RESEARCH
Systematic Review

Impact of Resveratrol Supplementation on Human Sirtuin 1: A Grading of Recommendations Assessment, Development and Evaluation–Assessed Systematic Review and Dose-Response Meta-Analysis of Randomized Controlled Trials

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ABSTRACT

Background Resveratrol, a natural polyphenol compound, possesses anti-aging, anti-tumor, and vascular protective properties. These attributes are believed to stem from its influence on Sirtuin 1 (Sirt1), a member of the human Sirtuin family and a nicotinamide adenine dinucleotide–dependent histone deacetylase.

Objective The aim of this study was to quantitatively investigate the impact of resveratrol supplementation on Sirt1 levels in adults by conducting a systematic review and meta-analysis of randomized controlled trials (RCTs) involving resveratrol supplementation.

Methods This Grading of Recommendations Assessment, Development and Evaluation –assessed systematic review involved a comprehensive search of PubMed, Embase, MEDLINE, Scopus, Web of Science, Cochrane Central Register of Controlled Trials, and Google Scholar databases using related keywords and was conducted from March 14, 2024, to April 15, 2024, to identify all RCTs investigating resveratrol's effects on Sirt1. Effect sizes were quantified as mean differences (MDs) or standardized mean differences (SMDs), with standard deviations of outcomes. An overall effect estimate was derived using a random-effects model when 2 or more studies reported similar outcomes. Statistical heterogeneity was assessed through the calculation of I^2 statistics. In addition, a dose-response analysis was performed to assess potential dose-response relationships. Risk of bias was assessed using the Cochrane risk-of-bias tool for RCTs (RoB 2). Publication bias was evaluated using Begg's test and a meta-regression using the year of publication as a moderator.

Results Eleven RCTs examining the effects of resveratrol on Sirt1 gene expression (4 RCTs), protein expression (5 RCTs), and serum levels (3 RCTs) were included in the meta-analysis. The results showed no significant impact of resveratrol on Sirt1 gene expression (SMD = 0.05; 95% CI −0.24 to 0.344; $P = .73$), protein expression (SMD = 0.3; 95% CI −0.15 to 0.77; $P = .18$), or serum levels (MD = −0.04; 95% CI −0.235 to 0.16; $P = .7$). However, subgroup analyses suggested a significant increase in Sirt1 gene expression in studies with an intervention duration of <12 weeks and evaluating blood tissue. Furthermore, the impact of resveratrol on Sirt1 appeared to be influenced by the dosage regimen, with a significant effect for intervention duration.

Conclusions Study results indicate that resveratrol supplementation does not significantly influence human Sirt1 based on the overall meta-analysis. However, the dose-response analysis suggests that the effect of resveratrol on Sirt1 depends on the dosage regimen.
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SIRTUINS, A FAMILY OF NICOTINAMIDE ADENINE dinucleotide (NAD+)-dependent class III histone deacetylases, are found throughout the body's tissues, with 7 isoforms ranging from sirtuin 1 (Sirt1) to sirtuin 7, distributed across the cytoplasm, nucleus, and mitochondria of cells.[1-3] Renowned for their regulatory functions, particularly in metabolism and aging, sirtuins have garnered considerable attention.[4] Sirt1, a prominent member

of the Sirtuin family, is primarily located within the nucleus and cytoplasm of mammalian cells. Its primary role involves regulating metabolic homeostasis, cellular differentiation, gene expression, maintaining genome stability, controlling apoptosis, autophagy, senescence, proliferation, aging, tumorigenesis, and promoting cell survival.[1,5,6] In addition, Sirt1 exerts regulatory influence on immune responses[7] and is implicated in a spectrum of pathologies, including various types of cancer,[6,8-13] diabetes and its complications,[14-17] and cardiovascular diseases.[18] Sirt1 plays a pivotal role in numerous molecular processes. One such process entails its facilitation of NAD+-dependent lysine deacetylation across a spectrum of proteins. This activity results in the generation of nicotinyl derivatives. Consequently, these derivatives induce cellular oxidative stress, serving as a defensive mechanism against deleterious metabolic byproducts.[19,20] Interestingly, sirtuins, functioning as histone deacetylases, hold a notable position in epigenetic regulation. Within this family, Sirt1 garners considerable attention as the extensively researched isoform that is pivotal for maintaining tissue equilibrium and impacting diverse diseases. Its role extends beyond histone deacetylation to modulate chromatin function and includes the deacetylation of nonhistone targets and various transcription factors. Through these mechanisms, Sirt1 regulates the expression of target genes, exerting both positive and negative effects.[6,21,22] Given the substantial roles of sirtuins, they have emerged as attractive therapeutic targets, leading to considerable efforts aimed at developing specific sirtuin activators and inhibitors.[23]

Resveratrol, known scientifically as 3,5,4′-trihydroxy-trans-stilbene, is a naturally occurring polyphenol compound that can potentially have anti-aging, antitumor, and vascular protection properties, achieved through the activation of Sirt1.[24,25] Some studies have been undertaken to assess the impact of resveratrol on Sirt1 modulation. For instance, an in vitro study conducted by Borra and colleagues[26] suggested that resveratrol might mimic endogenous regulators, potentially modifying Sirt1 structure and function, resulting in an apparent "up-regulation" of Sirt1. Another study proposed that the up-regulation of Sirt1 is one of the mechanisms involved in resveratrol's role in reducing superoxide production under high-glucose conditions.[27] Recent animal studies have also found that resveratrol has an activating impact on Sirt1 and on its associated pathways. For instance, administering resveratrol improved cardiac function in mice with heart failure by activating the Sirt1/p53 pathway, alongside other advantageous effects.[28] Moreover, administering resveratrol activates Sirt1 in male Sprague-Dawley rats with injured arteries,[29] and it may also trigger SIRT1/nuclear factor erythroid 2-related factor 2 signaling, mitigating inflammatory response and endoplasmic reticulum stress and ultimately postponing ovarian aging in a short-lived fish.[30] There are also clinical trials that suggested resveratrol exhibits an activating influence on Sirt1.[31-36] Conversely, certain clinical studies evaluating the effects of resveratrol supplementation found no evidence of resveratrol activating Sirt1.[37-41] Moreover, there are also inconsistencies regarding the effect of resveratrol on Sirt1 activation among other types of study design. For example, Huang and colleagues[25] discovered an inconsistency between the in vivo and in vitro effects. They observed that resveratrol treatment in vivo can promote the binding of liver kinase B1 to Sirt1, leading to the release of Sirt1 activity. However, this effect cannot be observed directly in vitro. In another study conducted by Beher and colleagues,[42] resveratrol did not trigger Sirt1 activation in in vitro and cell-based assays.

RESEARCH SNAPSHOT

Research Question: How does resveratrol supplementation affect human Sirtuin 1?

Key Findings: This systematic review and meta-analysis encompasses 11 randomized controlled trials published between 2011 and 2023. Resveratrol consumption did not have a statistically significant overall effect on Sirtuin 1 gene expression, protein expression, or serum levels in the pooled meta-analysis. However, results of the dose-response analyses evaluating the effect of dose and intervention duration showed that the effect of resveratrol on Sirtuin 1 was significantly influenced by the duration of the intervention. The certainty in these findings was graded from moderate to low.

Despite the abovementioned examples of evaluating the impact of resveratrol administration on Sirt1 and its associated pathways, to the best of our knowledge, only 2 systematic reviews[43,44] have specifically evaluated the impact of resveratrol on Sirt1. One review by Najafi and colleagues[43] examined the potential effects of resveratrol supplementation on SIRT1 and found beneficial influences on both SIRT1 gene expression and protein expression, although the results were inconsistent. In addition, another systematic review of randomized controlled trials (RCTs) assessed 7 studies to evaluate resveratrol's modulatory effects on sirtuins, suggesting a possible activation of Sirt1 in humans.[44] Although these systematic reviews indicated a potential role for resveratrol in modulating Sirt1 expression, the researchers did not perform a meta-analysis, leaving the effects without quantitative evaluation.

The inconsistencies in the existing literature, the lack of quantitative assessments, and the critical role of Sirt1 in metabolic regulation and various health conditions[6,13,14] underscore the pressing need for further research, particularly a meta-analysis, to better understand the relationship between resveratrol and human Sirt1. Hence, the objective of the present study was to assess the precise impact of resveratrol supplementation on Sirt1 gene expression, protein expression, and serum levels in adults via a systematic review and dose-response meta-analysis evaluated using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach.[45,46]

METHODS

Search Strategy

From March 14, 2024 to April 15, 2024, 2 reviewers conducted a systematic search across 7 scientific databases—PubMed, Embase, MEDLINE, Scopus, Web of Science, Cochrane Central Register of Controlled Trials, and Google scholar—as well as a manual search of related articles, which involved systematically reviewing references from relevant articles, bibliographies, and databases to identify additional studies that may not be captured through automated

searches. The search strategy for identifying relevant studies is shown in Figure 1 (available at www.jandonline.org). *EndNote* software, version 21.2[47] was used for the management of citations, removal of duplicates, and enhancement of the review process. This systematic review and meta-analysis strictly follow the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines.[48] The study protocol is registered in the International Prospective Register of Systematic Reviews (https://www.crd.york.ac.uk/PROSPERO/; registration no. CRD42024524424).

Inclusion and Exclusion Criteria

This study encompassed all of the parallel or crossover RCTs that evaluated the effects of resveratrol supplementation on Sirt1 levels, which were measured through serum levels, protein expression, or gene expression as either primary or secondary outcomes. The intervention was oral resveratrol supplementation, in any form and dosage, as the sole intervention compared with placebo, calorie-restricted diets, or no intervention among adults 18 years or older. Studies that did not use randomization or were conducted on populations younger than 18 years were excluded.

Article Screening and Selection

Two reviewers independently screened all records and assessed each report retrieved. In cases of disagreement regarding study selection, the reviewers collaboratively resolved conflicts to ensure consensus. Out of 21 potentially related studies, 11 studies met the eligibility criteria for inclusion in this systematic review and meta-analysis.[31-41]

Data Extraction

A custom data extraction form developed in Excel (Microsoft) was used by 2 reviewers to systematically collect and organize the relevant data from the included studies. The 2 reviewers worked independently during the data extraction process, and any disagreements were resolved through discussion to reach a consensus. The following data were extracted: surname of the first author, country of origin, year of publication, number of participants, sex, mean age with standard deviation (SD)[31-33,35,36,38-41] or standard error of the mean,[34,37] study design (parallel[31-33,35-40] or crossover[34,41]), intervention details (type of resveratrol and the dosage administered), control details, study duration, the types of outcomes (ie, Sirt1 serum levels, Sirt1 protein expression levels or Sirt1 gene expression levels, tissue evaluated, method, and unit of measurement), the mean value with SD[31,32,34,35,39-41] or standard error of the mean,[33,37] geometric mean with minimum and maximum values,[38] or median with interquartile range[36] of the outcomes, body mass index (BMI; calculated as kg / m^2), and health status of study participants. In 7 studies,[32-34,37,39-41] the levels of Sirt 1 were available only as graphical representations. Therefore, the authors were contacted to obtain missing quantitative data.

Quality Assessment and Risk of Bias

Two authors independently assessed the quality of the studies using the Cochrane risk-of-bias tool for RCTs (RoB 2).[49] This tool determines study bias in several domains, including risk of bias arising from the randomization process, risk of bias arising from period and carryover effects (crossover RCTs), risk of bias due to deviations from the intended interventions, risk of bias due to missing outcome data, risk of bias in measurement of the outcome, and risk of bias in selection of the reported result. Consequently, terms including "low," "some concerns," or "high" were used to assess each domain for study bias. In instances when independent reviewers disagreed on the degree of study bias within each domain, final assessments were made based on author consensus.

Statistical Analysis

To ensure a high level of precision in conducting the meta-analysis, Sirt1-related outcomes were categorized into the following 3 distinct measures based on the method of assessment: Sirt1 gene expression assessed via polymerase chain reaction,[50] Sirt1 protein expression evaluated through western blotting,[51] and Sirt1 serum levels measured using the enzyme-linked immunosorbent assay[52] method. Meta-analysis was then carried out separately for each measure whenever data from at least 2 studies were accessible. This meta-analysis used the dplyr, meta, metafor, and dosresmeta packages in RStudio, version 2023.12.1+402.[53] The effect size was reported as mean difference (MD) for Sirt1 serum levels and standardized mean difference (SMD) for Sirt1 protein expression levels and Sirt1 gene expression levels, with 95% CIs. Data were collected as mean (SD) change. If mean changes in both intervention and control groups were not reported for each study, the mean change was determined by subtracting the postintervention mean from the baseline mean. The SD for this calculated mean was determined according to the method specified in the Cochrane guideline.[54] When only SDs were unavailable, they were derived from the 95% CIs and standard errors (SEs).[54] In 1 study, in which change in outcomes was reported as geometric mean with minimum and maximum values,[38] the mean with SD was calculated.[54,55] However, another included RCT reported the outcomes as median with interquartile range.[36] Thus, the mean with SD was calculated for this study according to the method specified in the Cochrane guideline.[52] In order to consider heterogeneity in study methodology, random-effects models, using the restricted maximum likelihood estimator, were used to calculate the pooled effect size. The restricted maximum likelihood method was chosen because it provides unbiased estimates of variance components, particularly in models with random effects. In addition, the weighting of individual studies was based on inverse-variance weighting, where each study's contribution to the overall pooled estimate is inversely proportional to the variance of its effect size, thus giving more weight to studies with more precise (lower variance) estimates.[54] Seven studies[31,33,36-39,41] were treated as multiple separate trials in the meta-analysis and included more than once, due to variations in resveratrol dosages, different control groups, diverse outcomes measured, or the use of different tissues for evaluation. Crossover trials were included in the meta-analysis alongside parallel trials by comparing measurements from the intervention periods with those from the control periods. Although this method may introduce a unit-of-analysis error, it is deemed a conservative approach.[54]

Furthermore, sensitivity analyses were performed by conducting paired analyses of crossover trials, incorporating various correlation coefficients (0.25, 0.5, and 0.75) to investigate potential underweighting of crossover studies.

Sensitivity and subgroup analyses were used to investigate sources of heterogeneity. Sensitivity analyses, using the one-study remove (leave-one-out) approach, were conducted to assess the impact of each individual study on the overall pooled effect by systematically excluding 1 study at a time and recalculating the summary estimates. Subgroup analyses were conducted for study duration ($<12/\geq12$ weeks), age of participants ($<60/\geq60$ years), study design (parallel/crossover), dosage of resveratrol ($<$ 500 mg/d/$\geq$500 mg/d), type of control groups (low-calorie diet as control group with or without placebo/any control or placebo without calorie restriction), and same outcome evaluated in different tissues, when applicable. The between-study heterogeneity was evaluated using the I^2 statistic, categorized per Cochrane guidelines: low heterogeneity ($I^2 = 0\%$ to 40%); moderate heterogeneity ($I^2 = 30\%$ to 60%); substantial heterogeneity ($I^2 = 50\%$ to 90%); and considerable heterogeneity ($I^2 = 75\%$ to 100%).[56]

The dose-response meta-analyses were carried out using a random-effects model, incorporating the restricted maximum likelihood estimator for heterogeneity and using a 1-stage approach.[57-59] These analyses explored potential linear or nonlinear associations between the resveratrol dose or duration of intervention and the influence on outcomes of interest. Furthermore, another dose-response analysis was conducted to examine potential relationships between the cumulative dose of resveratrol and changes in Sirt1 levels. The calculation of the cumulative resveratrol dose followed the method used by Pitre and colleagues[60]; the cumulative dose was determined by multiplying the administered dose by the duration of the trial in days. Subsequently, the measurement unit was converted from milligrams to grams, representing the total amount of resveratrol consumed by study participants throughout the entire intervention period. Among 3 dose-response models, including linear model, nonlinear quadratic model, and nonlinear restricted cubic spline model, those with the lowest Akaike's information criterion[58,61] were considered as the best-fitting models.

To assess the potential for publication bias in the meta-analysis, we used Begg's test, a rank-based method that assesses the correlation between effect estimates and their variances. This test was selected over Egger's test and funnel plot analysis, as it is more robust for smaller datasets and does not depend on the assumptions of linear regression required by Egger's test.[54,62-64] Given that our analysis includes fewer than 10 studies, the statistical power of Egger's test and the funnel plot is limited in detecting bias, making Begg's test a more appropriate choice. However, the results should be interpreted cautiously, given the limited number of studies and, as a result, limited power.[62,63]

In addition, given the limited number of studies, we conducted a meta-regression using the year of publication as a covariate to explore any potential temporal trends in the effect sizes, which could indicate bias over time. This approach allows for the detection of publication bias indirectly by evaluating whether newer studies reported different results than older studies.[65]

Certainty Assessment

The collective certainty of evidence across studies was evaluated according to the guidelines established by the GRADE working group[45,46] (https://www.gradeworkinggroup.org/). Subsequently, the quality of evidence was categorized into 4 tiers based on the respective evaluation criteria, encompassing high, moderate, low, and very low classifications.[66]

RESULTS

Study Selection

Figure 2 presents the Preferred Reporting Items for Systematic Reviews and Meta-Analyses[48] diagram outlining the systematic search and literature selection process. Initially, from the 577 records retrieved through databases, 168 duplicates were removed. After screening the titles and abstracts of the remaining 409 records, 388 were deemed ineligible based on predefined criteria. Full-text assessment was performed on 21 records, leading to the exclusion of 10 studies that did not meet the criteria. In addition, all 10 records obtained through manual searches were targeted for retrieval. Full-text assessments were conducted on each record, but none met the criteria for inclusion in this study. Ultimately, out of 21 potentially related studies, 11 studies[31-41] met the eligibility criteria for inclusion in this systematic review and meta-analysis.

Study Characteristics

Table 1 provides a comprehensive overview of the specific characteristics of the trials included in this study. Among the 11 studies incorporated into this systematic review and meta-analysis, research was conducted across the following 8 countries: Brazil (9%),[31] Denmark (18%),[37,41] United States (9%),[39] Iran (18%),[32,38] Mexico (9%),[33] The Netherlands (18%),[34,40] Singapore (9%),[35] and Italy (9%).[36] The studies were published from 2011 to 2023. Two studies used a crossover design[34,41] and the remaining trials used a parallel design.[31-33,35-40] Nine studies used a double-blind design[32-37,39-41] and 1 study[38] used a partially blind design. One study[31] did not incorporate any levels of blinding. The trial duration varied from 4 to 24 weeks. The dosage of resveratrol administered in the studies varied between 40 mg/d and 3000 mg/d. Three studies examined 2 distinct dosages of resveratrol[33,36,37] and the remaining studies evaluated a single dose of resveratrol.[31,32,34,35,38-41] In terms of outcomes, 3 studies[32,37,39] examined Sirt1 gene expression across various tissues after resveratrol supplementation using polymerase chain reaction. One study reported the results as fold change relative to the control sample[32]; another expressed them in arbitrary units normalized to a housekeeping gene[37]; and the third presented the data as relative RNA abundance, also normalized to a housekeeping gene.[39] Two studies[33,38] assessed serum Sirt1 levels using the enzyme-linked immunosorbent assay method, reporting the results as nanograms per milliliter (which can be converted to International System of Units by multiplying by 1 to yield micrograms per liter[67]). One study[31] examined both gene expression, presented as arbitrary units normalized to a housekeeping gene, and serum Sirt1 levels, expressed as nanograms per milliliter. Furthermore, Sirt1 protein expression in various tissues was analyzed in another 5 studies[34-36,40,41] using western blotting. The results were

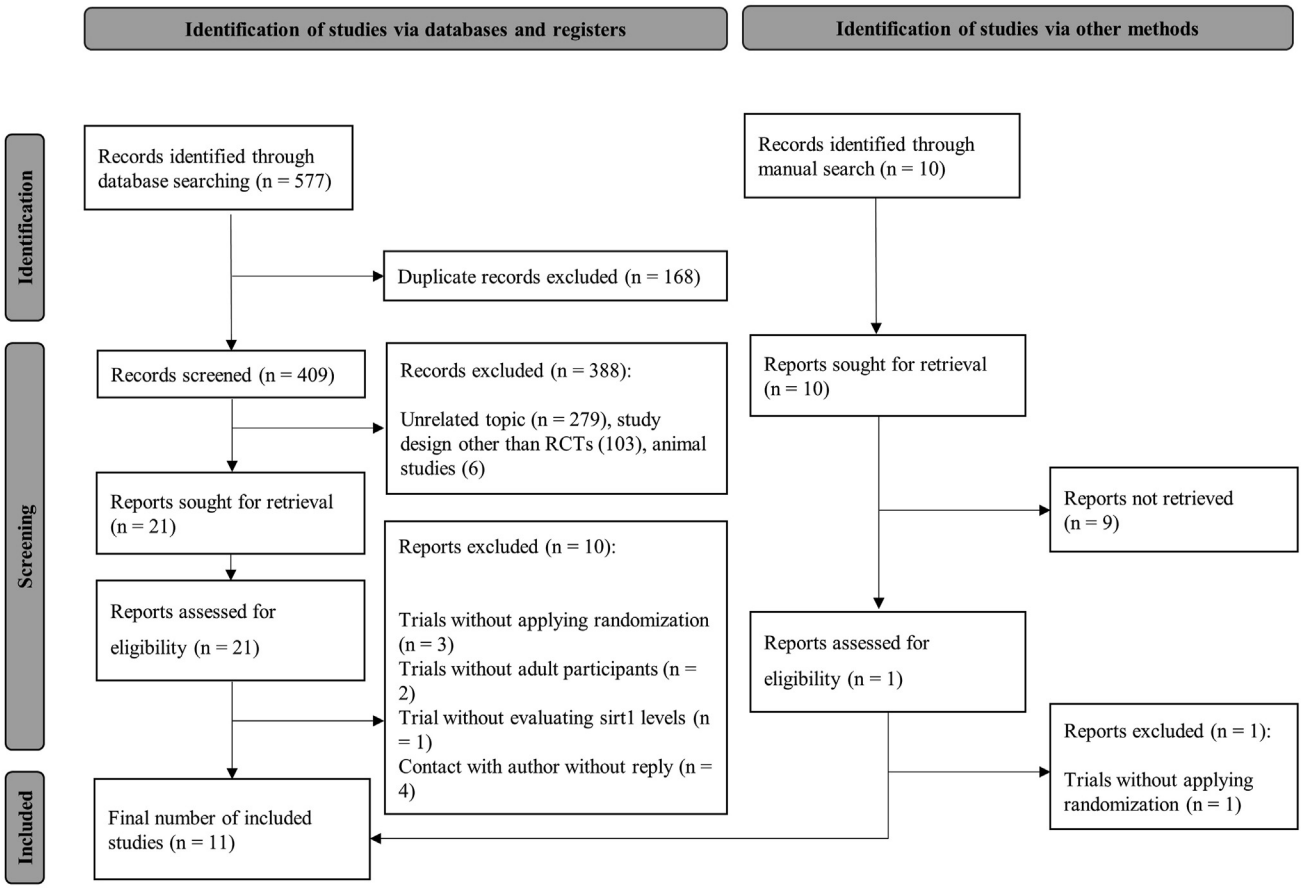

Figure 2. Flow diagram of the literature search and filtering results. RCT = randomized controlled trial.

reported in the following formats: as relative expression of SIRT1/tubulin,[34] as arbitrary units normalized to a housekeeping gene,[35,40] as relative amount,[36] and as relative expression.[41] Except for 4 studies,[32,34,35,40] the rest were included more than once in the meta-analyses due to the presence of multiple control groups,[38,41] multiple intervention groups,[33,36,37] multiple outcomes,[31] or evaluation across multiple tissues.[37,39]

Participant Characteristics

The analysis comprised 11 studies with a combined enrollment of 632 participants. Among these studies, 3 enrolled male participants exclusively[34,35,37] and 1 enrolled female participants exclusively.[39] In addition, 2 studies did not provide a clear delineation regarding the sex distribution among participants.[32,33] The mean age of participants, randomly assigned, ranged from 39[38] to 66[40] years. The sample sizes varied across studies, ranging from 10[35] to 192[36] participants. Mean BMI values ranged from 24[39] to 34.[37] Participants' health statuses included type 2 diabetes mellitus,[33,35,36] type 2 diabetes mellitus combined with coronary heart disease[32] or nonalcoholic fatty liver disease,[38] chronic obstructive pulmonary disease,[40] mitochondrial myopathies,[41] and metabolic syndrome, which was present in all study participants[37] or in a subset of them.[39] In studies conducted by Mansur and colleagues[31] and Timmers and colleagues,[34] participants were classified as healthy; Mansur and colleagues included individuals who were slightly overweight (BMI $<$ 30), and Timmers and colleagues included participants who were healthy but obese (BMI $\geq$ 30).

Quality Assessment and Risk of Bias

The quality and risk of bias in the eligible trials were evaluated using the Cochrane risk-of-bias tool (RoB 2),[49] and results are outlined in Figure 3. The study by Mansur and colleagues[31] raised "some concerns" regarding the randomization process and selection of reported results, and the study by Timmers and colleagues[34] raised "some concerns" in the selection of reported results. Overall, the risk of bias across all included studies ranged from "low" to "some concerns."

Meta-Analysis Results

Effect of Resveratrol Supplementation on Sirt1 Gene Expression. Sirt1 gene expression was evaluated across 4 RCTs [31,32,37,39] incorporated into this analysis. The RCT conducted by Kjær and colleagues[37] is represented 4 times in this meta-analysis due to its examination of 2 different dosages of resveratrol across 2 different tissues. In addition, the study conducted by Yoshino and colleagues,[39] focusing on Sirt1 gene expression in 2 different tissues, contributes 2 instances to this meta-analysis. Ultimately, the meta-analysis, which

Table 1. Characteristics of included studies in the systematic review and meta-analysis evaluating the impact of RSV[a] supplementation on human Sirt1[b]

First author, year	Country	Duration, wk	Health status	Group	n[c]	Sex	Age, y	BMI[d]	Outcome	Assessed tissue
							←mean ± SD[e]→			
Mansur, 2017[31]	Brazil	4	Healthy and overweight	RSV, 500 mg/d Low-calorie diet	24 24	12 M[f]/12 F[g] 12 M/12 F	58.5 ± 3.44 58.6 ± 3.65	27.6 ± 4.24 26 ± 3.22	Sirt1 gene expression (AU[h]) and Sirt1 blood concentration (ng/mL[i])	Peripheral leukocytes and plasma
Hoseini, 2019[32]	Iran	4	T2DM[j] and CHD[k]	RSV, 500 mg/d Placebo	28 28	Unreported	61 ± 8.6 63 ± 10.1	29 ± 3.1 28 ± 3.4	Sirt1 gene expression (fold change)	PBMC[l]
García-Martínez, 2023[33]	Mexico	24	T2DM	RSV, 500 mg/d RSV, 1000 mg/d Placebo	32 37 28	Men and women	63 ± 7 66 ± 6 64 ± 5	28 ± 3.6 28 ± 4.4 28 ± 3.4	Sirt1 blood concentration (ng/mL[i])	Plasma
							←mean ± SEM[m]→			
Timmers, 2011[34]	The Netherlands	4	Healthy and obese	Trans-RSV, 150 mg/d Placebo	11	Men	52.5 ± 2.1[n] 52.5 ± 2.1	31 ± 0.82 31 ± 0.74	Sirt1 protein expression (relative expression of SIRT1/tubulin)	Vastus lateralis muscle
							←mean ± SD→			
Goh, 2014[35]	Singapore	12	TD2M	Trans-RSV, 3000 mg/d Placebo	5 5	Men	56 ± 7.3 57 ± 5.3	29 ± 6.8 24 ± 3.6	Sirt1 protein expression (AU)	Skeletal muscle
Bo, 2018[36]	Italy	24	TD2M	RSV, 40 mg/d RSV, 500 mg/d Placebo	65 65 62	38 M/ 27 F 41 M/ 24 F 47 M/ 15 F	65 ± 8.6 65 ± 7.6 65 ± 8.8	29.5 ± 3.8 29 ± 3.9 28 ± 3.9	Sirt1 protein expression (relative amount)	PBMC
							←mean ± SEM→			
Kjær, 2017[37]	Denmark	16	MetS[o]	RSV, 150 mg/d RSV, 1000 mg/d Placebo	21 21 24	Men	49 ± 1.46 52 ± 1.28 48 ± 1.30	33 ± 0.858 34 ± 0.668 34 ± 0.77	Sirt1 gene expression (AU)	Skeletal muscle and SAT[p]
							←mean ± SD→			
Asghari, 2018[38]	Iran	12	NAFLD[q]	RSV, 600 mg/d Low-calorie diet Placebo	30 30 30	18 M/7 F 17 M/9 F 17 M/7 F[r]	40 ± 7.74 39 ± 5.51 40 ± 7.08	31 ± 3.10 30 ± 3.39 31 ± 3.31	Sirt1 blood concentration (ng/mL[i])	Plasma

(*continued on next page*)

Table 1. Characteristics of included studies in the systematic review and meta-analysis evaluating the impact of RSV[a] supplementation on human Sirt1[b] (*continued*)

First author, year	Country	Duration, wk	Health status	Group	n[c]	Sex	Age, y	BMI[d]	Outcome	Assessed tissue
Yoshino, 2012[39]	United States	12	Lean and overweight post-menopausal	RSV, 75 mg/d Placebo[s]	15 15	Women	58 ± 4.0 60 ± 4.3	24 ± 2.8 24 ± 2.7	Sirt1 gene expression (relative RNA abundance)	Skeletal muscle and adipose tissue
Beijers, 2020[40]	The Netherlands	4	COPD[t]	RSV, 150 mg/d Placebo	11 10	7 M/4 F 5 M/5 F	68 ± 9.0 65 ± 9.1	24 ± 3.7 25 ± 3.0	Sirt1 protein expression (AU)	Skeletal muscle
Løkken, 2021[41]	Denmark	8	Ambulatory patients with genetically verified MM[u]	Trans-RSV, 1000 mg/d Placebo, Untreated control group	11	6 M/5 F	49.5 ± 14.27	24.6 ± 6.21	Sirt1 protein expression (relative expression)	Muscle tissue

[a]RSV = resveratrol.
[b]Sirt1 = Sirtuin 1.
[c]n = Number of participants in the control and intervention groups for each study. For crossover randomized controlled trials, the total number of participants is reported.
[d]BMI = body mass index; calculated as kg / m^2.
[e]SD = standard deviation.
[f]M = male.
[g]F = female.
[h]AU = arbitrary unit.
[i]To convert ng/mL to μg/L, multiply by 1.
[j]T2DM = type 2 diabetes mellitus.
[k]CHD = coronary heart disease.
[l]PBMC = peripheral blood mononuclear cell.
[m]SEM = standard error of the mean.
[n]In this crossover randomized controlled trial, baseline characteristics are reported only in the 0 day of first phase of experiment.
[o]MetS = metabolic syndrome.
[p]SAT = subcutaneous adipose tissue.
[q]NAFLD = nonalcoholic fatty liver disease.
[r]The number of male and female participants does not equal the total sample size due to missing data for this variable.
[s]This study incorporated 2 control groups: 1 receiving a placebo and the other a low-calorie diet. The impact of RSV supplementation on Sirt1 gene expression was assessed only in the intervention and placebo groups.
[t]COPD = chronic obstructive pulmonary disease.
[u]MM = mitochondrial myopathies.

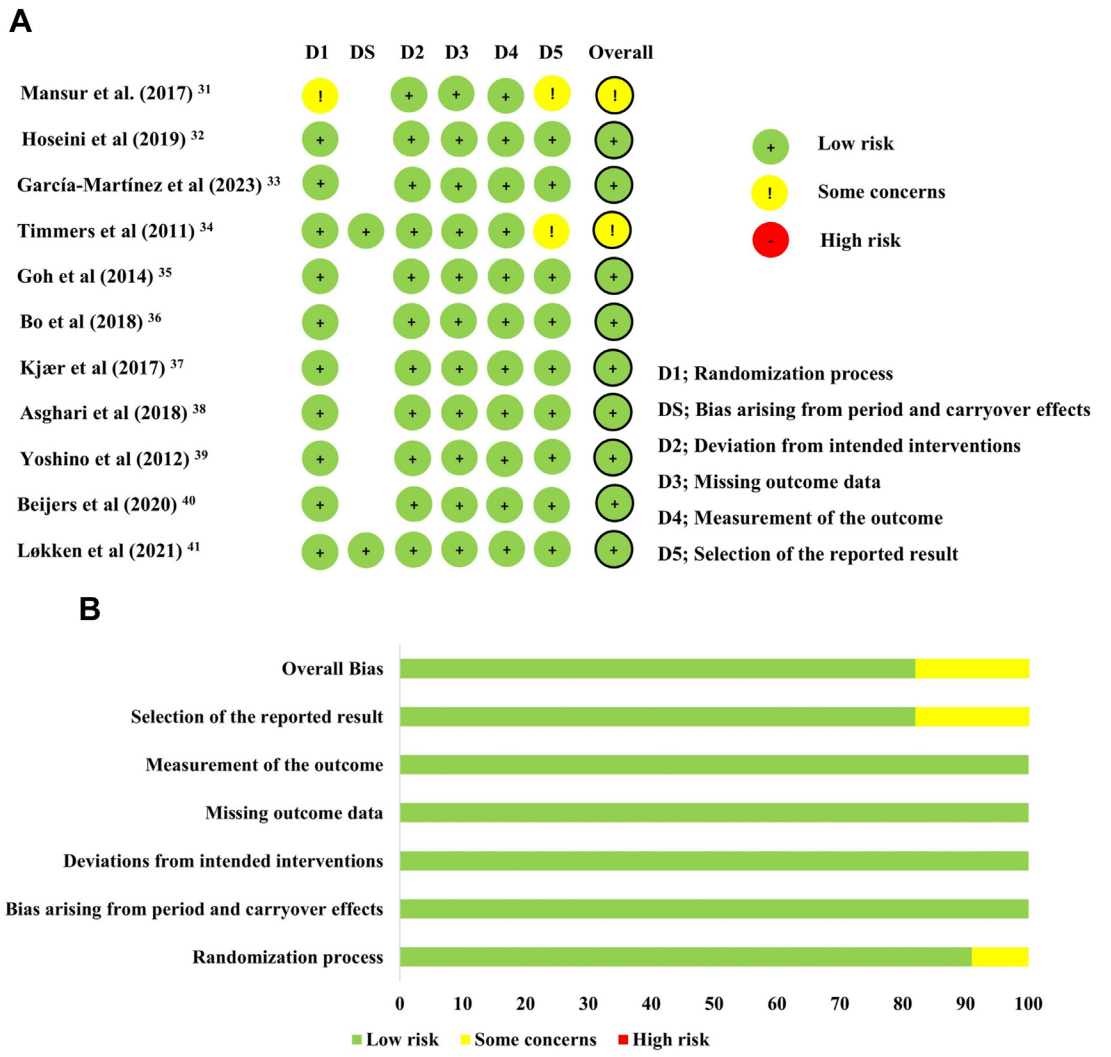

Figure 3. Cochrane risk-of-bias assessment: judgments on study quality and reliability across RoB 2 domains for each study included in the meta-analysis on the impact of resveratrol supplementation on human Sirtuin 1 (Sirt1). (A) Risk-of-bias assessment by individual study and (B) percentage distribution of risk-of-bias assessment across domain.

encompasses the Sirt1 gene expression data from the 4 studies after resveratrol supplementation (200 observations including 109 intervention and 91 control subjects), suggests that resveratrol supplementation does not significantly impact Sirt1 gene expression compared with the control groups (SMD = 0.05; 95% CI −0.24 to 0.344; $P = .73$) with moderate between-study heterogeneity ($I^2 = 46\%$; 95% CI 0% to 76%; $P = .07$) (Figure 4). To understand sources of heterogeneity, subgroup analyses were conducted based on trial duration (<12/≥12 weeks), dosage of resveratrol (<500/≥500 mg/d), and the specific tissue evaluated (adipose tissue/blood tissue/muscle tissue) (Table 2). Subgroup analyses by tissue evaluated and trial duration modified the heterogeneity, revealing a significant effect of resveratrol supplementation on Sirt1 gene expression in blood tissue and among trials lasting less than 12 weeks ($P = .046$). However, subgroup analysis based on the dosage of resveratrol did not modify the heterogeneity and did not show any significant effects within subgroups (Table 2).

Effect of Resveratrol Supplementation on Sirt1 Protein Expression. The meta-analysis encompassed RCTs assessing Sirt1 protein expression. The RCT conducted by Løkken and colleagues,[41] featuring 2 different control groups, and the study from Bo and colleagues[36] are each included twice in this meta-analysis because they investigated 2 different resveratrol dosages. Finally, results of the meta-analysis of 5 studies[34-36,40,41] evaluating Sirt1 protein expression after resveratrol supplementation (267 observations including 168 intervention and 99 control subjects) showed that resveratrol supplementation does not significantly affect Sirt1 protein expression compared with the control groups (SMD = 0.3; 95% CI −0.15 to 0.77; $P = .18$) with substantial between-study heterogeneity ($I^2 = 69\%$; 95% CI 32% to 86%; $P < .01$) (Figure 5). To understand sources of heterogeneity, subgroup analyses were conducted based on trial duration (<12/≥12 weeks), dosage of resveratrol (<500/≥500 mg/d), tissue assessed (muscle tissue/peripheral blood mononuclear cell), mean

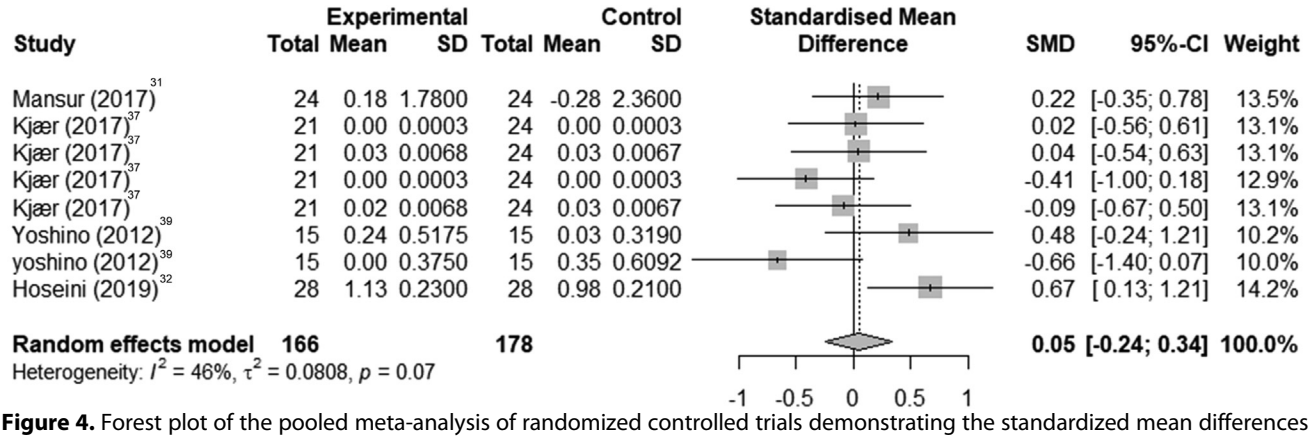

Figure 4. Forest plot of the pooled meta-analysis of randomized controlled trials demonstrating the standardized mean differences (SMDs) corresponding with 95% CI of the effect of resveratrol on Sirtuin 1 gene expression level.

age of participants (<60/≥60 years), and study design (parallel/crossover) (Table 2). Although between-study heterogeneity decreased among studies with a parallel design, those involving participants aged 60 years and older and studies using resveratrol doses <500 mg/d, subgroup analyses did not eliminate between-study heterogeneity. In addition, the effects of resveratrol supplementation did not achieve statistical significance in any of the subgroups (Table 2).

Effect of Resveratrol Supplementation on Serum Levels of Sirt1. Serum levels of Sirt1 were assessed across 3 RCTs[31,33,38] incorporated into this analysis. The RCT conducted by García-Martínez and colleagues,[33] which investigated 2 different resveratrol dosages, and the study conducted by Asghari and colleagues,[38] featuring 2 different control groups, are each represented twice in this meta-analysis. Results of the meta-analysis of these 3 studies (235 observations including 123 intervention and 112 control subjects) suggest that resveratrol supplementation does not significantly impact serum levels of Sirt1 compared with the control groups (MD = −0.04; 95% CI −0.235 to 0.16; $P = .7$), with low between-study heterogeneity ($I^2 = 23\%$; 95% CI 0% to 68%; $P = .27$) (Figure 6). A subgroup analysis based on the mean age of participants (<60/≥60 years) was conducted and completely resolved heterogeneity. The results are presented in Table 2.

Sensitivity Analysis

In order to determine whether the outcomes might have been underestimated in crossover trials, paired analyses of crossover RCTs were conducted with correlation coefficients of 0.25, 0.5, and 0.75 for assessing the effects of resveratrol supplementation on Sirt1 protein expression (Table 3). The inclusion of correlation coefficients indicated that the overall results for Sirt1 protein expression may deviate from the primary analyses and reach statistical significance (Table 3). Furthermore, sensitivity analyses using the leave-one-out method were conducted to identify studies that might have inflated effect sizes. However, no significant findings were revealed through this approach (data not shown). Subgroup analyses were performed to assess the duration of the trial, study design, mean age of participants, dosage of resveratrol, and specific tissue evaluated, when applicable. The results of the subgroup analysis are presented in Table 2.

Dose-Response Meta-Analysis Results

The investigation delved into the intricate relationship between resveratrol dosage, intervention duration, and cumulative dose of resveratrol through a dose-response meta-analysis, and the findings are illustrated in Figures 7 to 9. For all associations, a nonlinear quadratic model showed a higher value of Akaike's information criterion, compared with both linear and restricted cubic spline nonlinear models. Consequently, a discernible curve was not attained for this model.

For Sirt1 gene expression, the best-fitting model indicated a significant nonlinear effect of intervention duration (P nonlinearity = .0067; Figure 8); resveratrol dosage and cumulative dosage did not show significant effects.

In contrast, for Sirt1 protein expression, the dose-response analysis revealed significant relationships with resveratrol dosage (P linearity = .0007; Figure 7), intervention duration (P nonlinearity = .039; Figure 8), and cumulative resveratrol dose (P linearity < .0001; Figure 9).

For Sirt1 serum levels, a significant nonlinear relationship was found with intervention duration (P nonlinearity = .0024; Figure 8), but other factors, including resveratrol dosage and cumulative dose, were not statistically significant.

Publication Bias

After a thorough examination of the outcomes of Begg's rank correlation test, no indications of publication bias were discerned in studies exploring the impact of resveratrol supplementation on Sirt1 gene expression (bias estimate = −15, SE = 8.021; $P = .06$), Sirt1 protein expression (bias estimate = 1, SE = 6.5; $P = .88$), and Sirt1 serum level (bias estimate = 2, SE = 4.08; $P = .6$). Given the small sample size and the high degree of uncertainty reflected in the large SEs, these findings should be interpreted cautiously.

With the exception of Begg's test, a meta-regression was conducted using the year of publication as a moderator across the studies. The results showed that the year of publication was not a significant moderator across the studies examining the effect of resveratrol on Sirt1 gene expression (estimate = 0.06, SE = 0.065; $P = .34$) and Sirt1 protein expression

Table 2. Results of subgroup analyses for the meta-analysis evaluating the impact of resveratrol supplementation on human Sirt1[a]

Outcome	No. of trials[b]	SMD[c] or MD[d] (95% CI)	*P* value	I^2, %[e] (95% CI)	*P* value
Sirt 1 gene expression					
Overall	4[31,32,37,39]	0.05 (−0.24 to 0.344)	.73	46 (0 to 76)	.07
Trial duration					
<12 wk	2[31,32]	0.453 (0.007 to 0.9)	.046	23 (0 to 43)	.255
≥12 wk	2[37,39]	−0.1 (−0.38 to 0.18)	.49	19 (0 to 64)	.3
Resveratrol dosage					
<500 mg/d	2[37,39]	−0.018 (−0.43 to 0.396)	.93	38 (0 to 79)	.185
≥500 mg/d	3[31,32,37]	0.11 (−0.35 to 0.57)	.64	61 (0 to 87)	.053
Tissue assessed					
Adipose	2[37,39]	−0.3 (−0.69 to 0.07)	.113	10 (0 to 91)	.33
Blood	2[31,32]	0.45 (0.007 to 0.9)	.046	23 (0 to 43)	.255
Muscle	2[37,39]	0.1 (−0.26 to 0.46)	.58	0 (0 to 90)	.476
Sirt1 protein expression					
Overall	5[34-36,40,41]	0.3 (−0.15 to 0.77)	.18	69 (32 to 86)	.01
Trial duration					
<12 wk	3[34,40,41]	0.048 (−0.77 to 0.87)	.908	72% (19 to 90)	.014
≥12 wk	2[35,36]	0.55 (−0.001 to 1.104)	.05	70 (0 to 91)	.035
Study design					
Parallel	3[35,36,40]	0.445 (−0.01 to 0.9)	.055	60 (0 to 87)	.06
Crossover	2[34,41]	0.05 (−1.122 to 1.214)	.938	81 (41 to 94)	.005
Mean age of participants					
<60 y	3[34,35,41]	0.405 (−0.725 to 1.535)	.483	80.1 (47 to 92.5)	.001
≥60 y	2[36,40]	0.35 (−0.026 to 0.73)	.067	51.5 (0 to 86)	.127
Resveratrol dosage					
<500 mg/d	3[34,36,40]	0.32 (−0.16 to 0.8)	.187	37.2 (0 to 80)	.2
≥500 mg/d	3[35,36,41]	0.3 (−0.6 to 1.2)	.528	81 (49.5 to 93)	.001
Tissue assessed					
Muscle	4[34,35,40,41]	0.304 (−0.54 to 1.15)	.48	74 (34 to 89)	.004
PBMC[f]	1[36,g]	0.408 (−0.06 to 0.88)	.88	72 (0 to 94)	.06
Sirt1 serum levels					
Overall	3[31,33,38]	−0.04 (−0.235 to 0.16)	.7	23 (0 to 68)	.27
Mean age of participants					
<60 y	2[31,38]	−0.10 (−0.25 to 0.04)	.17	0 (0 to 90)	.934
≥60 y	1[33g]	0.59 (−0.037 to 1.2)	.065	0 (0 to 63)	.432

[a]Sirt1 = Sirtuin 1.
[b]In instances when the total number of trials included in subgroup analysis does not match the overall number of trials, it is due to variations in the tissues assessed or the dosages of resveratrol used in some of the trials.
[c]SMD = standardized mean difference. SMD used for Sirt1 gene expression and Sirt1 protein expression.
[d]MD = mean difference. MD used for Sirt1 serum levels.
[e]I^2 = test of heterogeneity. Low heterogeneity: I^2 = 0-40%, moderate heterogeneity: I^2 = 30-60%, substantial heterogeneity: I^2 = 50-90%, and considerable heterogeneity: I^2 = 75-100%.
[f]PBMC = peripheral blood mononuclear cell.
[g]One study was included in the meta-analysis as 2 trials because it evaluated 2 different dosages of resveratrol.

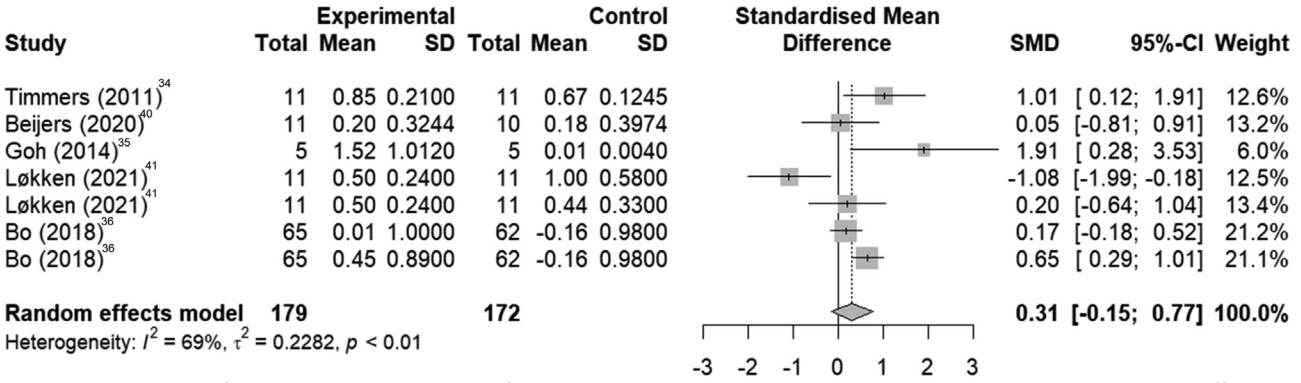

Figure 5. Forest plot of the pooled meta-analysis of randomized controlled trials demonstrating the standardized mean differences (SMDs) corresponding with 95% CI of the effect of resveratrol on Sirtuin 1 protein expression level.

(estimate = 0.017, SE = 0.08; P = .836), implying that the study results did not change over time. However, for the studies evaluating the effect of resveratrol supplementation on Sirt1 serum levels, the year of publication was found to be a significant moderator (estimate = 0.138, SE = 0.065; P = .03). This result indicates that more recent studies reported greater effects of resveratrol supplementation on Sirt1 serum levels, suggesting a possible temporal trend.

Certainty Assessment Results

The GRADE protocol was used to assess the certainty of evidence (Table 4). The evidence quality concerning Sirt1 serum levels was downgraded to moderate due to serious limitations in imprecision and publication bias, although it was enhanced by the presence of a dose-response gradient. Regarding the Sirt1 gene expression and Sirt1 protein expression, the quality of evidence was downgraded because of a very serious limitation in inconsistency and a serious limitation in imprecision, yet it was upgraded because of the existence of a dose-response gradient.

DISCUSSION

To our current understanding, this GRADE-assessed systematic review and dose-response meta-analysis represent the inaugural comprehensive and targeted investigation into the impact of resveratrol supplementation on Sirt1. It is worth noting that there are only 2 prior systematic reviews that examined the effects of resveratrol on Sirt1.[43,44] The current study encompassed all clinical trials with available data conducted between 2011 and 2023, ranging from 4 to 24 weeks in duration, involving a total of 632 adult participants. The findings diverge from those of the previous reviews,[43,44] suggesting that resveratrol supplementation does not significantly influence Sirt1 gene expression, Sirt1 protein expression, or Sirt1 serum levels, with low-to-moderate quality of evidence. Subgroup analyses, although limited by statistical power and the number of studies in each subgroup, indicate a significant impact on Sirt1 gene expression levels in trials lasting less than 12 weeks and those assessing Sirt1 gene expression in blood tissue. Sensitivity analysis using correlation coefficients within crossover trials suggested the potential for these study designs to have been undervalued, potentially leading to a significant effect of resveratrol on Sirt1 protein expression. However, these effects did not attain statistical significance in other subgroup or sensitivity analyses.

On one hand, the literature supports the activating effect of resveratrol on Sirt1. An in vitro study suggested that resveratrol may mimic natural regulators, modifying Sirt1's structure and leading to its upregulation.[26] Moreover, resveratrol administration activated Sirt1 in animal studies.[28-30] There are also clinical trials that have found that resveratrol exerts an activating influence on Sirt1.[31-36] Mechanistically, resveratrol is thought to activate Sirt1 indirectly by increasing intracellular NAD+ levels, a vital cofactor for Sirt1 activation. This process is driven by resveratrol's stimulation of AMP-activated protein kinase, which boosts NAD+ biosynthesis via enhanced activity of nicotinamide phosphoribosyltransferase. As NAD+ availability increases, Sirt1 deacetylase activity is enhanced, affecting various metabolic and cellular

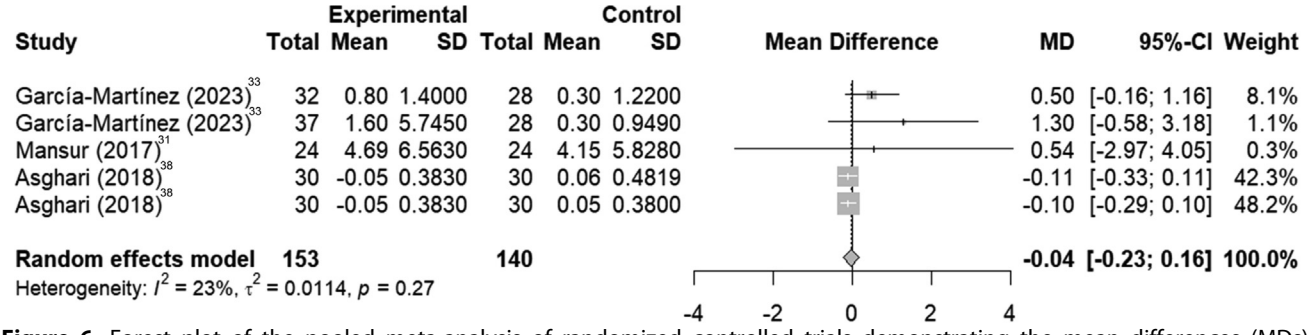

Figure 6. Forest plot of the pooled meta-analysis of randomized controlled trials demonstrating the mean differences (MDs) corresponding with 95% CI of the effect of resveratrol on Sirtuin 1 serum level.

Table 3. Sensitivity analyses using correlation coefficients[a] to assess the impact of crossover study design in the meta-analysis evaluating the impact of resveratrol supplementation on human Sirt1[b]

Outcome	Used correlation coefficients	Effect estimate (95% CI)	*P* value	I^2, %[c] (95% CI)	*P* value
Sirt1 protein expression	0.25	0.556 (0.3-0.8)	<.0001	70 (33.5-86)	.003
	0.5	0.655 (0.48-0.826)	<.0001	58 (3.5-82)	.026
	0.75	0.79 (0.75-0.832)	<.0001	41 (0-75)	.118

[a]This approach is conducted to evaluate whether the trials with crossover study design have been underweighted in primary meta-analysis. There are only 2 trials[34,41] with crossover study design evaluating the effect of resveratrol on Sirt1 protein expression.
[b]Sirt1 = Sirtuin 1.
[c]I^2 = test of heterogeneity. Low heterogeneity: I^2 = 0-40%, moderate heterogeneity: I^2 = 30-60%, substantial heterogeneity: I^2 = 50-90%), and considerable heterogeneity: I^2 = 75-100%.

processes, including glucose metabolism and mitochondrial function.[68,69]

On the other hand, inconsistencies exist regarding resveratrol's effect on Sirt1 activation across different study designs. For example, a research study found that resveratrol treatment facilitated the binding of liver kinase B1 to Sirt1 and enhanced Sirt1 activity in vivo, although this effect was not observed in vitro.[25] Moreover, an in vitro and cell-based assay indicated that resveratrol did not influence Sirt1 activation.[42] Furthermore, certain clinical studies assessing the effects of resveratrol supplementation reported no evidence of its ability to activate Sirt1.[37-41] Several factors may explain the observed lack of effect of resveratrol on Sirt1 activation in certain studies. One possible reason is the variability in resveratrol dosage and treatment duration, which may be inadequate to initiate Sirt1 activation.[70] In addition, differences in baseline Sirt1 levels, metabolic status, and health conditions of study participants (eg, type 2 diabetes and obesity) can affect their responsiveness to resveratrol.[71] Furthermore, bioavailability issues, such as poor absorption and rapid metabolism of resveratrol, might hinder its ability to reach sufficient concentrations necessary to activate Sirt1 in target tissues.[71]

Aside from the present study, 2 previous systematic reviews[43,44] have explored this topic, indicating that resveratrol supplementation may stimulate Sirt1 in humans. Findings from the current study diverge from these 2 systematic reviews. This discrepancy may be attributed to the integration of data through meta-analysis. Furthermore, although previous systematic reviews[43,44] considered the impact of resveratrol on certain Sirt1 outcomes to be noteworthy, our current study, after thorough communication with all authors and access to precise values regarding changes in Sirt1 outcomes, found that these effects were not statistically significant. Despite observable enhancements and positive alterations after resveratrol supplementation, these effects were not meaningful. Overall, this study can be considered more important because it has fewer methodological limitations and avoids publication bias. Moreover, it includes clinical trials with a relatively low risk of bias, offers more detailed data on Sirt1 changes, and applies the GRADE approach, which can provide more reliable insights into resveratrol's impact on Sirt1 in comparison with previous reviews.[43,44] This current study adds to a body of research indicating a less potent effect of resveratrol on Sirt1. Multiple studies, including clinical trials and in vitro and in vivo experiments, support our findings, confirming the lack of a noticeable impact of resveratrol on Sirt1.[25,37-42]

This meta-analysis pioneers an investigation into the dose-response relationship between resveratrol supplementation and Sirt1 levels, revealing a nonlinear correlation between the duration of resveratrol treatment and Sirt1 gene expression, Sirt1 protein expression, as well as the concentration of Sirt1 in serum. In addition, a linear relationship was observed between the dosage of administered resveratrol and Sirt1 protein expression. The results of the dose-response analysis underscore the significant impact of resveratrol dosage and treatment duration on activating Sirt1.

In addition to using the GRADE approach, conducting dose-response analysis, and incorporating trials with a high degree of blinding and low risk of bias, as mentioned previously, one of the notable strengths of this study was our meticulous evaluation of the specific effect of resveratrol on Sirt1 by categorizing the Sirt1 outcomes based on the method of measurement, ensuring high levels of precision. Furthermore, all comparisons between control and intervention groups within each trial were included in this meta-analysis, even if the variation was only in control groups, to avoid overlooking any evaluations. In addition, all possible subgroup and sensitivity analyses were conducted to assess various reasons for the diversity of effects among different trials and populations, as well as the effects of any confounding factors. However, this study was constrained by several limitations. It involves a few trials with a small number of participants, leading to evidence of low or moderate certainty regarding the outcomes. Furthermore, the limited number of studies included, the diversity of diseases among participants, and incomplete data within studies made it unfeasible to conduct certain subgroup analyses, such as those based on participants' health status or sex. Consequently, addressing between-study heterogeneity for specific outcomes of interest was challenging, and the impact of these factors was not assessed. In addition, most of the included population was obese or overweight, potentially limiting their representativeness of the general population. Another limitation arises from the limited variety in the dosage of resveratrol used across the included trials. This constraint may result in a failure to establish clear dose-response relationships between resveratrol dosage and Sirt1 gene expression, as well as Sirt1 serum concentration levels, despite the significant correlation observed for Sirt1 protein expression. Furthermore, literature searches were conducted during a 1-month timeframe, which means that for some of the databases, there is a small possibility that an eligible study published up until mid-April may have been missed.

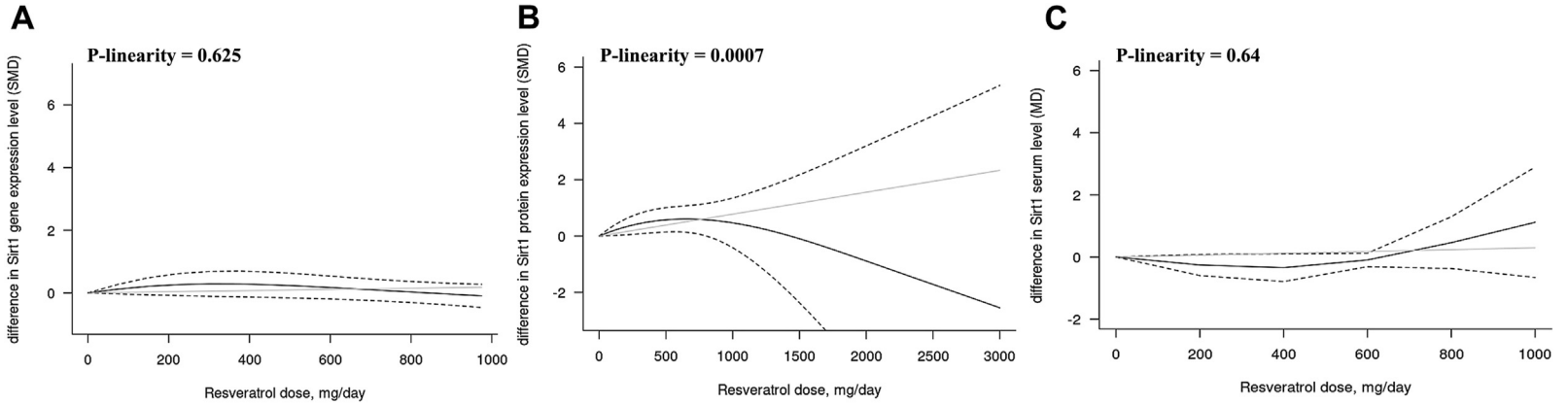

Figure 7. Dose-response relations between resveratrol dose (milligrams per day) and the observed effects of resveratrol supplementation on Sirtuin 1 (Sirt1) gene expression, protein expression, and serum levels, as measured by standardized mean differences (SMDs) for (A) Sirt1 gene expression level, (B) Sirt1 protein expression level, and measured by mean differences (MDs) for serum levels (C). The solid black curve represents a nonlinear association, with dashed lines indicating the 95% CI, providing a visual indication of the precision of the estimate. The solid gray line depicts the linear association. The *P* values indicated in each panel correspond to the statistical significance of the best-fitting model (either linear or nonlinear) for each outcome, helping assess whether the relationship is statistically meaningful.

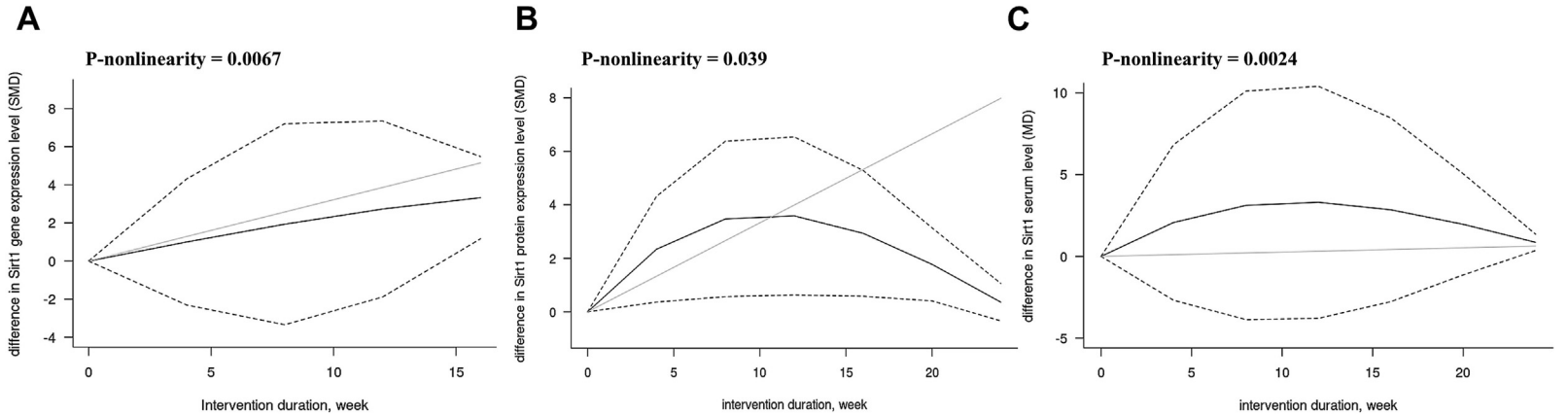

Figure 8. Dose-response relations between intervention duration (week) and the observed effects of resveratrol supplementation on Sirtuin 1 (Sirt1) gene expression, protein expression, and serum levels, as measured by standardized mean differences (SMDs) for (A) Sirt1 gene expression level, (B) Sirt1 protein expression level, and measured by mean differences (MDs) for serum levels (C). The solid black curve represents a nonlinear association, with dashed lines indicating the 95% CI, providing a visual indication of the precision of the estimate. The solid gray line depicts the linear association. The *P* values indicated in each panel correspond to the statistical significance of the best-fitting model (either linear or non-linear) for each outcome, helping assess whether the relationship is statistically meaningful.

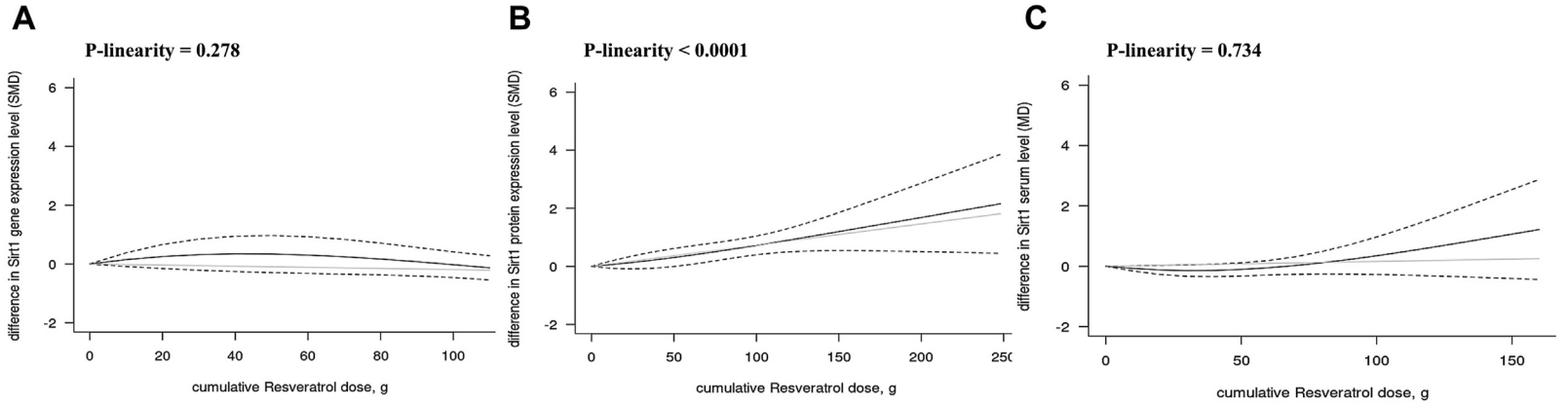

Figure 9. Dose-response relations between cumulative resveratrol dose (grams) and the observed effects of resveratrol supplementation on Sirtuin 1 (Sirt1) gene expression, protein expression, and serum levels, as measured by standardized mean differences (SMDs) for (A) Sirt1 gene expression level, (B) Sirt1 protein expression level, and measured by mean differences (MDs) for serum levels (C). The solid black curve represents a non-linear association, with dashed lines indicating the 95% CI, providing a visual indication of the precision of the estimate. The solid gray line depicts the linear association. The *P* values indicated in each panel correspond to the statistical significance of the best-fitting model (either linear or nonlinear) for each outcome, helping assess whether the relationship is statistically meaningful.

Table 4. GRADE[a] profile of included studies in the systematic review and meta-analysis evaluating the impact of resveratrol supplementation on human Sirt1[b]

Outcome	No. of studies	Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias	Dose-response	Sample size (intervention/control), n	Quality of evidence[c]
Sirt 1 gene expression	4[31,32,37,39]	No serious limitations	Very serious limitations	No serious limitations	Serious limitations	No serious limitations	Evidence of a gradient	200 (109/91)	⊕⊕◯◯[def] Low
Sirt1 protein expression	5[34–36,40,41]	No serious limitations	Very serious limitations	No serious limitations	Serious limitations	No serious limitations	Evidence of a gradient	267 (168/99)	⊕⊕◯◯[def] Low
Sirt1 serum levels	3[31,33,38]	No serious limitations	No serious limitations	No serious limitations	Serious limitations	Serious limitations	Evidence of a gradient	235 (123/112)	⊕⊕⊕◯[efg] Moderate

[a]GRADE = Grading of Recommendations Assessment, Development, and Evaluation.[45,46]
[b]Sirt1 = Sirtuin 1.
[c]⊕⊕⊕⊕ (High quality): There is a strong belief that the true effect lies close to estimated effect; ⊕⊕⊕◯ (moderate quality): There is a reasonable degree of certainty that the actual effect is near the estimated effect, while there exists a chance of significant difference; ⊕⊕◯◯ (low quality): the confidence in the estimated effect is restricted and there is a possibility of substantial disparity between the actual and estimated effects; ⊕◯◯◯ (very low quality): there is a minimal confidence in the estimated effect and the actual effect is probable to vary from the estimated one considerably.
[d]Heterogeneity, as indicated by the I^2, was moderate and substantial for Sirt1 gene expression and protein expression, respectively, and remained unexplained.
[e]CI crossed the clinical decision-making thresholds between recommending and not recommending treatment, as well as area of no effect.
[f]The involvement of participants is limited, with the most conservative criterion requiring a sample size exceeding 400 individuals.
[g]The presence of publication bias for Sirt1 serum level (asymmetric funnel plot).

CONCLUSIONS

This extensive analysis did not find a significant impact of resveratrol supplementation on Sirt1 levels based on the overall meta-analysis. However, subgroup and sensitivity analyses suggested signs of a potential stimulatory effect of resveratrol on Sirt1. Notably, this effect seems to depend on the dosage and duration of resveratrol intake. Therefore, further research involving larger and more diverse participant populations is warranted to refine the understanding of resveratrol's influence on human Sirt1 levels. Given that the current study underscores the importance of dosage and intervention duration in influencing resveratrol's impact on Sirt1, future investigations should prioritize exploring a range of dosages and intake durations to elucidate this relationship more comprehensively.

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STATEMENT OF POTENTIAL CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors.

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AUTHOR CONTRIBUTIONS

F. Mansouri: conceptualization, search, data extraction, data analysis, data interpretation, risk of bias analysis, manuscript drafting and revision. G. Feliziani: search, risk of bias analysis. L. Bordoni: conceptualization, supervision of the work and manuscript revision. R. Gabbianelli: supervision of the work, manuscript revision and funds acquisition.

Database name	Search strategy	Last search update	Number of retrieved studies
PubMed	(("Sirtuin1"[All Fields] OR "Sirtuin-1"[All Fields] OR "SIRT-1"[All Fields] OR "SIRT1"[All Fields] OR (("Sirtuins"[MeSH Terms] OR "Sirtuins"[All Fields] OR "sirtuin"[All Fields]) AND "s"[All Fields]) OR "SIRT/s"[All Fields] OR "Sirtuins"[MeSH Terms]) AND ("Resveratrol"[All Fields] OR "Resveratrol"[MeSH Terms]) AND ("randomized controlled trial"[All Fields] OR "randomised controlled trial"[All Fields] OR "RCT"[All Fields] OR "crossover"[All Fields] OR ("parallel"[All Fields]))	March 14th, 2024	29
EMBASE	#1 ('Sirtuin1:ab.ti' OR 'Sirtuin-1:ab.ti' OR 'SIRT-1:ab.ti' OR 'SIRT1:ab.ti' OR 'Sirtuins:ab.ti ' OR 'sirtuin:ab.ti') AND 'Resveratrol:ab.ti' #2 ('randomized controlled trial:ab.ti' OR 'randomised controlled trial:ab.ti' OR 'RCT:ab.ti') #3 #1 AND #2	March 23rd, 2024	31
MEDLINE	1- Sirtuin1.mp. 2- Sirtuin-1.mp. 3- SIRT-1.mp. 4- SIRT1.mp. 5- Sirtuin/ (MeSH) 6- SIRT/ (MeSH) 7- 1 or 2 or 3 or 4 or 5 or 6 8- Resveratrol.mp. 9- Resveratrol/ (MeSH) 10- 8 or 9 11- randomized controlled trial.tiab. 12- randomised controlled trial.tiab. 13- RCT.tiab. 14- Randomized Controlled Trial/ (MeSH) 15- 11 or 12 or 13 or 14 16- 7 and 10 and 15	March 27th, 2024	59
Scopus	TITLE-ABS-KEY ((sirt-1 OR sirtuin1 OR sirt1 OR sirtuin-1 OR sirtuin) AND (resveratrol) AND('randomized AND controlled AND trial' OR 'randomised AND controlled AND trial' OR rct))	April 1st, 2024	81
Web of Science	TS=("Sirtuin1" OR "Sirtuin-1" OR "SIRT-1" OR "SIRT1" OR "Sirtuin/s" OR "SIRT/s") AND TS="Resveratrol" AND TS=("randomized controlled trial" OR "randomised controlled trial" OR "RCT")	April 5th, 2024	13
Cochrane CENTRAL	("Sirtuin1" OR "Sirtuin-1" OR "SIRT-1" OR "SIRT1" OR "Sirtuin/s" OR "SIRT/s") AND "Resveratrol" AND ("randomized controlled trial" OR "randomised controlled trial" OR "RCT")	April 11th, 2024	17
Google scholar	"Sirtuin1" OR "Sirtuin-1" OR "SIRT-1" OR "SIRT1" OR "Sirtuin/s" OR "SIRT/s" "Resveratrol" "randomized controlled trial" OR "randomised controlled trial" OR "RCT"	April 15th, 2024	347

Figure 1. Search strategy for identifying relevant studies in databases.