



PHYSIOLOGY of the Skin

Resveratrol: A Real Anti-aging Product

The biological process of aging is a mystery. Many definitions are applied to and much controversy surrounds the discussion about aging, yet its true nature is still uncertain. A number of scientists believe that aging is not a biological process that beings are subject to undergo throughout time; rather, they believe that aging is mainly multiple environmentally induced changes in the basic biological system. True, there are some genetic components, but only a few, because approximately 80% of the aging process seems to be self-induced. A major cause is overeating, followed by smoking, sunbathing, lack of exercise and psychological stress. Because there

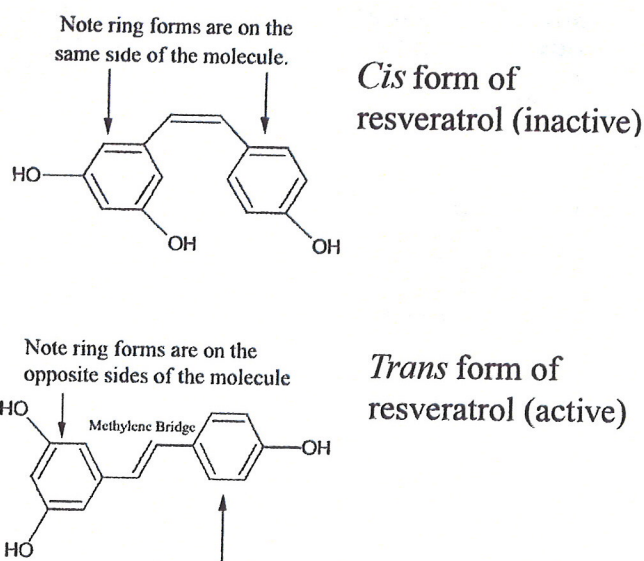
are many repair mechanisms in the body, it seems unlikely that there would also be a death gene or something that is designed to terminate life at a relatively early age. Of all the anti-aging methods proposed, and all the drugs and chemicals advertised, only one method appears to work, at least in mice, and that is dietary restriction.

One group of scientists working to understand aging mechanisms and age intervention discovered that a certain chemical, when taken orally, could provide similar benefits as offered by dietary restriction.^{1,2} Essentially, three groups of mice were studied for the effects of dietary restriction. One group was allowed unlimited food known

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Figure 1: Molecules of resveratrol



as “ad lib;” a second group was given an ad lib diet plus a natural chemical from red wine; and a third group was put on a caloric-restriction diet. The results showed that the caloric-restriction group and the ad lib group with the natural chemical from red wine lived the same length of time and enjoyed a similar health benefit. The ad lib group all died early. The natural chemical from red wine used in this study was resveratrol, which is the topic of this article.

What is resveratrol?

Resveratrol is a plant compound, chemically known as 3,5,4'-trihydrostilbene, a member of the stilbene family, which is a group of compounds that is made up of two aromatic rings and is joined by a link known as a

methylene bridge. (See **Figure 1**.) There are more than 30 stilbenes in the plant kingdom, but resveratrol is the parent molecule of a group of compounds known as viniferins^a, a family of phytoalexin polymers that prevents bacterial and fungal infections in plants. The phytoalexin are produced by the plant as a defense mechanism.

Although resveratrol is found in at least 72 plants, it is not found in many of the edible ones; peanuts and grapes seem to be the major edible sources. A weed that grows in Eastern Asia known as *Polygonum cuspidatum* is the richest natural source of resveratrol.

^a Viniferins are complex molecules that develop in grape leaves and wood in response to fungal infections.

Figure 2: Acetylation process

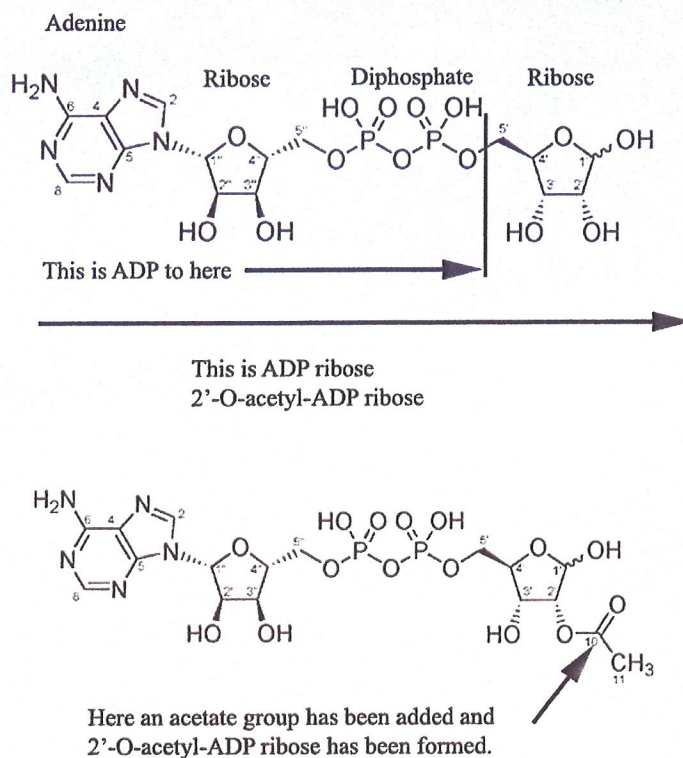
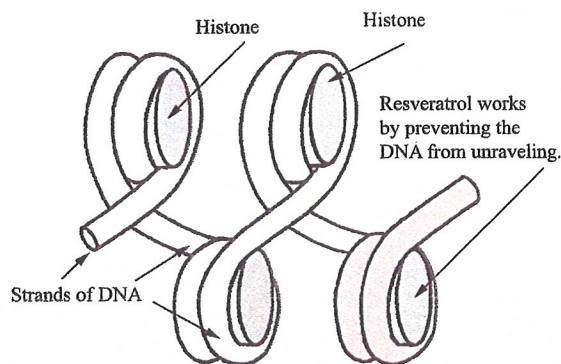


Figure 3: Histones wrapped with DNA strands



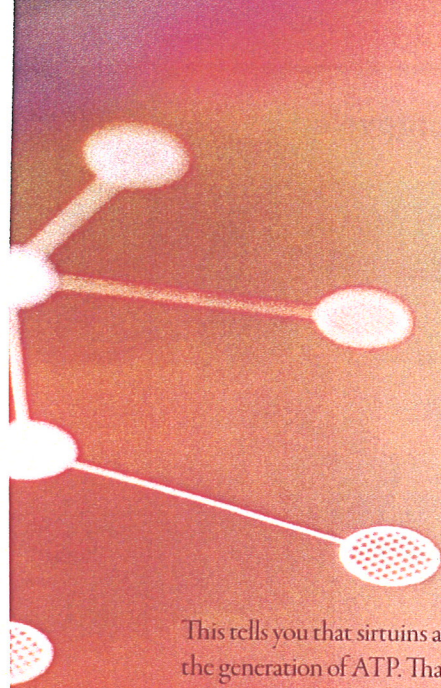
Sirtuins and resveratrol

Much of the research into aging mechanisms centers on cell growth and the control of cell growth. One gene that has been studied extensively in lower life forms in this regard is silent information regulator 2 (Sir2) proteins, or sirtuins. They are enzymes characterized as deacetylases/mono-ADP-ribosyltransferases and are found in many organisms, ranging from bacteria to humans.

The first concept to understand is that sirtuins remove acetyl groups^b from proteins. They do this in the presence of nicotinamide adenine dinucleotide (NAD⁺); that is

how it gets its name as a NAD⁺-dependent deacetylases. Now it must do something with this acetyl group, so the sirtuins then take the acetyl group from the protein and add it to the ADP-ribose part of NAD⁺ to form adenosine diphosphate (O-acetyl-ADP), a lower energy form of the high energy form known as adenosine triphosphate (ATP), a major source of energy in biology. Here is the key phrase: energy-linked. When you see sirtuins and ADP, you know ATP—or energy—is not far behind. This is how it works.

^b An acetyl group is a two carbon compound with formula CH₃-COO-. It is essentially vinegar.



This tells you that sirtuins are linked in some manner to the generation of ATP. That is the first key concept.

The hydrolysis^c of this protein, whatever it may be, yields three compounds: O-acetyl-ADP-ribose, the deacetylated substrate and nicotinamide. The dependence of sirtuins on NAD links their enzymatic activity to the energy state of the cell through the cellular NAD:NADH ratio.^d You can see this reaction that shows the acetylation process. (See **Figure 2**.) Due to this relationship with energy, sirtuins have been associated in the regulation of aging, transcription, apoptosis and stress-resistance. The regulation of many metabolic processes and cellular defense mechanisms could easily be the key to a possible lifespan-extending role for sirtuins in mammals. This reaction ties in with the histones and deacetylation and finally, resveratrol. I shall take you on a walk through this minefield and hopefully we shall not step on a mine. Keep in mind that all this discussion relates to turning on a cell's metabolic mechanisms, as well as turning them off. It is very similar to a traffic light that has green for go, red for stop and yellow for caution.

A brief cell review

A cell consists of a membrane, cytoplasm, a nucleus and organelles, such as the mitochondria. Deoxyribose nucleic acid (DNA) is the body's master template. Any time a protein—or any complex substance in the body—is made, DNA must open its two strands and expose the code for that substance. The code is copied in a process called transcription, and the copy is sent to the reticuloendothelium, an organelle in the cytoplasm, which then translates the copied code into the substance or protein. So transcription and translation are the two key words in molecular biology. All of this is written in

many basic textbooks in biology, and also in *Advanced Professional Skin Care, Medical Edition* (The Topical Agent, LLC, 2005).³ If you are rusty in this area, please review it before you read further in order to have a better understanding of this article. If you can remember the acts of transcription and translation, you have a grasp on biology. The DNA code can only be initiated by transcription.

Histones and deacetylation

This concept is critical to understanding resveratrol action. DNA^e, which is the genetic-containing molecule in every body cell, is a very large, very complex and very long molecule. In each cell, it is 1.8 m long when unwound. Think about that: A cell is only about 80–100 microns in diameter, while a strand of DNA is 1,800 mm, or 1,800,000 microns long. That is 18,000 times longer than the cell. Now, to get the DNA into the cell, it needs to be wound up to fit into a very tiny space. The cell uses tiny protein balls called histones to serve as the surface on which to wind the DNA. **Figure 3** shows a segment of DNA wrapped around some histones. Actually, they assemble to form one nucleosome core particle by wrapping 146 base pairs^f of DNA around the protein spool in a spiral. These histones with DNA wrapped around them form a string of beads with some space between, called linker DNA. It looks much like a very long rosary and is called chromatin. (See **Figure 4**.) Before any message can be transmitted to the DNA, this giant molecule must open up, unwind and expose the code. If the DNA does not unwind, there can be no cellular action. Now here is where the deacetylation comes in.

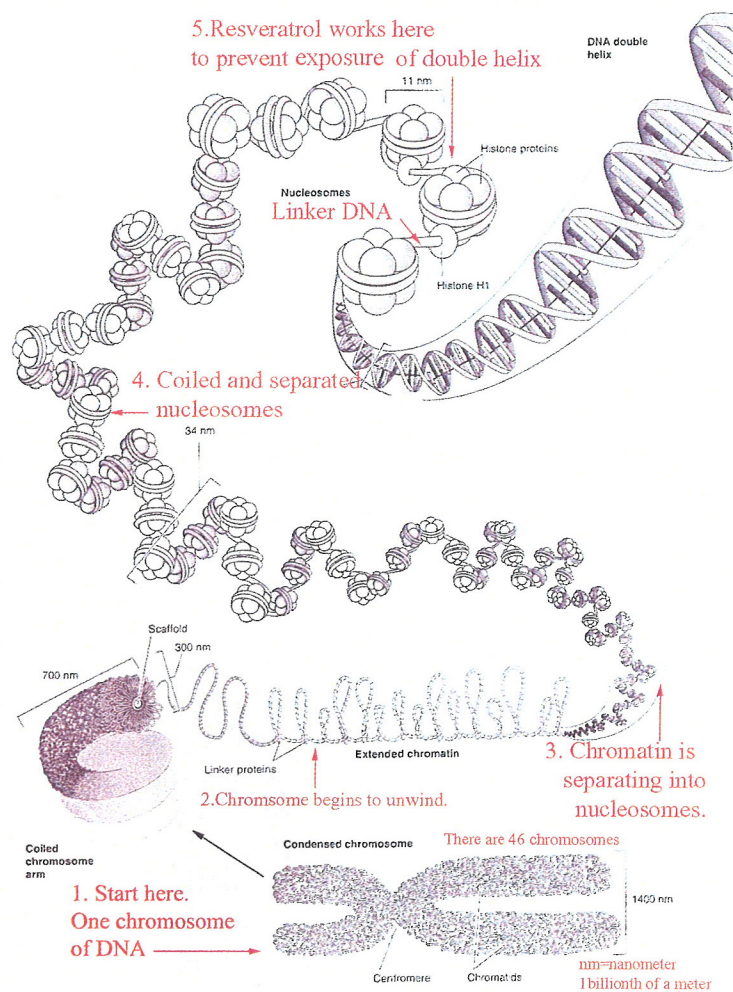
^e DNA is short for deoxyribose nucleic acid, the protein that contains all the genetic material needed to develop a human from conception, and maintain the body throughout its life.

^f Bases in DNA are known as thymine, adenine, guanine and cytosine. A base pair consists of two of these bases chemically bonded across from each other in a strand of DNA. Cytosine can only bind to guanine and thymine can only bind to adenine, and vice versa.

^c Hydrolysis is a chemical process in which a water molecule is removed from a protein.

^d NAD:NADH is called a redox reaction because it shuttles protons and electrons back and forth.

Figure 4: Chromosome unraveled



Acetic acid can be added to a molecule under certain circumstances and this is called acetylation. If it is removed, this is called deacetylation. It is known that when DNA is active, the histones are acetylated; if they are deacetylated, they become less active. Following is a short explanation for this phenomenon. Histones have positive ends due to amine groups present on their lysine and arginine amino acids. The positive charges help the amines to interact with and bind to the negatively charged phosphate groups on the DNA back. Being acetylated, which is the normal state in a cell, they neutralize these positive charges on the histone and change the amines into amides, thereby decreasing the ability of the histones to bind to DNA. What this action does is to allow chromatin expansion, causing more genetic transcription to occur. Enter histone deacetylase. This enzyme removes those acetyl groups, increases positive charges to the histone and causes it to bind tightly to the histones and DNA structure. Net result: condensing of the DNA structure,

which prevents transcription of DNA.⁸ With all of that background, how does resveratrol fit into the picture?

Enter resveratrol

Remember that sirtuins are the initiators of the deacetylation of the histones, and thus inactivate certain segments of DNA. It is this very action of resveratrol that makes it so effective as an anti-aging and anti-cancer agent. Resveratrol is capable of the activation of Sir2, which turns on the deacetylation process. This is the same result seen as an effect of caloric restriction (CR) in the life-extension process. This process is still not completely understood, but at least some progress has been made. A new phase of aging control is being entered, and you are in on the ground floor. The activation of Sir2 is only one aspect of the benefits of resveratrol, so let's look at some others.

Oral absorption. Resveratrol has been found to be highly absorbed—at least 70% in human beings—through oral doses. The compound is metabolized to resveratrol sulfate, but resveratrol glucuronide is the most common metabolite. There does not seem to be a problem with the absorption of resveratrol when taken orally.⁴

As an antioxidant. Many organic compounds, such as resveratrol, contain aromatic groups (six carbon ring structures), making them able to function as antioxidants. They achieve this action by forming stable radical structures, thereby preventing continuous oxidation. Resveratrol contains two aromatic groups that provide higher antioxidant protection than vitamin C and vitamin E.⁵ The antioxidant activity of resveratrol has been associated with protection against the initiation of atherosclerosis, often associated with oxidation of low-density lipoproteins (LDL). In early atherosclerosis lesions, blood platelets are seen that, when activated, can generate reactive oxygen species (ROS). Resveratrol can inhibit ROS formation, and thus reduce lipid peroxidation.⁶

Resveratrol and heart disease. The French are noted for having a relatively low incident of coronary heart disease. This phenomenon has been attributed to the high consumption of wine—the red varieties include resveratrol—in France.⁷ The most accepted explanation for how this heart protection occurs by resveratrol is the inhibition of platelet aggregation.⁸ Platelets are tiny blue (Wright's stain) cells without nuclei that function to stop bleeding by clumping at the site of a wound. They

are activated by adenosine diphosphate (ADP), collagen, thrombin and other factors, but when activated, they change their shape and clump around the damaged area in an attempt to seal off leaking blood. When this clumping becomes excessive, it can lead to the beginning of cardiovascular disease.

An additional cardioprotective effect of resveratrol has been related to its vasorelaxation properties. Investigators concluded that resveratrol causes vasorelaxation in endothelium-intact and endothelium-dependent aortic rings by the action of nitric oxide-dependent and nitric oxide-independent mechanisms.⁹ Nitric acid is a major vasodilator substance in the body.

Resveratrol and cancer. Cancer is one of the ugliest words in the English language. More is known about cancer now than ever before, but scientists are still a long way from solving this complex disease. One characteristic of cancer cells is that they have escaped the normal growth-control mechanisms in the body. Normal cells are controlled by many genetic systems that maintain a series of checks and balances on all the cells to keep just the right number available at all times. When a cell becomes worn out, nonfunctional or abnormal, the body will usually destroy it. When dealing with a cancer cell, this is not the case; the cell becomes autonomous, and will continue to divide and grow more cancer cells. This is known as abnormal cellular proliferation. Cancer therapy is directed at killing the cancer cell or inhibiting its proliferation: either way, the cancer cell is destroyed.

When a cell is abnormal, it is literally destroyed by being taken apart by a genetic mechanism called apoptosis. The anti-proliferative activity of resveratrol that occurs in some cancer cell lines is believed to be due to the induction of apoptosis.¹⁰ It is suggested that the proliferation inhibition of resveratrol is caused by the arrest of the cell cycle. When a cell cannot be destroyed by an existing genetic mechanism, it will stop the division of the cell in one of four stages: G1, S, G2 or M. The molecular mechanisms associated with the anti-proliferative effects relate to two mechanisms—one is the activation of p53 and the other is the suppression of nuclear factor- κ B (NF- κ B) and activator protein-1 (AP-1). These are complex systems, but you can learn more about them by checking out these references.^{11,12}

Resveratrol and inflammation. Inflammation is a biological reaction that represents a complex host's normal defense reaction to insult and stress, which can be either physiological or nonphysiological, including such agents as chemicals, drugs, oxidants and microbial entities. All inflammatory responses, whether acute or

⁹ All genetic information must undergo transcription and translation to effect a result, whatever it may be.

chronic, must be activated by well-coordinated, sequential events controlled by humoral and cellular reactions. ROS, including superoxide, hydroxyl radical, hydrogen peroxide and reactive nitrogen species (RNS), such as nitric oxide, are implicated in the inflammatory process.

The destructive effects of these ROS depends on their concentration and the microenvironment in which they are released. Normally the body can handle the ROS production, but when they are overproduced, such as when the body experiences sunburn or overeating, they become a major factor in tissue inflammation. Inflammatory responses usually proceed as follows: intracellular activation; infiltration of proinflammatory macrophages and lymphocytes, which are blood cells; increased vascular permeability; and finally tissue damage and cell death. In aging, the same process occurs, but it is slower and less violent.

Current research suggests that resveratrol blocks the inflammatory process in a rather complex way. NF- κ B is a key step in the process of inflammation.¹³ Inhibition of NF- κ B activity is a possible mechanism by which resveratrol exerts its anti-inflammatory activity. The exact mechanism by which resveratrol inhibits NF- κ B activation remains uncertain, but studies have clearly shown that this action is real.¹⁴

Resveratrol and aging skin. Aging, although studied intensively, remains a mystery. Many physical and biochemical aspects of the aging process are known, but no single unifying concept has emerged that explains it all. For example, it is known that individuals undergo aging changes at different rates; that is, some faster than others. It is understood that aging is associated with certain physical changes, such as grey hair and wrinkled skin, but it is also known that these changes are not absolute signs of aging. Biochemical changes, such as loss of muscle and bone mass are evident, along with hormonal changes—mainly reproductive ability. It is becoming clear that aging is not inevitable, and that some steps can be taken to slow it down, one of the most important being the reduction of food intake.

CR brings about a great many positive changes in a person's physiological make up, all of which make for longer, healthier life. CR triggers sirtuins, as well as reduces the strain on the body's mitochondria. The less oxygen a person breathes, the longer their lifespan because the body produces fewer free radicals.

This fact points to free radicals and oxidative damage as one of the major mechanisms in the aging process. At the core of free radical damage is inflammation, and inflammation is responsible for many of the dysfunctional aging changes and many diseases. Here is an interesting and astonishing fact: humans actually age daily, bit by bit.

Sure, there are few big happenings, either enzymatic or hormonal, but the daily damage has the biggest impact on aging changes. Why is this important? For one reason: It appears that the body cannot repair this damage as long as it is continuous. That is important! Stop the damage, and the repair will happen. It has been shown that 20-month-old mice (about 80-years-old in human years) can repair serious aging changes to become nearly normal in just two weeks.¹⁵ What this means is that aging can be controlled if the damage inflicted on biological systems every day is controlled. The changes seem to occur in 10-year increments. Look at the changes from age 10 to 20, and ages 40 to 50. After age 70, the changes are a bit more pronounced, and this may reflect an accumulation of many biological insults. The good news is that this process can be slowed down with diet and exercise. No one really wants to hear this, but there is strong evidence to support this concept.¹⁶

Caloric restriction

CR is a more palatable way of saying eating less, or dieting. It is considered a sound concept and enjoys widespread acceptance in the scientific community. As mentioned above, the gene Sir2 that promotes lifespan in lower organisms is called SIRT1 in mammals. A major function of this gene is to promote cellular resistance to stress-induced death, but it also stimulates many changes in the cells, including metabolic changes in both glucose and lipid metabolism. The bottom line is that SIRT1 is up-regulated (a fancy word meaning "increased action") with CR. This is the heart of a new theory about anti-aging called the Hormesis Hypothesis. Its core concept is that diet restrictions impose a mild stress on an organism. This stress then elicits a defense response that protects the organism against the causes of aging.^{17,18} There are four key predictions made by the Hormesis Hypothesis:

1. Caloric restriction induces intracellular cell-autonomous signaling pathways that respond to biological stress and low nutrition;
2. These pathways help defend the organism against the causes of aging;
3. The pathways alter glucose, fat and protein metabolism to enhance survival during times of adversity; and
4. The pathways are under the control of endocrine-signaling pathways that ensure that cells in the organism act in a coordinated fashion.



Keep this concept in mind as you will see and hear about it from time to time. It forms much of the basis for the positive effects of CR.

Resveratrol, CR and aging

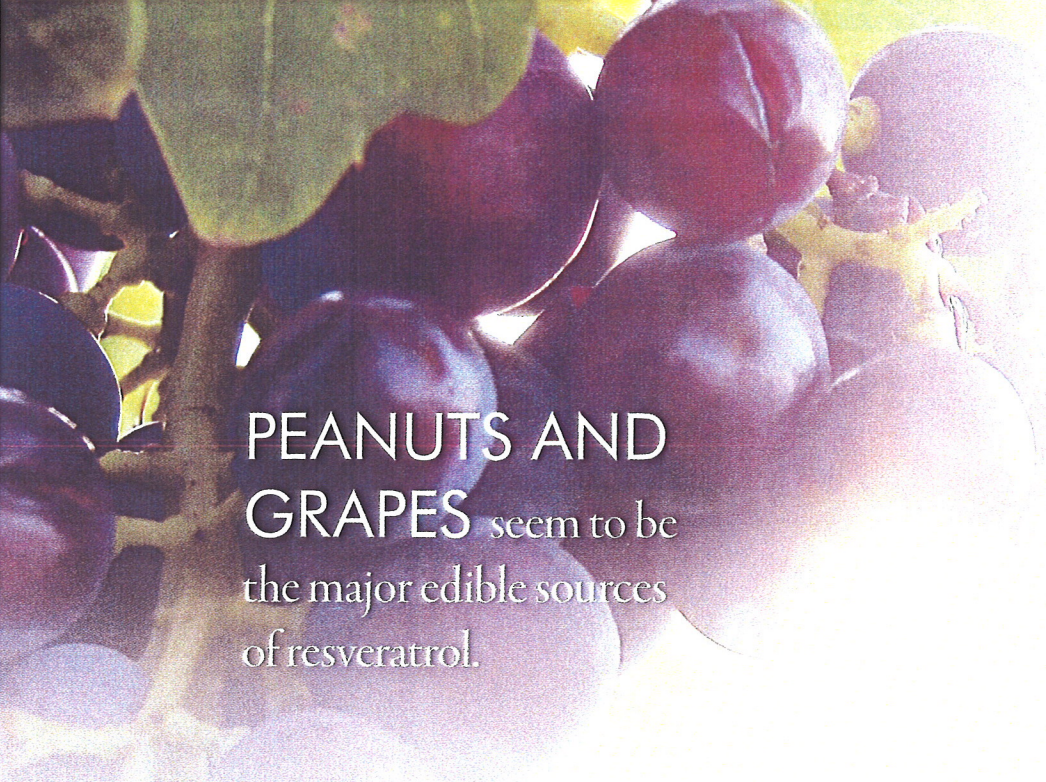
CR holds the greatest potential to delay human aging, based on current knowledge. Resveratrol is the most studied natural product for potential use as a mimic of CR, or as a potential therapeutic product to slow the aging process. At present, the mechanisms CR uses to extend lifespan are not known. It seems to slow down parts of the genetic program that relate to aging and to disrupt certain hormonal levels. Anorexia nervosa, for example, caused by severe dietary restriction, markedly affects the estrogenic hormones.

The ability of resveratrol to extend lifespan in animal models, such as flies and mice, made it very interesting to scientists as a potential anti-aging product. The science supporting this action is based on studies in yeast, flies and worms that show the levels of sirtuins are increased. Resveratrol was found to be one of the strongest candidates and was shown to extend yeast lifespan.¹⁹ The work on mammals is less clear. The gene in mammals that resembles Sir2 is called SIRT1, which CR is known to activate in some tissues. The role of SIRT1s in mammalian aging remains unknown.²⁰

So far, the evidence suggests that taking modest doses of resveratrol—from 100–200 mg—may help the body to metabolize fat better and activate the pathways that are turned on by CR. The other benefits of resveratrol make it worth taking as a supplement, even if the anti-aging aspect is not proven at this time.

Resveratrol and NF- κ B blockade

The esthetician should be familiar with this biological compound mentioned above. It is a transcription factor, so it activates some genes in the DNA molecule. This is a term that sooner or later you will need to know if you stay in the professional skin care field. Current evidence points out that by blocking the agents that turn on NF- κ B, you will prevent the inflammatory action that is associated with aging and actually allow the body to reverse the aging changes.²¹ Now that is a profound statement because it describes a major biological discovery. This is not the whole story, by far. Two camps exist in science on the topic of anti-aging. One is the negative group, the naysayers who disagree with everything, and the other is the positive group who are making real strides in understanding the aging process, and at the same time are discovering the cause of many diseases and finding ways to treat them.



PEANUTS AND GRAPES seem to be the major edible sources of resveratrol.

If you want to take resveratrol, it is important to read all the literature supplied by the vendor because it is a good source of information on resveratrol and to also speak with your physician. At present, with CR and resveratrol supplements, you have a good start on slowing down your aging rate.

Topical products will soon be available for skin care and should be very effective, but I strongly suggest you fully understand resveratrol and demand data for efficacy before you use or purchase these products. }

REFERENCES

A note on references: Some of these references you will be able to get directly from the Internet and others you may have to get a reprint from a university library. A good starting point is PubMed: www.ncbi.nlm.nih.gov/pubmed/.

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