Kaddurah-Daouk R, Beal MF. Neuroprotective effects of creatine and cyclocreatine in animal models of Huntington's disease. *The Journal of Neuroscience*. 1998;**18**:156-163
- [246] Lawler JM, Barnes WS, Wu G, Song W, Demaree S. Direct antioxidant properties of creatine. *Biochemical and Biophysical Research Communications*. 2002;**290**:47-52. DOI: 10.1006/bbrc.2001.6164
- [247] Jun DW, Cho WK, Jun JH, Kwon HJ, Jang KS, Kim HJ, Jeon HJ, Lee KN, Lee HL, Lee OY, Yoon BC, Choi HS, Hahm JS, Lee MH. Prevention of free fatty acid-induced hepatic lipotoxicity by carnitine via reversal of mitochondrial dysfunction. *Liver International*. 2011;**31**:1315-1324. DOI: 10.1111/j.1478-3231.2011.02602.x
- [248] Gulcin I. Antioxidant and antiradical activities of L-carnitine. *Life Sciences*. 2006;**78**:803-811. DOI: 10.1016/j.lfs.2005.05.103
- [249] Castilho RF, Kowaltowski AJ, Meinicke AR, Vercesi AE. Oxidative damage of mitochondria induced by Fe(II)citrate or t-butyl hydroperoxide in the presence of Ca²⁺: Effect of coenzyme Q redox state. *Free rad. Biologie et Médecine*. 1995;**18**:55-59. DOI: 10.1016/0891-5849(94)00098-5