NORBERT LATRUFFE - ALLAN LANÇON

RESVERATROL & OPHTHALMOLOGY

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Norbert LATRUFFE, PhD in 1977, and appointed in 1989 is currently full Professor in Biochemistry at the University of Burgundy, where he installed and headed the Laboratory of Molecular and Cellular Biology until 2006. Then he was in charge of the team of Biochemistry of Metabolism and Nutrition in the INSERM reserarch center, UMR 866 of Dijon until 2011. He undergraduated at the University of Besançon, graduated at the University of Lyon I. Then he became interested on the following research topics: - energetic metabolism of lipids (UA CNRS 531, Besançon) ; - phospholipid-dependent membrane enzymes (Post-doc at Vanderbilt University, Nashville TN, USA) ; - collaboration as visitor in different foreign universities (Stockholm, Bern, Himeji…) ; - and on the toxicology of peroxisome proliferators when he arrived in Dijon. Starting 1998, he lunched a new challenge on the preventing role of resveratrol, a well known phytophenol, against age-related pathologies: cancer, inflammation cardiovascular. With his collaborators, he was one of the first to explore resveratrol metabolism (2004), its pro-apoptotic properties (2004), and recently discovered a new resveratrol signaling pathway through micro RNA's modulation (2010). To date, Norbert LATRUFFE is the author of near 150 international papers and more than 110 lectures.

Norbert LATRUFFE is currently (or past) expert member of several national evaluation councils (CNRS, AFSSA, AERES, CNU…) and at international level (UE). He awarded several distinctions (Prize at the 16th Oncology and Molecular Medicine at Rhodes, laureat of the APICIL foundation and s.o.).

Allan LANÇON, obtained its PhD degree from the University of Burgundy in 2006 after a thesis on Resveratrol and its Human hepatic metabolism. He pursued his work from 2007 to 2012 by various Post-Doc positions in the INSERM research center, UMR 866. More than a pure scientist he is also a business man trained in 2009 to Management, Strategy and Commercial Negotiation, Finance and Accounting, at the French IFG-CNOF (Institute for Training in Management). His experience allowed him to take part in research and development of several nutritional supplements formulated with resveratrol: "one designed to fight against metabolic syndrome", "one intended to strengthen the antioxidant capacity of the body", "one to fight against type 2 diabetes and associated inflammation" and finally another "designed to limit joint inflammation in osteoarthritis".

HISTORY OF THE DISCOVERY OF THE RESVERATROL

The resveratrol or 3,4',5 trihydroxy-trans-stilbene is a natural polyphenol (Fig. 1) classified in the group of stilbenes.

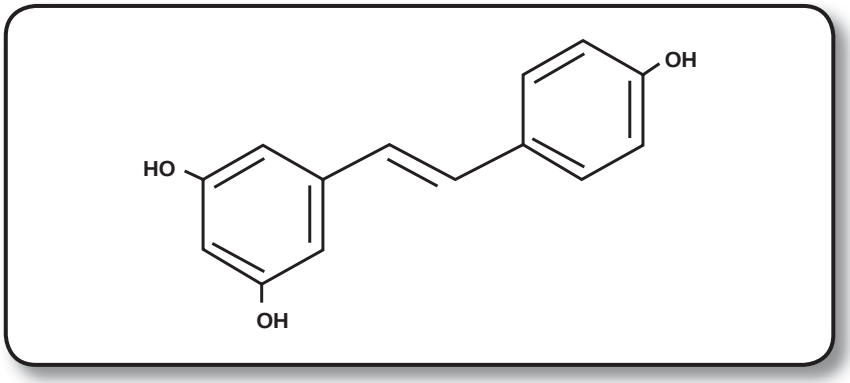

Figure 1 **Chemical structure of trans-resveratrol or 3,4',5 trihydroxy-trans-stilbene.**

Figure 2

Illustration showing the white hellebore *(Veratrum grandiflorum)*.

It was first identified in 1940 in the root of the white hellebore (Fig. 2) also called Varaire *(Veratrum grandiflorum)*, a species of plant which grows at 1000 m above sea level and higher in most mountain ranges in central and southern Europe, in the Caucus, in temperate Asia, in Siberia and as far away as Japan [1].

This is the plant which gave its name to "resveratrol". Indeed, the Latin prefix **"res"** means **"from"**, **"veratr"** is derived from *Veratrum* and finally the suffix **"-ol"** comes from the phenol functions of the molecule.

history

Later, in 1963, resveratrol was also found in large quantities in the root of the Japanese knotweed *(Polygonum cuspidatum)* (Fig. 3), used since 2000 BC in traditional Chinese (Hu-Chang) and Japanese (Ko-jo-kon) medicine [2]. The root of the *Polygonum* is currently the main source of natural resveratrol on the market.

Figure 3

Illustration and photograph of the Japanese knotweed *(Polygonum Cuspidatum)*.

In 1976, Langcake and Pryce discovered resveratrol in the vineyard. They described it as a phytoalexin, that means, a defense molecule the plant. Resveratrol is produced by the vine in response to biotic infections like that caused by the fungus *Botrytis cinerea* which is responsible for "gray decay" in grapes, a widespread disease well-known to winemakers. This phytoalexin is also synthesized by the vine during abiotic stresses such as UV irradiation or exposure to ozone [3].

Today, we know that resveratrol is also produced by a wide variety of plants which are edible or not by humans. It is found in trees such as the butterfly tree or pine, in legumes such as rhubarb, in peanuts as well as in a number of berries: grapes, blackberries, blackcurrants, blueberries and cranberries.

Nevertheless, it is in the grape and more precisely in grape skin that the concentration of resveratrol is the highest.

Thus, in the production of red wine, the fermentation of the grape increases the alcohol content, leading to the efficient extraction of polyphenols and resveratrol from the skin of the fruit. As the skins are not included in the fermentation of the juice of the fruit during the vinification of white wines, it is easy to understand that the final content of resveratrol is very low compared to that of red wines (Table 1).

In the present state of our knowledge, red wine is the most concentrated food source of resveratrol found in the diet of man.

Table I

Examples of trans-resveratrol concentrations in different food sources (Those can vary according to the origins of the vegetable sources).

Sources	Trans-resveratrol (µg/g)	References
Hop	0.5	[7]
Peanuts	5.1	[8]
Peanut butter	0.3	[8]
Grape skin	27.5	[9]
Ko-Jo-Kon	523	[8]
Blueberries	0.03	[10]
Beverages	**Trans-resveratrol (mg/L)**	**References**
Pinot noir (red)	10.6	[8]
Gamay (red)	28.3	[11]
Regent (red)	10.0	[11]
Gamay (rosé)	4.8	[11]
Chardonnay (white)	0.43	[11]
Chasselas (white)	0.86	[11]
Ko-Jo-Kon (infusion)	0.68	[8]

CHRONOLOGY OF MAJOR SCIENTIFIC DISCOVERIES

Resveratrol and the prevention of cardiovascular disease

The history of resveratrol, in terms of the interest it has generated in the scientific and medical communities, began in the 1990s. It was first associated with the phenomenon known as the "French Paradox", which links wine consumption to reduced cardiovascular risk (Fig. 4).

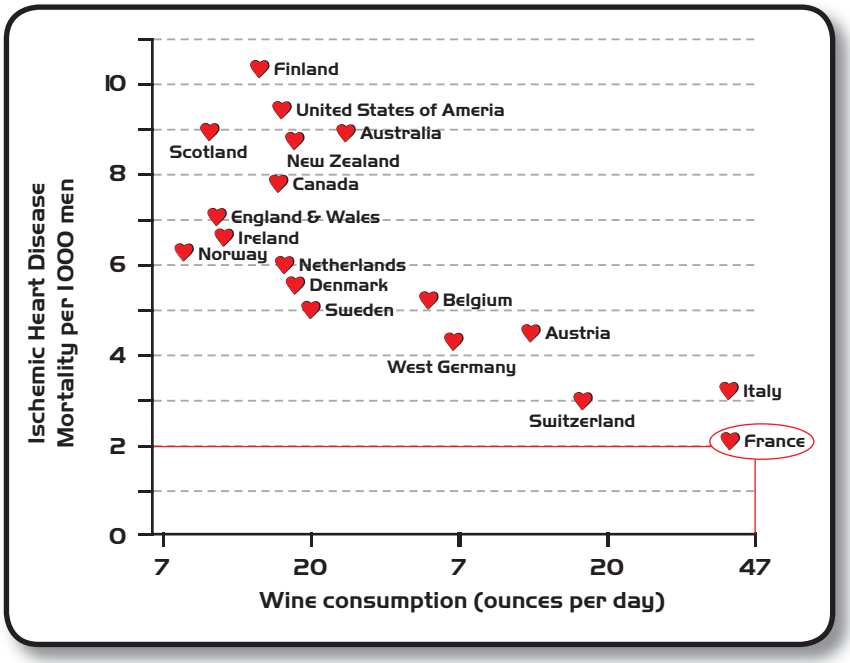

Figure 4

Wine consumption effect on Ischemic Heart Disease mortality (9).

(From Goldberg DM, Clinica Chimica Acta. 1995).

Indeed, it was shown that the situation in France is paradoxical in the sense that, despite a high consumption of saturated fat, the population boasts a low mortality from cardiovascular diseases.

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It was shown that this paradox could be partly attributed to the high consumption of wine, capable of inhibiting platelet adhesion and promoting vasorelaxation. Resveratrol thus became a potential candidate among the other polyphenols to explain the beneficial effects of wine. It has thus aroused great interest in many scientific studies to date. Such studies show, for example, that resveratrol is anti-atherogenic, that it reduces the levels of "bad cholesterol" (LDL) while increasing the levels of "good cholesterol" (HDL), that it reduces oxidative stress, it improves glucose homeostasis, it limits platelet adhesion, it promotes coronary vasorelaxation and even that it reduces arrhythmia [10] (Fig. 5).

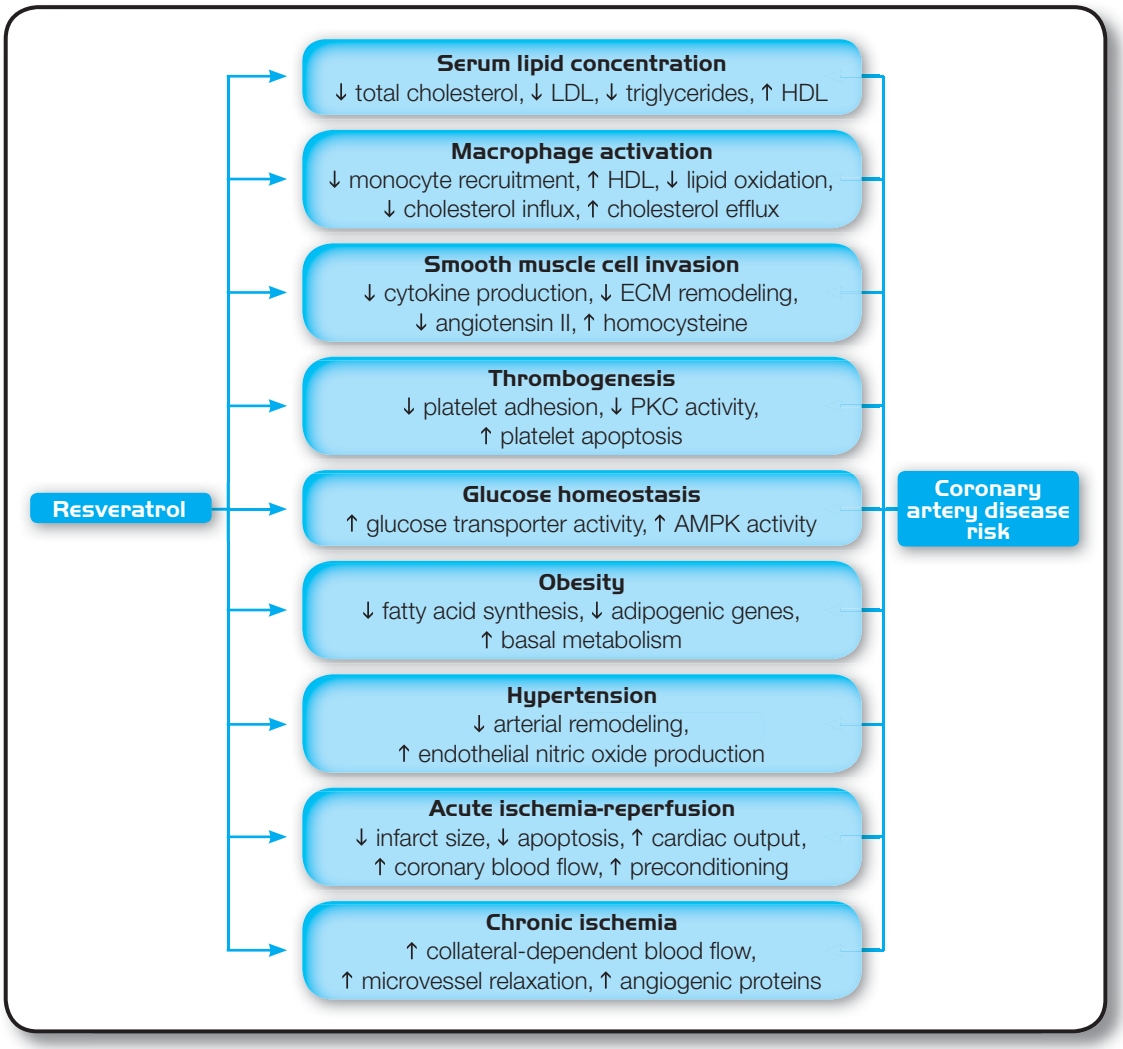

Figure 5

The many effects of resveratrol on coronary artery disease risk [11].

(From Chu LM *et al.*, Current atherosclerosis reports. 2011).

Anti-cancer potential of resveratrol

The year 1997 marked a turning point for resveratrol, which at that moment became a molecule extending new hope in the search for anticancer drugs. Indeed, Jang et al. published a study in the prestigious journal "Science" focusing for the first time on the chemopreventive potential of resveratrol in an *in vivo* model of carcinogenesis of the skin. The findings revealed that this polyphenol, when applied topically, was able to inhibit 98% of tumors [12]. From that time forward, although a very large proportion of publications relate to results obtained *in vitro*, hundreds of original articles have been published on the anticancer effects of resveratrol (for a recent review see [13]). It seems that the molecule is able to block the different stages of carcinogenesis, including initiation, promotion and progression (Fig. 6).

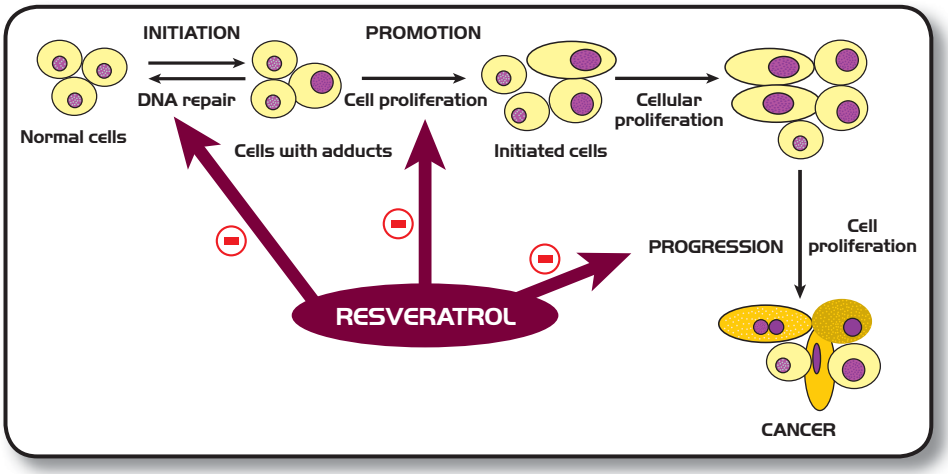

Figure 6

Inhibitory effects of resveratrol in the carcinogenesis process.

These effects have been demonstrated in mice and/or rats on numerous occasions and for various cancers such as skin, breast, digestive tract (stomach, liver and colon) and prostate cancer. If resveratrol penatrates easily in animal and human cells [14], its metabolism is nevertheless fast and it is eliminated in glucurono-or sulfo-conjugated form [15]. More recently and in a very interesting way, it was revealed that resveratrol could also sensitize cancerous tumors to chemotherapy [16], [17].

Neuroprotective effects of resveratrol

Since the early 2000s, several *in vitro* studies have advocated the neuroprotective use of resveratrol. It has indeed been shown capable of reducing the disastrous effects of ischemia /brain reperfusion in animal models [18, 19], a phenomenon leading to hypoxia/reoxygenation and oxidative shock in the surrounding tissue, eventually resulting in nerve cell death.

This polyphenol also showed that it could help in the fight against certain neurodegenerative diseases such as Alzheimer's and Parkinson's.

For example, it has been shown *in vitro* that resveratrol can both inhibit the formation of ß-amyloid peptides, neurotoxic molecules considered to be one of the causes of Alzheimer's, and promote their degradation via the proteasome. Furthermore, it appears that neuronal cell death induced by these ß-amyloid peptides is also limited by resveratrol [20].

In Parkinson's disease, resveratrol has been shown to limit the death of dopaminergic neurons induced by oxidative stress and inflammation by limiting the production of free radicals of oxygen (ROS) by NADPH oxidase and by inhibiting production of pro-inflammatory factors such as TNFα, IL-1ß and nitric oxide (NO) [21].

Finally, studies in *in vivo* models have shown that activation of the sirtuin SIRT1 and the induction of pathways that mimic "caloric restriction" seemed to be the cause of the protective effects of this polyphenol against such neurodegenerative diseases [22-24].

Effects of resveratrol on metabolic diseases

Following these numerous publications on resveratrol, other scientific areas have taken an interest in this small natural molecule. Thus, teams working on metabolic disorders have sought potential beneficial effects of resveratrol in the fight against type 2 diabetes and obesity, two conditions often triggered by a pre-existing "metabolic syndrome".

The metabolic syndrome represents the association of a series of metabolic malfunctions. This is not really a disease but rather a combination of risk factors. The syndrome is defined by the simultaneous presence of at least three of the following problems in the same person: hyperinsulinemia, hypercholesterolemia, hyperglycemia, hypertension and/or abdominal obesity. These are dysfunctions of the metabolism that can cause serious diseases such as type 2 diabetes, obesity and cardiac or cerebral vascular accidents.

Resveratrol has shown some interesting properties in the fight against this widespread syndrome. For example, in a recent (2011) study conducted on a rat model developing metabolic syndrome, continuous administration of resveratrol succeeded in preventing glucose intolerance and peripheral insulin resistance, thus avoiding the onset of diabetes [25].

Concerning more specifically type 2 diabetes, the effect of resveratrol on insulin sensitivity has been the subject of several studies using diabetic rat and mice models. Almost all experiments have shown a reduction in circulating insulin or an increased sensitivity to the hormone after an exposure period of 1 to 6 months.

Other studies have examined the effect of resveratrol on blood glucose in obese mice and rats or in whom diabetes was chemically induced. All these studies showed a reduction in blood glucose levels following treatment with resveratrol [26].

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Finally, a recent study published in August 2011 also confirms for the first time that resveratrol is able to improve insulin sensitivity in humans [27].

Concerning the problem of obesity, some studies have indicated that resveratrol consumption could limit weight gain caused by a high-fat diet in animal models exposed to high doses of the product (200 or 400mg/kg/day) [28, 29]. Low doses, up to 60 mg/kg/day showed no effect on the weight of the animals but nonetheless modulated lipid accumulation. Findings reported a reduction in the size of adipose tissue, associated with a reduction of abdominal obesity in obese rats [26].

Resveratrol and inflammation

Inflamation is the result of chemokines emission (still called interleukines), small chimiotactic proteins which mobilize the leucocytes by making them migrate from blood circulation towards the place of inflammation. These chemokines bind to their receptors at the membrane of monocytes to activate them in macrophages in order to eliminate pathogens or damaged cells and tissues. These events are generally associated with pain. One way to reduce inflammation is to inhibit the production of these chemokines. Also, some studies reported analgesic [30] and anti-inflammatory properties of resveratrol [31]. Moreover, it has been recently shown for the first time that resveratrol inhibited the expression of proinflammatory genes in human monocytes by stimulating the synthesis of anti-inflammatory microRNAs [32].

These data show that some opportunities exist for medical indications based on natural polyphenols like resveratrol by substituting, at least in part, steroidal or non-steroidal anti-inflammatory drugs to alleviate pain.

Resveratrol and aging

Resveratrol thus seems to be a protective molecule in many ways. Protection against harmful phenomena (oxidation, chronic inflammation) and protection against multiple diseases are the examples we have reported in the preceding lines. There is nevertheless still another surprising bioactivity in this polyphenol that goes beyond mere protection: the lengthening of lifespan [33].

This exceptional and unexpected finding was first reported on a simple single-cell model, the yeast *Saccharomyces cerevisiae*, with a gain of 70% in lifespan.

This observation was subsequently confirmed and extended to other, more complex organisms: the worm *Caenorhabditis elegans*, the fly *Drosophila melanogaster,* and the fish *Nothobranchius furzeri* (Fig. 7).

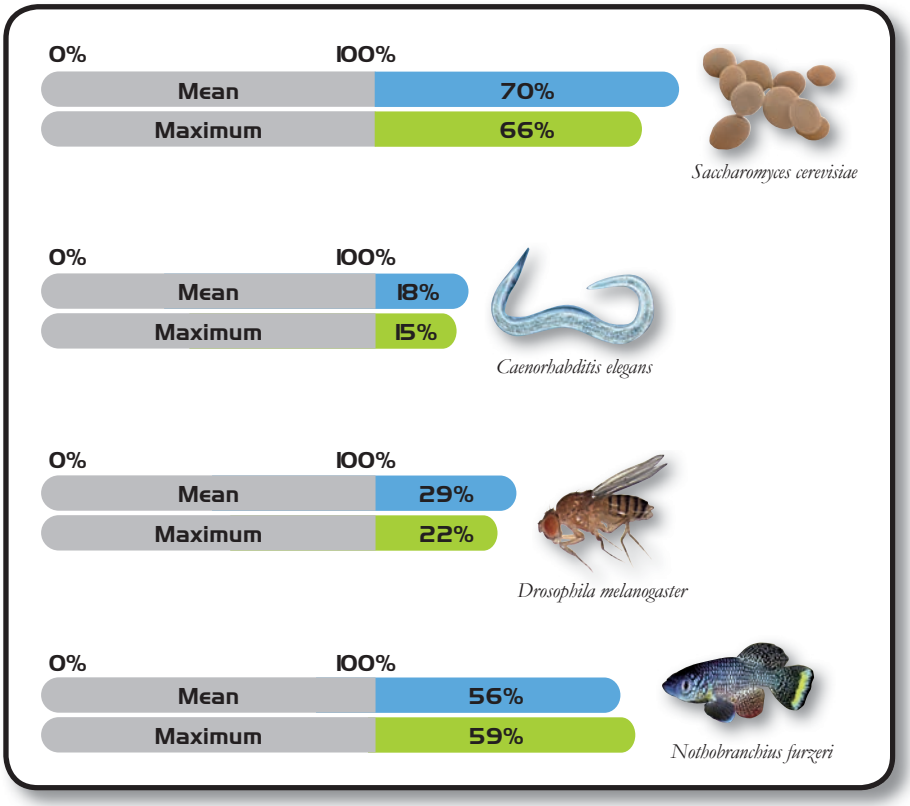

Figure 7

Resveratrol's effect on lifespan extention.

(From Sinclair DA *et al.*, Nature Reviews Drug Discovery, 2006)

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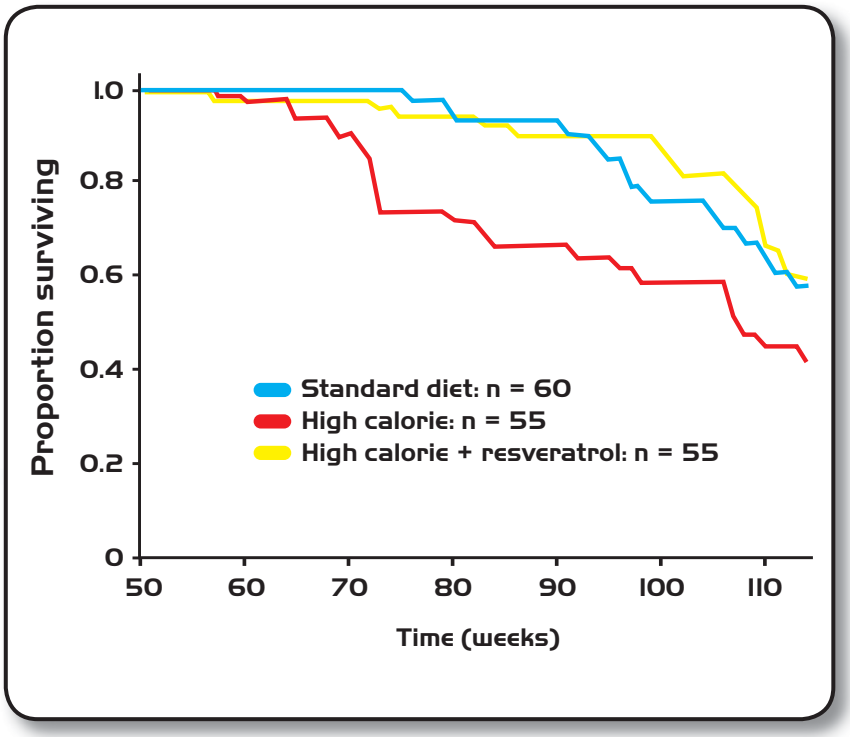
Although mammals such as mice seem little or not at all sensitive to this effect of resveratrol, the molecule has nonetheless been shown to extend the life of sick animals. A study conducted in 2006 reported that mice dying "young" because rendered obese by a high-calorie diet, regained a normal lifespan by consuming resveratrol [34], despite their morbid obesity (Fig. 8).

Figure 8

Lifespan extention effect on obese mice.

(From Joseph A. Baur *et al.*, 2006).



This particular effect of resveratrol is mediated in part by the activation of Sirtuin 1 (SIRT1), an enzyme activated during caloric restriction and also known to increase lifespan.

RESVERATROL AND OPHTHALMOLOGY

By acting on a wide range of organs or tissues, resveratrol is at the origin of many beneficial effects in animals and humans. But what about the eye?

In what follows, we will discuss, in the light of recent scientific articles, the effects of resveratrol on the phenomena involved in eye diseases by focusing on the antioxidant, anti-inflammatory and anti-angiogenic effects of the molecule.

Antioxidant effect of resveratrol

Age-related macular degeneration (AMD) is one of the main causes of the deterioration of vision in adults in developed countries. Although its exact pathogenesis is not completely clear, most studies indicate an early role of the retinal pigment epithelium (RPE) in the onset of the disease, with smoking as the most important environmental risk factor. RPE cells are involved in phagocytosis and the degradation of photoreceptor outer segments, a phenomenon which necessitates large amounts of energy and oxygen. As a result, the tissue undergoes a continuously high level of oxidative attack which stresses the epithelial cells and which can lead to their death by apoptosis. This is the early stage of AMD whose initiation is strongly suspected to be favored by the ingestion of cigarette smoke [35]. Indeed, the products of tobacco combustion contain a high concentration of free radicals and toxic compounds such as benzopyrene or acrolein which increase oxidative damage.

Due to its recognized antioxidant activity, resveratrol has been used in some studies to determine its ability to protect cells of the retinal pigment epithelium from oxidation and thus estimate the potential value of this molecule as a preventive and curative treatment of AMD. Thus, in 2010, a team worked on the toxic effects of acrolein associated with hydrogen peroxide (H_2O_2), a powerful oxidant molecule. Results showed a beneficial effect of resveratrol in relatively low concentrations (10 μM) on the RPE cells [36], including a lifting of the inhibition of phagocytosis induced by acrolein and protection against the toxicity of the acrolein/H_2O_2 cocktail.

To complement these data, in August 2011, a team working on RPE cells in culture reported a protective effect of polyphenols against the cytotoxicity induced by hydrogen peroxide [37]. Used in pretreatment, resveratrol was able to induce a significant and dose-dependent increase in the enzyme activity of the antioxidant defense system: superoxide dismutase, glutathione peroxidase and catalase. In addition, resveratrol was able in normal or oxidative stress conditions, to increase the level of reduced glutathione, an antioxidant molecule naturally synthesized within the cells. Finally, an inhibition of the production of reactive oxygen species (ROS) by resveratrol in RPE cells was also demonstrated, a finding which supports the hypothesis that it may also participate in antioxidant defenses by capturing free radicals directly *in cellulo* (a phenomenon also known as scavenging).

Similar effects were observed in the lens. This tissue is particularly sensitive to oxidative damage because the fiber cells that constitute it are not renewable and have a limited lifespan. The accumulation of damage to these cells throughout the life of the individual causes the degradation of proteins and eventually leads to cataracts.

A 2010 study performed on human lens epithelial cells [38] showed that resveratrol reduced cell death (Fig. 9) as well as the accumulation of ROS (Fig. 10), when they were subjected to the oxidative attack of H_2O_2. This cell protection appears to be mediated by the increased expression of defense enzymes such as superoxide dismutase-1 (SOD-1), catalase, and heme oxygenase-1 (HO-1).

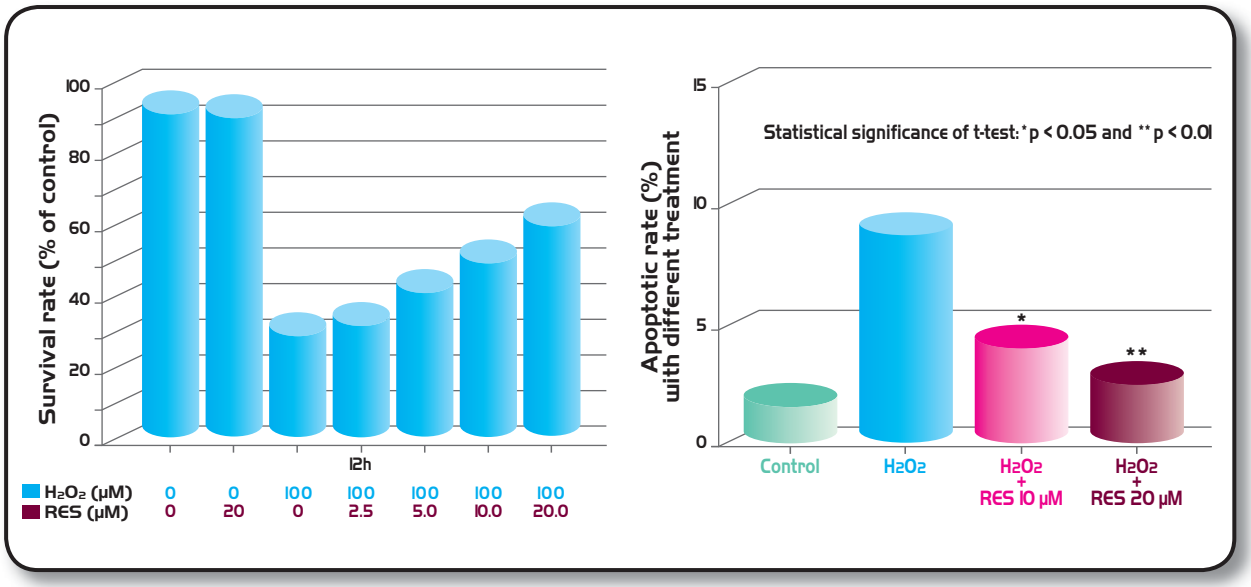


Figure 9 **Resveratrol protection against H_2O_2-induced cell damage in human lens cells. (From Zheng *et al.*, Mol. Vis., 2010).**

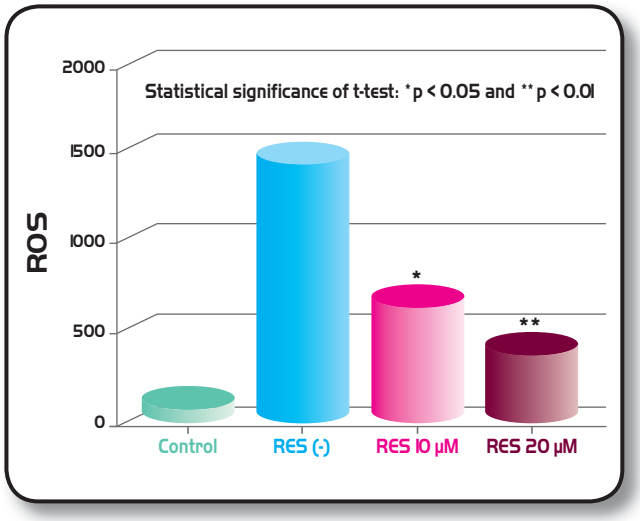


Figure 10

Resveratrol protection against H_2O_2-induced intracellular ROS production in human lens cells.

(From Zheng *et al.*, Mol. Vis., 2010).

Glaucoma is another pathology which can be caused in part by oxidative attack. Although inflammation seems to be the main trigger of the disease (see next section) oxidative stress is a contributing factor in altering the operation of the trabecular meshwork. This alteration leads to a lack of circulation of aqueous humor and finally to ocular hypertension.

In 2009, a study focusing on markers of glaucoma [39] showed that resveratrol operated a protective effect by normalizing the production of ROS in cells of the trabecular mesh when they were subjected to oxidative stress by hyperoxygenation (Fig. 11).

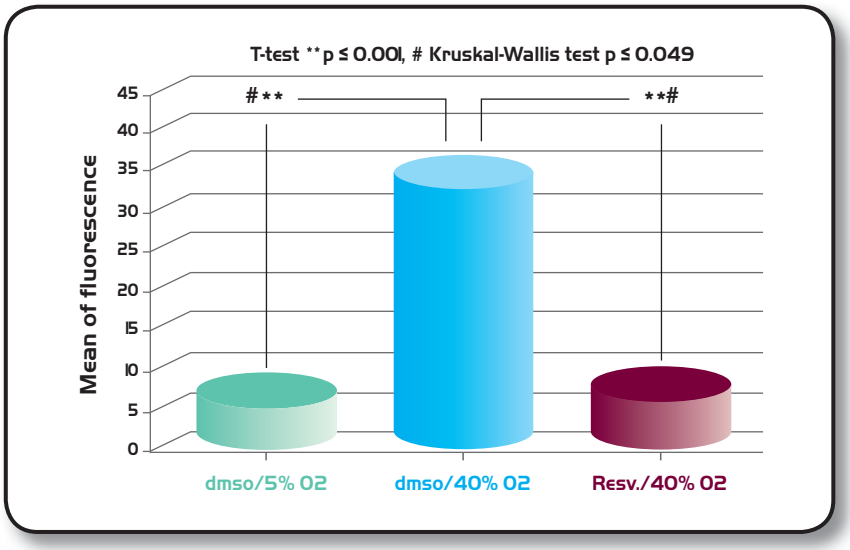


Figure II

Resveratrol protection against hyperoxygenation-induced ROS production in human trabecular meshwork.

(From Luna *et al.*, Food Chem. Toxicol., 2009).

Resveratrol is thus a molecule capable of acting in various cell types of the eye by increasing the level of natural antioxidant defenses.

It seems to increase the *in vitro* activity of defense enzymes and the rate of intracellular antioxidant molecules to limit the formation of free radicals (ROS) and the onset of irreversible damage, initiators of diseases such as AMD, cataracts, or glaucoma.

Anti-inflammatory effect of resveratrol

Inflammation is a phenomenon which is often associated with oxidation or at least which is frequently its consequence. It is a central process in the onset of diseases such as arthritis, psoriasis, Crohn's Disease, cardiovascular diseases or cancer.

In 2003 and 2004, it was widely recognized that inflammatory processes were also involved in the mechanisms of retinal diseases such as diabetic retinopathy [40] and AMD [41].

In 2009 an *in vivo* study in mice examined ocular inflammation during uveitis induced by endotoxins (EIU) and the protective effect of resveratrol [42].

The authors demonstrated that 5 days of prevention by oral supplementation with resveratrol was able to inhibit the production of two proteins crucial in the course of the inflammatory process: ICAM-1 and MCP-1. The MCP-1 protein (Monocyte Chimoattractant Protein 1) is a chemokine expressed by endothelial cells lining the vasculature.

Its role is to attract immune cells such as leukocytes to the inflammatory site.

These attracted leukocytes are then hooked by ICAM-1 proteins (Inter Cellular Adhesion Molecule-1) expressed on the surface of the endothelium, to extract and distribute them from the bloodstream to the target tissue.

Resveratrol thus has an anti-inflammatory effect by reducing the adhesion of leukocytes to the wall of retinal vessels (Fig. 12 and 13).

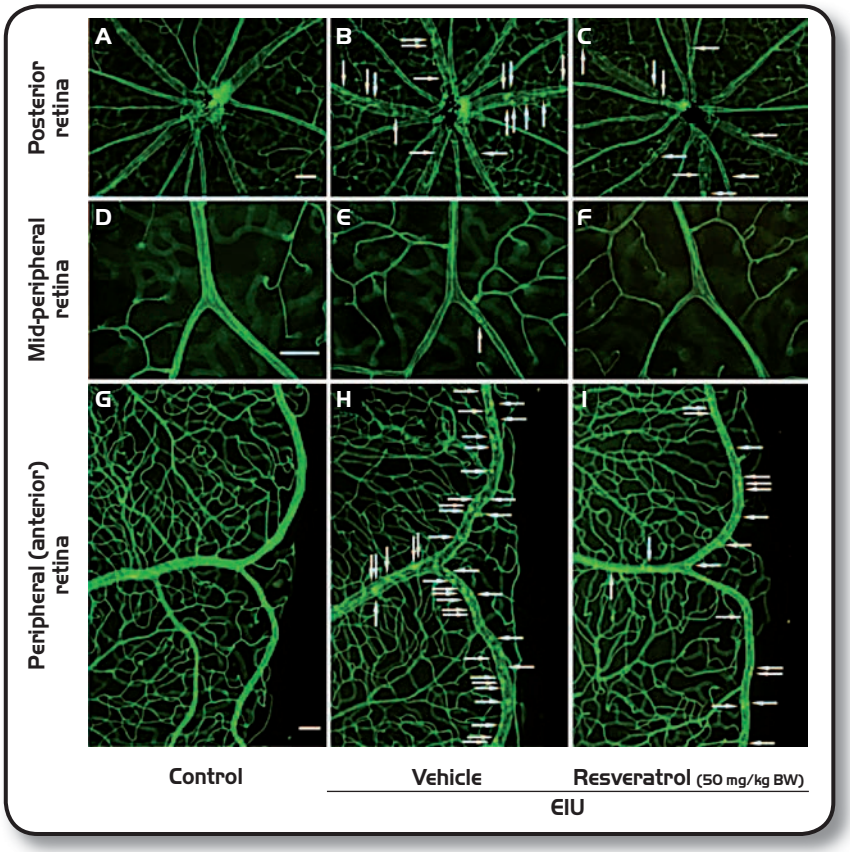


Figure 12

Suppression of retinal leukocyte adhesion with resveratrol.
Flatmounted retinas from vehicle-treated control mice (A, D, G), vehicle-treated EIU mice (B, E, H) and resveratrol-treated (50 mg/kg BW) EIU mice (C, F, I). Arrows = Adherent leukocytes.

(From Kubota *et al.* IOVS, 2009).

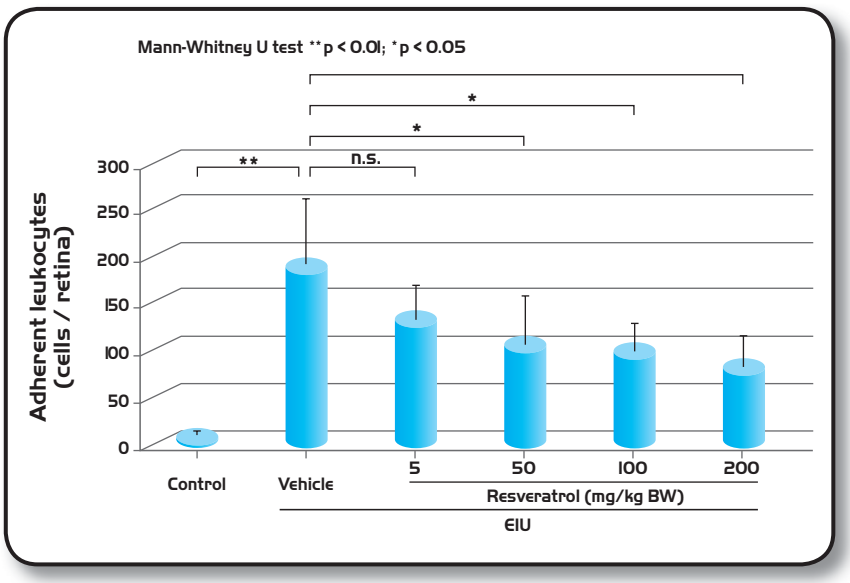


Figure 13

Quantification of adherent retinal leukocytes from figure 12.

(From Kubota *et al.* IOVS, 2009).

In diabetes, the persistence of high levels of blood glucose causes chronic inflammation with a slow but progressive deterioration of RPE cells leading to the impairment of the blood-retinal barrier and the loss of central vision. In 2010, an *in vitro* study led on retinal pigment cells was concentrated on this inflammatory phenomenon triggered by a condition of hyperglycemia [43]. The authors showed that cells subjected to this stress secrete proinflammatory cytokines such as interleukin 6 and interleukin 8 and that resveratrol is able to inhibit this reaction in a dose-dependent manner (Fig. 14).

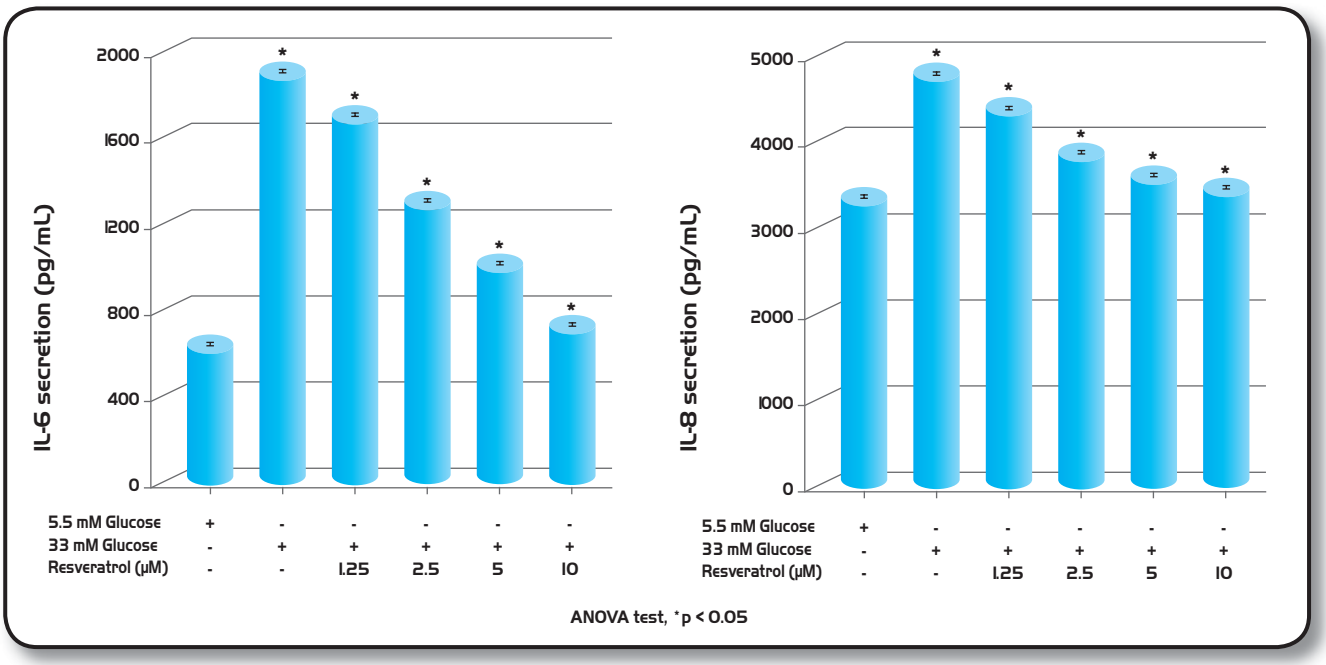


Figure 14 **Effect of resveratrol on cytokine expression during hyperglycemia-induced inflammation in RPE cells. (From Losso *et al.*, J. Agric. Food Chem., 2010).**

At the same time, the activity of cyclooxygenase-2 (COX2) responsible for the production of proinflammatory prostaglandins is also inhibited by resveratrol, whereas the protein expression of cellular interaction connexin 43 and Gap-junction is preserved. Cellular cohesion is maintained, thus preventing the degradation of the blood-retina barrier.

We observed in the previous section that resveratrol had a protective effect by inhibiting the production of ROS in cells of the trabecular meshwork when they were subjected to oxidative stress by hyperoxygenation, a factor that can promote the initiation of glaucoma. The same study [39] revealed that resveratrol also had the ability to decrease the expression of inflammation markers such as messengers of interleukin-1α, interleukin-6, interleukin-8 and E-selectin. This latter, also known as ELAM-1 for Endothelial Leukocyte Adhesion Molecule-1 is involved in the same way as ICAM-1 in recruiting leukocytes to the site of inflammation (Fig. 15).

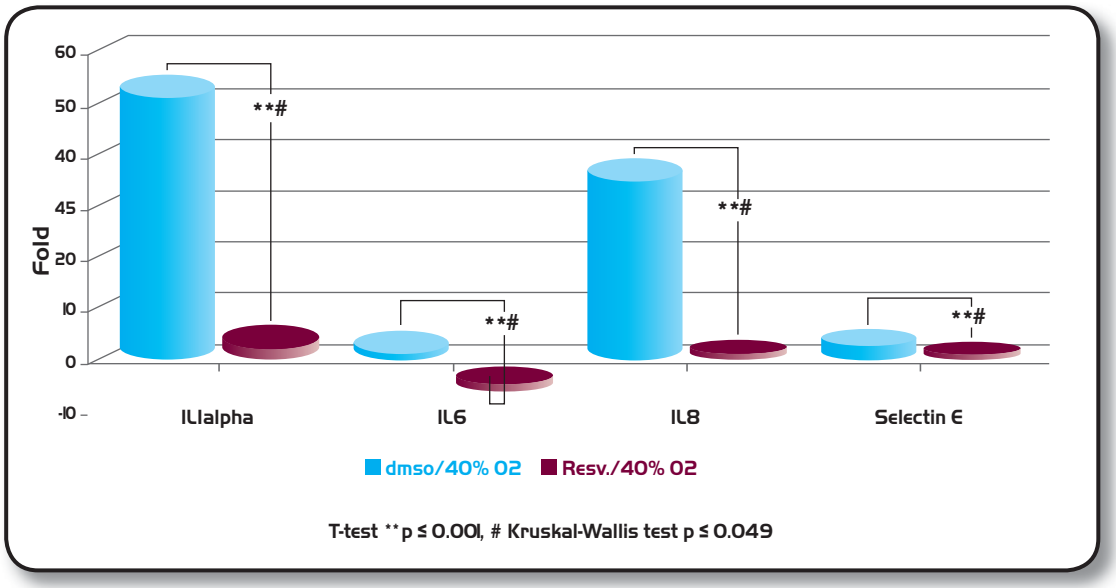


Figure 15 **Effect of resveratrol on the expression of inflammatory markers induced by chronic oxidative stress. (From Luna *et al.*, Food Chem. Toxicol., 2009).**

Resveratrol thus reveals anti-inflammatory capacities in ocular tissues *in vitro* and *in vivo*, first at the molecular level by limiting the expression of pro-inflammatory factors such as interleukins and prostaglandins but also at the cellular level by decreasing the chemo-attraction and recruitment of immune cells to the inflammatory site.

Anti-angiogenic effect of resveratrol

Angiogenesis is a normal and necessary process in the development of the organism and in tissue repair. This process becomes abnormal when, for example, it is diverted to further the growth of cancerous tumors or when activated in an uncontrolled manner in AMD.

An article published in 2001 reported the effect of resveratrol in a model of corneal neovascularization in mice [44]. The induction of angiogenesis was performed by implantation in corneal tissue of wicks soaked in growth factors (yellow arrows Fig. 16). The effect of vascular endothelial growth factor (VEGF) and fibroblast growth factor type 2 (FGF-2) is observable in the appearance of a dense neovascularization around the periphery of the cornea (photos A and B). Consumption of resveratrol in the drinking water for 3 days before implantation of the wicks limited the area of neovascularization induced by VEGF and FGF-2 but also the density of the network (photos C and D).

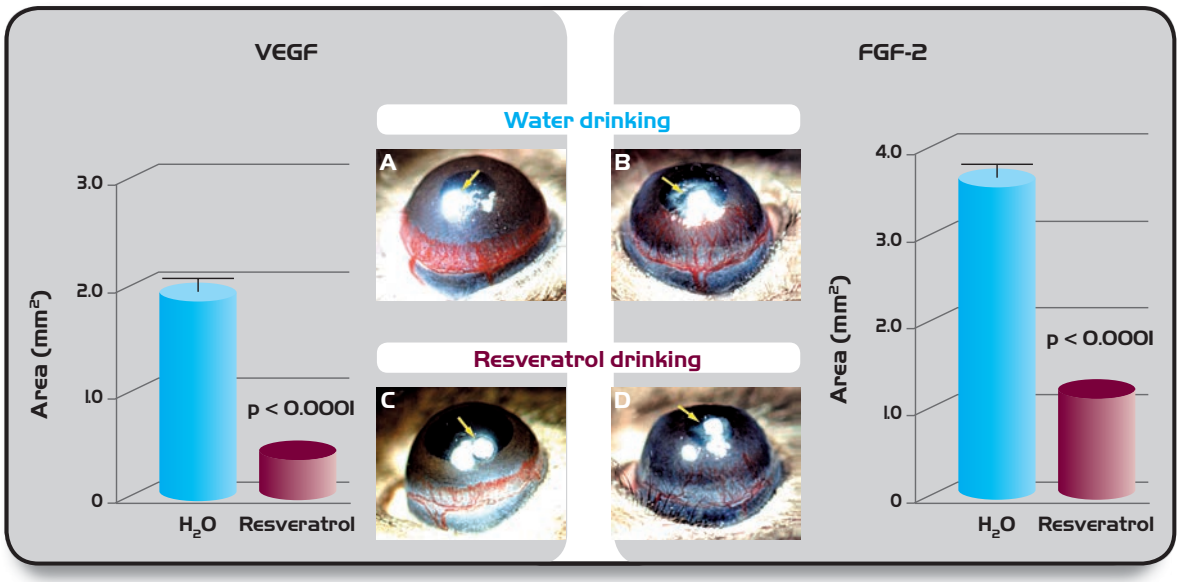


Figure 16 **Anti-angiogenic effect of resveratrol in mouse corneal micropocket assay. (From Brakenhielm *et al.*, FASEB J., 2001).**

The 2010 article from Losso *et al.* which studied the cells of the retinal pigment epithelium (RPE) during hyperglycemia-induced stress [43] confirmed this inhibitory activity on VEGF secretion (Fig. 17).

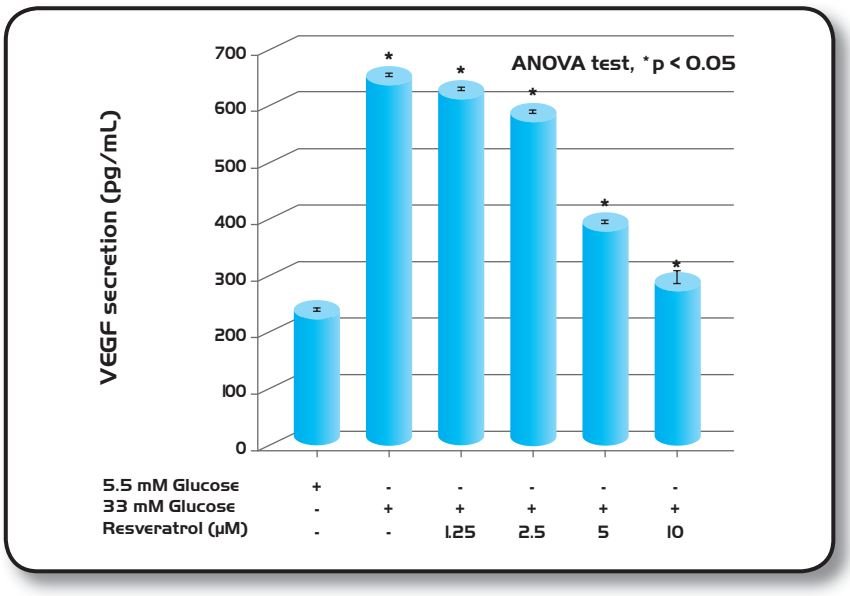


Figure 17

Effect of resveratrol on VEGF secretion during hyperglycemia-induced inflammation in RPE cells.

(From Losso *et al.*, J. Agric. Food Chem., 2010).

Very recently, in April 2011, another model of pathological development of neovascular lesions *in vivo* was used to evaluate the therapeutic effects of resveratrol. Unlike the corneal neovascularization model presented above, researchers at the prestigious Harvard Medical School in Boston used a line of mice which were mutant for the receptor of Very Low-Density Lipoprotein (VLDLR). Surprisingly and unexpectedly, after 15 days (P15) these mutant VLDLR$^{-/-}$ mice spontaneously developed retinal neovascularization (NV) and photoreceptor degeneration, similar to those encountered in macular telangiectasia and retinal angiomatous proliferation in man [45]. The study showed that consumption of resveratrol in mice, starting 5 days before the first appearance of neovascularization, prevents the development of approximately 70% of all lesions observed on the entire retina at 30 days (Fig. 18).

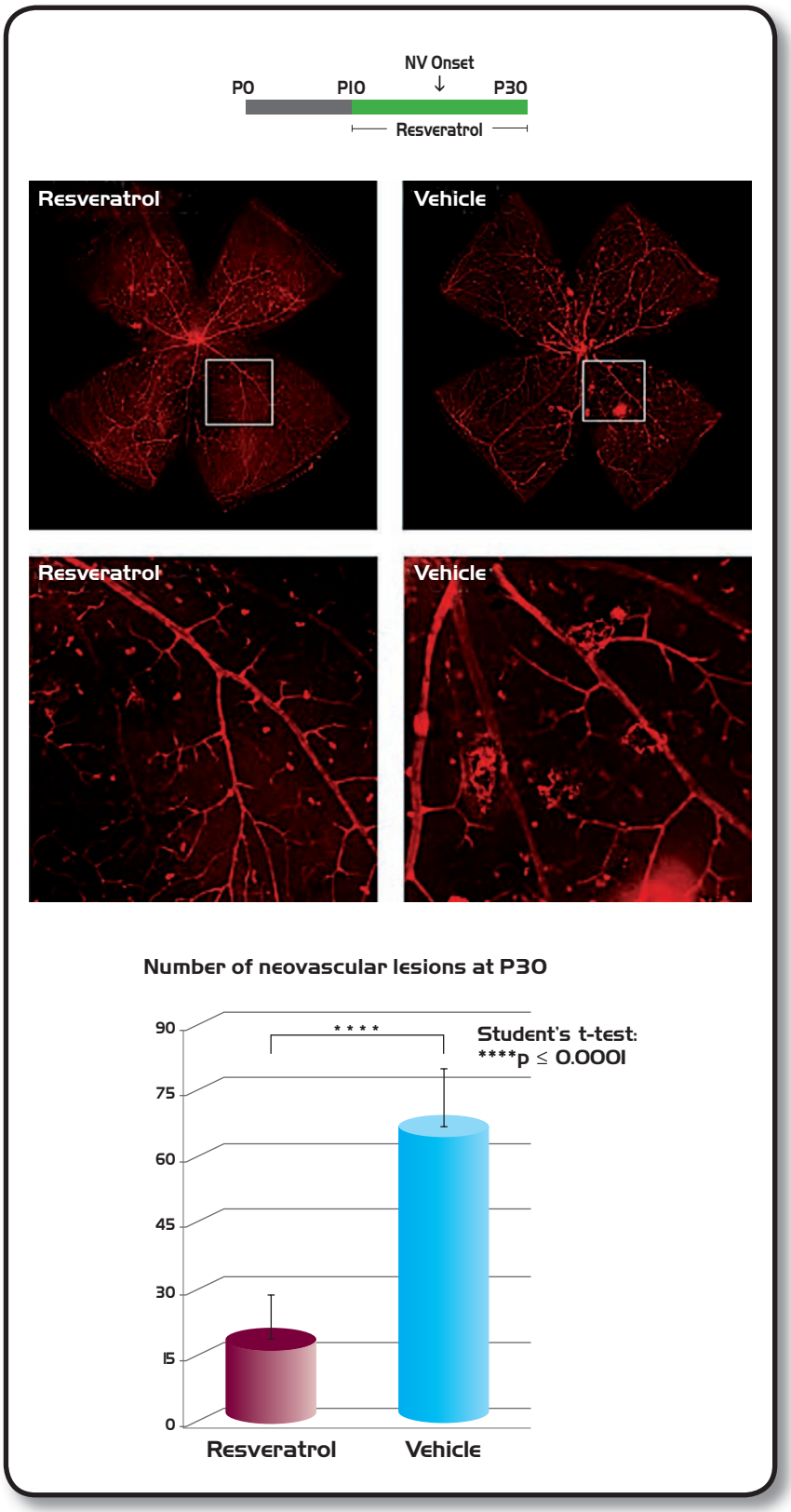


Figure 18

Effect of resveratrol prevention on spontaneous angiogenesis in whole retina of VLDLR $^{-/-}$ mice.

(From Hua *et al.*, IOVS, 2011).

In addition to a preventive effect, the molecule also showed a therapeutic potential. Indeed, supplementation in resveratrol beginning 6 days after the initial injury was still able to slow the progression of neovascularization by approximately 40% at 60 days (Fig. 19).

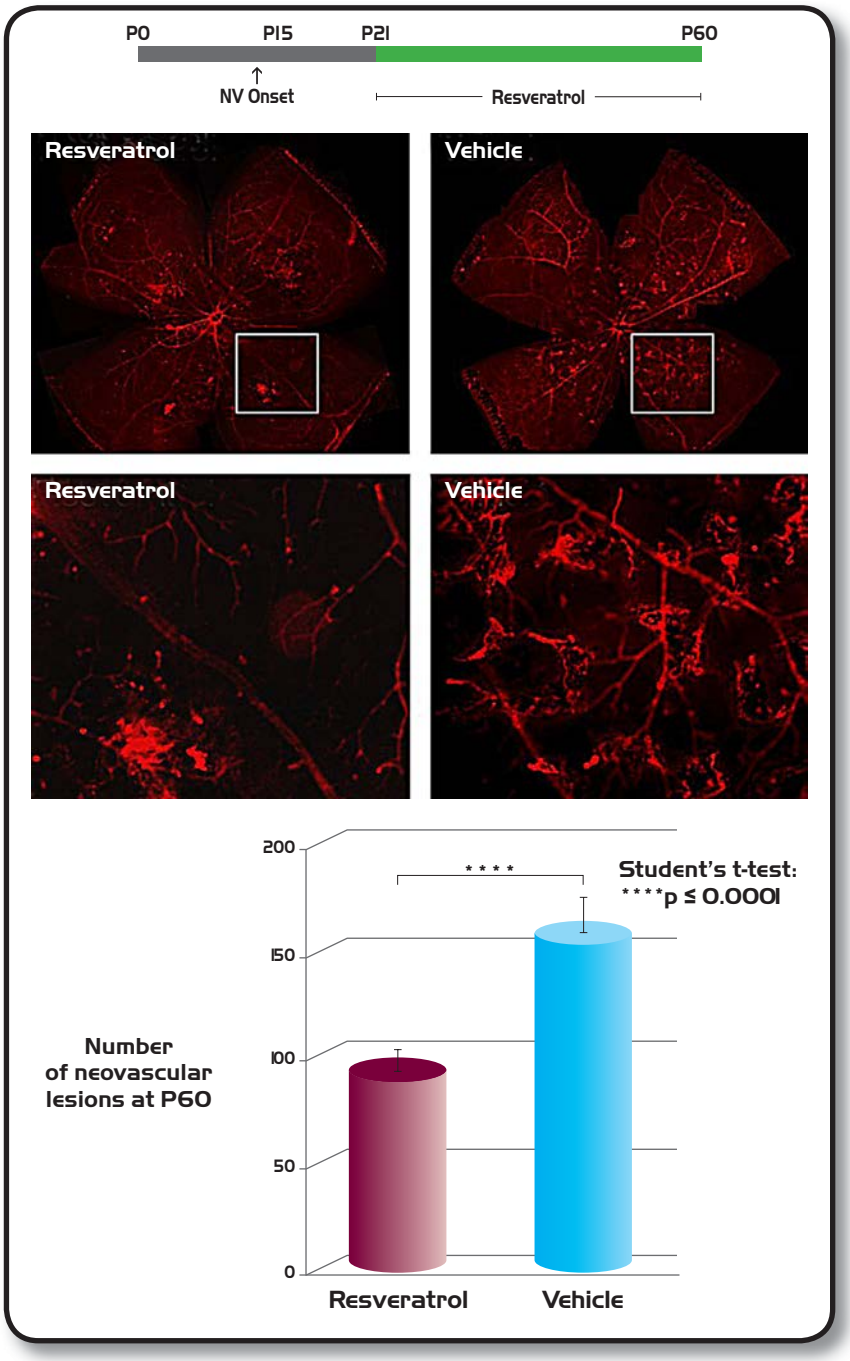


Figure 19

Effect of resveratrol treatment after spontaneous angiogenesis in whole retina of VLDLR $^{-/-}$ mice.

(From Hua *et al.*, IOVS, 2011).

Again, the anti-angiogenic effects of resveratrol seem mediated by its anti-VEGF action. This hypothesis was validated by the normalization of the VEGF mRNA level by resveratrol in VLDLR $^{-/-}$ mouse retina at 60 days. At this point we can observe a level comparable to that observed in the retina of normal mice (Fig. 20).

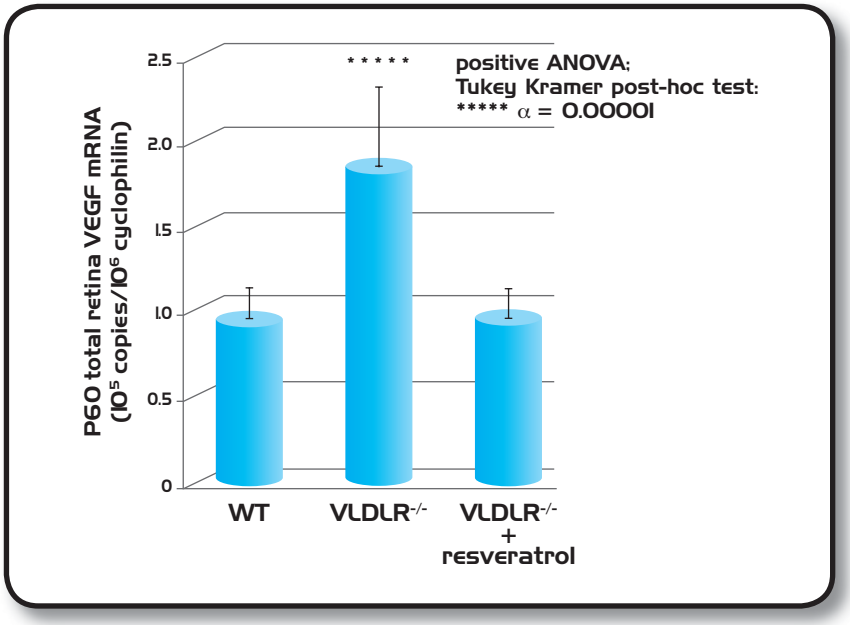


Figure 20

Effect of resveratrol on VEGF mRNA levels in whole retina of VLDLR $^{-/-}$ mice.

(From Hua *et al.*, IOVS, 2011).

This anti-VEGF property *in vivo* may be associated with an anti-proliferative and anti-migrative effect on endothelial cells, as demonstrated by the authors in an *in vitro* experiment on human microvascular kidney cells (Fig. 21).

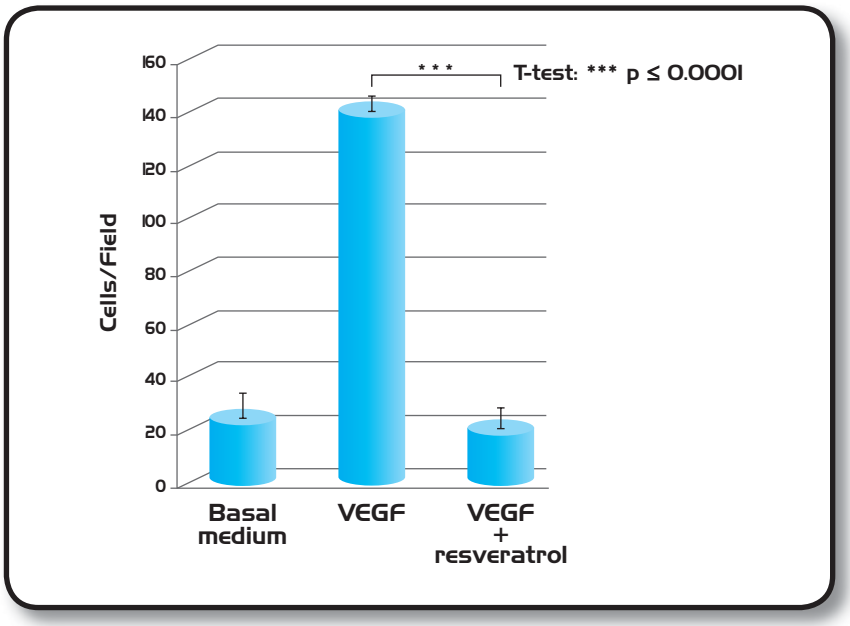


Figure 21

Effect of 50 µM resveratrol on Human Renal Microvascular Endothelial Cell Migration in Boyden chamber.

(From Hua *et al.*, IOVS, 2011).

The anti-angiogenic effect of resveratrol demonstrated *in vivo* and *in vitro* by this recent research has also been demonstrated in cancer research in its anti-metastatic aspect. Indeed, beyond its anti-VEGF, anti-proliferative and anti-migrative effects on vascular endothelial cells, resveratrol also has the ability to inhibit the degradation of the extracellular matrix by metalloproteinases, necessary for the development of new vessels.

This suggests that the molecule may be considered as a potential therapeutic agent in angiogenic eye diseases such as AMD, macular telangiectasia and retinal angiomatous proliferation.

CONCLUSION

Resveratrol is a naturally occurring molecule known for over 70 years but that has revealed, little by little since the 1990s, its therapeutic potential. Protecting plants from attacks and infections, resveratrol also surprisingly seems to protect humans. Many research areas have thus examined its potential in the treatment of common diseases: cardiovascular, neuro-degenerative, metabolic diseases and even cancer. The eye is no exception and could benefit as well as other organs from the plethoric effects of resveratrol in the fight against eye diseases. These primarily occur in an environment of oxidative and inflammatory stress, some of them with neovascular complications.

It has been shown in the scientific literature that resveratrol is able to act on various cell types of the eye by increasing the level of natural antioxidant enzymatic and molecular defenses. It has also been shown to possess an anti-inflammatory capacity by limiting the expression of pro-inflammatory factors like interleukins and prostaglandins but also by decreasing the chemo-attraction and recruitment of immunitary cells to the inflammatory site. Finally, it was shown to possess an anti-VEGF effect, to be anti-proliferative and anti-migrative on vascular endothelial cell. Three effects combined in an overall anti-angiogenic action.

Although all these discoveries have been made *in vitro* or *in vivo* in mice, resveratrol use in humans could be considered. Thus, a strategy for the treatment or prevention of angiogenic eye diseases by means of a resveratrol-based treatment could be particularly interesting because involving three fronts: anti-oxidation, anti-inflammation and anti-angiogenesis.

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