

Influence of Squalene on Cholesterol Levels in Albino Rats

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Addition of 2% squalene to diets of albino rats was not found to decrease the total cholesterol levels in their serum, heart or liver. It resulted in an increase in cholesterol levels in serum, heart and liver. No beneficial effect was seen in HDL cholesterol levels also. Squalene seems to have no effect on the relative sizes of heart and liver and also on the weight gain of rats. Significance of these findings is discussed.

Key words: Squalene, hypocholesterolemic effect, albino rats.

The importance of the quality and quantity of the major food constituents like protein, fat, minerals and fiber in controlling the levels of serum cholesterol is well known. (Kritchevski, 1959; Viswanathan Nair, 1985; Linder, 1985; Ammu *et al.* 1989, 1994). It has been established that saturated fats in the diet generally tend to increase serum cholesterol levels, while fats rich in polyunsaturated fatty acids have hypocholesterolemic effect (Piefer *et al.* 1962; Jones, 1974). However reports on the effects of unsaponifiable constituents of dietary oils on serum cholesterol are scanty. Squalene $C_{30}H_{50}$, a highly unsaturated hydrocarbon present in the unsaponifiable matter of the liver lipids of some species of sharks like *Centrophorus* sp. and *Squalus mitsukurii*, when administered to one day old chicks was found to lower the serum cholesterol levels (Brakken *et al.* 1961). However since squalene is an intermediate in the cholesterol biosynthesis, its hypocholesterolemic effect is doubtful. A study was therefore undertaken to assess the effect of squalene on serum

cholesterol levels when fed to albino rats at 2% level of the diet.

Materials and Methods

Six weeks old male albino rats (wistar strain) weighing around 100g were used for the study with four rats in each group. The basic diet consisted of 15% protein (protein rich casein, Sisco Laboratories), 10% fat, 4% salt mixture U.S.P. (Sisco), 1% vitamin mixture (Chapman *et al.* 1959), 5% cellulose and 0.1% methionine. Squalene at 2% level was added to two test groups. Cholesterol at 0.5% was added to one of the basic diets and one of the test diets. Corn starch was added to balance the diets. The rats after an initial adaptation period of 4 days on experimental diet, were fed on the respective diets for 4 weeks. Feed and water were given *ad libitum*. At the end of the feeding period rats were sacrificed. Heart, liver and blood were collected from all specimens. Serum was separated and total and HDL cholesterol estimated using Sigma Diagnostic Kit (Sigma Procedure Nos. 352, 352-3). Liver and heart of each

group were pooled and extracted using chloroform-methanol mixture (Bligh & Dyer, 1959). Lipids were saponified and unsaponifiable matter extracted by the method of AOAC (1990). Total cholesterol in the nonsaponifiable matter was estimated by the ferric chloride method as described by Rudel & Morris (1973).

Results and Discussion

The changes in the size and weight of the liver and heart and the weight gain of the four groups of rats are presented in Tables 1 and 3. Different types of lipids in the diets are known to influence the size and weight of organs like liver and heart. There was a significant increase in the relative size of liver of the animal given cholesterol supplemented diet. Difference between the control and squalene supplemented groups in the relative size of liver was not significant whereas the difference between the control and cholesterol supplemented groups was highly significant (Table-3). The effect of squalene in determining the relative size of liver appears to be not important. Song & Wander (1991) found that feeding of fish oil increased the liver weight and relative liver sizes in rats. Gilles *et al.* (1988) observed that the heart weight were not significantly different for the rats on different diets.

Total cholesterol level in serum was found to be significantly higher in the group of rats fed on diets containing squalene, with or without added cholesterol (Table 2 & 3). There was no significant increase in the level of serum total cholesterol as a result of addition of cholesterol (0.5% of the diet), whereas increase caused by squalene (2% of the diet) was highly significant. This shows that squalene is a strong hypercholesterolemic agent under these conditions. The ratio of HDL cholesterol to total cholesterol was significantly low when squalene or cholesterol was added to the control diet which also shows that squalene is not effective in preventing hypercholesterolemia and related problems. Compared to cholesterol, squalene has more detrimental effect as the latter is showing a highly significant t-value (Table 3)

Cholesterol levels of heart and liver showed increase when squalene was added to the diet. But the effects on the two organs were not to the same extent. Liver total cholesterol increased by 88% as a result of dietary squalene, whereas the increase in heart was only 9%. Total cholesterol levels in liver of the rats given diets supplemented with cholesterol and squalene was less than that in the animals given diets supplemented with cholesterol alone. It

Table 1. Effects of squalene and cholesterol on the size and weight of liver and heart of rats

Diets	Without added cholesterol			With added cholesterol at 0.5% level		
	Heart size*	Liver size	Wt. Gain	Heart size	Liver size	Wt. Gain
Coconut oil 10%	0.330	2.61	125	0.328	2.93	127.4
Coconut oil 10% + 2% squalene	0.315	2.77	132	0.322	3.12	131.9

* Heart/Liver size = weight of heart/Liver x 100/weight of rat

Table 2. Effect of squalene on cholesterol levels in serum, liver and heart in rats in the presence and absence of dietary cholesterol

Diets	Without added cholesterol			With added cholesterol at 0.5% level			
	Serum (mg/100ml)	Liver (mg/100g)	Heart (mg/100g)	Serum (mg/100ml)	Liver (mg/100g)	Heart (mg/100g)	
	Total	HDL		Total	HDL		
Coconut oil 10%	43.6	25.9	210.5	182.7	46.5	21.7	1575
10% coconut oil + 2% squalene	63.6	17.3	396.7	199.3	72.4	24.1	1393
							184.7
							175.1

Table 3. T-test for comparison of variables between four groups of rats - t values

Variable	Gr 1&2	Gr 1&3	Gr 3&4	Gr 2&4
Heart size	1.899	0.179	0.445	0.865
Weight gain	1.313	0.226	0.474	0.015
Liver size	1.522	4.458**	2.639*	3.408*
Total cholesterol	4.097**	0.755	3.254*	1.044
HDL cholesterol as % of Total cholesterol	8.823**	3.163*	2.245	1.733

Gr.1, control; Gr.2, squalene-supplemented; Gr.3, cholesterol-supplemented; Gr.4, squalene +cholesterol supplemented.

* Significant at 5% level

** Significant at 1% level

is interesting to note that the hypercholesterolemic action of squalene was not noticeable in the presence of cholesterol. This may be due to the fact that biosynthesis of cholesterol is reduced when there is added cholesterol in diet.

Rats fed with cholesterol supplemented diet had a high level of cholesterol deposited in the liver. This accumulation of cholesterol in the liver may be due to the redistribution of the total cholesterol between liver and serum as cholesterol is known to be in dynamic equilibrium in the system (Trussel, 1977).

Brakken *et al.* (1961) report a hypocholesterolemic effect for squalene when administered to chicks for two weeks. But squalene was not found to

be beneficial to any significant extent in this experiment where albino rats were used. However results from studies on albino rats are more readily extrapolated to man and hence this observation that squalene does not exert any beneficial hypocholesterolemic effect is more significant in human nutrition. It is also possible that squalene has its beneficial effect over a long feeding period only. Squalene is likely to increase serum, heart and liver cholesterol levels in animals fed on normal diets and it reduces serum HDL cholesterol levels. It is found that dietary squalene has no effect on heart and liver sizes as well as on weight gain of rats.

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