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N-Acetylcysteine increases glutathione levels in experimental models of heart diseases: a systematic review and meta-analysis

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ABSTRACT

Introduction: Cardiovascular diseases (CVD) are the leading cause of death worldwide, with oxidative stress playing a significant role in their progression. The use of N-acetylcysteine (NAC), a well-known antioxidant and a precursor to glutathione (GSH), has shown potential in preventing different types of CVD. Yet, there are limited data assessing NAC's impact on GSH levels in animal models of CVD. **Objective:** To estimate the effects of NAC supplementation on reduced glutathione (GSH) and total glutathione levels in cardiac tissue or serum/plasma from animals with established heart disease. **Methods**: We searched PubMed, Embase, Web of Science, Scielo, Lilacs/BINACIS, MedRxiv, and BioRxiv databases (the search was completed in February 2025). We included studies in animal models (of any species) of CVD receiving NAC (via any route or dose) and performed a random-effects meta-analysis and Risk of Bias assessment (PROSPERO register: CRD42023382024). **Results**: We identified 738 articles, fully reviewed 48, and included 8 in the meta-analysis. Meta-analysis showed that NAC supplementation resulted in a statistically significant increase in GSH (SMD = 1.54, CI 95% = 0.13–2.94; p = 0.03), with high heterogeneity (I^2 = 88%) and in total glutathione (SMD = 1.22, CI 95% = 0.41–2.02; p = 0.003) with moderate heterogeneity (I^2 = 49%). Subgroup analyses suggested a dose-dependent effect, while sensitivity analyses indicated that species- and model-related differences influenced the magnitude of the GSH response. **Conclusion:** NAC supplementation increases glutathione levels in animal models of heart disease, although the magnitude of the effect appears dose- and model-dependent.

Keywords: *Cardiovascular Disease; Oxidative Stress; Animal Model; Antioxidant; N-Acetylcysteine; Glutathione*

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INTRODUCTION

Cardiovascular diseases (CVD) are the leading cause of death worldwide, accounting for approximately 20.5 million deaths in 2021, or one-third of all global deaths[1]. A consistent body of evidence indicates that most CVDs share behavioral (unhealthy diet and smoking) and metabolic (high blood pressure and dyslipidemia) risk factors, which are often associated with an increased state of oxidative stress and inflammation[2]. To combat oxidative stress, the body relies on antioxidant systems composed of both enzymatic and non-enzymatic molecules. While enzymatic antioxidants are endogenous, non-enzymatic antioxidants can be either endogenous or exogenous, such as polyphenols and vitamins. A key endogenous non-enzymatic antioxidant is glutathione (GSH), a tripeptide containing a cysteine moiety, which serves as a cofactor for antioxidant enzymes, such as glutathione peroxidase and glutathione S-transferase. Additionally, GSH is an antioxidant and exhibits anti-inflammatory properties by suppressing NF-κB-mediated release of TNF-α, interleukin-6, and interleukin-1β, and plays a role in regulating cell signaling through post-translational modifications, such as glutathionylation[3]. Cells maintain antioxidant balance by preventing the accumulation of the GSH oxidation product, oxidized glutathione (GSSG). To achieve this, glutathione reductase, a NADPH-dependent enzyme, recycles GSH from GSSG[4].

Increasing intracellular concentrations of GSH mitigates the harmful effects of oxidative stress[5]. N-Acetylcysteine (NAC) is a widely used antioxidant supplement that has shown beneficial effects in various health conditions, including CVD[6], primarily due to its ability to donate a cysteine residue, which is essential for GSH synthesis. *In vivo*, however, evidence for an increase in GSH through NAC treatment is still controversial. For instance, chronic obstructive pulmonary disease patients[7] or schizophrenia patients[8] treated with NAC did not show increased GSH levels or clinical benefits. On the other hand, some reports showed increased GSH levels after NAC treatment in youth with autism spectrum disorder[9] and in either normolipidemic or hyperlipidemic subjects[10]. The inconsistency seen in clinical studies is less frequent in preclinical animal research, which often shows increased GSH concentrations after NAC treatment[3]. Nonetheless, the diversity of models, dosing strategies, and treatment regimens makes the comparisons challenging.

Thus, we conducted this systematic review and meta-analysis to evaluate whether NAC treatment effectively influences total and reduced glutathione levels in experimental models of cardiovascular diseases. This synthesis aims to clarify the role of NAC in modulating oxidative stress in cardiovascular diseases.

MATERIALS AND METHODS

Protocol pre-registration

This study was registered on PROSPERO (CRD42023382024) in March 2023[11]. One amendment to the original protocol needs to be reported: We included outcomes evaluated in heart tissue, serum, or plasma due to the initially small number of hits found.

Search strategies

In December 2022, we searched for articles in seven databases (PubMed, Embase, Web of Science, Scielo, Lilacs/BINACIS, BioRxiv, and MedRxiv) using the terms N-acetylcysteine, glutathione, cardiac disease, cardiac disorder, cardiovascular disease, heart failure, and heart disease. BioRxiv and MedRxiv were included to capture the so-called grey literature - papers that have not yet been published in peer-reviewed journals and might otherwise be overlooked. We updated the search in February 2025. A detailed search strategy can be found in Supplement 1.

Study selection

The eligibility criteria for studies were as follows. i) *In vivo* studies with animals of any species, age, and sexes with established - characterized by anatomical, functional, or biochemical signs - heart disease, published in any language; ii) presence of NAC as intervention - alone or in combination with other drugs - after the onset of the disease; iii) presence of a control group treated with placebo or saline. If NAC was combined with other drugs, control animals should receive the same co-treatments; iv) presence of data (mean, standard deviation/error, and sample size) on plasma, serum, and tissue levels of reduced (GSH) and/or oxidized glutathione (GSSG) and/or ratio between reduced/total glutathione and/or oxidized/total glutathione.

The exclusion criteria were as follows. i) Articles that contain data exclusively from human beings, *in vitro* or *ex vivo* experimentation; ii) articles that employed NAC as a preventive or prophylactic agent; iii) lack of an equivalent control group; iv) absence of author response to our data request.

Data extraction

Two authors independently performed data extraction (A.S.F. and T.S.) using standardized data extraction forms constructed on Jotform (https://www.jotform.com). Then, we cross-checked the results and resolved the disagreements with a third author (M.A.). We extracted the following data: Animal species and strain, age, sex, heart disease model, disease development protocol, type of euthanasia, interventions (mode and route of administration of NAC and time elapsed until euthanasia, and outcome data (as described above). When the study did not report the outcome in numbers, the graphic data were analyzed using ImageJ[12].

Risk of Bias (RoB)

The RoB tool used in this study was based on the previously published SYRCLE framework[13] and consisted of 9 items, which evaluated: 1) random sequence generation; 2) baseline characteristics; 3) random housing; 4) treatment blinding; 5) assessment blinding; 6) incomplete outcome data; 7) selective outcome reporting; 8) description of the intervention route; and 9) description of euthanasia. Each indicator was scored as either low, unclear, or high risk of bias independently by two authors (A.S.F. and T.S.), and disagreements were resolved by a third author (M.A.).

Statistical analysis

Data are presented as standardized mean differences (SMDs) with corresponding 95% confidence intervals (CIs) for each included study. The meta-analysis was conducted using a random-effects model (inverse variance method) to account for expected heterogeneity across studies. The Wald test was used to determine whether the overall pooled effect size (SMD) is significantly different from zero. Subgroup analyses were performed according to the NAC daily dose (Low: 120–150 mg/kg/day; intermediate: 200–300 mg/kg/day; high: >500 mg/

kg/day), provided there were more than three studies available. Sensitivity analysis was performed in two ways: i) a leave-one-out approach was used to identify which study contributed most to the overall heterogeneity; ii) an additional analysis restricted to rodent models was conducted to evaluate whether interspecies physiological or pharmacokinetic differences influenced the pooled estimates and heterogeneity. We estimated heterogeneity using I^2, with values between 25% to 50% considered moderate, and those above 50% considered high. The between-study variance (Tau^2) was estimated using the Restricted Maximum Likelihood (REML) method, which provides more accurate and less biased estimates of heterogeneity, particularly when the number of studies is small or heterogeneity is substantial. The analysis was performed using the Cochrane Review Manager (RevMan) software.

RESULTS

Study selection

We found 738 studies, which we later reduced to 571 by eliminating 167 duplicates. After screening titles and abstracts, we excluded 526 articles and thoroughly read 48. After reviewing the complete text, we excluded 40, and eight were considered suitable for inclusion (Figure 1). A detailed list of the evaluated articles and their respective exclusion reasons is provided in Supplement 2.

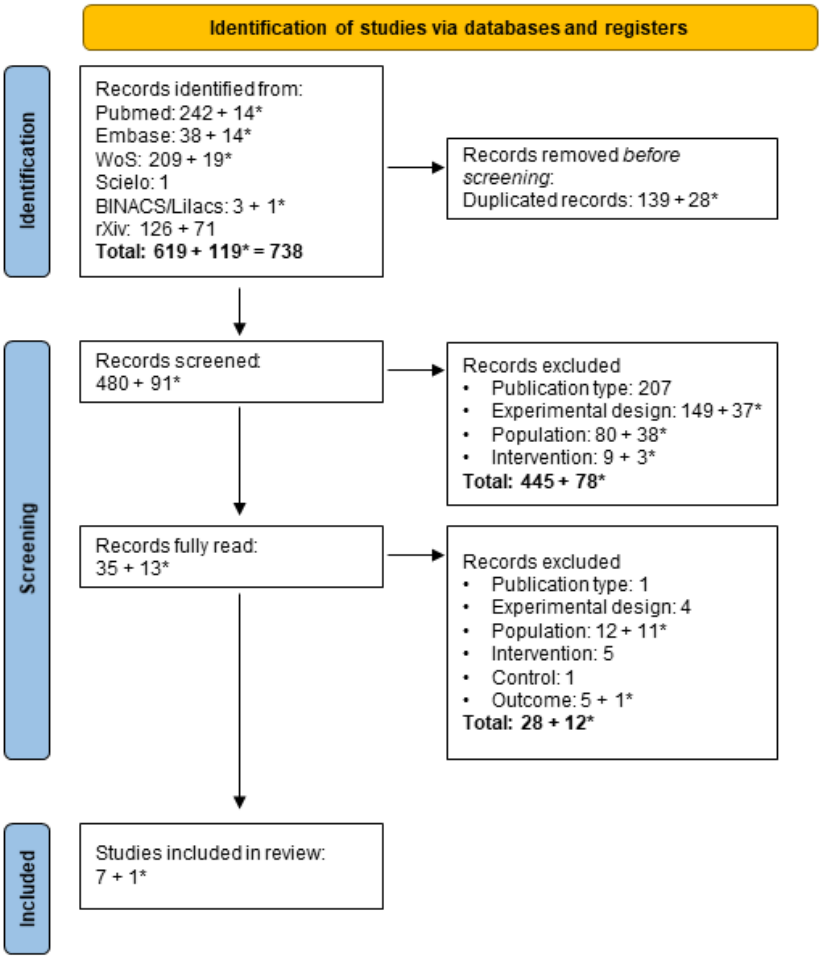

Figure 1: The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram shows study selection. * denotes hits obtained in the update search in February 2025.

Study characteristics

We found eight studies that met all the selection criteria. Three studies were included twice[14-16]: i) Adamy et al. (2007) presented two experimental designs with different follow-ups and separate sets of animals[14]; ii) Forman et al. (1988) assessed total glutathione in either infarcted or non-infarcted myocardium from the same animals - we split the sample size in the meta-analysis to avoid overestimating the effects[15]; iii) Lombardi et al. (2009) assessed the

GSSG/total glutathione ratio in either myocardium and plasma[16]. Some outcomes could not be meta-analyzed because they were reported in fewer than three studies. This was the case for GSSG/total glutathione, which was reported only by Lombardi et al. (2009)[16], and for GSSG and the GSH/GSSG ratio, which were reported only by Subrahmanian et al. (2024)[17] (Table 1). The studies were published between 1988 and 2024 by teams located in the USA[15-17], France[14,18], Taiwan[19], China[20], and Brazil[21]. Regarding animals used, we found rats were the most prevalent models, followed by mice, rabbits, and dogs. We also noticed a prevalence of models of ischemic cardiomyopathy[14,15,19]. NAC daily dose and regimen varied among studies, and we separated them into three ranges: low (120–150 mg/kg/day)[14,18,21], intermediate (200–300 mg/kg/day)[15,19,20], and high (500 mg/kg/day)[16,17]. As the follow-up time also differed between studies, the cumulative dose also varied, which could impact glutathione levels. We identified low cumulative doses ranging from 300 to 360 mg/kg[14,15], intermediate cumulative doses ranging from 3,600 to 8,400 mg/kg[14,18-21], and two studies with high cumulative doses ranging from 130,434 to 182,500 mg/kg[16,17] (Table 1). Although the euthanasia method can influence metabolic and detoxification outcomes[22], most of the studies included in this systematic review did not provide details on their euthanasia methods. Only two studies specified the use of a potassium chloride overdose in animals under anesthesia[15,20], while another two described the euthanasia that occurred under anesthesia, without specifying the method[14,21] (Supplement 3). Regarding main outcomes, the most commonly found was GSH in myocardium or serum[17–21], followed by total glutathione measured in the myocardium[14,15,21].

Table 1: Basic characteristics of the studies included.

Study	Country	Species, Strain	Sex of animals	Age of animals	CVD model	NAC dose	Follow-up	Outcome
Adamy et al., 2007[14]	France	Rat, Wistar	Male	11 wk	Ischemic cardiomyopathy	120 mg/kg/day (food)	1 mo	Total glutathione | Myocardium
							3 d	Total glutathione | Myocardium
Forman et al., 1988[15]	USA	Dog, Mongrel	Male/ Female	NI	Ischemic cardiomyopathy	150 mg/kg/h for 1h (loading, i.v.) + 50 mg/kg/h for 3h (maintenance, i.v.)	24h	Total glutathione | Myocardium IZ
							24h	Total glutathione | Myocardium NIZ
Lee et al., 2010[19]	Taiwan	Rat, Wistar	Male	NI	Ischemic cardiomyopathy	250 mg/kg/day (gavage)	4 wk	GSH | Myocardium
Lombardi et al., 2009[16]	USA	Rabbit, NI	NI	24 mo	Hypertrophic cardiomyopathy	500 mg/kg/day (drinking water)	12 mo	GSSG/total glutathione | Myocardium
							12 mo	GSSG/total glutathione | Plasma
Reyes et al., 2017[21]	Brazil	Rat, Wistar	Male	NI	Aortic stenosis	120 mg/kg/day (food)	8 wk	GSH and total glutathione | Myocardium
Rodriguez et al., 2018[18]	France	Mouse, NI	Female	6 mo	Dilated cardiomyopathy	140 mg/kg/day (drinking water)	1 mo	GSH | Myocardium and serum
Subrahmanian et al., 2024[17]	USA	Mouse, C57BL/6J	NI	7 wk	Aortic stenosis	2% (drinking water)*	1 mo	GSH, GSSG, and Do | Serum
Wu et al., 2014[20]	China	Rabbit, NI	NI	NI	DOX-induced heart failure	300 mg/kg/day (route NI)	4 wk	GSH | Serum

NI, not informed; d, days; wk, weeks; mo, months; DOX, doxorubicin; i.v., intravenous; IZ, analysis in infarcted myocardium zone; NIZ, analysis in non-infarcted myocardium zone. *For further analysis, we estimated a dose of 4,348 mg/kg/day based on a drinking volume of 5 mL/day and an average weight of 23 g for the C57BL/6J strain[23]. Full extraction table as Supplement 3.

Risk of bias assessment

We employed a 9-item scoring method based on the SYRCLE Risk of Bias Tool to evaluate the methodological quality of the studies[13]. The description of items and requirements is available as Supplement 4. We noticed a poor description of some items, mainly in those articles published before the first version of the ARRIVE guidelines[24]. Some items had limited descriptions, such as sequence generation, random housing, blinding of personnel, and blinding of outcomes, and were ranked as unclear risks. We did not find evidence of selective outcome reporting in the included studies. However, we found a high risk of bias associated with two items: incomplete data (i.e., sample size inconsistency when comparing methods and results) and description of euthanasia (i.e., lack of description) (Figure 2).

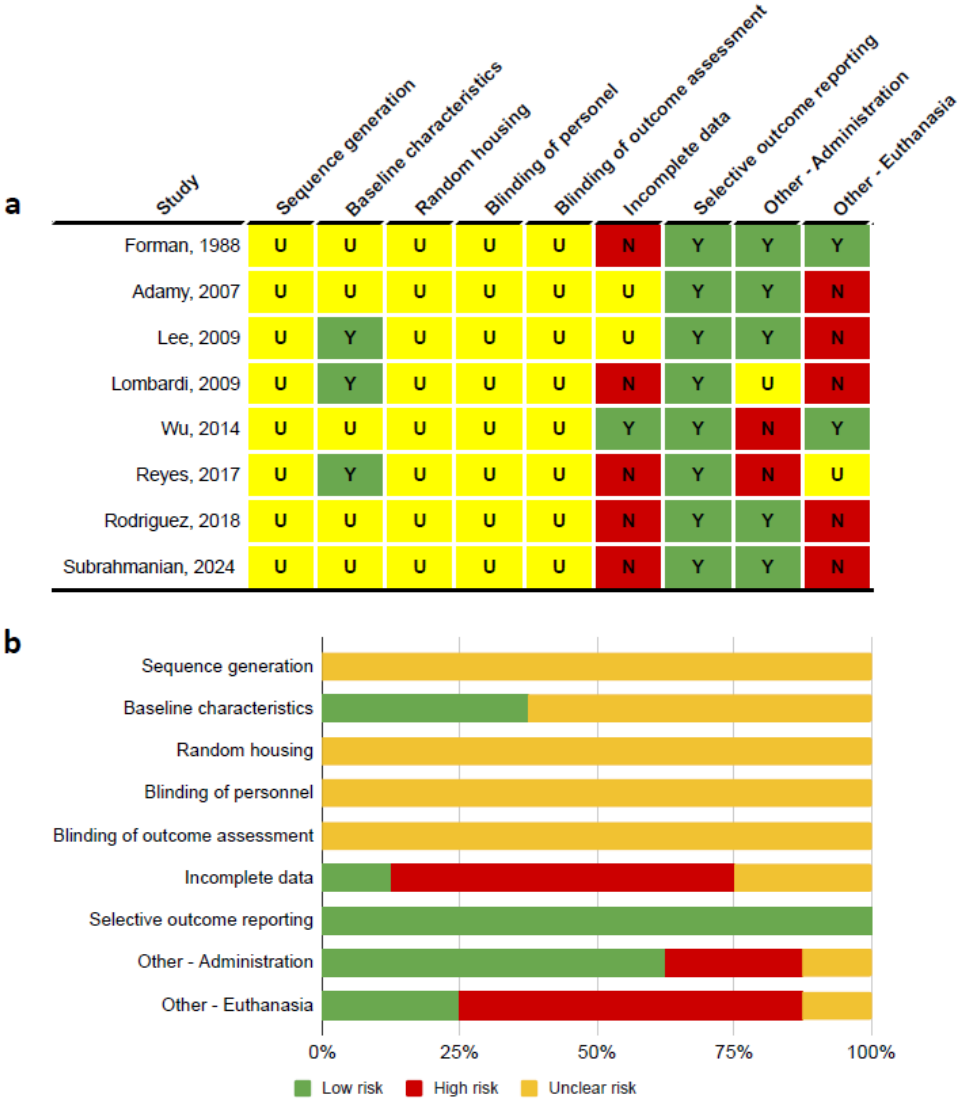

Figure 2: Risk of Bias assessment. A 9-item questionnaire adapted from the SYRCLE Risk of Bias (RoB) tool was used. **a**) Risk of bias classification for each study and item, based on available evidence: low risk (Y), high risk (N), or unclear risk (U). **b**) Proportion of studies classified as low, high, or unclear risk for each item. The complete dataset of the RoB assessment is provided as Supplement 4.

Meta-analysis results

According to our prospective register, a minimum of three studies were required for the outcome to enter the meta-analysis [11]. Thus, we meta-analyzed only two outcomes: GSH and total glutathione.

GSH meta-analysis consisted of four studies with 57 control and 52 NAC-treated animals and indicated an increase in GSH levels (SMD = 1.54, CI 95% = 0.13–2.94; p = 0.03). However, I^2 = 88% indicated high heterogeneity (Tau^2 = 2.68; Chi^2 = 36.17; df = 5; p < 0.00001) (Figure 3).

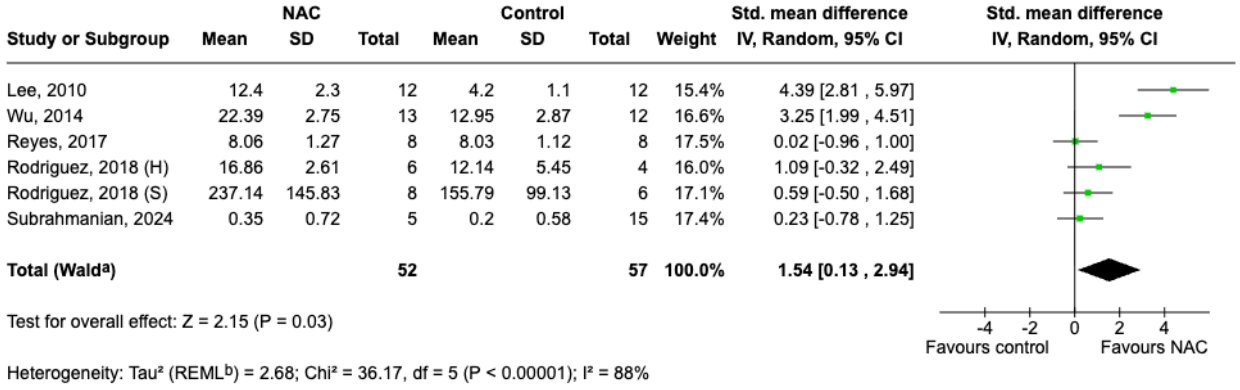

Figure 3: Forest plot of GSH meta-analysis. Data are expressed as standard mean difference (SMD) and were analyzed using the inverse variance method with a random-effects model via the online Cochrane RevMan software. (H) indicates data collected from heart analysis, and (S) indicates data collected from serum analysis.

In the subgroup analysis, we stratified the studies according to NAC dose. However, the results should be interpreted with caution, as some subgroups had fewer than three studies. For instance, NAC doses in the range of 200 to 300 mg/kg/day were associated with an increase in GSH (SMD: 3.72, CI 95% = 2.62–4.82; p < 0.00001), despite the small number of studies, whereas both the lower and the higher doses were not (Figure 4).

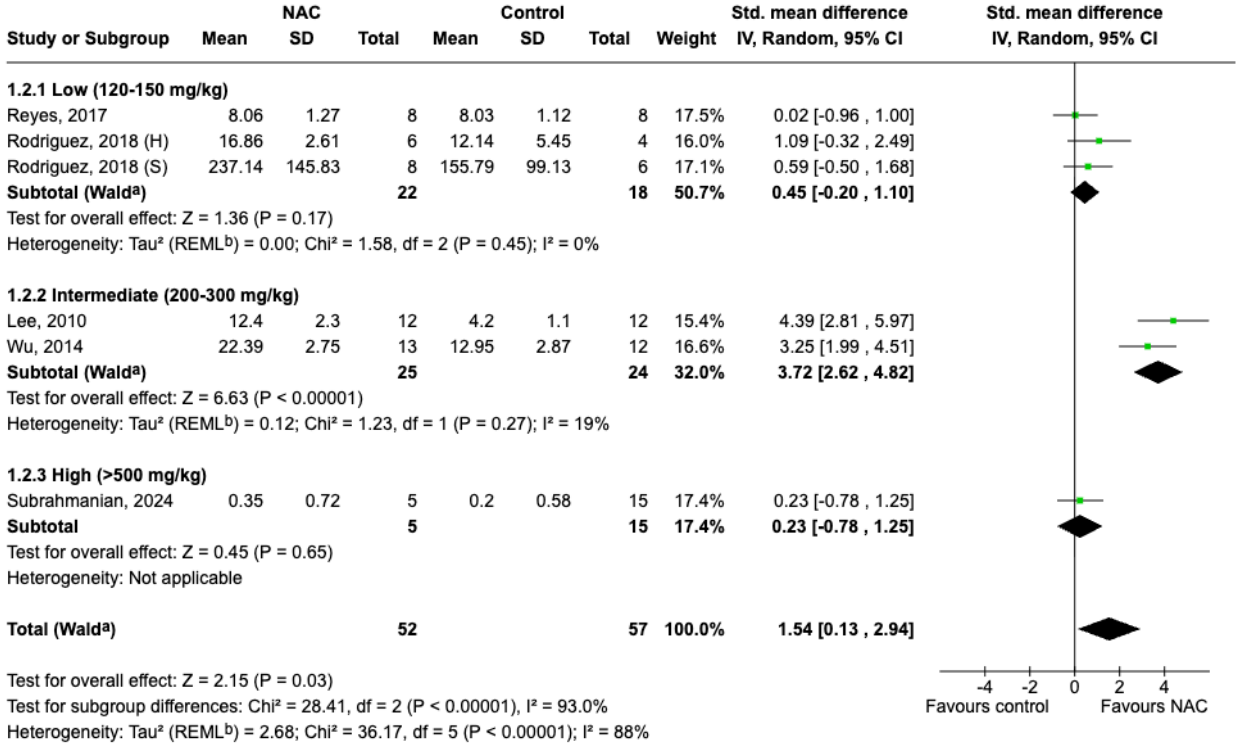

Figure 4: Forest plot of GSH subgroup meta-analysis. Studies were categorized according to the daily NAC dose and analyzed separately. Data are expressed as standard mean difference (SMD) and were analyzed using the inverse variance method with a random-effects model via the online Cochrane RevMan software. (H) indicates data collected from heart analysis, and (S) indicates data collected from serum analysis.

In the sensitivity analysis, the leave-one-out approach did not identify any single study as the primary source of heterogeneity (Supplement 5). However, when we restricted the analysis to rodent studies only, the beneficial effect of NAC on GSH levels was no longer observed, indicating that the overall effect may be driven by interspecies differences or by the inclusion of non-rodent models with distinct pharmacokinetic or methodological characteristics (Figure 5).

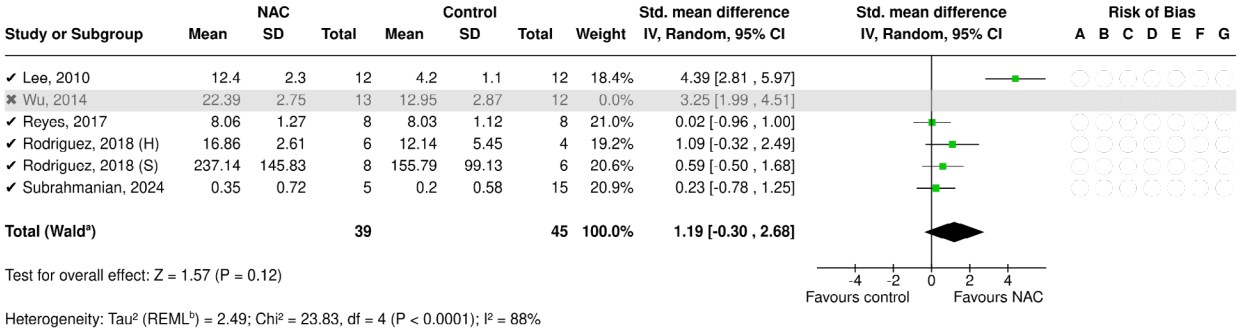

Figure 5: Sensitivity analysis of the meta-analysis on GSH levels. The analysis was conducted by restricting the dataset to rodent studies only**.** Data are expressed as standard mean difference (SMD) and were analyzed using the inverse variance method with a random-effects model via the online Cochrane RevMan software. (H) indicates data collected from heart analysis and (S) indicates data collected from serum analysis.

The total glutathione meta-analysis consisted of five approaches observed in three studies with 35 control and 38 NAC-treated animals. We found that NAC supplementation significantly increased total glutathione levels (SMD = 1.22, CI 95% = 0.41–2.02; p = 0.003). Heterogeneity was moderate, with an I^2 = 49% (Tau^2 = 0.39; Chi^2 = 7.88, df = 4; p = 0.10) (Figure 6).

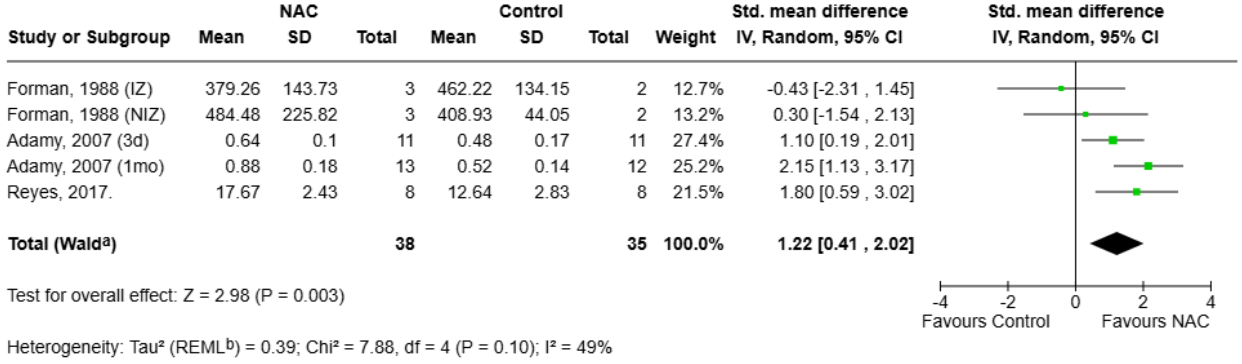

Figure 6: Forest plot of total glutathione meta-analysis. Data are expressed as standard mean difference (SMD) and were analyzed using the inverse variance method with a random effects model by online Cochrane RevMan software. (IZ), analysis in infarcted myocardium zone; (NIZ), analysis in non-infarcted myocardium zone; (3d), three days follow-up; (1mo) one month follow-up.

We performed a subgroup analysis considering daily NAC dose as a covariate. We found that low daily doses (120–150 mg/kg/day) were related to a significant increase in total glutathione levels (SMD = 1.64, CI 95% = 0.96–2.32; p < 0.00001) with low heterogeneity (I^2 = 22%; Tau^2 = 0.08; Chi^2 = 2.36; df = 2; p = 0.31). The intermediate daily NAC dose did not increase total glutathione, but we must consider this result with caution, as it derives from only one study 15 with a small sample size (4 control and 6 NAC-treated animals). Regarding the higher daily NAC dose, we did not find studies with this design to include in this meta-analysis (Figure 7).

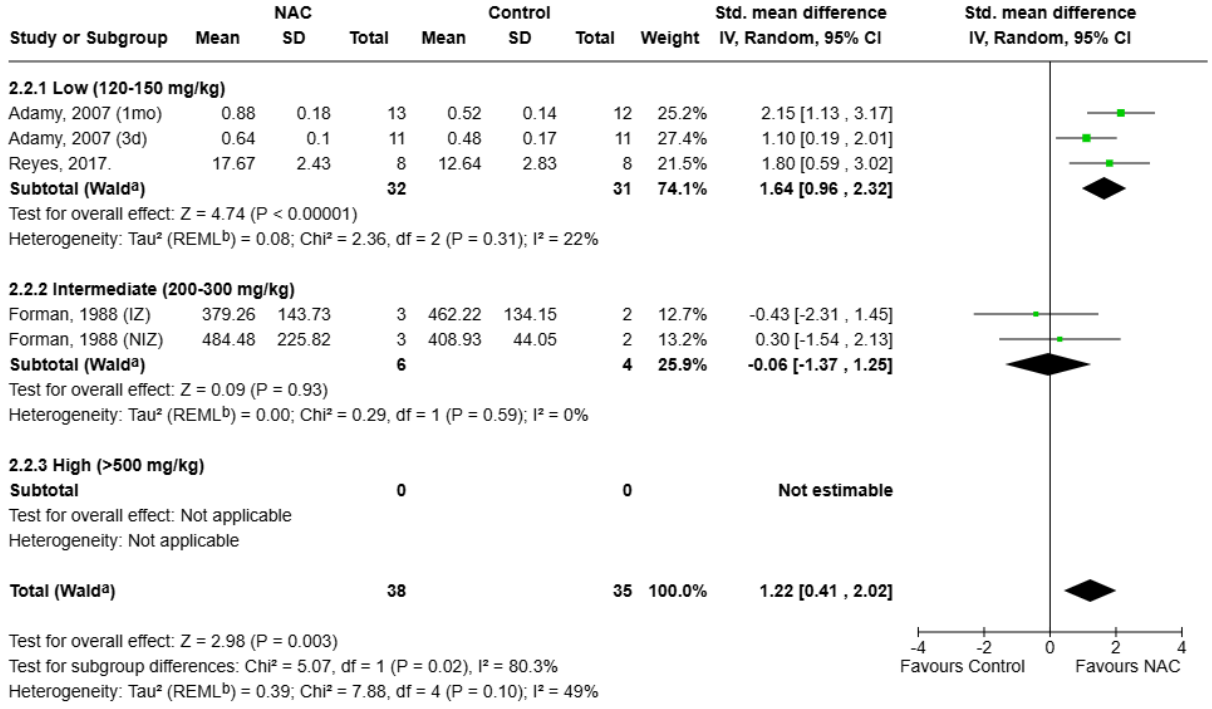

Figure 7: Forest plot of total glutathione subgroup meta-analysis. Studies were categorized according to the daily NAC dose and analyzed separately. No study with a high dose (>500 mg/kg/day) was found for this outcome. Data are expressed as standard mean difference (SMD) and were analyzed using the inverse variance method with a random-effects model via the online Cochrane RevMan software. (IZ), analysis in infarcted myocardium zone; (NIZ), analysis in non-infarcted myocardium zone; (3d), three days follow-up; (1mo) one month follow-up.

The leave-one-out sensitivity analysis approach indicated that removing the results from Forman et al. obtained from myocardial infarcted zone increased the SMD for total glutathione levels (SMD = 1.49, CI 95% = 0.82–2.15; p < 0.0001) with a small heterogeneity (I^2 = 24%; Tau² = 0.11; Chi² = 4.18, df = 3; p = 0.24) (Figure 8 and Supplement 5).

However, when we restricted the analysis to rodent studies only, we detected a further increase in total glutathione levels after the NAC treatment (SMD = 1.64, CI 95% = 0.96–2.32; p < 0.00001) with low heterogeneity I^2 = 22% (Tau² = 0.08; Chi² = 2.36, df = 2; p = 0.31) (Figure 9).

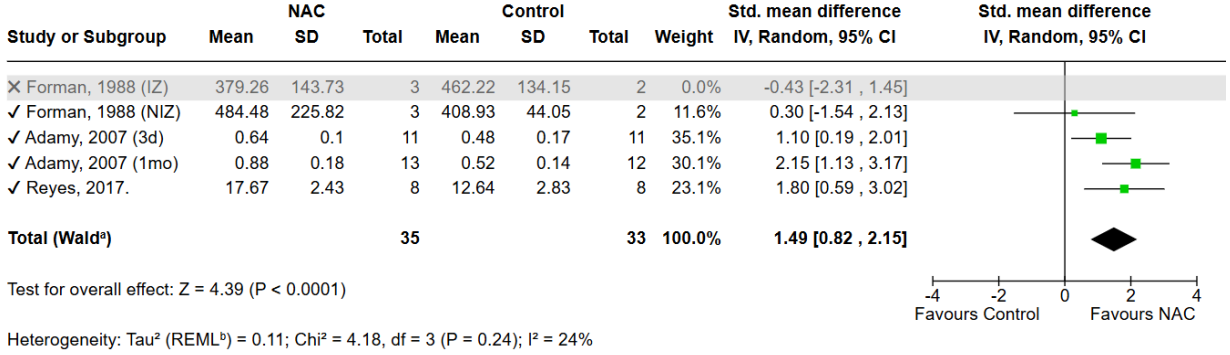

Figure 8: Leave-one-out sensitivity analysis for total glutathione. Each study was excluded from the analysis until the lowest I^2 was found. Data are expressed as standard mean difference (SMD) and were analyzed using the inverse variance method with a random effects model by online Cochrane RevMan software. (IZ), analysis in infarcted myocardium zone; (NIZ), analysis in non-infarcted myocardium zone; (3d), three days follow-up; (1mo) one month follow-up.

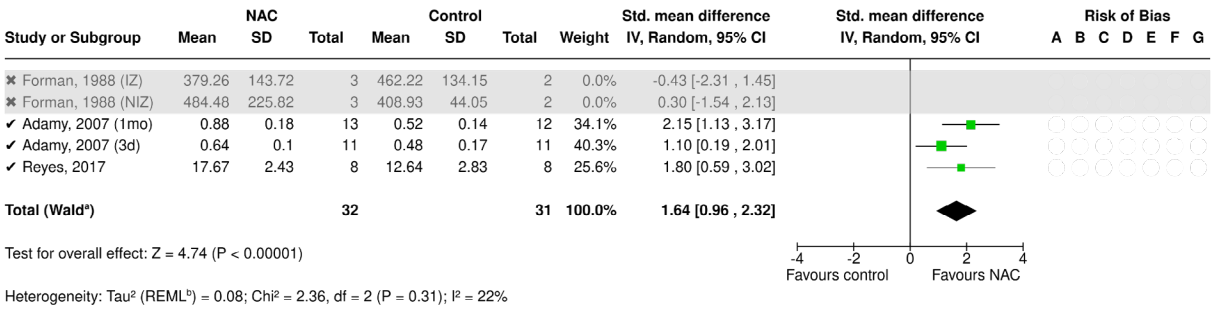

Figure 9: Sensitivity analysis of the meta-analysis on total glutathione levels. The analysis was conducted by restricting the dataset to rodent studies only. Data are expressed as standard mean difference (SMD) and were analyzed using the inverse variance method with a random effects model by online Cochrane RevMan software. (IZ), analysis in infarcted myocardium zone; (NIZ), analysis in non-infarcted myocardium zone; (3d), three days follow-up; (1mo) one month follow-up.

DISCUSSION

Here, we synthesized preclinical evidence across diverse cardiovascular models and identified a consistent effect of NAC on enhancing endogenous antioxidant capacity, particularly at intermediate daily doses (200–300 mg/kg/day). These findings strengthen the mechanistic rationale for NAC as an adjunctive therapy in cardiovascular disease and support further translational studies aiming to optimize antioxidant strategies in clinical settings.

We included eight studies and found that NAC supplementation increased both outcomes: reduced (GSH) and total (GSH + GSSG) glutathione. Our inclusion criteria encompassed various cardiovascular diseases, including ischemic, hypertrophic, and dilated cardiomyopathy, aortic stenosis, and chemotherapy-induced heart failure. To better reflect clinical practice, we included only studies in which NAC was administered as a treatment, i.e., after disease onset, rather than as a preventive measure. While NAC dosing regimens and administration routes varied across studies, the most common daily dose ranged from 120 to 250 mg/kg/day. Administration routes also differed, potentially affecting bioavailability: oral routes (via food, water, or gavage) depend on gastrointestinal absorption, with estimates suggesting that less than 10% of ingested NAC reaches systemic circulation[25]; in contrast, parenteral routes (intravenous, intraperitoneal, or intramuscular) offer greater precision. These variations in dosage and delivery likely contribute to the heterogeneity found in this meta-analysis.

Another factor contributing to the protocol variation found in preclinical studies is the partial reporting of methods and procedures[26], despite the availability of reporting guidelines since 2010[24], which may have two main consequences: First, subsequent studies may introduce methodological differences that can lead to varying outcomes. Second, the quality of the research cannot be adequately assessed if critical procedures, such as randomization, blinding, or euthanasia, are not fully described.

Several systematic reviews have also explored the relationship between NAC and cardiovascular disease patients, but they did not focus on mechanistic outcomes, such as glutathione levels. For instance, NAC has been shown to reduce in-hospital mortality in patients requiring cardiac catheterization[27] and the occurrence of all-cause mortality in ST-segment elevation myocardial infarction[28]. However, a meta-analysis evaluating NAC use in cardiac surgery also reported a lack of benefit, with no improvement in mortality, incident arrhythmia, or acute myocardial infarction[29]. The result could have been influenced by the wide variability of NAC doses (4–300 mg/kg/day), duration (1 hour–5 days), and administration route (oral, intravenous, and cardioplegia). Despite the controversial results observed in clinical studies, here we focus not on hard outcomes, but rather on the underlying molecular mechanism: can NAC increase glutathione levels in pre-clinical models?

Our meta-analysis revealed that NAC treatment favors an increase in both GSH and total glutathione. Some authors have questioned the assumed effects of NAC on GSH replenishment. From their viewpoint, such replenishment would only occur if at least two conditions were met: the enzymatic machinery involved is functional and GSH is genuinely depleted before the treatment[6]. While we cannot assess the first condition, all studies included in this meta-analysis - except Adamy et al.[14] - provided sham groups, allowing us to observe that the second condition, GSH depletion, is indeed present in the diseased models before the NAC supplementation. However, when meta-analysis was run considering the daily dose of NAC as a covariate, we saw a bell-shaped relationship for GSH, favoring the intermediate daily dose of NAC (200–300 mg/kg/day). Despite the small number of studies entering each sub-analysis group, we suggest the existence of an optimal NAC dose for GSH replenishment. This aligns with previous reports

indicating that NAC can exhibit pro-oxidant and toxic effects depending on the dose and environmental conditions, potentially leading to oxidative stress rather than preventing it[30,31].

We also sub-analyzed total glutathione according to the daily dose of NAC, but there were no studies in the high-dose group. Thus, our discussion is limited to the low and intermediate daily doses. We found an increase in the total glutathione levels in models using a low daily dose of NAC. As we did not find a corresponding increase in reduced glutathione (GSH), this finding may indicate an elevation in GSSG, suggesting the establishment of a pro-oxidant environment. In the same line, intermediate daily doses did not cause an increase in GSSG but caused an increase in GSH, reinforcing that these doses have the potential to replenish this antioxidant.

This meta-analysis has limitations that we must consider. We included studies with different experimental designs, considering either the disease (animal model) or the treatment scheme (dose and duration). This variability is expected in animal experimentation and is also observed in clinical settings[29]. To address this, we conducted subgroup and sensitivity analyses when feasible. Interestingly, in the GSH meta-analysis, the leave-one-out approach did not identify any single study as the sole driver of heterogeneity. In contrast, in the total glutathione meta-analysis, most of the heterogeneity was attributable to one sub-sample from Forman et al.[15], the only study conducted in dogs and using an intravenous NAC regimen. Given the possibility that interspecies physiological and pharmacokinetic differences could influence the pooled estimates, we conducted an additional sensitivity analysis excluding all non-rodent models. This restriction abolished the effect of NAC on GSH levels, while the increase observed in total glutathione remained unchanged. These findings underscore the importance of carefully considering species-related differences when interpreting pooled results in preclinical meta-analyses.

CONCLUSION

Our meta-analysis shows that NAC supplementation increases both GSH and total glutathione levels in animal models of heart disease. However, these effects vary according to experimental conditions. The rise in GSH was mainly observed at intermediate daily doses (200–300 mg/kg/day) and was no longer present when only rodent studies were analyzed, suggesting that interspecies or methodological differences influenced the pooled estimate. In contrast, the increase in total glutathione remained consistent across models, indicating a more robust effect.

These findings highlight that NAC's redox impact is dose- and model-dependent. Given the limited number of studies, substantial heterogeneity, and variability in experimental design, further well-controlled preclinical studies are needed to clarify the conditions under which NAC most effectively modulates cardiac glutathione levels.

Conflict of interest

None to declare.

Availability of data and material (data transparency)

Raw data are available as supplementary material. A preprint version of this manuscript is available[32].

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Author contributions: CRediT

A.S.F.: Literature Search, Data Collection, Writing – Original Draft Preparation; **T.S.**: Data Collection; **G.C.A.C.:** Data Collection, Data curation; **L.E.R.**: Conceptualization, Methodology; **M.A.**: Conceptualization, Data Collection, Data Analysis and Curation, Writing – Original Draft Preparation; All authors read and approved the final version of this manuscript.

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