

## Squalene: a multi-task link in the crossroads of cancer and aging

Alvaro L. Ronco<sup>1,2</sup> and Eduardo De Stéfani<sup>3</sup>

<sup>1</sup>Department of Epidemiology and Scientific Methods, IUCLAEH School of Medicine, Prado and Salt Lake p.16, Maldonado 20100, Uruguay; <sup>2</sup>Unit of Oncology and Radiotherapy, Pereira Rossell Women's Hospital, Bvar. Artigas 1550, Montevideo 11300, Uruguay; <sup>3</sup>Epidemiology Group, Department of Pathology, Clinicas Hospital, Av. Italia s/n, Montevideo 11300, Uruguay

**Correspondent author:** Alvaro Ronco, MD, <sup>1</sup>Department of Epidemiology and Scientific Methods, IUCLAEH School of Medicine, Prado and Salt Lake p.16, Maldonado 20100, Uruguay

Submission date: September 5, 2013; Acceptance date: December 14, 2013; Publication date: December 20, 2013

### ABSTRACT

Since its discovery in the beginning of the XXth century, squalene has been recognized as an important link in metabolic pathways. More recently, it has been further recognized as an intermediate step in the biosynthesis of cholesterol. Its well known antioxidant capability, together with its ability to protect skin, improve the immune system, and modulate the lipid profile, confer a high potential to this natural substance, which is spread all across the body structure, though mainly in the epithelial tissues, and in particular the skin sebum. This review will focus mainly on its major properties, which are related to anticancer properties, the maintenance of the oxidation/antioxidation balance, and its antiaging capabilities. Although the substance was originally obtained from shark liver oil, it is currently possible to obtain useful amounts from vegetable sources like extra virgin olive oil, therefore avoiding the dependence on capturing the aforementioned animal species. Recognized as one of the key components of the Mediterranean dietary style, squalene is necessary to adequately manage oxygen and its derivatives in every cell of the body.

**Key words:** aging, antioxidants, cancer, cholesterol, diet, olive oil, squalene

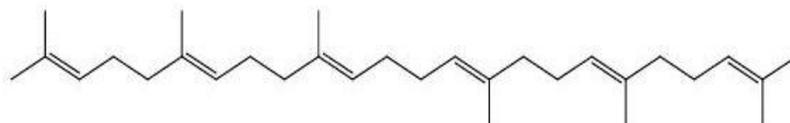
### Sources and structure

Inhabitants of the Japanese Peninsula of Izu used to drink an oil that was locally named "Samedawa," which means "cure all". In 1916, Dr. Mitsumaru Tsujimoto, a Japanese industrial engineer, described ten years after his initial discovery that Samedawa contained extremely large quantities of an unsaturated hydrocarbon [1]. He named the hydrocarbon squalene, due to its presence in the liver oil of sharks, from the Latin root "squalus" (shark).

Ten years later, its structure was established as a dihydrotriterpene, and the authors posited that it might be an intermediary in the biosynthesis of steroids [2].

In 1934, Robinson proposed a direct cyclization of squalene to the steroid molecule [3]. In 1936, Nobel laureate researcher Paul Karrer described the biochemical structure of squalene for the first time. He was already recognized for his discoveries on the chemical structures of Vitamins A and E [4].

The biochemical structure of squalene is  $C_{30}H_{50}$  ( $C_{30}:6n-\Omega_2$ ), a 30-carbon compound (polyprenyl, holding 6 prenyl groups, better known as isoprenoid or isoprene) (Figure 1).



**Figure 1.** Chemical structure of squalene

Due to a double bond structure of six  $CH_3$  groups, the isoprenoid has a strong natural antioxidative effect. Squalene has a similar structure to other isoprenoids as  $\beta$ -carotene, lycopene, vitamin A, vitamin E, and coenzyme Q10 (ubiquinone) [5].

Until today, shark liver oil is considered the richest source of squalene, with squalene accounting for at least 40% of its weight. It is also widely distributed in nature, in lesser proportions in amaranth oil (6-9%) [6], in wheat germ oil, and in olive oil (usually from 0.4% up to 1% in extra virgin olive oil) [7]. Evidence suggests that 60 to 80% of dietary squalene is absorbed from dietary intake [8,9]. Squalene was first found in the human body in the 1950's, when the metabolism of cholesterol was first identified [10]. Not long ago, knowledge spread that squalene is one of our body's own essential substances, which is present in small quantities in the skin and blood: the body synthesizes an average amount of 1.5 g/day [11]. Although the skin of primates has virtually no squalene, 12% of overall bodily squalene content in humans is in the sebum secreted by the skin. Furthermore, the daily secretion of squalene from the total skin surface ranges from 125 up to 475 mg/day [12].

### **Actions on the lipid profile: the best known history**

Squalene is an intermediate step in the biosynthesis of cholesterol and all steroid hormones. It is an inhibitor of the 3-hydroxy-3-methylglutaryl Coenzyme A (HMG-CoA) reductase activity and increases the activity of acyl CoA cholesterol acyltransferase [12]. In fact, the endogenous synthesis of squalene begins with the production of HMG-CoA. The initial reduction of HMG-CoA results in the formation of mevalonate [13]. Cholesterol is synthesized in a complex series of biochemical steps, known as the mevalonate pathway. After some biochemical reactions, featured by the addition of prenyl groups, the molecules of the intermediate group farnesyldiphosphate are gathered and reduced, resulting in the formation of squalene. After its biosynthesis, squalene can be transported to other regions of the body to be incorporated in tissues, or to be metabolized, resulting in the eventual synthesis of cholesterol and its steroid metabolites [14].

The presence of squalene in the cells affects the HMG-CoA reductase synthesis rate, which in turn affects the whole synthesis of isoprenoids and cholesterol. This offers possibilities for the prevention and treatment of high levels of "bad cholesterol," a risk factor for cardiovascular disease. However, "bad cholesterol" is not the only factor in

cardiovascular disease risk: when “bad cholesterol” is attacked by free radicals, it becomes an even more harmful substance, oxidized cholesterol (oxLDL), which is known as the worst cholesterol [15].

### **Squalene’s workplace: the cell membranes**

Currently, squalene protects the cellular and the cytoplasmatic organoids’ biomembranes from oxidative stress, as well contributing to maintaining order in the cholesterol metabolism and keeping harmful LDL-cholesterol at minimal levels [16]. Biomembranes are themselves highly vulnerable to the harm caused by free radicals, especially in the hydrophobic band between the two lipidic layers. Squalene is outstanding because of its great capability to receive or donate electrons without suffering a molecular alteration. Such stable and effective antioxidant configuration is given by its six isoprenoid units. Squalene can have two different forms: cyclic and noncyclic. The noncyclic form is entirely hydrophobic and is not engaged to the biological membrane. It is attracted by the band between both lipidic layers, the intermembrane. It accumulates there and performs its antioxidant task, capturing free radicals [5].

Certain experimental models propose that hydrocarbons such as squalene, specifically located in the middle of the lipid bilayer, inhibit proton leaks, which are events that consume cellular energy in an unproductive way [17]. In particular, polyunsaturated fatty acids (including those in foods containing lipids) are prone to suffering from oxidation, and this plays an important role concerning the integrity of biological membrane. The capability of squalene to protect the linoleic, linolenic, docosahexaenoic, and eicosapentaenoic acids against temperature-dependent self-oxidation is outstanding [18].

A relevant point related to cholesterol synthesis and antioxidant actions is the cyclization of squalene to lanosterol, already described in the 50’s [19], and dependent on the oxygen catchment by squalene [20] –in fact, it is oxydosqualene. Under certain given conditions, the noncyclic squalene loses its stability and curls itself, becoming cyclic and losing an important part of its antioxidant potential. It instead becomes the steroid nucleus. The molecules of cyclic squalene are known as hopanoids. It is not exactly known how this transformation takes place, but the noncyclical molecule enters into a cavity of a protein complex, it is divided into its six isoprenoids and then further reassembled into a new configuration. This is mandatory for being able to become the nucleus of cholesterol and other sterols [5].

In order to stabilize, squalene also attaches hydrogen ions from water and acids in the body, and in the process, releases oxygen to the body by the following reaction:  $C_{30}H_{50}$  (squalene) + 6  $H_2O$  (water)  $\rightarrow$   $C_{30}H_{62}$  (squalane) + 3  $O_2$  (oxygen) [20].

Squalene facilitates oxygen to reach the cellular level, causing further improvement in organ function through aerobic metabolism, which prevents acidotic cell syndrome, where cells become acidic, deteriorate, and die due to hypoxia [5].

### **Actions as anticancer agent**

Extra virgin olive oil contains small amounts of bioactive substances, which usually do not exceed 2% of its weight. Nevertheless, in spite of their small amount, these substances have a large series of properties which are beneficial. Among them, the anticarcinogenic and antioxidant capabilities are outstanding. These minor components include polyphenols within a range of 100-500 mg/kg according to different authors [21]. The major part is formed by

alcohols (tyrosol, hydroxytyrosol), but there are also other compounds such as flavonoids, lignans, and triterpenes. In regards to triterpenes, squalene is remarkable. A series of reviews have accepted that minor components of olive oil are potentially anticarcinogenic [22].

We recently performed a review of the relevant literature in Pubmed (5/July/2013) with the term “squalene” producing 3281 results, which have reached from the year 1926 up through 2013, most of which proceed from basic research. When the search added the term “cancer,” results were restricted to 203, with the oldest one being from 1956, mainly involving studies linking squalene to the protection against skin cancer. Undoubtedly, the links between squalene and cancer are more recent than most of the previous studies, which relate this substance with cholesterol, steroid molecules, and cardiovascular and metabolic areas.

We have mentioned that squalene is chemically an isoprenoid, sharing that structure with carotenoids like lycopene, with vitamins A and E, and also with other endogenous antioxidants as glutathione, superoxide dismutase, and ubiquinone (coenzyme Q10). All of these are powerful antioxidants. The previous paragraphs have remarked the extreme vulnerability of biomembranes to oxidative harm due to oxygen free radicals, especially in the hydrophobic band between the lipid bilayer. Squalene, usually located in this cellular zone, has an outstanding antioxidant capability recognized because of its highly stable structure. This capability is of major importance concerning the protective action against cancer (mainly thinking about reducing DNA damage), which has been remarked by the scientific literature.

The literature also recognizes in squalene an optimizing factor for the immune system. This has been observed in macrophages as well as in lymphocytes. Five decades ago, a published paper showed that squalene stimulates macrophages [23]. The evidence suggests that the biomembrane of immune cells is protected by squalene during phagocytosis, which makes them resistant to oxidative stress elements that such cells receive as an attack. The synthesis and consumption of squalene might be increased in those moments, like other antioxidants such as glutathione do. It has been found that two squalene precursors (geranyl and farnesyl) influence the synthesis and secretion of cytokines, which regulate the immune response. Interestingly, squalene precursors also regulate cellular growth and proliferation.

*Ras* is the most common family of oncogenes, found in 30% of cancers. Cancer cells activate the *ras* gene, which in turn synthesizes *ras* proteins and performs *ras* functions. The oncogenic *ras* protein is in a continuous activation state, leading to an uncontrolled cell division in the absence of growth signals [24]. The activation of these proteins depends on the isoprenoid metabolism in the neoplastic cells. Through isoprenylation, farnesylpyrophosphate fixes the *ras* protein to the cell membrane, and it also fixes some other important proteins to the cell membrane or to the nuclear cover. Without this vital step, the *ras* oncogene finds severe limitations for its activity [25]. Regarding the cell cycle, a G1 phase moving towards S phase requires two products of the mevalonate pathway for the protein isoprenylation: geranyl- and farnesylpyrophosphate [26,27]. Research results support the idea that inhibition of lipid metabolic pathways may be valuable as a possible molecular target for collaborating in the treatment of some cancers, e.g. lung [28], breast [29], esophagus [30], and sarcomas [31].

Farnesylpyrophosphate is of interest, because of its place in the isoprenoid synthesis pathway and its dependence on the HMG-CoA reductase. The presence of exogenous squalene establishes a negative feedback by inhibition of HMG-CoA reductase, then reducing

the farnesylpyrophosphate synthesis, disrupting the synthesis of mevalonate pathway and inhibiting the protein isoprenylation. The latter is even more important in tumor cells than in normal ones, especially when the *ras* oncogene is hyperactivated [32]. As a consequence, its disruption is deleterious for the cancer cell. If squalene achieves a stop in the G1 phase of this cell, it prevents cellular growth and proliferation [5]. A daily intake of squalene can inhibit the isoprenoid production in neoplastic cells and present an obstacle for their growth and development. Some authors have found this to be a possible explanation for the anticancer properties of olive oil [25].

The presence of ROS (reactive oxygen species) is involved in several biological facts which typically take place in the initiation and progression of cancer, according to the hypothesis of persistent oxidative stress in cancer [33]: a) they can originate a persistent activation of transcription factors as Nuclear Factor kappa B (NFκB); b) they can induce the expression of proto-oncogenes such as c-fos, c-jun and c-myc; c) oxidative stress can result in tumor cells developing resistance to chemotherapy; d) reactive oxygen can damage certain proteins and enzymes, including protease inhibitors, probably facilitating tumor invasion and metastases.

Experimental research has shown a protective activity of olive oil against chemically induced carcinogenesis in the breast gland [34], colon [35], skin [36], and liver [37]. In particular, topical application of squalene showed anticarcinogenic activity [38,39]. Concerning the involved mechanisms, researchers have found that minor compounds have an impact on cell death and proliferation. Besides this, most nutrigenomic studies of olive oil were conducted in cancer research [40-44].

A dietary enrichment with the unsaponifiable fraction of olive oil reduces the damage of acute colitis, alleviating the oxidative events and returning the expression of proinflammatory proteins to basal levels, probably through inhibition of signaling pathways of p38, Mitogen-activated protein kinase (MAPK), Cyclo Oxygenase-2 (COX-2), Nitric oxide synthase (iNOS), NFκB, and by enhancement of the Peroxisome Proliferator-Activated Receptor-gamma (PPAR-γ) pathway [45,46]. Since this disease is a potential risk factor for colorectal cancer, the increase of unsaponifiable fraction could contribute to the prevention of such a tumor.

Two leading scientists in the field of nutrition and cancer, Theresa Smith and Harold Newmark, have suggested that the protective effect of extra virgin olive oil could be due to its squalene content [8, 25], a concept based on a considerable amount of experimental studies in animals. Most of these studies investigated the effect of squalene when topically applied or in a systemic way on chemically induced cancers of skin, colon, or lungs in rodents. Rao et al. supported Newmark's hypothesis, also suggesting that squalene, as a component of olive oil, could be partially responsible for the chemopreventive effect of the oil [47]. Considered together, these results clearly show that dietary squalene has different anticarcinogenic effects [38, 47-50].

In 1999, D.A. Hertzler et al. cited a review which [51] proposed that squalene had the potential to be part of a chemotherapy regimen for human pancreatic cancer.

Squalene is capable of inhibiting aberrant hyperproliferation, a cellular marker associated with preneoplastic transformation [52]. Other authors have investigated the effect of squalene on antitumoral activity and immune response of the host in female rodents bearing sarcoma 180. The intraperitoneal administration of squalene was seen to enhance the

function of the reticuloendothelial system, in particular antibodies IgM, and a longer survival was observed in the animals [53].

The administration of squalene at 2% in the diet of rodents 14 days before and 30 days after a lethal total body irradiation with  $\gamma$ -rays, was followed by a cellular and systemic radioprotection. White blood cell count was consistently higher among the animals treated with squalene. This treatment was associated with a significant extension of survival time compared to the control group [54]. Beside this, the genotoxicity of doxorubicin has been reduced through administration of complementary squalene in rats [55]. The protection of bone marrow against the action of some antineoplastic drugs has been also documented [56]. Furthermore, this author's results suggest that squalene has a cytoprotective effect in vitro on pluripotential hematopoietic cells derived from bone marrow but does not protect cell lines of neuroblastoma from the toxicity induced by cisplatin [57]. According to this study, squalene would have a *selective* cytoprotective activity in normal cells against toxicity produced by chemotherapy, without protecting tumor cells.

Although the evidence supporting the use of squalene in combination with chemotherapy agents against cancer in humans is somehow reduced, such evidence suggests that squalene can potentiate the cytotoxic activity of some of those agents. The effect of squalene combined with ACNU (a nitrosourea) in a murine tumoral model, at doses of 4.2 g/kg has shown capabilities of potentiating the effect of ACNU against lymphoid leukemia. The authors reported a longer survival of some animals, without toxicity [48]. Survival in cultured cell lines indicates that squalene also increased cytotoxicity and antitumoral activity of adriamycin, cyclophosphamide, 5-fluoruracil, bleomycin, and cisplatin. The strongest potentiation effect was seen in bleomycin [58]. Basic research about squalene in breast cancer cell lines was recently carried out, emphasizing the increase in the cytotoxicity of adriamycin as well as an increase of antioxidant activity for normal cells [24]. Myelo- and nephrotoxicity due to agents like cisplatin and cyclophosphamide take place because of the generation of free radicals or because of the enhancement of oxidative metabolism. It is there that squalene is able to minimize certain tissue damages, in addition to potentiating cytotoxicity in cancerous cells [59]. We can recognize, therefore, that there seem to be significant powerful antioxidant and cytoprotective effects in squalene.

The convenience of combining the nutritional-pharmacologic effects in which squalene participates had already been suggested some years ago [60], regarding a combination of olive oil together with sulindac (as a COX-2 inhibitor), which represents an advantage considering the practical feasibility of this combination.

In a recent publication on nutrition and breast cancer [61], we have emphasized the potentially high usefulness of squalene as supplement with a double purpose: On one hand, collaborating with a nutritional style for the prevention of the disease; on the other hand, collaborating with different oncospecific therapies that patients must usually receive.

From surgery through radiotherapy and also chemotherapy, there is a space for coadjuvance with squalene in the treatment. Tumor cells live better in environments reached by small amount of oxygen (anaerobic ones), and oxygenation is harmful for them: this is obviously well known by specialists. A sustained acidosis is a potential upstream and indirect trigger in a multifactorial cascade of molecular events associated with carcinogenesis. The extracellular space of most tumors is mildly acidic, due to an exuberant production of lactic acid. There is growing evidence that extracellular acidity per se boosts the invasiveness and metastatic capacity of cancer cells [62]; moreover, this acidity renders cancer cells relatively

resistant to the high proportion of chemotherapeutic drugs that are mildly basic, and may impede immune rejection of tumors [63]. If the whole body is better oxygenated, the cytotoxicity of radio- and chemotherapy on malignant cells will be enhanced, and the oxygen will contribute to the protection of healthy cells (which are the majority). The quoted ability of squalene to neutralize a proton excess derived from metabolism could be somehow associated to the alkalizing action of fruits and vegetables, which has scientific support [64].

Squalene appears to have twice the capability of wiping the harmful oxygen and distributing the useful oxygen. Hence, there is an opportunity to profit from such potentiality without the risks of a pharmaceutical drug.

As an adjuvant therapy for cancer in humans, the information about the optimal doses is not available either. Anyway, if liver steatosis emerges as a consequence of some state-of-the-art cancer therapy, squalene appears as a helpful tool with the ability to normalize the situation, since it contributes to hepatic detoxification. Some experimental researchers have reported that animals fed with squalene show an enhanced capacity to excrete toxins such as hexachlorobenzene or strychnine [65-67]. Squalene can attract xenobiotic products which are highly lipophilic, contributing then to their body elimination [68]. As an extrapolation from available animal data, a dose between 2-5 g/day seems to be the therapeutic window. The experience in the therapy of chronic lead toxicity in Uruguayan children has been very useful [69], profiting from the high detoxifying capability of squalene. The researchers employed doses up to 6.5 g/day without any signs of toxicity or intolerance. The aforementioned experience without adverse side effects could be useful as a reference for its clinical administration, also taking into account the different ratio dose (g)/body weight (kg) in children.

In conclusion, we could say that the epidemiological, experimental and clinical evidence about the anticancer properties of squalene are promising. This substance could have a place as an adjuvant together with specific oncologic therapies. Furthermore, in the light of the existing evidence, we do not find any strong arguments against the intake of squalene among cancer patients within a range of 2-6 g/day.

### **Actions as an antiaging agent**

According to the World Health Organization, aging, or senescence, is “the series of morphologic and physiologic changes which appear as a consequence of the action of time on living beings, which supposes a reduction of adaptive capabilities in each one of the organs and systems, as well as in the capability of response to harmful agents with incidence in the individual” [70].

Aging is a complex process which is expressed in an organism at several levels: genetic, molecular, cellular, in organs, and in systems. Albeit, the fundamental mechanisms are still not well known, there is increasing evidence pointing to the “reactive species” of oxygen and nitrogen, as one of the primary determinants of aging. The theory of oxidative stress states that a progressive and irreversible accumulation of oxidative damage caused by reactive species impacts critical aspects of the aging process, contributing to damaged physiological functions, a higher incidence of diseases, and a reduction in life extension [71].

Research has generated very strong data which supports the theory of oxidative stress. Nevertheless, a direct cause-effect relationship between the accumulation of damage due to oxidation and aging has not been clearly established [72]. The oxidative damage of cellular and tissular components is located among the primary or secondary factors in the aging

process as well as in different human diseases (e.g. cardiovascular, cancer, diabetes, neuromuscular disfunctions, and degenerative processes) [73].

Oxidative stress refers to a serious imbalance between the production of reactive species and antioxidant defenses. The degree of stress is determined by the quoted balance or imbalance level. Regardless of how or where reactive species are generated, an increase of intracellular concentrations of oxidants has two important and potential effects: a) several cellular components are damaged [74,75]; and b) the activation of specific signaling pathways is triggered [74-76].

Both effects can influence numerous cellular processes related to aging and the development of diseases related to age. Oxidative stress status is under a strong regulatory control for most people, based on a wide range of lifestyle variables, which include diet and physical activity.

It has been shown that a high level of oxidative stress in humans could be reduced to a normal level through antioxidant supplements [73]. This fact has clinical implications but also gives another important message: a daily dietary which is simply rich in natural antioxidants could prevent and defend our organism against the incidence of diseases which are age-associated.

A Mediterranean-type diet was already observed in the 90's as good for human health, reducing the incidence of coronary diseases [77] and certain types of cancer [78]. Because of its abundant foods which were rich in vitamins, antioxidants, and monounsaturated fatty acids—all of them being potentially active in the protection against diseases associated to age--the Mediterranean diet was recognized as a “functional diet” which protects human health [79,80]. Experimental studies carried out in the past decade in human volunteers [81-84] showed that some of the minor components of olive oil (polyphenols, squalene) contributed significantly towards its benefits on health. After a meal rich in fats, an oxidative stress takes places and triggers inflammation, endothelial dysfunction, hypercoagulability, and a cascade of other atherogenic changes [85]. However, the intake of fatty foods with adequate sources of antioxidants, like a Mediterranean-type meal, can minimize the postprandial oxidative stress [86].

In this review we have already mentioned that squalene works as a powerful scavenger of oxygen free radicals, hence inhibiting the lipoperoxidation induced e.g. by UV radiations [87]. Besides this, its capability for the inhibition of HMG-CoA reductase stops cholesterol biosynthesis while it facilitates its excretion through bile [88]. In addition, squalene generates a modulation on the action of immune system on the arteries, something fundamental in order to stop atherogenesis [89,90]. This has also been recognized for other minor components of olive oil [91,92]. The arterial protection determined by these facts, together with the recognized antioxidant capability of dietary squalene, somehow represents an antiaging action, since arterial age is closely associated to its level of atherosclerosis. Aging makes the maintenance of the oxidation/antioxidation balance even more difficult, because the body's own antioxidant synthesis—in the same way the one of other important nutrients—declines with the advance of age.

It is interesting to remark that the interventions with components rich in antioxidants at dietary doses in healthy volunteers exert only marginal effects. In addition, detecting those effects could be difficult due to the current status of oxidative biomarkers [93]. In fact, the protective effect of minor components of olive oil on the oxidative damage in humans was better displayed in participants with a compromised oxidative status (men having a low-

antioxidant diet, postmenopausal women) or in patients with a high status of oxidative stress (hyperlipidemic, coronary disease, hypercholesterolemic, etc).

The same facts were observed by us in epidemiological studies related to nutrition and cancer [94,95]. For example, the increase of intake of  $\Omega$ -3 fatty acids through diet as well as through supplementation (and the same in the case of vegetables and fruits intake) suggest that there would not be a protective effect (or perhaps it might be strongly reduced) among those who have higher intake of them. On the contrary, when their negative risk association (protective effect) was stronger, the higher was the deficit prior to diagnosis. Observations suggest that when the oxidant/antioxidant status is adequate, it cannot be significantly improved by the addition of antioxidant foods. Conversely, the potentially beneficial effect of the latter is higher, as higher is the previous imbalance (oxidative stress).

Olive oil, as the complex food it is, can have a series of direct and indirect effects on genetic expression. The direct effect is the interaction with a series of transcription factors which are responsible for the positive-negative regulation of genic expression. The indirect effects refer to the interaction related to the metabolism on the cascades of cellular signaling which modify the genic transcription [96-98]. Several genes have been identified in experimental animal models as responders to the intake of olive oil [99].

### Concluding remarks

Squalene, an unsaturated compound, appears to contribute to the formation of useful oxygen when combined with water, and also to the efficient transportation of that useful oxygen to the whole body, helping in that way to maximize the catchment of such element. Having that capability, the likelihood of generating underoxygenated microenvironments which produce metabolic acidosis using anaerobic metabolism is reduced. The main problem derived from those anaerobic environments is that they facilitate the development of degenerative pathologies and cancer, among other alterations. These concepts are equivalent to saying that an adequate intake of squalene is preventive of cell aging, since it adequately maintains the nutritional levels of the requested oxygen.

The present review intended to briefly outline and discuss some important roles that squalene plays in the human body. It can be distinguished by the common property of antioxidant and oxygen administrator and it could be considered as a biological response modifier, regarding oxygen, the immune system and steroid synthesis. The impact on oxygen behavior also determines a control on inflammation processes, tightly linked to the development of several prevalent chronic diseases as cancer. If all these were not even considered enough to accept squalene as an outstanding substance, its detoxifying capability against xenobiotic products is another advantage as regards its performance. In view of the high concentration differences of squalene between shark liver oil ( $\geq 60\%$ ) and olive oil ( $\leq 1\%$ ), and also taking into account the ecological impact of capturing marine species, it would be desirable to have higher squalene concentrations in vegetable sources like olive oil (in which it is also enhanced by the action of other minor components), as an eco-friendly alternative.

According to the scientific literature and to our knowledge, no specific medical syndrome due to squalene deficiency has been described. In the opinion of the authors, this fact does not preclude us to think of squalene as essential for life and, furthermore, to consider it a *must* of healthy nutrition in modern times.

**List of abbreviations:** ACNU: amino methyl nitrosourea hydrochloride; COX : cyclooxygenase; HMG-CoA : 3-hydroxy-3-methylglutaryl Coenzyme A; iNOS : nitric oxide synthase; LDL : low-density lipoprotein; MAPK : mitogen-activated protein kinase; PPAR- $\gamma$ : peroxisome proliferator-activated receptor-gamma

#### Authors' contributions

A.L.R. designed the manuscript, performed the bibliographic research and wrote most of the text. E.D.S. made technical suggestions and reviews of the text.

#### Acknowledgments

The authors wish to thank Dr. Magdalena De Stéfani for her technical review on the English style and grammar of the present paper.

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