

The Potential of Resveratrol to Act as a Caloric Restriction Mimetic Appears to Be Limited: Insights from Studies in Mice

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ABSTRACT

Caloric restriction (CR) has been shown repeatedly to prolong the lifespan in laboratory animals, with its benefits dependent on molecular targets forming part of the nutrient signaling network, including the NAD-dependent deacetylase silent mating type information regulation 2 homologue 1 (SIRT1). It has been hypothesized that the stilbene resveratrol (RSV) may counteract age- and obesity-related diseases similarly to CR. In yeast and worms, RSV-promoted longevity also depended on SIRT1. While it remains unclear whether RSV can prolong lifespans in mammals, some studies in rodents supplemented with RSV have reported lowered body weight (BW) and fat mass, improved insulin sensitivity, lowered cholesterol levels, increased fitness, and mitochondrial biogenesis. Molecular mechanisms possibly leading to such changes include altered gene transcription and activation of SIRT1, AMP-activated kinase (AMPK), and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PPARGC1A). However, some mouse models did not benefit from RSV treatment to the same extent as others. We conducted a literature search on PubMed (15 April, 2020) for trials directly comparing RSV application to CR feeding in mice. In most studies retrieved by this systematic PubMed search, mice supplemented with RSV did not show significant reductions of BW, glucose, or insulin. Moreover, in some of these studies, RSV and CR treatments affected molecular targets differently and/or findings on RSV and CR impacts varied between trials. We discuss those RSV-induced changes in gene transcription hypothesized to partly counteract age-related alterations. Although there may be a moderate effect of RSV supplementation on parameters such as insulin sensitivity toward a more CR-like profile in mice, data are inconsistent. Likewise, RSV supplementation trials in humans report controversial findings. While we consider that RSV may, under certain circumstances, moderately mimic some aspects of CR, current evidence does not fully support its use to prevent or treat age- or obesity-related diseases. *Adv Nutr* 2021;12:995–1005.

Keywords: sirtuin, polyphenol, healthspan, diabetes mellitus type II, lunularin, dihydroresveratrol

Introduction

Resveratrol sources and structure

Resveratrol (RSV) is a secondary plant metabolite found in dietary sources such as berries and other fruits, including

peanuts and cocoa (1–4), with considerable amounts in grapes and, consequently, in wine (5). The stilbene RSV forms *cis* and *trans* derivatives that are mostly found as glucosides in plants (5). While wine drinkers can consume more than 1 mg/d RSV (6), in the US population the average RSV uptake is estimated to be around 80 µg/d (7). Supplementation of synthetic RSV up to 150 mg/d has been regarded as safe by the European Food Safety Authority (8). However, at doses of 2.5 g or higher, RSV supplementation may cause mild to moderate gastrointestinal symptoms (9). RSV is metabolized by gut microbiota (to dihydroresveratrol and possibly other metabolites, such as lunularin) and is quickly sulfonated or glucuronidated after resorption from the intestines, leading to very low levels of nonconjugated RSV in mice and humans (10, 11).

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Abbreviations used: AGE, advanced glycation end products; AMPK, AMP-activated kinase; BW, body weight; CR, caloric restriction; CRM, caloric restriction mimetic; CRP, C-reactive protein; EOD, every other day feeding regimen; HbA1c, glycated hemoglobin; HFD, high-fat diet; IGF-I, insulin-like growth factor I; IGF-IR, IGF-I receptor; *Pdk4*, pyruvate dehydrogenase lipoamide kinase isozyme 4; PPARGC1A, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; RSV, resveratrol; SIRT1, silent mating type information regulation 2 homologue 1; T2DM, type II diabetes mellitus; TG, triglyceride; *Ucp3*, uncoupling protein 3; WAT, white adipose tissue; WC, waist circumference.

Caloric restriction as putatively lifespan-extending intervention

Studies employing model organisms ranging from yeast to mice found that RSV may possibly prolong the lifespan [reviewed in Barnett et al. (12)]. The only nongenetic intervention that has repeatedly promoted longevity is a reduction of feed intake [caloric restriction (CR)]. Compared to ad libitum feeding, CR prolonged lifespans in model organisms, including yeast, mice, and rhesus monkeys (13–15). In mammals, CR was shown to reduce adipose tissue mass and body weight (BW) and decrease leptin, insulin, insulin-like growth factor I (IGF-I), and cholesterol plasma levels. Furthermore, CR lowered markers of inflammation such as TNF, which is expressed under the control of nuclear factor kappa B (15, 16) and may even favor healthy aging in humans (17). The WHO defines healthy aging as “the process of developing and maintaining the functional ability that enables wellbeing in older age” (18). Aging, however, increases susceptibility toward many common diseases (19, 20), such as coronary artery disease, type II diabetes mellitus (T2DM), cancers, dementia, and painful conditions (21). Chronic inflammation may further contribute to the pathogenesis of age-related diseases (22) and, late in life, increased frailty (23).

Pathways implicated in aging

Nutrients activate insulin and IGF-I receptors (IGF-IR), as well as downstream signaling pathways. Interestingly, hetero-genetic knockout of *Igfr1* increased the lifespan in mice (24). Upon inhibition of IGF-IR downstream mechanistic target of rapamycin, the lysosomal degradation pathway autophagy is induced (25). Of interest, genetically inducing autophagy made mice longer-lived, as well as more insulin-sensitive and more tolerant toward oxidative damage (26). Another molecular nutrient-sensing enzyme is NAD-dependent deacetylase silent mating type information regulation 2 homologue 1 (SIRT1) (27). Its overexpression in yeast, worms, and mice increased their lifespans (28–32). Moreover, SIRT1 can activate peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PPARGC1A), a major regulator for mitochondrial biogenesis (27).

Consistent with CR counteracting age-related changes in cellular signaling, it improved insulin sensitivity and autophagy in aged organisms (33). Remarkably, the phenotypes of CR and transgenic SIRT1 mice resemble each other (28), and SIRT1 was necessary for CR-induced lifespan extensions (32, 34, 35). In long-lived mice with genetically distorted signaling of growth hormone, which controls IGF-I production, CR could not further promote lifespan extensions (36). Thus, CR appears to activate SIRT1 and inhibit IGF-I, thereby affecting their downstream signaling (37).

Since the number of overweight humans worldwide and, consequently, cases of premature onset of age-related diseases are increasing (38), molecules possibly mimicking CR [caloric restriction mimetics (CRMs)] without having to decrease nutrient intake may seem like a hopeful measure that can prolong the human lifespan. According to National

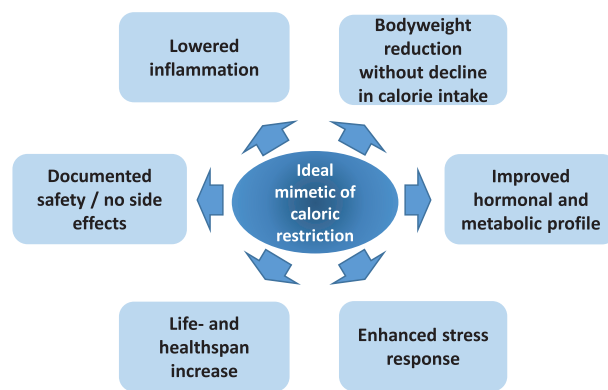


FIGURE 1 Desired properties of an “ideal” caloric restriction mimetic that would lead to health- and lifespan increases.

Institute on Aging—based researchers, a CRM candidate substance should not “significantly reduce long-term food intake”; mimic “metabolic, hormonal, and physiological effects of CR”; activate stress response pathways observed in CR; and promote CR-like effects on the lifespan and healthspan (39). In Figure 1, such effects and further desired properties (i.e., safety/lack of side effects) of ideal CRM candidates are depicted.

Resveratrol and putative lifespan extension

Of interest, RSV-induced lifespan increases in yeast, worms, and flies depended on their SIRT1 homologue (30, 32). Because RSV supplementation resembled CR-induced lifespan extensions in these settings, it has been hypothesized that RSV may mimic CR (32). However, it remains unclear whether RSV supplementation can increase the lifespan in mice (40–45). Therefore, we have reviewed the data from studies directly comparing CR with RSV that analyze aging- and obesity-related parameters. Since the number of studies in mammalian non-mouse models was too small to compare trials reasonably, we have limited our systematic search to CR-RSV trials in mice. Prior to reviewing this data, we give an overview of studies that have supplemented RSV in mice without including a CR control. At the end of this narrative review with a systematic component, we relate (inconsistent) findings from studies in rodents to data from human trials.

Molecular targets of resveratrol in rodents

In an early study on lifespans and RSV in male C57BL/6 mice, the stilbene prolonged the lifespan when supplemented at 0.4 g/kg to a high-fat diet (HFD) with 60% energy from fat. Moreover, stilbene supplementation lowered fed and fasted insulin, as well as fasted glucose levels, while increasing the number of mitochondria. In line with this finding, after 6 months of supplementation, hepatic PPARGC1A had a lower acetylation status in RSV-supplemented mice than in nonsupplemented mice. Yet, feed consumption and body temperature were unchanged (43). Moreover, RSV attenuated obesity-induced damage to the heart and liver in those mice on a HFD [60% calories from fat (43)].

Lagouge et al. (46) also found an increase in muscular mitochondria and brown adipose tissue, as well as improved insulin sensitivity, in RSV-supplemented C57BL/6 mice on a HFD with 40% energy from fat. In contrast to Baur et al. (43), Lagouge and colleagues (46) used a 10-time higher dose of RSV (0.4 g/kg and 4 g/kg diets, respectively) and found lower BWs in RSV-treated mice compared to the controls, since the stilbene-supplemented mice gained weight more slowly than the control mice. The RSV-fed Lagouge male C57BL/6J mice (46) also had more active muscular PPARGC1A, showing higher mRNA and protein levels alongside with SIRT-mediated deacetylation and therefore activation of PPARGC1A. While RSV supplementation of mice on a standard diet (in contrast to mice on 60% energy from a HFD) did not increase the lifespan, it induced transcriptional changes similar to the pattern detected in animals following an every other day feeding regimen (EOD) (47).

Using *Ampk* knockout mice, Um et al. (48) showed that RSV-induced benefits relied on this kinase responding to a lowered nutrient supply and forming part of the SIRT1 regulatory network (49). In contrast to *Ampk* knockout mice, RSV-supplemented wild-type mice showed an increased metabolic rate and a lowered BW, as well as improved insulin sensitivity and glucose tolerance. In skeletal muscle, mitochondrial biogenesis and *Ppargca1* mRNA levels were increased in RSV-supplemented mice as compared to non-supplemented animals (48). However, phosphorylation of AMPK as a measure of its activation shows high interindividual differences within experimental groups when analyzed in vivo and was not significantly altered in the liver, muscle, or white adipose tissue (WAT) of mice receiving RSV at 4 g/kg for 4 or 13 weeks (50). In vitro studies observed AMPK phosphorylation when RSV was administered at 25 μ M to Chinese hamster ovarian cells (43). This concentration is not reached in vivo since RSV fed at 4 g/kg led to concentrations below 2 μ M (41, 46).

In order to study whether RSV may slow down aging, the stilbene was applied to a mouse model for the premature-aging Werner syndrome. A mutation of the gene *Wrn* in a C57BL/6 background led to increased BW, visceral fat, liver steatosis, and triglyceride (TG) and glucose levels, as well as insulin resistance, when compared to wild-type mice (51). In the Werner mice, applying RSV at 0.4 g/kg from weaning to 5 or 9 months of age attenuated liver steatosis, hyperglycemia, and insulin resistance without lowering TG levels, visceral fat mass, or BW compared to nonsupplemented mutant mice. Moreover, RSV supplementation did not attenuate inflammation in the prematurely aging mice and did not expand their lifespan (51).

In a study with organ-specific *Ppargca1* knockout mice, RSV-supplemented (4 g/kg diet) animals depended on muscular PPARGCA1 for increased mitochondrial biogenesis. In wild-type mice, RSV showed a rather moderate influence on insulin and glucose levels, decreased total cholesterol plasma levels, and possibly decreased hepatic TGs. Dietary application of RSV (4 g/kg diet) was further compared to feeding a chemical SIRT1 inducer. Interestingly, improved

glucose homeostasis after supplementing the SIRT1 inducer did not depend on PPARGCA1. This indicates that RSV may not be a sole SIRT1 inducer. The effect of RSV in wild-type mice was highly tissue dependent, and *Ppargca1* mRNA levels in muscle did not seem to be affected significantly. While *Ppargca1* transcription was decreased in liver, it was increased in WAT (50).

Conversely, in another study in mice on a HFD, feeding RSV at 4 g/kg did not increase the mitochondria number or affect PPARGCA1 protein levels in the muscle. When the same authors used rats as a model, they similarly observed no changes in the mitochondria number or PPARGCA1 (41).

In a Wistar-based steatosis rat model, RSV at 0.2 g/kg BW for 18 weeks attenuated the HFD-induced rises in total cholesterol, TGs, and BW. Additionally, downregulation of autophagy-related *Map1lc3b* mRNA levels in animals on an HFD as compared to a standard diet was counteracted, and hepatic *Sirt1* mRNA levels were elevated by RSV feeding (52). An overview of molecular targets putatively affected by RSV, as well as RSV's microbial products and host-modified products, is depicted in **Figure 2**.

Neuroprotective properties of RSV have also been observed. CR in elderly humans may improve memory (53), and activation of SIRT1 could possibly counteract neurodegenerative disease (54). However, data on RSV improving neurodegeneration in mouse brains is not consistent (55).

Changes in phenotype, protein, and mRNA levels in mice

On 15 April, 2020, we searched PubMed (pubmed.ncbi.nlm.nih.gov) for original research articles using the search terms “restriction AND (mouse OR mice) AND resveratrol.” We screened the articles retrieved for studies that compared ad libitum-fed nonsupplemented animals to an RSV-supplemented group and to mice on CR and that were describing aging- or obesity-related parameters beyond changes in BW. We excluded genetic disease models and studies focusing on the brain. Since outcomes from studies on RSV often yield contradicting data, we concentrated on parameters that had been analyzed by at least 2 of these studies (**Figure 3**). This led to finding 7 studies that had evaluated BW, body composition, insulin sensitivity, serum hormone and/or lipid levels, activation of known molecular targets of CR, and/or changes in gene transcription (**Table 1**). Of these, 5 studies used C57BL/6 strains and the other 2 studies employed the F1 generation obtained from crossing C57BL/6 x C3H/He mice. All but 1 study, which included male and female rodents, studied male mice (56–62). Diets provided 10–60% of calories from fat. The RSV supplementation dose and duration varied from 18.6 mg to 4 g/kg and from 8 weeks to 16 months, respectively. In the majority of these studies, RSV application showed little to no effect on mouse phenotype. In contrast to CR, RSV did not decrease BW and only slightly blunted an adipose tissue mass increase in 1 out of 3 studies. The analyzed feed intake was not changed by RSV application. Cholesterol was measured in 3 studies, of which 2 showed

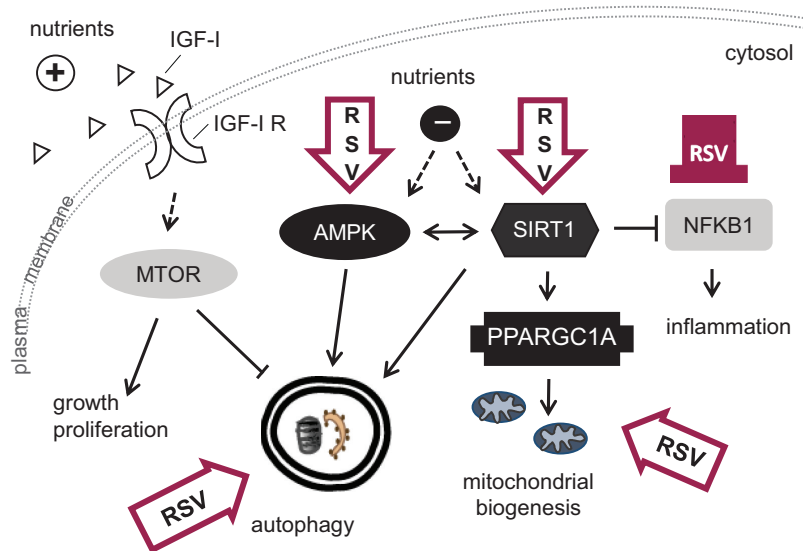


FIGURE 2 Molecular targets and cellular processes identified as being affected directly or indirectly by resveratrol. Contoured block arrows depict activation, the color-filled blunt arrow shows inhibition. AMPK, AMP-activated kinase; IGF-I, insulin-like growth factor I; IGF-I-R, IGF-I receptor; MTOR, mechanistic target of rapamycin; NFKB1, nuclear factor kappa B; PPARGC1A, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; RSV, resveratrol; SIRT1, silent mating type information regulation 2 homologue 1.

reductions by CR. However, RSV did not affect cholesterol levels (58, 59, 62). In 1 study, RSV increased TG levels but CR did not (62). However, in the mice from studies by Günther et al. (58) and Pallauf et al. (59), TG levels remained unchanged by RSV as well as by CR (63). Fasting glucose levels were monitored in 6 studies and decreased by most

CR interventions. RSV tended to decrease glucose levels in genetically heterogeneous mice but increased them in C57BL/6 mice on a low-dose RSV-supplemented standard diet (56, 62). The other 4 studies found no significant changes in blood glucose by RSV (56, 58–60, 63). Fasted insulin levels were measured in 5 studies, 2 of which showed a

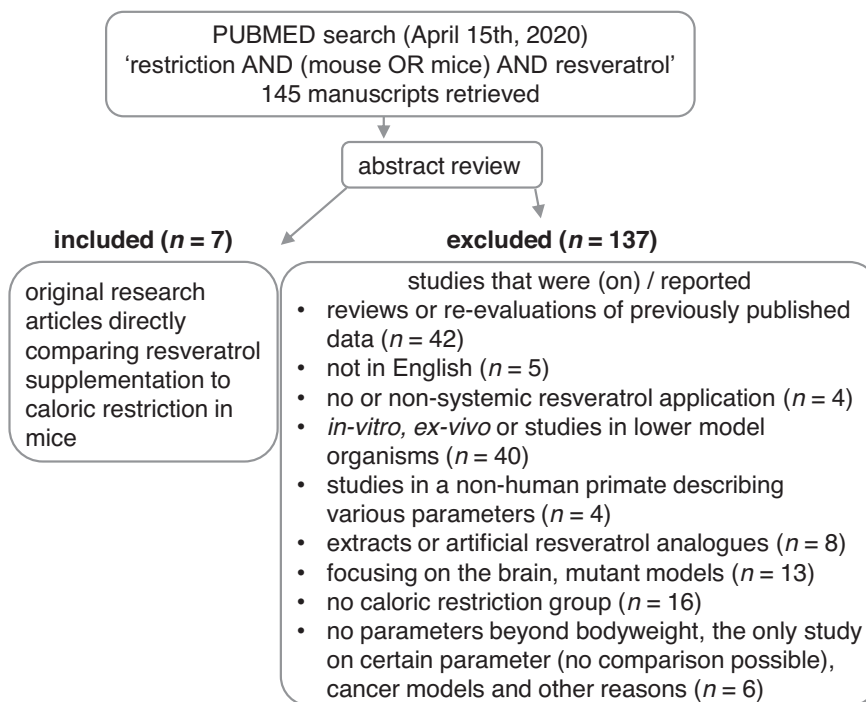


FIGURE 3 Selection of studies on resveratrol application compared to caloric restriction in mice.

TABLE 1 Studies comparing resveratrol supplementation to caloric restriction and ad libitum control in mice

Mice and diet	Male 14-mo-old C57BL/6xC3H/He F1 hybrid on AIN-93M; 10% energy from fat (57)			Male 2-mo-old C57BL/6xC3H/He F1 hybrid on AIN-93M; 10% energy from fat (56)			Male 7-wk-old C57BL/6J on 60% energy from fat; CR, RSV, and controls (61)			Female & male 6-mo-old C57BL/6J on a standard diet (62)			Male 1-y-old C57BL/6Rj on 40% energy from fat (59)			Male 18-wk-old C57BL/6Rj on 40% energy from fat (58)			Male 4-mo-old C57BL/6Rj on AIN-93G (17% energy from fat) (60)							
	16 mo	3 mo	12 wk	70% HFD	CR	RSV	CR	RSV	CR	RSV	CR	RSV	CR	RSV	CR	RSV	CR	RSV	CR	RSV	CR	RSV	CR	RSV		
Dose	75% ³ 0.05 g/kg diet	75% ³ 0.05 g/kg diet	70% HFD	70% HFD	CR	RSV	CR	RSV	CR	RSV	CR	RSV	CR	RSV	CR	RSV	CR	RSV	CR	RSV	CR	RSV	CR	RSV		
Phenotype																										
BW	n.a.	0.7 ^{1,7}	0.8 ^{1,7}	0.6 ^{1,7}	0.6 ^{1,7}	≈	≈	≈	0.9 ^{1,7}	1.1 ^{2,7}	0.6 ¹	≈	≈	0.6 ¹	≈	≈	0.6 ¹	≈	0.6 ¹	≈	≈	≈	≈	≈	≈	
ATM	n.a.	n.a.	0.5 ¹	0.3 ¹	≈	≈	≈	≈	n.a.	n.a.	0.2 ¹	≈	≈	0.2 ¹	≈	≈	0.2 ¹	≈	0.2 ¹	≈	≈	≈	≈	≈	≈	
Energy intake	CR	n.a.	≈	CR	CR	≈	≈	≈	n.a.	n.a.	CR	≈	≈	CR	≈	≈	CR	≈	CR	≈	≈	≈	≈	≈	≈	
Cholesterol	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	≈	≈	0.5 ¹	≈	≈	0.5 ¹	≈	≈	0.7 ¹	≈	0.7 ¹	≈	≈	≈	≈	≈	≈	
Glucose ⁸	0.8 ^{2,7,9}	0.8 ^{2,7,9}	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	≈	1.3 ^{1,7}	0.7 ¹⁻¹⁰	≈	≈	0.7 ¹⁻¹⁰	≈	≈	0.3 ^{1,8,10}	≈	0.3 ^{1,8,10}	≈	≈	≈	≈	≈	≈	
Insulin	≈ ^{8,9}	≈ ^{8,9}	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	≈ ⁸	1.2 ^{1,7,8}	0.5 ^{1,11}	≈	≈	0.5 ^{1,11}	≈	≈	0.2 ^{1,11}	≈	0.2 ^{1,11}	≈	≈	≈	≈	≈	≈	
IGF-I mRNA levels	0.7 ^{1,7,9}	≈ ⁹	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	≈	≈	n.a.	≈	≈	n.a.	≈	≈	n.a.	≈	n.a.	≈	≈	≈	≈	≈	≈	
Liver																										
<i>Pparg</i>	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
<i>Sirt1</i>	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Muscle																										
<i>Pparg</i>	3.4 ^{1,7}	≈	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Heart																										
<i>Pparg</i>	≈	≈	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Protein levels																										
Muscle																										
<i>Sirt1</i>	0.7 ^{1,7}	0.6 ^{1,7}	≈	1.3 ^{1,7}	≈	≈	≈	≈	≈	≈	≈	≈	≈	≈	≈	≈	≈	≈	≈	≈	≈	≈	≈	≈	≈	≈
Liver	≈	≈	1.5 ^{1,7}	1.6 ^{1,7}	≈	≈	≈	≈	≈	≈	≈	≈	≈	≈	≈	≈	≈	≈	≈	≈	≈	≈	≈	≈	≈	≈

Endpoints with assumed relevance to aging measured in more than 1 study are listed. Values are given related to the controls and therefore do not show units. Values < 1 show decreases and values > 1 show increases by the treatment. Since many values were derived from graphs rather than the original values, they are approximations and no SDs or SEMs can be shown. The amount of feed consumed by the CR group is given as the percentage of feed consumed by the controls. The LED had 10% energy from fat. The ≈ symbol indicates data that are not statistically significant different compared to non-supplemented/non-CR animals. Abbreviations: AIN-93M, American Institute of Nutrition (AIN) diet formulation 93 for maintenance (M); ATM, adipose tissue mass; BW, body weight; CR, caloric restriction; HFD, high-fat diet; IGF-I, insulin-like growth factor I; LFD, low-fat diet; n.a., not analyzed or value not given; *Pparg*, peroxisome proliferator-activated receptor gamma coactivator 1- α ; *Pparg*, peroxisome proliferator-activated receptor gamma coactivator 1- β ; *Sirt1*, sirtuin-1 (murine mRNA); *Sirt1*, NAD-dependent protein deacetylase sirtuin-1 (murine protein).

¹ Statistically significant difference to the control at $P < 0.05$

² P value is < 0.1 .

³ Compared to intake of the control group, control was -10% ad libitum.

⁴ The diet for the CR group consisted of 58% control diet + 34% dietary fiber + 8% soy.

⁵ 24 mg/kg BW injected 3 times per week.

⁶ With increasing BW of the mice, the RSV concentration in the diet was increased from 0.3 g/kg to 0.4 g/kg halfway through the trial.

⁷ Approximated from figure.

⁸ Measured in fasted mice.

⁹ Measured in 5-month-old mice after 3 months of supplementation.

¹⁰ Previously unpublished data obtained by using methods and mice described in Günther et al. (68) and Pallauf et al. (59).

¹¹ Measured in glucose-challenged mice (after application of a glucose bolus).

reduction by RSV as well as CR (58, 60). For fasting insulin, 2 trials revealed no influence by CR or RSV and 1 showed an increase by RSV supplementation but not by CR (56, 57, 62). Insulin levels after glucose application as an indicator of insulin sensitivity were decreased and thus improved in the CR groups of both studies that measured challenged insulin (58, 59). However, RSV only improved insulin sensitivity in 1 of these 2 studies (58). CR lowered IGF-I levels in 1 out of 2 studies, while RSV did not affect the growth factor (57, 62). In 30-month-old mice, both CR and RSV decreased muscular SIRT1 (57), while in 4–5-month-old mice, CR increased SIRT1 levels (61). SIRT1 protein in the muscle was not impacted by 2 g/kg RSV and was upregulated by 4 g/kg RSV, similar to the results of CR in the same study (61) (Table 1).

Supplementation of RSV was shown to attenuate the damage of a HFD on the mouse heart (43), possibly by somewhat mimicking CR (56). Since CR induced phosphorylation of the putative RSV target AMPK (64), we analyzed phosphorylated AMPK (p-AMPK) levels in the hearts of mice described in Günther et al. (58). While RSV-fed mice showed high variations of AMPK phosphorylation within the group, ad libitum control mice, CR mice, and RSV mice did not differ from each other in cardiac levels of activated AMPK [unpublished results (63)].

Various studies explored the changes in gene expression induced by aging and investigated whether CR and/or RSV could partly attenuate these changes. In the hearts of 30-month-old C57BL/6xC3H/He F1 hybrid mice after 16 months of dietary intervention, CR as well as RSV [50 mg/kg American Institute of Nutrition diet formulation 93 for maintenance (AIN-93M) diet or 4.9 mg/(kg mouse BW · d)] opposed the majority of age-related changes in gene transcription. Differential gene expression because of aging was observed for 1029 genes (57). However, in younger (5-month-old) mice supplemented with 1.25 mg RSV/(kg mouse BW · d), transcription of less than 10% of the 304 genes differentially transcribed in corresponding CR mice was changed toward a more CR-like pattern (56). Park et al. (65) studied the variations in gene expression in 5- and 25-month-old mice of 7 inbred strains. They found that age changed 6–15% of more than 22 000 gene transcripts measured in the hearts of the mice. However, which genes were affected by age depended greatly on the strain. Of the differentially regulated genes, only 20 genes were altered consistently over at least 6 out of 7 strains. Interestingly, in a study with Balb/C mice at 4 and 28 months of age, individual variation in hepatic gene transcription increased significantly with age. In studies by Park et al. (65) and White et al. (66), gene ontology terms obtained by functional annotation analysis of the transcriptome indicated that the immune response was upregulated in aged mice. Analyzing cardiac gene transcription, RSV at 50 mg/kg was as effective as CR in inhibiting age-dependent gene upregulation in the majority of the strains studied when applied from 15–30 months of age. Yet in the cerebellum, when measuring the transcription of 5 genes that were analyzed as markers

for dietary interventions attenuating age-related changes in mice, RSV supplementation only affected the transcription of complement C1q subcomponent subunit A, which codes for a protein involved in the complement system (65). Moreover, PCR data from mouse livers showed CR-mediated upregulation of *Ppargca1*, *Sirt1*, and phosphoenolpyruvate carboxykinase 1 (a gene that codes for a central protein in gluconeogenesis), while RSV supplementation left the mRNA levels of these genes unaffected (Table 1) (58, 59). Of interest, Svensson et al. (50) found decreased *Ppargca1* mRNA levels in the livers of RSV-supplemented mice (4 g/kg diet for 4 and 13 weeks), implying that RSV may even act contrarily to CR on *Ppargca1* gene regulation. In the heart, *Ppargca1* transcription showed no changes by CR in old, genetically heterogeneous mice on a standard diet (56); in young C57BL/6 mice on a HFD (40% energy from fat), *Ppargca1* was transcriptionally upregulated by CR (Table 1) (58, 63). However, RSV supplementation did not influence *Ppargca1* transcription in young or old mouse hearts (56, 57, 63). The pyruvate dehydrogenase lipoamide kinase isozyme 4 (*Pdk4*; a gene coding for an enzyme that inhibits the use of glucose for metabolism) and mitochondrial uncoupling protein 3 (*Ucp3*) were transcriptionally upregulated in the hearts of old and young genetically heterogeneous mice under CR. Young but not old RSV-supplemented mice also showed increased cardiac mRNA levels of *Pdk4* and *Ucp3* (56, 57). However, in young C57BL/6 mice on an HFD, neither CR nor RSV affected cardiac *Pdk4* mRNA levels (63). These findings indicate that, similar to CR-induced transcriptional changes, RSV supplementation effects may also depend on mouse strain, age, diet, and dose.

In C57BL/6NIA mice on an AIN-93G diet supplemented with 0.4 g RSV/kg, Pearson et al. (47) detected transcriptional changes in the liver, heart, muscle, and WAT of mice supplemented from 12–18 and 12–27 months of age. They found that RSV, similar to EOD, counteracted age-related changes in the liver. However, they did not detect such antiaging transcriptional alterations by RSV or EOD in the heart. In muscle, RSV but not EOD slowed down age-related changes. Contrarily, in adipose tissue, RSV and EOD (67) enhanced age-related changes. Yet, RSV improved parameters that decline with age and obesity. In old mice, supplementation of the stilbene increased bone strength (tissue mineral density in the distal femur) and improved cataracts (30-month-old mice), prolonged the time before falling off a rotarod (21- and 24-month-old mice), and, at doses of 0.24%, enhanced endothelial function, measured as acetylcholine relaxation and reduced oxidative stress (18-month-old mice). However, in mice with a leptin-deficient background, RSV supplementation could not improve murine motor functions and possibly decreased endurance, as was shown in a treadmill test (68).

RSV supplementation in humans renders inconclusive data

Future and ongoing studies could possibly show a moderate impact of RSV on human health, yet discouraging findings

from studies in rodents are somewhat reflected in humans. A PubMed search on 30 August, 2020, for recent (published 2014 or later) meta-analyses of human trials supplementing RSV and using glucose, insulin, TG, cholesterol, BW, or inflammatory markers as endpoints retrieved 19 publications [search terms “resveratrol AND (glucose OR insulin OR lipid OR inflammation OR body weight)”]. Of these 19 publications, 2 were on preclinical models and were thus not considered. Total cholesterol levels were analyzed by 8 articles. No influence of RSV supplementation was found in 4 reports (69–72), while 2 found a reduction (73, 74) and 2 focusing on patients with obesity and nonalcoholic fatty liver disease concluded that RSV could increase cholesterol levels (75, 76). Of 7 meta-analyses examining the impact of RSV supplementation on TG levels, only 1 study discovered reliable evidence for a reduction after treatments longer than 6 months in diabetic patients (70–74, 76, 77). Waist circumference (WC) and/or BW were investigated by 4 studies (69, 77–79). While 3 of these found a reduction of WC by RSV supplementation, 2 studies also included fat mass in their analyses. Although both reports detected reduced BWs, only Tabrizi et al. (79) furthermore observed a decreased fat mass. In contrast, Elgebaly et al. (77) found unchanged BW upon RSV supplementation. There were 7 meta-analyses that covered data on glucose levels after RSV supplementation. Of these, 3 reported no influence on glucose levels after stilbene application (71, 72, 77) and 3 analyses concluded that RSV could decrease blood glucose (69, 74, 80). However, Liu et al. (80) observed only diabetic patients to be responsive. Intriguingly, the authors of a meta-analysis from 2020 in patients with T2DM stated that data were insufficient for the evaluation of health benefits, since, after excluding trials with incomparable interventions and controls, only 3 studies remained for their assessment (81). Further glucose homeostasis-related parameters, such as insulin levels, the HOMA-IR index, or glycated hemoglobin (HbA1c), were analyzed by 4 publications (71, 74, 77, 80). Interestingly, the marker for average glucose levels during the last 3 months, HbA1c, appeared to be responsive toward stilbene treatment. While measuring insulin levels and determining the HOMA index yielded few promising results, all 3 meta-analyses evaluating HbA1c concluded that patients may benefit from RSV supplementation (71, 74, 80).

During aging, low-grade, chronic inflammation occurs (22). By counteracting the expression of inflammatory cytokines such as TNF, IL6, and acute-phase C-reactive protein (CRP), RSV could possibly counteract development of age-related illnesses (82). Of the meta-analyses retrieved by our PubMed search, 6 studied inflammation markers after RSV supplementation. While 4 of 6 studies found reduced CRP levels in RSV-supplemented human subjects (72, 74, 83–86), all 4 studies evaluating IL6 found no difference between stilbene-supplemented and nonsupplemented individuals (83–86). Levels of TNF were decreased by RSV in 2 of 3 analyses (84–86).

The contradicting data on RSV benefits in human trials may indicate that although there are some well-controlled

and well-designed studies, many trials lack appropriate controls and are difficult to compare with each other. Furthermore, studying different patients (gender, health status, age) and types of interventions (dose, time point, duration) might have contributed to these controversial outcomes.

In a small number of original research articles, laborious muscle biopsies and analyses of AMPK, SIRT1, and PPARGCA1 (molecular targets that may mediate putative CR-like properties of RSV, as mentioned above in the section “Resveratrol and putative lifespan extension”) were conducted (87–89). In 2 of these trials with 10–11 obese men and/or men suffering from T2DM, CR-like effects were shown by RSV supplementation, with elevated activation of AMPK and SIRT1 in the muscle (87), increased mitochondrial function, improved insulin sensitivity, and lowered plasma levels of proinflammatory cytokines and TGs (88). In contrast, with a larger number ($n = 45$) of nonobese women, none of these CR-like outcomes were observed after RSV supplementation (89).

Of interest, RSV could also affect patients negatively. In human subjects suffering from nonalcoholic fatty liver disease, 3 g of RSV per day increased hepatic stress (90). High doses may even lead to increased levels of TNF, as did a single dose of 5 g of RSV in healthy men (91). Furthermore, RSV supplementation may blunt the positive effects from health-improving interventions such as exercise (92). A negative impact of supplements on exercise-induced benefits has been reported before for antioxidant vitamins (93). Of interest, application of vitamin C and vitamin E at high doses has also been discussed as putatively favoring the healthspan. However, lifespan studies with these vitamins have yielded discouraging data (94, 95).

Another factor possibly contributing to controversial outcomes in human RSV supplementation trials could be their duration, since weeks or months may not suffice to adequately monitor metabolic, hormonal, and physiological changes caused by an intervention, or to evaluate how these changes may affect aging during years or decades. While studies in laboratory animals can render endpoints such as the lifespan and can provide easy access to organ tissues, choosing the correct biomarker in human trials can be challenging (96). The Targeting Aging with Metformin (TAME) trial, which is evaluating whether metformin may also benefit nondiabetic patients (97), has studied various possible biomarkers for aging research. They emphasize that a suitable biomarker needs to show a measurable change with age and be age-dependently associated with the all-mortality risk. Furthermore, it should be robust across data sets and populations, as well as reliable and reproducible across labs. Of interest, blood IGF-I levels show U-shaped concentration patterns for mortality risk, since both high and low levels of IGF-I are associated with increased risks of cancer and cardiovascular mortality (98). Candidate biomarkers for inflammation such as IL2, IL1B, interferon gamma (IFNG), or TNF may be unstable during storage or present at very low levels (97, 99). Here, IL6, CRP, and TNF receptor II seem

more suitable for measuring inflammation. Fasting insulin and IGF-I levels respond to changes in nutrient signaling, and HbA1c appears to be useful for monitoring metabolic aging (97). A recent 6-month supplementation trial in overweight adults found that although insulin sensitivity was not improved, HbA1c levels were decreased by 150 mg of RSV per day (67). Interestingly, nonenzymatically glycosylated tissue proteins [advanced glycation end products (AGEs)] also reflect increased glucose levels over time. AGEs can induce inflammation and increase oxidative stress. RSV supplementation may lower AGE toxicity (100). Since RSV may, similar to CR, affect numerous pathways implicated in aging, analyzing the serum metabolome as an -omics approach in human subjects (characterizing all metabolites affected by RSV treatment) could also produce relevant and feasibly reproducible data (101).

Conclusion

RSV supplementation studies in humans yield less promising data for aging-related benefits than epidemiological data on consumption of RSV-rich food (102, 103). Studies in model organisms comparing CR to RSV and studying age- and obesity-related biomarkers have found small, nonexistent, and even contrary effects of RSV on CR targets. Based on our literature review, we consider that RSV's CR-mimicking properties are rather moderate or are only applicable under certain circumstances. Therefore, we conclude that RSV supplementation cannot replace restricting dietary intake and may not be suitable for the prevention of age- or obesity-related diseases. With diet-derived molecules, hopes were that they might have fewer side effects than pharmaceutical drugs. However, non-plant derived small molecules such as metformin may be superior to polyphenols when counteracting age-related disease (104, 105).

In lifespan studies with RSV in mice, genotype or diet may influence the experimental outcome (42, 43, 47). Responsiveness toward RSV supplementation in humans may depend on the genome and dietary matrix. Genome-wide association studies searching for longevity genes and analyzing gene-diet interactions may identify different alleles of genes coding for proteins in nutrient-sensing cascades that are affected by RSV supplementation. To further evaluate whether RSV promotes healthy aging in human trials, long-term studies with high enough numbers of participants to detect the possible subgroups benefitting or not benefitting from RSV (106), using adequate biomarkers (96), and applying doses that have been regarded as safe (8) are needed. Supplementation of a single polyphenol compared to consumption of polyphenol-rich food may be less effective or even abolish positive effects because of side effects, such as toxicity issues, as well as nutrient-drug interactions (107). Moreover, the health benefits from consuming a plant-based diet appear to not solely rely on secondary plant metabolite uptake but on other factors, such as fiber consumption (108, 109). Therefore, further research on these other components found in plant-derived food and how they may positively affect aging is warranted. To date, to prolong the healthspan,

it seems that trying to increase the compliance for known lifespan-prolonging interventions, such as dietary changes and exercise, may yield more success than supplementation of single dietary factors such as RSV.

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