# The role of acid-base imbalance in statin-induced myotoxicity

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**Disturbances in acid-base balance, such as acidosis and alkalosis, have potential to alter the pharmacologic and toxicologic outcomes of statin therapy. Statins are commonly prescribed for elderly patients who have multiple comorbidities such as diabetes mellitus, cardiovascular, and renal diseases. These patients are at risk of developing acid-base imbalance. In the present study, the effect of disturbances in acid-base balance on the interconversion of simvastatin and pravastatin between lactone and hydroxy acid forms have been investigated in physiological buffers, human plasma, and cell culture medium over pH ranging from 6.8–7.8. The effects of such interconversion on cellular uptake and myotoxicity of statins were assessed in vitro using C2C12 skeletal muscle cells under conditions relevant to acidosis, alkalosis, and physiological pH. Results indicate that the conversion of the lactone forms of simvastatin and pravastatin to the corresponding hydroxy acid is strongly pH dependent. At physiological and alkaline pH, substantial proportions of simvastatin lactone (SVL; ~87% and 99%, respectively) and pravastatin lactone (PVL; ~98% and 99%, respectively) were converted to the active hydroxy acid forms after 24 hours of incubation at 37°C. At acidic pH, conversion occurs to a lower extent, resulting in greater proportion of statin remaining in the more lipophilic lactone form. However, pH alteration did not influence the conversion of the hydroxy acid forms of simvastatin and pravastatin to the corresponding lactones. Furthermore, acidosis has been shown to hinder the metabolism of the lactone form of statins by inhibiting hepatic microsomal enzyme activities. Lipophilic SVL was found to be more cytotoxic to undifferentiated and differentiated skeletal muscle cells compared with more hydrophilic simvastatin hydroxy acid, PVL, and pravastatin hydroxy acid. Enhanced cytotoxicity of statins was observed under acidic conditions and is attributed to increased cellular uptake of the more lipophilic lactone or unionized hydroxy acid form. Consequently, our results suggest that comorbidities associated with acid-base imbalance can play a substantial role in the development and potentiation of statin-induced myotoxicity. (Translational Research 2016;174:140–160)**

**Abbreviations:** cDNA = complementary DNA; Ct = cycle threshold; DMEM = Dulbecco's modified eagle medium; *Gapdh* = glyceraldehyde-3-phosphate dehydrogenase; *Hprt* =

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hypoxanthine phosphoribosyl transferase; HQC = high concentration quality control; IS = internal standard; LDH = lactate dehydrogenase; LLOQ = lower limit of quantification; LOV-A = lovastatin hydroxy acid; LOV-L = lovastatin lactone; LQC = low concentration quality control; *MHC* = myosin heavy chain; MQC = medium concentration quality control; mRNA = messenger RNA; *MRP* = multiresistant protein; MTT = thiazolyl blue tetrazolium bromide; NA = nonapplicable; *OATP* = organic anionic transporting polypeptide; PBS = phosphate buffer saline; PVA = pravastatin hydroxy acid; PVL = pravastatin lactone; RSD = relative standard deviation; RE = relative error; *Rps12* = ribosomal protein S12; SVA = simvastatin hydroxy acid; SVL = simvastatin lactone; *Tbp* = TATA box-binding protein

## AT A GLANCE COMMENTARY

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### Background

Statins are commonly prescribed for elderly patients who are at risk of developing acid-base imbalance as a result of multiple comorbidities such as diabetes mellitus, cardiovascular, and renal diseases. Disturbances in acid-base balance, such as acidosis and alkalosis, have potential to affect the ratio between the lactone and acid forms of statins and alter the pharmacologic and toxicologic outcomes of statin therapy.

### Translational Significance

This work provides a novel translational insight into the role of disturbances in acid-base balance in development of statin-induced muscle toxicity. The effect of acidosis on statin-induced muscle toxicity is particularly important in case of lipophilic statins, such as simvastatin. Enhanced cytotoxicity of statins was observed under acidic conditions as a result of increased cellular uptake of the more lipophilic lactone form or unionized hydroxy acid form. On the other hand, alkaline conditions were found to have a protective effect against statin-induced myotoxicity because of inability of statin to achieve adequate intracellular concentrations as a result of conversion to the more hydrophilic ionized hydroxy acid form.

## INTRODUCTION

Statins are cholesterol-lowering drugs commonly used to reduce morbidity and mortality associated with atherosclerotic cardiovascular diseases.[1] Recent data from the National Center for Health Statistics reveals that 27.9% of American men and women of 40 years and older are taking statins.[2] In recent years, a significant increase in the number of statin prescriptions has been reported by the British Heart Foundation with more than 7 million people currently taking either prescribed or over the counter statins in the UK.[3] According to the American College of Cardiology and American Heart Association guidelines[4,5] for prediction of cardiovascular risk factors, more than 1 billion people worldwide are now estimated to use statins.[6]

Statins are generally well tolerated, but muscular adverse effects considerably influence drug tolerability and patient adherence especially with long-term use.[1] The exact mechanism by which these drugs induce their myotoxic effects is not fully understood. Simvastatin, a highly lipophilic statin, is the most commonly prescribed cholesterol-lowering medication, and 42% of American adults who are using cholesterol-lowering drugs are prescribed this drug.[2] It has been postulated that lipophilic statins are more myotoxic than hydrophilic ones, most probably because of their ability to penetrate skeletal muscle tissues and alter membrane structure.[7-9] Nonetheless, the ability of lipophilic statins to penetrate hepatic cells makes them more potent in reducing elevated cholesterol levels.[9] This property might explain their wider use comparing to hydrophilic statins.

Several risk factors have been suggested to predispose patients to statin-associated myotoxicity including advanced age, high dose, female gender, drug interactions, genetic variability of drug metabolizing enzymes and transporters, lipophilicity of statins, and coincident morbidities.[10] Statins are administered either as lactone or hydroxy acid forms. The lactone form is pharmacologically inactive, whereas the hydroxy acid is the active form that lowers plasma cholesterol levels.[8] Substantial differences exist between these forms in term of their lipophilicity. The lactone form is highly lipophilic, whereas hydroxy acid has poor lipid solubility.[9,11] It has been reported that lactone form is more myotoxic than the active acid form owing to its lipophilicity.[8,12,13] In vivo, interconversion between both forms is mediated by enzymatic as well as pH-dependent chemical reaction in plasma, liver, and other tissues.[14-17] Therefore, acid-base imbalance can potentially alter the lipophilicity of statins by affecting their interconversion between lactone and hydroxy acid forms. The higher lipophilicity of statins in the lactone form can potentially facilitate their penetration into muscle cells