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## BENEFITS VERSUS COSTS OF STATIN DRUGS

Sara Shilcrat

### ABSTRACT

Statins have been prescribed to the masses as primary and secondary prevention for coronary disease caused by hypercholesterolemia after their initial discovery in the late 1980s. Their actions in reducing low-density lipoproteins and increasing high-density lipoproteins are well documented; however, many negative effects have been reported related to muscle pathology and kidney function. The goal of this study is to investigate whether the benefits of this class of drugs outweigh the costs. Intense review of the literature was conducted using scholarly articles with original research findings that were located via electronic databases such as Medline, Science Direct, Proquest Medical Library, and Google Scholar. Research findings on the benefits of statins extended beyond their lipid-related effects and included benefits to the immune system and inflammatory response, sepsis prevention, and improved endothelial cell functions, among others. Negative side effects of statins are many, including damage related to skeletal muscle tissue, such as rhabdomyolysis, myofiber necrosis, myotoxicity, myopathy, myalgia, reduced muscle resting chloride membrane potential (gCl), vacuolization of the T-tubule system, sarcolemma detachment, and targeting of the muscle's mitochondria. Differences between type I oxidative myofibers and type IIB glycolytic myofibers are discussed as well as the lipophilic and hydrophilic tendencies of the statins in relation to the damage inflicted on skeletal muscle tissue. In some rare cases of statin administration, motor neurons displayed Amyotrophic Lateral Sclerosis (ALS)-like symptoms that progressed up until muscle denervation. Additional negative side effects were seen to the circulatory and excretory systems, including altered chemical composition of both the blood plasma and urine, and rare renal failure due to rhabdomyolysis. The inquiry as to whether statins affect cardiac muscle as they do skeletal muscle is also addressed with the minimal findings that seemed to indicate that cardiac muscle is not targeted by statins.

After taking into account the benefits versus the costs of statins, in addition to the lack of a better drug on the market for combating coronary disease, it was suggested that statin administration should continue due to its proven cholesterol-related effects. However, statin users should be limited to patients with coronary disease triggered by high cholesterol. Patients with proven treatment options, such as patients with cancer or autoimmune diseases, were cautioned not to take statins for the possible benefits of unproven pleiotropic effects due to the likelihood of damage to skeletal muscle and kidney functioning. Monthly blood work and urinalysis were also suggested for patients on statins, and patients should be advised to speak to their physicians if they feel muscle pain or encountered changes in the ease of manipulating their muscles, as these are possible signs of muscle and nerve problems.

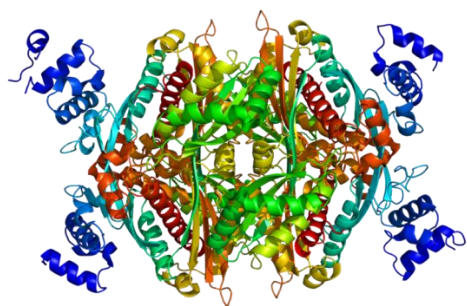
### INTRODUCTION

In 1987, lovastatin, commonly known as Mevacor, Altacor, or Altoprev, was released into the public market. This new drug, isolated from the fermentation of the fungus *Aspergillus terreus*, was the beginning of a new class of drugs, marketed by the name of 'statins,' that focused on lowering cholesterol levels (Statin 2012; Torbert 2003). At the time, knowledge of the connection between cholesterol and the formation of plaques, or atheromas, in blood vessels was just beginning to develop. Although today it is common knowledge that cholesterol escaping from ruptured atherosclerotic plaques is pinpointed as the culprit of many heart attacks, this was

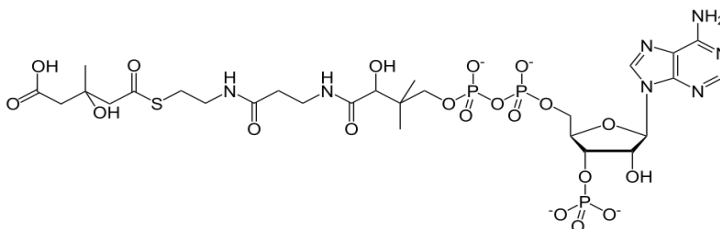
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yet unknown. Statins evolved from the skepticism that surrounded the lipid hypothesis, a controversial idea which associated coronary heart disease with increased levels of low-density lipoprotein (LDL) cholesterol and decreased levels of high-density lipoprotein (HDL) cholesterol (Statin 2012). Statins, or HMG-CoA reductase inhibitors, effectively stop the metabolic pathway ending in the synthesis of cholesterol. This class of drugs inhibits the functioning of an enzyme known as HMG-CoA reductase, and as a result, HMG-CoA, or 3-hydroxy-3-methylglutaryl-Coenzyme A, is not converted into mevalonic acid (Figures 1 and 2). Since this is the first step, also known as the rate-limiting step, in the pathway that leads to cholesterol, blocking this transformation will stop the entire cholesterol synthesis pathway (Figure 3). With the formation of cholesterol at a halt, fewer plaques will form, and subsequently rupture, decreasing the risk of heart attacks and other adverse effects of cardiovascular disease. However, in addition to blocking the formation of cholesterol which improves the cardiovascular disease prognoses, using statins also prevents the formation of other cholesterol derivatives such as isoprenoids and sterols including testosterone, estradiol, and cortisol, among others which may result in additional repercussions.



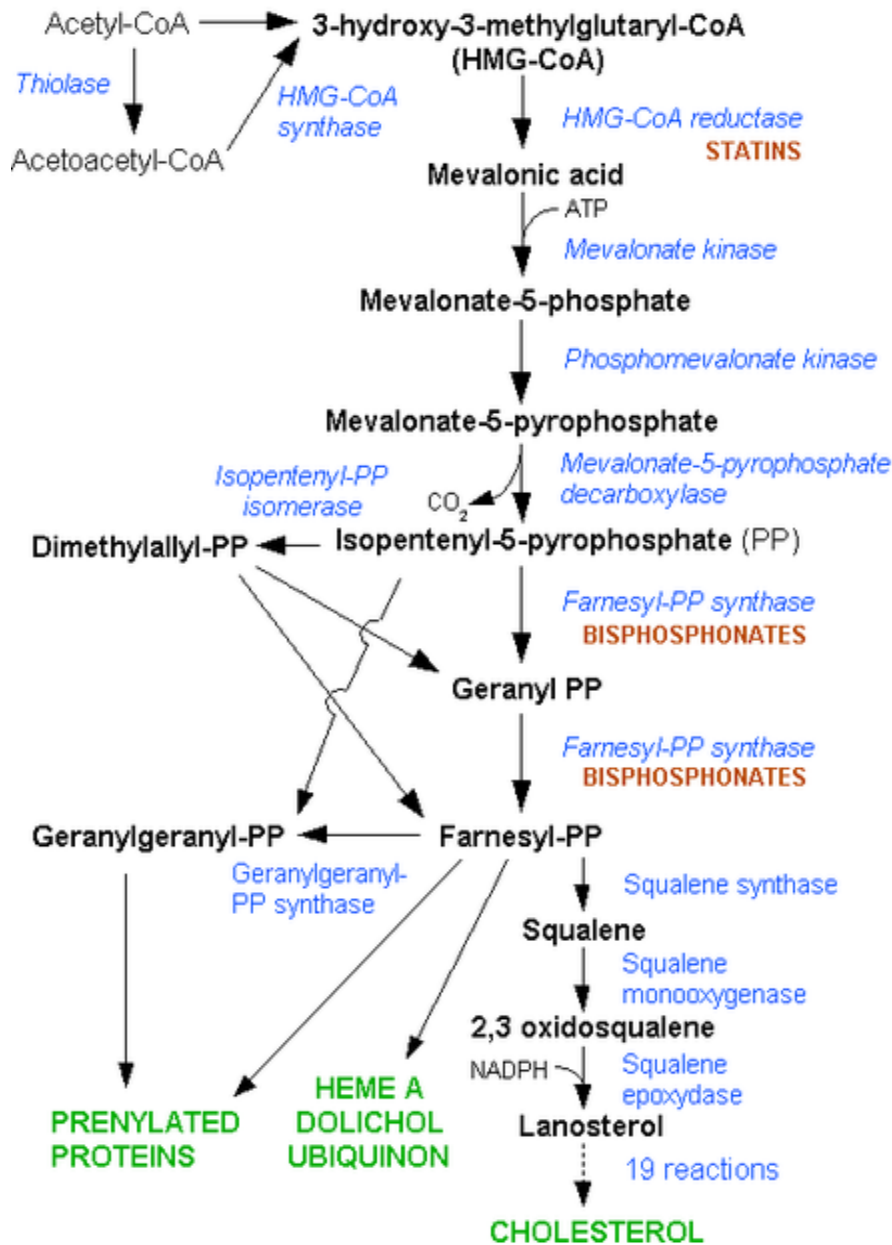
**Figure 1:** HMG-CoA Reductase Ribbon Model. Source: HMG-CoA reductase 2012



**Figure 2:** 3-Hydroxy-3-methylglutaryl-coenzyme A (HMG CoA) Structure. Source: HMG-CoA 2012

Today, statins are commonly prescribed as primary and secondary prevention for cardiovascular diseases associated with elevated cholesterol levels, or hypercholesterolemia (Merx and Weber 2006; Statin 2012). Since the discovery of lovastatin, other statins have successively entered the market including simvastatin (1988, as Zocor and Lipex), pravastatin (1991, as Prevachol, Selektine, and Lipostat), fluvastatin (1994, as Lescol and Lescol XL), atorvastatin (1997, as Lipitor and Torvast), cerivastatin (1998, as Lipobay and Baycol), and rosuvastatin (2003, as Crestor) (Torbert 2003) (Figure 4).

Other prescription drugs on the market, such as Vytorin (simvastatin and ezetimibe), Advicor (lovastatin and niacin extended release), Caduet (atorvastatin and amlodipine besylate), and Simcor (simvastatin and niacin extended release) combine one of the statins with another drug for multiple therapeutic effects (Statin 2012). Also, statins may be prescribed with concurrent use of fibrates, immunosuppressants, corticosteroids, antifungals, blood thinners like warfarin, and other prescription drugs (Mohaupt et al. 2009; Nicholls et al. 2011).



**Figure 3:** Cholesterol Synthesis Pathway. Source: Statin 2012

Each of the statins differs in its ability to reduce LDL cholesterol. Ranging from most effective to least effective, the statins can be arranged in the following order: cerivastatin > rosuvastatin > atorvastatin > simvastatin > lovastatin > pravastatin > fluvastatin (Statin 2012). Recommended dosages of the statin drugs differ based on their potency, with less potent statins prescribed at higher dosages and vice versa. Statins can also be classified on a continuum of being more hydrophilic or more lipophilic, affecting their LDL cholesterol reducing power. Differences in a particular statin's hydrophilicity are thought to cause different physiological effects on the body, especially when discussing the effects of statins on skeletal muscle (Pierno et al. 2006; Sidaway et al. 2009).

## BENEFITS VS. COSTS OF STATIN DRUGS

Examining the chemical structure of statins shows general similarities that exist in this class of drugs (Figure 4). Some key differences do exist, however, between statins that are derived from fermented natural substances (mevastatin, lovastatin, pravastatin, and simvastatin<sup>1</sup>) and laboratory-created synthetic statins (atorvastatin, cerivastatin, fluvastatin, pitavastatin, and rosuvastatin). Generally, the statins consist of two or more ring structures; in synthetic statins, one of the rings is a nitrogen-containing heterocyclic ring not found in naturally-derived statins. In addition, lacking in the fermentation derivatives, a fluorinated benzene ring is found in the synthetic statins as well as a seven-carbon fatty acid chain branching off a ring structure terminating in a carboxylic acid. The fatty acid chain is also characterized as a diol, with two hydroxyl groups coming off the chain at positions C3 and C5 counting from C1 of the -COOH group.. In the naturally occurring statins two fused cyclohexene rings share two carbon atoms and have double bonds located a single carbon away from one another. These statins lack the fatty acid chain and instead, a cyclic ester with a hydroxyl group attached 2 carbon atoms away is present. Additionally, they contain a second ester attached further away on the molecule that is followed by a *sec*-butyl group. Finally, the synthetic statins may contain one or more isopropyl groups branching off of the heterocyclic N-containing rings (Table 1).

The variations in statins' structures have generated a common assortment of side effects, but the individual structure of the statin has been shown to yield differences in the severity of the side effects when they are experienced. In 2001, cerivastatin was removed from the market due to the severe side effects, specifically rhabdomyolysis, or the breakdown of muscle tissue, which led to numerous deaths (Obayashi et al. 2011; Sidaway et al. 2009; Statin 2012). A few more common, less severe side effects seen in skeletal muscle after using statins include myotoxicity, myopathy, any abnormal condition or disease of muscle tissue, myalgia, or muscle pain, and limb weakness. Other adverse side effects seen with statins include increased creatinine kinase (CK) activity, increased ryanodine receptor 3 (RYR3) mRNA expression (Mohaupt et al. 2009), sarcolemma detachment (Mohaupt et al. 2009), vacuolization of muscle fibers (Mohaupt et al. 2009; Obayashi et al. 2011), increased myoglobinemia and myoglobinuria (Pierno et al. 2006), reduced sarcolemma resting chloride membrane potential (gCl) (Pierno et al. 2006; Pierno et al. 2009), muscle fiber necrosis, neuromuscular damage with ALS-like (amyotrophic lateral sclerosis) symptoms (Edwards et al. 2007), and kidney damage (Campese and Park 2007).

Despite these adverse effects, statins have been proven to be very effective at reducing LDL cholesterol and boosting HDL cholesterol. Other beneficial actions of statins include pleiotropic effects unrelated to lipid mobilization such as sepsis prevention (Hackam et al. 2006 cited in Merx and Weber 2006), increased KLF2 expression (T cells) (Bu et al. 2010), improved endothelium function including increased nitric oxide (NO) production (Liao and Laufs 2005; Merx and Weber 2006), and other possible applications for treating autoimmune and inflammatory disorders (Bu et al. 2010; Weitz-Schmidt 2003).

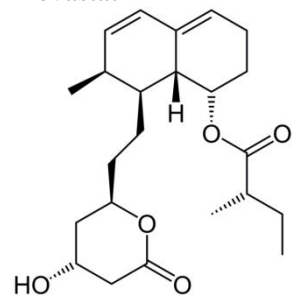
The sale of Lipitor™ (atorvastatin) swept the market, netting Pfizer an unprecedented yield in the pharmaceutical industry of more than \$12 billion dollars (Statin 2012). Yet whether the benefits of statins are really worth the adverse side effects experienced is still under debate. Doctors seem bent on continuing to prescribe the statin drugs and tend to taper the dosage as needed to mitigate the side effects. But when do the ill effects of statins go so far that it is no longer possible to justify their use? To what degree must the body's chemistry be altered in order to stop using statins?

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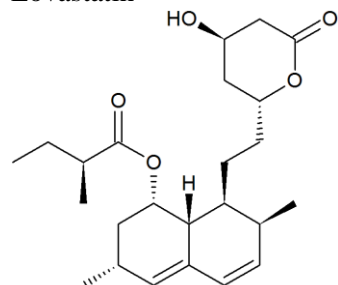
<sup>1</sup>Although simvastatin is actually synthetically made from a substance produced by fermenting *Aspergillus terreus*, it closely resembles naturally-derived statins in its structure.

**Naturally-Derived Statins**

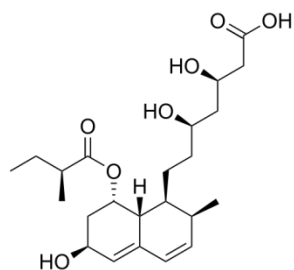
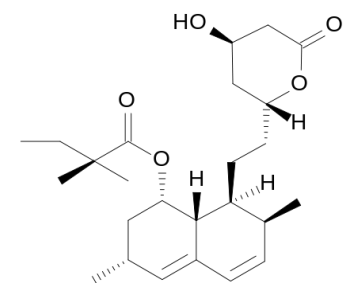
Mevastatin



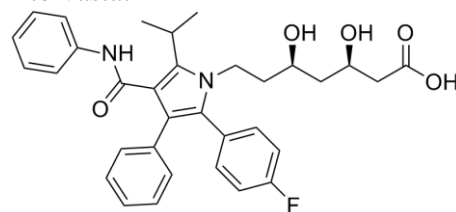
Lovastatin



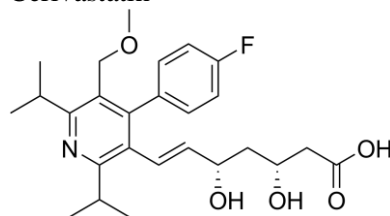
Pravastatin

Simvastatin<sup>1</sup>**Synthetic Statins**

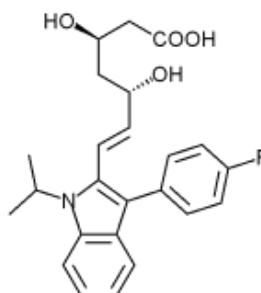
Atorvastatin



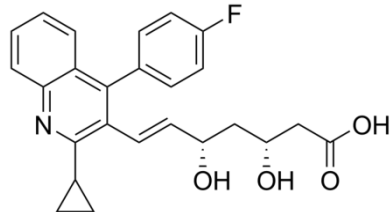
Cerivastatin



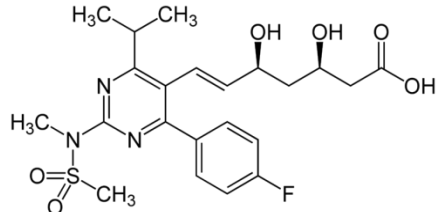
Fluvastatin



Pitavastatin



Rosuvastatin

**Figure 4:** Structures of the statins. Source: Statin 2012

## BENEFITS VS. COSTS OF STATIN DRUGS

**Table 1:** Features of the natural and synthetic statins.

Statins	Poly Cyclic	N-containing Heterocyclic Ring	<i>para</i> - Fluorobenzene Ring	Fused Cyclohexene Rings (Decene structure)	Fatty Acid with Diol /COOH	Cyclic Ester with -OH	Ester with <i>sec</i> - butyl	Isopropyl Groups	Additional Structures
<b>Natural (Fermentation derived)</b>									
<b>Mevastatin</b>	Y	N	N	Y	N	Y	Y	N	
<b>Lovastatin</b>	Y	N	N	Y	N	Y	Y	N	
<b>Pravastatin</b>	Y	N	N	Y	N	Y	Y	N	
<b>Simvastatin</b>	Y	N	N	Y	N	Y	*	N	
<b>Synthetic</b>									
<b>Atorvastatin</b>	Y	Y-pentane	Y	N	Y	N	N	Y	Cyclic Amide
<b>Cerivastatin</b>	Y	Y- cyclohexane	Y	N	Y	N	N	Y (2)	Alkene at C6- C7 of fatty acid chain
<b>Fluvastatin</b>	Y	Y-pentane	Y	N	Y	N	N	Y	Heterocyclic pentane attached to a benzene ring
<b>Pitavastatin</b>	Y	Y- cyclohexane	Y	N	Y	N	N	N	Cyclopropane and fused heterocyclic and non- heterocyclic 6C rings
<b>Rosuvastatin</b>	Y	Y- cyclohexane with 2 N's	Y	N	Y	N	N	Y	Methyl- Sulfur dioxide attached to a secondary amine

**METHODS**

Review of the literature on statins was done using electronic databases, such as Medline, Science Direct, Proquest Medical Library, and Google Scholar to procure articles on or related to statins using keywords like 'statins,' 'HMG-CoA reductase inhibitors,' and the like.

**DISCUSSION****Benefits of Statins:*****Reduction of LDL cholesterol and increase of HDL cholesterol***

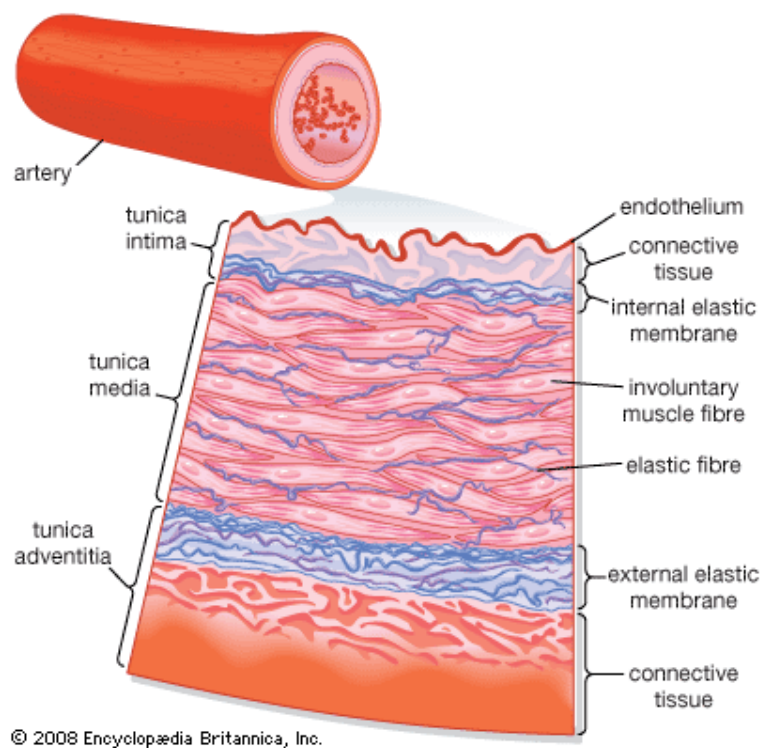
Statins were originally intended for their abilities to reduce LDL cholesterol and increase HDL cholesterol. Some studies have shown that modulations of LDLs and HDLs brought about by statins may result in regression of coronary disease (Nicholls et al. 2011; Nissen et al. 2004; Nissen et al. 2006). In an extended study spanning more than 2 years, 1039 patients with coronary disease were treated with one of two statin drugs, atorvastatin (80 mg daily) or rosuvastatin (40 mg daily), in a randomized clinical trial to determine and compare their individual effects on the progression of atherosclerotic plaques (Nicholls et al. 2011).

### ***Percent atheroma volume and normalized total atheroma volume regression***

Before and after the 104-week period, ultrasounds were recorded of a particular artery with stenosis. The external elastic membrane of the vessel and the lumen size were measured (Figure 5), and the following formulas were used to determine the percent atheroma volume (PAV) and the normalized total atheroma volume ( $TAV_{normalized}$ ), that allowed for comparison between participants who had different atheroma sizes:

$$PAV = \frac{\Sigma (\text{External Elastic Membrane}_{area} - \text{Lumen}_{area})}{\Sigma \text{External Elastic Membrane}_{area}} \times 100$$

$$TAV_{normalized} = \frac{\Sigma (\text{External Elastic Membrane}_{area} - \text{Lumen}_{area})}{\text{median no. of no. of images in pullback images in cohort.}}$$



**Figure 5:** Diagram of an Artery in Cross Section. Source: Diagram of an artery in cross section 2008

The changes in PAV and  $TAV_{normalized}$  were calculated as the PAV or  $TAV_{normalized}$  at week 104 minus the initial PAV or  $TAV_{normalized}$ . Interpreting the formulas, an increase in PAV corresponds to a decrease in the opening size of the lumen, or the higher the PAV value, the more closed the coronary artery is. The  $TAV_{normalized}$  follows the same methodology as the PAV, with a larger  $TAV_{normalized}$  related to more closed arteries in the sample of participants. During the 104 week period, the HDL and LDL cholesterol and triglyceride levels of the participants were measured at 24, 48, 72, and 104 weeks (Nicholls et al. 2011).

The study showed that the two intensive statin regimens lead to statistically significant results. Both atorvastatin and rosuvastatin lowered LDL cholesterol levels

and increased HDL cholesterol, yet, rosuvastatin was more effective statistically at achieving an overall lower LDL to HDL ratio, bringing down LDL cholesterol levels below 70 mg/deciliter in many participants, and decreasing the percentage of individuals with LDL cholesterol levels above 100 mg/deciliter compared to atorvastatin (Table 2). The PAV and  $TAV_{normalized}$  values decreased significantly corresponding to an increase in the lumen size of the participants' blocked arteries due to shrinkage of the plaques.

## BENEFITS VS. COSTS OF STATIN DRUGS

**Table 2:** LDL and HDL Cholesterol Levels After Intensive Statin Regimens. Source: Nicholls et al. 2011

	Statin (mg/deciliter least-squares mean values $\pm$ SD)	
	Atorvastatin	Rosuvastatin
<b>LDL cholesterol levels</b> ( $p < 0.001$ )		
at baseline	119.9 $\pm$ 28.9	120.0 $\pm$ 27.3
at 104 weeks	70.2 $\pm$ 1.0	62.6 $\pm$ 1.0
<b>HDL cholesterol levels</b> ( $p = 0.01$ )		
at baseline	44.7 $\pm$ 10.7	45.3 $\pm$ 11.8
at 104 weeks	48.6 $\pm$ 0.5	50.4 $\pm$ 0.5

Although the PAV showed a slightly greater reduction with rosuvastatin than atorvastatin, it was not statistically significant, yielding similar effectiveness in both statins. With respect to the  $TAV_{normalized}$ , rosuvastatin did significantly reduce the  $TAV_{normalized}$  value more than atorvastatin. Rosuvastatin was also more effective in reducing the PAV in women, participants with higher initial HDL cholesterol levels, and in participants with higher initial LDL levels. Two interesting abnormalities found in the participants' lab work were increased levels of a liver enzyme, alanine aminotransferase, in the atorvastatin group and more proteinuria in the rosuvastatin group (Nicholls et al. 2011). Alanine aminotransferase, or alanine transaminase, is an enzyme found in both hepatocytes and myocytes that reversibly converts glutamate to  $\alpha$ -ketoglutarate, leading to the formation of pyruvate. Gluconeogenesis converts pyruvate to high-energy glucose; the glucose can then be utilized by the cell. Alanine aminotransferase is used in enzymatic assays and indicates signs of liver damage and/or myopathy (Nelson and Cox 2005; Alanine Transaminase 2012).

This experiment is a clear indicator of the efficiency of statins at yielding mean LDL cholesterol levels below the recommended 70 mg/deciliter for secondary prevention of coronary disease. HDL cholesterol levels also came close to the recommended 50 mg/deciliter, leading the researchers to believe that if given enough time, the statin regimen would meet the desired levels for HDL cholesterol and LDL cholesterol and facilitate the regression, or at least deter the progression, of coronary disease (Nicholls et al. 2011).

Other research has found similar results with pravastatin and atorvastatin (Nissen et al. 2004), and rosuvastatin (Nissen et al. 2006). Still, a major consideration is that although the PAV reflects reduction in the size of a particular atherosclerotic plaque, it does not necessarily translate into preventing an impending cardiovascular episode. Second,  $TAV_{normalized}$  regression has not been linked to any clinical significance. Finally, although disease advanced in one third of the participants even with the heavy statin regimen, results indicate the beneficial aspects of statins with regard to cholesterol and plaque regression and demonstrate the general safety of statins even at high doses (Nicholls et al. 2011).

### ***Pleiotropic effects***

Statins have been found to aid in a variety of other functions (Liao and Laufs 2005).

### **Immune responses and inflammation:**

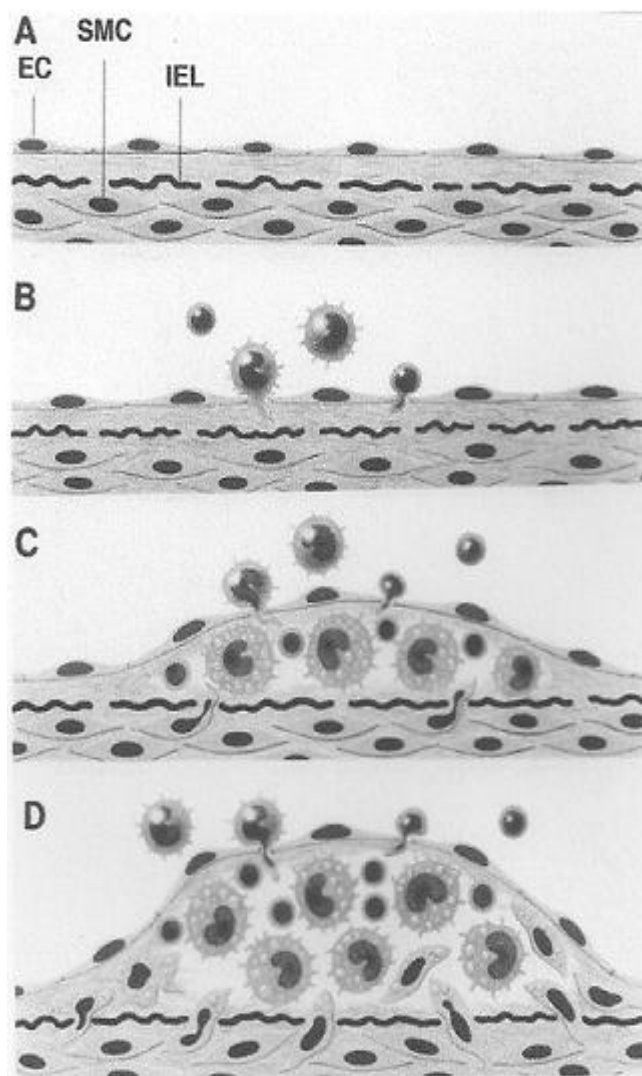
#### ***Effects on T lymphocytes and KLF-2 gene expression***

T cells are important actors in the inflammatory responses of the body. Statins were proven to upregulate the expression of the Kruppel-like factor 2 (KLF-2) gene in activated, or

effector, CD4<sup>+</sup> helper T cells and CD8<sup>+</sup> cytotoxic T cells, and prevent the downregulation of KLF-2 that normally occurs in recently activated T cells (Bu et al. 2010). The KLF-2 gene is thought to inhibit T cell proliferation that occurs with inflammatory responses and keeps T cells in a resting state as KLF2 mRNA is expressed in naïve and memory T cells (Buckley et al. 2001 and Kuo et al. 1997 cited in Bu et al. 2010).

#### ***Effects on immune cells and LFA-1 binding site***

A second pleiotropic effect seen with lovastatin in particular is its ability to bind to a novel site on LFA-1, lymphocyte function-related antigen 1, an integrin molecule found on T lymphocytes and macrophages (Weitz-Schmidt 2003). Acting as an allosteric inhibitor, lovastatin changes the conformation of the LFA-1 and decreases its affinity for its substrate intercellular adhesion molecule-1 (ICAM). ICAM, is a molecule found on endothelial cells that binds to integrins (the adhesion molecules found on immune cells). During diapedesis, macrophages and T lymphocytes will roll and attach to selectin molecules expressed on the endothelial surface (Tortora and Derrickson 2012; Watanabe and Fan 1998). Subsequently, the immune cells will strengthen their attachment to the endothelium using  $\beta 2$  integrins on their surface, such as LFA-1 and bind to members of the immunoglobulin family, like ICAM-1, located on the endothelium. Binding at these two sites will lead the immune cell to squeeze between adjacent endothelial cells and reach the site of inflammation. ICAM-1 has been found to be expressed by endothelium where cholesterol-induced plaques are beginning to form. Atherosclerotic plaques are thought to be induced by the sticking of T cells and other immune cells to the endothelium lining blood vessels (Figure 6) (Watanabe and Fan 1998). If statins change the binding site shape of LFA-1 receptors on immune cells, there will be less attachment of the immune cells to the linings of blood vessels, possibly leading to less atherogenesis (Weitz-Schmidt 2003). This may explain the plaque regression seen in clinical trials, however, in previous studies lovastatin was not the statin being studied



**Figure 6:** A postulated hypothesis for the pathogenesis of atherosclerosis.

(A) A normal arterial wall. (B, C) Monocytes and T lymphocytes adhere to the endothelial cell surface and subsequently enter subendothelial space. Monocytes are transformed into macrophages and some become foam cells after uptake of lipids. (D) Most macrophages become foam cells and smooth muscle cells in the media start to migrate into the media and proliferate. These cells constitute the typical fatty streak lesion. EC: endothelial cell, SMC: smooth muscle cell, IEL: internal elastic lamina. Source: Watanabe and Fan 1998

(Nicholls et al. 2011; Nissen et al. 2004; Nissen et al. 2006). It is therefore possible that other statins may change additional receptors besides LFA-1 on immune cells that are involved in attaching them to the endothelium. Alternatively, it is also possible to conjecture that statins may modulate adhesion molecules on the endothelial surface or that statins other than lovastatin may have another entirely different method for diminishing the size of plaques.

Statins are also involved in increasing the synthesis of nitric oxide gas by stimulation and upregulation of endothelial nitric oxide synthase (Laufs et al. 1998 and Kureishi et al. 2000 cited in Liao and Laufs 2005).

### ***Sepsis prevention***

Some research has found evidence of fewer cases of sepsis, including moderate to severe, and even fatal, sepsis cases when patients were taking statins compared to matched controls (Hackam et al. 2006).

### **Costs of Statins:**

Statins have been proven to cause a wide range of negative side effects many of which target muscles, but damage can also occur to nerves innervating skeletal muscles and the kidneys as well.

### ***Damage to skeletal muscle***

There are two possible mechanisms for how damage occurs. These will now be discussed.

### ***Targeting the mitochondria***

It is necessary to understand how statins lead to skeletal muscle degradation in order to fully grasp the extent of their effects on the body. A study of cerivastatin in male rats implicates its targeting of mitochondria as a plausible cause of muscle toxicity (Obayashi et al. 2011). The researchers studied effects to the soleus muscle, a muscle rich in type I fibers, and the extensor digitorum longus and the tibialis anterior, muscles rich in type II fibers, throughout the course of cerivastatin treatment. While no particular skeletal muscle is purely made up of one type of muscle fiber (i.e. type I, IIA or IIB) (Swenson 2006), a particular muscle may contain a larger percentage of one of the three types of fibers; researchers tend to choose these muscles to study in order to determine trends in muscle fiber types after statin administration. While light microscopy did not show any visible signs of damage to any of the three muscles on day 6, electron microscopy of the soleus muscle revealed mitochondria that were swollen, electron dense, deteriorated, and contained inclusion bodies. Other abnormalities included autophagic vacuoles, some of which were consuming membrane-bound organelles, activated lysosomes, myeloid structures, and disorderly myofibrils. By day 8, the soleus muscle of the cerivastatin-treated rats showed enlarged mitochondria in addition to vast differences in the diameter of the myofibers and some very darkly staining myofibers.

Overall, the mitochondria in this study showed changes in shape, becoming rounded as opposed to oval, and were sought out and destroyed by lysosomes. Only after the destruction of the mitochondria were myofibrils jumbled and autophagic vacuoles active. These findings led to the logical premise that mitochondria are targeted by cerivastatin (Obayashi et al. 2011). While this and other studies zero in on the damaging effects of statins to mitochondria as the primary targets, this idea has been challenged (Waclawik et al. 1993, Schaefer et al. 2004, and Westwood et al. 2008 cited in Obayashi et al. 2011).

### ***Statins' effects on mitochondria in relation to susceptibility of muscle fiber type to damage***

Examination of the mitochondria activity in Hanai et al.'s experiment (2007), discussed below, reinforces a likely conclusion as to why type IIB fibers are more susceptible to statin-

associated muscle damage and why type I fibers are resistant to such damage. Since type IIB fibers lack two protective factors found in type I fibers, specifically more numerous mitochondria and greater expression of a gene that halts tissue atrophy known as PGC-1 $\alpha$ , they are likely more vulnerable to damage by statins. Alternatively, if statins target mitochondria in particular, type IIB fibers are at a loss, already having fewer mitochondria with the compounded problem of the statins depleting the few mitochondria that these fibers have left.

Nonetheless, the argument that type IIB fibers are more vulnerable to statins was not seen in Obayashi et al.'s study of cerivastatin in rats (2011). Damage in this experiment was delivered solely to the soleus muscle, a predominantly type I oxidative, slow twitch muscle. Both the tibialis anterior and the extensor digitorum longus muscles, fast twitch, glycolytic type IIB-predominant muscles, did not show the damage seen to the soleus' mitochondria. This contradiction may be reconciled by stressing which statin was used in Obayashi et al.'s study compared to that by Hanai et al. in 2007, namely cerivastatin versus lovastatin. It is possible that these two statins have different methods of inducing skeletal damage. Extrapolating a step further, it seems logical that cerivastatin's potency, leading ultimately to its removal from the public market, may be linked to changes in the mitochondria seen in type I fibers. It may be that rhabdomyolysis of type I myofibers specifically may be more serious overall, both from a physiological and function-related point of view. Anatomically, these muscle cells contain large amounts of myoglobin, which will be released into the blood plasma, to be dealt with by the kidneys if the muscle breaks down. Functionally, type I fibers resist fatigue from long term exercise, maintain sustained contracture for long time periods, act when only weak muscle contracture is needed, and finally, may compromise up to half of the fibers in a particular muscle (Tortora and Derrickson 2012). Conversely, lovastatin and other statins may act in a different manner that causes muscle damage primarily to type IIB fibers (Hanai et al. 2007; Schaefer et al. 2004; Smith et al. 1991 cited in Sidaway et al. 2009; Westwood et al. 2005; Pierno et al. 2006).

Alternatively, mitochondria contain the necessary components of oxidative phosphorylation, one of which is CoQ<sub>10</sub>. CoQ<sub>10</sub> is a protein produced by the prenylation pathway that stems from HMG-CoA being converted to mevalonic acid (Figure 3). Since statins inhibit HMG-CoA reductase, less CoQ<sub>10</sub> is made than may be needed by the mitochondria. When there is abundant PGC-1 $\alpha$  expression, it is possible that the more massive mitochondria that are produced by expression of this gene will intrinsically have more CoQ<sub>10</sub> and will not need to rely on the synthesis of new CoQ<sub>10</sub> from HMG-CoA reductase activity, inhibitable by statins. As of yet, this idea is still under assessment (Hanai et al. 2007). Again, if CoQ<sub>10</sub> is the problem, the same logic applies as to why type IIB fibers are more susceptible to statins compared to type I, namely the limited amounts of mitochondria present. If the amount of CoQ<sub>10</sub> is similarly lessened in all mitochondria, still the overall quantity of CoQ<sub>10</sub> in type I muscles will likely exceed that found in type IIB muscles just by a greater number of mitochondria. The increased mitochondria, and as a result CoQ<sub>10</sub>, in the type I fibers will then not be as severely affected by the statin-induced shortage of the CoQ<sub>10</sub>, a mainstay of the electron transport system.

### ***Potency of cerivastatin***

Cerivastatin was determined as the most potent statin since a dose less than 20 mg/kg, of cerivastatin, which does not cause myopathy with other statins, generated myopathy (Sidaway et al. 2009). While cerivastatin does cause severe rhabdomyolysis, it must be remembered that this is a rare side effect that was not found in preliminary testing but seen after release into the mass market. Since it is truly an unusual side effect of statins and linked mainly to the retracted cerivastatin, it would be wont to discontinue use of this entire class of drugs due to fear of this

particular adverse reaction. Therefore, it would not be reasonable to stop prescribing statins to the masses, yet this side effect should be monitored closely in the rare chance that there are signs of myopathy in a particular patient.

#### ***Atrogin-1 expression inducing muscle atrophy***

Another study discusses a second mechanism for statins' effects on skeletal muscle. In this mechanism, statins are thought to switch on the expression of a gene involved in a pathway leading to atrophy in body tissues (Hanai et al. 2007). The gene, atrogin-1/MAFbx, is part of the ubiquitin proteasome pathway (UPP), a pathway involved in protein breakdown in the body, and codes for an enzyme called ubiquitin-protein ligase that is specific to muscle tissue. Elevated atrogin-1 mRNA levels were found in skeletal muscle biopsies<sup>2</sup> of patients with statin-associated myopathy and in patients with myopathy that were not taking statins compared to healthy controls. Lovastatin was introduced to C2C12 myotubes (skeletal muscle cell precursors) and zebrafish embryos to determine whether there would be a similar abundance of atrogin-1 expression in these organisms after treatment. In the myotubes, increasing amounts of lovastatin resulted in commensurate increases in the amount of atrogin-1 mRNA, its corresponding protein, and muscle proteolysis. Larger amounts of lovastatin lead to markedly shrunk myotubes. The cells progressively deteriorated displaying evidence of vacuoles and extreme distortion ending in the loss of the myotubes after 5 days (concentrations of lovastatin included 0.0, 0.25, 1.0, 2.5, 5.0, and 10.0  $\mu\text{M}$ ). Vacuolization of the myotubes may reflect the vacuolization that is seen in skeletal muscle tissues in the T-tubule system reported by Mohaupt et al. (2009). The researchers also proved that the atrogin-1 gene was needed to cause lovastatin-induced morphology changes in the myotubes; myotubes bred lacking the atrogin-1 gene and then dosed with a particular concentration of lovastatin (either 0.0, 0.01, 0.05, 0.25, 1.0, or 2.5  $\mu\text{M}$ ) did not show changes to the diameter and morphology of the myotubes unlike matched atrogin-1-containing myotubes at the parallel dose of lovastatin (Hanai et al. 2007). What is interesting to note is a slight dip in myotube diameter when atrogin-1 null myotubes were dosed with 2.5  $\mu\text{M}$  of lovastatin. Whether this would decrease enough to become significant with 5.0  $\mu\text{M}$  or 10  $\mu\text{M}$  concentration will remain unknown as the researchers did not continue to dose the myotubes with these increasing concentration levels. It is also possible that  $\geq 5.0$   $\mu\text{M}$  lovastatin concentration greatly exceeds the amount of lovastatin that would be given to a patient in a clinical setting per kilogram. Yet this rationale is hard to justify as wild-type for atrogin-1 myotubes were initially dosed at these concentrations to determine the effects to the myotubes.

Depending on the dosage, lovastatin triggered specific morphological changes in the skeletal muscle of zebrafish embryos which were dosed 20 hours post fertilization (Hanai et al. 2007). Changes to zebrafish skeletal muscle morphology were determined by exogenously-prepared antibodies that, upon reacting with skeletal muscle tissue, would latch on to myosin found in the thick filaments. These morphological changes in the muscle were classified based on their severity. Class 1 changes to muscle consistent with 0.025-0.05  $\mu\text{M}$  lovastatin treatment included bowing, gap formation, and disruption of the muscle fibers. Increasing the lovastatin dosage, class 2 morphological changes (0.05-0.5  $\mu\text{M}$ ) comprised thin/irregular or diffuse appearance of the myosin strands. Finally, irregular muscle segment boundaries were categorized under class 3 changes due to lovastatin treatment (1.0-5.0  $\mu\text{M}$ ).

#### ***Confirmation of statins' inhibition of HMG-CoA reductase in zebrafish embryos***

The effects of lovastatin were confirmed to be the result of HMG-CoA reductase inhibition. After knocking out the HMG-CoA reductase gene and eradicating any corresponding,

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<sup>2</sup> Biopsies were taken from the quadriceps.

loose mRNA in the cell with antisense technology, the skeletal muscle showed similar morphological characteristics to the class 1 ‘disrupted’ muscle fibers of the zebrafish treated with lovastatin (wild-type for HMG-CoA reductase and atrogen-1 genes (Hanai et al. 2007).

### ***Muscle myopathy***

The relationship between histological damage of skeletal muscles and painful muscles thought to be caused by statins has been studied (Mohaupt et al. 2009). The vastus lateralis of 83 participants were biopsied. Participants were divided into 5 experimental groups (Table 3). Participants in group 4 (n=29) were presently taking atorvastatin (17%), simvastatin (41%), fluvastatin (7%), pravastatin (31%), or rosuvastatin (3%), and they had previously been prescribed 4 out of the 5 statins currently being used<sup>3</sup>. Group 5 participants (n=19) currently on statins without symptoms of myopathy reported prescriptions for simvastatin (74%) and pravastatin (21%), while in the past, one of the participants had been on simvastatin. As for the participants in group 3 whom had ceased their statin regimens (n=15), the statins previously prescribed included atorvastatin (40%), simvastatin (53%), fluvastatin (7%), pravastatin (53%), and cerivastatin (7%). A careful record of other drugs being used alongside statins were documented including fibrates, immunosuppressants, corticosteroids, blood thinners, macrolid antibiotics, antifungals, and HIV-protease inhibitors. The biopsies were studied for microscopic anatomical variances in the skeletal muscle’s structure. For the skeletal muscle damage to be considered significant, the researchers mandated that a minimum of 2% of the myofibers from the biopsy needed to show clear evidence of destruction (Mohaupt et al. 2009).

**Table 3:** Groups 1-5 for Experiment Relating Statin-Induced Myopathy to Muscle Injury.

Source: Mohaupt et al. 2009

<b>Group</b>	<b>Condition</b>	<b>Currently On Statin Regimen Y/N</b>	<b>Pre-existing Statin-Induced Myopathy Y/N</b>	<b>No. of Participants</b>	<b>Gender</b>
<b>1</b>	Healthy	N	N	10	Male
<b>2</b>	Hypercholesterolemia (Unrelated to muscles)	N	N	10 (Age matching to Groups 3 & 4 )	7 Males 3 Females
<b>3</b>	Clinically Diagnosed Myopathy	N (At least 3 weeks off treatment regimen)	Y	15	8 Males 7 Females
<b>4</b>	Clinically Diagnosed Myopathy	Y	Y	29	22 Males 7 Females
<b>5</b>	Hypercholesterolemia	Y	N (reported no muscle problems)	19	12 Males 7 Females

<sup>3</sup> Rosuvastatin was not prescribed previously (Mohaupt et. al. 2009).

***Patient-reported symptoms of myopathy in groups 3, 4, and 5***

About two thirds (67%) of those who discontinued statin use (group 3) and approximately half (48%) of the current statin users (group 4) were presently suffering from myalgia, or muscle pain (Table 4). Another symptom of myopathy expressed by one fifth of past statin users and 38% of current statin users was muscle weakness in the torso and upper arms. A lesser noted symptom of myopathy found was muscle cramping (13% in group 3 participants and 7% in group 4). Finally, 3 out of 15 (20%) participants in group 3 who discontinued statin use mentioned experiencing myalgia, muscle weakness, and/or cramping lasting more than a month after discontinuing statin use (Mohaupt et al. 2009).

**Table 4:** Results of Experiment Relating Statin-Induced Myopathy to Muscle Injury for Groups 3-5. Source: Mohaupt et al. 2009

Group	3	4	5
Myopathy Symptoms			
Myalgia No. (%)	10 (67)	14 (48)	N/A
Weakness No. (%)	3 (20)	11 (38)	N/A
Cramping No. (%)	2 (13)	2 (7)	N/A
Myopathy Symptoms Lasting More than 1 month after Discontinuing Usage of Statins No. (%)	3 (20)	N/A	N/A
No. of Weeks Since Discontinuing Statin Usage, Median (Range)	12 (3-300)	N/A	N/A
No. of Participants with Significant Muscle Damage	9	16	1
No. of Damaged Myofibers in Participant(s) with Significant Muscle Damage No. (%) {Percentage Range of Damaged Myofibers Having Lesions}	9 (60) {2.8-100%}	9 (60) {3.3-43%}	1 (5)
Percentage of Fibers Injured Median value	9.0%	9.5%	N/A

***Experimental results showing specific skeletal muscle damage linked to myopathy***

With regard to damaged muscle fibers, participants in group 3 and group 4 showed evidence of significant muscle fiber damage in the form of lesions to their vastus lateralis muscles compared to the control group. Furthermore, of the 25 participants with skeletal muscle injury, 21 (84%) were actively using statins<sup>4</sup>. When viewing the muscles using light and electron microscopy, there was evidence of intact sarcolemmas detaching from the contracting part of the muscle. Other findings specific to statin users with myopathy (and not found in matched controls) included ghost cells (deteriorated cells with hollow T-tubules), inconsistency in muscle cells' sizes, and vacuolization of the T-tubules.

This experiment reveals that many patients presenting with statin-induced myopathy did have structural muscle damage. This is an alarming result as now myopathy may need to be

<sup>4</sup> This article (Mohaupt et al. 2009) is problematic as only 16/25 participants with skeletal muscle injury are current statin users (group 4).

considered as a more serious “red flag”, indicating the start of muscle damage. The authors also pinpointed the appearance of myofibers with damage being limited to the T-tubules and the detachment of the sarcolemma. They hypothesized that the vacuoles formed in the T-tubule passageways may lend themselves to making the muscle susceptible to greater damage. Vacuoles in the T-tubule system may prevent the even transmission of an action potential to all myofibers, impeding proper muscle contraction. Further investigation is needed to clearly define how vacuoles in the T-tubules affect muscle fiber function. The other major finding, detachment of the sarcolemma, may also be problematic as it may prevent the proper depolarization of the membrane, leading to inconsistencies in muscle contraction. Further, the researchers suggested that the creatine phosphokinase did not leak into the blood stream, preventing a rise in blood creatine phosphokinase levels, due to the intact nature of the sarcolemma.

***Expression of calcium homeostasis genes in myopathy patients’ vastus lateralis muscles***

In addition, the expression of mRNA for 8 different genes coding for proteins found in the T-tubules and adjoining sarcoplasmic reticulum was studied (Mohaupt et al. 2009). All of the genes chosen by the researchers correspond to proteins that are involved in intracellular calcium ion homeostasis. Calcium’s importance lies in the fact that it is essential for muscle contraction (Tortora and Derrickson 2012). Calcium release from the sarcoplasmic reticulum is carefully regulated by proteins in muscle cells to prevent unwanted contractions; the researchers chose to study the expression of these genes to determine fluctuations in their concentrations related to myopathy in patients (Mohaupt et al. 2009).

Of the 8 genes related to calcium homeostasis that were studied, only one of the genes, the ryanodine receptor-3 (RYR3) gene, was expressed in greater quantities in participants with structural muscle injury (n=25). Ryanodine receptor-3 is found in variable amounts in adult skeletal muscle tissue along with ryanodine receptor-1 (RYR1). The high amounts of ryanodine receptor-3 mRNA were thought to be linked to problems with calcium homeostasis; however, the experiment could not prove if the increased amounts of the mRNA were caused by statin-induced muscle damage or by increased expression of the gene before using statins. mRNA for a different gene coding for sarcoplasmic reticulum transporting  $\text{Ca}^{2+}$  ATPase 3 was also found in greater quantities in participants with muscle injury, however, it was not found statistically significant, which the authors attribute to the diversity in the expression of this gene (Mohaupt et al. 2009).

This study is inherently problematic. Limitations of this study include

- a) the small size of the experimental groups,
- b) the lack of data indicating an average amount (with a range) of myofibers biopsied from participants in a particular group and,
- c) neglecting to mention the average size of the myofibers,
- d) ambiguousness and miscalculations mentioned previously,
- e) the presumably small amount of myofibers biopsied,
- f) determination of the significance of 2% of myofibers being damaged in the biopsy sample, which was thought to be low for the amount of myofibers sampled<sup>5</sup>,
- g) the variety of statins that the participants were taking, and lastly,
- h) failing to follow up with participants.

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<sup>5</sup> Participants did not report feeling any pain from this muscle (Mohaupt et al. 2009).

The significance level of 2% of the myofibers displaying damage is most problematic in this study. One myofiber may range from 100 microns to a few centimeters in length once it matures (Skeletal Muscle Fiber Structure 2005; Tortora and Derrickson 2012); in this experiment, a 3 mm x 6 mm biopsy yielded only 15-20 cells, totaling about 2.5-5 cells/mm. This translates to less than a third of one myofiber from those biopsied had to show structural damage to be considered significant. Finally, the researchers discussed that

i) no clear definition was established for what constituted statin-induced myopathy (i.e. certain symptoms etc.) before beginning the study, a very serious oversight. From this study, numerical data should not be used to support any conclusions due to the ambiguities and inconsistencies in the way in which the article was written. Nonetheless, the electron micrographs are still valid, and anatomical changes to the muscle fibers can be believed as these changes were similar to those seen in other experiments (Obayashi and colleagues in 2011 and Hanai and colleagues in 2007).

***Muscle myopathy in relation to statin accumulation in muscle fibers and/or systemic tissues***

Studies of statins also fixate on whether the amassing of statins has a toxic effect on muscle and systemic tissues. A study performed with rodents with statin-induced myopathy focused on determining how statins buildup in muscle and body tissues over time in order to explain unusual cases of delayed onset of myopathy (Sidaway et al. 2009). Further, a comparison of the accumulation of statins in skeletal muscle with predominantly type I versus type IIB fibers was assessed. In previous studies, slow-twitch, oxidative, type I skeletal muscle fibers were found resistant to necrosis caused by statins, while fast-twitch, glycolytic, type IIB skeletal muscle fibers were more susceptible to cell death due to statin usage (Smith et al. 1991; Schaefer et al. 2004; Westwood et al. 2005 cited by Sidaway et al. 2009).

***Experimental methods for testing statin accumulation***

Statin-induced myopathy was induced in female rats by treatment with one of the following three statins: cerivastatin, simvastatin, or rosuvastatin. A fourth group of rats was given a smaller dose of rosuvastatin which was not anticipated to cause myopathy. After anywhere from 5-16 days, blood was drawn for creatinine kinase activity testing. Creatinine is a byproduct of metabolized creatine that is found in muscles and is filtered by the kidneys. Creatinine is an indicator of renal functioning, specifically the glomerular filtration rate (Creatinine 2012). Blood samples and skeletal muscle samples (from the soleus muscles and right gastrocnemius) were collected and preserved throughout the 16 day period at scheduled intervals. The muscle samples were inspected under light microscopy for signs of myopathy based on necrosis found in 2 or more muscles or if the plasma creatinine kinase levels exceeded  $1000 \text{ IU l}^{-1}$ .

Additionally, the muscle samples were tested for statins and cholesterol metabolites using the HMG-CoA reductase enzyme inhibition assay. On days 1 and 5, cerivastatin and simvastatin were distributed in doses large enough to cause myopathy, but no myopathy was determined at this time. Further monitoring of the rats during days 5-8 still showed no evidence of myopathy in any of the three statins. At days 10-16, the first signs of myopathy were evident in half of the rats in each of the three experimental statin groups (Sidaway et al. 2009).

***Location of statin exposure***

While the soleus and gastrocnemius muscles during the 16 day experiment showed very similar statin exposure for the three experimental groups, comparison of the muscles to the blood plasma revealed an unequal distribution of active statin metabolites. Accumulation of statin metabolites favored the skeletal muscles over the blood plasma. Studying the ratio of the active

statin drug in the gastrocnemius muscle compared to the amount of active drug found in the plasma, the three statins differed with the largest ratio calculated for simvastatin<sup>6</sup> > cerivastatin > rosuvastatin. The results denoted a greater amount of simvastatin concentrated in the gastrocnemius muscle relative to the blood plasma compared to the other statin drugs. Similar to the muscle/blood plasma ratio of statin exposure, a ratio of statin buildup in the liver was compared to the blood plasma as well. For cerivastatin, the ratio was very high (96.85) compared to the ratio seen with simvastatin (4.02) (Sidaway et al. 2009).

***Conclusions disproving statin accumulation correlated to myopathy in both systemic and skeletal muscle tissues***

Important conclusions were deduced from this study. Specifically, the method by which statins generated myopathy was not related to the previously-held notion of statin accumulation, either in the skeletal muscles or the systemic tissues. Accumulation of statins was ruled out as the cause of statin-induced myopathy due to stable levels of statin exposure in the body tissues from the initial dosage to the dosage on day 5. The trend continued in days 5-12 with no significant accumulation of statins in the systemic tissues during this time, yet signs of myopathy were starting to develop. This means that before and during myopathy no differences were seen in statin exposure levels. It is therefore a logical conclusion to attribute delayed onset of statin-induced myopathy to some other mechanism besides prolonged statin exposure in systemic tissues (Sidaway et al. 2009).

***Conclusions about whether statin accumulation differs in muscle fiber type***

A second important finding was related to the type of muscle fiber affected by the statin treatment. Past studies have isolated the fast-twitch, glycolytic, type IIB skeletal muscle fibers as the most susceptible to necrosis from statin-associated myopathy; in this research study, no difference was found in the amount of statin accumulation between the two types of muscle fibers for any of the three statin therapies. The similarity in statin buildup in the muscles lead to the conclusion that differences in the susceptibility of muscle fibers to necrosis from statins is based on the biochemistry and physiology of the fibers and not their individual statin-accumulating tendencies (Sidaway et al. 2009).

***Lipophilicity vs. hydrophilicity in statins and its effect on statin accumulation in muscle fibers***

The researchers also brought up the important concept of lipophilic versus hydrophilic tendencies of the statins. While no significant difference was found between muscle fiber types and statin buildup, there was some accumulation of statins in the muscle cells. When creating a ratio between the statins' exposure in muscle compared to the blood plasma, the ratio was tipped more in favor of the muscle cells for cerivastatin and simvastatin. The penetrance of these two statins reflects their characteristic propensity towards being slightly more lipophilic, and the researchers conjecture that the method by which these two statins cross the phospholipid bilayer of the myofiber and enter the cells is based on diffusion and not transporters<sup>7</sup> (Sidaway et al. 2009).

***Lipophilic statins and increased myopathy***

While lipophilic and hydrophilic statins have led to myopathy in skeletal muscle, another study verified the hypothesis that lipophilic statins in particular increase the risk of myopathy due to their ability to cross the phospholipid bilayer of the cells' plasma membrane. A study was

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<sup>6</sup> The ratio for simvastatin was the same for both the 80 mg and 20 mg simvastatin experimental groups (Sidaway et al. 2009).

<sup>7</sup> Conversely, when the researchers studied the liver, transporters were thought to bring statins into the hepatocytes (Sidaway et al. 2009).

conducted on rats that tested two different statins, fluvastatin and atorvastatin. First, the statins were examined for their typical lipid-related results<sup>8</sup>. Then the rodents were euthanized, and the tibialis anterior, soleus muscle, heart, liver, and kidneys were extracted to determine their weight. In the fluvastatin rats taking 20 mg/kg/day, the tibialis anterior showed a significant reduction in weight, however, the soleus muscle did not show this reduction. Furthermore, the heart and kidney of the high dose fluvastatin rats were significantly heavier than the control rodents. This finding was dose-dependent as the rats on 5 mg/kg/day dosage of fluvastatin did not show differences in the sizes of their muscles or organs. Rats on atorvastatin did not show significant differences in organ sizes or muscle except for an increase in muscle size of the tibialis anterior muscle (Pierno et al. 2006).

#### **Alteration to resting chloride membrane potential (gCl). *gCl reduction***

Pierno et al. in 2006 proved that vast changes occur to the resting membrane chloride conductance (gCl) and the overall ability for sarcolemma excitement when using statins. Resting chloride membrane potential/conductance is an important indicator of sarcolemma functioning in muscle tissue. The gCl stabilizes the membrane after an action potential and assists in repolarization of the membrane for future action potentials (Bryant and Conte Camerino 1991 and Jentsch et al. 2002 cited in Pierno et al. 2006; Aromatans and Rychkov 2006 cited in Pierno et al. 2009). Rats on the 20 mg of fluvastatin showed a significant decrease in myofiber diameter of the extensor digitorum longus muscle and gCl (29% reduction), similar to atrophy seen in the tibialis anterior mentioned previously. Although rats on atorvastatin and 5 mg fluvastatin had larger myofiber diameters than the control rats, the gCl showed the same trend as in the high dose fluvastatin rats with atorvastatin rats having a 24% reduction in gCl and 5 mg fluvastatin rats having a 20% reduction in gCl.

#### ***Changes in four key factors related to muscle excitability due to reduced gCl***

With the reduced gCl, the muscle fibers after exposure to statins tended to display more excitable behavior that was determined by measuring the following factors:

- a) the smallest current needed to procure an action potential,
- b) the amount of elapsed time between turning on the current and the first depolarizing spike that indicates the start of an action potential,
- c) the maximum number of action potentials that could be elicited by a myofiber when stimulated by a current of a particular value in a 100 millisecond time period, and
- d) just how depolarized a myofiber became after an action potential.

The following table reflects the changes to these four factors in each of the experimental groups (Table 5).

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<sup>8</sup> An increase in HDL cholesterol was found in the rats on atorvastatin only, but for these rats, the total amount of cholesterol remained the same. Fluvastatin, with both the 5 and 20 mg/kg/day dose, showed modest reduction of total cholesterol of the rats, but no significant increase in HDL cholesterol in particular (Pierno et al. 2009).

**Table 5:** Effects of atorvastatin and fluvastatin on membrane excitability of extensor digitorum longus muscle fibers in rats. Source: Pierno et al. 2006

Statin Treatment	Muscle Fiber Excitability Changes			
	A	B	C	D
Fluvastatin, 20 mg	Decrease	Increase	Increase	Slight increase
Fluvastatin, 5 mg	No change	Increase	Increase	Slight increase
Atorvastatin, 10 mg	No change	Increase	Increase	No change

A – the smallest current needed to procure an action potential; B – the amount of elapsed time between turning on the current and the first depolarizing spike that indicates the start of an action potential; C – the maximum number of action potentials that could be elicited by a myofiber when stimulated by a current of a particular value in a 100 millisecond time period; D – just how depolarized a myofiber became after an action potential. All information is based on comparison to control.

#### ***Changes in voltage threshold for mechanical activation in muscle cells due to statins***

Another factor examined was the voltage threshold for mechanical activation in myofibers extracted from the extensor digitorum longus muscles of the 4 rodent experimental groups (Pierno et al. 2006). The voltage threshold for mechanical activation relates to the amount of time needed at a specific current value to cause depolarization of the myofiber, ranging anywhere from 5-500 milliseconds. After subjecting the myofibers of control and statin-treated rats with a particular current for a set amount of time, contraction occurred more readily in the statin-treated rats than the controls. What was more interesting is the fact that the statin-treated rats had more negative resting potentials to begin with compared to the controls, yet they could reach the threshold for depolarization and produce an action potential more easily than control rats with less negative resting potentials when both groups were stimulated by exposure to the same current. In other words, an electrical pulse that could depolarize a statin-treated myofiber of a large negative resting potential would not be able to induce an action potential in a myofiber at the same negative resting potential when not treated prior with statins; to depolarize this non-statin treated myofiber at this negative value, the same size current would need to be applied to the myofiber for a longer duration (Pierno et al. 2006).

#### ***Implications of changes to muscle fiber excitability***

Signs of serious changes to the excitability of the muscle fiber are evident in this study. Muscle fibers treated with statins will now contract readily when excited by a current whereas before the statin treatment, the same current would elicit an action potential. As a result, this change may lead to cramping or repeated contracting of a muscle in patients on statins. These results proved the original hypothesis that these lipophilic statins show a greater propensity for changing the muscle function evidenced by a decrease in the resting chloride membrane potential (gCl), an increase in the sarcolemma depolarization, leading to more action potentials, and finally, a more negative voltage threshold for mechanical activation (Pierno et al. 2006). Agreeably, the authors also suggest that since no changes were seen morphologically in the myofibers of the statin-treated rats besides for the decrease in fiber diameter, changes to the blood plasma composition may be a better warning sign of impending myopathy, possibly terminating in rhabdomyolysis (Table 6). An interesting finding was the increase in muscle mass in the tibialis anterior of the atorvastatin rats, not necessarily indicating hypertrophy, but may relate to an increase in protein production due to statins (Pierno et al. 2006). This, however, is speculation, but may have some truth.

## BENEFITS VS. COSTS OF STATIN DRUGS

**Table 6:** Effects of chronic treatment with atorvastatin and fluvastatin on biochemical parameters in rat plasma. Source: Pierno et al. 2006

Plasma parameter	Control	Atorvastatin 10mg·kg <sup>-1</sup>	Fluvastatin 5mg·kg <sup>-1</sup>	Fluvastatin 20mgkg <sup>-1</sup>
Myoglobin (ng ml <sup>-1</sup> )	0.14±0.02	0.23±0.01 *P<0.02	0.16±0.02	0.28±0.05 *P<0.005
LDH (mU ml <sup>-1</sup> )	587±76	1255±202 *P<0.005	550±77	915±151
CK (mU ml <sup>-1</sup> )	1238±217	2118±202 *P<0.005	1468±40	1795±189 *P<0.05
Creatinine (mg ml <sup>-1</sup> )	7±0.3	8±0.5	7±0.8	8±0.6
Potassium (m Eq <sup>-1</sup> )	5.9±0.3	6.8±0.7	5.5±0.6	5.3±0.6
Azotemia (mg ml <sup>-1</sup> )	0.48±0.02	0.55±0.04	0.45±0.03	0.62±0.05 *P<0.005
N (samples)	10	6	7	7

CK, creatine kinase; LDH, lactate dehydrogenase. Each row shows the mean ± SEM of the plasma parameters measured from the number of samples as indicated.

\*Significantly different with respect to control (by Bonferroni's t-test) (Pierno et al. 2006).

### ***Conclusions about atorvastatin potency and the possibility of statin accumulation leading to toxicity***

Atorvastatin was found more potent than fluvastatin as only 10 mg of atorvastatin (compared to 20 mg of fluvastatin) resulted in elevated levels of muscle components in the blood plasma (Table 6). Elimination of atorvastatin took longer compared to fluvastatin (Corsini et al. 1995 cited in Pierno et al. 2006). The researchers also conjectured that results with atorvastatin support the view that statins produce myopathy by accumulation, yet accumulation of statins leading to toxicity was disproven in the experiment of Sidaway et al. (2009). The question that now arises is whether the results that Sidaway and colleagues found after this study in 2006 are generalizable findings. A possible reconciliation between the differences in opinion could be that Sidaway and colleagues proved that accumulation leading to toxicity did not occur particularly with simvastatin, rosuvastatin, and cerivastatin, while Pierno and colleagues tested atorvastatin and fluvastatin. While it would be much simpler if the accumulation of the statins could be related to their origin (i.e. naturally-derived statins cause accumulation and synthetic statins do not or vice versa), this does not resolve the issue as Sidaway and colleagues (2009) used both synthetic (rosuvastatin and cerivastatin) and a naturally-derived (simvastatin) statins. Also, the differences in opinion on statin accumulation cannot be attributed to lipophilicity as all five of the statins tested are thought to be more lipophilic by both sets of researchers. More research is needed to determine whether proof exists in the statin buildup theory from more current research studies done within the last few years. Coming back to the potency of atorvastatin in particular, it is most curious that even with muscle proteins in the plasma and myoglobinuria discharged from the kidneys in the atorvastatin-treated rats, no gross, microscopic damage was seen in the myofibers, and no decrease in muscle weight was noted (Pierno et al. 2006).

### ***Electromyography findings of statin treatment on skeletal muscle***

Fluvastatin (20 mg/kg/day and 5 mg/kg/day) and atorvastatin (10 mg/kg/day) were tested to determine how statins lower the gCl of muscle fibers, leading to myotoxicity. Biweekly, electromyography using micro electrodes inserted into the rats' gastrocnemius muscles was performed. Recordings of the electrical activity lasted 3-4 minutes in duration. Abnormalities in activity spikes (attributed to myotoxicity) in the muscles of statin-treated rats were determined by comparison to controls. Examination of the electromyographs showed that after 7-8 weeks of statin treatment, 10% of rats on both the high and low doses of fluvastatin and 20% of those on atorvastatin showed additional electrical spikes 500 milliseconds in length, not seen in the control rats, occurring after spikes related to muscle movement (Pierno et al. 2009).

### ***gCl reduction and muscle fiber type***

Results showed between 20-35% decrease in the gCl of the extensor digitorum longus muscles of rats on statins compared to the matched controls, yet the soleus muscle did not show any significant change in gCl due to statins (Pierno et al. 2009).

### **Reversing changes to gCl:**

#### **Chelerythrine as a protein kinase c inhibitor**

#### ***Effects on slow and fast twitch muscles after statin administration***

Chelerythrine, a known protein kinase C inhibitor, was added to the extensor digitorum longus muscles to see if it stopped the drop in gCl due to subsequent administration of statins. The effects of chelerythrine were studied both ex vivo and in vitro. Ex vivo administration of 1  $\mu\text{mol/L}$  of chelerythrine to the extensor digitorum longus muscles of the control rats showed a small increase to the gCl. The results were appropriate as a large increase in the gCl was not expected to occur since the extensor digitorum longus muscle is a fast twitch muscle, and the CIC-1 channels are already in an open state<sup>9</sup>. Chelerythrine increased the gCl in all three statin groups compared to statin administration alone. Of the three statin experimental groups, atorvastatin displayed the most significant restoration to the gCl, increasing it by 40% compared to control muscles with statin treatment only and raising it to the gCl level of muscles from the control rats not on statins (Pierno et al. 2009).

### ***Decreased body mass and mobility***

Damage to muscle by statins can affect overall health and may limit mobility. Rodents on the 20 mg/kg/day dose (higher dose) of fluvastatin began to eat less by weeks 3 and 4 and showed a decline in their gross weight. Two out of the ten rats in the experimental group on the higher dose of fluvastatin showed difficulty with the righting reflex<sup>10</sup> and demonstrated evidence of paralysis in their lower bodies. The other groups of rats (5 mg/kg/day fluvastatin and 10 mg/kg/day atorvastatin) fared like the control rats and did not show these movement-related issues (Pierno et al. 2006). People taking statins, especially those on high doses should therefore be aware of possible disturbances to their weight, gait, and general ease of manipulation of their muscles.

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<sup>9</sup> When the researchers tested the effects of atorvastatin 50  $\mu\text{mol/L}$  dose on the soleus muscle in vitro, no reduction occurred to the gCl reflecting the already lowered gCl that is found in slow twitch muscles. Further study was then focused solely on fast twitch muscles with higher baseline gCl's (Pierno et al. 2009).

<sup>10</sup> The righting reflex tests rodents for the speed at which they are able to return to their natural, ventral position (resting on their paws) after being flipped onto their backs.

***Effects to the excretory system: Alterations in blood plasma leading to changes in urine composition***

The excretory system is also affected by statins. Protein that is released into the blood by dying muscle is filtered by the kidneys. To determine the effects of the statins (fluvastatin and atorvastatin) on both muscle and kidneys, the composition of both the blood plasma and urine were studied (Pierno et al. 2006). Damage isolated to muscle tissue was evident when elevated levels of the following compounds were seen only in the blood plasma (and not urine): myoglobin, lactate dehydrogenase, creatine kinase, potassium, azotemia, or the measure of how much nitrogen originating from urea is found in the blood, and/or creatinine. If these compounds were found in both the plasma and the urine, kidney filtration was also thought to be impaired. The following results were noted (Table 7).

**Table 7:** Blood plasma and urine composition after statin treatment on rats. Source: Pierno et al. 2006

Statin	20 mg/kg/ day Fluvastatin	5 mg/kg/day Fluvastatin	10 mg/kg/day Atorvastatin
<b>Blood plasma</b>			
Myoglobinemia	Increase	No change	Increase
Lactate Dehydrogenase	No change	No change	Increase
Creatine Kinase	Increase	No change	Increase
Creatinine	No change	No change	No change
K <sup>+</sup>	No change	No change	No change
Azotemia	Increase	No change	No change
<b>Urine</b>	*	No change	No change
Myoglobinuria		No change	Increase
Creatinuria		No change	No change
Urinary electrolytes		No change	No change
Na <sup>+</sup>		Decrease	Decrease
K <sup>+</sup>		Decrease	No change
Cl <sup>-</sup>		Decrease	No change
Phosphate		Decrease	Decrease
Proteinuria		Decrease	No change

Changes to the blood (increase, decrease, or no change) are related to the criteria in the control rats not on statins.

\*No testing was done on the urine of rats on the 20 mg/kg/day fluvastatin dose.

The different treatments with the statins led to varied compositions of the blood plasma and urine. Fluvastatin strictly at the higher dose led to increased levels of myoglobinemia. Myoglobinemia is the release of the heme-containing pigment called myoglobin into the blood. Normally found in skeletal muscle to aid in delivering additional oxygen to these tissues during vigorous activity, myoglobin that is released into the blood is filtered by the kidneys. Excessive secretion of myoglobin can result in myoglobinuria, urine rich in myoglobin, and ultimately, can damage the kidneys and renal failure may result (Shankar et al. 2002). In this experiment, other increases in substances and chemicals in the blood due to fluvastatin included plasma creatine kinase and azotemia. Atorvastatin led to significant increases in myoglobin, lactate dehydrogenase, and creatine kinase in blood plasma (Pierno et al. 2006).

***Motor Neuron Damage: Reports of Amyotrophic Lateral Sclerosis-like symptoms in statin users***

A more alarming side effect of statins is the development of lesions on motor neurons. Reports from Vigibase, the database for WHO for International Drug Monitoring, picked up on over 40 profiles of patients on HMG-CoA reductase inhibitors that contained reports of ‘upper motor neuron lesion’ or ‘amyotrophic lateral sclerosis’ symptoms. Amyotrophic Lateral Sclerosis (ALS) is a rare, fatal disease in which motor neurons degenerate; the possibility of statins causing these effects is very worrisome. If statins do cause damage to motor neurons, muscles may become atrophied or weak from lack of proper innervation. Many statins were reportedly used that produced ALS-like symptoms by Vigibase including simvastatin, atorvastatin, cerivastatin, lovastatin, and rosuvastatin. Other frequently compounded symptoms experienced by these patients while taking statins included myalgia, myopathy, falling and balance problems, and difficulty with speech and manipulation of the tongue, as well as others patients (Edwards et al. 2007). This information is relevant to the caution that must be exercised when taking statins. More research is needed to further assess the connection between neuromuscular issues and statins.

**Prevention of statin damage to muscle**

***PGC-1 $\alpha$  inhibits atrogen-1 expression and its implications***

Prevention of lovastatin-induced muscle damage was achieved by the regulation of the PGC-1 $\alpha$  gene mentioned previously. PGC-1 $\alpha$  was determined in other studies (Sandri et al. 2006 cited in Hanai et al. 2007) to inhibit atrogen-1 expression thereby lessening muscle atrophy. The experimenters tested this premise with zebrafish embryos. After injecting the embryos with cDNA segments containing the PGC-1 $\alpha$  gene, expression of the protein coded for by PGC-1 $\alpha$  prevented the side effects of lovastatin alone including muscle damage, atrogen-1 expression, and muscle cell shrinkage.

Comparison of mitochondrial activity between embryos given lovastatin with and without added PGC-1 $\alpha$  cDNA was also examined. Cells with injected PGC-1 $\alpha$  showed more active mitochondria and increased mitochondria activity than cells without the added gene. Similarly, myotubes treated with PGC-1 $\alpha$  genes when given lovastatin showed no changes in muscle morphology, atrogen-1 expression ceased, and oxidative phosphorylation genes were turned on, indicating mitochondria activity (Hanai et al. 2007).

This experiment suggests the possibilities for PGC-1 $\alpha$  to be used to counter the negative effects that statins have on muscle tissue. The authors speculate that using a drug to trigger the expression of PGC-1 $\alpha$  may be a viable option<sup>11</sup> (Hanai et al. 2007). Yet, this study is specific to

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<sup>11</sup> The authors mention the drug metformin, used to treat type 2 diabetes, as a specific example of a drug that increases PGC-1 $\alpha$  expression (Hanai et al. 2007)

lovastatin and may not allow for generalization to other statins. A second consideration is whether this study can be extended beyond myotubes and zebrafish embryos to clinical practice on humans. It must also be mentioned, however, that increasing PGC-1 $\alpha$  expression may be detrimental to other body tissues, and it may reverse the beneficial outcomes that statins have on coronary disease, namely increasing HDL cholesterol and lowering LDL cholesterol. More research is needed to clarify the side effects of abundant PGC-1 $\alpha$  expression on systemic tissue and cardiac muscle. Still, the prospect of using a drug that increases PGC-1 $\alpha$  expression to prevent muscle damage due to statins is very promising.

### ***Results of knocking out atrogen-1***

Interestingly, the absence of the atrogen-1 gene in zebrafish embryos prevented muscle damage due to lovastatin administration or HMG-CoA reductase knock out (Hanai et al. 2007). In order to verify that atrogen-1 is the reason for the changes to zebrafish embryos' skeletal muscle seen after lovastatin administration (as opposed to another gene), the atrogen-1 gene was knocked out allowing for a survey of the lovastatin-treated muscle. The researchers mimicked the procedure used to knock out the HMG-CoA reductase gene (and corresponding mRNA) for the atrogen-1 gene<sup>12</sup>. The results demonstrated a significantly lower degree of lovastatin-induced damage to the zebrafish's skeletal muscle with the knock out atrogen-1 gene compared to the wild-type that was homozygous for atrogen-1. Further, when the zebrafish lacked both the HMG-CoA reductase gene and the atrogen-1 gene, distortions to muscle morphology were significantly less, leading to the conclusion that eliminating the atrogen-1 gene reverses muscle defects that would otherwise be present due to HMG-CoA reductase knock out (Hanai et al. 2007). Targeted knockdown of zebrafish HMG-CoA reductase has a muscle phenotype similar to that with lovastatin treatment and can be rescued by zebrafish atrogen-1 knockdown).

### ***Clinical application viability for atrogen-1***

It is questionable as to whether knocking out atrogen-1 can be used clinically to prevent statin-induced muscle damage. This process is likely much simpler in less complex and/or less developed organisms such as zebrafish embryos compared to humans. Additionally, knocking out the atrogen-1 gene may have other repercussions on the body that may far outweigh the benefits seen from eliminating the possibility for tissue atrophy. If atrogen-1 targeting was to be employed to help statin users, more research and testing would be necessary to determine all of the outcomes that result from its expression.

### ***Statins and cardiac muscle***

Since statins affect skeletal muscle, concern arises as to whether these same effects will appear in cardiac muscle tissue. Little of the research presented here indicated any effects, positive or negative, to cardiac muscle tissue. However, preliminary findings from the research mentioned here seem to indicate that statins do not target cardiac muscle tissue. In the study of Mohaupt et al. (2009), ryanodine receptor 3 showed increased expression in skeletal muscle, but its analog, ryanodine receptor 1, that is found to a lesser degree in skeletal muscle but principally in cardiac muscle, was not significantly expressed as mRNA more than that seen in the control. Also, atrogen-1 knock out did not generate defects in cardiac muscle tissue so this method may be a possible candidate for negating statins' effects on skeletal muscle (Serrano et al. 2010 cited in Hanai et al. 2007). More examination of the research is needed to determine effects seen to cardiac muscle after statin usage.

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<sup>12</sup> Zebrafish embryos without the atrogen-1 gene did not show any significant differences in muscle morphology compared to atrogen-1-expressing muscle cells before administering lovastatin, maintaining the integrity of the trial (Hanai et al. 2007).

## CONCLUSION

Much research has been done and continues to be performed on statins, which could not all be discussed here. Other findings not discussed included statins effects on Ras and Rho proteins (Liao and Laufs 2005), applications with cancer patients (Demierre et al. 2005), and effects on coagulation and fibrinolysis processes (Krysiak et al. 2003), and much more. Due to the lack of a better option to reduce cholesterol levels, statin usage will continue, but it is advised that statin usage should be limited to patients with a history of coronary heart disease due to high cholesterol levels. It is strongly discouraged for use on patients with heart disease unrelated to cholesterol as well as autoimmune and cancer patients if other treatments are viable that have been known to improve the condition. Use of statins by these patients may not help their original disease and may cause further complications such as muscle breakdown and possible kidney damage. For patients with coronary disease and high cholesterol, it is advised that patients should have their blood and urine regularly tested for changes mentioned here, preferably within 1 month of beginning statin usage and once a month, subsequently. Changes in chemicals, specifically creatine kinase, found in the blood does not necessarily mean that a patient should cease statin usage on the basis of creatine kinase levels. Although participants with myopathy had increased creatine kinase levels, this increase was not limited to the patients taking statins (Mohaupt et al. 2009).

Muscle pain should be reported to the physician immediately, since pain could be a sign of damage to muscles (Mohaupt et al. 2009). Also, difficulty with controlling muscle movements or speech could be a sign of the rare, but serious, motor neuron damage and should be reported. Quality of life and mobility may be reduced with statins if muscle damage is not caught early. In addition, statins may not prevent a cardiovascular episode and do not reverse coronary disease (Nicholls et al. 2011). Yet, overall statins accomplish their lipid-related tasks well and may even produce regression in atheroma size.

## REFERENCES

- Alanine transaminase. Retrieved May 13, 2012 from: [http://en.wikipedia.org/wiki/Alanine\\_aminotransferase](http://en.wikipedia.org/wiki/Alanine_aminotransferase).
- Bu D, Tarrio M, Grabie N, Zhang Y, Yamazaki H, Stavrakis G, Maganto-Garcia E, Pepper-Cunningham Z, Jarolim P, Aikawa M, García-Cardena G, Lichtman A. 2010. Statin-induced Krüppel-like factor 2 expression in human and mouse T cells reduces inflammatory and pathogenic responses. *Journal of Clinical Investigation* 120(6):1961-1970.
- Campese VM, Park J. 2007. HMG-CoA reductase inhibitors and the kidney. *Kidney International* 71(12):1215-1222.
- Creatinine. 2012. Retrieved May 19, 2012 from: <http://en.wikipedia.org/wiki/Creatinine>.
- Demierre, MF, Higgins PDR, Gruber SB, Hawk E, Lippman SM. 2005. Statins and cancer prevention. *Nature Reviews Cancer* 5(12):930-942.
- Diagram of an artery in cross section. 2008. *Encyclopedia Britannica*. Retrieved May 13, 2012 from: <http://www.google.com/imgres?q=artery&um=1&hl=en&sa=N&biw=1366&bih=575&tbm=isch&tbnid=j7IOv6eMYy5ErM:&imgrefurl=http://m.eb.com/assembly/121565&docid=qKOENwZuoeOKGM&imgurl=http://media.web.britannica.com/eb-media/16/55016-004-3F443F76.gif&w=410&h=400&ei=3cOFT8K8F4nw0gGoruTcBw&zoom=1&iact=rc&d>

- ur=333&sig=116465313956319292164&page=3&tbnh=123&tbnw=126&start=43&ndsp=29&ved=1t:429,r:8,s:43,i:250&tx=49&ty=91.
- Edwards RI, Star K, Kiuru A. 2007. Statins, neuromuscular degenerative disease and an amyotrophic lateral sclerosis-like syndrome: an analysis of individual case safety reports from vigibase. *Drug Safety* 30(6):515-525.
- Hackam DG, Mamdami M, Li P, Redelmeier DA. 2006. Statins and sepsis in patients with cardiovascular disease: A population-based cohort analysis. *Lancet* 367(9508):413-418.
- Hanai J, Cao P, Tanksale P, Imamura S, Koshimizu E, Zhao J, Kishi S, Yamashita M, Phillips P, Sukhatme V, Lecker S. 2007. The muscle-specific ubiquitin ligase atrogin-1/MAFbx mediates statin-induced muscle toxicity. *Journal of Clinical Investigation* 117(12):3940-3951.
- HMG-CoA. 2012. Retrieved May 13, 2012 from: <http://en.wikipedia.org/wiki/HMG-CoA>.
- HMG-CoA reductase. 2012. Retrieved May 13, 2012 from: [http://en.wikipedia.org/wiki/HMG-CoA\\_reductase](http://en.wikipedia.org/wiki/HMG-CoA_reductase).
- Krysiak R, Okopień B, Herman ZS. 2003. Effects of HMG-CoA reductase inhibitors on coagulation and fibrinolysis process. *Drugs* 63(17):1821-1854.
- Liao JK, Laufs U. 2005. Pleiotropic effects of statins. *Annual Review of Pharmacology and Toxicology* 45:89-118.
- Merx MW, Weber C. 2006. Statins: A preventive strike against sepsis in patients with cardiovascular disease? *Lancet* 367(9508):372-373.
- Mohaupt M, Karas R, Babiychuk E, Sanchez-Freire V, Monastyrskaya K, Iyer L, Hoppeler H, Breil F, Draeger A. 2009. Association between statin-associated myopathy and skeletal muscle damage. *Canadian Medical Association Journal* 181(1-2):E11-E18.
- Nelson DL, Cox MM. 2005. *Lehninger Principles of Biochemistry*. 664.
- Nicholls S, Ballantyne C, Barter P, Chapman M, Erbel R, Libby P, Raichlen J, Uno K, Borgman M, Wolski K, Nissen S. 2011. Effect of two intensive statin regimens on progression of coronary disease. *New England Journal of Medicine* 365(22):2078-2087.
- Nissen SE, Nicholls SJ, Sipahi I, Libby P, Raichlen JS, Ballantyne CM, Davignon J, Erbel R, Fruchart JC, Tardif J, Schoenhagen P, Crowe T, Cain V, Wolski K, Goormastic M, Tuzcu EM. 2006. Effect of very high-intensity statin therapy on regression of coronary atherosclerosis: The ASTEROID trial. *Journal of the American Medical Association* 295(13):1556-1565.
- Nissen SE, Tuzcu EM, Schoenhagen P, Brown BG, Ganz P, Vogel RA, Crowe T, Howard G, Cooper CJ, Brodie B, Grines CL, DeMaria AN. 2004. Effect of intensive compared with moderate lipid-lowering therapy on progression of coronary atherosclerosis: A randomized controlled trial. *Journal of the American Medical Association* 291(9):1071-1080.
- Obayashi H, Nezu Y, Yokota H, Kiyosawa N, Mori K, Maeda N, Tani Y, Manabe S, Sanbuissho A. 2011. Cerivastatin induces type-I fiber-, not type-II fiber-, predominant muscular toxicity in the young male F344 rats. *Journal of Toxicological Sciences* 36(4):445-452.
- Pierno S, Camerino G, Cippone V, Rolland J, Desaphy J, De Luca A, Liantonio A, Bianco G, Kunic J, George A, Conte Camerino D. 2009. Statins and fenofibrate affect skeletal muscle chloride conductance in rats by differently impairing CIC-1 channel regulation and expression. *British Journal of Pharmacology* 156(8):1206-1215.
- 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. 2006. Effects

- of chronic treatment with statins and fenofibrate on rat skeletal muscle: A biochemical, histological and electrophysiological study. *British Journal of Pharmacology* 149(7):909-919.
- Schaefer WH, Lawrence JW, Loughlin AF, Stoffregen Da, Mixson LA, Dean DC, Raab CE, Yu NX, Lankas GR, Frederick CB. 2004. Evaluation of ubiquinone concentration and mitochondrial function relative to cerivastatin-induced skeletal myopathy in rats. *Toxicology and Applied Pharmacology* 194:10-23.
- Shankar S, Tuncali K, vanSonnenberg E, Seifter JL, Silverman SG. 2002. Myoglobinemia after CT-guided radiofrequency ablation of a hepatic metastasis. *American Journal of Roentgenology* 178(2):359-361.
- Sidaway J, Wang Y, Marsden A, Orton T, Westwood F, Azuma C, Scott R. 2009. Statin-induced myopathy in the rat: Relationship between systemic exposure, muscle exposure and myopathy. *Xenobiotica* 39(1):90-98.
- Skeletal muscle fiber structure. 2005. University of California Regents. Retrieved May 18, 2012 from: <http://muscle.ucsd.edu/musintro/myofiber.shtml>.
- Statin. 2012. Retrieved February 7, 2012 from: <http://en.wikipedia.org/wiki/Statins>.
- Steroid. 2012. Retrieved May 13, 2012 from <http://en.wikipedia.org/wiki/Steroid>.
- Swenson R. 2006. Review of Clinical and Functional Neuroscience-Swenson chapter 5 spinal cord. Retrieved May 6, 2012 from: [http://www.dartmouth.edu/~rswenson/NeuroSci/chapter\\_5.html](http://www.dartmouth.edu/~rswenson/NeuroSci/chapter_5.html).
- Torbert JA. 2003. Lovastatin and beyond: The history of the HMG-CoA reductase inhibitors. *Nature Reviews Drug Discovery* 2:517-526.
- Tortora GJ, Derrickson B. 2012. Principles of Anatomy and Physiology. Hoboken: John Wiley & Sons 331-340, 351-352, 740.
- Watanabe T, Fan J. 1998. Atherosclerosis and inflammation: Mononuclear cell recruitment and adhesion molecules with reference to the implication of ICAM-1/LFA-1 pathway in atherogenesis. *International Journal of Cardiology* 66(1):S45-S53.
- Weitz-Schmidt G. 2003. Lymphocyte function-associated antigen-1 blockade by statins: Molecular basis and biological relevance. *Endothelium* 10:43-47.
- Westwood FR, Bigley A, Randall K, Marsden AM, Scott RC. 2005. Statin-induced muscle necrosis in the rat: distribution, development, and fibre selectivity. *Toxicologic Pathology* 33(2):246-257.