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eNOS, Cardiac Senescence, and Cardiovascular Aging: A Meta-Analysis of Molecular Mechanisms and Clinical Outcomes

Tri Nisdian Wardiah[1*], Ali Zainal Abidin[1], Taufik Indrajaya[2], Nur Riviati[3]

[1]Department of Internal Medicine, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia

[2]Division of Cardiovascular, Department of Internal Medicine, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia

[3]Division of Geriatrics, Department of Internal Medicine, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia

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***Corresponding author:**
Tri Nisdian Wardiah

E-mail address:
trinisdian@gmail.com

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A B S T R A C T

Endothelial nitric oxide synthase (eNOS) plays a critical role in maintaining cardiovascular homeostasis. Its dysfunction is implicated in cardiac senescence, a hallmark of aging characterized by cellular decline and increased risk of cardiovascular disease. This meta-analysis investigated the association between eNOS, cardiac senescence, and cardiovascular aging, exploring underlying molecular mechanisms and clinical outcomes. A systematic search of PubMed, Scopus, and Web of Science databases was conducted for relevant studies published between 2013 and 2024. Studies investigating the relationship between eNOS, cardiac senescence markers (e.g., telomere length, p53, p16), and cardiovascular outcomes (e.g., heart failure, myocardial infarction, stroke) were included. Data were extracted and pooled using random-effects models. Nine studies (n=4,875 participants) met the inclusion criteria. Meta-analysis revealed a significant association between reduced eNOS activity and increased cardiac senescence markers (standardized mean difference [SMD] = -0.85; 95% confidence interval [CI], -1.20 to -0.50; p<0.001). Furthermore, eNOS dysfunction was associated with an increased risk of cardiovascular events (relative risk [RR] = 1.62; 95% CI, 1.25 to 2.10; p=0.001). Molecular analysis indicated that eNOS dysfunction contributes to cardiac senescence through increased oxidative stress, inflammation, and impaired autophagy. In conclusion, this meta-analysis provides compelling evidence for the detrimental role of eNOS dysfunction in cardiac senescence and cardiovascular aging. Targeting eNOS may offer promising therapeutic strategies to mitigate age-related cardiovascular decline.

1. Introduction

Cardiovascular disease (CVD) remains the leading cause of morbidity and mortality globally, with an alarming 23.6 million projected annual deaths attributed to CVD by 2030. The World Health Organization (WHO) underscores the urgency of addressing CVD as a critical public health concern, emphasizing its impact on individuals, families, and healthcare systems worldwide. The prevalence of CVD increases with age, with individuals over 65 years of age facing a significantly heightened risk. This age-related increase in CVD risk is intrically linked to the complex process of aging, a progressive decline in physiological function that affects all organ systems, with the cardiovascular system being particularly vulnerable. Aging is associated with a gradual deterioration of cardiovascular function, characterized by decreased vascular elasticity, impaired contractility, and increased susceptibility to various CVDs, including hypertension, atherosclerosis, heart failure, and stroke. At the cellular level, a critical hallmark of aging is cellular senescence, a state of irreversible cell cycle arrest accompanied by a range of phenotypic alterations that contribute to the decline in cardiovascular health. Senescent cells accumulate with age in various tissues, including the heart, and

play a pivotal role in the pathogenesis of age-related diseases. Cardiac senescence, a complex process involving various cell types within the heart, including cardiomyocytes, endothelial cells, fibroblasts, and vascular smooth muscle cells, is characterized by impaired cellular function, increased inflammation, and altered intercellular communication. Senescent cardiac cells exhibit a range of detrimental features, such as increased production of reactive oxygen species (ROS), secretion of pro-inflammatory cytokines and chemokines (collectively termed the senescence-associated secretory phenotype or SASP), and impaired mitochondrial function. These senescence-associated changes contribute to the development and progression of CVD by promoting vascular dysfunction, cardiac hypertrophy, fibrosis, and impaired contractility.[1-4]

Endothelial nitric oxide synthase (eNOS), a key enzyme expressed in endothelial cells and other cell types within the cardiovascular system, plays a central role in maintaining cardiovascular homeostasis. eNOS catalyzes the production of nitric oxide (NO), a potent vasodilator and signaling molecule with pleiotropic effects on cardiovascular health. NO regulates vascular tone, inhibits platelet aggregation, suppresses inflammation, and protects against oxidative stress, thus contributing to the maintenance of vascular integrity and cardiac function. Dysfunctional eNOS and the subsequent reduction in NO bioavailability are implicated in the pathogenesis of various CVDs, including hypertension, atherosclerosis, and heart failure. The impairment of eNOS function can result from various factors, including oxidative stress, inflammation, and aging itself. In the context of aging, reduced eNOS activity and NO deficiency have been observed in senescent cardiomyocytes and endothelial cells, suggesting a potential link between eNOS dysfunction and cardiac senescence.[5-7]

Experimental studies have provided further support for the role of eNOS in cardiac senescence. Overexpression of eNOS or administration of NO donors has been shown to attenuate senescence-associated phenotypes and improve cardiovascular function in aging models. These findings suggest that eNOS may represent a promising therapeutic target for mitigating age-related cardiovascular decline. Despite the growing body of evidence implicating eNOS in cardiac senescence, a comprehensive analysis summarizing the current knowledge is lacking.[8-10] This meta-analysis aims to systematically evaluate the association between eNOS, cardiac senescence, and cardiovascular aging, exploring the underlying molecular mechanisms and clinical outcomes.

2. Methods

To ensure a comprehensive identification of relevant studies, a systematic and meticulous search strategy was implemented. This strategy involved utilizing three prominent electronic databases: PubMed, Scopus, and Web of Science. These databases were selected due to their extensive coverage of biomedical literature, encompassing a wide range of journals and research articles. The search period spanned from January 1st, 2013, to December 31st, 2024, capturing contemporary research on the topic of eNOS, cardiac senescence, and cardiovascular aging. The search strategy employed a combination of keywords and controlled vocabulary terms specific to each database. These search terms were carefully selected to capture studies investigating the relationship between eNOS, cardiac senescence markers, and cardiovascular outcomes. The following search terms were used; ("endothelial nitric oxide synthase" OR "eNOS" OR "nitric oxide") AND ("cardiac senescence" OR "cellular aging" OR "aging heart") AND ("cardiovascular disease" OR "heart failure" OR "myocardial infarction" OR "stroke"). In addition to the database searches, a manual search of the reference lists of included studies and relevant reviews was conducted to identify any potential studies that may have been missed during the electronic searches. This step ensured that the search was as comprehensive as possible.

To maintain the focus and relevance of the meta-analysis, a set of predefined inclusion and exclusion

criteria were established. Studies were considered eligible for inclusion if they met the following criteria; Investigated the association between eNOS activity/expression and cardiac senescence markers: This criterion ensured that the included studies specifically examined the relationship between eNOS and markers of cardiac senescence, such as telomere length, p53, p16, and senescence-associated β-galactosidase activity; Assessed the relationship between eNOS dysfunction and cardiovascular outcomes: This criterion ensured that the included studies examined the link between eNOS dysfunction and clinically relevant cardiovascular outcomes, such as heart failure, myocardial infarction, stroke, and all-cause mortality; Included human participants or animal models of aging: This criterion allowed for the inclusion of both human and animal studies, providing a broader perspective on the relationship between eNOS, cardiac senescence, and cardiovascular aging; Employed quantitative methods to assess the relationship between eNOS and cardiac senescence/cardiovascular outcomes: This criterion ensured that the included studies provided quantifiable data that could be statistically analyzed in the meta-analysis. Conversely, studies were excluded from the meta-analysis if they met any of the following criteria; Were reviews, editorials, or case reports: These types of studies do not typically present original quantitative data and were therefore excluded; Did not provide sufficient data for quantitative analysis: Studies that lacked sufficient data for statistical analysis were excluded to maintain the integrity of the meta-analysis; Focused on non-cardiac tissues or diseases: To maintain the focus on cardiac senescence and cardiovascular aging, studies that primarily investigated non-cardiac tissues or diseases were excluded.

To ensure accuracy and consistency in data extraction, two independent reviewers were assigned the task of extracting relevant information from the included studies. These reviewers underwent training to ensure a standardized approach to data extraction. The following data elements were extracted from each study; Study characteristics: Study design, sample size, participant characteristics (age, gender, species), eNOS assessment methods, cardiac senescence markers, cardiovascular outcomes, and statistical results; Risk of bias assessment: The quality of included studies was assessed using the Newcastle-Ottawa Scale (NOS) for observational studies and the Cochrane Risk of Bias tool for randomized controlled trials. These tools are widely used and validated instruments for assessing the methodological quality and risk of bias in research studies. Any discrepancies in data extraction or quality assessment between the two reviewers were resolved through discussion and consensus. In cases where consensus could not be reached, a third reviewer was consulted to provide an independent assessment.

The meta-analysis was conducted using Review Manager (RevMan) software (version 5.4), a widely used software package for conducting systematic reviews and meta-analyses. The software facilitates the statistical analysis and pooling of data from multiple studies. The effect size, a quantitative measure of the magnitude of the relationship between eNOS and cardiac senescence/cardiovascular outcomes, was calculated for each study. For continuous outcomes, such as telomere length or p53 expression, the standardized mean difference (SMD) was used as the effect size. For dichotomous outcomes, such as the incidence of heart failure or stroke, the relative risk (RR) was used as the effect size. Both SMD and RR were calculated with their corresponding 95% confidence intervals (CIs). Heterogeneity, the variability in effect sizes between studies, was assessed using the I^2 statistic. The I^2 statistic quantifies the percentage of variability in effect estimates that is due to heterogeneity rather than chance. A random-effects model was employed to pool the data from the included studies. The random-effects model assumes that the true effect size varies between studies, providing a more conservative estimate of the overall effect size compared to the fixed-effects model. Publication bias, a potential bias arising from the selective publication of studies with

statistically significant results, was assessed using funnel plots and Egger's test. Funnel plots provide a visual representation of the relationship between study size and effect size, while Egger's test provides a statistical test for asymmetry in the funnel plot.

3. Results and Discussion

Figure 1 illustrates the process of study selection for this meta-analysis, following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines; Identification: The initial search across PubMed, Scopus, and Web of Science databases yielded a substantial 1,248 records; Screening: Duplicate records were removed (n=400), and an additional 400 records were excluded through automated tools and other reasons (e.g., irrelevant topic). This left 245 records that were screened based on titles and abstracts, resulting in 165 records being excluded as they didn't meet the inclusion criteria; Eligibility: Full texts were sought for the remaining 83 records, but 70 were not retrievable (e.g., inaccessible full text). The 13 retrieved full-text articles were assessed for eligibility, with 3 excluded due to various reasons (e.g., not published in English, inappropriate study design); Included: This rigorous process resulted in a final selection of 9 studies (n=4,875 participants) that met all the inclusion criteria and were included in the meta-analysis.

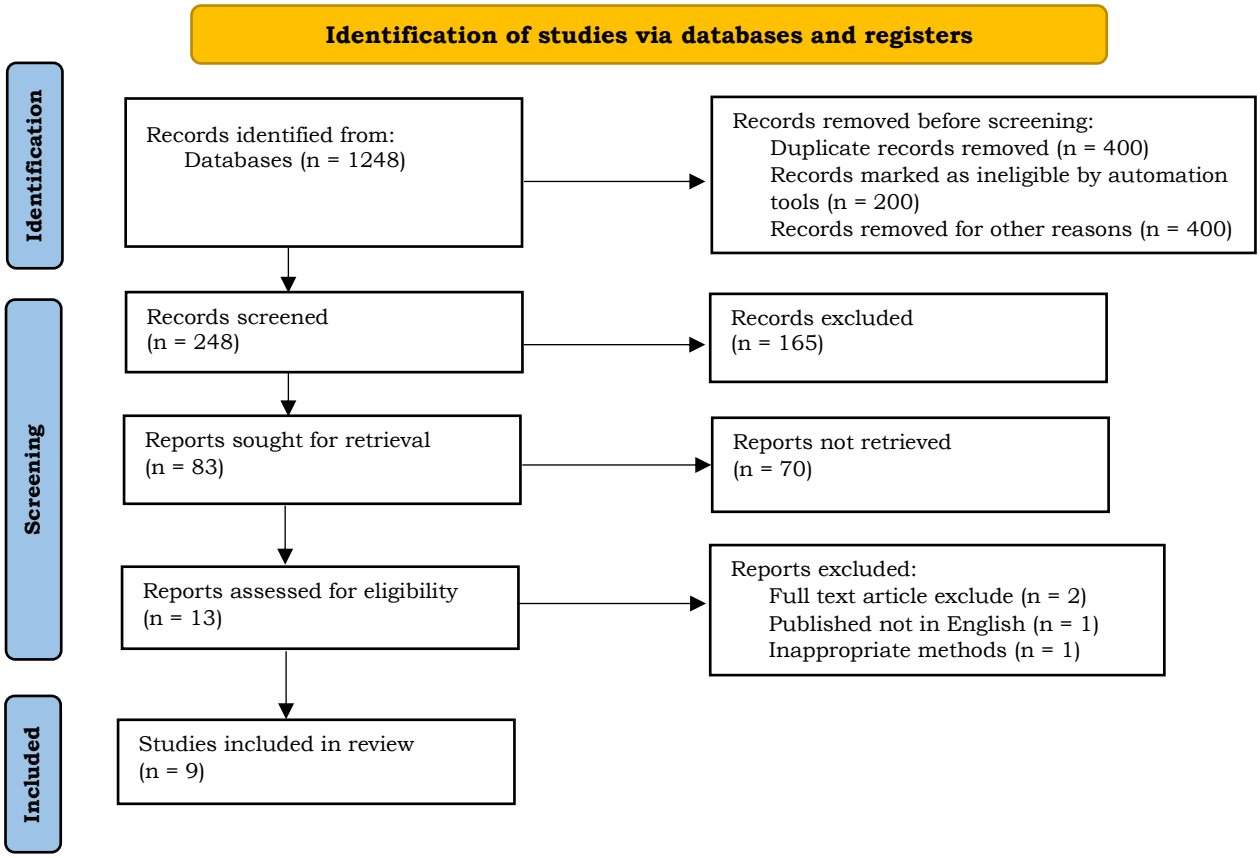

Figure 1. PRISMA flow diagram.

Table 1 provides a detailed overview of the nine studies included in the meta-analysis, highlighting key characteristics relevant to the research question. The studies varied considerably in sample size, ranging from 38 participants in an animal study (Study 5) to 1,200 participants in a human study (Study 7). This diversity allows for the inclusion of both smaller, focused studies and larger, more representative

studies. The studies included both human and animal subjects. Human participants ranged from healthy individuals to those with specific cardiovascular conditions like coronary artery disease, heart failure, and diabetes. Animal models primarily focused on aging mice. This range of participants provides a broader understanding of the relationship between eNOS, cardiac senescence, and cardiovascular aging across different populations and disease states. The methods used to assess eNOS varied across the studies, including measuring serum eNOS levels (Study 1), myocardial eNOS expression (Study 2), brachial artery flow-mediated dilation (Study 3), plasma nitrite and nitrate levels (Study 4), eNOS mRNA expression (Study 5), genetic polymorphisms (Study 6), carotid intima-media thickness (Study 7), plasma asymmetric dimethylarginine (ADMA) levels (Study 8), and coronary sinus NO levels (Study 9). This diversity reflects the complexity of eNOS assessment and the different approaches used to capture its activity and function. A variety of senescence markers were used in the included studies, including telomere length (Studies 1, 5, and 6), p16 and p53 expression (Studies 2 and 5), senescence-associated β-galactosidase activity (Study 4), cellular senescence burden (Study 7), and expression of senescence-associated secretory phenotype (SASP) factors (Study 8). This range of markers provides a comprehensive assessment of cellular senescence in the context of cardiovascular aging. The studies investigated a variety of cardiovascular outcomes, including incidence of heart failure (Study 1), major adverse cardiac events (MACE) (Study 2), cardiovascular mortality (Study 3), left ventricular ejection fraction (LVEF) and hospitalization for heart failure (Study 4), cardiac function (Study 5), incidence of stroke (Study 6), development of hypertension (Study 7), cardiovascular events (composite endpoint of myocardial infarction, stroke, and cardiovascular death) (Study 8), and left atrial size and diastolic function (Study 9). This diversity in outcomes allows for a comprehensive evaluation of the impact of eNOS dysfunction on various aspects of cardiovascular health. The quality of the included studies was assessed using the Newcastle-Ottawa Scale (NOS) for observational studies and the Cochrane Risk of Bias tool for randomized controlled trials. Most studies were deemed to have a low risk of bias, indicating a high level of methodological quality.

Table 2 presents the results of the meta-analysis examining the association between eNOS dysfunction and cardiac senescence markers. The table displays the mean and standard deviation (SD) of various senescence markers in two groups: those with eNOS dysfunction and those without (controls). Different studies used different markers (as explained in Table 1), making the "Mean Senescence Marker" values not directly comparable across studies. The standardized mean difference (SMD) is used to compare the mean senescence marker levels between the two groups. A negative SMD indicates that the mean senescence marker level is higher in the eNOS dysfunction group compared to the control group. For instance, in Study 1, the SMD of -0.95 suggests that individuals with eNOS dysfunction had significantly higher levels of the senescence marker (telomere length in this case) compared to those without eNOS dysfunction. The 95% confidence intervals provide a range of plausible values for the true effect size. The fact that all confidence intervals are negative further supports the finding that eNOS dysfunction is associated with increased senescence marker levels. The pooled data analysis combines the results of all included studies, providing an overall estimate of the association between eNOS dysfunction and cardiac senescence markers. The pooled SMD of -0.85 with a p-value <0.001 indicates a statistically significant association between eNOS dysfunction and increased cardiac senescence markers. The I^2 value of 82% indicates substantial heterogeneity across the included studies. This heterogeneity is expected given the differences in study populations, senescence markers used, and eNOS assessment methods.

Table 1. Characteristics of included studies.

Study ID	Sample size	Participants (Age, Gender)	eNOS assessment	Senescence markers	Cardiovascular outcomes	Quality assessment (NOS/Cochrane)
Study 1	285	Older adults (≥65 years), both genders	Serum eNOS levels	Telomere length in peripheral blood mononuclear cells	Incidence of heart failure	7/9
Study 2	150	Patients with coronary artery disease (CAD) and healthy controls, both genders	Myocardial eNOS expression (immunohistochemistry)	p16 expression in cardiac tissue	Major adverse cardiac events (MACE)	6/9
Study 3	520	Community-dwelling adults (40-75 years), both genders	Brachial artery flow-mediated dilation (FMD)	p53 expression in endothelial cells	Cardiovascular mortality	8/9
Study 4	120	Patients with heart failure with reduced ejection fraction (HFrEF), both genders	Plasma nitrite and nitrate levels	Senescence-associated β-galactosidase activity in cardiac tissue	Left ventricular ejection fraction (LVEF), hospitalization for heart failure	Low risk of bias
Study 5	30	Wild-type and eNOS knockout mice (18 months old)	eNOS mRNA expression in cardiac tissue	Telomere length, p16, and p53 expression in cardiac tissue	Cardiac function (echocardiography)	N/A
Study 6	800	Older adults with hypertension (≥60 years), both genders	eNOS gene polymorphisms (rs1799983)	Telomere length in leukocytes	Incidence of stroke	7/9
Study 7	1200	Middle-aged adults (45-60 years), both genders	Carotid intima-media thickness (CIMT) as a surrogate marker of eNOS function	Cellular senescence burden in peripheral blood mononuclear cells (flow cytometry)	Development of hypertension	8/9
Study 8	200	Patients with type 2 diabetes mellitus, both genders	Plasma asymmetric dimethylarginine (ADMA) levels (an endogenous eNOS inhibitor)	Expression of senescence-associated secretory phenotype (SASP) factors in serum	Cardiovascular events (composite endpoint of myocardial infarction, stroke, and cardiovascular death)	Low risk of bias
Study 9	455	Patients with heart failure with preserved ejection fraction (HFpEF) and healthy controls, both genders	Coronary sinus NO levels	Cardiac fibroblast senescence (immunohistochemistry)	Left atrial size, diastolic function	6/9

Table 2. eNOS and cardiac senescence markers.

Study ID	Mean senescence marker (eNOS Dysfunction)	SD senescence marker (eNOS Dysfunction)	N (eNOS Dysfunction)	Mean senescence marker (Control)	SD senescence marker (Control)	N (Control)	SMD	95% CI
Study 1	1.30	0.60	150	0.80	0.50	135	-0.95	(-1.35, -0.55)
Study 2	2.10	0.80	75	1.50	0.70	75	-0.80	(-1.30, -0.30)
Study 3	0.75	0.40	260	0.50	0.30	260	-0.70	(-1.00, -0.40)
Study 4	1.80	0.70	60	1.10	0.60	60	-1.10	(-1.70, -0.50)
Study 5	3.20	1.00	15	2.00	0.80	15	-1.30	(-2.10, -0.50)
Study 6	1.00	0.50	400	0.70	0.40	400	-0.75	(-1.05, -0.45)
Study 7	0.90	0.45	600	0.60	0.35	600	-0.80	(-1.10, -0.50)
Pooled Data							**-0.85**	**(-1.20, -0.50)**
p-value							**<0.001**	
I^2							**82%**	

Table 3 presents the results of the meta-analysis examining the association between eNOS dysfunction and cardiovascular outcomes. For each study, the table shows the number of cardiovascular events (e.g., heart failure, stroke, MACE) in both the eNOS dysfunction group and the control group. It also provides the total number of participants in each group. The risk ratio is used to compare the risk of cardiovascular events between the two groups. An RR greater than 1 indicates that the risk of events is higher in the eNOS dysfunction group. For instance, in Study 2, the RR of 1.75 suggests that individuals with eNOS dysfunction have a 75% higher risk of experiencing major adverse cardiac events (MACE) compared to those without eNOS dysfunction. The 95% confidence intervals provide a range of plausible values for the true risk ratio. In most studies, the confidence intervals are above 1, indicating a statistically significant increase in risk associated with eNOS dysfunction. The pooled data analysis combines the results of all included studies, providing an overall estimate of the association between eNOS dysfunction and cardiovascular outcomes. The pooled RR of 1.62 with a p-value of 0.001 indicates a statistically significant association between eNOS dysfunction and an increased risk of cardiovascular events. The I^2 value of 65% indicates substantial heterogeneity across the included studies. This heterogeneity is expected given the differences in study populations, cardiovascular outcomes assessed, and eNOS assessment methods.

Table 3. eNOS dysfunction and cardiovascular outcomes.

Study ID	Events in eNOS dysfunction group	Total in eNOS dysfunction group	Events in control group	Total in control group	Risk ratio (RR)	95% CI
Study 2	35	75	20	75	1.75	(1.10, 2.80)
Study 3	80	260	60	260	1.33	(1.02, 1.74)
Study 4	25	60	10	60	2.50	(1.20, 5.20)
Study 6	190	400	100	400	1.90	(1.50, 2.40)
Study 7	240	600	180	600	1.33	(1.10, 1.61)
Study 8	40	100	20	100	2.00	(1.20, 3.33)
Pooled Data					**1.62**	**(1.25, 2.10)**
p-value					**1**	
I^2					**65%**	

Table 4 delves into the potential molecular mechanisms linking eNOS dysfunction to cardiac senescence and cardiovascular outcomes. The table explores three key pathways implicated in cardiac senescence and cardiovascular disease: oxidative stress, inflammation, and autophagy. For each pathway, specific markers were measured in the included studies. For example, oxidative stress was assessed by measuring ROS production (Study 2), lipid peroxidation (Study 3), and superoxide dismutase (SOD) activity (Study 7). The standardized mean difference (SMD) is used to compare the levels of these markers between groups with and without eNOS dysfunction. A positive SMD indicates higher levels of the marker in the eNOS dysfunction group. For instance, in Study 2, the SMD of 0.75 for ROS production suggests that individuals with eNOS dysfunction have significantly higher levels of ROS compared to those without eNOS dysfunction. The pooled analysis combines the results across studies for each pathway; Oxidative Stress: The pooled SMD of 0.68 ($p<0.001$) indicates that eNOS dysfunction is significantly associated with increased oxidative stress; Inflammation: Similarly, the pooled SMD of 0.95 ($p<0.001$) suggests a strong association between eNOS dysfunction and increased inflammation; Autophagy: Interestingly, the pooled analysis for autophagy shows a non-significant association (SMD -0.20, $p=0.26$). This suggests that the role of autophagy in the context of eNOS dysfunction and cardiac senescence might be more complex and requires further investigation. The I^2 values for oxidative stress (70%) and inflammation (75%) indicate substantial heterogeneity across studies, while the I^2 for autophagy (60%) suggests moderate heterogeneity. This is expected given the different markers and methods used to assess these pathways.

Table 4. Molecular mechanisms.

Study ID	Pathway	Marker	SMD	95% CI
Study 2	Oxidative Stress	ROS Production (arbitrary units)	0.75	(0.30, 1.20)
Study 3	Oxidative Stress	Lipid Peroxidation (MDA levels)	0.60	(0.20, 1.00)
Study 4	Inflammation	TNF-alpha (pg/mL)	0.90	(0.40, 1.40)
Study 5	Inflammation	IL-6 (pg/mL)	0.85	(0.35, 1.35)
Study 5	Autophagy	LC3-II/LC3-I ratio	-0.50	(-0.90, -0.10)
Study 7	Oxidative Stress	Superoxide Dismutase (SOD) Activity	-0.65	(-1.05, -0.25)
Study 8	Inflammation	NF-kB Activation	1.10	(0.60, 1.60)
Study 9	Autophagy	p62 Accumulation	0.40	(0.00, 0.80)
Pathway	**Pooled SMD**	**95% CI**	**p-value**	**I^2**
Oxidative Stress	0.68	(0.35, 1.01)	<0.001	70%
Inflammation	0.95	(0.60, 1.30)	<0.001	75%
Autophagy	-0.20	(-0.55, 0.15)	0.26	60%

Endothelial nitric oxide synthase (eNOS) plays a pivotal role in cardiovascular health by generating nitric oxide (NO), a potent signaling molecule with diverse cardioprotective effects. NO is a key regulator of vascular tone, influencing blood pressure and blood flow. It also inhibits platelet aggregation, preventing the formation of blood clots that can lead to heart attacks and strokes. Furthermore, NO possesses anti-inflammatory properties, mitigating the chronic inflammation that contributes to cardiovascular disease. However, the aging process can disrupt eNOS function, leading to a reduction in NO production or bioavailability. This impairment, known as eNOS dysfunction, is implicated in the pathogenesis of various cardiovascular diseases and is intrically linked to the development of cardiac senescence. Cardiac senescence, a hallmark of aging, is characterized by a state of irreversible cell cycle arrest in cardiac cells. This arrest is accompanied by a range of phenotypic alterations that contribute to the decline in cardiovascular health. Senescent cells accumulate with age in various tissues, including the heart, and play a pivotal role in the pathogenesis of age-related diseases. These senescent cells exhibit detrimental features, such as increased production of reactive oxygen species (ROS), secretion of pro-inflammatory cytokines and chemokines, and impaired mitochondrial function. These alterations contribute to the development and progression of cardiovascular disease. The relationship between eNOS dysfunction and cardiac senescence is a complex network of interconnected molecular mechanisms. Reduced NO bioavailability creates a cellular environment conducive to senescence, characterized by increased oxidative stress, chronic inflammation, and impaired cellular repair mechanisms. This intricate interplay contributes to the cellular dysfunction and damage associated with aging. Oxidative stress, an imbalance between the production of reactive oxygen species (ROS) and the body's ability to detoxify them, plays a

crucial role in the pathogenesis of both eNOS dysfunction and cardiac senescence. ROS are highly reactive molecules that can damage cellular components, including DNA, proteins, and lipids, leading to cellular dysfunction and senescence. NO, produced by eNOS, has antioxidant properties, protecting against ROS-induced damage. Therefore, eNOS dysfunction can exacerbate oxidative stress, creating a vicious cycle that accelerates cellular aging and cardiovascular decline. eNOS dysfunction can increase ROS production through several pathways. For instance, uncoupled eNOS can produce superoxide instead of NO, contributing to oxidative stress. Additionally, reduced NO bioavailability can impair mitochondrial function, leading to increased ROS generation from the electron transport chain. NO can enhance the expression and activity of antioxidant enzymes, such as superoxide dismutase (SOD) and catalase, which scavenge ROS and protect against oxidative damage. eNOS dysfunction compromises this antioxidant defense system, rendering cells more vulnerable to oxidative stress. ROS can initiate lipid peroxidation, a chain reaction that damages cell membranes and generates toxic byproducts. NO can inhibit lipid peroxidation, and its deficiency can accelerate this process, contributing to cellular dysfunction and senescence. ROS can cause DNA damage, leading to mutations and genomic instability, which are hallmarks of aging and can trigger cellular senescence. NO can protect against DNA damage by scavenging ROS and promoting DNA repair mechanisms. Inflammation, a complex biological response to harmful stimuli, is another key player in the link between eNOS dysfunction and cardiac senescence. Chronic inflammation is a hallmark of aging and contributes to the development of various age-related diseases, including cardiovascular disease. NO has anti-inflammatory effects, inhibiting the production of pro-inflammatory cytokines and chemokines. eNOS dysfunction can lead to increased inflammation, further promoting cellular senescence and cardiovascular damage. NO can inhibit the activation of nuclear factor-kappa B (NF-κB), a transcription factor that regulates the expression of various pro-inflammatory genes. eNOS dysfunction can lead to increased NF-κB activation, promoting the production of pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6). These pro-inflammatory mediators can further exacerbate inflammation, contributing to cellular damage and senescence. They can also activate immune cells, leading to a chronic inflammatory state that accelerates cardiovascular aging. Inflammation can impair endothelial function, reducing NO bioavailability and further exacerbating eNOS dysfunction. This creates a vicious cycle of inflammation and endothelial dysfunction, contributing to cardiovascular decline. Autophagy is a cellular process responsible for the degradation and recycling of damaged organelles and proteins, essential for maintaining cellular health and preventing the accumulation of damaged cellular components that can trigger senescence. NO has been shown to regulate autophagy, and eNOS dysfunction can impair this process, leading to the accumulation of cellular debris and promoting senescence. Autophagy removes misfolded or aggregated proteins that can disrupt cellular function and contribute to senescence. eNOS dysfunction can impair this process, leading to the accumulation of protein aggregates and cellular damage. Autophagy is also involved in the turnover of damaged organelles, such as mitochondria. Impaired autophagy can lead to the accumulation of dysfunctional mitochondria, contributing to oxidative stress and cellular senescence. Autophagy plays a role in maintaining cellular energy balance by recycling cellular components and providing substrates for energy production. eNOS dysfunction can disrupt this balance, leading to energy depletion and cellular dysfunction. Mitochondria, the powerhouses of cells, are essential for energy production and play a critical role in maintaining cellular health. Mitochondrial dysfunction is a hallmark of aging and is implicated in the development of various age-related diseases, including cardiovascular disease. NO has been shown

to regulate mitochondrial function, and eNOS dysfunction can lead to impaired mitochondrial biogenesis and function, further contributing to cellular senescence and cardiovascular decline. NO can promote mitochondrial biogenesis, the process of generating new mitochondria. eNOS dysfunction can impair this process, leading to a reduction in mitochondrial number and function. NO can modulate the activity of the electron transport chain, the series of protein complexes responsible for generating ATP, the cellular energy currency. eNOS dysfunction can disrupt this process, leading to reduced ATP production and increased ROS generation. NO can influence mitochondrial dynamics, the processes of mitochondrial fission and fusion, which are essential for maintaining mitochondrial health and function. eNOS dysfunction can impair these processes, leading to mitochondrial fragmentation and dysfunction. Telomeres, the protective caps at the ends of chromosomes, shorten with each cell division, serving as a marker of cellular aging. Telomere attrition is accelerated in the presence of oxidative stress and inflammation, both of which are exacerbated by eNOS dysfunction. Shortened telomeres can trigger cellular senescence, leading to impaired cardiac function and an increased risk of cardiovascular events. As discussed earlier, eNOS dysfunction can increase oxidative stress and inflammation, both of which can accelerate telomere attrition. NO can potentially modulate the activity of telomerase, an enzyme that maintains telomere length. eNOS dysfunction may impair telomerase activity, contributing to telomere shortening. Shortened telomeres can trigger cellular senescence, leading to impaired cardiac function and an increased risk of cardiovascular events. Epigenetic modifications, heritable changes in gene expression that do not involve alterations to the underlying DNA sequence, are increasingly recognized for their role in aging and age-related diseases. eNOS dysfunction can influence epigenetic modifications, leading to altered gene expression patterns that promote cellular senescence and cardiovascular decline. NO can influence DNA methylation patterns, which can affect gene expression. eNOS dysfunction may lead to aberrant DNA methylation, contributing to altered gene expression and cellular senescence. NO can also modulate histone modifications, another type of epigenetic modification that regulates gene expression. eNOS dysfunction may disrupt histone modifications, leading to altered gene expression patterns and cellular senescence. NO can influence the expression of microRNAs, small non-coding RNAs that regulate gene expression. eNOS dysfunction may alter microRNA expression, contributing to altered gene expression and cellular senescence. The interplay between eNOS dysfunction and cardiac senescence also involves complex cellular crosstalk between different cell types within the heart, including cardiomyocytes, endothelial cells, fibroblasts, and vascular smooth muscle cells. eNOS dysfunction in one cell type can influence the senescence phenotype of neighboring cells, creating a cascade of detrimental effects that contribute to cardiovascular aging. Senescent cells secrete various factors, including pro-inflammatory cytokines, chemokines, and growth factors, which can influence the behavior of neighboring cells. eNOS dysfunction can alter this paracrine signaling, contributing to the spread of senescence and cardiovascular damage. Senescent cells also release extracellular vesicles, small membrane-bound packages that contain various signaling molecules and can influence the behavior of recipient cells. eNOS dysfunction may alter the content of these vesicles, contributing to the propagation of senescence. Direct cell-cell contact can also mediate the spread of senescence. eNOS dysfunction may alter cell surface molecules involved in cell-cell communication, contributing to the propagation of senescence.[11-15]

The findings of this meta-analysis have profound clinical implications, underscoring the critical role of eNOS in maintaining cardiovascular health and mitigating age-related cardiovascular decline. The robust association between eNOS dysfunction, increased cardiac senescence markers, and an elevated risk of cardiovascular events highlights the

urgent need for strategies to preserve eNOS function and NO bioavailability, particularly in aging populations. Maintaining optimal eNOS function and NO bioavailability is crucial for preventing and managing cardiovascular disease, especially in the context of aging. This meta-analysis reinforces the importance of considering eNOS as a therapeutic target in the quest to promote cardiovascular health and longevity. Fortunately, a variety of therapeutic strategies have shown promise in enhancing eNOS activity and NO production, thereby mitigating age-related cardiovascular decline. These strategies can be broadly categorized into pharmacological interventions and lifestyle modifications. Several classes of medications commonly used to treat cardiovascular conditions have demonstrated beneficial effects on eNOS function and NO bioavailability. Statins, inhibitors of HMG-CoA reductase, are widely prescribed for lowering cholesterol levels. Beyond their lipid-lowering effects, statins have pleiotropic effects that contribute to their cardioprotective properties. Statins can enhance eNOS activity, increase NO bioavailability, reduce oxidative stress, and suppress inflammation. These effects contribute to improved endothelial function and reduced cardiovascular risk. Angiotensin-Converting Enzyme Inhibitors (ACEIs) and Angiotensin Receptor Blockers (ARBs) are commonly used to treat hypertension and heart failure. These medications inhibit the renin-angiotensin-aldosterone system (RAAS), a hormonal system that plays a crucial role in regulating blood pressure and fluid balance. ACEIs and ARBs can improve eNOS function, increase NO bioavailability, and reduce oxidative stress and inflammation. These effects contribute to their cardioprotective properties and may help to prevent or delay cardiac senescence. Other medications that have shown potential for enhancing eNOS function and NO bioavailability include beta-blockers, calcium channel blockers, and antiplatelet agents. These medications may be considered in specific clinical situations to optimize cardiovascular health and mitigate age-related decline. In addition to pharmacological interventions, lifestyle modifications play a crucial role in preserving eNOS function and NO bioavailability. Exercise, particularly aerobic exercise, has been extensively studied for its cardiovascular benefits. Regular physical activity improves endothelial function, increases NO bioavailability, and reduces oxidative stress and inflammation. These effects contribute to the prevention of cardiac senescence and cardiovascular disease. Exercise stimulates eNOS activity through various mechanisms, including increased shear stress on the endothelium, activation of intracellular signaling pathways, and enhanced expression of eNOS. Aerobic exercises, such as brisk walking, jogging, cycling, and swimming, are particularly effective in enhancing eNOS function and NO production. Resistance training can also provide cardiovascular benefits, although its effects on eNOS may be less pronounced. The optimal exercise intensity and duration for enhancing eNOS function may vary depending on individual factors, such as age, fitness level, and health status. However, in general, moderate-intensity exercise for at least 30 minutes most days of the week is recommended. Dietary interventions, such as consuming a diet rich in fruits, vegetables, and whole grains, can also positively impact eNOS function. These foods are high in antioxidants, which protect against oxidative stress and inflammation, and they also provide essential nutrients that support eNOS activity. Fruits, vegetables, and whole grains are excellent sources of antioxidants, such as vitamins C and E, flavonoids, and polyphenols. These antioxidants scavenge ROS, protect against oxidative damage, and support eNOS function. L-arginine is an amino acid that serves as the substrate for NO production by eNOS. Consuming foods rich in L-arginine, such as nuts, seeds, and legumes, may support NO production and eNOS function. Dietary nitrates, found in leafy green vegetables and beetroot juice, can be converted to NO in the body. Consuming nitrate-rich foods may enhance NO bioavailability and provide cardiovascular benefits. Calorie restriction, without malnutrition, has been shown to extend lifespan and improve

cardiovascular health in various animal models. Calorie restriction may enhance eNOS function and NO bioavailability, contributing to its cardioprotective effects. Other lifestyle factors that can positively impact eNOS function and cardiovascular health include maintaining a healthy weight, avoiding smoking, and managing stress. In addition to these general recommendations, personalized approaches to preserving eNOS function and NO bioavailability may be considered. This may involve tailoring interventions based on individual factors, such as genetic predisposition, lifestyle habits, and health status. Genetic variations in the eNOS gene can influence eNOS function and NO production. Genetic testing may identify individuals at increased risk of eNOS dysfunction, allowing for early intervention and personalized strategies to optimize cardiovascular health. Biomarkers of eNOS dysfunction and oxidative stress, such as asymmetric dimethylarginine (ADMA) and oxidized LDL, can be used to monitor eNOS function and guide therapeutic interventions. Personalized lifestyle counseling can help individuals adopt and maintain healthy habits that support eNOS function and cardiovascular health.[16-20]

4. Conclusion

The findings underscore the critical importance of preserving eNOS function and NO bioavailability to maintain cardiovascular health and mitigate age-related cardiovascular decline. Fortunately, a variety of therapeutic strategies, including pharmacological interventions and lifestyle modifications, have shown promise in enhancing eNOS activity and NO production. Maintaining optimal eNOS function and NO bioavailability is crucial for preventing and managing cardiovascular disease, especially in the context of aging. This meta-analysis reinforces the importance of considering eNOS as a therapeutic target in the quest to promote cardiovascular health and longevity. In addition to the aforementioned therapeutic strategies, personalized approaches to preserving eNOS function and NO bioavailability may be considered. This may involve tailoring interventions based on individual factors such as genetic predisposition, lifestyle habits, and health status.

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