A Literature Review of Dietary Resveratrol Supplements: Do they Mimic the Effects of Endurance Exercise?

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A Literature Review of Dietary Resveratrol Supplements:

Do they Mimic the Effects of Endurance Exercise?

Abstract

Resveratrol is a polyphenol whose best dietary sources are red wine, red grapes, and peanuts. It has become a popular dietary supplement because it has been suggested to be responsible for the cardioprotective effects of red wine. Resveratrol may also mimic the effects of calorie-restriction to increase longevity, as shown in several animal studies. Resveratrol is thought to exert its effects primarily by activating a class of proteins called sirtuins. These proteins are NAD$^+$-dependent deacetylases that potentially regulate metabolism and gene expression. A further increase in the popularity of resveratrol supplements occurred following reports that it may also function as an exercise mimetic, inducing some of the same physiological adaptations in skeletal muscle that are observed with endurance exercise training. Through the AMPK-SIRT1-PGC-1α pathway, resveratrol has been shown to induce transcription of nuclear encoded mitochondrial proteins, and thereby induce mitochondrial biogenesis. In animal studies, the amount of resveratrol needed to induce these effects is considerably high. Commercially available supplements may not contain enough resveratrol to be effective as exercise mimetics. However, some supplements may improve glucose tolerance and insulin sensitivity, as shown in human studies.
Introduction

Phenolic compounds contain one or more aromatic rings with a hydroxyl group attached to at least one of these rings (2). Phenolics are generally divided into two categories, flavonoids and non-flavonoids. Flavonoids are polyphenolic compounds comprising of 15 carbons, with 2 aromatic rings connected by a 3-carbon bridge (2). There are several categories of non-flavonoid phenolic compounds; resveratrol is a member of a group of non-flavonoid polyphenolics known as stilbenes that have a general structure of two 6-carbon phenolic rings joined by a two carbon bridge (29). These compounds tend to be produced by plants in defense against pathogens. High intake of dietary phenols has been associated with a reduction in degenerative diseases, such as cardiovascular disease, cancer, obesity, and diabetes (29).

Resveratrol (3,5,4′-trihydroxystilbene) is a polyphenol commonly found in foods such as red wine, grapes, and peanuts and has been shown to have several health benefits (6, 52). Resveratrol was first discovered in the 1940′s and has hence forth been used to explain the “French paradox” (52). The “French paradox” is a term used to refer to the phenomenon in which although the French consume a diet high in saturated fat they have a two- to three- fold lower incidence of cardiovascular related diseases when compared to other countries, such as the U.S. (11). This theory suggests that resveratrol has cardioprotective effects which may be attributed to resveratrol's ability to improve vascular function by inhibiting platelet aggregation, reducing blood pressure, and increasing the expression of the enzyme responsible for the synthesis of the vasodilator nitric oxide (NO) (12, 43, 47, 57). Bradamante et al. discovered that resveratrol supplementation creates a “preconditioning” affect which reduces the damage caused by acute ischemia and reperfusion injuries, which could be a result of improvements in
cardiovascular function (5). These improvements in cardiovascular function may also explain why resveratrol has also been shown to delay/prevent neurodegenerative disease and stroke-induced brain damage (4, 44).

Resveratrol has been shown to increase longevity. Resveratrol supplementation has been shown to increase lifespan of fish, flies, and worms, and has natural antioxidant properties (3). It is thought that resveratrol affects longevity through sirtuin activation. Sirtuins act on gene transcription, DNA repair, cell signaling, and apoptosis allowing them to play a key role in longevity (11). Through sirtuin activity, resveratrol supplementation has also been shown to improve longevity in mice by protecting against cancer (6).

Resveratrol may mimetic the effects of endurance exercise and serve as a remedy for age-related chronic diseases. Like endurance training, resveratrol has been suggested to improve endurance capacity by enhancing lipid metabolism through an increase in mitochondrial content and a reduction in reactive oxygen species (ROS) produced by mitochondria (40, 55). The outcome of resveratrol supplementation improves cellular metabolic processes in humans and may possibly prevent or delay many chronic diseases (53).

Understanding the mechanism of action of resveratrol is critical for development of drugs to combat chronic diseases such as cardiovascular disease, diabetes, cancer, and obesity. Development of more potent and molecularly specific resveratrol-derived agents could produce a drug to treat metabolic disorders (45, 54).

**Mechanism of physiological adaptations in response to endurance exercise**

Endurance exercise activates an array of metabolic pathways. “Skeletal muscle is a malleable tissue capable of altering its type and amount of protein in response to disruptions to
cellular homeostasis” (14). Endurance exercise is known to enhance cardiovascular function and aerobic capacity through increases in mitochondrial content within muscle as well as increasing the capillary-to-muscle fiber ratio (24). Increased contractile activity is the initial signal in pathways responsible for exercise induced adaptations (24). Contractile activity causes ATP breakdown and calcium influx, both of which signal activation of downstream kinases (24).

Increases in the AMP:ATP ratio within the cell, as seen during exercise, stimulates the energy sensing enzyme AMP-protein activated kinase (AMPK) (24). Activation of AMPK induces activation of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) (27).

PGC-1α is a coactivator for several nuclear transcription factors, such as peroxisome proliferator-activated receptor gamma (PPAR-γ) and nuclear respiratory factor 1 and 2 (NRF1 & 2) (42). These nuclear transcription factors are assembled on the DNA promoter region and stimulate the expression of nuclear encoded mitochondrial proteins (NEMP), which are required for mitochondrial gene expression, replication, and assembly.

AMPK activation also triggers silent mating type information regulation 2 homolog 1 (SIRT1) activation by AMPK-derived increases in its substrate, NAD⁺ (9, 10). SIRT1 is a NAD⁺-dependent deacetylase enzyme and is therefore considered an energy sensing enzyme (58). SIRT1 is known to deacetylate histones as well as non-histone proteins rich in lysine in an NAD⁺ consuming reaction (13). SIRT1 deacetylates PGC-1α and positively regulates its activity (19, 48). SIRT1 also has the potential to deacetylate kinases responsible for the phosphorylation of AMPK (28). Therefore, SIRT1 and AMPK are considered to reciprocally activate each other (25).
PGC-1α is a key regulator of adaptations to endurance training (36). PGC-1α coactivates nuclear transcription factors responsible for gene transcription of NEMP involved in mitochondrial biogenesis (49). Post-translational modification of PGC-1α affects its activation status (24). Deacetylation of PGC-1α by SIRT1 improves its ability to promote expression of NEMP (48). PGC-1α can also be activated via phosphorylation by AMPK (30).

PGC-1α transcription and activation have been shown to increase during metabolic stress, exercise and calorie restriction (19). PGC-1α regulates mitochondrial biogenesis through post-translational modification of existing PGC-1α protein and increases in PGC-1α protein expression (8, 60).

Overexpression of PGC-1α correlates with increases in mitochondrial content and a shift towards a more oxidative fiber type composition (23, 46). Increases in mitochondrial content were not seen in PGC-1α-knockout mice in response to energy deprivation (62). The studies previously discussed support the conclusion that PGC-1α is the final regulator of mitochondrial biogenesis. Therefore, anything that artificially increases PGC-1α activity may lead to an increase in mitochondrial biogenesis.

**Mechanism of resveratrol supplementation**

The mechanism through which resveratrol induces metabolic adaptations involves a number of cellular responses. By activating SIRT1 and/or AMPK, increased PGC-1α activation follows and transcription of NEMP occurs. Figure 1 shows how endurance exercise and exercise mimetics, such as resveratrol, might target the same cellular pathways; therefore inducing the same physiological adaptations.
SIRT1 and mitochondrial biogenesis

Mitochondrial biogenesis is SIRT1-mediated. Price et al. examined the effects of SIRT1 overexpression on mitochondrial biogenesis. Price used transgenically altered mice to induce SIRT1 overexpression. Figure 2A shows the results of SIRT1 overexpression on PGC-1α mRNA content. A significant increase in PGC-1α mRNA content was shown in SIRT1 overexpression mice compared to wild type mice (p<0.05). SIRT1-mediated activation of existing PGC-1α protein increases PGC-1α expression, due to self-induced promoter activation (24, 60). Therefore increases in PGC-1α mRNA in response to SIRT1 overexpression suggests increased PGC-1α activation. Increases in PGC-1α activity are associated with increased mitochondrial biogenesis.
Mitochondrial DNA content was also significantly increased in SIRT1 overexpression mice, figure 2B (p<0.05). SIRT1 plays a key role in acute and chronic exercise-mediated mitochondrial biogenesis (16, 21). The data in figure 2 provides evidence that SIRT1 overexpression is sufficient to induce mitochondrial biogenesis, allowing SIRT1 to function as an exercise mimetic.

SIRT1 mRNA levels are elevated following endurance exercise. In humans, SIRT1 mRNA has been shown to increase after marathon running and after a three hour cycling session (18, 38). SIRT1 protein content increases in humans after a single bout of cycling and in rats following 45 minutes treadmill running (20, 51). Therefore, anything that increases SIRT1 activity and content may also increase PGC-1α activity, resulting in mitochondrial biogenesis.

Resveratrol induces mitochondrial biogenesis through SIRT1. To determine the role of SIRT1 in resveratrol-mediated mitochondrial biogenesis SIRT1-knockout mice were used.
Figure 3 shows the effects of SIRT1-knockout on mitochondrial DNA content in mice treated with resveratrol. Wild type resveratrol-treated mice display a significant increase in mitochondrial DNA content (p<0.05). On the contrary, SIRT1-knockout mice did not display a significant change in mitochondria content when treated with resveratrol. These results imply that SIRT1 plays a key role in resveratrol-mediated mitochondrial biogenesis, since adaptations were not seen in SIRT1-knockout mice.

This study is consistent with the finding that SIRT1 and PGC-1α are activated by resveratrol, altering muscle gene expression and inducing mitochondrial biogenesis (61). In other studies, SIRT1-knockout mice show a decrease in PGC-1α mRNA content and increased PGC-1α acetylation levels (1, 19). Treatment with SIRT1 inhibitors, such as nicotinamide, decreases the expression of mitochondrial genes regulated by PGC-1α (19). Resveratrol induces SIRT1-
mediated mitochondrial biogenesis by increased deacetylation of PGC-1α (13). This leads to the conclusion that resveratrol-mediated physiological adaptations may be SIRT1-dependent.

**AMPK and SIRT1 activation**

Since AMPK is an activator of SIRT1, then perhaps resveratrol-mediated SIRT1 activation is not a direct effect, but rather an indirect outcome of resveratrol-mediated AMPK activation (9). To test this hypothesis and determine if AMPK is required for resveratrol-induced mitochondrial biogenesis, Um et al. used genetically modified AMPK-knockout mice.

In wild type mice, resveratrol treatment significantly increased PGC-1α mRNA content (p<0.05), shown in figure 4A. AMPK increases SIRT1 activity in response to increases in NAD⁺ levels, which leads to the activation of PGC-1α, resulting in increased PGC-1α mRNA (9, 27). There was no significant change in PGC-1α mRNA content in AMPK-knockout mice. This corresponds to significant decreases in PGC-1α deacetylation in AMPK-knockout mice (10). AMPK activation induces SIRT1-mediated activation of PGC-1α as a result of elevated NAD⁺ levels, the substrate for SIRT1 (9). SIRT1-mediated deacetylation of PGC-1α requires functional AMPK (10).

Figure 4B shows significant increase in mitochondrial DNA content in resveratrol-treated wild type mice (p<0.05). However, AMPK-knockout mice did not show a significant change in mitochondrial DNA content when treated with resveratrol. These data suggest that resveratrol cannot activate SIRT1 without AMPK (52). The ability of resveratrol to function as an exercise mimetic may be AMPK-dependent.
Physiological Adaptations Seen With Resveratrol Supplementation

Lagouge et al. demonstrated that resveratrol supplementation in mice induces mitochondrial biogenesis and improves aerobic capacity (figure 5). Metabolic adaptations can be attributed to regulation of gene transcription (17). Figure 5 provides evidence that resveratrol induces mitochondrial biogenesis, confirmed by the increase in VO$_2$max and exercise endurance ($p<0.05$). Resveratrol-mediated activation of AMPK elevates intracellular NAD$^+$ levels (45, 54). Through SIRT1 deacetylation, resveratrol reduces the level of PGC-1$\alpha$ acetylation in wild type mice (10). Induced mitochondrial biogenesis and function is attributed to PGC-1$\alpha$ activation and mediates muscle fiber-type switching (37). Resveratrol supplementation in mice was shown to increase aerobic capacity as demonstrated in figure 5A & B, due to significant increases in both VO$_2$max and distance run at exhaustion.

Resveratrol has been shown to stimulate a shift towards a more oxidative phenotype. This is achieved by a reduction in the amount of type IIb highly glycolytic fast-twitch fibers and a rise
in the amount of type IIa and IIx oxidative fast-twitch fibers (45). The conversion of glycolytic fast-twitch fibers to oxidative fast-twitch fibers, which are mitochondria rich, improves physical endurance (33). Resveratrol-treated mice demonstrated a 2-fold increase in distance run at exhaustion (figure 5B), suggesting an increase in exercise endurance.

Other adaptations induced by resveratrol supplementation include increased metabolic rate, decreased fat mass, decreased body weight, increased glucose tolerance, and increased insulin sensitivity (41, 54).

In humans, resveratrol supplementation leads to several cellular adaptations. To determine if resveratrol-mediated adaptations seen in animal studies are also seen in humans, a double-blind cross-over study was performed (53). Eleven obese but otherwise healthy men were given either placebo or resveratrol, 150mg/day, for thirty days. Figure 6 shows the effects of resveratrol on citrate synthase activity, which is used as a measure of mitochondrial content. Decreased citrate synthase activity is associated with mitochondrial dysfunction, as seen in diabetes (32). Prolonged activation of AMPK increases citrate synthase activity (59). However,
total mitochondrial content did not increase (53). Resveratrol supplementation in humans appears to improve insulin sensitivity, reduce plasma triglyceride levels, increase total fat oxidation, and reduce systolic blood pressure (53).

Timmers et al. suggest that the adaptations induced by resveratrol are a result of increased mitochondrial efficiency. This is due to increased citrate synthase activity rather than an increase in mitochondrial abundance, since mitochondrial DNA copy number did not increase. These data suggest that thirty days may not be long enough to see the full effects of resveratrol supplementation. Also, the mechanism of resveratrol-mediated mitochondrial biogenesis may be more complex in human models than the animal models previously studied.

Dosage

The adaptations seen with resveratrol supplementation greatly depend on the dosage used. Resveratrol is rapidly absorbed by enterocytes within the small intestines (52). An oral dose of 25 mg resveratrol results in plasma concentrations in the order of nanomolars (15, 31, 56).
Resveratrol is rapidly metabolized by the liver and plasma concentrations peak within approximately 30 minutes (52). Red wine has a resveratrol concentration of approximately 84 ng per 100 g of wine (50). The concentration of resveratrol required to activate SIRT1 is in the micromolar range and is probably not achievable by diet alone (6). Resveratrol concentrations in vivo may not be high enough to effectively induce a metabolic response (6).

Adaptations seen in animal studies were dependent on the dose of resveratrol used. In obese rodent models, a “high” dose of resveratrol, 400 mg/kg/day, was able to improve insulin sensitivity and reduce body weight (34). Treatment with a “moderate” dose, 25-30 mg/kg/day, induced a SIRT1-dependent shift towards a more oxidative phenotype (45). Muscle fiber type switching is attributed to increased mitochondrial biogenesis and improved mitochondrial function (24). These results suggest that a moderate dose of resveratrol is more effective at stimulating mitochondrial biogenesis.

The resveratrol dosage required for physiological adaptations in humans may be different than that required for animals. Timmers et al. used a dose of 150 mg/day. Although, this dosage is approximately 200-fold lower than the dosage used by Lagouge et. al, (200-400 mg/kg/day), plasma resveratrol concentrations in humans showed a 2-fold or greater increase compared to plasma concentrations seen in animal studies when using the higher dosage (34). This could be due to differences in the rate of metabolism of resveratrol between humans and mice. With a dosage of 150 mg/day of resveratrol, improvements in glucose tolerance and insulin sensitivity were seen, however, increases in mitochondrial biogenesis were not.

The precise dosage required to see resveratrol-induced mitochondrial biogenesis in humans has yet to be determined. However, if the effective dosages of resveratrol used in animal
studies were extrapolated to a reference 70kg person, the dosage required to see results would be 1.7 g/day to 28 g/day. Commercial resveratrol supplements typically contain 500 mg/capsule, with a daily dosage of one capsule. This supplement may not provide a high enough dosage of resveratrol to induce the physiological adaptations seen in animal studies. Of the commercially available resveratrol supplements, one particular liquid supplement provided only 100 mg/day with the recommended dose. The amount of resveratrol present in this supplement may not be enough to induce the same physiological adaptations seen in animal or human studies.

**Efficacy of Resveratrol as an Exercise Mimetic**

Resveratrol has been shown to function as an exercise mimetic. Physiological adaptations seen in response to endurance exercise include weight-loss, improved glucose tolerance and insulin sensitivity, increased mitochondrial biogenesis, and increases in VO$_2$max and resistance to fatigue. Several animal studies have shown that resveratrol induces all of these adaptations as well as others (34, 45, 54). Currently, human studies have shown improvements in glucose tolerance and insulin sensitivity as well as several other factors, but an increase in mitochondrial biogenesis or aerobic capacity have not been observed (53). However, the adaptations that were observed in human studies are still consistent with adaptations seen in response to endurance exercise. Therefore, resveratrol does function as an exercise mimetic, but as of yet, human studies have not been able to attain all of the resveratrol-induced adaptations observed in animals studies. Nonetheless, commercially available resveratrol supplements may not induce any adaptations due to the low levels of resveratrol within the supplement.
Conclusion.

The mechanism through which resveratrol functions as an exercise mimetic is complex. The pathway is not linear, but rather continual. Based on the studies examined in this paper, several conclusions can be made.

PGC-1α activation is the determining factor regulating expression of NEMP and mitochondrial biogenesis. This is supported by the lack of mitochondrial biogenesis in PGC-1α knockout mice and the enhanced mitochondrial biogenesis in PGC-1α overexpression mice (7, 35). Furthermore, increases in transcription of NEMP and mitochondrial biogenesis results in a shift towards a more oxidative muscle fiber phenotype (23).

Resveratrol directly activates SIRT1 resulting in subsequent activation of PGC-1α. Resveratrol supplementation has been shown to increase SIRT1 activity by allosteric interactions with SIRT1, establishing an increased affinity of SIRT1 for NAD⁺ and acetylated substrates (26). This allows for an increase in the deacetylation and partial activation of PGC-1α.

Resveratrol directly activates AMPK causing an increase in PGC-1α activity. Resveratrol is shown to increase phosphorylation of AMPK, although the exact mechanism is unclear (4). AMPK phosphorylates PGC-1α, increasing its protein stability and partially activating it (30).

SIRT1 and AMPK activation are reciprocally induced. AMPK induces SIRT1 activation by increasing available NAD⁺ for use in deacetylation reactions, leading to increased deacetylation of PGC-1α (9, 58). This supports the theory that resveratrol indirectly regulates SIRT1 activity. Meanwhile, SIRT1 is also shown to activate AMPK by deacetylating LKB1 (liver kinase B1), the primary kinase responsible for phosphorylating and activating AMPK (28).
This suggests the possibility that SIRT1 is the major target of resveratrol. The proposed mechanism of resveratrol-mediated mitochondrial biogenesis can be seen in figure 7.

The majority of the literature reviewed in this paper supports the theory that resveratrol directly activates both SIRT1 and AMPK. SIRT1 overexpression and SIRT1-knockout data strongly imply that SIRT1 is essential for mitochondrial biogenesis as well as resveratrol-mediated AMPK activation (45). Although, a high dosage of resveratrol has been shown to activate AMPK in a SIRT1-independent manner, mitochondrial adaptations do not occur (45). This supports the hypothesis that SIRT1 is necessary for resveratrol-induced mitochondrial biogenesis. AMPK-knockout mice provide strong evidence that AMPK is imperative for resveratrol-induced mitochondrial adaptations, since mitochondrial biogenesis did not occur in
AMPK-knockout mice (54). SIRT1-dependent deacetylation of PGC-1α was shown to be partially blocked in AMPK-knockout mice (10).

These results support the idea that AMPK and SIRT1 each partially activate PGC-1α, and without one or the other, there is no significant increase, or full activation, in PGC-1α activity; therefore mitochondrial biogenesis is not induced. Activation of AMPK has been shown to precede and regulate alterations in SIRT1 activity (10). It has been implied that phosphorylation of PGC-1α by AMPK may serve as a signal for SIRT1-induced deacetylation of PGC-1α (30). In conclusion, the mechanism of action responsible for resveratrol’s ability to function as an exercise mimetic is dependent upon direct activation of both AMPK and SIRT1.

Future research in resveratrol and exercise mimetics

Future research needs to be done to determine the principal dosage of resveratrol required to exploit its physiological adaptations. Determining the correct dosage can lead to developing a more potent exercise mimetic which could be used to treat several diseases, including metabolic disorders and cardiovascular disease.

References


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