Annals of Clinical and Medical Case Reports[R] ISSN 2639-8109 |Volume 15

Case Report Open Access

Impact of a Glycine and N-Acetyl Cysteine Ethyl Ester Complex on Antioxidant and Inflammation Status and Related Quality of Life Parameters in Healthy Adults

Gerald M Haase[1]*, Neil E Wolkodoff[2], Joyce A Curry[3], Aimee W Houghton[4]

[1]Clinical Professor of Surgery, University of Colorado School of Medicine, Aurora, CO

[2]Medical Program Director, Colorado Center for Health and Sports Science, Denver, CO

[3]Assistant Professor of Anatomy & Exercise, Colorado Christian University, Lakewood, CO

[4]Assistant Director of Nursing, Colorado Christian University, Lakewood, CO

***Corresponding author:**

Gerald M. Haase,
University of Colorado School of Medicine,
Aurora, Colorado, USA

Received: 01 Feb 2026
Accepted: 10 Feb 2026
Published: 14 Feb 2026
J Short Name: ACMCR

Keywords:

NAC Ethyl Ester; Glutathione; Antioxidant Status; TNF-α; Quality of Life

Citation:

Gerald M. Haase, Impact of a Glycine and N-Acetyl Cysteine Ethyl Ester Complex on Antioxidant and Inflammation Status and Related Quality of Life Parameters in Healthy Adults. Ann Clin Med Case Rep® 2026; V15(1): 1-9

1. Abstract

1.1. Aim

This pilot investigation aimed to assess the impact of a proprietary glycine and N-acetyl cysteine (NAC) ethyl ester complex on quality-of-life (QOL) measures as well as blood levels of antioxidant, inflammatory and toxicant status. Recent evidence suggests that esterification of NAC enhances its bioavailability compared to NAC alone. The addition of glycine is thought to further promote glutathione synthesis, potentially amplifying antioxidant effects. The primary goal of the current trial was to evaluate both the subjective (experiential) and objective (laboratory) outcomes associated with oral consumption of this encapsulated supplement over an eight-week open-label study in middle-aged and older adults.

1.2. Methods

Nineteen (N=19) healthy subjects between the ages of 43 and 72 years were prospectively and randomly selected from the study applicant pool. The research protocol prospectively excluded individuals with specific neurological, oncologic, or cardiometabolic conditions, as well as those prescribed medications that could interfere with laboratory testing. Participants consumed the supplement daily for eight weeks and completed an at-home, hybrid lifestyle questionnaire derived from validated QOL surveys both at baseline and at the study's conclusion. Blood samples were also collected at these times to assess relevant biomarkers. Interim weekly personal contacts were made to ensure compliance and gather the subjects' experiential input.

1.3. Results

Eighteen subjects completed the QOL assessments, and sixteen provided all the required blood samples. After the 8-week supplementation period, the comprehensive QOL rating scores improved from 3.36 to 4.18, highly statistically significant at the $p<0.0001$ level. The weekly interim survey scores improved similarly from 3.0 to 3.81 ($p<0.0001$). The overall glutathione levels increased by eight percent from 698 to 751 µM, and tumor necrosis factor-alpha (TNF-α) levels decreased by seven percent from 0.76 to 0.71 pg/mL. In the older subject cohort, these two parameters statistically significantly improved ($p<0.05$). Also, in this subgroup, those with lower initial glutathione values increased by 18 percent from 612.6 to 720.9 µM. Gamma-glutamyl transferase and C-reactive protein levels were unchanged. The study supplement was generally well tolerated, and no serious adverse effects were observed.

1.4. Conclusion

This pilot trial demonstrated the effectiveness of a proprietary supplement complex on QOL parameters and correlative blood biomarkers. The overall comprehensive QOL factors and the weekly interim lifestyle parameters all improved in a highly statistically significant manner. Showing beneficial trends, the overall glutathione levels increased, and the overall TNF-α levels decreased. When evaluated in the older subjects, these two biomarkers statistically significantly improved. Even in the participants who did not show benefits in blood markers, the perceived QOL factors still improved.

2. Introduction

Normal human metabolism related to oxygen utilization generates free radicals, which are potentially harmful by causing oxidative stress. A variety of external factors, such as job con-

ditions, physical activity, smoking, radiation, extreme environments, mental stress, and traumatic events, can further increase free radical production and impact overall health. This oxidative damage can injure body proteins, lipids and DNA, negatively affecting cells, tissues and organs. Acute and chronic inflammation and exposure to various toxins can exacerbate the spectrum of injury. Collectively, these factors contribute to the initiation and progression of adverse health conditions including most chronic diseases, as well as a premature and accelerated aging process [1-3].

Due to its high oxygen consumption, the human brain is particularly vulnerable to reactive oxygen species and resultant oxidative stress. This damage affects tissue integrity and induces the production of pro-inflammatory cytokines, further harming neuronal structures, lipid membranes, synaptic function, as well as cellular proteins and DNA [4]. The accumulation of free radical-induced injury is strongly associated with the onset and progression of mild cognitive impairment, dementia, and Parkinson's and Alzheimer's diseases [5,6]. In addition, oxidative stress-related tissue damage contributes to metabolic disorders such as diabetes and its resulting micro- and macrovascular complications [7,8].

To counter this downward cascade, the human species has developed an elaborate protective system. The system utilizes antioxidants and anti-inflammatory substances to directly neutralize excess free radicals, decrease inflammatory reactions, and minimize the effects of toxicants. Glutathione is the most abundant low-molecular-weight antioxidant substance in aerobic cells [9-11]. It is a critical non-enzymatic agent that detoxifies and scavenges excess free radicals and pro-oxidants, protecting cell cycle regulation, protein metabolism, gene expression, immune, and neuronal function [12-16]. Given its central role in brain homeostasis and function, glutathione has shown broad therapeutic benefits across various medical applications. It also provides a biomarker for enhanced health status and diminishing major age-related chronic diseases [17,18]. Reduced amounts of this molecule relate to the development of most common serious diseases, especially neurodegenerative and neuropsychiatric conditions [19,20]. Elevating levels of this key antioxidant prevented experimentally induced heavy metal neurotoxicity and protected in vitro brain tissue cell lines [21].

Glutathione's potent antioxidant properties are due to its high intracellular concentration (millimolar levels rather than micromolar), strong reactivity potential, and ubiquitous nature throughout the human body [22]. This molecule is a tripeptide synthesized intracellularly from the amino acids cysteine, glycine, and glutamate, specifically resulting in gamma-L-glutamyl-L-cysteinyl-glycine [23,24]. Thus, researchers still widely regard N-acetyl cysteine (NAC) to be an effective antioxidant, although clinical trials have produced inconsistent results. The key metabolic pathway that converts NAC into glutathione within cells is responsible for its primary antioxidant effects [25]. However, this amino acid itself may not directly protect against diseases caused by oxidative stress. Producing an ethyl ester of NAC through carboxyl group esterification boosts its lipophilicity, bioavailability, and pharmacokinetic activity [26]. Cells and tissues, such as red blood cells and the brain, quickly absorb this form, which then defends against dangerous exposures.

Therefore, the ethyl ester form is considered the optimal choice for analytical chemistry, as it maximizes the pharmacological effects of the amino acid for glutathione synthesis [27]. Animal models and human cell line studies demonstrated the superiority of this esterified pattern [28,29]. These studies showed maintenance of healthy brain tissue and endothelial cells and the ability to cross the plasma membrane barrier. The maximal therapeutic benefits are achieved by maintaining the correct peak levels of NAC-ethyl ester. These lipophilic glutathione prodrugs are particularly effective at penetrating specific target tissue and cellular environments, such as retinal pigment epithelium, as well as in diabetic retinopathy and age-related macular degeneration [30,31].

As glutathione is a tripeptide, the glycine and NAC complex is likely to mitigate the oxidative stress and mitochondrial dysfunction that contribute to aging. In vivo models show that supplementation resulted in a 24% longer lifespan in treated mice compared to controls. It also increased glutathione synthesis and corrected abnormal mitophagy and genomic damage in the heart, liver and kidneys [32]. These findings were consistent with the timing of several confirmatory clinical trials. The central concept in these studies was that older adults suffer impaired aging from oxidative damage, inflammatory reactions, endothelial abnormalities, metabolic defects, and cognitive decline. Supplementation with glycine and NAC corrects glutathione deficiency, decreases inflammation, and improves metabolic and endothelial function [33].

A pilot human trial in diabetic patients demonstrated this proof of concept in that the intervention decreased insulin resistance and optimized glucose, mitochondrial, and fatty acid metabolism (34). Supplementation has been shown to prevent multiple signs of aging due to oxidative damage. The positive clinical trials extended over a range of designs from observational and open- label studies to prospective, randomized placebo-controlled trials. These pilot investigations showed that the aging health biomarkers and related clinical abnormalities could be either enhanced or corrected [35-37]. While overall glutathione levels did not always consistently increase, subsets of older subjects with high oxidative burden and the lowest baseline levels generally improved to some degree.

Considering the promising findings from previous studies, it appeared rational to pursue this line of research with potentially confirmatory clinical experience. Therefore, this investigative trial was undertaken to evaluate the impact of a unique glycine and NAC ethyl ester supplement on blood levels of glutathione, inflammatory measures, and toxicant status. Most importantly, this prospective human trial included assessing a broad range of related quality of life (QOL) factors and symptoms to demon-

strate an “experiential” perspective. Such insights into personal impact are highly valuable for the potential consumer audience.

3. Materials and Methods

The trial group consisted of 19 healthy adult participants of all genders (5 males, 14 females), with ages ranging from 43 to 72 years (mean age 54.4). The research protocol excluded vulnerable populations from this investigation. Before enrolling anyone, the researchers screened potential subjects for current medication or supplement use and conducted clinical and safety assessments. Exclusion criteria included a personal history of neurological, cardiometabolic, or oncological disease as well as the use of medications that could interfere with blood testing results. Seven individuals were excluded from consideration due to one or more of these factors.

The remaining eligible participants were prospectively and randomly selected for inclusion into the study. Because of the open-label design of the trial and to ensure consistency, the subjects were counseled to maintain stable nutrition, medications or supplements, lifestyle habits, exercise regimens, and working environments. The Institutional Review Board of the Colorado Center for Health and Sports Science reviewed and approved the trial’s background, schema, risk profile, and range of potential outcomes. All participants acknowledged their understanding of the study parameters, risks, requirements, and potential benefits, and provided written informed consent.

The study supplement employed in this investigation was Neuro-NAC™ (Nutri by Nature’s Fusions, LLC; Orem, UT). This proprietary trademarked complex provides a 2,175 mg combination of glycine and NAC ethyl ester (Figure 1). It also contains the Daily Value ranges for the mineral co-factors selenium and molybdenum, further supporting the desired enzymatic reactions. The recommended daily dosage, delivered in three capsules, could be taken with food in a single morning or evening dose or in two or three divided doses throughout the day. The participants consumed the study supplement for the entire eight weeks of the trial.

At the start of the trial, subjects completed a lifestyle rating survey that examined their observations regarding 12 parameters of their daily lives (Table 1). The components of this hybrid instrument were derived from factors highlighted in previously published and validated scales, assessments, questionnaires, and surveys (38-41). Broad descriptors were provided, and each participant could select the description that was most closely related to their current situation. A five-point Likert rating score (1 to 5, worst to best) was then constructed from their responses and was used to indicate everyone’s baseline QOL status prior to consuming the study supplement (42). Each subject completed a second iteration of the same survey at the termination of the trial. In this system, a rating of “3” designates an average response, with higher numbers indicating better metrics and lower numbers being worse appraisals. The raw scores for each item and the composite mean values were compared between the baseline and final measurements and analyzed statistically.

One additional parameter, nutritional status, was monitored only to ensure that there were no marked changes in dietary intake. This factor was not included in the composite data analysis.To further enhance compliance with the trial conditions, each subject was also contacted weekly. This conversation provided a platform for continuous serial subjective observations regarding changes in QOL factors as related to six dimensions. These factors were selected from the initial full 12-question survey, and included energy, mood, cognition, body condition, sleep, and stress (Table 2). The Likert scale “1-5” rating method was maintained for these interim checkpoints, and the statistical analysis followed the same approach as for the baseline and final surveys. Significant outcomes or notable trends were determined by employing a two-tailed Student’s t-test.Besides the lifestyle survey, each subject underwent evaluation of selected blood testing at the beginning of the trial and end of the eight-week supplement consumption period. The water-soluble endogenous compound, glutathione, reflects the oxidative stress status and total antioxidant capacity of the individual. Given this central role, measuring its levels seemed a logical first step in the analytic platform of this trial, despite emerging evidence of new counter associations for its oxidant and antioxidant function (43,44). Total glutathione in whole blood was determined with these ambivalent implications in mind (BioAgilytix Diagnostics; Boston, MA). The analysis employed thiol-scavenging agents and spectrophotometric techniques using a validated colorimetric detection process (ImmunoChemistry Technologies, LLC; Davis, CA). Gamma-glutamyl transferase (GGT) metabolizes glutathione and has been associated with the risk of liver disease, heart disease, diabetes, stroke, and alcohol intake [45,46). However, its physiological role in oxidative stress has led to its recognition as an indicator of toxicant, pollutant, and xenobiotic burden (47-49). In parallel, biomarkers of inflammation are related to oxidative damage. Immune cells release pro-inflammatory cytokines during the response to injury. Among these, tumor necrosis factor alpha (TNF-α), produced by macrophages and natural killer cells, plays a key role as both a critical immune-stimulant and inflammatory response mediator (50,51). Substantial research has further established its importance in chronic diseases, including neurological conditions (52,53).

Scientific researchers have long described C-reactive protein (CRP) as an acute-phase protein synthesized by liver cells. It represents a non-specific physiological and biochemical response to pathological stimuli. However, the short-lived and temporary nature of its blood levels and lack of specificity limit its usefulness in clinical practice (54). Nevertheless, it may provide some value in combination with other markers. GGT, TNF-α, and CRP were determined utilizing spectrophotometry and immunoassay techniques (Quest Diagnostics; St. Louis, MO). Blood samples were collected at baseline and after the eight-week supplement period. Statistical analysis of these biomarkers followed the same methodology used for evaluating the “experiential” lifestyle measures.

Figure 1: Study Supplement.

Table 1: Full Quality of Life Survey – Baseline and Final Assessment.

1	General Fatigue	7	Body Tissue Condition
2	Breathing Ability/Function	8	General Health/Well-Being
3	Memory, Focus, Brain "Fog"	9	Gastric Distress
4	Overall Energy Level	10	Stress/Life Event Management
5	General Mood/Outlook	11	Exercise Performance/Recovery
6	Quality of Sleep	12	Joint/Muscle Soreness/Inflammation

4. Results

Eighteen participants (5 males, 13 females) successfully completed the entire eight weeks of product consumption and all survey and blood testing. One subject withdrew from the study due to an unrelated personal event that prevented completion of the final assessments. At her request, she was excluded from the trial data set. Two further participants completed the final QOL survey but left the study site for an extended time and could not provide the final blood sampling. Thus, they were included in the "experiential" data set but not in the blood test analysis, leaving 16 evaluable subjects in this latter "objective" analysis section.

Figure 2 depicts the baseline and final Likert Scale ratings for the weekly analysis of the six highlighted lifestyle factors. The mean composite score significantly increased from the initial "3" value to 3.81 ($p<0.0001$). All individual parameters improved in a similar manner, with cognition and sleep pattern being the most positively impacted (Figure 3). Significant QOL factor changes were noted as early as three to four weeks after trial onset, and these perceived benefits were sustained throughout the study.

The mean comprehensive 12-question QOL survey (omitting nutritional status) improved in composite rating from a score of 3.36 to 4.18, a 24 percent increase. This was highly statistically significant at the $p<0.0001$ level (Figure 2). Assessments of individual factors also uniformly and significantly increased by 0.18 points to 1.54 points (Table 3). The factors that appeared to be most improved were related to fatigue, body tissue status, gastric distress, and inflammation. No adverse effects were observed or reported except in one subject who experienced temporary diarrhea.The blood test outcomes varied depending on which analyte was considered. The initial total glutathione levels ranged from 513 to 965 µM (mean 698 µM). The mean composite value at week 8 showed an improving trend with an eight percent increase to 751 µM. The raw values increased in ten subjects, were unchanged in one, and decreased in five. Initial TNF-α levels ranged from 0.33 to 1.03 pg/mL (mean 0.76 pg/mL). An equal number of participants decreased and increased their levels. However, the mean composite levels also showed some benefit

with a seven percent decrease to 0.71 pg/mL. The initial GGT levels were all low and in the normal range, from 9 to 36 U/L (mean 16 U/L), representing a healthy group with minimal toxicant burden. Accordingly, the final mean composite levels were essentially unchanged. The raw values decreased in eight subjects, were unchanged in two, and increased in six. Similarly, the initial CRP values were also all low and in the normal range (< 3.0 mg/L) and remained stable at this level throughout the trial.

NAC supplementation is associated with blood marker improvement in older age and higher oxidative stress or inflammation status. To assess these effects of age, subgroup analyses were undertaken for the two biomarkers, glutathione and TNF-α, that improved in the overall group (Table 4). In those subjects 54 years of age and older, the mean glutathione value significantly increased from 673 to 734.7 µM ($p<0.05$). For this same cohort, the mean TNF-α significantly decreased from 0.821 to 0.725 pg/mL ($p<0.05$). In addition, those with lower (<698 µM) initial glutathione levels represented a cohort that had higher baseline oxidative stress. For this subgroup, the mean glutathione values also trended beneficially and increased from 612.6 to 720.9 µM, an 18 percent improvement.

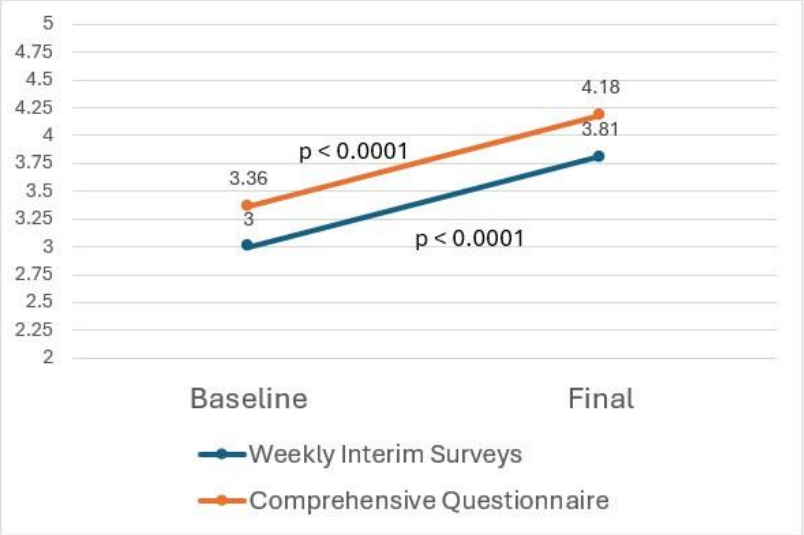

Figure 2: Mean Composite Ratings-Weekly and Comprehensive QOL Surveys.

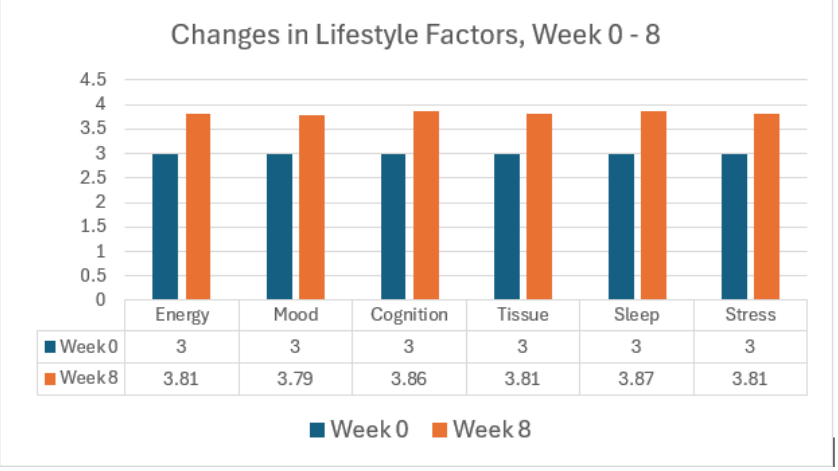

Figure 3: Mean Individual Factor Rating - Weekly Interim Surveys.

Table 2: Weekly Likert Scale Assessment – Key Lifestyle Domains.

1	Energy: related to activities, work, exercise, recreation
2	Feeling of Well-Being, Mood
3	Cognitive Status, Memory, Focus
4	Condition of hair, skin, nails; Pain/Inflammation in joints, muscles
5	Sleep Pattern, Fatigue Level
6	Stress Level, Illness

Numerical rating is based on weekly status since the study onset, when the participant began consuming the supplement. The value can be a fraction in decimal form, such as 2.75 or 3.5.

Table 3: Mean Outcome Scores for Comprehensive QOL Survey.

1	Poor or markedly worse
2	Below average or somewhat worse
3	Average or about the same
4	Good or somewhat better
5	Great or markedly better

Table 4: Mean Biomarker Changes in Older Subjects.

Question	Baseline	Week 8	Question	Baseline	Week 8
Fatigue	2.77	4.26	Body Tissue	3.28	4.82
Breathing	4.11	4.29	Well Being	3.72	4.29
Cognition	3.39	4	Stomach Issues	2.83	4.29
Energy	3.28	4.11	Stress	3.61	4.06
Mood	3.61	4	Exercise	3.89	4.41
Sleep	3.22	3.88	Inflammation	2.61	3.76

	Glutathione (µM)		TNF-α (pg/mL)	
Subject	Week 0	Week 8	Week 0	Week 8
1	735	735	1.03	0.87
2	615	620	0.97	0.82
3	591	670	0.88	0.72
4	697	769	0.68	0.74
5	513	637	0.99	0.62
6	836	792	0.79	0.85
7	645	858	0.56	0.54
8	686	717	0.58	0.62
9	739	814	0.91	0.75
MEAN	673	734.7	0.821	0.725

5. Discussion

This prospective pilot observational trial is among the few human studies to describe the impact of a proprietary glycine and NAC-ethyl ester complex on both participant subjective experience and blood biomarkers. It is also the first clinical trial to serially monitor a comprehensive panel of QOL factors with repeated assessments throughout the study period. These observations hold meaningful potential clinical relevance across a broad adult age range and support reasonable generalizability of the results. This investigation was a limited eight-week experience. Nevertheless, the outcomes demonstrated highly significant "experiential" improvement in QOL measures and positive trends in biomarker changes, especially significant among older subjects. Those with lower initial glutathione levels also received some benefit.

Scientific research has long focused on identifying natural products capable of positively influencing antioxidant, inflammatory, and toxicant status. NAC was one of the most prominent amino acids to exhibit this promise, but its clinical results have been inconsistent [25]. Esterification of NAC, with the addition of glycine, has been shown to enhance bioavailability and efficacy, providing the rationale for the current study [26,33]. The positive lifestyle outcomes observed were satisfying and supported this approach, while the blood biomarker results warrant more nuanced interpretation.

A notable strength of this trial was the comprehensive scope of the QOL evaluations, which were conducted in two distinct formats. Initially, an extensive hybrid questionnaire was administered at both baseline and study completion, assessing 12 dimensions of daily life. This instrument was derived from several previously validated QOL surveys. Participants responded utilizing a 5-point Likert Scale, allowing for uniform comparisons of ratings from the beginning to the end of the trial. Final analysis showed a highly statistically significant improvement in composite scores at the $p<0.0001$ level, indicating a clinically meaningful positive effect. It was critical that each subject maintained their pre-study general lifestyle, nutrition status, activity routine, and exercise regimen. Thus, the noted benefits could be ascribed to the supplementation and not to other external factors.

These findings were further enhanced by the second set of evaluations. Each participant was interviewed weekly and queried on six specific factors that were derived from the main survey. This approach also allowed monitoring of any changes related to other external factors. Individual and composite scores tabulated from these interval assessments reinforced the overall comprehensive survey, lending more robustness to the conclusions.

Each individual measure improved, and the composite rating was also highly statistically significant at the $p<0.0001$ level. Of interest, the interim analysis noted that many of the subjects reported a perceivable enhancement in QOL factors quite early in the trial, as soon as the three-to-four-week mark. It was also particularly relevant that these improvements were sustained throughout the duration of the study.

Considering the positive "experiential" findings, the blood biomarkers offered notable parallels and yet some contrasts. These apparent distinctions require careful consideration. All sampling was performed through Quest Diagnostics Laboratories and its collaborative facilities. This entity is federally licensed and certified by the Clinical Laboratory Improvement Amendments (CLIA) program of the Centers for Medicare & Medicaid Services, the agency that regulates all laboratory testing performed on individuals in the U.S. The CLIA certification ensures consistent standardized procedures, reliable result accuracy and minimizes any confounding technical variability.

The participant group was generally healthy, exhibiting robust baseline total glutathione levels with a mean composite value of 698 μM. Nevertheless, the levels still increased in most subjects, and in the five whose levels did not increase, the QOL scores still all improved. This is notable since previous research shows that glutathione levels may not uniformly change with supplementation, although older individuals and those with oxidative stress generally derive more benefit (36). Decreases in the inflammatory cytokine TNF-α followed a similar pattern. Seven of the nine subjects whose levels did not decrease still reported improved QOL scores. The measurement of GGT was likewise mixed with no overall increase or reduction in toxicant status. CRP, consistent with its role as a highly variable and non-specific acute phase protein, contributed little to the biomarker panel. Its individual and composite mean values remained essentially unchanged over the eight-week interval.

In concert with previous reports, the blood glutathione and inflammatory marker value of the supplementation regimen was most appreciated in the older participants. Only one such subject failed to improve their glutathione level, while the overall cohort mean level statistically significantly increased. Similarly, the mean TNF-α level for this subgroup also improved, significantly decreasing overall. Likewise, in the cohort whose initial glutathione level was below the groupwide mean, the values for this biomarker also demonstrated a strong improvement trend.

Several important considerations emerged from this trial. The two-month "real world" study environment, where life events such as unrelated illness, unexpected injury, job change, and personal loss occurred, only enhanced the generalizability of the results. Nevertheless, these events did not alter the overall compliance of the study cohort in terms of consuming the supplement or completing all interval assessments and blood samplings. The benefits of personal connection with all participants weekly clearly served to enhance the interest and commitment of the subjects. This is particularly critical in decentralized trials performed outside of a controlled setting (55,56). This success was facilitated by 1) prospective, randomized recruitment, 2) employing a credible therapeutic intervention, 3) ensuring consistent lifestyle/nutrition, and 4) collecting and evaluating relevant data.

While the trial outcomes were generally positive, the study had some limitations. There was a relatively modest number of eligible subjects, and the intervention exposure period was only eight weeks. While these factors may engender some doubts about the findings, the breadth of beneficial results may be considered compelling. The open-label design of the trial without a placebo group may also present potential concerns. However, considerable efforts were taken to mitigate the placebo and Hawthorne effects in the subjective data collection [57,58]. All participants were personally and frequently interviewed and monitored. This was employed to ascertain supplement consumption compliance and diligent QOL parameter assessments. Life events were recorded but did not change any of the study conditions. Future investigations of longer duration with larger subject numbers and a placebo cohort may be warranted to further validate and more broadly generalize the current findings.

6. Conclusion

The proprietary glycine and N-acetyl cysteine ethyl ester supplement highly significantly improved comprehensive quality of life factors after an 8-week pilot trial. The study formulation also significantly improved these parameters in a serial manner during weekly interim surveys. Benefits were perceived as early as three to four weeks after supplementation and were sustained throughout the study. Overall, glutathione levels increased, and tumor necrosis factor-alpha levels decreased, and these improvements achieved statistical significance in the subgroup of older individuals. No serious adverse effects were encountered during the trial.

7. Acknowledgements

The authors are fully responsible for the content of the manuscript. GMH and NEW provided the overall study design, subject compliance, data assembly and analysis, and final editing. JAC and AWH contributed initial research background, subject recruitment, and editorial review. The sponsoring entity, Nature's Fusions LLC (NF), provided study products and logistical support. Quest Diagnostics Laboratories (QDL) provided blood testing support. NF and QDL had no role in study design or execution, data collection or analysis, or manuscript preparation.

References

1. Sekhar RV, Patel SG, Guthikonda AP. Deficient synthesis of glutathione underlies oxidative stress in aging and can be corrected by dietary cysteine and glycine supplementation. Am J Clin Nutr. 2011; 94(3): 847-853.
2. Dwivedi D, Megha K, Mishra R. Glutathione in brain: overview of its conformations, functions, biochemical characteristics, quantitation and potential role in brain disorders. Neurochem Res. 2020; 45(7): 1461-1480.
3. Singh RJ. Glutathione: a marker and antioxidant for aging. J Lab Clin Med. 2002; 140(6): 380-381.
4. Jomova K, Vondrakova D, Lawson M. Metals, oxidative stress and neurodegenerative disorders. Mol Cell Biochem. 2010; 345(1-2): 91-104.
5. Pocernich CB, Bader Lange ML, Sultana R. Nutritional approaches to modulate oxidative stress in Alzheimer's disease. Curr Alzheimer Res. 2011; 8(5): 452-469.
6. Pocernich CB, Butterfield DA. Elevation of glutathione as a therapeutic strategy in Alzheimer disease. Biochim Biophys Acta. 2012; 1822(5): 625-630.
7. Sekhar RV, McKay SV, Patel SG. Glutathione synthesis is diminished in patients with uncontrolled diabetes and restored by dietary supplementation with cysteine and glycine. Diabetes Care. 2011; 34(1): 162-167.
8. Tuell D, Ford G, Los E. The role of glutathione and its precursors in type 2 diabetes. Antioxidants (Basel). 2024; 13(2): 184.
9. Franco R, Schoneveld OJ, Pappa A. The central role of glutathione in the pathophysiology of human diseases. Arch Physiol Biochem. 2007; 113(4-5): 234-258.
10. Lu SC. Regulation of glutathione synthesis. Mol Aspects Med. 2009; 30(1-2): 42-59.
11. Owen JB, Butterfield DA. Measurement of oxidized/reduced glutathione ratio. Methods Mol Biol. 2010; 648: 269-277.
12. Forman HJ, Zhang H, Rinna A. Glutathione: overview of its protective roles, measurements, and biosynthesis. Mol Aspects Med. 2009; 30(1-2): 1-12.
13. Zhang H, Forman HJ. Glutathione synthesis and its role in redox signaling. Semin Cell Dev Biol. 2012;23(7):722-728.
14. Lu SC. Glutathione synthesis. Biochim Biophys Acta. 2013; 1830(5): 3143-3153.
15. Morris G, Anderson G, Dean O. The glutathione system: a new drug target in neuroimmune disorders. Mol Neurobiol. 2014; 50(3): 1059-1084.
16. Averill-Bates DA. The antioxidant glutathione. Vitam Horm. 2023; 121: 109-141.
17. Aoyama K, Nakaki T. Impaired glutathione synthesis in neurodegeneration. Int J Mol Sci. 2013; 14(10): 21021-21044.
18. Gasmi A, Nasreen A, Lenchyk L. An update on glutathione's biosynthesis, metabolism, functions, and medicinal purposes. Curr Med Chem. 2024; 31(29): 4579-4601.
19. Gu F, Chauhan V, Chauhan A. Glutathione redox imbalance in brain disorders. Curr Opin Clin Nutr Metab Care. 2015; 18(1): 89-95.
20. Perez LM, Hooshmand B, Mangialasche F. Glutathione serum levels and rate of multimorbidity development in older adults. J Gerontol A Biol Med Sci. 2020; 75(6): 1089-1094.
21. James SJ, Slikker 3rd W, Melnyk S. Thimerosal neurotoxicity is associated with glutathione depletion: protection with glutathione precursors. Neurotoxicology. 2005; 26(1): 1-8.
22. Giustarini D, Milzani A, Dalle-Donne I. How to increase cellular glutathione. Antioxidants (Basel). 2023; 12(5): 1094.
23. Wu G, Fang Y-Z, Yang S. Glutathione metabolism and its implications for health. J Nutr. 2004; 134(3): 489-492.
24. Lapenna D. Glutathione and glutathione-dependent enzymes: from biochemistry to gerontology and successful aging. Ageing Res Rev. 2023; 92: 102066.
25. Rushworth GF, Megson IL. Existing and potential therapeutic uses for N-acetylcysteine: the need for conversion to intracellular glutathione for antioxidant benefits. Pharmacol Ther. 2014; 141(2): 150-159.
26. Giustarini D, Milzani A, Dalle-Donne I. N-acetylcysteine ethyl ester (NACET): a novel lipophilic cell-permeable cysteine derivative with an unusual pharmacokinetic feature and remarkable antioxidant potential. Biochem Pharmacol. 2012; 84(11): 1522-1533.
27. Tsikas D, Schwedhelm KS, Surdacki A. S-nitroso-N-acetyl-L-cysteine ethyl ester (SNACET) and N-acetyl-L-cysteine ethyl ester (NACET)-cysteine-based drug candidates with unique pharmacological profiles for oral use as NO, H2S and GSH suppliers and as antioxidants: results and overview. J Pharm Anal. 2018; 8(1): 1-9.
28. Uemura T, Watanabe K, Ko K. Protective effects of brain infarction by N-acetylcysteine derivatives. Stroke. 2018; 49(7): 1727-1733.
29. Giustarini D, Galvagni F, Dalle Donne I. N-acetyl cysteine ethyl ester as GSH enhancer in human primary endothelial cells; a comparative study with other drugs. Free Radic Biol Med. 2018; 126: 202-209.
30. Kularatne RN, Bulumulla C, Catchpole T. Protection of human retinal pigment epithelial cells from oxidative damage using cysteine prodrugs. Free Radic Biol Med. 2020; 152: 386-394.
31. Tosi GM, Giustarini D, Franci L. Superior properties of N-acetylcysteine ethyl ester over N-acetylcysteine to prevent retinal pigment epithelial cells oxidative damage. Int J Mol Sci. 2021; 22(2): 600.
32. Kumar P, Osahon OW, Sekhar RV. GlyNAC (glycine and N-acetylcysteine) supplementation in mice increases length of life by correcting glutathione deficiency, oxidative stress, mitochondrial dysfunction, abnormalities in mitophagy and nutrient sensing, and genomic damage. Nutrients. 2022; 14(5): 1114.
33. Sekhar RV. GlyNAC supplementation improves glutathione deficiency, oxidative stress, mitochondrial dysfunction, inflammation, aging hallmarks, metabolic defects, muscle strength, cognitive decline, and body composition: implications for healthy aging. J Nutr. 2021; 151(12): 3606-3616.
34. Sekhar RV. GlyNAC (glycine and N-acetylcysteine) supplementation improves impaired mitochondrial fuel oxidation and lowers insulin resistance in patients with type 2 diabetes: results of a pilot study. Antioxidants (Basel). 2022; 11(1): 154.

35. Kumar P, Liu C, Hsu JW. Glycine and N-acetylcysteine (GlyNAC) supplementation in older adults improves glutathione deficiency, oxidative stress, mitochondrial dysfunction, inflammation, insulin resistance, endothelial dysfunction, genotoxicity, muscle strength, and cognition: results of a pilot clinical trial. Clin Transl Med. 2021; 11(3): e372.
36. Lizzo G, Migliavacca E, Lamers D. A randomized controlled clinical trial in healthy older adults to determine efficacy of glycine and N-acetylcysteine supplementation on glutathione redox status and oxidative damage. Front Aging. 2022; 3: 852569.
37. Kumar P, Liu C, Suliburk J. Supplementing glycine and N-acetylcysteine (GlyNAC) in older adults improves glutathione deficiency, oxidative stress, mitochondrial dysfunction, inflammation, physical function, and aging hallmarks: a randomized clinical trial. J Gerontol A Biol Sci Med Sci. 2023; 78(1): 75-89.
38. Ware Jr JE. Standards for validating health measures: definition and content. J Chron Dis. 1987; (6): 473-480.
39. Dijkers MP. Individualization in quality of life measurement: instruments and approaches. Arch Phys Med Rehabil. 2003; 84(4 Suppl 2): S3-S14.
40. Rosen SI, Reuben DB. Geriatric assessment tools. Mt Sinai J Med 2011; 78(4): 489-497.
41. Klaus D, Engstler H, Mahne K. Cohort profile: the German ageing survey (DEAS). Int J Epidemiol. 2017; 46(4): 1105-1105g.
42. Sullivan GM, Artino Jr AR. Analyzing and interpreting data from Likert-like scales. J Grad Med Educ. 2013; 5(4): 541-542.
43. Pompella A, Visvikis A, Paolicchi A. The changing face of glutathione, a cellular protagonist. Biochem Pharmacol. 2003; 66(8): 1499-1503.
44. Ali SS, Ahsan H, Zia MK. Understanding oxidants and antioxidants: classical team with new players. J Food Biochem. 2020; 44(3): e13145.
45. Whitfield JB. Gamma glutamyl transferase. Crit Rev Clin Lab Sci. 2001; 38(4): 263-355.
46. Lee D-H, Blomhoff R, Jacobs Jr DR, Is serum gamma glutamyltransferase a marker of oxidative damage? Free Radic Res. 2004; 38(6): 535-539.
47. Lee D-H, Jacobs Jr DR. Is serum gamma-glutamyltransferase a marker of exposure to various environmental pollutants? Free Radic Res. 2009; 43(6): 533-537.
48. Lee D-H, Jacobs Jr DR. Serum gamma-glutamyltransferase: new insights about an old enzyme. J Epidemiol Community Health. 2009; 63(11): 884-886.
49. Brennan PN, Dillon JF, Tapper EB. Gamma-glutamyl transferase (gamma-GT) – an old dog with new tricks? Liver Int. 2022; 42(1): 9-15.
50. Peristeris P, Clark BD, Gatti S. N-acetylcysteine and glutathione as inhibitors of tumor necrosis factor production. Cell Immunol. 1992; 140(2): 390-399.
51. Wang J-Y, Wen L-L, Huang Y-N. Dual effects of antioxidants in neurodegeneration: direct neuroprotection against oxidative stress and indirect protection via suppression of glia-mediated inflammation. Curr Pharm Des. 2006; 12(27): 3521-3533.
52. Clark IA. How TNF was recognized as a key mechanism of disease. Cytokine Growth Factor Rev. 2007 Jun-; 18(3-4): 335-343.
53. Zindler E, Zipp F. Neuronal injury in chronic CNS inflammation. Best Pract Res Clin Anaesthesiol. 2010; 24(4): 551-562.
54. Pepys MB, Hirschfield GM. C-reactive protein: a critical update. J Clin Invest. 2003; 111(12): 1805-1812.
55. Hernandez AF, Lindsell CJ. Ensuring virtual vigilance in decentralized clinical trials. JAMA. 2025; 333(2): 119-120.
56. Chen J, Di J, Daizadeh N. Decentralized clinical trials in the era of real-world evidence: a statistical perspective. Clin Transl Sci. 2025; 18(2): e70117.
57. Annoni M. Better than nothing: a historical account of placebos and placebo effects from modern to contemporary medicine. Int Rev Neurobiol. 2020; 153: 3-26.
58. Wickstrom G, Bendix T. The “Hawthorne effect” – what did the original Hawthorne studies actually show? Scand J Work Environ Health. 2000; 26(4): 363-367.