# The Role of Squalene in the Prevention of Atherosclerosis through its LDL-Reducing Effects: A Review

## I Putu Edra Putra Indrawan

Master Program in Biomedical Science,
Faculty of Medicine, Udayana University, Bali, Indonesia

*Corresponding author details: I Putu Edra Putra Indrawan; andrewedra11@gmail.com

**ABSTRACT**

***Introduction:*** Atherosclerosis is the most common cause of mortality and morbidity in the world. Various efforts to prevent atherosclerosis, one of which is squalene supplementation. This review will discuss the role of squalene in preventing atherosclerosis through its effect on reducing LDL levels. ***Discussion:*** Squalene can prevent the formation of atherosclerotic plaques through the mechanism of suppressing the expression of the CD36 ox-LDL receptor on the surface of monocytes and macrophages, thereby reducing the uptake of ox-LDL without causing cytotoxicity. In addition, squalene also provides an antiproliferative effect on macrophages and monocytes resulting in a decrease in the formation of atherosclerosis. ***Conclusion:*** In theory, administration of Squalene can prevent atherosclerosis by reducing total cholesterol, LDL, triglycerides, and increasing HDL levels.

***Keywords:*** Atherosclerosis; LDL; squalene

## INTRODUCTION

Atherosclerosis is a complex chronic disease caused by the accumulation of fibrous fatty deposits in the intima layers of the arteries and is the most common cause of mortality and morbidity in the world. High levels of low-density lipoprotein (LDL) in the blood, low levels of high-density lipoprotein (HDL) and increased triglycerides are risk factors for atherosclerosis (Wang & Butany, 2017; Chiu et al., 2017).

There are various ways to prevent atherosclerosis, such as lifestyle changes, physical activity, and diet to maintain lipid levels in the body (Michos et al., 2019). One way to regulate the diet is to add supplements, one of which is squalene. Squalene was first discovered in 1916 by Tsujimoto Mitsumaru in shark liver oil (*Squalus milsukurii* and other *squaloids*). Squalene is a polyunsaturated triterpene that mediates the biosynthesis of phytosterols or cholesterol in plants, animals and humans (Popa et al., 2015).

The main source of squalene is the liver of marine animals, but according to recent research this molecule can be found in plants (Lozano-Grande et al., 2018; Micera et al., 2020). Based on its bioactive properties, squalene has various functions, including as an antioxidant and cardioprotector through controlling cholesterol (Lozano-Grande et al., 2018). Based on a number of studies cited by Ibrahim et al, squalane shows potential as an alternative therapy for hyperlipidemia. However, there are also several studies which state that consumption of squalene does not prevent increases in cholesterol and triglycerides and it is suspected that long-term consumption of squalene can cause hypercholesterolemia (Ibrahim et al., 2020).

### Blood Vessel Degeneration

Several studies have demonstrated structural changes in the arterial wall that occur with aging. The main change is degeneration of elastin which is thinned, clipped, and fragmented.

In addition, there is also an increase in collagen and calcium deposition and degeneration of elastic fibers. Metabolic changes such as hyperglycemia and glycation of arterial wall proteins also contribute to arterial stiffness due to aging. Chronic low-grade inflammation can also accelerate the aging process (Nilsson et al., 2013).

Aging also induces progressive endothelial dysfunction even in the absence of cardiovascular disease. Endothelial dysfunction due to an imbalance between vascular protection against deterioration factors from the endothelium (Laina et al., 2018). The mechanism is still not clearly understood, but several pathways that play a role in senescense (such as: irreversible arrest of the cell cycle due to telomere shortening, oxidative stress conditions, or DNA damage) and the vasodilator Nitric Oxide (NO) pathway are thought to cause loss of vascular function due to aging. (Laina et al., 2018; Thijssen et al., 2016).

Nitric Oxide (NO) has potent anti-atherogenic properties and plays an important role in the regulation of vascular smooth muscle cells (Laina et al., 2018; Thijssen et al., 2016) NO has vasoprotective, cardioprotective, and vasodilator effects which have anti- inflammatory, anti-thrombotic, and antioxidant (Laina et al., 2018). A decrease in the synthesis of tetrahydrobiopterin (a cofactor for NO production) with aging is one of the factors that disrupts the NO-pathway in the elderly (Thijssen et al., 2016).

Another mechanism of vascular dysfunction in the elderly can also be caused by increased vascular oxidative stress and inflammation which causes pro-inflammatory phenotypic changes. In the elderly, increased levels of Reactive Oxygen Species (ROS) and decreased or lost compensatory abilities of antioxidants are the causes of increased oxidative stress (Thijssen et al., 2016). The high concentration of singlet oxygen under conditions of oxidative stress causes NO inactivation (Laina et al., 2018).

**Atherosclerosis**

Atherosclerosis is believed to be the result of passive accumulation of cholesterol-carrying lipoproteins, especially LDL in the arterial wall (Narasimhulu et al., 2016). In 1961, researchers in the Framingham Heart Study identified serum cholesterol as one of the risk factors for coronary heart disease. Since then, a large number of epidemiological studies and clinical trials have proven that increased serum LDL is the main cause of atherosclerosis (Khatana et al., 2020; Michos et al., 2019) According to the "infiltration theory", the development of atherosclerosis is triggered by subendothelial influx and retention of lipoproteins (especially LDL and apolipoprotein B) on the arterial wall (Khatana et al., 2020). LDL must undergo a process of structural change to have atherogenic properties, and the circulating LDL uptake by macrophages is not fast enough to overload cells with the cholesterol needed for the formation of foam cells (Poznyak et al., 2021).

The beginning of the formation of atherosclerosis is endothelial injury which causes infiltration and accumulation of LDL in the subendothelial space (Kattoor et al., 2017). Under conditions of oxidative stress, LDL will be oxidized by ROS through a lipid peroxidation process involving phospholipid molecules to form ox-LDL (Khatana et al., 2020). Oxidized LDL will cause increased expression of cell adhesion molecules on endothelial cells resulting in leukocyte recruitment in the subendothelial space (Kattoor et al., 2017). With endothelial disruption in arterial bends and branches, more and more LDL and residual VLDL are retained in the extracellular matrix of the tunica intima (Wengrofsky et al., 2019).

LDL and VLDL accumulation will trigger endothelial activation of the NF-κB pathway so that inflammatory cells will migrate to the intima layer which enhances endothelial expression of adhesion proteins such as vascular cell adhesion molecules-1 (VCAM-1), selectin P and E, as well as proinflammatory receptors and cytokines that cause migration of monocytes and T lymphocytes (Kattoor et al., 2017; Wengrofsky et al., 2019). Monocytes differentiate into macrophages that express scavenger receptors and internalize modified lipoproteins (Kattoor et al., 2017).

Oxidized-LDL will interact with macrophage receptors to prevent negative feedback on scavenger receptor expression, causing repeated and sustainable uptake of ox-LDL and cholesterol to form foam cells in the arterial intima lining. The presence of these foam cells on the arterial wall is a hallmark of early atherosclerotic lesions (Kattoor et al., 2017). Atherosclerotic plaque is a large necrotic core of foam cells, smooth muscle cells, collagen, calcium, and a thin fibrous cap (which blocks plaque from entering the bloodstream). Oxidized-LDL encourages smooth muscle cells to produce collagen and elastin which form a necrotic core around atherosclerotic plaques so that the plaque becomes more extensive. Thus, ox-LDL plays an important role in every stage of atherosclerosis (Khatana et al., 2020).

**Squalene**

Squalene (2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene) is a triterpene which has the formulation $C_{30}H_{50}$ with 6 double bonds. This compound is a natural lipid with an isoprenoid structure that belongs to the terpenoid family and is known as an intermediate in the biosynthesis of phytosterols in plants or cholesterol in animals and humans (Popa et al., 2015).

Squalene is an odorless, colorless and liquid oil (Micera et al., 2020). The carbon double bond (C=C) makes this molecule an unsaturated fat and sensitive to oxidation.

Squalene is not susceptible to peroxidation processes so it can act as an antioxidant by binding to singlet oxygen (Lozano-Grande et al., 2018).

About 60 to 85% of squalene from food is absorbed by the body and then carried by chylomicron into circulation. Squalene is then taken to the liver where it will undergo a cyclic process to form sterols and bile acids. Post-absorptive plasma in normal individuals was found to contain squalene bound to the lipoprotein fraction: 50.8% in VLDL, 25.6% in LDL, and 23.6% in HDL and triglycerides (Güneş, 2013; Micera et al., 2020). Because of its good absorption efficiency, squalene is applied as a carrier for oral administration of therapeutic molecules (Güneş, 2013).

Serum squalene is obtained from endogenous cholesterol synthesis and can also be obtained from consuming a diet rich in squalene. About 60 to 85% of squalene from food is absorbed by the body and then carried by chylomicron into circulation. Squalene is then taken to the liver where it will undergo a cyclic process to form sterols and bile acids (Güneş, 2013). Post-absorptive plasma in normal individuals was found to contain squalene bound to the lipoprotein fraction: 50.8% in VLDL, 25.6% in LDL, and 23.6% in HDL and triglycerides (Güneş, 2013; Micera et al., 2020). Because of its good absorption efficiency, squalene is applied as a carrier for oral administration of therapeutic molecules (Güneş, 2013).

**Role of Squalene in LDL and Atherosclerosis**

Hypercholesterolemia is a metabolic disorder characterized by total cholesterol levels above 200 mg/dL and LDL levels higher than 100 mg/dL (Micera et al., 2020). LDL accumulation occurs in the walls of blood vessels and then undergoes modification to become ox-LDL. This modified LDL will be carried by macrophage scavenger receptors and form foam cells which lead to the formation of atherosclerotic plaques. High LDL conditions are a contributing factor to the formation of atherosclerotic lesions (Ibrahim et al., 2020; S. Liu et aI., 2018).

Squalene is directly involved in terminating cholesterol synthesis through negative feedback by reducing the action of the HMG-CoA reductase enzyme (the main enzyme in cholesterol biosynthesis), increasing the activity of the enzyme acyl CoA cholesterol acyltransferase and excretion of bile acids and cholesterol (Ronco & de Stéfani, 2013; Micera et al., 2020) In its role of reducing the action of the HMG-CoA reductase enzyme, squalene is thought to cause an increase in cholesterol synthesis which is a derivative of squalene (Ronco & de Stéfani, 2013).

A study by Chan et al (1996) showed that 860 mg of squalene showed a decrease in LDL levels after 20 weeks of administration. The same thing was also found from a study by Farvin et al (2006) in rats with myocardial infarction given 2% squalene which showed prevention of an increase in LDL. Research by Preobrazhenskaya et al (2015) in rats with cardiomyopathy given 6% squalene or 0.25ml/kg amaranth oil showed a decrease in total cholesterol, triglycerides, LDL cholesterol, and free fatty acids (Ibrahim et al., 2020) Research by Spanova and Daum (2011) stated that only a small amount of dietary squalene is converted to cholesterol, and the higher the consumption of squalene will cause an increase in squalene levels in the serum, thereby providing a hypocholesterolemic function.

Squalene can prevent the formation of atherosclerotic plaques through the mechanism of suppressing the expression of the CD36 ox-LDL receptor on the surface of

monocytes and macrophages thereby reducing the uptake of ox-LDL without causing cytotoxicity. In addition, squalene also provides an antiproliferative effect on macrophages and monocytes resulting in a decrease in atherosclerosis (Granados-Principal et al., 2012).

Squalene is considered to have various functions as both a nutrient and a medicine, such as cholesterol control and cardioprotective, antioxidant, moisturizing and skin protection, detoxification agent, anticancer, and drug administration agent (Lozano-Grande et al., 2018; Micera et al., 2020; Popa et al., 2015). Squalene shows the effect of lowering total cholesterol, LDL, triglycerides, and increasing HDL while keeping levels normal (Lozano-Grande et al., 2018; Popa et al., 2015). In addition to these hypolipidemic effects, squalene can also fight lipid peroxidation (Lozano-Grande et al., 2018).

## CONCLUSION

Squalene theoretically can prevent atherosclerosis through the effect of reducing total cholesterol, LDL, triglycerides, and increasing HDL levels.

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