Resveratrol

Resveratrol (3,4',5-trihydroxystilbene) is a natural substance with multiple medicinal applications and health benefits due to its preventive and therapeutic potential in various health conditions.

Resveratrol is a polyphenolic compound naturally occurring in many plants including fruits, herbs and vegetables. It is considered a plant's phytoalexin (antibiotic) produced in response to infections by various pathogens, injury or stress such as exposure to ultraviolet radiation.

The name "resveratrol" is derived from resorcinol (dihydroxybenzene) a compound first isolated from *Veratrum grandiflorum* by M. Takaoka in 1939[30]. In 1963 it was found in the Chinese and Japanese medicinal plant *Polygonum Cuspidatum* (aka Japanese Giant Knotweed). Interest in this molecule has been growing gradually since 1992 when it was suggested that its presence in wine could have cardio protective effects[19].

In 1997 the anti-carcinogenic effects of resveratrol were demonstrated and in 2003 the journal Nature reported its ability to significantly extend lifespan in the yeast *Saccharomyces cerevisiae*[13]. Later, other species were added to this list including the nematode *Caenorhabditis elegans*, the fruit fly *Drosophila melanogaster* and the short-lived *Nothobranchius furzeri* fish[34].

Today we know that resveratrol has a wide range of biological properties including antioxidant, anti-inflammatory, antimicrobial as well as anti-cancer, anti-hyperglycemic and antidiabetic. However, its anti-aging effects still remain controversial. Resveratrol has demonstrated cardiovascular and neuroprotective effects and it works in synergy with other naturally

occurring compounds as well as pharmaceutical drugs. All of this and much more makes resveratrol unique and worthy to explore in medical science as a potential "silver bullet" against many pathologies.

Sources of Resveratrol:

Resveratrol is found in a variety of plants and foods. The richest sources include grapes, blueberries, pistachios, peanuts, dark chocolate and many others. Red wine appears to be the most common resveratrol consumption source (Fig. 1)[3].

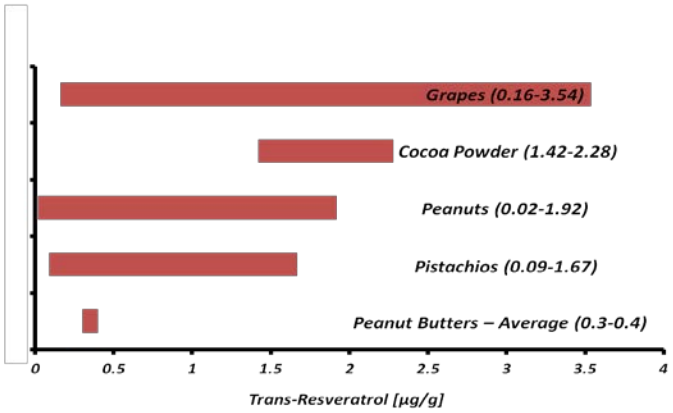


Fig.1. *Trans*-Resveratrol content in various foods

Resveratrol predominantly accumulates in the skin of grapes. Its concentration in wine, however, depends on a number of factors including the type of grape, geographic origin, environmental stressors (such as fungal infections) and production methods (Fig.2). In general, white wines contain less resveratrol than red wines which is primarily due to the preparation method. Red wine is fermented together with the grape skin whereas white wine is fermented without the skin[19].

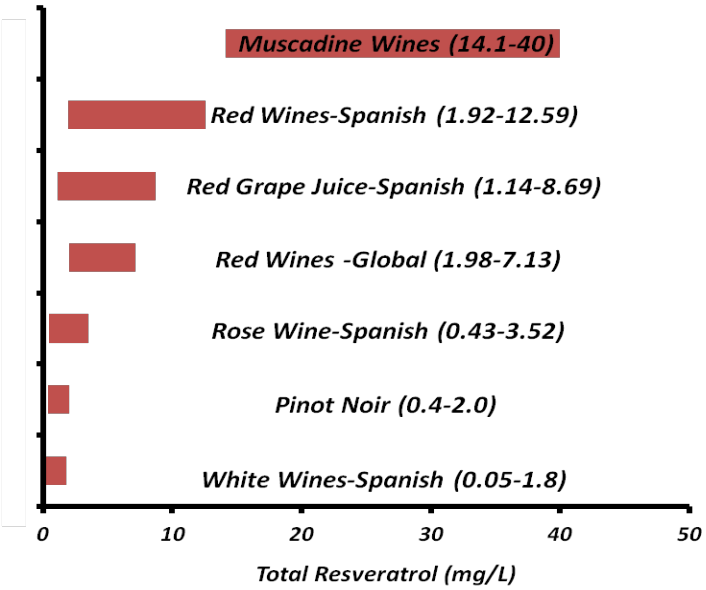
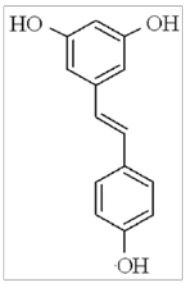


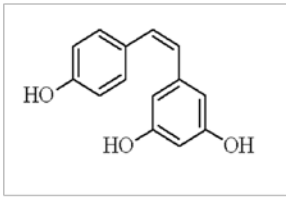
Fig.2. Total Resveratrol content in wines

Metabolic forms and bioavailability:

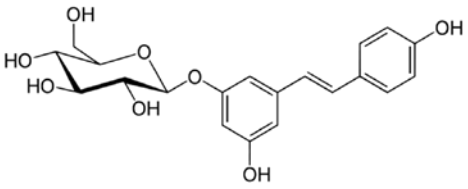
Resveratrol exists in two forms in plants: geometric isoforms called trans-resveratrol, and cis-resveratrol. Both isoforms can be either free or bound to glucose. In a bound form resveratrol is called piceid (see Fig.3).



trans-Resveratrol



cis-Resveratrol

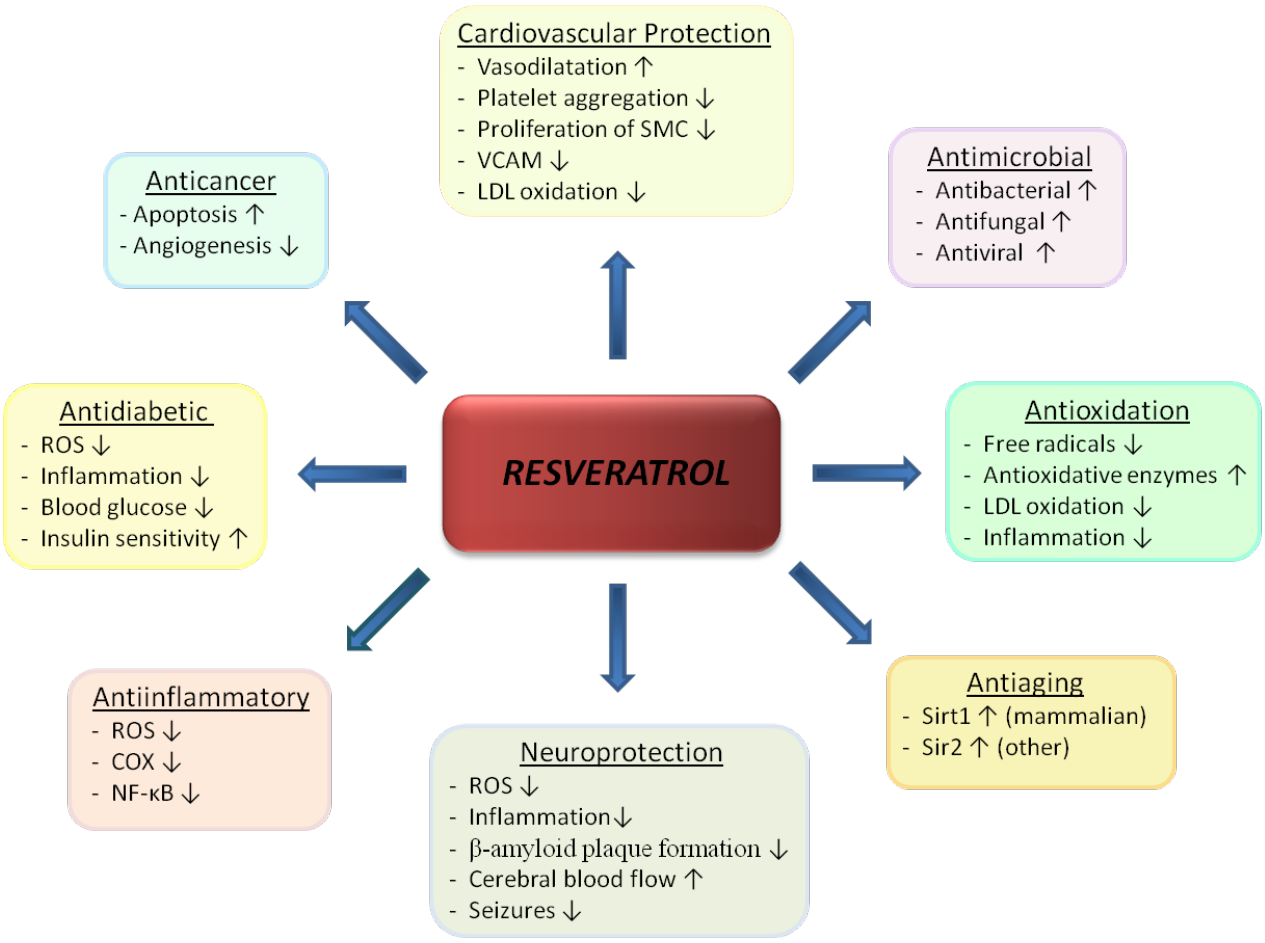


Resveratrol-3-O-β-D-glucoside
(Piceid, Polydatin)

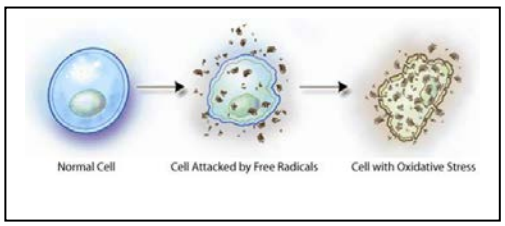
Various studies have demonstrated that resveratrol is well absorbed and quickly metabolized to sulfates and glucuronides by enterocytes (intestinal cells) and hepatocytes (liver cells). This rapid metabolism is one of the reasons why its bioavailability (proportion of a substance that enters the circulation and is able to be utilized by cells) is very low[27]. A study of 40 healthy volunteers who received from 0.5 g to 5 g of resveratrol in a single dose, demonstrated its peak (maximum) plasma (blood) concentration between 0.83 and 1.5 hours after consumption and a very low mean plasma concentration from 73 ng/ml to 539 ng/ml for a 0.5 g and 5 g resveratrol intake, respectively[6]. Corresponding concentrations of metabolites exceeded those of free resveratrol by approximately 20-fold. Therefore, there is a growing interest in studying the activities of these metabolites[5]. Both resveratrol and its metabolites are eliminated from the body in the urine and feces[7].

Finally it is important to take into consideration that most studies which have reported promising health benefits of resveratrol were conducted *in vitro* (in cell culture) and used high concentrations that are rather unachievable via oral supplementation due to its low bioavailability.

Health Benefits of Resveratrol



Antioxidant: Thousands of resveratrol studies have revealed its multiple medical applications.

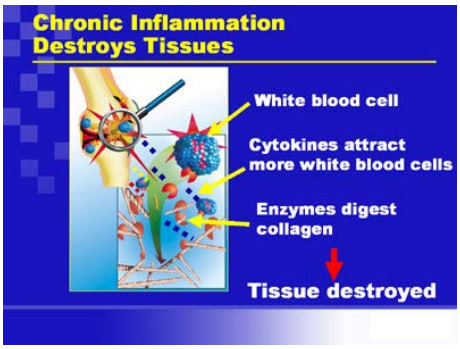


Much of this is attributed to its antioxidant properties and its ability to prevent and control oxidative stress (see description box). Resveratrol exhibits antioxidant properties/effects either as a potent free radical scavenger and/or by up-regulating cellular mechanisms of oxidative defense by increasing the expression of antioxidant enzymes[1, 28].

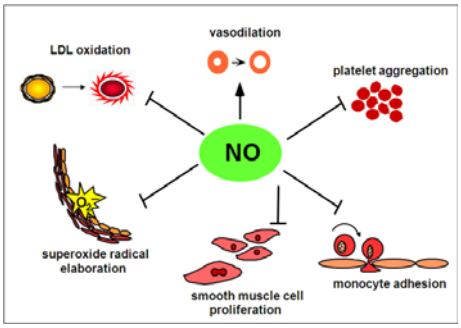
These enzymes, such as superoxide dismutase (SOD) catalase, or glutathione peroxidase, protect the cell's organelles and molecules like lipids, proteins, and DNA, against damage caused by free radicals, thus preserving the structural and functional cell integrity. Resveratrol has also been found to prevent low density lipoprotein (LDL) oxidation by chelating (binding) copper as well as by directly scavenging free radicals[10].

Oxidative stress
Free radicals such as reactive oxygen species (ROS) are constantly generated in our body. Due to their high reactivity, ROS adversely alter lipids, proteins, and DNA consequently underlying many diseases. Excessive production and/or inability to eliminate ROS lead to the condition known as oxidative stress

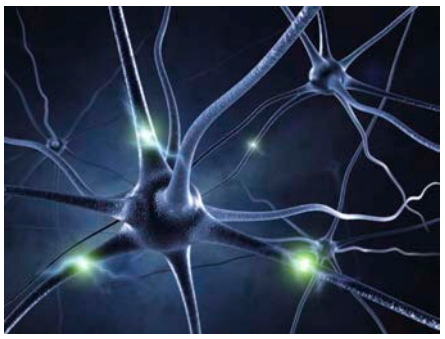
Inflammation: As an anti-inflammatory agent resveratrol presents its unique pleiotropic (multipoint) mechanism of action. First, as an antioxidant it inhibits the activation of pro-inflammatory cascade caused/initiated by free radicals. Second, it blocks the activity of the molecules (e.g., transcription factors, enzymes) responsible for the production of inflammatory mediators. Resveratrol does this either by repressing the protein complex called nuclear factor kappa B (NFkB), which is a transcription factor for many pro-inflammatory molecules including cyclooxygenase (COX) enzymes and/or by direct inactivation of COXs[1, 26]. COX enzymes are involved in the production of inflammatory mediators (e.g., prostaglandins) and therefore are a primary target for classic non-steroidal anti-inflammatory drugs (NSAIDs). Thus, resveratrol appears to be beneficial in the control of inflammatory conditions and autoimmune disorders where inflammation plays a key role such as arthritis, allergies, multiple sclerosis (MS), and in managing/coping with diabetes and arteriosclerosis.



Cardiovascular benefits: Resveratrol stands behind a phenomenon known as the "French paradox" which describes a reduced risk of heart disease in France in spite of a diet rich in fats. Studies show that moderate consumption of wine may benefit the cardiovascular system and resveratrol is thought to be a primary molecule responsible for this effect. It can involve



different mechanisms, which together contribute to cardio-protective outcomes. One of the most important features of resveratrol is its ability to stimulate nitric oxide (NO) production that results in vasodilation (widening of blood vessels) which is crucial for healthy blood flow. Additionally, resveratrol can inhibit platelet aggregation (clumping together of blood platelets) and that has huge health implications since excessive blood clotting may facilitate clogging of the blood vessels thereby restricting blood flow and causing a heart attack or stroke. Furthermore, resveratrol has been shown to inhibit proliferation of vascular smooth muscle cells (VSMC)[22] and vascular cell adhesion molecule expression (VCAM)[8] and to protect against LDL oxidation. All of these hinder atherosclerotic plaque formation and promote cardiovascular health.



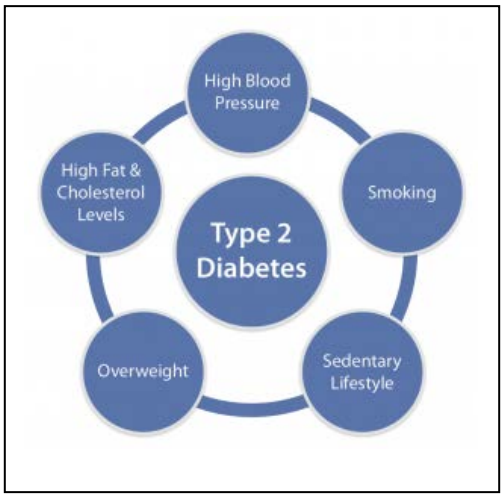
Nervous system: Many *in vitro* as well as animal and human studies have tested the neuro-protective effects of resveratrol. It has been suggested that its neuro-protective effects result from its antioxidant properties since oxidative stress and mitochondrial (mitochondria are the powerhouses of the cell) dysfunction are the main causes of several neurodegenerative diseases. Resveratrol's protective function against ischemic brain injury as well as its ability to prevent seizures and to act as an anticonvulsant have been demonstrated in many animal studies[1, 3]. The studies concluded that its antioxidant activity is a major factor behind these positive results.

Brain function: Resveratrol has been found to delay the onset of β-amyloid plaque formation, the cause of Alzheimer's disease (AD), where oxidative stress is proposed to be a primary reason for amyloid precursor protein (APP) alternation[1]. In mice resveratrol significantly reduced plaque formation in different sections of the brain: in media cortex (-48%), striatum (-

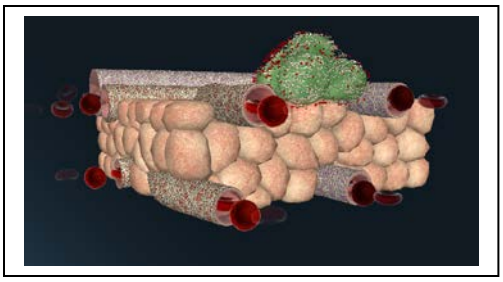
89%), and hypothalamus (-90%)[14]. Suggested mechanisms explaining these results include: reduction of oxidative stress, chelation of copper enriched in β-amyloids and removing amyloid deposits by increasing intracellular proteosomal activity that was observed *in vitro*[18]. Also, an *in vitro* study on cortical astrocytes (star shaped neurons) showed that resveratrol applied at low concentration increased both glutamate uptake and glutathione content. Glutamate is the major excitatory neurotransmitter and its excess is associated with neuro-degeneration/neurodegenerative diseases, whereas glutathione is the body's main antioxidant to combat free radicals. In the same experiment, the higher concentration of resveratrol gave opposite effects[1].

In a human study, oral supplementation with 250 mg or 500 mg of trans-resveratrol resulted in an increase in cerebral blood flow, explained by increased nitric oxide release[15].

Other metabolic aspects: Resveratrol has demonstrated potential in prevention and management of obesity, metabolic syndrome and diabetes. It was discovered that hyperglycemia leads to increased ROS production and subsequently to oxidative stress that eventually results in pancreatic β cell dysfunction and diabetes complications[1]. Moreover, oral administration of resveratrol enhanced the activity of antioxidant enzymes and decreased oxidative stress, which destroys β cells[1] as well as lowered blood glucose and triglycerides in diabetic rats[29]. In addition, it was noticed that hepatic glycogen synthesis and the utilization of glucose in a variety of tissues can be stimulated by resveratrol which has been associated with an improvement in insulin sensitivity[29]. Also, postprandial glucose levels in humans could be lowered without an increase in insulin production, indicating the insulin-sensitizing effect of resveratrol[27].



Cancer: Resveratrol displays promising antitumor properties since it has been found to inhibit the proliferation of various human cancer cell lines, including prostate, breast, stomach, colon,



thyroid and pancreatic[11, 31]. Animal cancer models have shown its efficacy in preventing and treating skin, esophageal, intestinal, and colon tumors[19]. Various anti-cancer cellular mechanisms of resveratrol have been suggested including accelerated apoptosis (programmed cell death) of tumor cells, and inhibition of angiogenesis (new blood vessel formation) which contributes to tumor starvation as well as its anti-metastatic effect by preventing the spread of cancer cells[16, 19]. Resveratrol has promising anti-cancer potential but because of its poor bioavailability the best efficacy has been limited to the tumors it can come into direct contact with (e.g., skin cancers or gastrointestinal tract cancers).

Aging: Based on animal studies resveratrol gained fame as an anti-aging molecule which has so far been associated with caloric restriction (CR), a dietary regime using low caloric intake without malnutrition. Both resveratrol and CR can activate a so-called longevity gene SIRT1.

SIRT1 is one of the key genes activated during calorie restriction which leads to a number of biological adaptations associated with longer lifespan[1]. Resveratrol was found to extend the lifespan of several species including yeast *Saccharomyces cerevisiae*, the nematode *Caenorhabditis elegans*, the fruit fly *Drosophila melanogaster* and the short-lived *Nothobranchius furzeri* fish[19]. In mice resveratrol counteracted the effects of a high-caloric diet in 144 out of 153 biochemical pathways, thus delaying or inhibiting many unfavorable changes and preventing early mortality in obese animals[4]. Recent studies suggest that increased longevity may be mediated by calorie restriction combined with the beneficial properties of resveratrol supporting healthy aging and a prolonged lifespan[1, 24].

Anti-microbial properties: Resveratrol is a plant polyphenolic phytoalexin (antibiotic) with

antiviral, antifungal and antibacterial properties. It has been shown to be effective against the herpes simplex virus (HSV) type 1 and 2, varicella-zoster virus, human

cytomegalovirus, certain influenza and respiratory viruses viruses, and it may synergistically enhance the efficacy of some anti-HIV drugs[19]. It has been shown that resveratrol can inhibit growth of some bacteria like *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa,* and *Helicobacter pylori* as well as the fungi *Aspergillus niger, Aspergillus flavus,* and *Candida albicans*[16].

Other: Resveratrol has shown a potential in activating expression of gamma-globin genes which regulate the production of hemoglobin HbF, which is produced by a fetus but ceases soon after birth. HbF can be very helpful in the treatment of beta thalassemia (inherited blood disorder)[5, 9].

Human Clinical Trials with Resveratrol

Tables 1 and Table 2 present short descriptions of clinical trials involving resveratrol supplementation. These recently conducted trials were selected from a review article "Resveratrol supplementation: Where are we now and where should we go?" by Marta G. Novelle et al. 2015[20]. For more information go to www.clinicaltrials.gov.

Abbreviations used in Table 1 and Table 2.

RSV - resveratrol, T2DM - type 2 diabetes mellitus, CVD - cardio vascular disease, LDL - low density lipoprotein, CRP - C-reactive protein, TNF-α - tumor necrosis factor alpha, PAI-1 - plasminogen activator inhibitor-1, IL-6 - interleukin 6, IL-10 - interleukin 10.

Table 1. Results from clinical trials involving resveratrol supplementation in participants with medical conditions [20].

125 OBJECTIVE	SUBJECTS	FORM & DOSE OF RESVERATROL	PRIMARY RESULTS AND CONCLUSIONS	TIMING OF ADMINISTRATION
To determine if RSV increases insulin sensitivity in T2DM.	19 males between the ages of 41.4–65.8 previously diagnosed with T2DM.	Placebo or 5 mg twice daily for one month.	Low RSV doses both improved insulin resistance and decreased blood glucose levels.	Participants were instructed to refrain from eating polyphenol rich foods 4 weeks before initiation of the study.
To determine if RSV improves glucose metabolism and vascular function in	Ten subjects (7 women) with mean age of 72 years diagnosed with	Daily intake of 1, 1.5 or 2 g of RSV for 4 weeks.	Daily doses of RSV between 1 and 2 g improved insulin sensitivity and post-meal plasma glucose.	RSV was divided into multiple doses following meals.

adults with impaired glucose tolerance (IGT).	impaired glucose tolerance.			
To determine if RSV has cardio protective effects on patients with history of myocardial infarction.	40 Caucasian patients (14 women) between the ages of 42 and 80 with a history of myocardial infarction.	Placebo or daily intake of 10 mg capsule of RSV for 3 months.	RSV significantly improved endothelial function and left ventricular diastolic function, while lowering LDL levels. Platelet aggregation and red blood cell deformability were also decreased by RSV.	Not specified.
To evaluate the effects of RSV treatment on bone turnover markers, mass, and structure in obese men with metabolic syndrome.	74 middle aged obese men between the ages of 41.8 and 56.8, all previously diagnosed with metabolic syndrome.	Placebo or intake of 500 mg/day, or 75 mg twice daily for 16 weeks.	Bone density increased in a dose-dependent manner by stimulating formation and mineralization.	Participants were instructed to refrain from consuming other nutritional supplements during the study period.
After ingestion, measure concentrations of RSV and its metabolites in the colorectal tissue of humans.	Twenty patients (11 women) between the ages of 46 and 83 with histologically confirmed colorectal cancer.	Daily intake of 0.5 or 1.0 g of RSV for 8 days before surgery.	Ki-67 level (proliferation marker) was reduced by 5% and 7% in cancer and normal tissue, respectively.	RSV consumed during the evening.
To investigate the role of RSV on inflammatory and fibrinolytic status of patients with high risk of CVD.	75 volunteers (41 women) between the ages of 45 and 72 taking statin medications.	Daily intake of 8 mg for six months and 16 mg for the next six months. Capsules were taken in the morning.	RSV-rich grape supplement improved the inflammatory and fibrinolytic status in patients who were on statins for primary prevention of CVD and at high CVD. RSV decreased high-sensitivity C-reactive protein, TNF- α, PAI-1, and IL6/IL-10 and increased IL-10 levels.	Subjects told not to take any other supplements and abstain from drinking alcohol for the duration of the study.
To investigate the effect of RSV on insulin signaling and function in patients with metabolic syndrome.	24 patients previously diagnosed with metabolic syndrome between the ages of 30 and 50 (number of women not noted).	Placebo or 500 mg RSV 3 times a day for 90 days.	In the RSV group, there were significant reductions in total weight, BMI, fat mass, and weight circumference. There were also significant differences in insulin sensitivity.	RSV capsules were taken 3 times a day before meals.

Table 2. Results from clinical trials involving resveratrol supplementation in obese "healthy" participants [20].

OBJECTIVE	SUBJECTS	FORM & DOSE OF RESVERATROL	PRIMARY RESULTS AND CONCLUSIONS	TIMING OF ADMINISTRATION
To investigate whether RSV has beneficial effects on inflammation and oxidative stress markers in smokers.	50 healthy volunteers (35 women) between the ages of 20 and 50 with current and past smoking histories. Under no medication.	500 mg daily for three months, followed by a 3-month washout period, followed by placebo (or vice versa).	Decreased levels of C-reactive protein and triglycerides, and increased total antioxidant levels. Weight circumference, blood pressure, and cholesterol did not significantly change.	Thirty-day washout period in which participants were instructed not to consume polyphenols.
To investigate if RSV supplementation decreases markers of oxidative and inflammatory stress.	2 groups of 10 normal, healthy age matched adult subjects with mean age of 36 ± 5. Under no anti-inflammatory drugs.	Placebo or daily intake of 40 mg RSV for 6 weeks.	RSV supplementation decreased markers of oxidative stress and inflammation (TNF- α, IL-6, C-reactive protein) after a high-fat, high calorie meal.	Subjects were informed to refrain from anti-inflammatory drugs for the duration of the study.
To determine if cerebral blood flow and cognitive performance improve after oral RSV supplementation.	Bioavailability assessment: 9 healthy men between the ages of 21–29. Cognitive performance: 24 healthy adults (20 women) between the ages of 18	Doses of 250 mg and 500 mg of RSV on separate days. Doses were given in a single administration.	RSV dose-dependently increased cerebral blood flow, without cognitive changes.	Participants were fasted and 45 min was allowed for absorption.

	and 25. Under no medication.			
To investigate the effects of a 30-day RSV supplementation on metabolic profile.	11 obese, but otherwise healthy men with a mean age of 52.5 ± 2.1. Under no medication.	Daily intake of 150 mg RSV for a 30-day period.	Clinical measurements were significantly improved after RSV consumption, including blood pressure and respiratory quotient. Lowered postprandial energy expenditure and adipose tissue lipolysis, and increased oxidative phosphorylation.	Subjects told not to take any other supplements and abstain from drinking alcohol for the duration of the study.
To determine whether RSV supplementation enhances memory in older adults and, if so, investigate the underlying mechanisms.	46 overweight but otherwise healthy adults (18 women) between the ages of 50 and 75. Under no antidepressant drugs.	Placebo or daily intake of 200 mg RSV for 26 weeks.	Memory retention was significantly increased in the RSV group and functional connectivity of the hippocampus with the parietal, frontal, and occipital areas was improved.	4 capsules a day. 2 before the first main meal and 2 before the second main meal.

Synergy

All biochemical reactions and metabolic pathways in our body are interrelated and involve various compounds (e.g. substrates, cofactors) and as well they are influenced by external factors (e.g., temperature, pH). Often the same result may be accomplished through alternative pathways. Therefore it is essential that optimal conditions and required compounds are present in the cells at the same time in order to avoid any missing links and to achieve maximum biological effects.

Metabolism is based on biological synergy between substances that are directly involved in the same pathway or indirectly through alternative pathways that eventually result in the same physiochemical response. Synergistic interactions between different compounds can benefit various cellular processes, such as increasing absorption or bioavailability of molecules involved in the process (helping them to get to the reaction place at the right moment, in the required amounts). Thus, synergy allows for maximizing biological effects and maintaining metabolic balance without using megadoses of individual nutrients.This principle of biological synergy was pioneered by Dr. Rath's research and applied in designing various nutritional supplement formulations. Its advantage is better efficacy through the use of moderate nutrient doses compared to a single compound application, thus maintaining cellular metabolic balance - the basis of health.

Table 3 presents select natural and synthetic compounds proven to work in synergy with resveratrol. In addition, resveratrol has a protective effect and can alleviate toxic side effects of many drugs such as arsenic trioxide or doxorubicin[7, 26].

Table3. Resveratrol synergy with natural compounds and drugs.

Property	Results of resveratrol synergy	Synergy with natural compounds	Synergy with drugs
Antioxidant	[21] Membrane lipids oxidation ↓ (in vitro on human erythrocytes)	Quercetin Pterostilbene	
	[1] Heme-enhanced oxidation ↓	Curcumin	
Anti-inflammatory	[14] Inflammatory markers ↓ (mice study)	Quercetin	
Cardiovascular protection	[16] Ischemic injury ↓ Autophagy ↑(rats study)	γ-tocotrienol	
	[14] Vascular smooth muscle cell (VSMC) proliferation ↓ (in vitro)	Quercetin	
	[14] Stenosis ↓ (mice study)	Quercetin	
Chemoprevention / Anticancer	[19][20] Lung cancer ↓ (mice study)	Curcumin	
	[10] Human lung adenocarcinoma↓ (in vitro) [7] Cardiotoxicity decreased		Arsenic trioxide (As_2O_3)
	[9] Skin cancer ↓ (mice study)	Black tea polyphenol (BTP)	
	[15] Skin cancer ↓ (mice study)	Ellagic acid Grape seed extract (topically or in the diet)	
	[6] Skin cancer ↓ (in vitro and in vivo)		5-fluorouracil (5-FU)
	[13] Melanoma cell viability ↓ (in vitro)	Ursolic acid	
	[4] Human liver cancer cells (HepG2) ↓ (in vitro)	Tanshinone IIA (Tan IIA)	
	[7] Acute promyelocytic leukemia (APL) ↓ (in vitro)		Arsenic trioxide (As_2O_3)
	[18] Colon cancer ↓(in vitro and in vivo)	Curcumin	
	[11] Head and neck squamous cell carcinoma (HNSCC) cell lines ↓		Etoposide
	[17] Human liver cancer cells (HepG2) ↓ HeLa cells (immortal cell line) ↓	Artemisinin (ART)	
	[12] Breast cancer cells ↓ (in vitro on MCF-7)	γ-tocotrienol	
	[24] Ovarian cancer ↓ (in vitro)	Indole-3 Carbinol (I3C)	
Anti-obesity	[23] Adipogenesis ↓ Adipocyte apoptosis ↑ (in vitro on primary human adipocytes)	Genistein+Quercetin	
	[3] Fatty acid oxidation ↑ AMPK, Sirt1, Sirt3 activity ↑ (in vitro on mouse 3T3-L1 adipocytes)	Leucine	
	[3] Adipose Sirt1 activity ↑ Muscle glucose and palmitate uptake↑ Insulin sensitivity ↑ (mice study)	Leucine	
	[2] Adipose tissue triglyceride (TG) accumulation ↓ (rats study)	Quercetin	
Immunomodulation	[27] Stimulation of immune system ↑	β-1,3-Glucan	

Antimicrobial	[25] Chlamydia pneumonia ↓ (in vitro)		Clarithromycin Ofloxacin
	[8] Leishmania amazonesis ↓ (in vitro)		Amphotericin B
	[24] HIV-1 ↓ (in vitro)		Decitabine
	[22] Streptococcus mutans biofilms ↓	Fraction separated from Polygonum cuspidatum (resveratrol 16.2%, emodin 18.9%, physicon 2.07%)	Fluoride
Anti-aging	[5] Mitochondrial mass ↑ Mitochondrial DNA content ↑ Mitochondrial biogenesis factors (PGC1-α, TFAM, NRF-1) ↑ SIRT1 enzymatic activity ↑ (in vitro on HUVECs)	Equol	

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Resveratrol Safety and Supplements

Most resveratrol studies have been conducted using *in vitro* and animal models, with a relatively low number of human studies. All clinical trials with resveratrol are widely diverse regarding the doses and time of supplementation. In addition, they involve individual variability such as sex, age, diet, concomitant medication and physical activity levels all of which might contribute to its bioavailability and physiological responses[7, 20].

Available data suggest that resveratrol is well tolerated and non-toxic for daily consumption up to 450 mg for a 132 lb (60-kg) person[25]. The highest safe resveratrol intake reported was 5 g in a single dose[6].

Since resveratrol can interact with other substances and has pleiotropic physiological effects, there are some aspects which need to be considered:

- Resveratrol might decrease blood clotting, and people with bleeding disorders and those taking anticoagulants, anti-platelet drugs or NSAIDs should take that into consideration.
- As an estrogen agonist it might affect some hormone-sensitive conditions.
- Resveratrol can affect detoxification pathways in the body (cytochrome P450 enzyme system) and individuals on medications metabolized by the liver system should consult a physician regarding adjusting their medication doses.

Due to a variety of resveratrol supplements available on the market today, a few facts should be taken in consideration before making a purchase.

- Only *trans*-resveratrol (not *cis*-resveratrol) has been associated with health benefits [5]. Therefore, the manufacturer should clearly declare on the label the total content of

trans-resveratrol. Terms like "Red Wine Complex" or "Grape Extract" seem to be useless and misleading.

- Grapes are not the best source of resveratrol in supplements because of two primary reasons:
    - The concentration of resveratrol in grape skin is relatively low to produce a high quality and potency product.
    - Due to a wide use of toxic chemicals in the grape cultivation process, it is very difficult to obtain a supplement free of contaminants.

For these reasons, in most of the *in vitro* and *in vivo* studies the source of resveratrol was Japanese Giant Knotweed, which grows in the wild and without artificial fertilizers. This plant is a source for Transmax resveratrol, which is the concentrated pure resveratrol (synthetic and GMO free and without fillers, additives, or expanders) and it has been used in most clinical trials[35].

Research Insight Behind Resveratrol Effects at the Cellular and Molecular Levels

Thousands of *in vitro*, *ex vivo* and *in vivo* studies have shown that resveratrol can act as an activator and inhibitor of numerous pathways and it affects various factors inside and outside the cell.

A**ntioxidant:** Resveratrol is a potent scavenger of hydroxyl, superoxide, superoxide anion, singlet oxygen, nitrogen oxide, and metal-induced radicals[1],and also up-regulates cellular mechanisms of oxidative defense. It activates antioxidant enzymes such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), glutathione S-transferase (GST), and glutathione reductase (GR)[16, 27, 28].

In human studies resveratrol supplementation significantly increased nuclear factor (erythroid-derived 2)-like 2 binding activity, also known as (Nrf-2) as well as mRNA expression of the NAD(P)H dehydrogenase [quinone]1 (NQO-1) and glutathione S-transferase pi 1 (GST-π1) genes, suggesting its strong antioxidant effect[27].

Resveratrol also shows protective effects against cell membrane lipid peroxidation and DNA damage caused by ROS[1] as well as LDL oxidation by free radicals and cupric ions that are chelated by resveratrol[10].

Inflammation: Resveratrol's anti-inflammatory properties may be mediated by its effects on nuclear factor kappa B (NFkB) and the Inhibitor of kappa B (I-kB) kinase activity[16]. When NFkB is activated, it migrates to the nucleus where as a transcription factor it induces expression of multiple pro-inflammatory genes such as COX-1, COX-2, TNF-α, IL-1, IL-8, inducible nitric oxide synthase (iNOS), VCAM-1, and many others[24, 26]. It has been shown that resveratrol reduced the transcriptional activity of the p65 subunit of NFkB and inhibited IkBα (the inhibitor of NFkB) degradation[23]. Moreover resveratrol blocked the ubiquitination of NEMO (subunit of IkB kinase) and inhibited IkB kinase(beta)-mediated NFkB activation[23].

Since NFkB is involved in inflammation and inflammatory related disorders and as well is linked to almost all types of cancer this indicates therapeutic potential of resveratrol. Interestingly, resveratrol can also directly inactivate cyclooxygenase (COX) enzymes at both cyclooxygenase and peroxidase active sites, whereas most non-steroidal anti-inflammatory drugs (NSAIDs) target the cyclooxygenase reaction only[1]. Furthermore, resveratrol can reduce the production of prostaglandin E2 (PGE2) in LPS-activated microglial cells by inhibiting the expression of microsomal PGE2 synthase-1 (mPGES-1)[14]. Also, in a mouse model of chronic colitis it decreased inflammatory cytokines TNF-α, IL-1β as well as COX-2 and iNOS activity and increased anti-inflammatory cytokine IL-10[24].

It is important to note that in lipopolysaccharide-activated macrophages, resveratrol can increase the production of IL-10, while significantly decreasing the secretion of interferon-gamma (IFN-γ), TNF-α, IL-1, IL-4,and IL-6[24].

SIRT1 mediated effects: Resveratrol may activate the SIRT1 which stands for sirtuin (silent mating type information regulation 2 homolog) 1 which is an enzyme that deacetylates proteins that contribute to cellular regulation such as reaction to stressors and longevity. It was found that resveratrol can enhance the binding between Sirtuin1 and Lamin A, which is a direct cellular activator of Sirtuin1[2].

SIRT1 can modulate p53 functions as a transcription factor. Both participate in cell survival under oxidative stress conditions as well as regulate cell metabolism, stress signaling, genome stability and cell cycle control. SIRT1 also controls the forkhead O (FOXO) family as a p53-independent cell protective pathway. Interestingly, FOXO1, FOXO3a, and FOXO4 were found to be involved in SOD2's (Superoxide Dismutase) up-regulation upon resveratrol treatment[12]. Furthermore, the SIRT1/FOXO factor axis is involved in resveratrol-induced eNOS transcriptional activation. It is well known that many cardiovascular protective effects of resveratrol are linked to enhanced production of NO (Nitric Oxide) by eNOS and resveratrol has been shown to enhance both eNOS gene expression (via SIRT1/FOXO axis) and eNOS enzynatic activity[36].

Another mechanism of resveratrol via SIRT1 (in moderate doses) and SIRT1-independent (in high doses) pathways is induction of AMPK activation (AMP-activated protein kinase)[21]. AMPK is a central metabolic sensor and regulator of many intracellular systems such as glucose uptake, oxidation of fatty acids, biogenesis of GLUT4 and mitochondria. It maintains the energy balance of the cell by modulating ATP levels.

Cellular energy. In addition to SIRT1 mediated effects on cellular energy metabolism, resveratrol may affect cellular energy by its effect on peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), which results in an increase of PGC-1α activity. PGC-1α increases mitochondrial biogenesis and function decreasing oxidative stress in many tissues and organs including the brain, heart, liver, and skeletal muscle, and hence improves their function[1]. In this aspect it has been suggested that resveratrol can be considered in developing natural approaches to obesity and type 2 diabetes. (Liang H, Ward WF, PGC-1alpha: a key regulator of energy metabolism, Adv. Physiol.Educ. 2006; 30(4), 145-151)

Anti-cancer effects: Among many anti-cancer mechanisms of resveratrol, it has been shown that it can decrease anti-apoptotic proteins expression as well as inhibit signal transduction through the mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K), or NFkB mediated pathways in cancer cells[1]. The investigation of anti-angiogenic effects of resveratrol using HUVEC (Human Umbilical Vein Endothelial Cells) demonstrated that at higher concentrations resveratrol can suppress PKG activity (cGMP-dependent protein kinase). Subsequently, this enzyme decreases the expression of the inhibitor of pro-apoptotic proteins (IAPs) such as c-IAP1, c-IAP2, livin and XIAP which results in the inhibition of HUVEC's tube formation and consequently, angiogenesis[33].

In addition, resveratrol has been shown to inhibit protein kinase C (PKC), Akt (PKB), Bcl-2 phosphorylation, focal adhesion kinase (FAK), and matrix metalloprotease-9 (MMP-9)[1], thereby affecting various cancer mechanisms. In T-cell acute lymphoblastic leukemia cells it increased a pro-apoptotic Bax, p53, and p21waf, whereas it decreased anti-apoptotic Bcl-xL, Bcl-2, cyclin D1, and TNF receptor-associated factor 2[31]. In a human study the decreased level of IGF-1 and IGFBP-3 after ingestion of high doses (2.5g/daily) of resveratrol has been observed[19].

The study in breast cancer showed that resveratrol can interfere with estrogen synthesis as a potent aromatase inhibitor[32]. Interestingly, resveratrol can have both agonistic and antagonistic activity. It can act as an estrogen receptor antagonist in the presence of estrogen and as an agonist in its absence[17].

Its anti-cancer effects may also be mediated by its antagonistic effects on aryl hydrocarbon receptors (AhRs), which are ligand-activated transcription factors bound to inactive chaperones. Various animal and human data suggested that AhR is involved in various signaling pathways critical to cell normal homeostasis, such as cell proliferation and differentiation, gene regulation, cell motility and migration, inflammation and others. Dysregulation of these physiological processes is known to contribute to tumor initiation, promotion, and progression. Resveratrol is a competitive antagonist against some AhR ligands such as TCDD which is highly toxic dioxin[1]. Inhibition of AhRs prevents the expression of cytochrome P450 enzymes involved in phase I liver detoxification and are considered as activators of procarcinogens[3].

The recent study published in Nature (December 2014) reported that resveratrol can mimic tyrosine due to structural similarity and bind to the enzyme TyrRS (Tyrosyl tRNA synthetase). After translocating to the nucleus, this enzyme can activate poly-ADP-ribose polymerase (PARP1) which is an important multifunctional enzyme. One of the PARP1 functions is to repair damaged DNA and since resveratrol is involved in its activation, this could be another mechanism explaining its anti-cancer benefits.

In summary, the above scientific data showing the efficacy of resveratrol on the cellular level reflect only a small part of ongoing research. Resveratrol is the example of a natural molecule with wide potential in prevention and therapy of many health conditions.

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