



# The Effects of N-acetylcysteine on Inflammatory Markers and Homocysteine: A Systematic Review and Meta-analysis of Randomized Controlled Trials

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## Abstract

**Background:** Recently a number of trials investigated the effect of N-acetylcysteine (NAC) administration on inflammatory markers and homocysteine (Hcy). However, their findings are controversial. The aim of this paper is to present a meta-analysis and give a review of all randomized controlled trials (RCTs) in order to determine the effects of NAC on inflammatory markers and Hcy.

**Methods:** An electronic search was conducted using PubMed, Scopus, Cochrane Library, Google scholar and Web of Science databases from inception until April 2019. A study quality assessment was performed using the Jadad scale and heterogeneity between studies was statistically computed using Cochrane's Q test and I-square (I<sup>2</sup>). Data were pooled using a random-effects model and weighted mean difference (WMD) was considered as the overall effect size.

**Results:** Out of 1115 potential citations, 10 studies (reported 13 effect sizes for different parameters) met the inclusion criteria and were eligible for this meta-analysis. NAC supplementation resulted in a significant reduction in Hcy levels (WMD: -2.05; 95% CI: -3.73, -0.37). NAC administration did not affect C-reactive protein (CRP) levels (WMD: 0.06; 95% CI: -0.21, 0.34), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) levels (WMD: 0.07; 95% CI: -0.27, 0.40), and interleukin 6 (IL-6) (WMD: -0.23; 95% CI: -1.23, 0.77).

**Conclusion:** It could be concluded that this meta-analysis of RCTs demonstrated that NAC administration to various patients significantly improved circulating Hcy, but did not affect CRP IL-6 and TNF- $\alpha$  levels.

## Introduction

N-acetylcysteine (NAC) is a sulfhydryl group (SH) compound with the chemical formula C<sub>5</sub>H<sub>9</sub>NO<sub>3</sub><sup>1</sup> and is precursor, i.e. the acetylated form of L-cysteine. Acetyl group in this molecule protects it from oxidation.<sup>2</sup> The acetylation, also, facilitates reduced polarity and tissue uptake.<sup>3</sup> NAC has been reported to play a role in wide range of disorders, including HIV infection,<sup>4</sup> cancer,<sup>2</sup> chelation in metal toxicity,<sup>5</sup> acetaminophen poisoning,<sup>6</sup> Sjögren's syndrome,<sup>7</sup> psychiatric disorders, sepsis, influenza,<sup>8</sup> inflammatory bowel disease<sup>9-11</sup> and cardiovascular disease (CVD).<sup>12</sup> NAC has its therapeutic application due to its direct and indirect antioxidant and anti-inflammatory activity. Direct antioxidant effect of NAC occurs because

of reaction with free radicals and interaction with reactive oxygen species (ROS), precisely with its free thiol group.<sup>13</sup> The indirect antioxidant function of NAC is related to its role as glutathione (GSH) precursor, resulting in increase of intracellular GSH concentration, particularly in liver cells where it is an important intracellular antioxidant.<sup>14</sup> Namely NAC acts mainly as the supply of cysteine for GSH synthesis.

A plethora of studies have demonstrated anti-inflammatory effects of NAC. This activity is related to suppression of nuclear factor- $\kappa$ B (NF- $\kappa$ B) activity. Coupling I $\kappa$ B to NF- $\kappa$ B prevents from translocation of NF- $\kappa$ B to nucleus. IKK $\beta$  with phosphorylation of I $\kappa$ B and dissociation of

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I $\kappa$ B facilitates degradation of I $\kappa$ B by the proteasome, nuclear translocation of NF- $\kappa$ B and transcription of genes involved in inflammation such as interleukin-1 $\beta$  (IL-1 $\beta$ ), IL-6, IL-8, and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ).<sup>15</sup> NAC suppresses the activation of IKK $\beta$ /NF- $\kappa$ B axis by two ways. First, NAC directly inhibits the 26s proteasome activity.<sup>16</sup> Secondly, NAC scavenges ROS by thiol group or indirectly by increasing synthesis of reduced GSH that causes suppression of IKK $\beta$ .<sup>17</sup> Homocysteine (Hcy) is a sulfhydryl group containing amino acid related to methionine metabolism. Hcy can be have various fate in the body. Hcy may be converted to methionine by folic acid, B12 and betaine-dependent remethylation pathways or can be converted, via the trans-sulfuration pathway, into cysteine.<sup>18</sup> Hyperhomocysteinemia is considered as a risk factor for CVD and stroke since Hcy is known to have adverse effects on vascular endothelium and smooth muscle cells with resultant alterations in subclinical arterial structure and function. High-dose folic acid, vitamin B6 or B12, serine, or betaine cannot normalize hyperhomocysteinemia in the individuals that are resistant to conventional treatment.<sup>19,20</sup> However, it has been shown that administration of NAC orally or intravenously dramatically decreases Hcy levels.<sup>21-23</sup> Nevertheless, the effects of NAC on circulating inflammatory markers and Hcy are controversial and no studies have been done to analyze the effects of NAC on both inflammatory biomarkers and Hcy. Therefore, the aim of this study was to perform a meta-analysis and summarize systematically the present evidence from RCTs concerning the effects of NAC on inflammatory markers and Hcy levels in various diseases.

## Methods

### Search strategy

Two authors independently searched electronic databases including PubMed, Scopus, Cochrane Library, Google scholar and Web of Science databases from inception up to April 2019 for relevant RCTs investigating the associations between NAC administration and inflammatory markers and Hcy. The search strategy was limited to RCTs in humans and published in English. The following MeSH and text keywords were used to identify primary RCTs: intervention (“N-acetylcysteine” OR “NAC”), and parameters [“C-reactive protein (CRP)” OR “tumor necrosis factor- $\alpha$  (TNF- $\alpha$ )” OR “interleukin 6 (IL-6)” OR “homocysteine (Hcy)”. The reference lists of related RCTs and previous reviews were manually searched to detect further studies which were not captured in our primary search.

### Inclusion and exclusion criteria

RCTs with the following criteria were included in meta-analysis: human trials with either parallel or cross-over design, data on the effects of NAC on inflammatory markers and Hcy extracted from RCTs with standard deviation (SD) and related 95% confidence intervals (CIs)

for both intervention and placebo groups. Other studies such as animal experiments, in vitro studies, case reports, observational studies, investigations without control group, trials with  $\leq 2$  weeks period and studies that did not achieve the least quality score were excluded.

### Data extraction and quality assessment

Two independent authors (JH and AM) screened the retrieved articles based on the eligibility criteria. As the first step the title and abstract of studies were reviewed. Then, the full-text of relevant studies was analyzed to ascertain the suitability of a study for the meta-analysis. Any disagreement was resolved through the judgment of the third author (ZA).

The following data extracted from the selected studies: the first authors' name, year of publication, study location, sample size, study design, dosage of supplementation, duration of the intervention, type of disease, the mean and SD for inflammatory markers and Hcy in each intervention group. The same two authors assessed the studies' quality independently using the Jadad scale.

### Data synthesis and statistical analysis

The pooled effects of NAC supplementation on inflammatory markers and Hcy were calculated using change score approach. Weighted mean difference (WMD) with 95% CI was used for pooling data to determine the pooled effect sizes by utilizing the random-effect model.

### Heterogeneity and publication bias

Heterogeneity across included studies was evaluated using Cochran's Q test (with significant P-value  $< 0.1$ ) and I-square test (I<sup>2</sup> greater than 50 percent showing significant heterogeneity). The funnel plot, as well as the Beggs's and Egger's regression tests was used to determine the publication bias. Both STATA 11.0 (Stata Corp., College Station, TX) and Review Manager 5.3 (Cochrane Collaboration, Oxford, UK) were applied for data analysis. Assessment of study quality was conducted using the Jaddad scale.

## Results

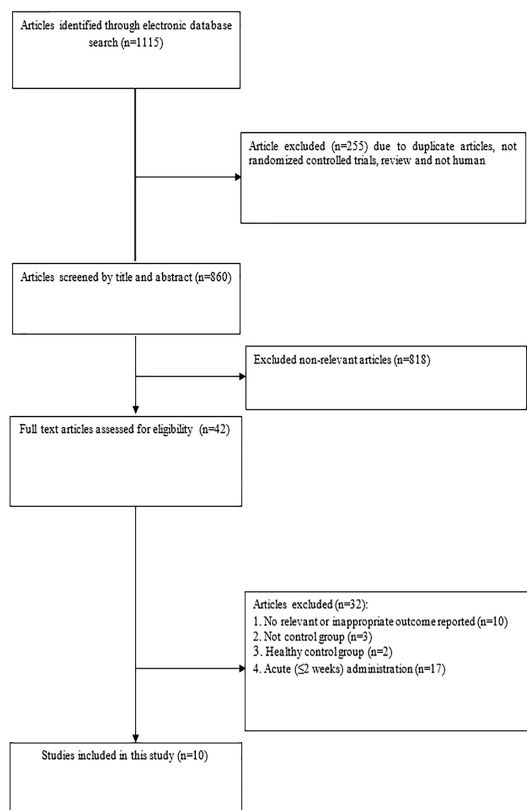
### Characteristics of the included studies

General characteristics of included studies are presented in Table 1. Ten studies which reported 13 effect sizes were included. Flowchart of study selection procedure is shown in Figure 1. These studies were published between 2003 and 2019. A total of 587 participants, including 340 individuals in the intervention and 247 persons in the control groups, were enrolled in these trials. The studies were conducted in Iran, Turkey, Poland, Australia, Italy, Island and Spain. Included trials were done among healthy participants, workers with lead exposure, depressive patients, patients with T2DM, dialysis subjects and patients with ulcerative colitis. Duration of intervention was varied from 1 to 6 months and the dosage of NAC supplements was varied between 200 mg/d to 2400 mg/d. In four studies, NAC

**Table 1.** Characteristics of included studies.

Authors (Ref)	Publication year	Sample size (control/intervention)	Type of study	Country	Health status	Intervention (name and daily dose)	Duration	Age (y) (control, intervention)	Presented data(placebo)	Presented data(intervention)
Panizzutti et al. <sup>24</sup>	2018	15/13	Placebo-controlled randomized trial	Australia	Bipolar depression received 2000 mg/d NAC in past 2 months	2000 mg/d NAC + usual treatment	6 months	22-70	TNF- $\alpha$ ↓	TNF- $\alpha$ ↑
Vural et al. <sup>25</sup>	2018	17/23	Prospective, randomized, controlled, and open label study	Turkey	Peritoneal dialysis	1200 mg/d NAC	1 month	49±13, 46±15	IL-6↑, TNF- $\alpha$ ↑	IL-6↓, TNF- $\alpha$ ↓
Martina et al. <sup>26</sup>	2008	12/12	Randomized, double-blind, placebo-controlled study	Italy	Male patients with T2DM and HTN	1200 mg/d NAC + 1200 mg/d L-arginine	6 months	67±16.5, 62.5±13.4	CRP↔, IL-6↔, TNF- $\alpha$ ↓, Hcy↓	CRP ↓, IL-6↑, TNF- $\alpha$ ↔, Hcy↓
Guijarro et al. <sup>27</sup>	2008	18/19	Randomized, placebo-controlled pilot study	Spain	Ulcerative colitis	800 mg/d NAC + mesalamine	4 weeks	42.2 ± 13, 51.4 ± 14	IL-6 ↓, TNF- $\alpha$ ↑	IL-6↓, TNF- $\alpha$ ↑
Hasebe et al. <sup>28</sup>	2017	63/58	Prospective, randomized, double-blind, placebo-controlled study	Australia	Unipolar depression	2000 mg/d NAC + usual treatment	12 weeks	20-77	CRP↓, IL-6↓	CRP↓, IL-6↑
Bashardoust et al. <sup>29</sup>	2018	25/26	Randomized placebo-controlled clinical trial	Iran	Hemodialysis patients	1200 mg/d NAC	1 month	62.76±14.47, 65.50±11.05	CRP↓	CRP↓
Abasi Larki et al. <sup>30</sup>	2019	19/21	Double-blind, randomized clinical trial	Iran	Hemodialysis patients	1200 mg/d NAC	8 weeks	61.05±19.09, 60.61±16.61	CRP↓, IL-6↓	CRP↓, IL-6↓
Friedman et al. <sup>31</sup>	2003	16/18	Randomized placebo-controlled trial	Island	Hemodialysis	2400 mg/d NAC	4 weeks	70±4, 68±3	Hcy↓	Hcy↓
Ventura et al. <sup>32</sup> (a)	2003	6/14	Not reported	Italy	Healthy subjects	600 mg/d NAC	1 month	44±19, 42±11	Hcy↑	Hcy↓
Ventura et al. <sup>32</sup> (b)	2003	7/14	Not reported	Italy	Healthy subjects	1800 mg/d NAC	1 month	44±19, 46±14	Hcy↑	Hcy↓
Kasperczyk et al. <sup>33</sup> (a)	2016	16/40	Not reported	Poland	Lead-exposed workers	200 mg/d NAC	12 weeks	42.5±8.66	Hcy↓	Hcy↔
Kasperczyk et al. <sup>33</sup> (b)	2016	17/44	Not reported	Poland	Lead-exposed workers	400 mg/d NAC	12 weeks	42.5±8.66	Hcy↓	Hcy↓
Kasperczyk et al. <sup>33</sup> (c)	2016	16/38	Not reported	Poland	lead-exposed workers	800 mg/d NAC	12 weeks	42.5±8.66	Hcy↓	Hcy↓

CRP; C-reactive protein; IL-6; interleukin-6; TNF- $\alpha$ ; tumor necrosis factor- $\alpha$



**Figure 1.** Literature search and review flowchart for selection of studies.

was used at a daily dosage of 1200 mg. In some studies, NAC consumption was along with usual treatment or the administration of other compounds like mesalamine or L-arginine. Measured outcomes were: CRP (4 studies), IL-6 (5 studies), TNF- $\alpha$  (4 studies) and Hcy (4 studies, 7 effect size).

**The effects of NAC on inflammatory cytokines**

Analysis of 4 studies did not show any significant effect on CRP concentrations following NAC supplementation

(WMD: 0.06; 95% CI: -0.21, 0.34) (Figure 2A and Table 2). This finding remained unchanged after subgroup analysis by the study sample size (Table 3).

Analysis of 4 studies showed no significant changes in TNF- $\alpha$  levels (WMD: 0.07; 95% CI: -0.27, 0.40) following NAC supplementation (Figure 2B and Table 2). Subgroup analyses by the study location and study duration showed no significant effects of NAC supplementation on serum TNF- $\alpha$  concentrations, however, we found a significant elevation of TNF- $\alpha$  levels in studies done in European countries (WMD: 0.14; 95% CI: 0.11, 0.17) and those with a duration of < 8 weeks (WMD: 0.14; 95% CI: 0.11, 0.17) (Table 3).

NAC administration did not significantly change IL-6 levels, as shown in the meta-analysis of 5 studies (WMD: -0.23; 95% CI: -1.23, 0.77) (Figure 2C and Table 2). In the subgroup analyses, a significant reduction of IL-6 levels was seen among studies done in European countries, studies with a duration of <8 weeks, and those done in subjects aged <60 years (for all: WMD: -0.22; 95% CI: -0.26, -0.18) (Table 3).

**The effects of NAC on Hcy**

Pooling findings of 7 effect sizes showed a significant reduction in Hcy levels following NAC supplementation (WMD: -2.05; 95% CI: -3.73, -0.37) (Figure 2D and Table 2). This finding did not change after subgroup analysis by the study duration and dosage (Table 3).

Findings from dose-response analysis about NAC intake on inflammatory markers and Hcy revealed that the overall pooled estimates on inflammatory markers and Hcy were independent of NAC dosage. We failed to detect a significant effect of specific dosage of NAC on inflammatory markers and Hcy, as examined by non-linear dose-response meta-analysis (Figure 3A-D).

Regarding the study quality, presented in Table 4, four publications had a Jadad score of 2 (Vural et al, Panizzutti et al, Ventura et al and Kasperczyk et al) and others had a score of  $\geq 3$ .

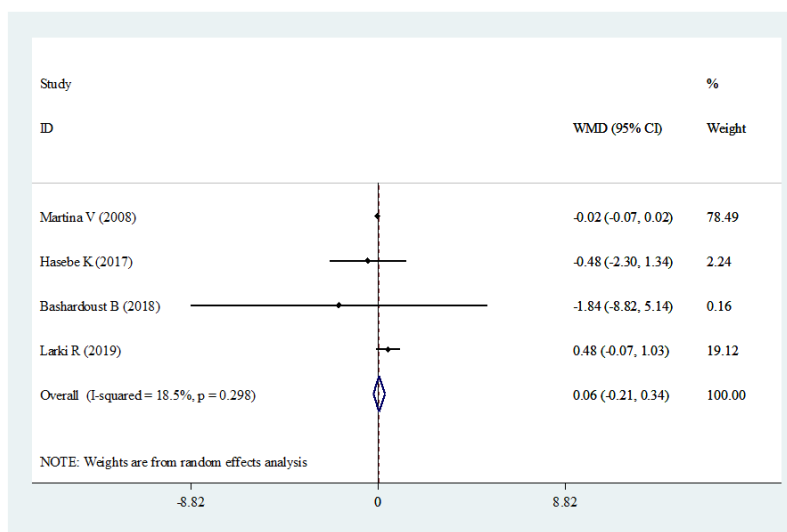


Fig.2 A

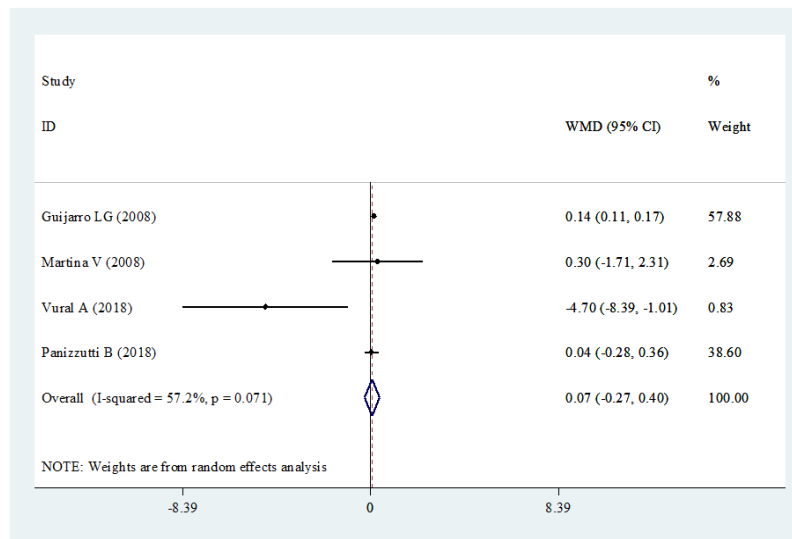


Fig.2 B

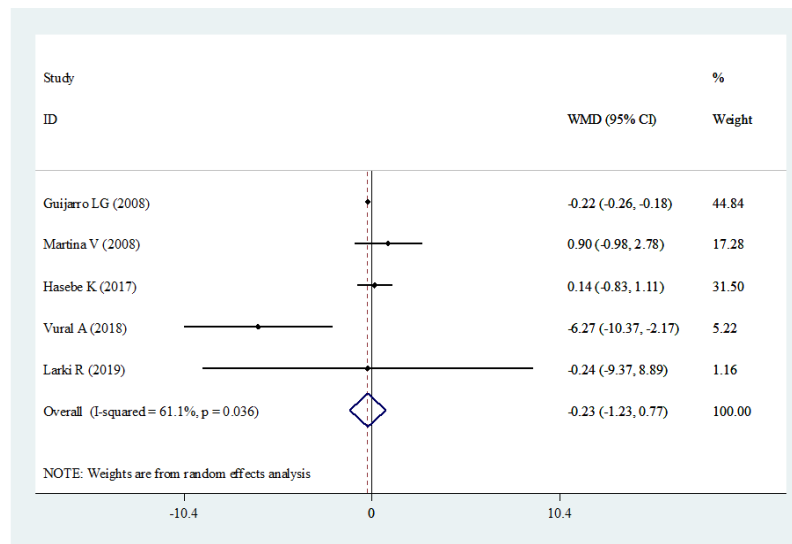


Fig.2 C

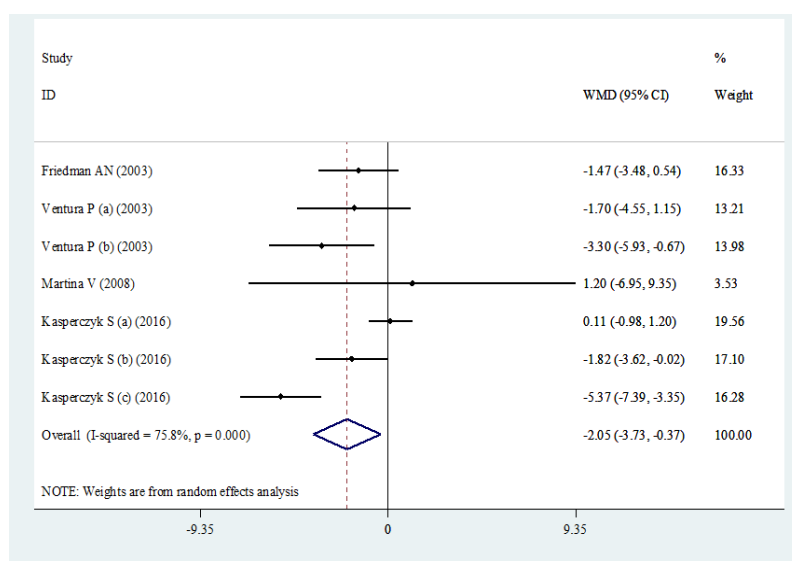


Fig.2 D

**Figure 2.** Meta-analysis on outcome standardized mean differences estimates for CRP in N-acetylcysteine and placebo groups (CI=95%).

**Table 2.** The effects of N-acetylcysteine on inflammatory markers and homocysteine.

Variables	Number of effect sizes	Weighted Mean difference	CI 95%	Heterogeneity	
				I <sup>2</sup> (%)	P- value heterogeneity
CRP	4	0.06	-0.21, 0.34	18.5	0.29
IL-6	5	-0.23	-1.23, 0.77	61.1	0.03
TNF-α	4	0.07	-0.27, 0.40	57.2	0.07
Homocysteine	7	-2.05	-3.73, -0.37	75.8	<0.001

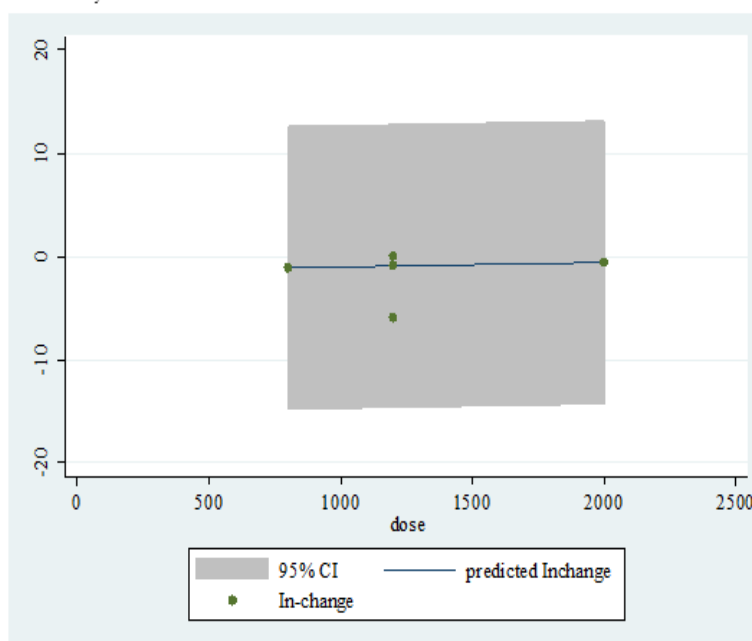
CRP; C-reactive protein; IL-6; interleukin-6; TNF-α; tumor necrosis factor-α

**Table 3.** Subgroup analyses for the effects of N-acetylcysteine on inflammatory markers and homocysteine.

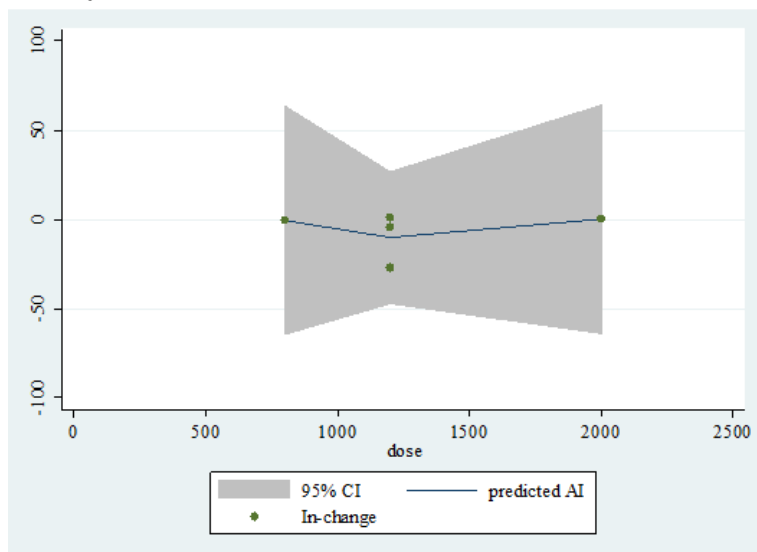
Variables	Subgroups	Number of effect sizes	Pooled WMD	95% CI	I <sup>2</sup> (%)	Between-study I <sup>2</sup> (%)	
							CRP
		≥50	2	-0.57	-2.32, 1.19	00.0	
	Study location	European countries	2	-0.22	-0.26, -0.18	26.7	0.96
		Non-European countries	3	-0.20	-1.14, 0.74	77.5	
IL-6	Study duration	<8 weeks	2	-0.22	-0.26, -0.18	88.1	0.23
		≥8 weeks	3	0.29	-0.56, 1.15	00.0	
	Participants' age	<60 years	3	-0.22	-0.26, -0.18	77.6	0.25
		≥60 years	2	0.85	-0.99, 2.69	00.0	
TNF-α	Study location	European countries	2	0.14	0.11, 0.17	00.0	0.40
		Non-European countries	2	0.01	-0.31, 0.32	84.1	
	Study duration	<8 weeks	2	0.14	0.11, 0.17	84.9	0.55
		≥8 weeks	2	0.05	-0.27, 0.36	00.0	
Homocysteine	Study duration	<8 weeks	3	-2.04	-3.43, -0.64	00.0	0.34
		≥8 weeks	4	-1.25	-2.09, -0.41	86.8	
	Participants' age	<60 years	5	-1.49	-2.26, -0.71	83.6	0.87
		≥60 years	2	-1.32	-3.27, 0.63	00.0	
Supplementation dosage	<1000 mg/d	4	-1.31	-2.13, -0.50	86.6	0.43	
	≥1000 mg/d	3	-2.02	-3.59, -0.45	0.40		

CRP; C-reactive protein; IL-6; interleukin-6; TNF-α; tumor necrosis factor-α.

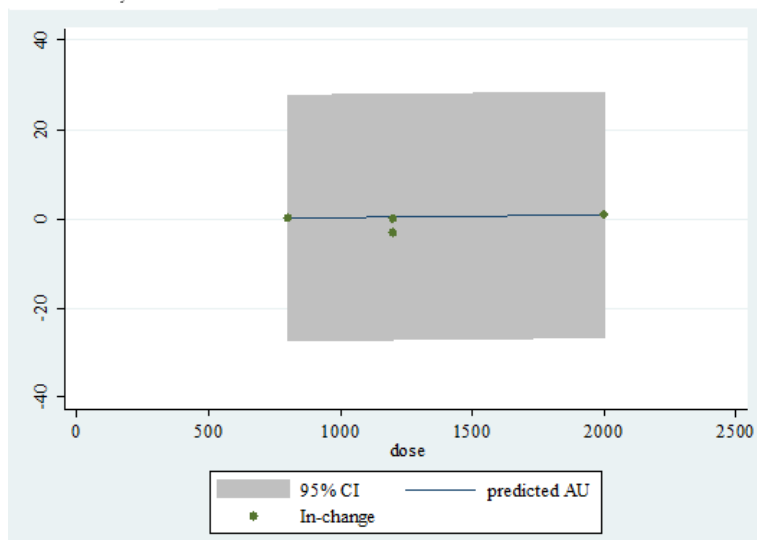
A: CRP, P=0.67



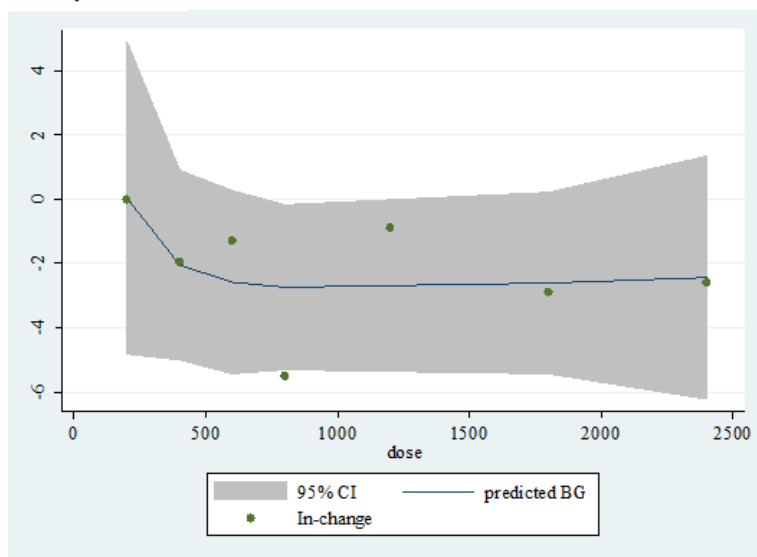
B: IL-6, P=0.55



C: TNF- $\alpha$ , P=0.50



D: Hcy, P=0.60



**Figure 3.** Non-linear dose-response relations between NAC dosage and inflammatory markers and Hcy in (A) CRP, (B) IL-6, (C) TNF- $\alpha$  and (D) Hcy levels. The 95% CI is demonstrated in the shaded regions.

**Table 4.** Methodological quality scores for included studies using Jadad scale.

Authors (Year)	Randomization	Blinding	Account of all patients	Total Score
Panizzutti et al. <sup>24</sup> 2018	1	1	0	2
Vural et al. <sup>25</sup> 2018	1	0	1	2
Martina et al. <sup>26</sup> 2008	1	2	1	4
Guijarro et al. <sup>27</sup> 2008	2	0	1	3
Hasebe et al. <sup>28</sup> 2017	1	1	1	3
Bashardoust et al. <sup>29</sup> 2018	2	2	1	5
Abasi Larki et al. <sup>30</sup> 2019	2	2	1	5
Friedman et al. <sup>31</sup> 2003	1	2	1	4
Ventura et al. <sup>32</sup> 2003	1	0	1	2
Kasperczyk et al. <sup>33</sup> 2016	1	0	1	2

## Discussion

This systematic review and meta-analysis analyzed the effect of orally or intravenously administration of NAC on circulating inflammatory markers and Hcy levels in subjects with different diseases. Our findings demonstrated that NAC significantly decreased circulating Hcy levels, but did not affect CRP, IL-6, and TNF- $\alpha$  levels.

### Effects of NAC administration on inflammation markers

There are a plethora of studies analyzing the beneficial impacts of NAC on CRP, IL-6, and TNF- $\alpha$  but their results are controversial. In a placebo-controlled randomized trial from Panizzutti et al.,<sup>24</sup> treatment with 2000 mg/day of adjunctive NAC in patients with bipolar depression during 8 weeks and 32 weeks did not significantly change TNF- $\alpha$ , CRP and IL-6 levels. In another study, intake of 600 mg twice daily of NAC significantly reduced TNF- $\alpha$  and IL-6 levels during 1 month, but in the same study, a significant difference was not observed in markers of TNF- $\alpha$  and IL-6 levels during 12 months.<sup>25</sup> Martin et al.<sup>26</sup> showed that the administration of NAC with a dose of 600 mg, twice a day, during 6 months in hypertensive patients with type 2 diabetes mellitus resulted in a significant reduction of CRP levels. Guijarro et al.<sup>27</sup> found that circulating IL-6 levels did not change significantly, with respect to baseline values, after treatment of ulcerative colitis patients with 0.8 g/d NAC in a randomized, placebo-controlled pilot study. Hasebe et al.<sup>28</sup>, in their study on patients with unipolar depression, showed that NAC supplementation with a dose of 2000 mg/day during 12-week detracted blood CRP, IL-6 values as compared to the placebo. A similar finding was seen in the study performed by Bashardoust et al.<sup>29</sup>, in which oral administration of 1200 mg daily of NAC supplementation during 1 month to patients undergoing hemodialysis showed a significant reduction in CRP levels. In the study of Abasi Larki et al.<sup>30</sup>, intake of 600 mg NAC every 12 hours for eight weeks significantly not change the IL-6 levels in the intervention group as compared to the control group. Increased inflammatory biomarkers and metabolic profiles increase the risk of CVD and diabetes.<sup>34-37</sup> Suppression of inflammation markers production by NAC could be caused by inhibition of the pro-inflammatory transcription factor activators protein-1 (AP-1) and NF- $\kappa$ B.<sup>38</sup> Induction of NF- $\kappa$ B and

AP-1 in response to oxidative stress clarify a fundamental role of NAC in the modulation of inflammation-related genes due to the action mechanism of NAC as free radical-scavenger. However, these discrepancies in the valence of NAC between studies might be to some extent too because of variation in dosage of NAC, the longitude of treatment, baseline levels of NAC and inflammation markers, and also differences in the type of the disease.

### Effects of NAC administration on Hcy levels

Hyperhomocysteinemia induces a large production of ROS in vascular cells and macrophages. These changes increase expression of several cytokines such as TNF- $\alpha$ , IL-1 $\beta$  and IL-6, monocyte chemoattractant protein-1, and intracellular adhesion molecule-1 due to activation of nuclear NF- $\kappa$ B and P2X7 pathways.<sup>39,40</sup> This can contribute to development of diseases such as stroke, aortic aneurysm, myocardial infarction, Alzheimer's disease and epilepsy. Therefore, normalization of Hcy concentrations in different conditions can be a therapeutic target especially for patients with hyperhomocysteinemia. NAC is a thiol-containing compound broadly applied for therapy of acetaminophen poisoning<sup>41</sup> and chronic obstructive pulmonary disease<sup>42</sup> without drastic side effects.<sup>43</sup> On the other hand, many other studies supported the idea that NAC administration orally or intravenously can acutely and dramatically decrease Hcy levels.<sup>21-23</sup> Kasperczyk et al.<sup>33</sup> showed that chronic oral treatment with NAC decreased Hcy levels and oxidative stress in a dose-dependent manner in lead-exposed workers. Similar with this study, NAC reduced Hcy levels in hemodialysis patients,<sup>44-46</sup> kidney transplant recipients,<sup>47</sup> patients with coronary artery disease,<sup>48</sup> and patients undergoing cardiac angiography.<sup>49</sup> These effects of NAC can be explained by removing of Hcy from protein binding sites, especially from circulating albumin. This could cause formation of disulfide forms which have a higher liver and kidney clearance that could facilitate their removal from plasma.<sup>32,50</sup> On the other hand, given the knowledge that the administration of NAC can also increase antioxidant capacity. Our meta-analysis study's results give prominence that NAC as an adjunctive approach along with other drugs can help to reduce homocysteine as an atherogenic agent.

It is important to note that first, most of the studies



investigated on the property of NAC have been conducted on a small scale and require large-scale studies for a more definitive conclusion. Second, the number of studies per effect size is low and further studies are needed to obtain a more convincing conclusion. Third, duration of intervention is relatively short and may not be sufficient to obtain beneficial effects. Fourth, heterogeneity of results is generally high for most autosomes, which may affect the efficacy of results. Due to the low number of studies, a sensitivity analysis for publication bias was not conducted.

### Conclusion

NAC has antioxidant properties and may diminishes inflammation. Meta-analysis of outcomes in our study indicated that NAC administration in different types of patients significantly lowered serum levels of Hcy, but did not affect inflammatory parameters including CRP, TNF- $\alpha$  and IL-6 levels.

### Conflict of Interests

The authors declare that they have no conflict of interest.

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