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Human Skeletal Muscle Drug Transporters Determine Local Exposure and Toxicity of Statins

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Rationale: The 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, or statins, are important drugs used in the treatment and prevention of cardiovascular disease. Although statins are well tolerated, many patients develop myopathy manifesting as muscle aches and pain. Rhabdomyolysis is a rare but severe toxicity of statins. Interindividual differences in the activities of hepatic membrane drug transporters and metabolic enzymes are known to influence statin plasma pharmacokinetics and risk for myopathy. Interestingly, little is known regarding the molecular determinants of statin distribution into skeletal muscle and its relevance to toxicity.

Objective: We sought to identify statin transporters in human skeletal muscle and determine their impact on statin toxicity in vitro.

Methods and Results: We demonstrate that the uptake transporter OATP2B1 (human organic anion transporting polypeptide 2B1) and the efflux transporters, multidrug resistance–associated protein (MRP)1, MRP4, and MRP5 are expressed on the sarcolemmal membrane of human skeletal muscle fibers and that atorvastatin and rosuvastatin are substrates of these transporters when assessed using a heterologous expression system. In an in vitro model of differentiated, primary human skeletal muscle myoblast cells, we demonstrate basal membrane expression and drug efflux activity of MRP1, which contributes to reducing intracellular statin accumulation. Furthermore, we show that expression of human OATP2B1 in human skeletal muscle myoblast cells by adenoviral vectors increases intracellular accumulation and toxicity of statins and such effects were abrogated when cells overexpressed MRP1.

Conclusions: These results identify key membrane transporters as modulators of skeletal muscle statin exposure and toxicity. (*Circ Res*. 2010;106:297-306.)

Key Words: statins ■ drug transporters ■ myopathy

HMG-CoA (3-hydroxy-3-methylglutaryl coenzyme A) reductase inhibitors, statins, are highly effective drugs for the treatment of hypercholesterolemia, a major risk factor of cardiovascular disease. Statins inhibit the synthesis of mevalonate, the rate-limiting step in cholesterol biosynthesis.[1,2] Although statins are generally well tolerated,[3] skeletal muscle side effects are commonly reported among those treated. One such side effect, myalgia, which is defined as muscle aches or weakness in the absence of blood creatine kinase elevation, occurs in 5% to 15% of statin-treated patients.[2,4–8] In rare cases, potentially life-threatening statin-induced rhabdomyolysis may occur, a condition characterized by acute muscle damage, resulting in pronounced elevation in creatine kinase levels and possible renal failure.[9]

The pathophysiology of statin-induced myopathy is not completely understood. The leading mechanism suggests a role for cellular depletion of secondary metabolic intermediates of mevalonate in the development of statin-induced myotoxicity.[10] In addition to decreased cholesterol synthesis, HMG-CoA reductase inhibition by statins causes a commensurate reduction in the levels of downstream metabolic products including isoprenoids, dolichol, and ubiquinone (coenzyme Q10).[10–13] Among these are the isoprenoid secondary metabolic intermediates geranylgeranylpyrophosphate and farnesylpyrophosphate that are involved in protein isoprenylation and activation of small GTPases such as Rho and Rab. The important role for diminished isoprenylation in the mechanism of statin myotoxicity is related to induction of the muscle atrophy-linked protein atrogin-1.[12] This is highlighted by the findings that supplementation of geranylgeranylpyrophosphate to cultured skeletal myotubes or isolated myofibers treated with statins leads to attenuation of toxicity,[11,13–15] whereas inactivation of a Rab and RhoA induces toxicity.[11,13] Decreased geranylgeranylation of small GT-

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Non-standard Abbreviations and Acronyms	
BCRP	breast cancer resistance protein
HMG-CoA	3-hydroxy-3-methylglutaryl coenzyme A
HSMM	human skeletal muscle myoblast
MRP	multidrug resistance–associated protein
OATP	organic anion transporting polypeptide
OAT	organic anion transporter
P-gp	P-glycoprotein

Pases by statins appears to stimulate the mitochondrial apoptotic cell death pathway in skeletal myotubes.[13,15] In addition to isoprenoids, coenzyme Q10 levels in plasma[16] and skeletal muscle[17] are decreased with statin treatment. Although depletion of coenzyme Q10 is thought to affect oxidative phosphorylation and protection from statin-induced oxidative stress, compelling clinical evidence is lacking regarding the efficacy of coenzyme Q10 treatment of statin myopathy.[18] There is recent evidence to indicate that fatty acid oxidation is perturbed in cultured myotubes of statin intolerant (myalgic) patients,[19] a finding that differs from patients with rhabdomyolysis.[20] Such data suggest that the mechanisms of statin toxicity are different between those affected with myalgia and rhabdomyolysis.

It is well documented that myotoxicity is statin dose-dependent, and myopathy risk increases when statins are coadministered with drugs that either interact to increase plasma statin levels or themselves have propensity for muscle damage.[21–24] Indeed, macrolide antibiotics and azole antifungals are well-known drug inhibitors of hepatic statin metabolism via cytochrome P450 enzymes, dramatically increasing plasma statin levels.[21,24,25] Moreover, inhibition of statin liver uptake (transport) mediated by multiple members of the organic anion transporting polypeptide (OATP) family by drugs such as gemfibrozil, cyclosporine A and rifampin can elevate drug levels.[24,26–29] Furthermore, we have previously reported that a common genetic polymorphism resulting in a single nucleotide difference in the *SLCO1B1* gene encoding hepatic OATP1B1 (521C>T, V174A; rs4149056)[30] is associated with increased plasma levels of a number of statins.[31] In a genome-wide association study, the rs4149056 polymorphism in *SLCO1B1* was found to be the most robust predictor of the risk for simvastatin-induced myopathy.[32] In addition, efflux transporters in liver that mediate secretion of statins into bile could play a role in risk for statin myopathy. For example, genetic variation in the statin biliary efflux transporters multidrug resistance–associated protein (MRP)2 and breast cancer resistance protein (BCRP) is associated with variability in pravastatin and rosuvastatin plasma levels, respectively.[33–35]

Despite that the currently marketed statins have varying physicochemical characteristics, membrane transporters that act to facilitate drug uptake or efflux in tissues appear to have significant influence on the pharmacokinetics of most statins. This is evidenced by marked changes in plasma drug levels following transporter inhibition or through their attendant

genetic polymorphisms.[36,37] Considerable attention has been given to drug transporters in the small intestine, kidney, and liver, which affect systemic exposure to statins. However, there is a paucity of studies that have examined statin transporters within skeletal muscle and their influence on myotoxic side effects of statins, despite that this has been considered conceptually.[4] Given that plasma drug levels do not entirely predict risk for statin myopathy,[38] we hypothesize that factors which control local skeletal muscle statin concentrations, such as muscle transporters, may be more relevant. Specifically, we propose that the interplay between statin uptake versus efflux transporters modulates the response to skeletal muscle statin exposure.

In this study, we identify drug transporters in human skeletal muscle that affect the distribution of 2 prototypic lipophilic and hydrophilic statins, atorvastatin and rosuvastatin. We demonstrate that the uptake transporter, OATP2B1, and the efflux transporters, MRP1, MRP4, and MRP5 are expressed in skeletal muscle and are capable of transporting atorvastatin and rosuvastatin. Importantly, we show that by affecting drug transporter activity in a model of human skeletal muscle, statin toxicity can be modulated.

Methods
An expanded Methods section is available in the Online Data Supplement at http://circres.ahajournals.org.

Gene Expression Analysis
Tissue and cell culture expression of transporters was determined by quantitative polymerase chain reaction, and Western blotting and immunofluorescence microscopy were performed as described in the Online Data Supplement.

Identification of Statin Transporters
Heterologous expression of transporters in human cervical cancer cells (HeLa) by recombinant vaccinia virus method and statin transport activity assays were performed as described previously[39] with modifications detailed in the Online Data Supplement.

Statin Accumulation and Toxicity in Skeletal Muscle Cells
Overexpression of transporters in cultured primary differentiated human skeletal muscle myoblast (HSMM) (Lonza, Walkersville, Md) cells by adenoviral gene delivery, statin cellular accumulation assays, as well as toxicity assays (ATP content, mitochondrial methylthiazolyldiphenyl-tetrazolium bromide reduction, caspase 3/7 activation) are described in detail in the Online Data Supplement.

Results
Identification of Statin Transporters in Skeletal Muscle
Little is known about the expression of drug transporters in human skeletal muscle. Therefore, we screened a cDNA library of human skeletal muscle for expression of a wide variety of drug transporters including OATPs, organic anion transporters (OATs), organic cation transporters, MRPs, BCRP, and P-glycoprotein (P-gp). The known statin uptake transporters such as OATP1B1, OATP1B3, OAT1, and OAT3,[40–45] as well as efflux transporters such as MRP2, P-gp, and BCRP,[33,42,46–48] were not detected in skeletal muscle (not shown). However, we detected mRNA expression of the known statin uptake transporter OATP2B1 (Figure
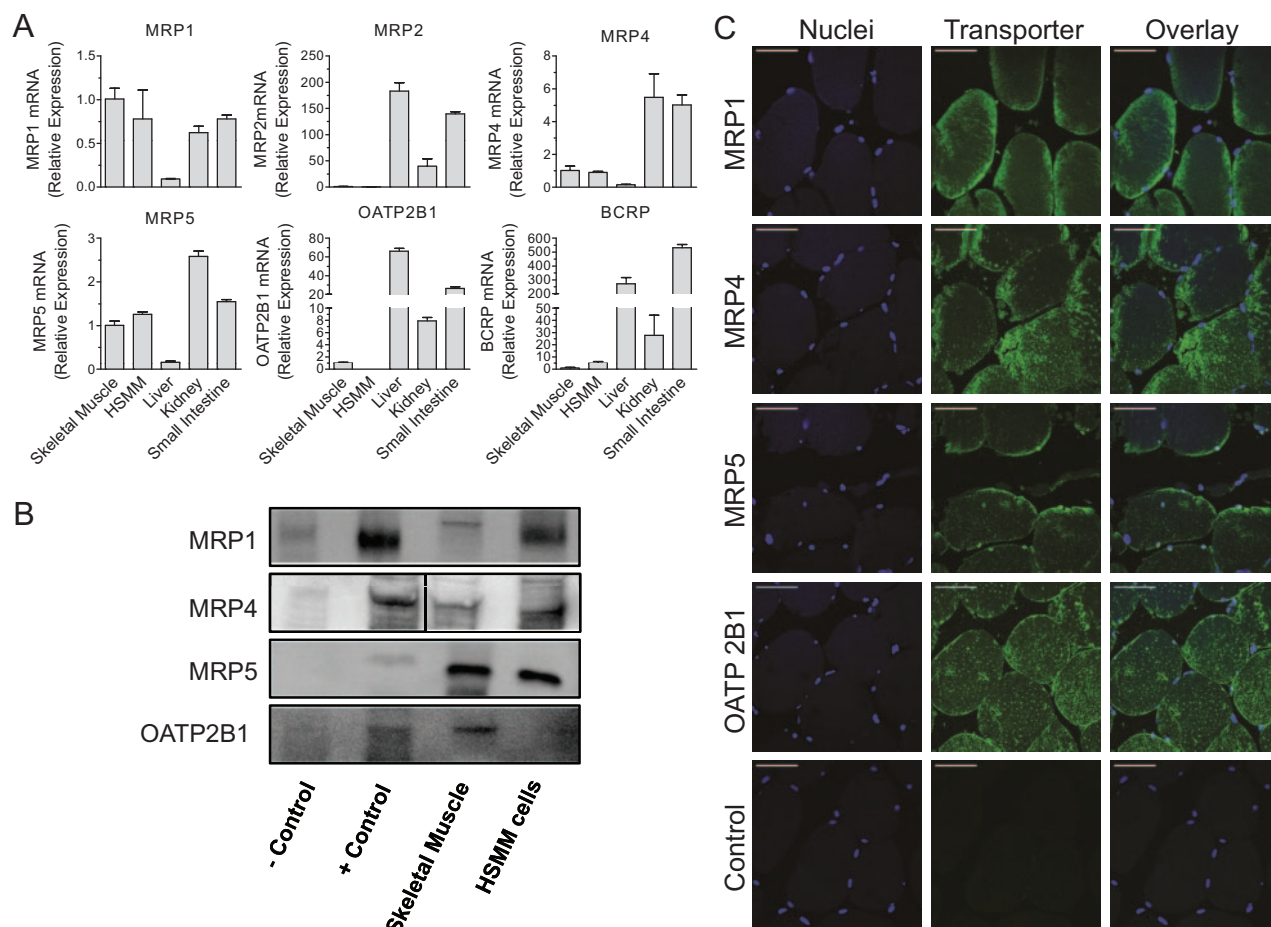
Figure 1. Expression of uptake and efflux transporters in various human tissues and HSMM cells. A, Relative mRNA gene expression of MRP1, MRP2, MRP4, MRP5, BCRP, and OATP2B1 in a range of human tissues and cultured HSMM cells. Expression was normalized to expression in human skeletal muscle. B, Protein expression of MRP1, MRP4, MRP5, and OATP2B1 in human skeletal muscle and cultured HSMM cells by Western blot. Lysates from HeLa cells transfected with blank vector or transporter cDNA served as negative and positive controls, respectively. In the MRP4 blot, one lane was cropped as denoted by the dividing line. C, Cellular localization of MRP1, MRP4, MRP5, and OATP2B1 in normal human skeletal muscle was determined by immunofluorescence confocal microscopy. Transporters are shown in green, whereas nuclei are shown in blue. Scale bar=50 μm.

1A).[40,49] The mRNA level of OATP2B1 was highly detectable in skeletal muscle, although it is significantly lower than tissues with very high expression (liver, kidney and small intestine). Given that MRP2 transports statins, the expression of other members of this efflux transporter family was examined revealing high expression of MRP1, MRP4, and MRP5 in skeletal muscle. Quantitative mRNA analysis and Western blot confirmed the expression of these drug transporters in human skeletal muscle (Figure 1A and 1B). Immunolocalization of OATP2B1, MRP1, MRP4, and MRP5 in normal human skeletal muscle by confocal microscopy demonstrated that each was expressed on the sarcolemmal membrane (Figure 1C).

We then determined whether the transporters identified in skeletal muscle were capable of statin transport using a recombinant (vtf-7) vaccinia virus overexpression system in a human cervical cancer cell line (HeLa).[39] As we previously described, OATP2B1 is capable of rosuvastatin transport[40] and here we confirm that atorvastatin is also a transport substrate (Figure 2).[49,50] Indeed, in this model, OATP2B1 increases the cellular accumulation of rosuvastatin and atorvastatin by 2-fold. Although OATP2B1 is the relevant trans-

porter for uptake of statins into skeletal muscle, we present new and confirming data that other OATPs (1B1, 1B3 and 1A2) transport both atorvastatin and rosuvastatin (Figure 2). It should be noted that the differences in statin transport between OATP2B1 and other transporters as shown in this model (Figure 2) likely do not reflect the relative statin uptake efficiencies in vivo, because transporter expression was not normalized and the absolute expression of these transporters in different tissues is undetermined.

Drug interactions involving inhibition of the major liver OATPs (1B1 and 1B3) are associated with elevated plasma statin levels. Similarly, OATP2B1 is susceptible to inhibition by coadministered medications.[51] Here, we show that stimulated intracellular accumulation of atorvastatin and rosuvastatin by OATP2B1 is attenuated after coincubation with cerivastatin, gemfibrozil, gemfibrozil-glucuronide, fenofibrate, rifampin, and glyburide (Figure 3). Interestingly, incubation with cyclosporine A caused a significant reduction in rosuvastatin accumulation but increased atorvastatin levels (Figure 3).

The transport efficiency of human OATPs compared to rodent OATPs is lower in this experimental system.[40] For
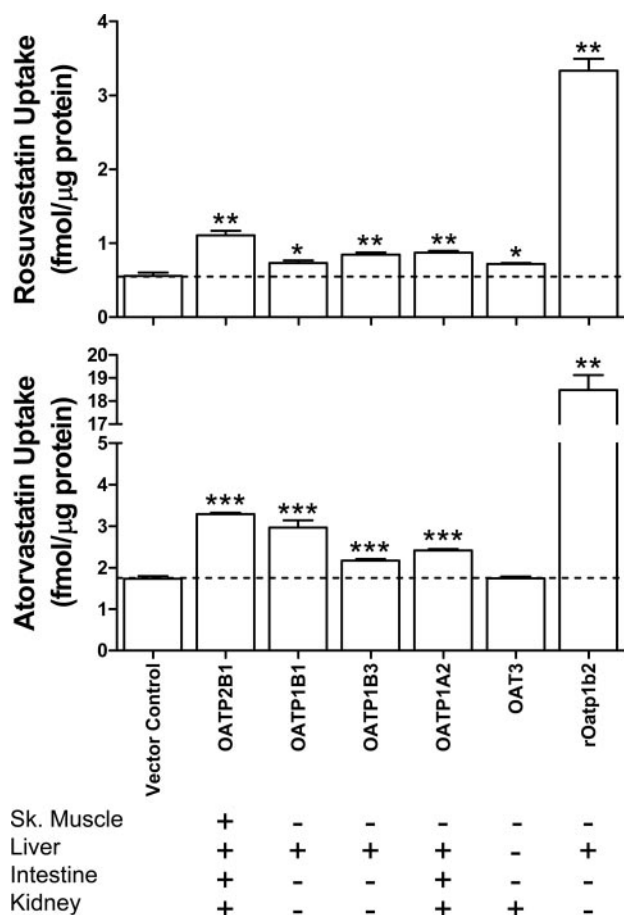
Figure 2. Intracellular accumulation of statins in HeLa cells transiently transfected with various uptake drug transporters. Intracellular accumulation of [³H]rosuvastatin and [³H]atorvastatin in cells expressing OATP2B1, OATP1B1, OATP1B3, OATP1A2, OAT3, and rOatp1b2. Results are presented as femtomoles per microgram of protein±SEM (n=3). *$P<0.05$, **$P<0.01$, ***$P<0.001$ compared to vector control. The presence (+) or absence (−) of transporter expression in various tissues is shown below.

Figure 3. Chemical inhibition of statin uptake by OATP2B1 in transiently transfected HeLa cells. Intracellular accumulation of [³H]rosuvastatin and [³H]atorvastatin in cells expressing OATP2B1 (gray bars) cotreated with cerivastatin, gemfibrozil, gemfibrozil-glucuronide, fenofibrate, rifampin, glyburide, or cyclosporine A at 100 μmol/L. Results are presented as femtomoles per microgram of protein±SEM (n=3). *$P<0.05$, **$P<0.01$, ***$P<0.001$ compared to OATP2B1 DMSO treatment.

instance, the rat ortholog of the human transporters OATP1B1 and OATP1B3, rOatp1b2, appeared capable of mediating a significantly greater accumulation of rosuvastatin and atorvastatin into cells using this technique, in relation to human OATP2B1 (Figure 2). For this reason, we used rOatp1b2 as the model transporter to maintain statin uptake into cells thereby allowing for the identification of pertinent transporters capable of statin efflux (see below).

Although highly expressed in skeletal muscle, it was not known whether MRP1, MRP4, or MRP5 transported statins. Hence, HeLa cells were double transfected with the uptake transporter rOatp1b2 and various efflux transporters. In this system, modulation of cellular retention of atorvastatin or rosuvastatin served as an indicator for efflux transport activity. Indeed, we confirm that rosuvastatin and atorvastatin are transported by MRP2, P-gp, and BCRP using this double transporter (uptake/efflux) transfection system (Figure 3). Rosuvastatin retention in cells expressing rOatp1b2 together with MRP1, MRP4, or MRP5 was lower (77%, 80%, and 27% lower, respectively) than cells expressing rOatp1b2 alone (Figure 4). This was also true for atorvastatin when
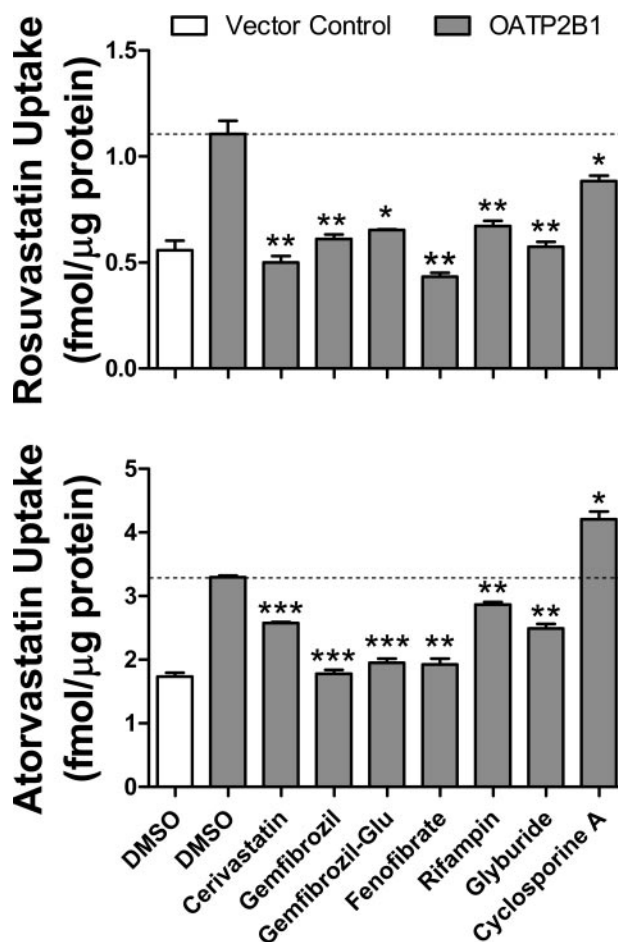
MRP1 and MRP4 (63 and 47% lower, respectively), but not MRP5, were double transfected with rOatp1b2. These findings demonstrate that MRP1, MRP4, and MRP5 are novel statin transporters.

Statin Disposition in an In Vitro Model of Human Skeletal Muscle

To evaluate the role of statin transport in toxicity, we used differentiated, primary human skeletal muscle myoblast (HSMM) cells as an in vitro model. First, we assessed whether HSMM cells expressed statin efflux transporters and found that MRP1, MRP4, and MRP5 are constitutively expressed (Figure 1A and 1B), whereas MRP2, BCRP, and P-gp are absent. Cellular localization studies revealed that some MRP1 is expressed on the cell surface of HSMM cells, although significant levels of the transporter are found in intracellular spaces. By contrast, MRP4 and MRP5 are not found on the plasma membrane, but localize within the Golgi in HSMM cells (Figure 5A). To test whether functional MRP activity is present in HSMM cells, statin accumulation was examined after chemical inhibition of efflux transport. When
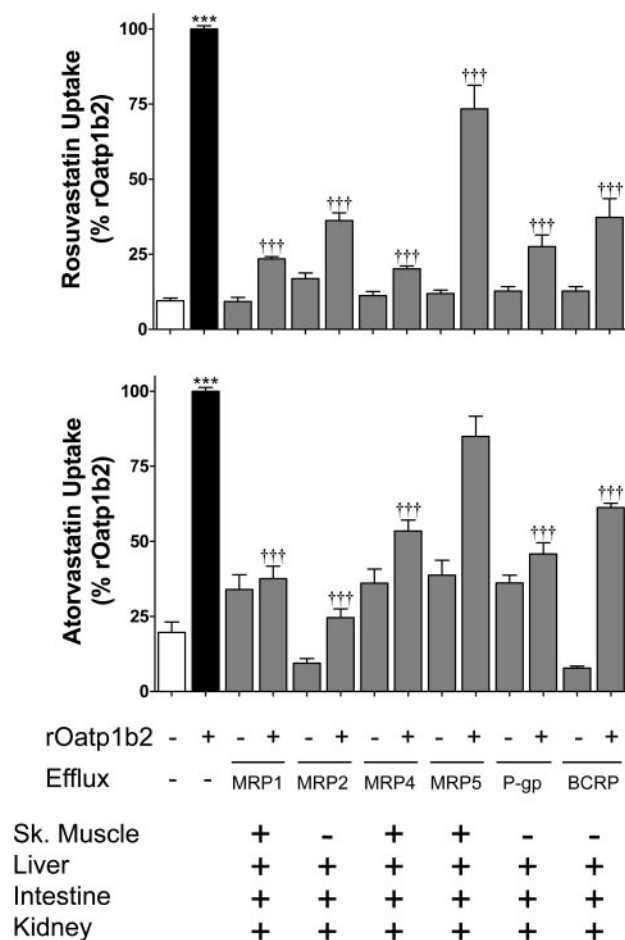
Figure 4. Intracellular accumulation of statins in HeLa cells transiently transfected with rOatp1b2 and various efflux drug transporters. Intracellular accumulation of [³H]rosuvastatin and [³H]atorvastatin in HeLa cells expressing rOatp1b2 and/or MRP1, MRP2, MRP4, MRP5, P-gp, or BCRP. Results are presented as percentages of rOatp1b2-mediated uptake±SEM (n=3 to 8). ***P<0.001 compared to vector control and efflux transporter alone; †††P<0.001 compared with rOatp1b2 mediated uptake. The presence (+) or absence (−) of transporter expression in various tissues is shown below.

HSMM cells were coincubated with known MRP inhibitors MK-571,[52] dipyridamole,[53] quercetin,[54] or verapamil,[55] there was a significant increase in cellular retention for both atorvastatin and rosuvastatin when compared to cells treated with vehicle (Figure 5B). Together with membrane localization studies, the results indicate that HSMM cells natively express functional MRP1 transporter whose activity determines the intracellular accumulation of statins. Coincubation with cerivastatin also significantly increased the cellular retention of atorvastatin and rosuvastatin in HSMM cells (Figure 5B), suggesting that this statin interacts with and is a possible substrate of MRPs. With respect to statin uptake, none of the known statin uptake transporters was expressed in HSMM cells, and, importantly, OATP2B1 is not expressed despite that it is present in vivo (Figure 1).

Role of Transporters in Statin Toxicity in an In Vitro Model of Human Skeletal Muscle

We next examined the effect of transporters on skeletal muscle exposure and toxicity of statins. In this experiment,

we overexpressed OATP2B1 and MRP1 in HSMM cells using adenoviral vectors (Ad-OATP2B1 and Ad-MRP1, respectively). Examination by confocal microscopy demonstrated robust overexpression of these transporters in HSMM cells (Figure 6). There was significant plasma membrane expression of MRP1, whereas for OATP2B1 there was some transporter on the cell membrane, but the majority was confined intracellularly.

Adenoviral overexpression of OATP2B1 in HSMM cells caused a significant increase in the cellular retention of both atorvastatin and rosuvastatin over 60 minutes (Figure 6B). After transduction with Ad-OATP2B1 and Ad-MRP1, the cellular retention of both rosuvastatin and atorvastatin were significantly attenuated compared to Ad-OATP2B1 alone. Transduction with Ad-MRP1 alone did not significantly reduce the levels of rosuvastatin in HSMM cells; however, there was a trend toward reduced atorvastatin accumulation. Consequently, the effect of statin efflux transporters on intracellular statin levels is not pronounced in the absence of influx transporters. These results indicate that overexpression of OATP2B1 and MRP1 leads to changes in intracellular statin levels in an HSMM cell model.

Preliminary studies were performed to define the time- and concentration dependency for atorvastatin and rosuvastatin cytotoxicity in HSMM cells. Cell viability, as evaluated by intracellular ATP levels, declined after 5 days of statin treatment. At comparable concentrations (eg, 100 μmol/L), atorvastatin was more toxic than rosuvastatin (Figure 7A). The cytotoxicity of atorvastatin and rosuvastatin in HSMM cells after adenoviral-mediated transporter gene delivery was assessed by 2 measures of mitochondrial function, ATP content and MTT reduction to formazan (Figure 7B through 7D), as well as activation of Caspases 3/7, a marker of apoptosis induction (Figure 7D). Transduction of HSMM cells with Ad-OATP2B1 sensitized HSMM cells to atorvastatin toxicity as demonstrated by signals from all 3 toxicity end-points (Figure 7B through 7D). There was a similar trend for rosuvastatin toxicity but only the increased activity of caspases 3/7 reached statistical significance (Figure 7D). This result is likely because overexpressed OATP2B1 was not well localized on the plasma membrane, leading to modest elevation of intracellular statin concentrations. Similarly, when HSMM cells were transduced with Ad-MRP1 alone, there was a lack of effects on statin cytotoxicity, consistent with the absence of significant changes in intracellular statin accumulation. However, when cells were transduced with both Ad-OATP2B1 and Ad-MRP1, there was protection against toxicity by both statins when compared to HSMM cells transduced with Ad-OATP2B1 alone to viabilities similar to those cells transduced with Ad-MRP1 alone (Figure 7B through 7D). Taken together, these results indicate that OATP2B1 expression promotes statin toxicity, whereas MRP1 is cytoprotective in human skeletal muscle cells.

Discussion

Drug transporters have recently been implicated in statin-induced myopathy. However, those that have been previously considered have been the transporters located in the liver and small intestine, which are largely responsible for controlling
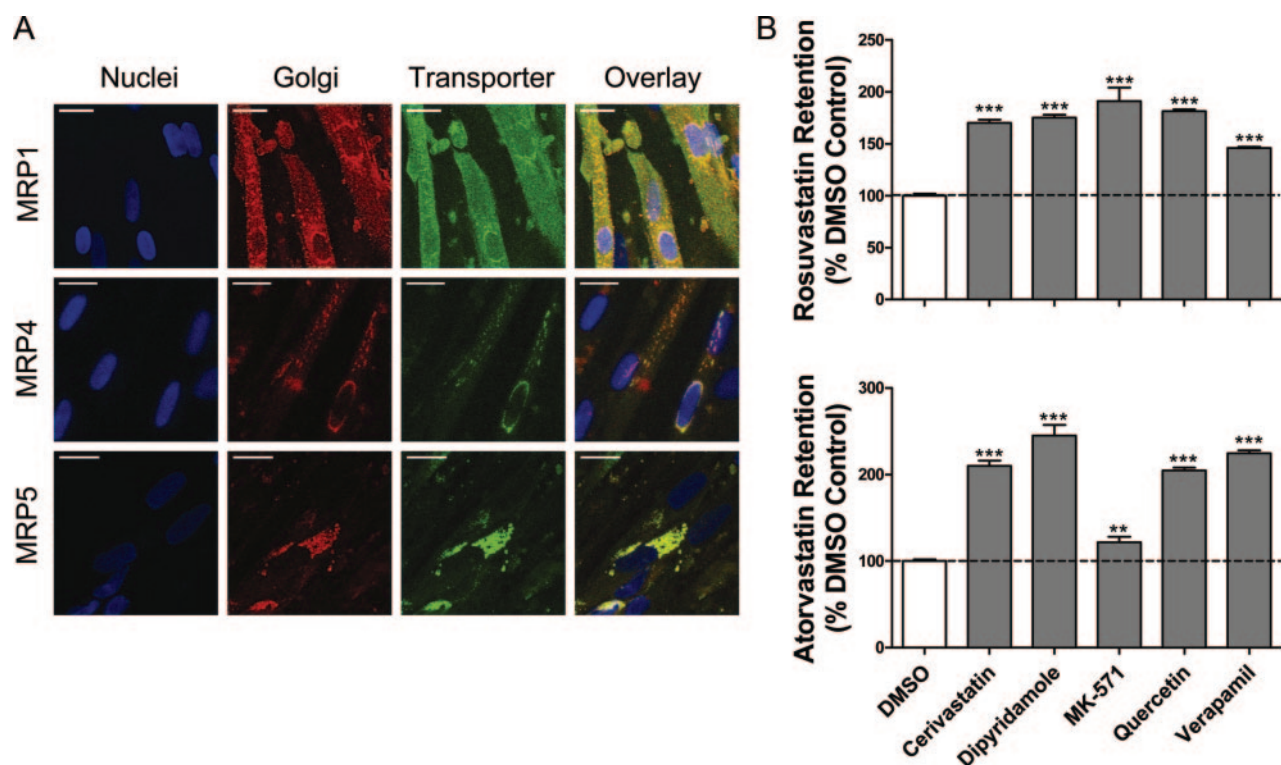
Figure 5. A, Immunofluorescence localization of MRP1, MRP4, and MRP5 (green), Golgi (red), and nuclei (blue) in differentiated HSMM cells using confocal microscopy. Scale bar=20 μm. B, Intracellular accumulation of [^{3}H]rosuvastatin and [^{3}H]atorvastatin after 30 minutes in HSMM cells cotreated with or without 100 μmol/L cerivastatin or the nonspecific MRP inhibitors dipyridamole, MK-571, quercetin, and verapamil at 100 μmol/L. Results are presented as percentages of DMSO control±SEM (n=3 to 4). **P<0.01, ***P<0.001 compared with DMSO control.

plasma statin concentrations. Indeed, genetic polymorphisms in the hepatic statin uptake transporter, OATP1B1, leading to reduced transport function are associated with a dramatic increase in risk for simvastatin-related myopathy.[32] Moreover, inhibition of both OATP1B1 and liver glucuronidation activity by concomitant treatment with the antilipidemic drug gemfibrozil causes elevation of cerivastatin plasma concentrations conferring greater predisposition to rhabdomyolysis.[29,56] Although high plasma statin level is thought to be a risk factor, it does not entirely predict myopathy. In fact, there are individuals who exhibit high statin plasma levels but do not develop myopathy, suggesting that other factors including skeletal muscle fiber statin concentration may have an impact on side effect risk.[38] Despite the recognition that drug transporters control intracellular statin concentrations, the relevant transporters in human skeletal muscle have long been overlooked.

In this report, we identified drug transporters in human skeletal muscle capable of transporting statins. Previous reports have shown that OATP2B1 is a high-affinity uptake transporter for both atorvastatin and rosuvastatin.[40,49] OATP2B1 is expressed on the apical and basolateral membranes of enterocytes and hepatocytes, respectively, and contributes to the oral absorption and hepatic distribution of statins. In addition, OATP2B1 is localized on the plasma membrane of cardiac endothelial cells,[49] as well as in platelets,[50] where it is thought to be involved in the pleiotropic cardiovascular effects of statins. Here, we show for the first

time, OATP2B1 is similarly expressed on human skeletal muscle sarcolemmal membrane. These findings are consistent with a report that suggested the presence of Oatp1a4 and Oatp2b1 in rat skeletal myofibers at the mRNA level.[57] However, demonstration of rat Oatp1a4 and Oatp2b1 protein expression in muscle was not confirmed, nor were data presented to show that these transporters mediate statin uptake.[57] Despite that direct measurement of statin accumulation was not monitored, cotreatment of rat skeletal myofibers with the OATP inhibitor estrone sulfate afforded protection against the toxicity of the hydrophilic and lipophilic statins pravastatin and fluvastatin, respectively.[57]

The known statin efflux transporters, namely P-gp, MRP2 and BCRP are not expressed in human skeletal muscle (Figure 1A). However, isoforms of the MRP transporter family such as MRP1, MRP4, and MRP5 are highly expressed in skeletal muscle, although previous to this report, their capacity for statin efflux was unknown. Here, we demonstrate that the 3 human skeletal muscle MRPs (MRP1, MRP4, and MRP5) transport rosuvastatin and/or atorvastatin. These transporters are expressed on the sarcolemmal membrane of muscle fibers, indicating a protective role against intracellular statin accumulation. There is wide substrate overlap among MRPs,[58] and this is certainly also the case for statins that are transported by the skeletal muscle MRPs, albeit at differing efficiencies. Recently, a role for rat Mrp1 in statin-induced myopathy has been suggested in studies that demonstrate precipitation of rosuvastatin-mediated skeletal
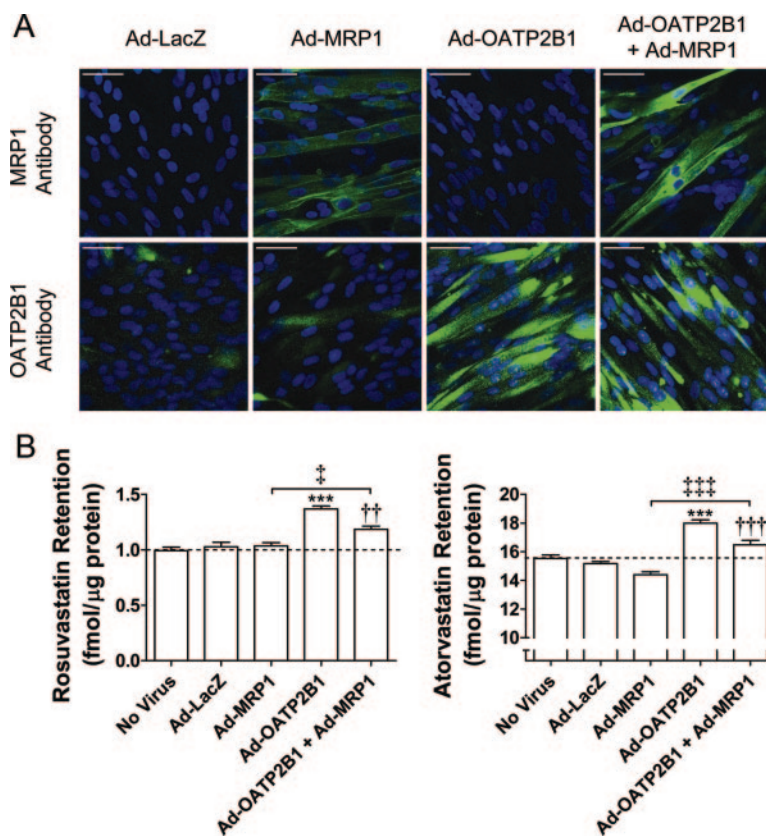
Figure 6. Statin accumulation in HSMM cells after adenoviral transduction of MRP1 and OATP2B1. A, Immunofluorescence localization of MRP1 or OATP2B1 (green) and nuclei (blue) in HSMM cells after adenoviral infection with Ad-LacZ, Ad-MRP1, Ad-OATP2B1, or Ad-OATP2B1 and Ad-MRP1 in differentiated HSMM cells using confocal microscopy. Scale bar=50 μm. B, Intracellular accumulation of [^{3}H]rosuvastatin and [^{3}H]atorvastatin in HSMM cells after adenoviral overexpression of OATP2B1 and MRP1. Results are presented as femtomoles per microgram of protein$\pm$SEM (n=4). ***$P<0.001$ compared with no virus, Ad-LacZ, or Ad-MRP1; $\dagger\dagger P<0.01$, $\dagger\dagger\dagger P<0.001$ compared with Ad-OATP2B1; $\ddagger P<0.05$, $\ddagger\ddagger\ddagger P<0.001$ compared with Ad-MRP1.

muscle toxicity in rats cotreated with the MRP inhibitor, probenecid.[59] Interpretation of these findings remains difficult for a number of reasons, including a lack of demonstration that rat Mrp1 transports rosuvastatin, absence of Mrp1 expression data in tissues such as skeletal muscle, and a deficiency of information regarding differences in plasma and tissue concentrations of rosuvastatin after probenecid cotreatment.[59]

The dynamic interplay between uptake and efflux transporter activities likely controls muscle fiber statin concentrations, which determines susceptibility to toxicity. We have shown that the toxicity of rosuvastatin and atorvastatin in primary human skeletal muscle cells is dependent on the achieved intracellular drug concentrations. This is highlighted by the findings that reduction of cellular statin accumulation by MRP1 overexpression in cultured skeletal muscle cells heterologously expressing OATP2B1 (Figure 6B) afforded cytoprotection against statin exposure (Figure 7C and 7D). In the guinea pig, skeletal muscle concentrations of rosuvastatin and atorvastatin are less than 10% of that found in plasma, suggesting that the balance is tipped toward higher efflux than uptake activity.[60] In our evaluation of the literature, the plasma-to-skeletal muscle concentration ratio of statins in humans is not known, but this value will undoubtedly be dependent on the relative expression and intrinsic activities of the attendant uptake (OATP2B1) and efflux (MRP1, MRP4, MRP5) transporters. The present results would also suggest that drug–statin interactions occurring not only at the level of the hepatocyte cell membrane but also in skeletal muscle fibers could contribute to myopathy. Certainly, a number of

clinically used drugs are substrates/inhibitors of the here identified skeletal muscle statin transporters (Figures 3 and 5B).[61,62] Our data suggest that skeletal muscle statin uptake by OATP2B1 can be inhibited by concomitantly administered drugs such as gemfibrozil, fenofibrate, and glyburide (Figure 3). This finding could be considered contradictory to the increased risk of statin myopathy in patients cotreated with gemfibrozil. However, it should be mentioned that the gemfibrozil inhibits hepatic statin clearance to increase systemic statin exposure and there remains the possibility that inhibition of skeletal muscle efflux transport could offset the protection provided by OATP2B1 inhibition. Indeed, we demonstrate that statin efflux can be blocked by concurrent treatment of HSMM cells with known inhibitors of MRPs (Figure 5B). Moreover, one must consider not only pharmacokinetic but also pharmacodynamic interactions in extrapolating the current transport inhibition findings to myopathy risk.

There are limitations with the differentiated HSMM cell model for the study of statin toxicity. Firstly, although MRP1, MRP4, and MRP5 are constitutively expressed in HSMM cells, only MRP1 localized to the plasma membrane (Figure 5A). This is in contrast to immunofluorescence data that show these MRPs are expressed on the sarcolemmal membrane of intact skeletal muscle fibers (Figure 1C). It is for this reason that we are only able to assess the effect of MRP1 but not MRP4 or MRP5 on cytoprotection against statins in this model of skeletal muscle. Furthermore, HSMM cells do not natively express OATP2B1, as is found in vivo; hence, we required viral gene delivery to assess the role of uptake
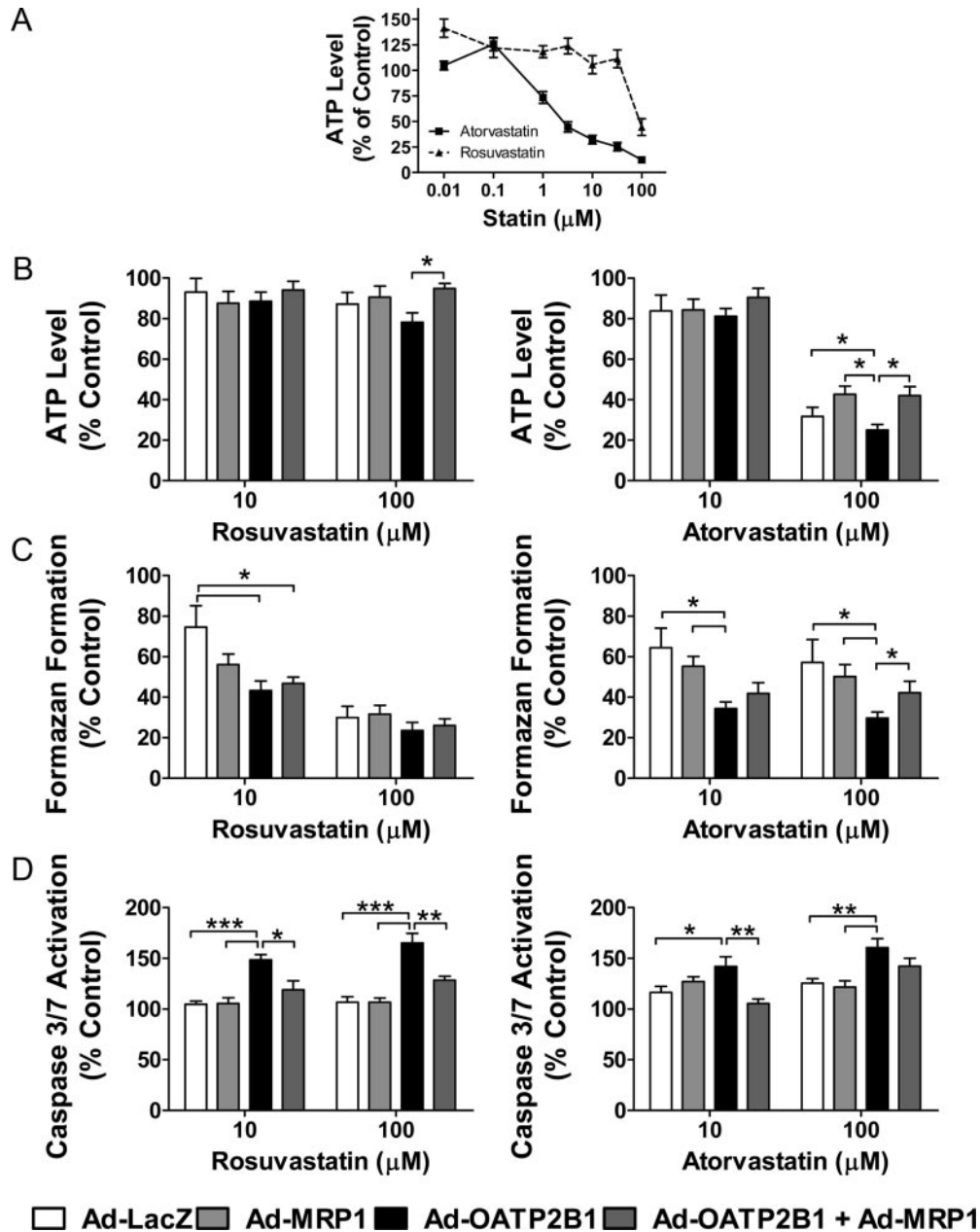
Figure 7. Statin cytotoxicity in HSMM cells after adenoviral transduction of MRP1 and OATP2B1. A, Dose-dependent cellular viability of HSMM cells after treatment with atorvastatin and rosuvastatin for 5 days, measured by intracellular ATP concentration. Results are presented as percentages of DMSO control±SEM (2 independent experiments with 3 determinations per experiment). B, Cellular viability of HSMM cells after treatment with atorvastatin and rosuvastatin for 72 hours, measured by intracellular ATP concentration. Results are presented as percentages of DMSO control±SEM (n=5). C, Cellular viability of HSMM cells after treatment with atorvastatin and rosuvastatin for 72 hours, measured by formazan formation using a MTT assay. Results are presented as percentages of DMSO control±SEM (n=4). D, Induction of apoptosis in HSMM cells after treatment with atorvastatin and rosuvastatin for 48 hours measured by activation of caspase 3 and 7. Results are presented as percentages of DMSO control±SEM (n=4). *$P<0.05$, **$P<0.01$, ***$P<0.001$.

transport on statin toxicity. That OATP2B1 is not expressed in HSMM cells compares well with other skeletal muscle genes that we have found at very low levels in relation to intact skeletal muscle, including creatine kinase M isoform (Online Figure I). Therefore, in interpreting the present toxicity findings, it should be considered that this in vitro model of skeletal muscle differs phenotypically to muscle fibers in vivo.

In conclusion, statin transporters are present in human skeletal muscle that control intracellular drug exposure. We propose a role for OATP2B1 in sensitizing skeletal muscle cells to statin toxicity and that the novel statin efflux transporters MRP1, MRP4, and MRP5 protect muscle from toxicity. The dynamic functional interplay between these uptake and efflux transporters in vivo likely determines risk for statin-induced myopathy.

Acknowledgments

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