

## Review

# Squalene, Olive Oil, and Cancer Risk: A Review and Hypothesis

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### Abstract

**Epidemiological studies of breast and pancreatic cancer in several Mediterranean populations have demonstrated that increased dietary intake of olive oil is associated with a small decreased risk or no increased risk of cancer, despite a higher proportion of overall lipid intake. Experimental animal model studies of high dietary fat and cancer also indicate that olive oil has either no effect or a protective effect on the prevention of a variety of chemically induced tumors. As a working hypothesis, it is proposed that the high squalene content of olive oil, as compared to other human foods, is a major factor in the cancer risk-reducing effect of olive oil. Experiments *in vitro* and in animal models suggest a tumor-inhibiting role for squalene. A mechanism is proposed for the tumor-inhibitory activity of squalene based on its known strong inhibitory activity of  $\beta$ -hydroxy- $\beta$ -methylglutaryl-CoA reductase catalytic activity *in vivo*, thus reducing farnesyl pyrophosphate availability for prenylation of the *ras* oncogene, which relocates this oncogene to cell membranes and is required for the signal-transducing function of *ras*.**

### Introduction

Certain epidemiological studies suggest a cancer-protective effect of dietary olive oil relative to other types of nonmarine fat sources. In Greece, women with approximately 40% of energy intake from fat, mainly as olive oil, have a breast cancer rate of only about one-third that of United States women, who have until recently also consumed about 40% of energy from fat (1-3). A case-control study in Spain showed a reduced risk for breast cancer in women with the highest olive oil consumption (4). In a large case-control study in Greece, a similar lack of overall association with total fat intake was seen, but breast cancer risk was 25% lower in women consuming olive oil more than once a day (5). In another case-control study in Spain (6), women in the highest third of monounsaturated fat intake (largely from olive oil) had reduced risk of breast cancer (relative risk = 0.30; 95% confidence interval, 0.1-0.8). A recent report of a case-control study performed in Italy (7) indicated a decreased risk of breast cancer with increased intake

of unsaturated fatty acids. In Italy, about 80% of edible oil is olive oil,<sup>2</sup> suggesting a protective effect of olive oil intake. In another Italian case-control study of diet and pancreatic cancer (8), increased frequency of edible oil consumption was associated with a trend toward decreased risk. Edible oil in Italy consists of about 80% olive oil, thus suggesting a protective effect of olive oil in pancreatic cancer.<sup>2</sup>

Animal studies on fat and cancer have generally shown that olive oil either has no effect or a protective effect on the prevention of a variety of chemically induced tumors. Olive oil did not increase tumor incidence or growth, in contrast to corn and sunflower oil, in some mammary cancer models (9-11) and also in colon cancer models (12, 13), although in an earlier report, olive oil exhibited mammary tumor-promoting properties similar to that of corn oil (14), in contrast to the later studies (15).

The protective or, at the least, nonpromoting activity of olive oil is often ascribed to its high content (about 72%) of the monoenoic unsaturated fatty acid oleic acid (C18:1,  $\omega$ 9). But this fatty acid is also found in the fat of beef and poultry in the range of 30-45% of the fat and is also found in appreciable levels in other vegetable oils, such as corn (30%), palm (43%), peanut (49%), soybean (25%), and sunflower seed (33%; Ref. 16). The other fats and oils rich in oleic acid are largely associated with increased risk of colon and breast cancer in humans and generally act as promoters of chemically induced tumors in rodents. Thus it seems that the monoenoic unsaturated fatty acid (oleic acid) content of olive oil cannot account for its protective effect or lack of promotion effect in cancer development.

A recent report of a case-control study of adipose tissue stores of individual monounsaturated fatty acids in relation to breast cancer in women also suggest that the oleic acid in olive oil does not account for any protective property of this oil (17).

A consideration was made of other components unique to olive oil, qualitatively or quantitatively different from other fats and oils, as a possible explanation for its protective or nonpromoting effects. Squalene, at up to 0.7%, is uniquely high in olive oil as compared to other common human food fats and oils (18, 19). Other vegetable oils and animal fats are considerably lower, in the range of 0.002-0.03% (18) squalene content. Food-regulatory agencies often rely on the squalene content to determine the purity of commercial olive oil. Squalene is a hydrocarbon of the triterpene type containing six isoprene units with a pleasant, bland taste. It is a key intermediate in the biosynthetic pathway to steroids in both plants and animals. Thus, it can be considered as almost ubiquitous in most plant and animal cells, although at enormously different levels.

There are only a few reports of an inhibiting effect of squalene itself in rodent cancer models. Van Duuren and Goldschmidt noted that squalene as well as oleic acid applied topically to mouse skin completely inhibited benzo(a)pyrene-induced skin carcinogenicity (20). D-Limonene only partly in-

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<sup>2</sup> C. LaVecchia, personal communication.

hibited skin tumors in the same experiment. In a later study, Murakoshi *et al.* (21) reported that topically applied squalene markedly suppressed the promoting effect of 12-*O*-tetradecanoylphorbol-13-acetate on mouse skin tumors initiated with 7,12 dimethylbenz(*a*)anthracene. In a recent study, we demonstrated potent inhibition of aberrant hyperproliferation in a mammary epithelial cell line *in vitro* (22). In another recent study using 1% squalene in the diet of rats treated with azoxymethane, a 45% suppression of induced aberrant crypt foci was seen.<sup>3</sup> This latter study, to my knowledge, is the first experimental demonstration of the inhibitory action of dietary squalene on carcinogenesis. Other studies on the antitumor activity of squalene have been reported in Japan (23–25).

In rats given 1% squalene in the diet for 5 days, serum squalene rose about 20-fold. This was accompanied by a strong inhibition (about 80%) of HMG-CoA<sup>4</sup> reductase activity in hepatic microsomes (26). It is not clear whether this inhibition stems from squalene itself or one or more of its metabolites, such as sterols and their oxygenated derivatives (27) produced endogenously. Inhibition of HMG-CoA reductase activity, the rate-limiting control step in the normal biosynthetic pathway to cholesterol, presumably also reduces the levels of a series of intermediates between HMG-CoA reductase and cholesterol (27) including mevalonate, geranyl pyrophosphate, and FPP. FPP is a source for the biosynthesis of ubiquinone, heme a, dolichol, and particularly for the prenylation (farnesylation) of certain oncogenes. Many oncogenes such as *ras* are proteins produced in the aqueous environment of the cell cytosol but require relocation to the lipophilic cell plasma membrane for activation. This is achieved by attachment of farnesol supplied from FPP via a specialized enzyme system (28, 29). Prevention of prenylation prevents the activation of these proteins as signal-transducing agents in the regulation of cell-transforming activity.

Several other dietary isoprenoids are also known to act as inhibitors of the mevalonate pathway and act similarly to reduce cellular availability of FPP, resulting in reduction of tumor growth (30).

There have been several research programs aimed at development of (drug) inhibitors of protein farnesylation as potential cancer chemotherapeutic agents (31). Some of these agents are targeted to the specific enzymes involved in protein farnesylation, without interfering with the downstream squalene and cholesterol biosynthesis. Dietary squalene presumably inhibits HMG-CoA reductase activity to reduce FPP formation in the cells and also does so without deprivation of any squalene to cholesterol precursors, presumably by enhancing these squalene metabolites.

The squalene inhibition effect on HMG-CoA reductase activity is probably part of normal feedback regulation control of cholesterol biosynthesis. Increasing dietary intake of squalene (*e.g.*, from increased intake of olive oil) to augment endogenous levels of squalene thus serves to lower FPP levels and therefore reduce oncogene product activation via prenylation, and thus it can potentially reduce tumor growth without markedly disturbing a normal biochemical pathway. Reduction of tumor growth and development could be expected in tumor types strongly dependent on oncogenes requiring prenylation for activation, such as *ras*. Colon, breast, melanoma, and pancreas develop such tumor types, particularly pancreatic adeno-

carcinomas, in which up to 90% are associated with activated *ras* oncogene mutations (28). All these tumors represent potential targets for squalene use as a chemopreventive agent.

Increased dietary squalene intake could theoretically augment cholesterol and bile acid production, resulting in enhanced atherosclerotic disease. However, a few studies in rabbits and humans indicate that a high intake does not seem to be associated with a high risk of atherosclerosis. Kritchevsky *et al.* (32) fed 3% squalene in a high-cholesterol diet to rabbits for 14 weeks, who failed to develop more atheromas than similar cholesterol-fed controls. Our recent study also indicates that 1% squalene in the diet had no effect on serum cholesterol in rats.<sup>3</sup> Strandberg *et al.* fed 900 mg of squalene daily to humans for 7–30 days, demonstrating about 60% absorption with a 17-fold increase in serum squalene, but produced no consistent increases in serum cholesterol levels (33). However, these short-term feeding studies are insufficient to fully answer questions of long-term effects of higher-than-normal dietary squalene intake on metabolism of cholesterol and other steroids, as well as adequacy of biosynthesis of ubiquinones, heme a, and dolichols for normal cell function.

Squalene in humans is supplied by both endogenous biosynthesis and dietary sources. The Rockefeller University group (18) measured squalene in normal human tissues and plasma and found it to be widely distributed, but at great variations in level. Sebum was reported to contain 12% squalene, and adipose tissue contained 0.01–0.04% squalene, with other tissues having lower levels. Plasma squalene levels rose strikingly with increased dietary squalene. Generally, there was a direct relationship between plasma levels of squalene and triglycerides, but not with cholesterol.

The average dietary intake of squalene in the United States has been estimated to be only about 30 mg/day (18), with a possible high intake up to 200 mg/day on a 2000-Kcal diet rich in fish and salads dressed with olive oil (18). In Mediterranean countries such as Greece, where olive oil intake is about 20 kg/year/person (60 g/day), or Italy, where olive oil intake is 12 kg/year/person (35 g/day; Ref. 34), average squalene daily intake is more likely to be in the range of 200–400 mg/day based on about 0.6–0.7% squalene content (19) of olive oil. This large difference in average dietary intake of squalene between Mediterranean countries and the United States (7–13-fold) may be related to lower cancer mortality in the Mediterranean basin countries (1–5).

A closely related hypothesis has been proposed by El-Soheby *et al.* (35), who demonstrated that dietary cholesterol inhibited chemically induced breast cancer in rats, and they proposed that the administered cholesterol inhibited HMG-CoA reductase activity, reducing the availability of mevalonate, which is required for DNA synthesis and cell proliferation.

Sharks, which have been claimed to be resistant to cancer (36), have unusually high tissue levels of squalene. Shark liver oil contains 40% or more squalene (18, 19). Dogfish liver oil is very high, reported to be over 90% squalene (19). It is interesting to speculate whether the high squalene content is related to the reputed cancer resistance in sharks.

In summary:

(a) Increased dietary intake of olive oil is associated with a small decreased risk or no increased risk of cancer, despite high lipid intake, in epidemiological studies of breast and pancreatic cancer in several Mediterranean populations.

(b) As a working hypothesis, it is proposed that the high squalene content of olive oil, as compared to other human

<sup>3</sup> C. V. Rao, B. S. Reddy, and H. Newmark, *Carcinogenesis*, in press.

<sup>4</sup> The abbreviations used are: HMG-CoA,  $\beta$ -hydroxy- $\beta$ -methylglutaryl CoA reductase; FPP, farnesyl pyrophosphate.

foods, is a major factor in the cancer risk-reducing effect of olive oil.

(c) A few experiments *in vitro* and in animal models suggest a tumor-inhibiting role for squalene.

(d) A mechanism is proposed for the tumor-inhibitory activity of squalene, based on its known strong inhibitory activity of HMG-CoA reductase activity, thus reducing FPP availability for prenylation of some oncogenes (e.g., *ras* prenylation as a step in relocation to cell membranes for function as signal-transducing agent).

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