

## **Statins.**

### **Learning Objectives.**

**At the end of this module, you should be able to :**

- 1. Give a brief description of the mechanisms by which statins produce their beneficial effects;**
- 2. Discuss the pleiotropic effects of statins;**
- 3. Understand the importance of mevalonate-inhibition;**
- 4. Describe what would be seen in a patient presenting with statin-related rhabdomyolysis.**

Statins are a group of drugs used to lower cholesterol levels by inhibiting the activity of the enzyme HMG-CoA reductase (3-Hydroxy-3-methylglutaryl coenzyme A reductase), which plays a central role in the production of cholesterol in the liver. Raised levels of cholesterol are associated with an increase in cardiovascular disease, and statins are therefore used in the prevention of these diseases. They appear to be most effective in individuals already suffering from cardiovascular disease (CVD), and as such are a form of secondary prevention. However, they are also advocated and used extensively in those without previous CVD but with elevated cholesterol levels and other risk factors, such as diabetes and high blood pressure.

Statins currently available are :

- Atorvastatin (Lipitor, Torvast) - the most widely used, and probably the best-selling pharmaceutical agent in history.
- Fluvastatin (Lescol)
- Lovastatin (Mevacor, Altacor, Altoprev)
- Pitavastatin (Livalo, Pitava)

- Pravastatin (Pravachol, Selektine, Lipostat)
- Rosuvastatin (Crestor)
- Simvastatin (Zocor, Lipex).

Some of these agents occur naturally, such as in some mushrooms and red yeast rice, others are fermented from bacteria, whilst some are purely synthetic. Some of them are also combined with other agents, such as niacin (Vit B3), amlodipine, or ezetimibe - referred to as Combination Therapy.

The beneficial effects of statins are usually attributed to their capacity to reduce endogenous cholesterol synthesis by competitive inhibition of the principal enzyme involved - HMG CoA reductase. Since mevalonate, the product of HMG CoA reductase reaction, is the precursor not only for cholesterol, but also for many other nonsteroidal isoprenoidic compounds, use of statins to inhibit this key enzyme may result in pleiotropic effects, i.e. actions other than those for which the drug/agent was specifically developed. Some of these beneficial effects include improvement of endothelial function by the up-regulation of endothelial synthase (eNOS), decrease in vascular smooth muscle cell proliferation and macrophage proliferation, reduction of platelet activity, stabilisation of atherosclerotic plaques, and antioxidant, anti-inflammatory and immunomodulatory effects.

### **Mechanisms of action of statins**

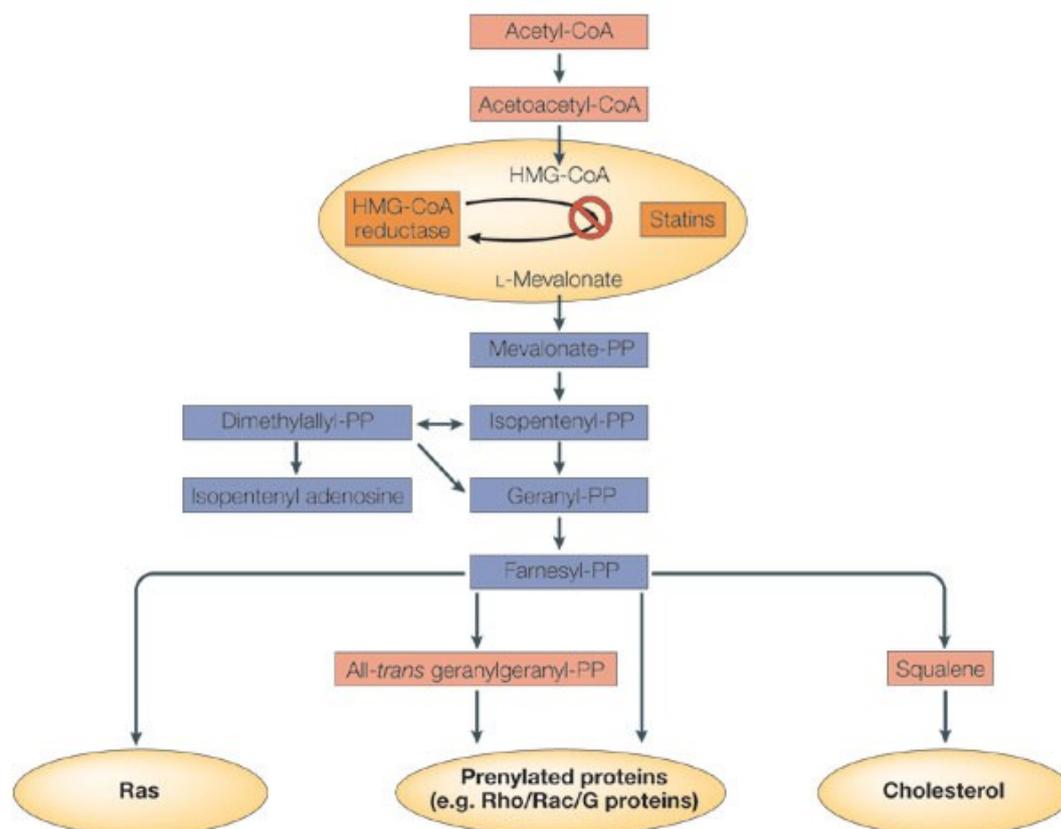
Dyslipidaemia and hypercholesterolemia are controlled by the liver cells. Hepatocytes take up around 50% of LDL cholesterol from the circulation. An increase in take-up in hepatocytes would be an efficient method to decrease plasma LDL cholesterol level.

#### ***Inhibition of HMG CoA reductase***

Statins target hepatocytes and inhibit HMG-CoA reductase, the enzyme that converts HMG-CoA into mevalonic acid, a cholesterol precursor. The statins alter the conformation of the enzyme by binding to its active site, thereby

preventing HMG-CoA reductase from attaining a functional structure. This ability to change conformation at the active site makes these drugs very effective and specific. Binding of statins to HMG-CoA reductase is reversible.

The inhibition of HMG-CoA reductase results in the reduction of intracellular cholesterol, which then induces the activation of a protease which separates the sterol regulatory element binding proteins (SREBPs) from the endoplasmic reticulum. The SREBPs are translocated at the level of the nucleus, where they increase the gene expression for LDL receptor. Therefore, the reduction of cholesterol in hepatocytes leads to the increase of hepatic LDL receptors, which then bind to the circulating LDL, reducing its' levels and that of it's precursors - intermediate density lipoproteins (IDL) and very low density lipoproteins (VLDL). All statins reduce LDL cholesterol in a dose dependent fashion, and are effective after administration of a single daily dose. Their effect on triglyceride reduction parallels LDL cholesterol reduction.



### ***Direct effects of HMG CoA reductase inhibition***

Statins inhibit hepatic synthesis of apolipoprotein B-100, causing a reduction of the synthesis and secretion of triglyceride-rich lipoproteins and an increase of receptor production for apolipoproteins B & E. This explains why atorvastatin and simvastatin are capable of reducing LDL in patients with homozygous family hypercholesterolemia where LDL receptors are not functional. Statins have a small effect on HDL increase, and no influence on lipoprotein concentration.

### ***Isoprenoids***

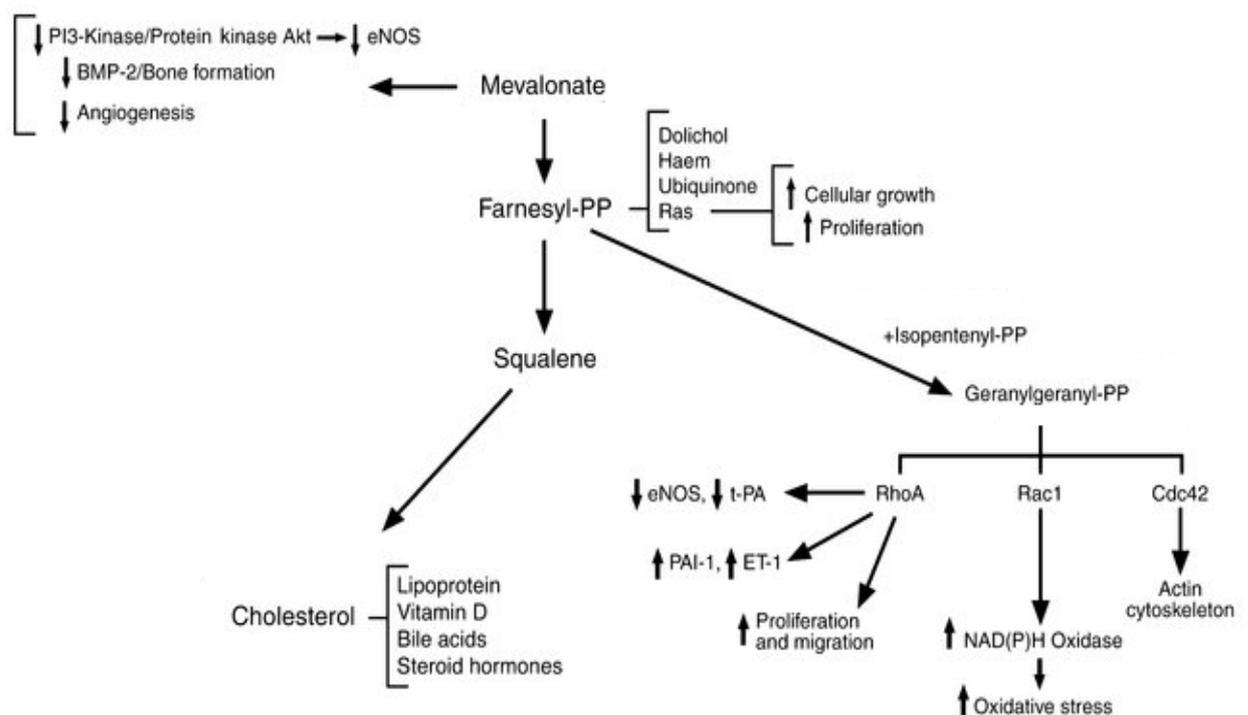
Metabolism of mevalonate ultimately leads to the synthesis of isoprenoid metabolites, which serve as lipid attachments for a number of intracellular signalling molecules. By inhibiting mevalonic acid synthesis, statins also prevent the synthesis of other important isoprenoid intermediates of the cholesterol biosynthetic pathway, such as farnesylpyrophosphate (FPP) and geranylgeranylpyrophosphate (GGPP). These intermediates serve as important lipid attachments for the post-translational modification of a variety of cell-signalling proteins. Protein isoprenylation permits the covalent attachment, sub-cellular localisation, and intracellular trafficking of membrane-associated proteins. Members of the Ras and Rho GTPase family are major substrates for post-translational modification by isoprenylation and may be important targets for inhibition by statins.

Inhibition of RhoA by statins increases endothelial nitric oxide synthase (eNOS) expression and has been shown to decrease severity of cerebral ischemia in ischaemic stroke experimental models. Similarly, statins also increase the expression of tissue-type plasminogen activator and inhibit the expression of plasminogen activator inhibitor-1 and endothelin-1 by mechanisms involving inhibition of geranylgeranylation.

Because Ras and Rho also regulate the cell cycle, they are, in addition, likely targets for the direct anti-proliferative effects of statins. Statins inhibit vascular smooth muscle cell proliferation in transplant-associated

arteriosclerosis and may have clinical benefits in inhibiting certain breast cancers.

Inhibition of Rac1 geranylgeranylation and Rac1-mediated NAD(P)H oxidase activity by statins attenuates angiotensin II-induced reactive oxygen species production in vascular smooth muscle cells and cardiac myocytes. These cholesterol-independent antioxidant effects of statins lead to the inhibition of hypertrophic responses in these tissues.



### ***Reduction of LDL susceptibility towards oxidation***

At least 4 mechanisms were proposed to explain statins' antioxidant properties.

1. The hypocholesterolaemic effect, resulting in reduced lipoprotein cholesterol, and thus, reduced level of oxidation substrate.
2. The decrease of cell oxygen production, by inhibiting the generation of superoxide by macrophages.

3. The binding of statins to phospholipids on the surface of lipoproteins (fluvastatin and lovastatin bind to LDL phospholipids) preventing the diffusion towards the lipoprotein core of free radicals generated during oxidative stress.
4. The potent antioxidative potential of the metabolites (i.e. atorvastatin and fluvastatin metabolites) also results in lipoprotein protection from oxidation.

## **Beneficial effects of statins**

### ***Effects on cholesterol esterification and its accumulation in macrophages***

Experimental studies in mouse macrophages have shown that fluvastatin and simvastatin, but not pravastatin, inhibit cholesterol esterification induced in cells by acetyl LDL. The efficacy of fluvastatin in inhibiting cholesterol esterification is more increased in cholesterol loaded cells than in normal ones, an effect that might be explained by the fact that the HMG CoA reductase is already inhibited in lipid-loaded cells, as compared with unloaded ones.

### ***Effects on endothelial cell function***

Endothelial dysfunction represents an early event in the initiation of an atherosclerotic lesion induced by hypercholesterolemia. Nitric oxide (NO) regulates the anti-atherosclerotic function of the endothelium. Hypercholesterolemia reduces the capacity of endothelial cells to produce NO (probably due to the reduced availability of L-arginine, the physiologic substrate of NO synthase) and determines an increased degradation of NO. Cholesterol reduction by statins leads to a significant increase in endothelial function. The effect of statins on the endothelial function can be partially independent of the reduction of the lipid level. Activation of eNOS (endothelial nitric oxide synthase) by statins takes place post-translationally and is prevented by the isoprenoid derivatives mevalonate and geranylgeraniol.

### ***Effects on the inflammatory process***

Adhesion to the endothelium and transendothelial diapedesis (cells moving out of blood vessels into the surrounding tissue space) of circulating monocytes and of T-lymphocytes, represent key events in formation of an atherosclerotic. Cytokines secreted by macrophages and lymphocytes can modify endothelial function, smooth muscle cells (SMC) proliferation, collagen degradation and thrombosis. Statins can reduce the expression and function of molecules on the leukocyte's surface. In addition, statins are able to inhibit transendothelial migration and chemotaxis of neutrophils, which can explain the anti-inflammatory effect of these compounds. A further anti-inflammatory effect of statins on monocytes and macrophages is decreased expression of intercellular adhesion molecule-1 and the secretion of interleukine-6 (IL-6).

### ***Effects on proliferation, migration and apoptosis of arterial SMC***

All statins, except for pravastatin, reduce aortic SMC proliferation. Mevalonate, trans-farnesol and trans-geranylgeraniol prevent the inhibitory effect of statins on SMC proliferation, suggesting that this effect derives from the inhibition of the mevalonate pathway. Fluvastatin, simvastatin and cerivastatin, but not pravastatin, inhibit arterial SMC migration induced by fibrinogen. Preclinical observations and *in vitro* studies suggest that apoptosis can modulate the arterial wall in proliferative lesions where SMC are dominant.

### ***Effects on the stability of the atherosclerotic plaque***

Coronary events are the result of unstable atherosclerotic lesion rupture and thrombus formation. The plaque instability, manifested as ulceration of the fibrous cap, the rupture of the plaque and internal haemorrhage, are characteristics of plaques with numerous lipid deposits and macrophages in the cap. Recently, it was demonstrated that statins (fluvastatin, simvastatin) can inhibit the gelatinolytic activity of metalloproteases, as well as their secretion by macrophages, reducing the chance of atherosclerotic cap breakdown. Angiographic studies showed that statins

reduce the progression and induce the regression of coronary atherosclerosis, and reduce the formation of new lesions and the incidence of coronary events.

### ***Effects on platelet activation***

Hypercholesterolemia is associated with hypercoagulability, as well as with increased platelet activation. An increased level of LDL determines an increase in platelet reactivity, associated with an increased thromboxane A<sub>2</sub> biosynthesis. In addition, platelet-dependent-thrombin generation is increased in hypercholesterolaemic subjects, and pravastatin treatment determines a restoration of thrombin formation. Statin therapy is accompanied by a reduction of platelet aggregation induced by ADP, collagen or fibrinogen, as well as a reduction of thromboxane production, in parallel with LDL cholesterol reduction.

### ***Other beneficial effects of statins***

The fact that mevalonate plays a key role in cell proliferation and that many malignant cells present an increased HMG-CoA reductase activity, suggests that a selective inhibition of this enzyme could lead to a new chemotherapy for cancer management. Results obtained *in vitro* have shown that statins can inhibit tumour cell growth, a fact confirmed by some *in vivo* experiments also. The obtained reduction of sterols synthesis by statins, suggests that inhibition of tumour cell growth can be related to the reduction of isoprenoid compounds. This effect can influence Ras protein farnesylation, thus inhibiting Ras-dependent tumour cell growth. Recent experimental evidence supports a role for the mevalonate pathway in murine and rabbit osteoclast formation and bone resorption. In addition, it was demonstrated *in vitro* and *in vivo* in rodents that statins enhance new bone formation. Statin administration is associated with a decrease of bone fracture risk in subjects over 50 years, probably because of the increase of the mineral density of the bones. Therefore, subjects with hyperlipidaemia known to present increased risk for osteoporosis (mostly post-menopausal women) may benefit from statin therapy.

## **Adverse effects of statin therapy**

Statins are generally well tolerated. The most important adverse effects are liver and muscle toxicity. Myopathy can happen if inhibitors of cytochrome P450 (CYP) or other inhibitors of statin metabolism are administered together with statins, causing an increase in their blood concentration. An example of a group of drugs which may do this are the azole antifungals.

Fibrates and niacin (VIT B3) enhance myopathy risk by a mechanism not involving increased statin blood concentration. Other risk factors are: hepatic dysfunction, renal insufficiency, hypothyroidism, advanced age and serious infections.

### ***Myopathy and Rhabdomyolysis***

Rhabdomyolysis is the rapid breakdown of skeletal muscle. Breakdown products of damaged muscle cells are released into the bloodstream and some of these, such as myoglobin, are harmful to the kidneys and may lead to kidney failure. The severity of the symptoms, which may include muscle pains, vomiting and confusion, depends on the extent of muscle damage and whether kidney failure develops. The damage may be caused by physical factors such as a crush injury, extreme strenuous exercise, medication such as statins, drug abuse, and infection.

Statins are associated with muscle complaints ranging from muscle weakness and cramps to myalgia with and without elevated creatine kinase (CK) levels, mild CK elevations, or myositis and rhabdomyolysis. Myalgia is the least severe but most common presentation of muscle toxicity.

Rhabdomyolysis and potential renal failure are the most severe but least common presentations. Rhabdomyolysis results from sarcolemmal injury that causes release of skeletal myocyte contents (myoglobin, CK, uric acid, and electrolytes) into the circulation. It can have a number of causes other than statin therapy. Fortunately the development of myopathy and rhabdomyolysis is relatively uncommon for all of the currently available statins

Although the precise mechanism for statin-induced myopathy is not fully understood, inhibition of the production of one or more precursors in the cholesterol biosynthetic pathway is likely to be involved, leading to a ubiquinone (co-enzyme Q10) deficiency. Since ubiquinone is an essential intracellular energy component in the mitochondria, cellular respiration may be affected. The isoprenoids farnesol and geranylgeraniol are involved in post-transcriptional modification of proteins (e.g., ras proto-oncogen, Rho-related proteins) that mediate pivotal cellular functions leading to cell signalling, differentiation, proliferation, and, ultimately, apoptosis. Statins can reduce circulating ubiquinone levels. However, their effect on levels in skeletal muscle is unclear, and the role of coenzyme Q10 supplementation in reducing the risk for myopathy remains controversial.

The most common risk factors for statin-associated muscle syndromes are:

- Increased aged (over 80 years)
- Female
- Small body frame and frailty
- Multi-system disease - diabetes, liver disease, chronic renal failure, hypothyroidism
- Peri-operative period
- Major trauma
- Hypothermia
- Electrical disturbances
- Metabolic acidosis
- Hypoxia
- Viral and bacterial infection
- Consumption of large quantities of grapefruit juice (2 pints / 1100mls) which suppresses CYP 450 enzymes
- Alcohol abuse
- Concomitant drug use involving drugs interacting with CYP enzymes

Most myopathic syndromes associated with statins appear to occur in patients who have one or more of these risk factors.

Caution should be used when starting statin therapy in patients with more than one risk factor (e.g., elderly with poor renal function). Patients should be informed about the risk of myopathy and advised to report any unexplained muscle discomfort or weakness that is not associated with physical exercise or other trauma to muscles. If muscle symptoms cannot be explained based on history and physical examination, then creatine kinase(CK) levels should be established. If the patient has symptoms of myopathy and CK is significantly increased, then statin therapy and other lipid-lowering therapy should be discontinued. While the hallmark of myopathy is CK elevation with muscle symptoms, patients with biopsy-proven myopathy without CK elevation have been reported.

## **Conclusions**

Statins are widely used for the treatment of hypercholesterolemia. They inhibit HMG-CoA reductase competitively, reduce LDL levels more than other cholesterol-lowering drugs, and lower triglycerides levels in hypertriglyceridaemic patients. Statins have anti-atherosclerotic effects which correlate positively with the decrease in LDL cholesterol. In addition, they can exert anti-atherosclerotic effects independently of their hypolipidaemic action. Because mevalonate metabolism generates a series of vital isoprenoids for different cellular functions, from cholesterol synthesis to the control of cell growth and differentiation, HMG-CoA reductase inhibition has beneficial pleiotropic effects. Consequently, statins significantly reduce the incidence of coronary events, both in primary and secondary prevention, being the most efficient hypolipidaemic compounds that have reduced the rate of mortality in coronary patients. Statins are well tolerated and have an excellent safety record. Independent of their hypolipidaemic properties, statins interfere with events involved in bone formation. In addition, it has been demonstrated that HMG-CoA reductase inhibitors impede tumour cell growth.

## KEY LEARNING POINTS.



1. Statins are a group of drugs used to lower cholesterol levels by inhibiting the activity of the enzyme HMG-CoA reductase , which plays a central role in the production of cholesterol in the liver.
2. The reduction of the production of mevalonate brought about in hepatocytes is the mechanism by which this occurs.
3. They appear to be most effective in individuals already suffering from cardiovascular disease (CVD), and as such are a form of secondary prevention.
4. Statins also have many pleiotropic effects, such as the improvement of endothelial function, a decrease in vascular smooth muscle cell proliferation and macrophage proliferation, reduction of platelet activity, stabilisation of atherosclerotic plaques, antioxidant, anti-inflammatory and immunomodulatory effects.
5. Myalgia and rhabdomyolysis have been reported in patients taking statins.
6. These adverse muscular effects are also associated with a number of other risk factors.
7. Statins significantly reduce the incidence of coronary events, both in primary and secondary prevention, being the most efficient hypolipidaemic compounds that have reduced the rate of mortality in coronary patients.