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Journal of Cellular Biochemistry 113:2057–2063 (2012)

Journal of Cellular Biochemistry

# 3-Hydroxy 3-Methylglutaryl Coenzyme A Reductase Inhibition Impairs Muscle Regeneration

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## ABSTRACT

Skeletal muscle has the ability to regenerate new muscle fibers after injury. The process of new muscle formation requires that quiescent mononuclear muscle precursor cells (myoblasts) become activated, proliferate, differentiate, and fuse into multinucleated myotubes which, in turn, undergo further differentiation and mature to form functional muscle fibers. Previous data demonstrated the crucial role played by 3-hydroxy 3-methylglutaryl coenzyme A reductase (HMGR), the rate-limiting enzyme of cholesterol biosynthetic pathway, in fetal rat myoblast (L6) differentiation. This finding, along with epidemiological studies assessing the myotoxic effect of statins, HMGR inhibitors, allowed us to speculate that HMGR could be strongly involved in skeletal muscle repair. Thus, our research was aimed at evaluating such involvement: in vitro and in vivo experiments were performed on both mouse adult satellite cell derived myoblasts (SCDM) and mouse muscles injured with cardiotoxin. Results demonstrate that HMGR inhibition by the statin Simvastatin reduces SCDM fusion index, fast MHC protein levels by 60% and slow MHC by 40%. Most importantly, HMGR inhibition delays skeletal muscle regeneration in vivo. Thus, besides complaining of myopathies, patients given Simvastatin could also undergo an impairment in muscle repair. J. Cell. Biochem. 113: 2057–2063, 2012. 

**KEY WORDS:** HMG-CoA REDUCTASE; MUSCLE REGENERATION; MYOSIN HEAVY CHAIN; SATELLITE CELLS; STATINS

Skeletal muscle is the most abundant tissue of our body. Apart from its essential role in locomotion, it is also the main store of carbohydrates and proteins in the body as well as a body heat generator. The proper maintenance and function of skeletal muscle are, therefore, essential for body homeostasis. A severe, acute loss of muscle function is potentially lethal and the debilitating effects of chronic decline in mobility are commonplace experience. The maintenance of a working skeletal musculature is conferred by its remarkable ability to regenerate. Indeed, upon muscle damage a finely orchestrated set of cellular responses is activated, resulting in the regeneration of a well innervated, fully vascularized, and contractile muscle apparatus [Charge and Rudnicki, 2004].

Following muscle damage, myofibers are sheared or torn exposing the intracellular contents to the extracellular environment. Activation of calcium-dependent proteases leads to the rapid disintegration of myofibrils, whereas the activation of the complement cascade induces chemotactic recruitment of neutrophils and then later of macrophages who begin the process of digestion of the necrotic myofibers and cellular debris by phagocytosis. Neutrophil- and macrophage-released cytokines amplify the inflammatory response and recruit satellite cells [Turner and Badylak, 2011] muscle-specific stem cells located under the basal lamina of muscle fibers [Mauro, 1961], responsible for muscle regeneration [Iani et al., 1994; Zammit et al., 2006].

Normally quiescent in adult skeletal muscle, satellite cells become activated when muscle is injured and proliferate to generate a pool of muscle precursor cells or myoblasts (SCDM). These cells can then either repair damaged segments of fibers or fuse together to generate entirely new multinucleated muscle fibers [Boldrin et al., 2010].

Muscle regeneration from satellite cells has long been believed to recapitulate, at least partially, the process of embryonic myogenesis. Supportive evidence includes the findings that proliferating and differentiating satellite cells re-express myogenic regulatory factors (MRFs, namely *MyoD*, *Myf5*, *Myogenin*, and *MRF4*) and embryonic myofibrillar genes (e.g., the embryonic isoform of myosin heavy chain, *eMHC*) during muscle regeneration [Zhao and Hoffman, 2004].

It was recently shown that 3-hydroxy 3-methylglutaryl Coenzyme A reductase (HMGR), the key and rate limiting enzyme of cholesterol biosynthetic pathway, and its main end-products

Grant sponsor: University of Roma Tre (CLAR).

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Manuscript Received: 1 December 2011; Manuscript Accepted: 17 January 2012
Accepted manuscript online in Wiley Online Library (wileyonlinelibrary.com): 23 January 2012
DOI 10.1002/jcb.24077 • © 2012 Wiley Periodicals, Inc.

(e.g. prenyls, dolichol) are crucial for the myogenesis to occur: indeed, in insulin-induced fetal rat myoblast (L6) differentiation, HMGR down-regulation or inhibition by the statin mevinolin lead both to a decreased expression of myogenin (myo) and eMHC and to a reduction of myoblast fusion into multinucleated syncitia [Martini et al., 2009]. In fact, HMGR inhibition could lead to a reduction of prenyls needed for the lipidation and the consequent activation of Rho small G protein family members, some of which (RhoA and RhoE) are known to be involved in the myogenic process [Fortier et al., 2008; Martini et al., 2009]. Moreover, the statin-induced reduction of dolichol, can affect the glycosylation of proteins implicated in myoblast fusion [Belo et al., 1993]. Although the involvement of HMGR in myoblast differentiation had been postulated and well characterized, little or nothing is known about the role-played by the enzyme in the differentiation and maturation of adult satellite cell derived myocytes (SCDM) responsible for muscle regeneration.

Statins, HMGR competitive inhibitors, besides the beneficial effects on plasma lipid profile in patients suffering from hypercholesterolemia, have been shown to cause myopathy characterized by weakness, pain, elevated serum creatine phosphokinase and, to a lesser extent (0.05%), rhabdomyolysis, which is a life-threatening condition [Cheng et al., 2005].

The hypothesized mechanisms underlying the statin myotoxicity include structural and functional membrane modifications, mitochondria dysfunction and alteration of intracellular signal transduction pathway due to the impairment of cholesterol, ubiquinone, and prenyl synthesis, respectively [Draeger et al., 2006]. Thus, statin-induced myopathy and the strict relationship between mevalonate pathway and myogenesis suggests that HMGR end-products are essential for skeletal muscle homeostasis maintenance. Indeed myoblast differentiation occurs not only in pathological conditions but also after common traumas occurring in many sports [Jarvinen et al., 2005]: in case of muscle damage, a dysregulation of HMGR pathway can interfere with the proper muscle repair.

Thus, this research was aimed at assessing the putative involvement of HMGR in muscle regeneration by analyzing the effects of the enzyme inhibition both in vitro on differentiating SCDM and in vivo on cardiotoxin (CTX) injured mouse muscles.

## MATERIALS AND METHODS

All materials used were obtained from commercial sources and of the highest quality available. All materials with no specified source are obtained from Sigma–Aldrich (Milan, Italy).

### CELLS

Adult primary satellite cells were a generous gift of Dr. Marco Crescenzi of Italian National Institute of Health. The cells were grown on gelatin-coated dishes in 77% nutrient mixture F-10 Ham (N6908, Sigma) with Pen/Strep solution (penicillin 100 U/ml and streptomycin 0.1 mg/ml), 20% fetal bovine serum, 3% chicken embryo extract (prepared in Hank's Balanced Solution), and 2.5 ng/ml basic fibroblast growth factor (Peprotech, Rocky Hill, NJ). Cells were cultured at 37°C in a humified incubator containing 5% $CO_2$. For differentiation, cells were seeded at high density ($2.5 \times 10^5$) in gelatin-coated 35 mm dishes and, after cell adesion, growth medium was replaced with DMEM (D5796, Sigma) and Pen/Strep solution (penicillin 100 U/ml and streptomycin 0.1 mg/ml) supplemented with 10% fetal bovine serum. Medium was changed every 24 h. In order to study the involvement of HMGR in cell differentiation, 1 μM Simvastatin (Sim) (in DMSO) and/or 100 μM mevalonate (Mva) were used daily. Control cells were treated with DMSO (Veh) at the same volume of Simvastatin. Cell lysates were carried out as previously described [Martini et al., 2007].

### IMMUNOFLUORESCENCE STAINING

For immunofluorescent staining, SCDM were grown on 18 mm glass coverslips in 6-well plates ($2.5 \times 10^5$ cells/well/coverslip). Treated cells were washed with phosphate-buffered saline (PBS, pH 7.4), fixed for 20 min in 4% paraformaldehyde and then permeabilized for 3 min with Triton X-100 (0.2% in PBS). Cells were washed, blocked in 2% BSA for 1 h at room temperature, and incubated with rhodamine–phalloidin (Invitrogen, Carlsbad, CA) (0.2% in BSA 2%) for 20 min. SCDM were then rinsed 3 times with PBS for 10 min each. Nuclear staining was performed incubating cells with DAPI (5% in PBS) for 15 min. Slides were mounted with Prolong antifade reagent (Invitrogen, Carlsbad, CA). Fluorescence images were obtained using an Olympus BX51 microscope. At least 10 fields for each sample were examined. Fusion index was calculated performing the ratio between nuclei number observed in phalloidin positive myotubes and total number of myotubes. Apoptotic index was calculated as the ratio between apoptotic nuclei and total number of nuclei observed. A minimum of 1,000 nuclei was counted for each experimental condition. For microscopic analysis, three independent experiments were performed for each condition.

### ANIMALS AND TREATMENTS

Five-month-old female C57BL/6 mice (Harlan Nossan, S. Pietro al Natisone, Italy) were housed under controlled temperature (20 ± 1°C), humidity (55 ± 10%), and illumination (lights on for 12 h daily, from 7 a.m. to 7 p.m.). Food and water were provided ad libitum. The experiments were performed according to the ethical guidelines for the conduct of animal research (Ministero della Salute, Official Italian Regulation No. 116/92, Communication to Ministero della Salute no. 391/121).

Mice (Total number = 18) were anaesthetized with ether fume woods, and 40 μl of 10 μM CTX (Cardiotoxin) (CalBiochem, Merck KGaA, Darmstadt, Germany) in PBS were injected into the right *tibialis anterior* muscle [Delaunay et al., 2008]. Three mice were treated daily for 21 days with IP injection of Simvastatin (1.5 mg/kg/die in DMSO), 3 mice with IP injection of Simvastatin (1.5 mg/kg/die) and Mevalonate (150 mg/kg/die in $H_2O$), 12 mice received DMSO (Veh) at the same volume of Simvastatin.

The animals were sacrificed with IP injection of urethane in $H_2O$ (1 g/kg). Control mice were sacrificed at 0, 3, 10, 21 days after being injured by CTX, whereas Simvastatin and Simvastatin plus Mva treated mice were sacrificed 21 days after CTX injection.

For morphological analysis, tibial muscles from both sides of the mice, were dissected and fixed by immersion in PBS containing 4% freshly depolymerized paraformaldehyde, overnight at 4°C.

### MORPHOLOGICAL ANALYSIS

Fixed muscles were dehydrated in graded ethanol, transferred to Bioclear (BioOptica, Milan, Italy), then to a 1:1 mixture of Bioclear and paraffin, and finally embedded in paraffin. Transverse, 7-μm-thick sections were cut by a microtome and collected on Superfrost Plus slides (BioOptica). For each treatment group, a minimum of 10 sections were deparaffinized, rehydrated, and stained by hematoxylin–eosin (H&E), then dehydrated and mounted with Eukitt (Kindler GmbH & Co., Freiburg, Germany). Slides were observed under an Olympus BX 51 microscope, equipped with a Leica DFC 420 camera; electronic images were captured by a Leica Application Suite system, and composed in an Adobe Photoshop CS2 format.

### PROTEIN ANALYSIS

**Western Blotting Analysis.** Protein profiles were analyzed by Western blotting. 20 μg of protein from lysates were resolved by 7% SDS–PAGE at 100 V for 60 min. The proteins were subsequently electrophoretically transferred to nitrocellulose for 90 min at 100 V. The nitrocellulose membrane was blocked at room temperature with 3% BSA in Tris-buffered saline (138 mM NaCl, 27 mM KCl, 25 mM Tris–HCl, 0.05% Tween-20, pH 6.8), and probed at 4°C overnight with primary antibodies (HMGR—Upstate, Lake Placid, NY; eMHC—Abcam, Cambridge, UK; fast MHC MY-32 and slow MHC NOQ7.5.4D—Sigma) followed by incubation for 1h with secondary IgG antibodies coupled to horseradish peroxidase (Bio-Rad Laboratories, Milan, Italy). The nitrocellulose membrane was then re-probed with anti-tubulin (α-tubulin DM-1A, Sigma) antibody. Bound antibodies were visualized using enhanced chemoluminescence detection (GE Healthcare). All images derived from Western blotting were analyzed with ImageJ (NIH, Bethesda, MD) software for Windows. Each reported value was derived from the ratio between arbitrary units obtained by the protein band and the respective tubulin band.

### STATISTICAL ANALYSIS

Data are expressed as mean ± SD. The difference of parameters was statistically tested for significance with one-way ANOVA followed by Dunnett post-test. $P < 0.05$ was considered statistically significant. Statistical analysis was performed using GraphPad Instat3 (GraphPad software, Inc., La Jolla, CA) software for Windows. The significance of the difference was indicated by $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.

## RESULTS

Our previous work highlighting the crucial role played by HMGR and its main end products in fetal rat myoblast (L6) myogenic process [Martini et al., 2009] allowed us to speculate that the enzyme inhibition could affect the differentiation of SCDM responsible for muscle regeneration and impair proper muscular repair.

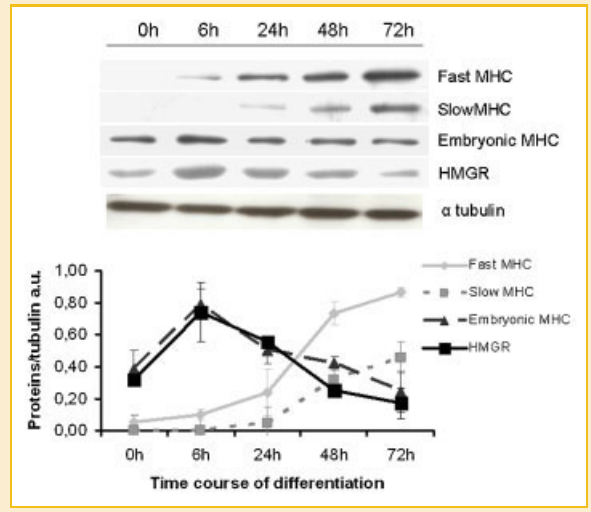


Fig. 1. Time course of differentiation of HMGR and embryonic, fast and slow MHC in SCDM. The figure shows a typical Western blotting and the densitometric analysis of a time course ranging from 0 to 72 h of HMGR, eMHC, fMHC, and sMHC protein levels in SCDM induced to differentiate through the replacement of the growth medium with that of differentiation. Data are expressed as the mean ± SD of at least three different experiments. For details see the main text.

Figure 1 illustrates that, in SCDM, both HMGR and eMHC protein levels increase 6 h after the induction of differentiation and later decrease up to 72 h. On the contrary, the rise of both fast and slow MHC isoforms (fMHC, sMHC) was observed 24 h after the induction of differentiation even though the fMHC was barely detectable at 6 h. The similar modulation of HMGR protein levels in both fetal L6 rat myoblasts [Martini et al., 2009] and in SCDM, allowed us to confirm an involvement of HMGR in SCDM differentiation. Our results were in agreement with the time dependent expression of the different MHC isoforms occurring in muscle regeneration [Ciciliot and Schiaffino, 2011].

Proliferating Simvastatin- and Vehicle (Veh)-treated SCDM had a very similar morphology in growth medium (data not shown), but 72 h after the induction of differentiation, the myotubes derived from both types of culture showed striking differences (Fig. 2A). Indeed, Veh-treated cells formed large branched myotubes with a high number of nuclei, whereas the myotubes from the Simvastatin-treated cells were much smaller and thinner and had a relatively small number of nuclei per myotube. On the contrary, the myotubes deriving from the differentiation and fusion of SCDM treated both with Simvastatin plus Mevalonate were morphologically very similar to the vehicles (Fig. 2A).

Interestingly, the fusion index (Fig. 2B) was much lower in Simvastatin-treated culture than in Veh at 24, 48, 72 h treatment. In fact 72 h after the replacement of the medium and the beginning of statin treatment, the fusion index decrease achieved 60%. Mevalonate supplemented to Simvastatin completely prevented the Simvastatin effect (Fig. 2B dark gray columns) sustaining the pivotal role of HMGR in skeletal muscle differentiation. No significant differences were detected either in total nuclei number (Fig. 2C) or apoptotic index (Fig. 2D) among the samples, indicating

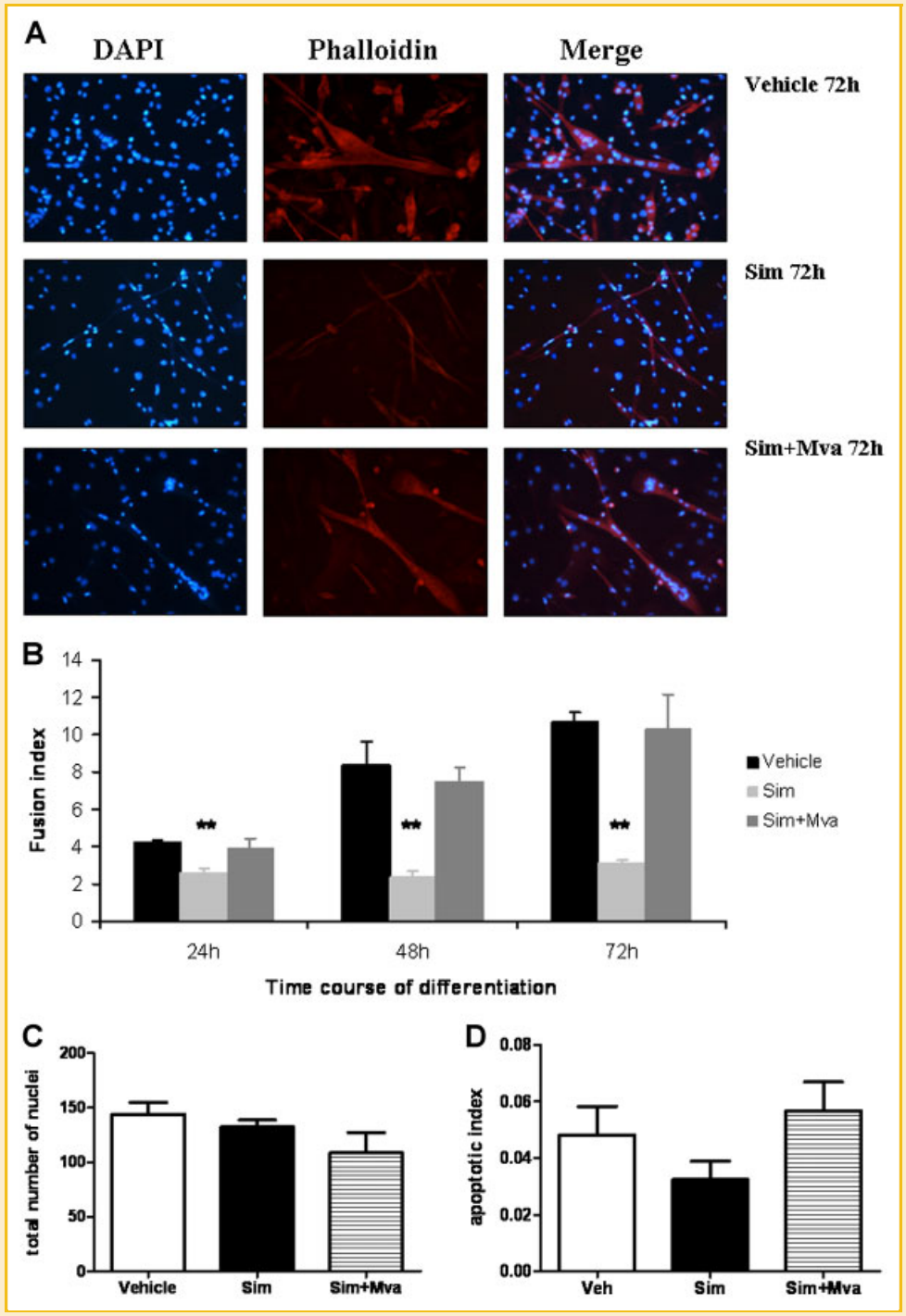


Fig. 2. Immunofluorescent staining, differentiation, and apoptotic indexes of Veh, Sim, and Sim + Mva treated SCDM. A: The figure shows phalloidin staining, Dapi staining and merged images of Veh, Sim, and Sim + Mva treated SCDM 72 h after the induction of differentiation. B: Fusion index of Veh, Sim, and Sim + Mva treated SCDM 24, 48, and 72 h after the induction of differentiation. $^{**}P < 0.01$ as from ANOVA followed by Dunnett post-test versus Veh sample of each time. C: Total number of nuclei of Veh, Sim, and Sim + Mva treated SCDM 72 h after the induction of differentiation. D: Apoptotic index of Veh, Sim, and Sim + Mva treated SCDM 72 h after the induction of differentiation. Each column represent the mean ± SD of at least three different experiments. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/jcb]

that the reduced fusion index detected in Simvastatin treated cells was not due to a different cell number.

The degree of SCDM differentiation and myotube maturation was determined by assaying the expression of eMHC, fMHC, and sMHC. As expected from fusion index results, Simvastatin treated SCDM maintained eMHC levels higher than in Veh samples (Fig. 3A) and showed a general decrease in the amount of both the adult contractile proteins fMHC and sMHC in comparison with Veh at 24,

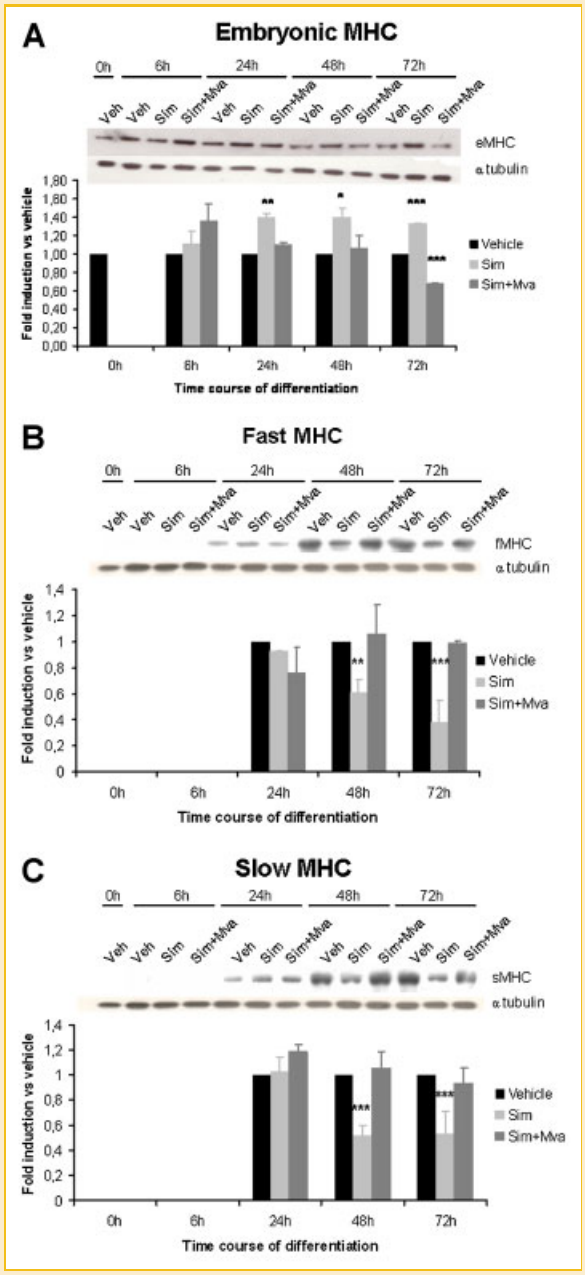


Fig. 3. Time course of differentiation of eMHC, fMHC, and sMHC protein expression in Veh, Sim, and Sim + Mva treated SCDM. A: Typical Western blot and densitometric analysis of eMHC detected in Veh, Sim, and Sim + Mva treated SCDM 0, 6, 24, 48, 72 h after the induction of differentiation. Data are expressed as the ratio between Sim or Sim + Mva versus Veh samples of each time considered. Each column represents the mean ± SD of at least three different experiments. B: Typical Western blot and densitometric analysis of fMHC detected in Veh, Sim, and Sim + Mva treated SCDM 0, 6, 24, 48, 72 h after the induction of differentiation. Data are expressed as the ratio between Sim or Sim + Mva versus Veh samples of each time considered. Each column represents the mean ± SD of at least three different experiments. C: Typical Western blot and densitometric analysis of sMHC detected in Veh, Sim, and Sim + Mva treated SCDM 0, 6, 24, 48, 72 h after the induction of differentiation. Data are expressed as the ratio between Sim or Sim + Mva versus Veh samples of each time considered. Each column represents the mean ± SD of at least three different experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ as from ANOVA followed by Dunnett post-test versus Veh sample of each time.

48, and 72 h (Fig. 3B and C). Rescue experiments abolished Simvastatin effect both on eMHC, fMHC, and sMHC protein expression; nevertheless, after 72 h, eMHC levels were lower compared with controls when SCDM were co-treated with Simvastatin and Mevalonate (Fig. 3A dark gray columns).

Since the results obtained in vitro suggested a delaying effect of Simvastatin on SCDM differentiation, in vivo experiments were performed in order to confirm whether Simvastatin could impair muscle regeneration.

The morphological features of *tibialis anterior* muscles from animals injured with CTX and chronically treated with Veh or Simvastatin or Simvastatin plus Mevalonate were analyzed. Muscles injected with CTX displayed a profoundly altered cytoarchitecture, at both day 3 and 10 after injury, compared to controls (Fig. 4A–C). In H&E stained sections from mice at 3 days after the toxic insult (Fig. 4B), a prominent inflammatory infiltrate was observed, and muscle fiber integrity was compromised. At 10 days (Fig. 4C), muscle regeneration ensues, as indicated by the presence of central nuclei in most muscle fibers. At 21 days after tissue injury (Fig. 4D), *tibialis anterior* muscles appeared completely recovered, as a regular cytoarchitecture was recognized in H&E stained sections. The large majority of muscle fibers showed a peripherally located nucleus, suggesting full cell differentiation. Histological analysis of Simvastatin treated mice demonstrated a delayed regeneration of CTX injured muscles. In fact, 21 days after the toxic insult, muscles were still in the process of regenerating their fibers, as assessed by the presence of numerous centrally located nuclei (Fig. 4E). Importantly, the effect exerted by Simvastatin was totally abolished by administration of Mevalonate (Fig. 4F). Quantitative analysis is provided in Table I.

## DISCUSSION

In damaged muscle, the necrosis of the fibers stimulates an inflammatory response with the invasion of macrophages, followed by activation of satellite cells which undergo proliferation, differentiation, and fusion to one another or to undamaged portions of the fiber [Charge and Rudnicki, 2004]. Since the inhibition and down-regulation of HMGR, was demonstrated to be crucial for the myogenic process to occur [Martini et al., 2009], we hypothesized an involvement of the enzyme in the differentiation of adult SCDM, responsible for muscle repair, and in the regenerative program of muscle tissue. Once ascertained that the initial increase and the subsequent decrease of HMGR drew a similar trend of that previously observed in rat fetal L6 myoblasts [Martini et al., 2009], we analyzed, in a time course ranging from 6 up to 72 h, eMHC, fMHC, and sMHC protein expression. The time dependent pattern of MHC isoform expression in agreement to that already reported for regenerating muscles, indicated that the activation, proliferation, and fusion of adult satellite cells implied an initial expression of MHCs typical of developing muscle, such as eMHC, whose subsequent decline was partially overlapped and followed by the increase of the adult contractile proteins fMHC and sMHC, respectively [Ciciliot and Schiaffino, 2011].

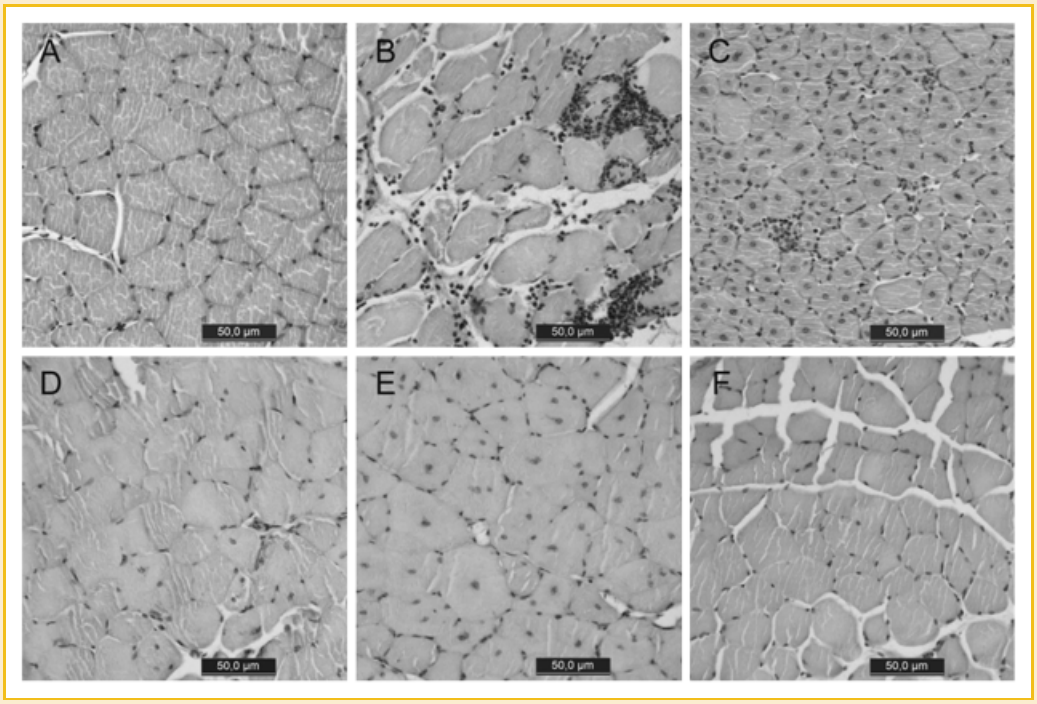


Fig. 4. Morphology of tibial muscles from mice of the different treatment groups. H&E stained paraffin sections. A: Noninjured muscle, showing regular histological features, including peripherally located cell nuclei. B: CTX-injected muscle, 3 days after injury. Damaged muscle fibers and large areas of inflammatory infiltrate are observed. C: CTX-injected muscle, 10 days after injury. Most muscle fibers show central nuclei, suggesting ongoing regeneration, while few areas of inflammatory infiltrate are still present. D: CTX-injected muscle, 21 days after injury. The tissue shows normal histological features. Most muscle fibers show peripheral nuclei, suggesting full differentiation. E: CTX-injected muscle from a Sim treated mouse, 21 days after injury. While the overall cytoarchitecture appears normal, several muscle fibers displaying a centrally located nucleus are still present, suggesting incomplete differentiation and/or delayed regeneration. F: CTX-injected muscle from a Sim and Mva treated mouse, 21 days after injury. The tissue shows normal histological features, similar to those shown in (A) and (D).

The lack of any decrease of eMHC observed in Simvastatin-treated SCDM together with the inhibition of fMHC and sMHC expression demonstrated a delay in myotube maturation and in fiber formation. This observation was further supported by morphological analysis which highlighted a reduced SCDM fusion index. In addition, myotubes derived from Simvastatin-treated SCDM showed a very immature phenotype as that reported in myotubes derived from satellite cells extracted from human fetuses affected by congenital myotonic dystrophy, an autosomal dominant disease characterized by myotonia and progressive muscle weakness [Furling et al., 2001]. Interestingly, a range of cases have now been reported in which statin use has "uncovered" previously clinically silent or clinically tolerated conditions, among others myotonic dystrophy [Tsivgoulis et al., 2006].

TABLE I. Quantitative Analysis of Muscle Regeneration

| | PNF | CNF | TF |
|---|---|---|---|
| Control | 87 ± 5 | 0 | 87 ± 5 |
| Injured Veh 21 days | 73 ± 8 | 6 ± 5 | 79 ± 13 |
| Injured Sim 21 days | 15 ± 3 | 77 ± 6 | 92 ± 6 |
| Injured Sim + Mva 21 days | 95 ± 8 | 2 ± 1 | 97 ± 8 |

Data are the mean ± SD of fibers displaying peripherally located nuclei (PNF), of fibers displaying centrally located nuclei (CNF), and of total fibers (TF) in 10 different fields for each experimental condition.

The results obtained in vitro, led us to ascertain whether HMGR inhibition could impair muscle regeneration in vivo. Thus, we investigated the response to CTX-induced muscle damage in female mice treated with Veh, or Simvastatin, or with both Simvastatin plus Mevalonate, since CTX is a highly reproducible way to induce muscle regeneration [d'Albis et al., 1988]. CTX, a peptide isolated from snake venoms, is known to induce the depolarization and contraction of muscular cells, to disrupt membrane organization, and to lyse muscle cells. Injected in adult mouse *tibialis anterior* muscle, CTX induces muscle degeneration leading to a wound coagulum with mononuclear cell infiltration within 1 day of injection. Inflammatory response and mononuclear cell proliferation is most active within 1–4 days of injection. By 10 days post injection, the architecture of the muscle is restored, although most regenerated myofibers are smaller and display central myonuclei. The return to a morphologically and histochemically normal mature muscle is observable 3 weeks after being damaged [Nakamura et al., 2010]. Under normal conditions 21 days after CTX injection the regenerated muscle was morphologically and functionally indistinguishable from undamaged muscle. Remarkably, under Simvastatin treatment the muscle was still regenerating. The rescue experiments assessed a completely restored cytoarchitecture of a fully repaired muscle.

The prevention of the harmful effect of Simvastatin by means of Mevalonate addition in vitro and in vivo, strongly demonstrated

that the delayed myogenic process was due to the inhibition of HMGR activity rather than to an effect exerted by the drug per se and highlighted the pivotal role played by HMGR in muscle regeneration and, in turn, in muscle homeostasis maintenance. Notably, Mammen et al. [2011] showed that in muscle biopsy, fibers undergone regeneration process express high levels of HMGR.

To explain the mechanisms underlying the observed effects we can speculate that they are likely ascribable to the impairment of prenylated protein activation (Rho protein family) and dolichol synthesis [Martini et al., 2009].

Thus, the data obtained strongly suggest that HMGR plays an essential role in physiological muscle regeneration.

Therefore, patients for whom statins are prescribed in clinical practice, might not only eventually complain of myopathy but also might not be able to repair any muscle damage because of the impairment in SCDM differentiation and fusion.

## ACKNOWLEDGMENTS

The financial support from the University of Roma Tre (CLAR) to VP and MM are gratefully acknowledged.

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