

RESEARCH MONOGRAPH . COMPOUND PROFILE

GlyNAC

A defined combination of glycine and N-acetylcysteine developed at Baylor College of Medicine as a glutathione-replenishing intervention targeting age-associated oxidative stress, mitochondrial dysfunction, and multiple hallmarks of biological aging.

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AUTHOR	Kodi <i>ac</i> bio <i>l</i> abs Research
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ABSTRACT

GlyNAC is a defined equimolar combination of glycine and N-acetylcysteine (NAC) that supplies the two amino acid precursors whose availability limits the intracellular biosynthesis of glutathione (gamma-glutamylcysteinylglycine, GSH), the most abundant endogenous non-protein thiol antioxidant in mammalian cells. The combination was developed and systematically investigated by Rajagopal V. Sekhar and colleagues at Baylor College of Medicine beginning approximately 2018, on the basis of prior observations that aging, HIV infection, and type 2 diabetes are associated with deficient intracellular glutathione concentrations traceable to diminished availability of both glycine and cysteine rather than to reduced activity of the synthetic enzymes glutamate-cysteine ligase and glutathione synthetase. The mechanistic rationale is that NAC undergoes deacetylation to cysteine in the intestinal mucosa and liver, while supplemental glycine directly enters the glutathione synthetic pathway; co-administration of both precursors at physiologically relevant doses corrects the dual substrate deficit and restores intracellular glutathione concentrations within two weeks in human subjects, with downstream improvements in oxidative stress biomarkers, mitochondrial fatty-acid oxidation, inflammation, insulin resistance, endothelial function, genomic integrity, and cellular senescence markers.

The principal clinical evidence derives from a series of trials conducted at Baylor College of Medicine. A 36-week open-label pilot trial in older adults (aged 61 to 80 years) demonstrated that 24 weeks of GlyNAC supplementation at approximately 100

milligrams per kilogram per day of each component (approximately 7 grams of glycine and 7 grams of NAC daily for a 70-kilogram adult) corrected red blood cell glutathione deficiency, reduced plasma markers of oxidative stress (thiobarbituric acid reactive substances, F2-isoprostanes), improved mitochondrial fatty-acid oxidation by indirect calorimetry, lowered fasting insulin resistance (homeostatic model assessment of insulin resistance), improved grip strength and gait speed, and improved cognitive scores on the Montreal Cognitive Assessment; benefits receded within 12 weeks of supplementation withdrawal. A subsequent double-blind, placebo-controlled randomized clinical trial in 24 older adults (aged 65 to 80 years) confirmed these findings over 16 weeks of supplementation, with additional demonstration of improvements in multiple hallmarks of aging including mitochondrial dysfunction, impaired mitophagy, inflammation, endothelial dysfunction, genomic damage, stem cell fatigue, and cellular senescence. An independent dose-finding randomized controlled trial conducted by the Nestle Institute of Health Sciences in 114 healthy older adults tested three dose tiers (2.4, 4.8, and 7.2 grams per day total in a 1:1 glycine-to-NAC ratio) over two weeks and confirmed dose-dependent increases in plasma glycine and cysteine concentrations and in erythrocyte glutathione.

Parallel investigations have extended the GlyNAC evidence base to HIV-infected adults (open-label, improvements in oxidative stress, mitochondrial function, inflammation, endothelial function, insulin resistance, strength, and cognition), type 2 diabetes (pilot study, 30 percent improvement in mitochondrial fatty-acid oxidation and 22 percent reduction in insulin resistance over 14 days), and COVID-19 (observational characterization of severe glutathione deficiency and oxidative stress in hospitalized

patients). A preclinical mouse lifespan study demonstrated that GlyNAC supplementation initiated at 65 weeks of age (corresponding to late middle age in humans) extended median lifespan by 24 percent and corrected glutathione deficiency, oxidative stress, mitochondrial dysfunction, impaired mitophagy, abnormal nutrient sensing, and genomic damage in liver, heart, and kidney tissues.

The combination is well tolerated in all published trials. No serious adverse events attributable to GlyNAC have been reported. The principal mild adverse events are gastrointestinal (nausea, bloating), attributable to the NAC component, and are dose-dependent and generally self-limiting. The glycine component has an extensive safety record as a dietary amino acid and as a research compound at doses up to 60 grams per day in schizophrenia trials. NAC has an established clinical safety profile as a mucolytic and acetaminophen-overdose antidote at doses substantially exceeding those used in GlyNAC research.

This monograph documents the composition, molecular pharmacology, and glutathione biosynthetic rationale of GlyNAC; the pharmacokinetics of the individual components; the complete preclinical and clinical evidence base across aging, HIV, diabetes, and COVID-19 indications; sourcing and quality verification; reconstitution and handling; stack interactions; the adverse-event profile; and a comparative assessment of five alternative glutathione-replenishing strategies (N-acetylcysteine alone, oral reduced glutathione, liposomal glutathione, alpha-lipoic acid, and whey protein concentrate) against GlyNAC on five competency standards (mechanistic completeness, effect size, clinical validation, side-effect profile, and practical accessibility).

1. Compound Identification

1.1 Names, synonyms, and registry identifiers

Designation: GlyNAC. The name is a portmanteau of the two constituent amino acids: glycine (Gly) and N-acetylcysteine (NAC). The designation was introduced by Sekhar and colleagues in the Baylor College of Medicine research program and has become the standard identifier in the published literature and in commercial supplement formulations [1, 2].

Component 1: Glycine. International Nonproprietary Name: Glycine. Chemical Abstracts Service registry number: 56-40-6. International Union of Pure and Applied Chemistry name: 2-aminoacetic acid. Molecular formula: $C_2H_5NO_2$. Molecular weight: 75.03 g/mol. Glycine is the simplest and smallest of the 20 proteinogenic amino acids, distinguished by a hydrogen atom as its sole side chain, rendering it the only common amino acid that is achiral [3].

Component 2: N-Acetylcysteine. International Nonproprietary Name: Acetylcysteine. Chemical Abstracts Service registry number: 616-91-1. International Union of Pure and Applied Chemistry name: (2R)-2-acetamido-3-sulfanylpropanoic acid. Molecular formula: $C_5H_9NO_3S$. Molecular weight: 163.19 g/mol. NAC is the N-acetylated derivative of the amino acid L-cysteine, containing a free sulfhydryl group that confers both its mucolytic activity and its role as a cysteine prodrug for glutathione synthesis [4].

Target product: Glutathione (GSH). Chemical Abstracts Service registry number: 70-18-8. Molecular formula: $C_{10}H_{17}N_3O_6S$. Molecular weight: 307.32 g/mol. Glutathione is the tripeptide gamma-L-glutamyl-L-cysteinyl-glycine, the most abundant low-molecular-weight thiol in mammalian cells, present at intracellular concentrations of 1 to 10 millimolar. It is synthesized in two ATP-dependent steps by glutamate-cysteine ligase (the rate-limiting enzyme) and glutathione synthetase [5].

1.2 Structural class and chemistry

GlyNAC is not a single molecular entity but a defined binary combination of two amino acid species administered simultaneously to provide both rate-limiting substrates for glutathione biosynthesis. The structural class is therefore that of a sub-

strate-replenishment strategy targeting the gamma-glutamyl cycle. The combination exploits the observation that intracellular glutathione deficiency in aging and in several disease states is attributable to diminished availability of both glycine and cysteine (the latter supplied as its acetylated prodrug NAC), rather than to impaired activity of the synthetic enzymes [1, 6].

Glycine is a white crystalline powder, freely soluble in water (25 g/100 mL at 25 degrees Celsius), with a sweet taste and a zwitterionic structure at physiological pH. N-Acetylcysteine is a white to slightly off-white crystalline powder with a characteristic sulfurous odor, freely soluble in water and in ethanol, with a pKa of approximately 9.5 for the sulfhydryl group.

1.3 Synthesis and manufacture

Both components are manufactured independently and combined at the formulation stage. Glycine is produced industrially by the Strecker synthesis (reaction of formaldehyde with ammonium chloride and hydrogen cyanide, followed by hydrolysis) or by the ammonolysis of chloroacetic acid; pharmaceutical-grade glycine is available at greater than 99 percent purity. N-Acetylcysteine is synthesized by acetylation of L-cysteine with acetic anhydride or acetyl chloride under controlled conditions; pharmaceutical-grade NAC is manufactured under current Good Manufacturing Practice and is available at greater than 98 percent purity by high-performance liquid chromatography [4]. Commercial GlyNAC supplements combine the two powders in a 1:1 mass ratio or in equimolar ratio in capsule or powder form. The Baylor College of Medicine clinical trials used individually weighed capsules of each component, not a fixed-dose combination product [1, 2].

2. Abstract

GlyNAC (glycine and N-acetylcysteine) is a combination amino acid supplement that provides the two precursor substrates whose co-deficiency limits intracellular glutathione biosynthesis in aging and in multiple chronic disease states. The combination was developed by Rajagopal V. Sekhar at Baylor College of Medicine on the basis of metabolic tracer studies demonstrating that older adults exhibit simultaneously reduced fractional synthesis rates of glutathione attributable to diminished availability of both glycine and cysteine, and that supplementation with both precursors (but not either alone) fully restores glutathione synthesis within two weeks [1, 6].

The clinical evidence base, while modest in total sample size, is consistent across multiple populations and study designs. A 36-week open-label pilot trial in older adults demonstrated that 24 weeks of GlyNAC supplementation corrected glutathione deficiency, reduced oxidative stress, improved mitochondrial fatty-acid oxidation, lowered insulin resistance, improved endothelial function, reduced genomic damage markers, and improved grip strength, gait speed, and cognitive scores [1]. A subsequent 16-week double-blind randomized placebo-controlled trial confirmed these findings and additionally demonstrated improvements in markers of mitophagy, cellular senescence, and stem cell fatigue [2]. Benefits receded within 12 weeks of supplementation withdrawal in both trials, consistent with a substrate-dependent mechanism rather than a durable epigenetic or structural modification.

Parallel investigations have demonstrated analogous benefits in HIV-infected adults [7], in patients with type 2 diabetes [8], and in a mouse lifespan study that reported 24 percent median lifespan extension with GlyNAC supplementation initiated at the rodent equivalent of late middle age [9]. An independent dose-finding trial by the Nestle Institute of Health Sciences confirmed dose-dependent glutathione repletion in healthy older adults across three dose tiers [10].

The combination is well tolerated, with no serious adverse events reported in any published trial. The principal mild adverse events are gastrointestinal symptoms (nausea, bloating) associated with the NAC component. Both individual components have extensive human safety records at doses equal to or exceeding those used in GlyNAC research.

3. Discovery and Development History

3.1 Conceptual origin

The GlyNAC combination derives from two converging lines of investigation in the Sekhar laboratory at Baylor College of Medicine. The first was a series of stable-isotope tracer studies beginning approximately 2009 that quantified the fractional synthesis rate of glutathione in erythrocytes of older adults compared to young adults [6, 11]. These studies demonstrated that older adults have significantly lower erythrocyte glutathione concentrations and significantly lower fractional synthesis rates, and that the synthesis deficit was attributable to reduced intracellular availability of both glycine and cysteine (the two amino acid substrates for which supply is rate-limiting in the gamma-glutamyl cycle) rather than to reduced activity of glutamate-cysteine ligase or glutathione synthetase [6].

The second line of investigation was the observation that supplementation with cysteine alone (as NAC) partially corrected the glutathione deficit but did not fully restore synthesis rates to young-adult levels, while supplementation with glycine alone had a smaller but measurable effect. Only the combination of both substrates fully normalized the fractional synthesis rate and the steady-state glutathione concentration [6, 11]. This dual-substrate-deficit model distinguished the GlyNAC approach from prior NAC-only or cysteine-only glutathione repletion strategies.

3.2 Preclinical studies in aged mice

The Sekhar laboratory extended the human tracer findings to an aged-mouse model. In C57BL/6J mice, GlyNAC supplementation in drinking water from 65 weeks of age (the approximate rodent equivalent of late middle age in humans) corrected age-associated glutathione deficiency, oxidative stress, and mitochondrial dysfunction in liver, heart, and kidney [9]. The mice receiving GlyNAC exhibited 24 percent longer median lifespan than unsupplemented age-matched controls. Mechanistic analysis demonstrated correction of impaired mitophagy, abnormal nutrient sensing (mTOR and AMPK pathway markers), and genomic damage (gamma-H2AX foci, 8-hydroxy-2-deoxyguanosine) in all three organs examined [9].

3.3 Pilot clinical trial in older adults

The first human clinical trial of GlyNAC was a 36-week open-label pilot study in 8 older adults (aged 61 to 80 years) and 8 young adults (aged 21 to 40 years) published in 2021 [1]. Older adults received GlyNAC (glycine at 1.33 milligrams per kilogram per day and NAC at 1.33 milligrams per kilogram per day, corresponding to approximately 100 milligrams per kilogram per day of each component, or approximately 7 grams of each for a 70-kilogram individual) for 24 weeks, followed by a 12-week washout period. Outcomes measured at baseline, 24 weeks, and 36 weeks included erythrocyte glutathione, plasma oxidative stress markers, mitochondrial fuel oxidation by indirect calorimetry, fasting insulin and glucose (HOMA-IR), endothelial function by flow-mediated dilation, genomic damage (8-OHdG), body composition by dual-energy X-ray absorptiometry, grip strength, gait speed, and cognitive function (Montreal Cognitive Assessment).

All measured parameters improved significantly after 24 weeks of GlyNAC supplementation. Erythrocyte glutathione concentrations rose from deficient to normal, plasma thiobarbituric acid reactive substances and F2-isoprostanes declined, mitochondrial fatty-acid oxidation increased, HOMA-IR decreased, endothelial function improved, genomic damage markers declined, grip strength and gait speed increased, and Montreal Cognitive Assessment scores improved [1]. After 12 weeks of washout, most parameters returned toward baseline, consistent with a substrate-dependent mechanism.

3.4 Randomized clinical trial in older adults

The pivotal trial was a 16-week double-blind, placebo-controlled randomized clinical trial in 24 older adults (aged 65 to 80 years) published in 2023 [2]. Twelve subjects received GlyNAC at the same dose used in the pilot trial; 12 received isonitrogenous alanine placebo. The trial confirmed the pilot findings and extended the outcome panel to include markers of aging hallmarks: mitophagy (PINK1, Parkin), inflammation (interleukin-6, tumor necrosis factor alpha), cellular senescence (p16, p21), stem cell fatigue (Ki-67), and telomere-related parameters. GlyNAC supplementation produced statistically significant improvements in all primary and secondary outcomes compared to placebo [2].

3.5 Extension to other populations and conditions

Subsequent and parallel studies extended the GlyNAC evidence to HIV-infected adults (open-label trial demonstrating improvements in oxidative stress, mitochondrial function, inflammation, endothelial function, insulin resistance, strength, and cognition; benefits receded after stopping supplementation) [7], to patients with type 2 diabetes (pilot study demonstrating 30 percent improvement in mitochondrial fatty-acid oxidation and 22 percent reduction in insulin resistance over 14 days) [8], and to COVID-19 (observational study documenting severe glutathione deficiency and elevated oxidative stress in hospitalized patients, with implications for GlyNAC supplementation) [12].

3.6 Independent replication

An independent dose-finding randomized controlled trial conducted by the Nestle Institute of Health Sciences in 114 healthy older adults (aged 60 to 85 years) tested three daily GlyNAC doses (2.4, 4.8, and 7.2 grams per day total, in a 1:1 glycine-to-NAC ratio) over two weeks [10]. All three doses produced significant increases in plasma glycine and cysteine and in erythrocyte glutathione, with dose-dependent magnitude. The study confirmed the substrate-repletion mechanism and provided external validation of the Baylor findings.

4. Mechanism of Action

4.1 Glutathione biosynthesis and the dual-substrate deficit

Glutathione is synthesized intracellularly in two sequential ATP-dependent reactions. In the first and rate-limiting step, glutamate-cysteine ligase (also known as gamma-glutamylcysteine synthetase) catalyzes the formation of gamma-glutamylcysteine from L-glutamate and L-cysteine. In the second step, glutathione synthetase adds glycine to gamma-glutamylcysteine to form the complete tripeptide [5]. The availability of L-cysteine is classically considered the principal rate-limiting substrate, and this assumption has driven decades of research on NAC and cysteine-donor interventions for glutathione repletion.

The Sekhar laboratory's stable-isotope tracer studies demonstrated that in older adults, both glycine and cysteine availability are diminished, and that the glycine deficit is an independent and quantitatively significant contributor to reduced glutathione fractional synthesis rate [6, 11]. This dual-substrate deficit model provides the mechanistic rationale for the combination: NAC supplies cysteine (via hepatic and intestinal deacetylation), glycine supplies itself, and the combination corrects both substrate deficits simultaneously, fully restoring glutathione synthesis to young-adult levels within approximately two weeks [6].

4.2 NAC as a cysteine prodrug

N-Acetylcysteine is deacetylated in the intestinal mucosa, in the hepatic first-pass circulation, and in peripheral tissues to yield free L-cysteine. The sulfhydryl group of the released cysteine is the critical functional moiety; it provides the thiol that is incorporated into glutathione and that confers the antioxidant and redox-buffering capacity of the tripeptide. NAC also has direct antioxidant activity through its free sulfhydryl group, though this is quantitatively minor compared to its role as a glutathione precursor at physiological concentrations [4, 13].

4.3 Glycine as a direct substrate and independent effector

Glycine participates in glutathione synthesis as the terminal amino acid added by glutathione synthetase. Beyond this role, glycine has independent anti-inflammatory, cytoprotective, and metabolic effects. Glycine is an inhibitory neurotransmitter (via strychnine-sensitive glycine receptors), an obligatory co-agonist at the N-methyl-D-aspartate (NMDA) receptor, a substrate for heme, purine, creatine, and collagen biosynthesis, and an activator of glycine-gated chloride channels on macrophages and Kupffer cells that suppress calcium-dependent proinflammatory signaling [14, 15]. The anti-inflammatory activity of glycine at macrophage glycine-gated chloride channels is independent of glutathione synthesis and may contribute to the anti-inflammatory effects of GlyNAC beyond what glutathione repletion alone would produce.

4.4 Downstream consequences of glutathione restoration

Restored intracellular glutathione produces multiple downstream effects that correspond to the clinical observations in GlyNAC trials:

Oxidative stress reduction. Glutathione in its reduced form (GSH) is the principal intracellular scavenger of reactive oxygen species, peroxides, and electrophilic metabolites, operating through glutathione peroxidase-catalyzed reduction of hydrogen peroxide and lipid hydroperoxides to water and corresponding alcohols [5]. Restoration of GSH concentrations reduces the steady-state burden of oxidative damage to lipids, proteins, and nucleic acids.

Mitochondrial function. GSH is present at 5 to 10 millimolar concentrations in the mitochondrial matrix (maintained by a dedicated transport system from the cytosol) and is essential for the function of mitochondrial glutathione peroxidase 4, for the maintenance of the electron transport chain, and for the prevention of mitochondrial permeability transition. GSH deficiency impairs mitochondrial fatty-acid beta-oxidation and increases reliance on glucose oxidation; restoration of GSH corrects this fuel-switching phenotype, as demonstrated by indirect calorimetry in GlyNAC trials [1, 2, 8].

Inflammation. Oxidative stress activates the NF-kappaB, NLRP3 inflammasome, and AP-1 proinflammatory transcription programs. Glutathione repletion attenuates this activation. In combination with glycine's independent anti-inflammatory

effects at macrophage chloride channels, GlyNAC supplementation reduces circulating concentrations of interleukin-6, tumor necrosis factor alpha, and C-reactive protein in older adults [1, 2, 14].

Insulin sensitivity. Oxidative stress and mitochondrial dysfunction impair insulin signaling through serine phosphorylation of insulin receptor substrate-1 and through accumulation of diacylglycerol and ceramide intermediates. Correction of the oxidative and mitochondrial deficits restores insulin sensitivity, as reflected in declining HOMA-IR in GlyNAC-supplemented subjects [1, 2, 8].

Genomic integrity. Glutathione is required for the activity of several DNA repair enzymes and protects DNA from oxidative base modifications (principally 8-hydroxy-2-deoxyguanosine). Restoration of glutathione reduces the steady-state burden of genomic damage, as measured by circulating 8-OHdG and by gamma-H2AX foci in the mouse lifespan study [1, 2, 9].

4.5 Effects on aging hallmarks

The 2023 randomized clinical trial additionally measured markers of several recognized hallmarks of aging as defined by Lopez-Otin et al. (2013, 2023) [2, 16]. GlyNAC supplementation improved markers of mitochondrial dysfunction (respiratory chain function), impaired mitophagy (PINK1 and Parkin expression), inflammation (inflammaging cytokines), insulin resistance (nutrient sensing), endothelial dysfunction, genomic damage, stem cell fatigue (Ki-67 proliferative index), and cellular senescence (p16INK4a, p21). The breadth of these improvements across multiple aging hallmarks, and their reversal upon supplementation withdrawal, is consistent with a model in which glutathione deficiency is a proximal node in the aging process that, when corrected, produces cascading improvements across multiple downstream pathways.

5. Pharmacokinetics and Routes of Administration

5.1 Glycine pharmacokinetics

Glycine is rapidly and efficiently absorbed from the gastrointestinal tract via active amino acid transport systems (principally the PAT1/SLC36A1 and GLYT1/SLC6A9 transporters in the intestinal epithelium). Oral bioavailability is high. Plasma concentrations rise within 30 minutes of oral administration and reach peak levels within 1 to 2 hours [17]. The plasma half-life of glycine is dose-dependent, ranging from approximately 0.5 hours at low doses to approximately 4 hours at doses above 5 grams, reflecting saturation of renal reabsorption and metabolic clearance pathways [17]. Glycine is distributed throughout all body compartments, with high concentrations in the central nervous system (where it serves as a neurotransmitter), in the liver (where it participates in conjugation reactions and in glutathione synthesis), and in connective tissues (where it is the most abundant amino acid in collagen).

Glycine is metabolized by the glycine cleavage system (a mitochondrial multienzyme complex that produces carbon dioxide, ammonia, and a methylene group transferred to tetrahydrofolate) and by serine hydroxymethyltransferase (interconversion with serine). It is also consumed in the synthesis of glutathione, creatine, heme, purines, and hippurate (glycine conjugation of benzoic acid). Renal clearance of free glycine occurs by glomerular filtration with extensive tubular reabsorption; at supraphysiological doses, reabsorption saturates and glycine appears in urine [17].

Endogenous glycine production is approximately 3 grams per day from serine and threonine metabolism in liver and kidney; dietary intake from protein averages approximately 3 to 5 grams per day in typical Western diets. GlyNAC supplementation at the Baylor protocol dose of approximately 7 grams per day therefore approximately doubles total daily glycine availability [14].

5.2 N-Acetylcysteine pharmacokinetics

N-Acetylcysteine has low oral bioavailability, estimated at 4 to 10 percent in healthy subjects and 6 to 12 percent in critically ill patients, attributable to extensive first-pass deacetylation in the intestinal mucosa and liver [4, 13, 18]. The deacetylation product, free L-cysteine, is the biologically active species; it enters the systemic cysteine pool and is available for glutathione synthesis, protein incorporation, and taurine/sulfate metabolic pathways.

Following oral administration, reduced NAC reaches peak plasma concentration within approximately 1 to 2 hours. The terminal elimination half-life of intact NAC is approximately 6.25 hours after oral dosing. Total cysteine (free plus protein-bound) plasma concentrations peak later and decline more slowly, reflecting the incorporation of NAC-derived cysteine into plasma proteins and intracellular glutathione [18].

NAC is not a significant substrate or inhibitor of cytochrome P450 enzymes and has minimal drug-drug interaction potential at the metabolic level [4]. The principal metabolic pathways are deacetylation (to cysteine), oxidation (to cystine and mixed disulfides), and sulfate conjugation. The ultimate excretory product is inorganic sulfate, excreted renally.

5.3 Routes of administration

GlyNAC is administered orally in all published clinical trials, as capsules containing individually weighed quantities of glycine and NAC. No intravenous, subcutaneous, or other parenteral GlyNAC formulation has been studied. The individual components (glycine and NAC) are each available in oral, intravenous, and topical formulations for their respective independent clinical applications [4, 14].

5.4 Special populations

Pharmacokinetic data specific to the GlyNAC combination in special populations (pediatric, geriatric, renal impairment, hepatic impairment) have not been published. However, the individual components have well-characterized pharmacokinetics in these populations. NAC clearance is reduced in severe hepatic impairment (the same population in which it is used therapeutically as an acetaminophen-over-

dose antidote). Glycine clearance is reduced in severe renal impairment owing to the renal contribution to glycine catabolism. Dose adjustments in these populations have not been formally studied for the GlyNAC combination.

6. Clinical Evidence Base

6.1 Aging: pilot clinical trial

The foundational human study was an open-label pilot trial (Kumar et al. 2021, Clinical and Translational Medicine) in 8 older adults (aged 61 to 80 years) and 8 young adults (aged 21 to 40 years) [1]. Young adults served as a reference group without intervention. Older adults received GlyNAC at approximately 100 milligrams per kilogram per day of each component for 24 weeks, followed by 12 weeks of washout.

Key outcomes at 24 weeks versus baseline in older adults included: erythrocyte glutathione concentration increased by approximately 200 percent (restoring to young-adult levels); plasma thiobarbituric acid reactive substances decreased by approximately 72 percent; F2-isoprostanes decreased significantly; fasting mitochondrial fatty-acid oxidation (measured by respiratory quotient on indirect calorimetry) improved significantly; HOMA-IR decreased by approximately 56 percent; flow-mediated dilation improved; 8-OHdG (genomic damage marker) decreased; waist circumference decreased; grip strength improved; six-minute walk distance improved; gait speed improved; and Montreal Cognitive Assessment scores improved [1].

After 12 weeks of washout (week 36), most parameters had returned toward or to baseline levels, supporting the interpretation that GlyNAC provides substrate-level correction rather than a permanent structural or epigenetic modification.

6.2 Aging: randomized controlled trial

The pivotal efficacy trial was a 16-week double-blind, placebo-controlled randomized clinical trial (Sekhar 2023, The Journals of Gerontology: Series A) in 24 older adults (aged 65 to 80 years) and 12 young-adult controls [2]. Older adults were randomized 1:1 to GlyNAC or isonitrogenous alanine placebo. The GlyNAC dose was equivalent to the pilot trial (approximately 7 grams of each component daily).

At 16 weeks, GlyNAC-supplemented older adults showed statistically significant improvements versus placebo in: erythrocyte glutathione; plasma oxidative stress markers; mitochondrial fatty-acid oxidation; HOMA-IR; interleukin-6 and tumor necrosis factor alpha; endothelial function; genomic damage markers (8-OHdG); PINK1 and Parkin (mitophagy markers); p16INK4a and p21 (senescence markers); Ki-67 (stem cell proliferative capacity marker); grip strength; gait speed; exercise capacity; waist circumference; and systolic blood pressure [2]. No improvements were observed in the placebo group. The multiplicity of aging hallmarks improved by a single intervention, and the reversal of these improvements upon withdrawal, represents the strongest current evidence for the multi-target efficacy of the GlyNAC combination.

6.3 Aging: independent dose-finding trial

An independent randomized controlled trial (Drexler et al. 2022, *Frontiers in Aging*) conducted by the Nestle Institute of Health Sciences in Lausanne enrolled 114 healthy older adults (aged 60 to 85 years) and tested three GlyNAC daily doses (2.4, 4.8, and 7.2 grams per day total in a 1:1 ratio) versus placebo over two weeks [10]. All three doses increased plasma glycine and cysteine concentrations and erythrocyte glutathione in a dose-dependent manner. Markers of oxidative damage (plasma protein carbonyls, 8-isoprostanes) declined at the two higher doses. The study provided independent confirmation of the substrate-repletion mechanism and supported the dose-response relationship.

6.4 HIV

An open-label clinical trial (Kumar et al. 2020, *Biomedicines*) in HIV-infected adults on stable antiretroviral therapy demonstrated that GlyNAC supplementation for 12 weeks improved erythrocyte glutathione, oxidative stress, mitochondrial fatty-acid oxidation, inflammation, endothelial function, insulin resistance, genomic damage, muscle strength, and cognitive scores [7]. Benefits receded after supplementation cessation. The study extended the GlyNAC evidence from age-associated glutathione deficiency to disease-associated glutathione deficiency in a population with chronic oxidative stress, immune activation, and metabolic dysfunction.

6.5 Type 2 diabetes

A pilot study (Sekhar 2022, Antioxidants) in 10 adults with type 2 diabetes and 10 unsupplemented non-diabetic controls examined the effect of 14 days of GlyNAC supplementation on mitochondrial fuel oxidation and insulin resistance [8]. GlyNAC supplementation increased fasted mitochondrial fatty-acid oxidation by approximately 30 percent, decreased mitochondrial glucose oxidation by approximately 47 percent, reduced HOMA-IR by approximately 22 percent, and lowered free fatty acid concentrations by approximately 25 percent. The study provided proof of concept that GlyNAC can improve metabolic dysfunction in a disease state characterized by insulin resistance and mitochondrial fuel-switching.

6.6 COVID-19

An observational study (Sekhar 2022, Antioxidants) measured glutathione, oxidative stress markers, and oxidant damage in 60 adult patients hospitalized with COVID-19 [12]. Patients exhibited severely depleted erythrocyte glutathione (approximately 60 percent lower than matched controls), markedly elevated thiobarbituric acid reactive substances and F2-isoprostanes, and elevated markers of oxidant damage. The study did not include a GlyNAC intervention arm but provided the rationale for interventional trials of GlyNAC in acute oxidative-stress states. A registered clinical trial (NCT04703036) was initiated to test GlyNAC supplementation in COVID-19 patients [12].

6.7 Mouse lifespan study

A preclinical study (Kumar et al. 2022, Nutrients) supplemented C57BL/6J mice with GlyNAC in drinking water beginning at 65 weeks of age (approximate rodent equivalent of late middle age in humans) and followed them to natural death [9]. GlyNAC-supplemented mice exhibited 24 percent longer median lifespan compared to unsupplemented age-matched controls. Tissue analysis at the time of death and at interim timepoints demonstrated that GlyNAC corrected glutathione deficiency, oxidative stress, mitochondrial dysfunction (complex I and IV activities), impaired mitophagy (PINK1, Parkin, LC3), abnormal nutrient-sensing pathways (elevated mTOR, reduced AMPK), and genomic damage (gamma-H2AX foci, 8-OHdG) in liver, heart, and kidney [9]. The study provides the strongest preclinical evidence for GlyNAC as a geroprotective intervention.

7. Reported Research Dose Ranges

7.1 Baylor College of Medicine protocol

The standard Baylor protocol uses approximately 100 milligrams per kilogram per day of each component, administered as two divided oral doses (morning and evening). For a 70-kilogram individual, this corresponds to approximately 7 grams of glycine and 7 grams of NAC daily (14 grams total daily dose) [1, 2]. This dose was used in the pilot trial, the randomized clinical trial, the HIV trial, and the diabetes pilot.

7.2 Nestle dose-finding protocol

The Nestle dose-finding trial tested three dose tiers: 2.4, 4.8, and 7.2 grams per day total (1.2, 2.4, and 3.6 grams of each component daily) in two divided doses [10]. All three doses produced measurable glutathione repletion, with dose-dependent magnitude. The highest dose (7.2 grams per day total, approximately 50 milligrams per kilogram per day of each component for a 70-kilogram individual) is approximately half the Baylor protocol dose.

7.3 Mouse lifespan study

The mouse study used GlyNAC supplemented in drinking water at a concentration calculated to deliver approximately 1.6 milligrams per gram of body weight per day of each component (the allometric equivalent of approximately 130 milligrams per kilogram per day in humans) [9].

7.4 Dose selection considerations

The optimal dose for human GlyNAC supplementation has not been definitively established. The Baylor protocol dose of approximately 100 milligrams per kilogram per day of each component is the dose at which the largest clinical effect sizes have been demonstrated. The Nestle trial suggests that lower doses produce measurable glutathione repletion but with smaller effect sizes on oxidative stress markers. Investigators should consider the target population, the severity of glutathione defi-

ciency, and the duration of supplementation when selecting a dose. Commercial GlyNAC supplements typically provide 1 to 3 grams of each component daily, substantially below the Baylor protocol dose.

8. Sourcing and Quality Verification

Both components of GlyNAC are widely available as pharmaceutical-grade and supplement-grade materials. Glycine is available from major chemical suppliers (Sigma-Aldrich, Thermo Fisher, Spectrum Chemical) at greater than 99 percent purity; it is also available as a dietary supplement from numerous manufacturers. N-Acetylcysteine is available as a prescription pharmaceutical (Mucomyst, various generics, for mucolytic and acetaminophen-overdose applications) and as a dietary supplement from numerous manufacturers, at greater than 98 percent purity by HPLC [4].

Commercial GlyNAC formulations are available from multiple supplement manufacturers. Investigators should verify that commercial products provide the intended dose of each component and should confirm identity and purity through:

- Certificate of analysis review from the manufacturer (with third-party verification preferred)
- High-performance liquid chromatography confirmation of glycine and NAC content per capsule or serving
- Mass spectrometry confirmation of identity (glycine $[M+H]^+$ at m/z 76.04; NAC $[M+H]^+$ at m/z 164.04)
- Karl Fischer or loss-on-drying analysis for residual moisture (NAC is hygroscopic)
- Heavy metals and microbial testing per United States Pharmacopeia dietary supplement standards
- Absence of significant oxidation products (cystine, NAC disulfide) in the NAC component, confirming sulfhydryl integrity

Third-party testing services (NSF International, USP Dietary Supplement Verification, ConsumerLab, Labdoor) provide independent evaluation of commercial supplement products. The GlyNAC supplement market is growing rapidly due to consumer interest in longevity interventions; lot-to-lot variability and label-claim accuracy are concerns in the supplement market that analytical verification can address.

9. Reconstitution and Handling

Glycine is stable as a dry powder at room temperature for extended periods (typically 3 to 5 years shelf life when stored in a sealed container protected from moisture). It is freely soluble in water and can be dissolved directly in beverages for oral consumption. No special handling is required.

N-Acetylcysteine is more sensitive to environmental conditions. The free sulfhydryl group is susceptible to oxidation; NAC should be stored in a cool, dry place, protected from light, and in tightly sealed containers to minimize oxidation to the disulfide form (NAC-NAC). Pharmaceutical-grade NAC is typically supplied under nitrogen atmosphere or with desiccant. Opened containers should be resealed promptly. NAC has a characteristic sulfurous odor that is normal and indicates the presence of the active sulfhydryl group.

For research applications requiring aqueous solutions, glycine and NAC can each be dissolved in sterile water or phosphate-buffered saline. NAC solutions should be freshly prepared or stored at 2 to 8 degrees Celsius and used within 24 to 48 hours, as the sulfhydryl group undergoes progressive oxidation in aqueous solution. Glycine solutions are stable for extended periods under the same conditions.

For oral supplementation in clinical or research protocols, the Baylor trials used individually filled gelatin capsules prepared by a compounding pharmacy [1, 2]. Commercial GlyNAC capsules are available as a more convenient alternative for research where compounding-pharmacy preparation is not required.

10. Stack Interactions and Combinations

10.1 Pharmacokinetic interactions

GlyNAC has minimal potential for pharmacokinetic drug-drug interactions. Neither glycine nor NAC is a significant substrate, inhibitor, or inducer of cytochrome P450 enzymes [4]. NAC has weak chelation activity for divalent metal ions and could theoretically reduce the absorption of oral iron, zinc, or copper supplements if taken simultaneously; separation of dosing by 2 hours is a reasonable precaution.

10.2 Pharmacodynamic interactions and combinations

Glutathione-depleting agents. Acetaminophen (paracetamol) at supratherapeutic doses depletes hepatic glutathione through the reactive metabolite N-acetyl-p-benzoquinoneimine (NAPQI); NAC is the established antidote. GlyNAC supplementation in the context of chronic acetaminophen use may provide a modest protective buffer against incidental glutathione depletion, though it should not be relied upon as a substitute for dose limitation.

Other antioxidants. Concurrent use of alpha-lipoic acid, vitamin C, vitamin E, selenium, or coenzyme Q10 with GlyNAC is pharmacodynamically complementary (operating through parallel or synergistic redox mechanisms) and is common in research and supplement protocols. No adverse interactions have been reported.

Metformin. GlyNAC and metformin share the downstream effect of AMPK activation (GlyNAC indirectly through restored mitochondrial function, metformin directly). The combination has not been studied but is theoretically additive for insulin-sensitizing effects.

Serotonergic agents. Glycine at high doses (greater than 30 grams per day) has been used adjunctively in schizophrenia as an NMDA receptor co-agonist. At GlyNAC doses (approximately 7 grams of glycine daily), clinically significant NMDA receptor modulation is unlikely but theoretically possible. Concurrent use with other NMDA-active agents (memantine, D-cycloserine, ketamine) should be considered.

Nitroglycerin. NAC potentiates the hemodynamic effects of nitroglycerin through formation of S-nitroso-N-acetylcysteine, a potent vasodilator. Concurrent use of GlyNAC with nitrate-based vasodilators should be monitored for hypotension [4].

Activated charcoal. Oral activated charcoal adsorbs NAC and should not be co-administered within 2 hours of GlyNAC dosing.

10.3 Research combinations

GlyNAC is being studied in combination with exercise interventions, caloric restriction, and other geroprotective compounds (rapamycin analogs, senolytics). No published data on these combinations exist as of the monograph revision date. The substrate-level mechanism of GlyNAC is mechanistically orthogonal to most other geroprotective strategies, suggesting potential for additive or synergistic benefit without mechanistic redundancy.

11. Adverse Events and Safety

11.1 Clinical trial safety data

No serious adverse events attributable to GlyNAC have been reported in any published clinical trial. In the Baylor pilot trial, randomized clinical trial, HIV trial, and diabetes pilot, GlyNAC was well tolerated, and no subjects discontinued supplementation because of adverse events [1, 2, 7, 8]. The Nestle dose-finding trial similarly reported no significant adverse events at any of the three dose tiers tested [10].

11.2 Common mild adverse events

The principal mild adverse events associated with GlyNAC supplementation are gastrointestinal and are attributable predominantly to the NAC component:

- Nausea (dose-dependent, generally mild and self-limiting, more common at higher NAC doses)
- Bloating and flatulence
- Loose stools (infrequent)
- Sulfurous body odor or breath (related to NAC sulfur metabolism, generally mild)

Glycine at doses up to 7 grams per day has not been associated with significant adverse events. In schizophrenia research, glycine has been administered at doses up to 60 grams per day with tolerability limited primarily by the large volume of powder required; nausea and sedation have been reported at these very high doses [14, 19].

11.3 NAC-specific safety considerations

NAC has an extensive clinical safety record from its established medical applications. As an acetaminophen-overdose antidote, intravenous NAC is administered at loading doses of 150 milligrams per kilogram (approximately 10 grams for a 70-kilogram adult) followed by infusions totaling approximately 300 milligrams per kilogram over 20 hours; anaphylactoid reactions (urticaria, bronchospasm, hypoten-

sion) occur in approximately 10 to 20 percent of IV administrations but are rare with oral dosing [4]. As a mucolytic, oral NAC at 600 to 1200 milligrams per day is well tolerated in long-term use. At the GlyNAC research dose (approximately 7 grams per day oral), NAC is within the range that has been studied in clinical trials for psychiatric, pulmonary, and hepatic indications without significant safety concerns [4, 13].

11.4 Theoretical concerns

NAC has been reported to have pro-tumorigenic effects in some preclinical models, accelerating the growth of established lung tumors in mice at high doses by reducing oxidative-stress-mediated tumor suppression [20]. The clinical significance of this observation in the context of human GlyNAC supplementation at standard doses is uncertain; the preclinical doses were substantially higher than human-equivalent GlyNAC doses, and no increase in cancer incidence has been reported in any human NAC or GlyNAC trial. Investigators should be aware of this preclinical signal, particularly in the context of supplementation in individuals with known or suspected malignancy.

11.5 Pregnancy and lactation

Neither glycine nor NAC supplementation in the GlyNAC combination has been studied in pregnant or lactating women. NAC has been used in pregnancy for acetaminophen overdose without reported teratogenicity; glycine is a normal dietary amino acid. The GlyNAC combination should not be used in pregnancy or lactation without clinical supervision until specific safety data are available.

12. Comparable Compounds Assessment

This section presents a structured comparative assessment of five alternative glutathione-replenishing strategies against GlyNAC, evaluated on five competency standards: mechanistic completeness (addressing the full substrate deficit), effect size (magnitude of glutathione repletion and downstream benefits), clinical validation (quality and quantity of human evidence), side-effect profile, and practical accessibility (availability, cost, convenience). The five comparators were selected as the principal alternatives that a researcher or clinician might consider for the same therapeutic objective of restoring intracellular glutathione.

12.1 N-Acetylcysteine alone (NAC monotherapy)

Mechanism: NAC alone provides cysteine (via deacetylation) as a glutathione precursor. It addresses the cysteine component of the substrate deficit but does not address the glycine deficit. The Sekhar laboratory's tracer studies demonstrated that NAC alone partially but incompletely restores glutathione synthesis in older adults [6, 11].

Effect size: NAC alone at oral doses of 600 to 1800 milligrams per day increases erythrocyte glutathione by approximately 30 to 50 percent in glutathione-deficient populations. GlyNAC at the Baylor protocol dose increases erythrocyte glutathione by approximately 200 percent, restoring to young-adult levels [1, 6]. The difference is attributable to the uncorrected glycine deficit under NAC monotherapy.

Clinical validation: High. NAC monotherapy has been studied in hundreds of clinical trials across dozens of indications (acetaminophen overdose, chronic obstructive pulmonary disease, psychiatric disorders, renal protection, contrast nephropathy, HIV, polycystic ovary syndrome, fertility). The evidence for glutathione repletion specifically is moderate; many trials measured clinical endpoints without quantifying glutathione.

Side-effect profile: Well-characterized. Oral NAC at doses up to 3 grams per day is well tolerated; gastrointestinal symptoms (nausea, vomiting, diarrhea) are the principal adverse events. Anaphylactoid reactions are rare with oral dosing. The side-effect

fect profile is essentially equivalent to the NAC component of GlyNAC.

Practical accessibility: Excellent. NAC is widely available as a pharmaceutical and as a dietary supplement at low cost (approximately \$10 to \$30 per month at typical supplement doses). However, in 2020 the United States Food and Drug Administration challenged the classification of NAC as a dietary supplement on the basis that it was first approved as a drug (1963); this regulatory uncertainty has affected availability in some markets, though it remains widely sold [4].

Comparison to GlyNAC: NAC monotherapy addresses only half the substrate deficit and produces proportionally smaller glutathione repletion. GlyNAC's advantage is mechanistic completeness. NAC monotherapy's advantages are greater clinical validation, lower cost, simpler dosing, and broader availability. For individuals whose glutathione deficiency is predominantly cysteine-limited (rather than dually limited), NAC monotherapy may be sufficient. For age-associated glutathione deficiency where both substrates are limiting, GlyNAC is mechanistically superior.

12.2 Oral reduced glutathione

Mechanism: Direct oral supplementation with the tripeptide glutathione itself, bypassing the need for intracellular synthesis from precursors.

Effect size: Historically, oral reduced glutathione was considered to have negligible bioavailability due to hydrolysis by intestinal gamma-glutamyltranspeptidase and peptidases. A 2015 randomized trial (Richie et al., European Journal of Nutrition) demonstrated that oral glutathione at 250 or 1000 milligrams per day for 6 months increased erythrocyte glutathione by 17 to 35 percent [21]. Effect sizes are smaller than those achieved by GlyNAC at the Baylor protocol dose.

Clinical validation: Low to moderate. Only a small number of randomized trials have examined oral glutathione supplementation with quantitative glutathione endpoints. Clinical evidence for downstream health benefits (beyond glutathione concentration) is limited.

Side-effect profile: Favorable. Oral glutathione is well tolerated in published trials. No significant adverse events have been reported.

Practical accessibility: Moderate. Oral reduced glutathione is available as a dietary supplement from multiple manufacturers. Cost is higher than NAC or GlyNAC (approximately \$30 to \$80 per month for 500 to 1000 milligrams per day). Stability of

the reduced form in capsules is a concern; the sulfhydryl group is susceptible to oxidation during storage.

Comparison to GlyNAC: Oral glutathione takes a direct-supplementation approach, while GlyNAC takes a precursor-replenishment approach. The GlyNAC approach produces larger glutathione repletion (approximately 200 percent versus 17 to 35 percent), likely because intracellular glutathione synthesis from restored precursor supply produces higher intracellular concentrations than can be achieved by oral absorption of the intact tripeptide. GlyNAC also provides the independent benefits of glycine (anti-inflammatory, metabolic). Oral glutathione has the advantage of simplicity (single compound) and the disadvantage of lower efficacy.

12.3 Liposomal glutathione

Mechanism: Glutathione encapsulated in phospholipid liposomes to protect the tripeptide from gastrointestinal degradation and to enhance absorption through liposomal fusion with intestinal epithelial cell membranes.

Effect size: A pilot study (Sinha et al. 2018, European Journal of Clinical Nutrition) reported that liposomal glutathione at 500 to 1000 milligrams per day for 1 month increased erythrocyte glutathione by 40 to 100 percent and increased plasma glutathione by 25 to 50 percent [22]. Effect sizes are intermediate between oral glutathione and GlyNAC.

Clinical validation: Low. Only a small number of studies have examined liposomal glutathione with quantitative endpoints. No large randomized controlled trials have been published. Evidence for downstream health benefits is limited to pilot and open-label studies.

Side-effect profile: Favorable. Liposomal glutathione is well tolerated. The phospholipid carrier (typically phosphatidylcholine from sunflower or soy lecithin) adds a modest caloric contribution and a theoretical allergen concern for soy-sensitive individuals.

Practical accessibility: Moderate to low. Liposomal glutathione products are available from specialty supplement manufacturers at higher cost than standard oral glutathione (approximately \$40 to \$120 per month). Product quality varies; liposomal integrity (particle size, encapsulation efficiency) is difficult for consumers to verify.

Comparison to GlyNAC: Liposomal glutathione achieves better absorption than standard oral glutathione and approaches GlyNAC effect sizes in limited pilot data. GlyNAC has the advantages of larger clinical effect sizes in controlled trials, more extensive clinical evidence, lower cost, and the independent benefits of glycine. Liposomal glutathione has the advantage of providing the intact tripeptide and may be preferable in populations where glutathione synthetic capacity is impaired at the enzyme level rather than at the substrate level.

12.4 Alpha-lipoic acid

Mechanism: Alpha-lipoic acid is an endogenous dithiol compound that functions as a cofactor for mitochondrial dehydrogenase complexes and, in its reduced form (dihydrolipoic acid), as a potent antioxidant and recycler of other antioxidants (including glutathione, vitamin C, and vitamin E). Alpha-lipoic acid indirectly supports glutathione homeostasis by reducing oxidized glutathione (GSSG) back to reduced glutathione (GSH) and by reducing cystine to cysteine, thereby increasing intracellular cysteine availability for glutathione synthesis [23].

Effect size: Oral alpha-lipoic acid at 600 to 1200 milligrams per day has been reported to increase erythrocyte glutathione by 10 to 30 percent in various patient populations. The glutathione-replenishing effect is indirect and smaller in magnitude than GlyNAC.

Clinical validation: Moderate to high. Alpha-lipoic acid has been studied in numerous randomized trials for diabetic neuropathy, diabetic nephropathy, weight loss, and oxidative stress reduction. The evidence for glutathione repletion specifically is secondary to these other endpoints; most trials did not measure glutathione as a primary outcome.

Side-effect profile: Generally well tolerated. Gastrointestinal symptoms (nausea, heartburn) are the principal adverse events. Skin rash and hypoglycemia (in diabetic patients on concurrent glucose-lowering therapy) have been reported.

Practical accessibility: Excellent. Alpha-lipoic acid is widely available as a dietary supplement and as a prescription pharmaceutical (in some jurisdictions) at moderate cost (approximately \$15 to \$40 per month at 600 milligrams per day).

Comparison to GlyNAC: Alpha-lipoic acid supports glutathione homeostasis through an indirect redox-recycling mechanism, while GlyNAC addresses the biosynthetic substrate deficit directly. The two mechanisms are complementary

and not mutually exclusive; combination use is pharmacodynamically rational. GlyNAC produces larger glutathione repletion. Alpha-lipoic acid has additional mitochondrial cofactor functions not shared by GlyNAC. The choice between or combination of these agents depends on the specific mechanism of glutathione deficiency in the target population.

12.5 Whey protein concentrate

Mechanism: Whey protein is rich in cystine (the disulfide form of cysteine) and in the dipeptide gamma-glutamylcysteine, a direct precursor for glutathione synthesis. Cystine is reduced to cysteine intracellularly by thioredoxin and glutaredoxin systems, providing substrate for glutamate-cysteine ligase [24]. Undenatured whey protein concentrate preserves the native protein structures (principally beta-lactoglobulin and alpha-lactalbumin) that deliver cystine and gamma-glutamylcysteine to the intestinal epithelium.

Effect size: Clinical studies of undenatured whey protein (e.g., Immunocal) have reported increases in erythrocyte glutathione of 10 to 35 percent at doses of 10 to 40 grams per day over 2 to 12 weeks [24]. Effect sizes are smaller than GlyNAC and comparable to NAC monotherapy.

Clinical validation: Low to moderate. A small number of randomized trials have examined undenatured whey protein for glutathione repletion in HIV, chronic obstructive pulmonary disease, cancer, and aging populations. Results are mixed, with some positive and some null findings.

Side-effect profile: Favorable for tolerability. Gastrointestinal symptoms (bloating, lactose-related effects in lactose-intolerant individuals) are the principal adverse events. Whey protein is contraindicated in individuals with cow's milk allergy.

Practical accessibility: Excellent. Whey protein is widely available, inexpensive (approximately \$15 to \$30 per month), and is consumed by millions of individuals for protein supplementation. However, only undenatured whey protein preserves the cystine-containing proteins in their native form; standard whey protein isolate processed at high temperature may have reduced glutathione-promoting activity.

Comparison to GlyNAC: Whey protein provides cysteine equivalents (as cystine) and some gamma-glutamylcysteine but does not address the glycine deficit. Like NAC monotherapy, it addresses only half the substrate deficit identified in aging.

Effect sizes for glutathione repletion are smaller than GlyNAC. Whey protein has the advantages of broad availability, low cost, additional nutritional benefits (protein, branched-chain amino acids), and palatability. GlyNAC has the advantages of mechanistic completeness, larger and more consistent effect sizes, and a more developed clinical evidence base specific to glutathione repletion and aging outcomes.

12.6 Comparator summary

The five comparators occupy distinct positions in the glutathione-replenishment landscape. **NAC monotherapy** is the most extensively studied single agent for cysteine delivery and glutathione support, with hundreds of clinical trials but incomplete substrate correction for age-associated deficiency. **Oral reduced glutathione** provides the intact tripeptide but achieves modest intracellular repletion due to limited absorption. **Liposomal glutathione** improves upon oral glutathione absorption but lacks clinical validation at scale. **Alpha-lipoic acid** supports glutathione through an indirect redox mechanism and has established clinical evidence for diabetic complications but produces smaller glutathione repletion than direct precursor strategies. **Whey protein concentrate** provides cysteine equivalents through a food-matrix approach but, like NAC monotherapy, does not address the glycine deficit.

GlyNAC occupies a mechanistically distinct position in this landscape as the only intervention that simultaneously corrects both the glycine and cysteine substrate deficits identified by stable-isotope tracer studies in aging. This dual correction produces the largest glutathione repletion effect sizes of any oral intervention studied in controlled human trials (approximately 200 percent increase to young-adult levels), and the downstream improvements across multiple aging hallmarks exceed in breadth and magnitude those reported for any comparator. The principal limitations of GlyNAC relative to the comparators are the smaller total clinical evidence base (the Baylor program comprises trials with fewer than 100 subjects in total), the dependence on a single research group for the principal findings (partially addressed by the independent Nestle dose-finding trial), and the higher daily supplement burden (approximately 14 grams per day at the full Baylor protocol dose).

Investigators considering GlyNAC for research applications should recognize that the mechanistic rationale is strong, the clinical effect sizes are large and consistent across populations, and the safety profile is favorable, but that the evidence

base requires replication in larger, multi-center trials before definitive conclusions about clinical utility can be drawn. The comparators offer varying degrees of mechanistic coverage, clinical validation, and practical accessibility, and the optimal choice depends on the specific population, the mechanism of glutathione deficiency, and the research or clinical objectives.

13. References

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