Glutathione For Detoxification and Promotion of Health

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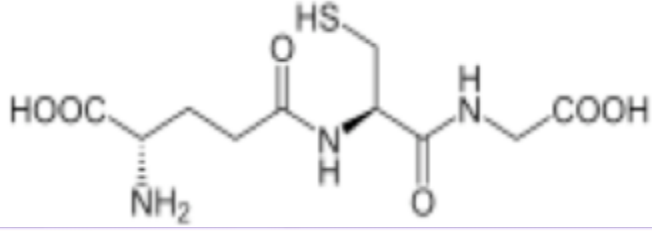
- **Academic**
  - Founding president (1978) of Bastyr University, first accredited, natural medicine university
  - Editor-in-Chief: *Integrative Medicine: A Clinician's Journal*
  - *Textbook of Natural Medicine*, 4th ed 2012; *Clinical Pathophysiology*, 2012
  - *Clinical Pathophysiology*, A Functional Perspective, 1st edition 2012
- **Policy**
  - Member Medicare Coverage Advisory Committee, 2003-2005
  - Member White House Commission on CAM Policy, 2000-2002
- **Public**
  - *Encyclopedia of Natural Medicine*, 3rd ed 2012 (2,000,000 copies in 6 languages)
  - *Encyclopedia of Healing Foods*, 2005
- **Example Awards and Recognitions**
  - Natural Products Association: Clinician of the Year, 2012
  - Juror for Roger's Prize: 2009, 2011, 2013
  - Institute for Functional Medicine: Linus Pauling Award, 2004
  - American Holistic Medical Association: *Pioneer in Holistic Medicine*, 2003
  - *Natural Health Magazine*: *Leading health educator in the past 30 years*. 2001
  - Alternative Healthcare Management: *1 of the 4 most influential CAM leaders*, 2000
  - *Seattle Magazine*: *1 of the top 20 national intellectual leaders from Seattle*, 1996

Outline

- Critical role of glutathione—detoxification, inflammation and so much more
- Review of physiology
- Glutathione production
- Clinical applications
- GGT as measure of toxic load
- GGT as predictor of disease and longevity
- Ways to increase intracellular levels



Hard to Overstate Importance of Glutathione

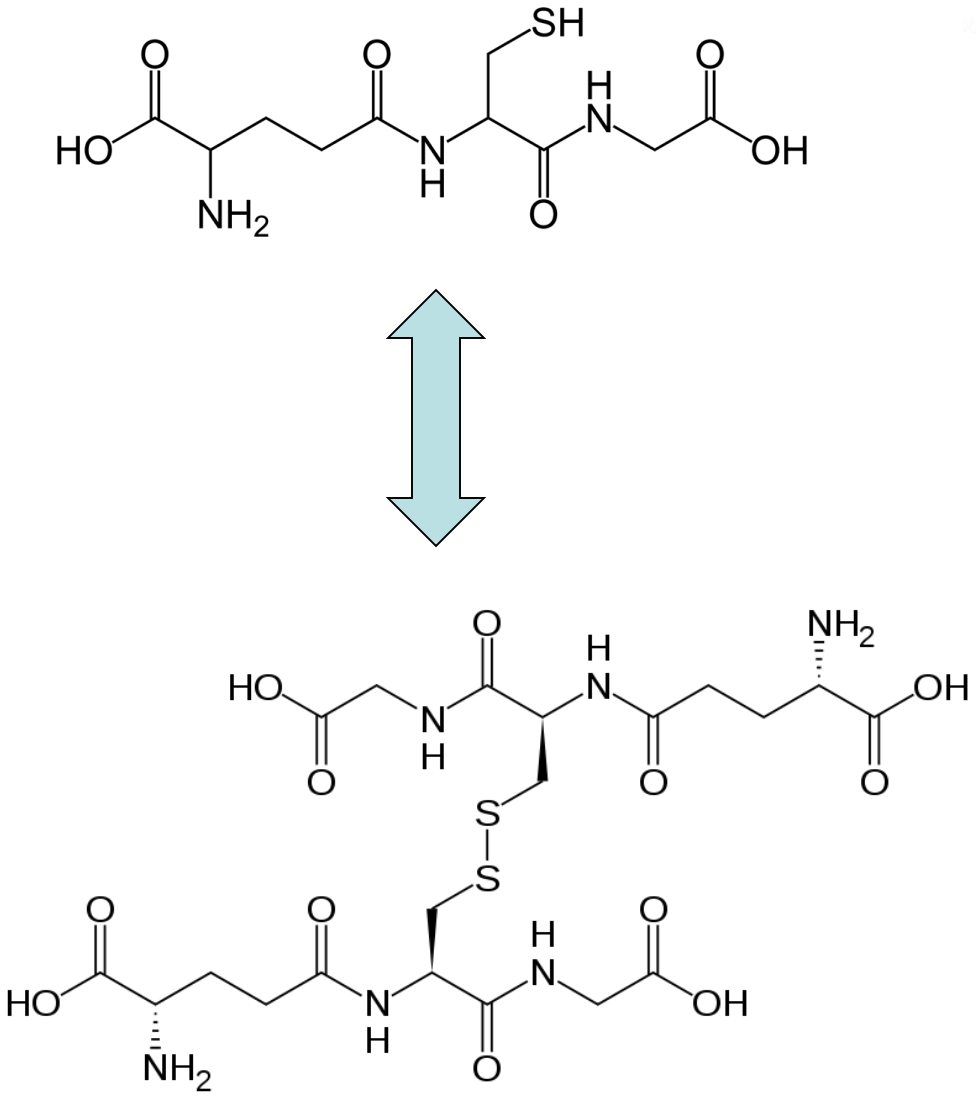
- Plays a crucial role in shielding cellular macromolecules from endogenous and exogenous reactive oxygen & nitrogen species.
- Decreases causes of inflammation
  - POPs
  - Mercury
- Quenches inflammation
- Levels directly predictive of health and longevity

Glutathione—Many Key Roles

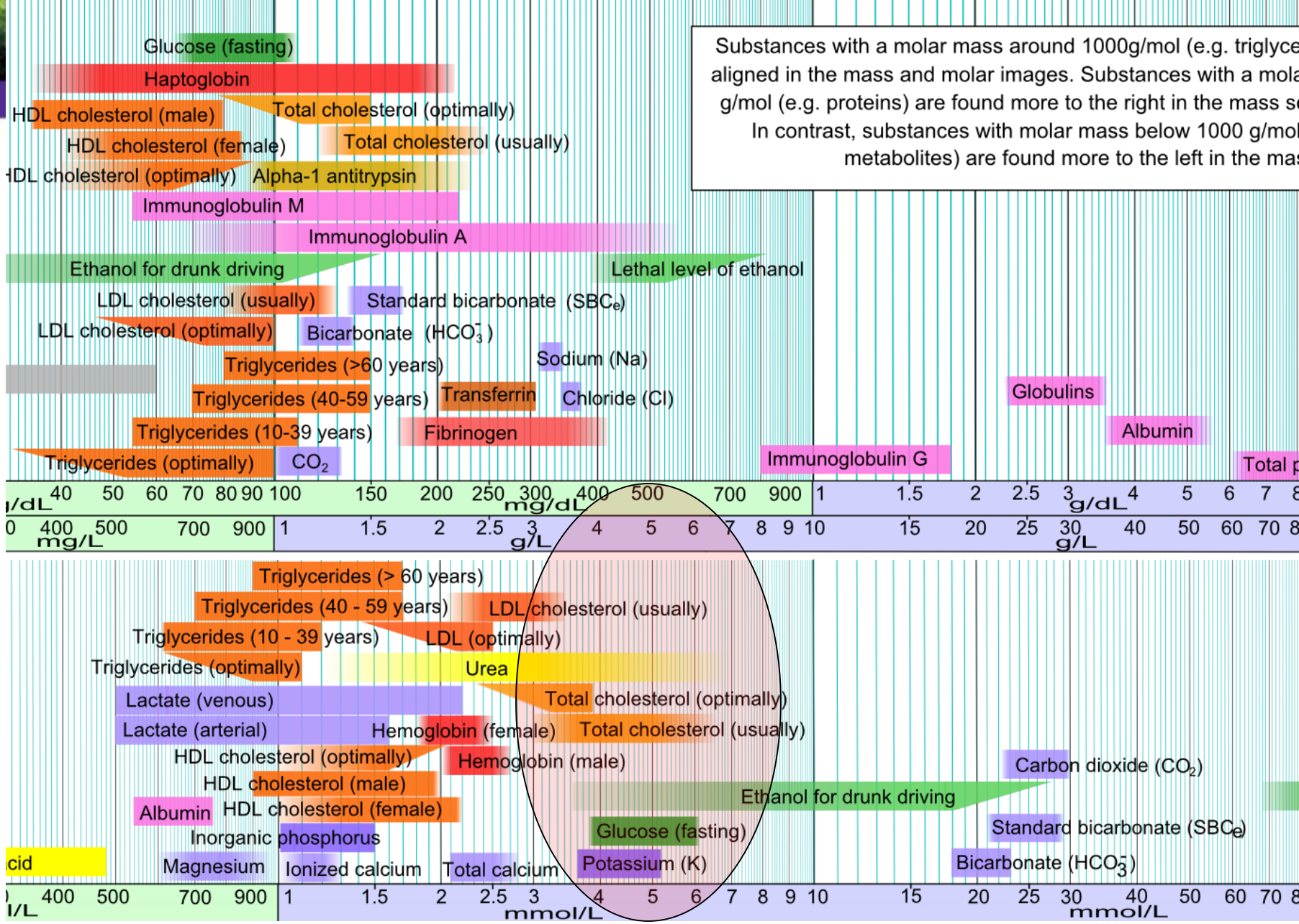
1. Direct chemical neutralization of singlet oxygen, hydroxyl radicals and superoxide radicals
2. Cofactor for several antioxidant enzymes
3. Regeneration of vitamins C and E
4. Neutralization of free radicals produced by Phase I liver metabolism of chemical toxins
5. One of approximately 7 liver Phase II reactions which conjugate the activated intermediates produced by Phase I to make them water soluble for excretion by the kidneys
6. Transports mercury out of cells and the brain
7. Regulation of cellular proliferation and apoptosis
8. Vital to mitochondrial function and maintenance of mtDNA

Glutathione

- Tripeptide (cysteine, glycine and glutamic acid)
- Relatively high (5 millimolar) concentrations in most cells
- Exists in reduced state (GSH) and oxidized state (GSSG)
- Ratio determines cell redox status
  - Healthy cells at rest have a GSH/GSSG ratio >100
  - Ratio drops to 1-10 in cells exposed to oxidant stress
- Thiol buffer maintaining sulfhydryl groups of many proteins in their reduced form
- Produced exclusively in the cytosol and actively pumped into mitochondria



Glutathione: 5 mmolar in Most Cells!



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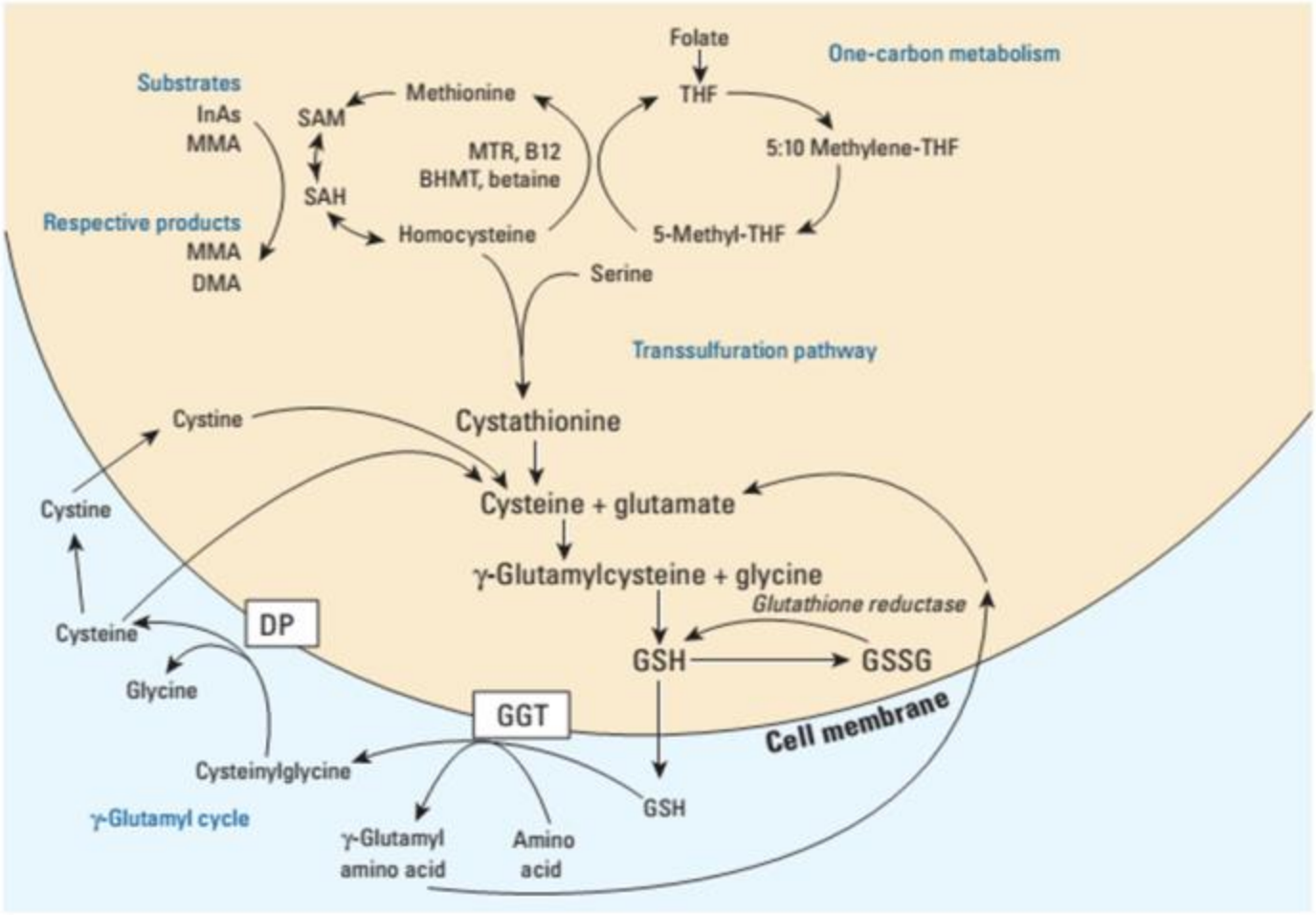
Synthesis, Regeneration & Recycling

- GSH is made available in 3 ways:
  - Synthesis via a 2 step process catalyzed by the enzymes GCL and glutathione synthetase—requires ATP
  - Regeneration of oxidized GSSG to reduced GSH by glutathione reductase—requires NADPH
  - Recycling of cysteine from conjugated glutathione—requires NADPH
- Regulation of synthesis, regeneration & recycling
  - Glutathione synthesis is primarily controlled by GCL because the rate-limiting step is the cellular level of the amino acid cystein
  - GCL is in part regulated by GSH feedback inhibition
- If GSH is depleted due to oxidative stress, inflammation, or exposure to xenobiotics, de novo synthesis of GSH is up regulated, as is cysteine synthesis.

Biswas SK, Rahman I. Environmental toxicity, redox signaling and lung inflammation: the role of glutathione. Mol Aspects Med. 2009 Feb-Apr;30(1-2):60-76

Townsend DM, Tew KD, Tapiero H. The importance of glutathione in human disease. Biomed Pharmacother. 2003 May-Jun;57(3-4):145-55

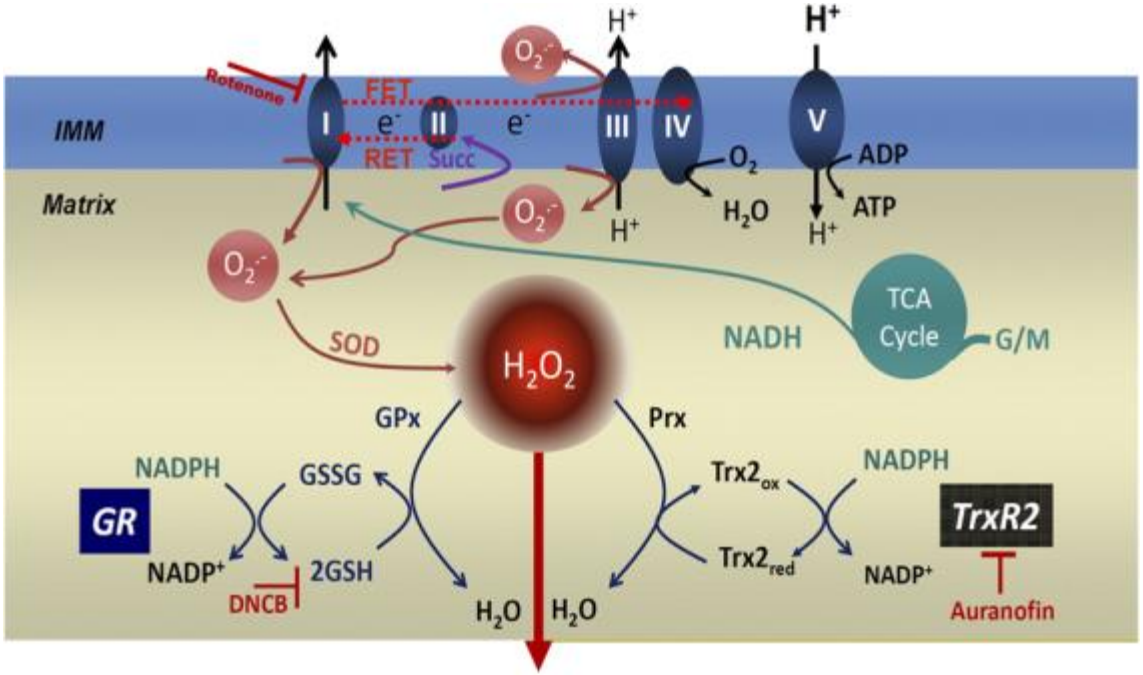
Synthesis, Regeneration and Recycling



Hall MN, Niedzwiecki M, Liu X, et al. Chronic arsenic exposure and blood glutathione and glutathione disulfide concentrations in bangladeshi adults. Environ Health Perspect. 2013 Sep;121(9):1068-74. doi: 10.1289/ehp.1205727. Creative Commons Attribution License

Glutathione Neutralizes Oxidants Involved in Inflammation

- Directly scavenges diverse oxidants:
  - Superoxide anion
  - Hydroxyl radical
  - Nitric oxide and carbon radicals
- Catalytically detoxifies:
  - Hydroperoxides
  - Peroxynitrite
  - Lipid peroxides



Franco R, Cidlowski JA. Apoptosis and glutathione: beyond an antioxidant. Cell Death Differ. 2009 Oct;16(10):1303-14

Marí M, et al. Mitochondrial glutathione, a key survival antioxidant. Antioxid Redox Signal. 2009 Nov;11(11):2685-700. doi: 10.1089/ARS.2009.2695

Aon MA, Stanley BA, et al. Glutathione/thioredoxin systems modulate mitochondrial H2O2 emission: an experimental-computational study. J Gen Physiol. 2012 Jun;139(6):479-91. doi: 10.1085/jgp.201210772. Epub 2012 May 14.

Glutathione Critical to Mitochondrial Protection

- Mitochondria contain 10-15% of total cellular glutathione, all of which is produced in the cytosol.
- A substantial decrease in glutathione levels precedes the mitochondrial dysfunction and neuronal death found in Parkinson's disease, suggesting GSH depletion may initiate the subsequent impairment

Marí M, et al. Mitochondrial glutathione, a key survival antioxidant. Antioxid Redox Signal. 2009 Nov;11(11):2685-700. doi: 10.1089/ARS.2009.2695

Merad-Boudia M, et al. Mitochondrial impairment as an early event in the process of apoptosis induced by glutathione depletion in neuronal cells: relevance to Parkinson's disease. Biochem Pharmacol. 1998 Sep 1;56(5):645-55

Glutathione in Detoxification

- Detoxification of both xenobiotic and endogenous compounds
  - Facilitates excretion from cells (Hg)
  - Facilitates excretion from body (POPs, Hg)
  - Directly neutralizes (POPs, many oxidative chemicals)
- Facilitate the plasma membrane transport of toxins by at least four different mechanisms most important of which is formation of glutathione S-conjugates
- GSH conjugation (Phase II) of activated intermediates from Phase I

Phase II Glutathione Conjugation

- Low levels of glutathione and/or transferase activity are also associated with chronic exposure to chemical toxins and alcohol, cadmium exposure, AIDS/ HIV, macular degeneration, Parkinson's disease, and other neurodegenerative disorders.
- Toxic metabolite (NAPQI), causes an increase in reactive oxygen species and nitrosamines, mitochondrial poisoning, lipid peroxidation, and covalent binding to proteins, with eventual necrosis. Toxicity amplified if glutathione is low (another effect of alcohol), because glutathione rapidly detoxifies NAPQI.

James LP, et al. Acetaminophen-induced hepatotoxicity. Drug Metab Dispos. 2003 Dec;31(12):1499-506

Glutathione: Critical for Detoxification and Protection from Mercury

- Binds and transports mercury out of cells
- Binds and transports mercury out of the brain across the blood brain barrier
- Irreversibly(?) binds to mercury in the brain
- Neutralizes oxidative damage from mercury

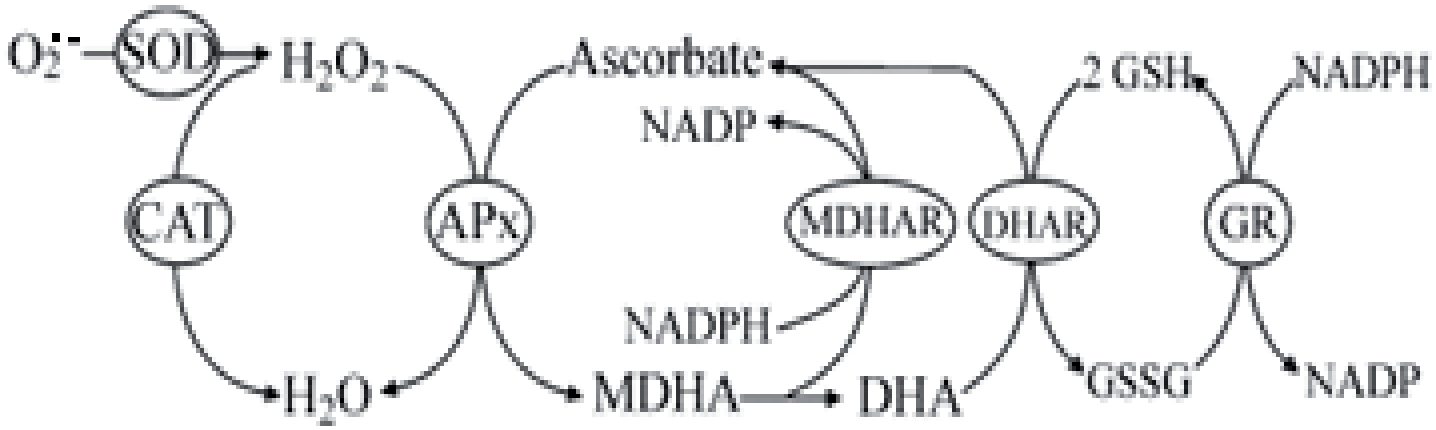
Baker, SM. The Metaphor of Oceanic Disease. IMCJ February, 2008;7:1.
Mosharov, E., Cranford, M.R., Baneriee, R. The Quantitatively Important Relationship between Homocysteine Metabolism and Glutathione Synthesis by the Transsulfuration Pathway and Its Regulation by Redox Changes. Biochemistry. 2000 Sept;39:13005-13011.

Genetic Polymorphisms Important

- Genetic polymorphisms in GSTs, GCL, and selenoprotein genes were shown to influence mercury body burdens among heavy fish consumers (measured as erythrocyte total mercury levels), as well as urine and hair mercury levels among dental professionals.

Schläwicke et al. Genetic variation in glutathione-related genes and body burden of methylmercury. Environ Health Perspect. 2008 Jun;116(6):734-9
Goodrich JM, et al. Glutathione enzyme and selenoprotein polymorphisms associate with mercury biomarker levels in Michigan dental professionals. Toxicol Appl Pharmacol. 2011 Dec 1;257(2):301-8

Glutathione Regenerates Vitamins C and E



Teixeira 2005; Creative Commons Attribution License

Packer L, et al. Molecular aspects of lipoic acid in the prevention of diabetes complications. Nutrition. 2001 Oct;17(10):888-95

Felipe Karam Teixeira, et al. Multigene families encode the major enzymes of antioxidant metabolism in Eucalyptus grandis L. Genet. Mol. Biol. vol.28 no.3 suppl.0 São Paulo 2005

GSH/GSSG Plays Key Role in Apoptosis

- Accumulation of GSSG due to oxidative stress is directly toxic to cells, inducing apoptosis by activation of the SAPK/MAPK pathway
- Glutathione depletion triggers apoptosis, although unclear whether it is mitochondrial or cytosol pools of GSH that are the determining factor

Filomeni G, et al. Activation of c-Jun-N-terminal kinase is required for apoptosis triggered by glutathione disulfide in neuroblastoma cells. Free Radic Biol Med 2005; 39: 345–354

Marí M, et al. Mitochondrial glutathione, a key survival antioxidant. Antioxid Redox Signal. 2009 Nov;11(11):2685-700

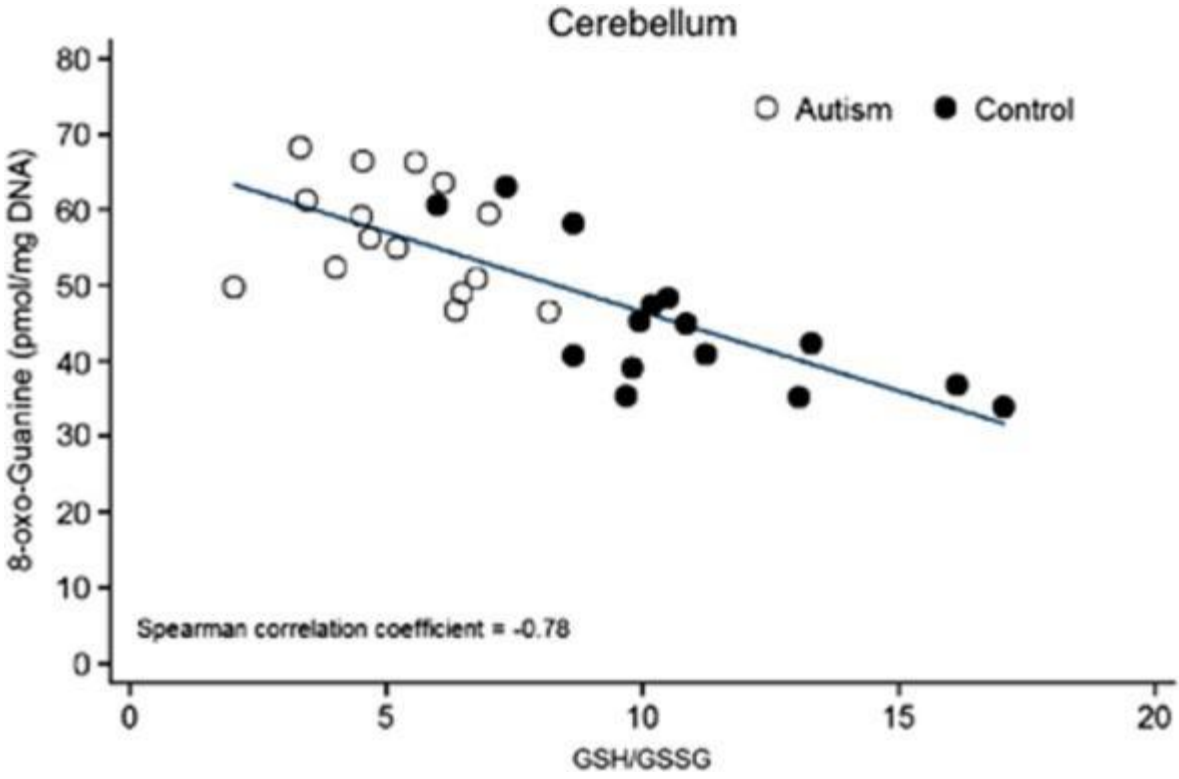
Depleted GSH Has Been Implicated In:

- Neurodegenerative disorders (Alzheimer's, Parkinson's and Huntington's diseases, amyotrophic lateral sclerosis, Friedreich's ataxia)
- Pulmonary disease (COPD, asthma, and acute respiratory distress syndrome)
- Immune diseases (HIV, autoimmune disease)
- Cardiovascular diseases (hypertension, myocardial infarction, cholesterol oxidation)
- Liver disease
- Cystic fibrosis
- Chronic age-related diseases (cataracts, macular degeneration, hearing impairment, and glaucoma)
- Aging process itself

Ballatori N, Krance SM, Notenboom S, Shi S, Tieu K, Hammond CL. Glutathione dysregulation and the etiology and progression of human diseases. Biol Chem. 2009 Mar;390(3):191-214

Glutathione Depletion Correlates with Oxidative Damage in the Brain

- Frozen samples from cerebellum and temporal cortex from individuals with autism and unaffected controls



Rose S, et al. Evidence of oxidative damage and inflammation associated with low glutathione redox status in the autism brain. Transl Psychiatry. 2012 Jul 10;2:e134

GSH Depletion Associated with Aging

- Decrease in GSH synthesis has been implicated as instrumental to the aging process
- Age-dependent decrease in intracellular GSH
- Supplementation with glutathione precursors were shown to fully restore GSH status.
- Sample of community-dwelling elderly, higher glutathione levels were associated with higher levels of physical health, fewer illnesses, and higher levels of self-rated health

Rebrin I, Sohal RS. Pro-oxidant shift in glutathione redox state during aging. Adv Drug Deliv Rev. 2008 Oct-Nov;60(13-14):1545-52

Voss P, Siems W. Clinical oxidation parameters of aging. *Free Radic Res. 2006 Dec;40(12):1339-49*

Sekhar RV, et al. Deficient synthesis of glutathione underlies oxidative stress in aging and can be corrected by dietary cysteine and glycine supplementation. Am J Clin Nutr. 2011 Sep;94(3):847-53

Julius M, et al. Glutathione and morbidity in a community-based sample of elderly. *J Clin Epidemiol. 1994 Sep;47(9):1021-6*

Glutathione and Telomere Length

- GSH status has been found to parallel telomerase activity, an important indicator of lifespan
- Strong association between telomere length and oxidative stress, interventions that increase glutathione levels are likely to protect both telomeres and mtDNA from damage

Borrás C, et al. Glutathione regulates telomerase activity in 3T3 fibroblasts. J Biol Chem. 2004 Aug 13;279(33):34332-5

Salpea KD, Talmud PJ, Cooper JA, et al. Association of telomere length with type 2 diabetes, oxidative stress and UCP2 gene variation. *Atherosclerosis.* 2010 Mar;209(1):42-50

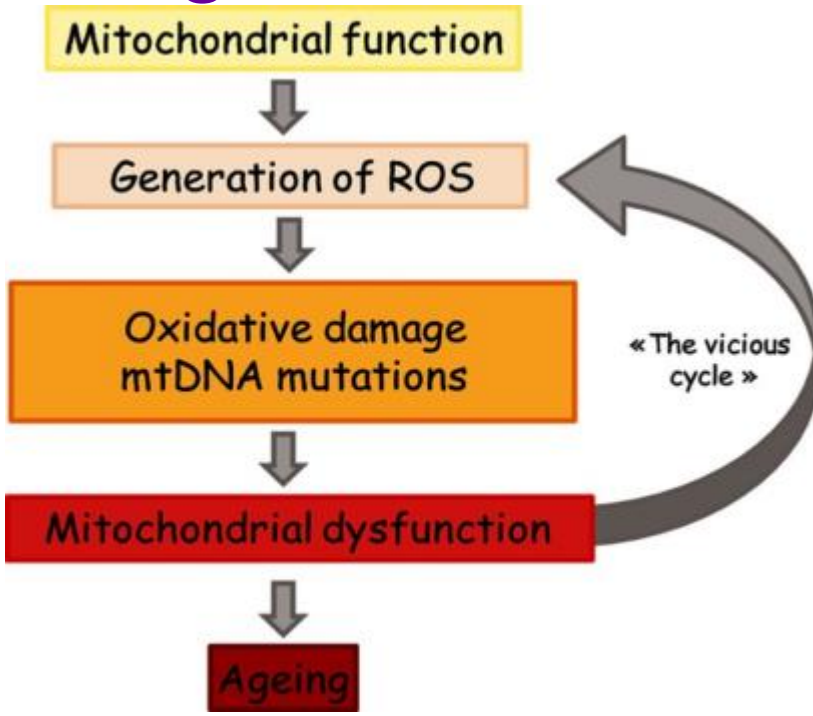
Mitochondria Aging and Mitochondria

- Accumulation of damage to mitochondria tissue and DNA (mtDNA) leads to aging of humans and animals.
- Age-related impairment in the ETC:
  - Decreases ATP synthesis
  - Increases production of reactive oxygen species (ROS) through increased electron leakage in the respiratory chain
  - H_2O_2 and oxidative damage to DNA and lipids increase with age
  - Mitochondrial glutathione declines with age
  - Energy depletion $\rightarrow$ cellular dysfunction, apoptosis, and inflammation lead to tissue dysfunction, aging, and degeneration

Wei YH, et al. Mitochondrial theory of aging matures--roles of mtDNA mutation and oxidative stress in human aging. Zhonghua Yi Xue Za Zhi (Taipei). 2001;64:259-70
Richter C, et al. Normal oxidative damage to mitochondrial and nuclear DNA is extensive. Proc Natl Acad Sci U S A. 1988 Sep;85(17):6465-7
Lee HC, Wei YH. Oxidative stress, mitochondrial DNA mutation, and apoptosis in aging. Exp Biol Med (Maywood). 2007 May;232(5):592-606.

Glutathione and mtDNA Damage

- Strong correlation between mtDNA damage and mitochondrial glutathione oxidation

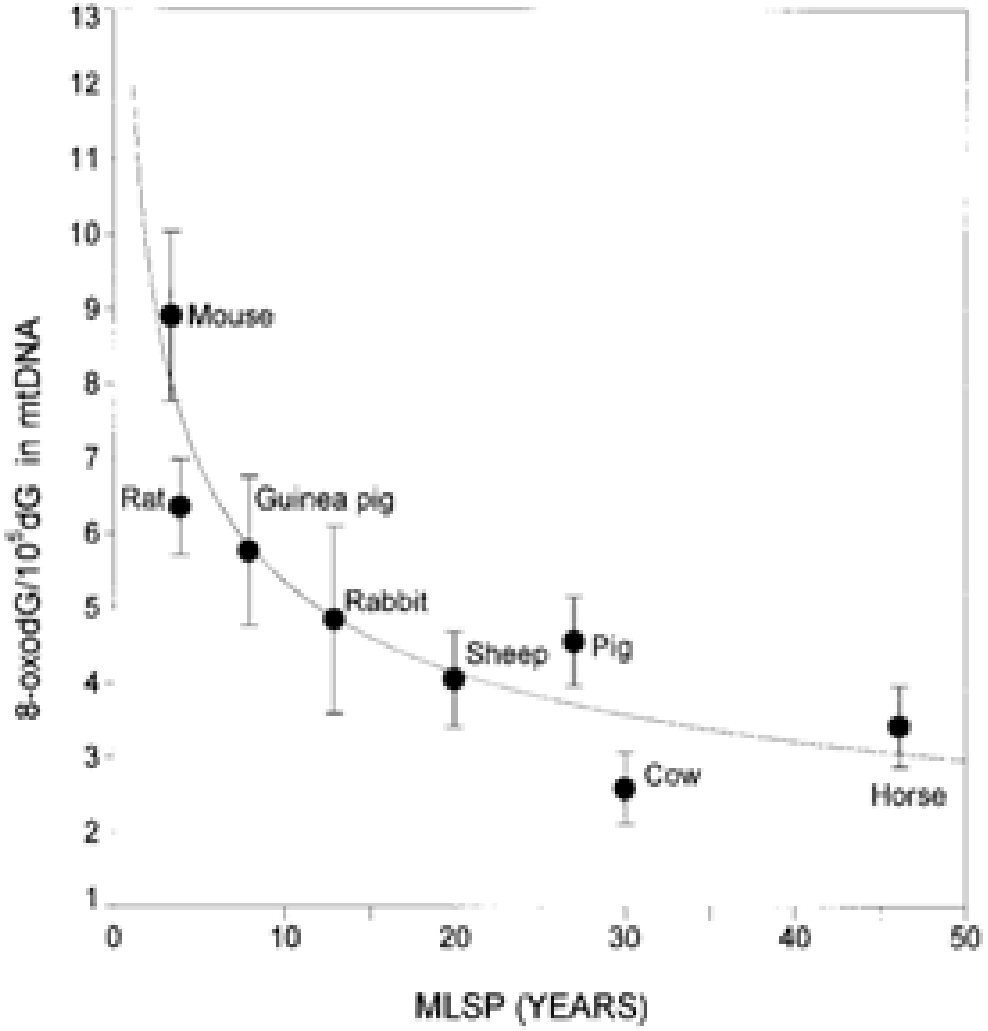


de la Asuncion JG, Millan A, Pla R, et al. Mitochondrial glutathione oxidation correlates with age-associated oxidative damage to mitochondrial DNA. *FASEB J. 1996 Feb;10(2):333-8*

Barja G, Herrero A. Oxidative damage to mitochondrial DNA is inversely related to maximum life span in the heart and brain of mammals. FASEB J. 2000 Feb;14(2):312-8

Lagouge M, Larsson NG. The role of mitochondrial DNA mutations and free radicals in disease and ageing. J Intern Med. 2013 Jun;273(6):529-43. doi: 10.1111/joim.12055

Strong Correlation Between mtDNA Damage and Species Longevity



Key role in aging process

Barja G, Herrero A. Oxidative damage to mitochondrial DNA is inversely related to maximum life span in the heart and brain of mammals. FASEB J. 2000 Feb;14(2):312-8

GGT as a Measure of Glutathione Demand and Toxic Exposure

- Gamma-glutamyl transferase (γ-glutamyl transpeptidase)
- Provides the rate-limiting cysteine through catabolic “salvage pathway”
- Increases in GGT correlate with: metabolic syndrome, both fatal and non-fatal coronary heart disease (CHD) events, atherosclerosis, fatty liver, gestational diabetes, cancer, hypertension, and carotid intima-media thickness

Jo SK, Lee et al. Serum gamma-glutamyl transferase activity predicts future development of metabolic syndrome defined by 2 different criteria. Clin Chim Acta. 2009 May;403(1-2):234-40.

Kozakova M, et al. The RISC Investigators. Fatty liver index, gamma-glutamyltransferase and early carotid plaques. Hepatology. 2012 Feb 15. doi: 10.1002/hep.25555.

Van Hemelrijck M, et al. Gamma-glutamyltransferase and risk of cancer in a cohort of 545,460 persons - the Swedish AMORIS study. Eur J Cancer. 2011 Sep;47(13):2033-41.

Lee DH, et al. Gamma-glutamyltransferase is a predictor of incident diabetes and hypertension: the Coronary Artery Risk Development in Young Adults (CARDIA) Study. Clin Chem. 2003 Aug;49(8):1358-66.

Eroglu S, et al. Association between serum gamma-glutamyltransferase activity and carotid intima-media thickness. Angiology. 2011 Feb;62(2):107-10

GGT: Indirect Measure of POPs

- Glutathione is key intracellular defense against oxidative stress induced by POPs
- Binds to POPs for detoxification in Phase II glutathione conjugation
- Exposure to POPs induces GGT to increase glutathione availability

Lee DH, et al (2003) Gamma-glutamyltransferase and diabetes—a 4 year follow-up study. Diabetologia 46:359–364

Pamela A, et al. Serum gamma-glutamyltransferase: linking together environmental pollution, redox equilibria and progression of atherosclerosis? Clin Chem Lab Med. 2009;47(12):1583-4.

Lee DH, et al. Serum gamma-glutamyltransferase: new insights about an old enzyme. J Epidemiol Community Health. 2009 Nov;63(11):884-6.

GGT as Measure of POPS – Normal Range?

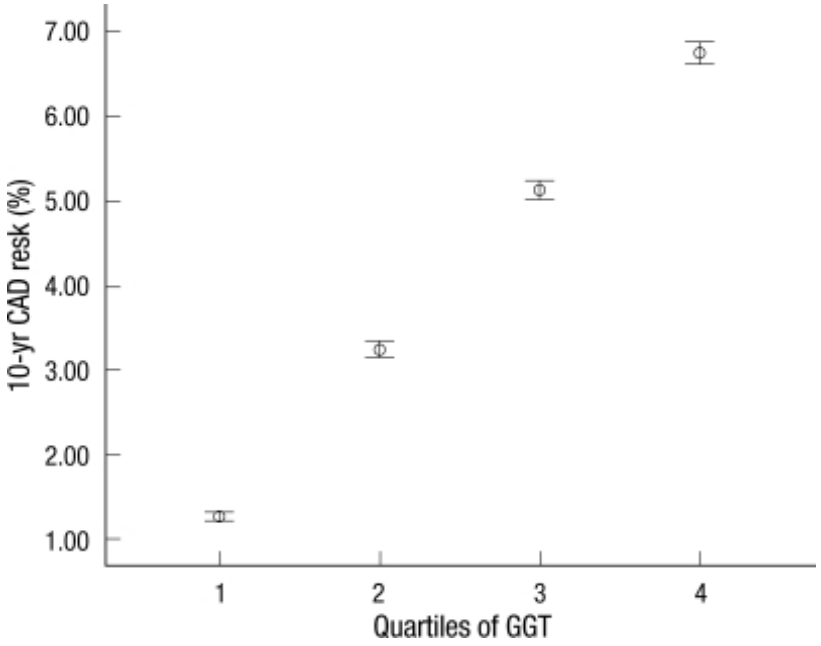
- Within normal range, GGT predicts type 2 diabetes, gestational diabetes, coronary heart disease, hypertension, stroke, dyslipidaemia, fatty liver, chronic kidney disease and cancer
- Men with GGT levels >50 U/l had ~26 fold risk for diabetes compared to those with <10. Those with 40-49 had a ~20 fold risk.
- Levels within normal range occur with obesity, xs alcohol, cigarette smoking, physical inactivity, high meat/low fruit and vegetable intake
- Cumulative biomarker for environmental pollutants

Lee DH, et al. Serum gamma-glutamyltransferase predicts non-fatal myocardial infarction and fatal coronary heart disease among 28,838 middle-aged men and women. Eur Heart J 2006;27:2170–6
Lee DH, et al. Gamma-glutamyltransferase and diabetes--a 4 year follow-up study. Diabetologia. 2003 Mar;46(3):359-64.
Lee DH, et al. Can persistent organic pollutants explain the association between serum gamma-glutamyltransferase and type 2 diabetes? Diabetologia. 2008 Mar;51(3):402-7.

GGT – Sensitive Marker of MetS

- Population based study
  - Increased GGT independently associated with IFG/IGT
  - CRP was not after adjusting for GGT
  - Serum GGT positively associated with IFG/IGT in spite of obesity

- 2 Cross-sectional studies
  - GGT tightly associated with HOMA-R and estimated insulin secretion
  - GGT correlates with Framingham score (CAD risk)

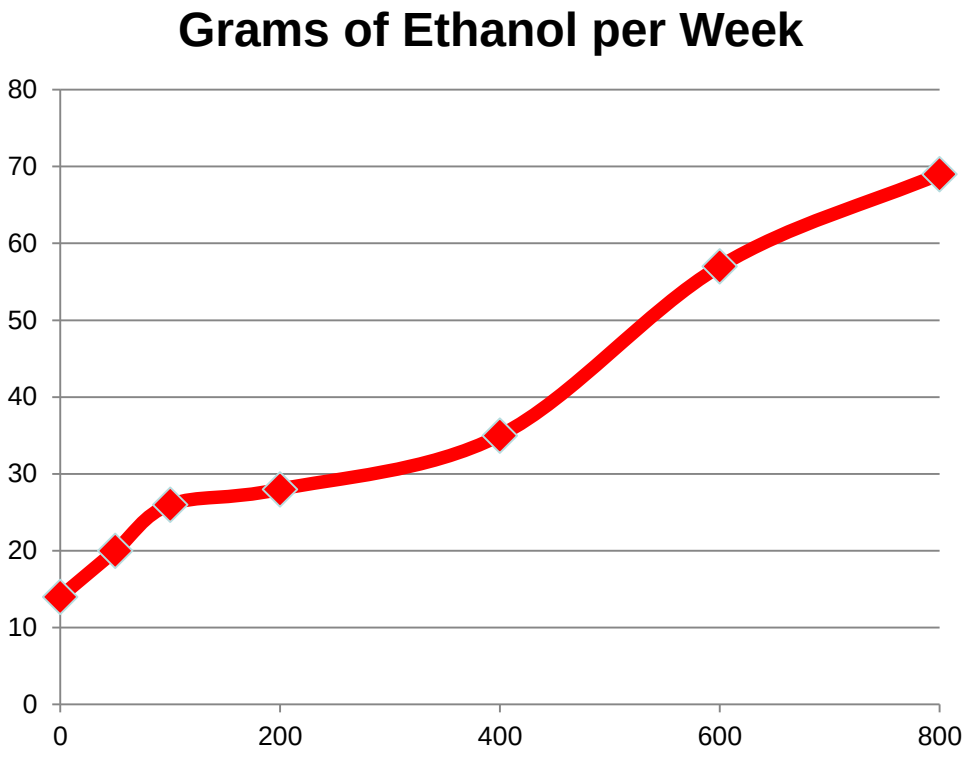


Kim 2011

Sun J, et al. The association of gamma-glutamyltransferase and C-reactive protein with IFG/IGT in Chinese adults in Qingdao, China. Clin Chim Acta. 2011 Aug 17;412(17-18):1658-61.
Bianchi C, et al. Serum gamma-glutamyltransferase levels are related to insulin sensitivity and secretion in subjects with abnormal glucose regulation. Diabetes Metab Res Rev. 2010 Mar;26(3):181-6.
Kim KN et al. Serum gamma-glutamyltransferase concentration correlates with Framingham risk score in Koreans. J Korean Med Sci. 2011 Oct;26(10):1305-9.

GGT and Alcohol Consumption

- GGT directly correlates with alcohol consumption
- In a non-uniform population, 40 g/d will elevate GGT ~15%
- Watch for false negatives
  - Inability to up regulate
  - Are these the ones most sensitive to/damaged by chemical toxins?
- Could up-regulation of GGT in light alcohol consumption be reason for benefit?

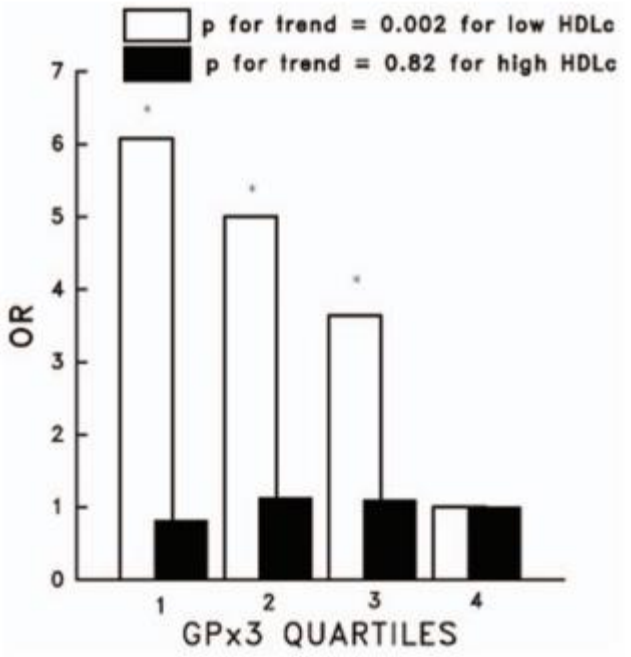


Adapted from: Nagaya T, et al. Dose-response relationships between drinking and serum tests in Japanese men aged 40–59 years. Alcohol 1999 Feb. 17(2): 133–8.

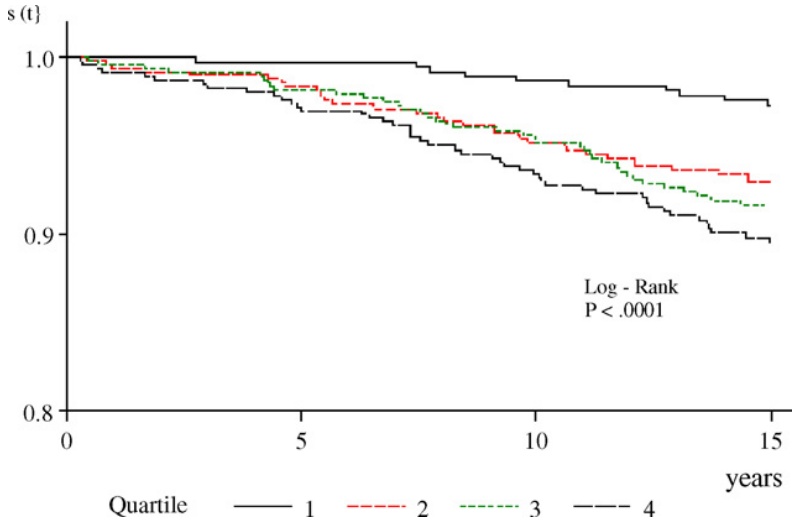
Glutathione as Predictor of Cardiovascular Disease

- **Glutathione peroxidase**
- Minnesota Heart Survey; 26 to 85 years old
- Low activity predictive of cardiovascular mortality, only among those with low HDL-C
- Lowest GP had OR of 6.08 for CV mortality

- **GGT**
- 18 year study,1,878 men
- GGT > 35 = 3.08 fold increased risk CVD!
- Similar for stroke, diabetes, etc.



Buijsse 2012; the Creative Commons

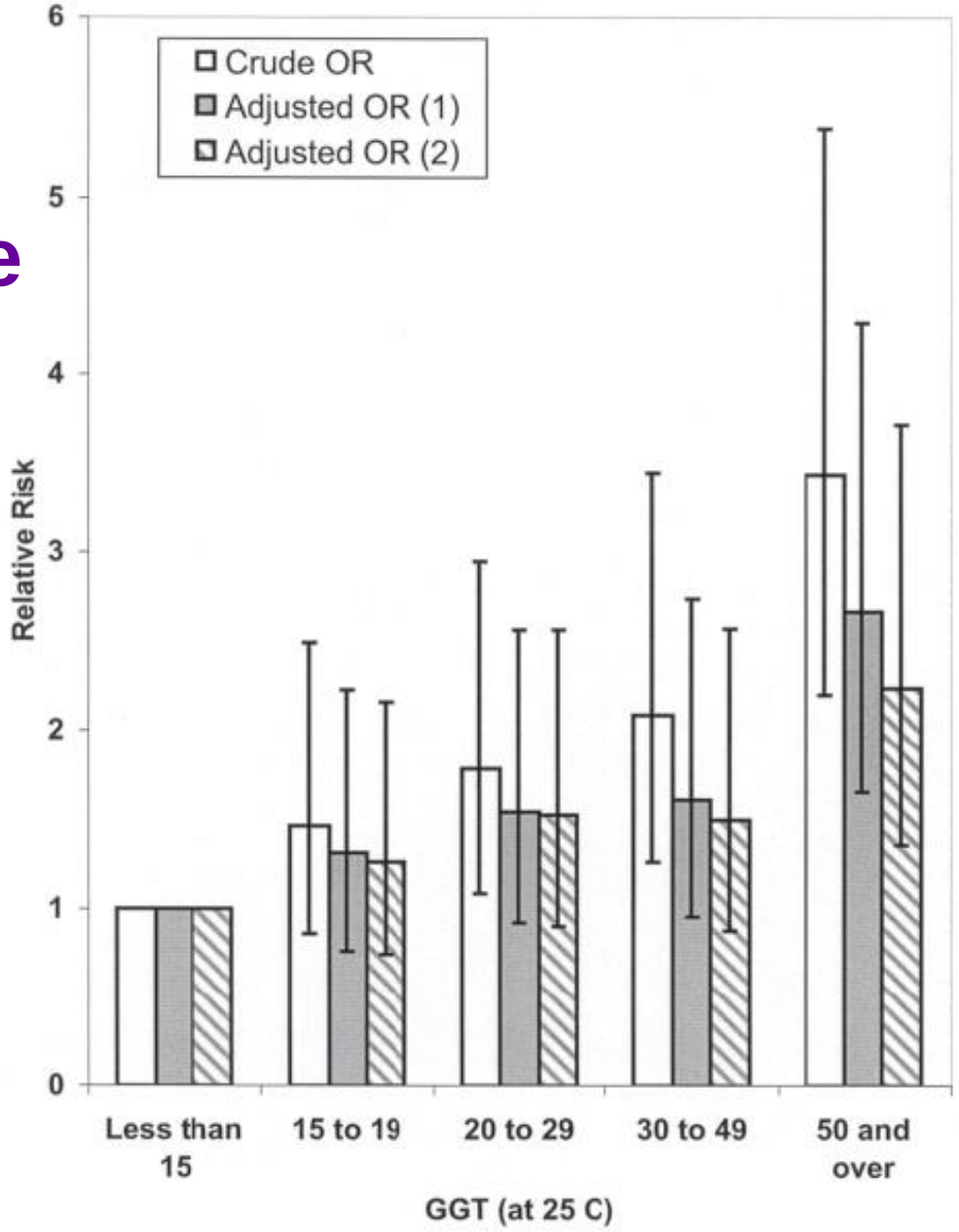


Meisinger C, et al. Serum gamma-glutamyltransferase is a predictor of incident coronary events in apparently healthy men from the general population. Atherosclerosis. 2006;189(2):297-302

Buijsse B, Lee DH, et al. Low serum glutathione peroxidase activity is associated with increased cardiovascular mortality in individuals with low HDLc's. PLoS One. 2012;7(6):e38901. doi: 10.1371/journal.pone.0038901

GGT Levels Correlate with Risk of Death

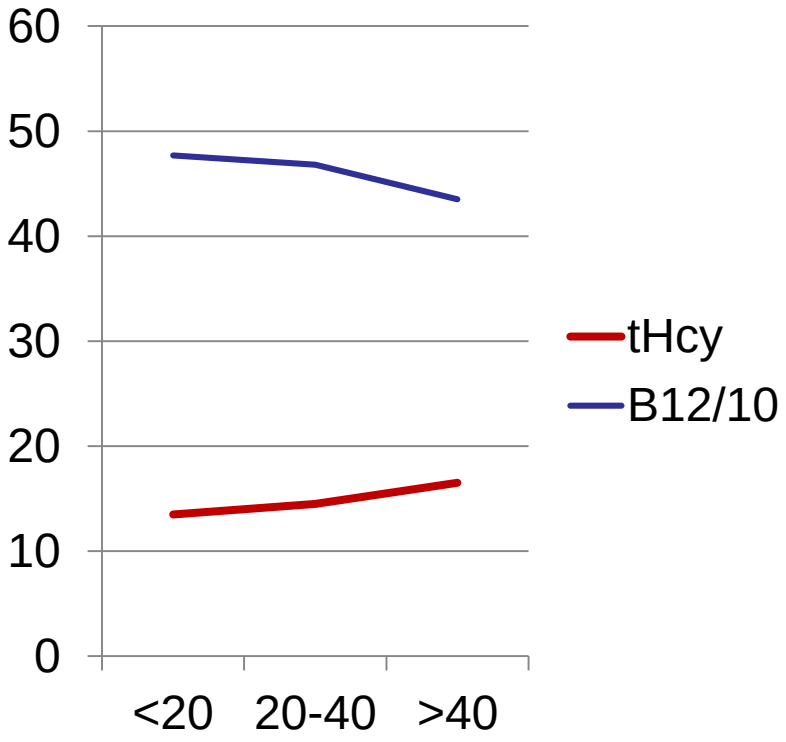
- GGT over 50 associated with tripling of death rate!
- 30-40 (normal range!) associated with doubling



Brenner H, Rothenbacher D, Arndt V, et al. Distribution, determinants, and prognostic value of gamma-glutamyltransferase for all-cause mortality in a cohort of construction workers from southern Germany. Prev Med 1997; 26: 305–10.

GGT Inverse Correlation with Homocysteine

- General: R = 0.29
- Diabetics: R = 0.22
- Also correlation with B12



Lippi G, et al. Plasma gamma-glutamyl transferase activity predicts homocysteine concentration in a large cohort of unselected outpatients. Intern Med. 2008;47(8):705-7
Sakuta H, et al. Serum gamma-glutamyl transferase is associated with plasma total homocysteine in Japanese patients with type 2 diabetes. Acta Diabetol. 2007 Dec;44(4):177-80

Factors that Affect GGT

Increase

- Excessive alcohol
  - Acetaminophen worsens
- Meat intake
- Fried food intake
- Smoking
- Elevated blood sugar
- Obesity
- Anticonvulsants, OCAs

Decrease

- Dietary vitamins C & E, carotenoids and fiber
- Fruit and vegetable intake
- Bean and lentil intake
- Whole grain intake
- Nut intake
- Physical activity
- Coffee!

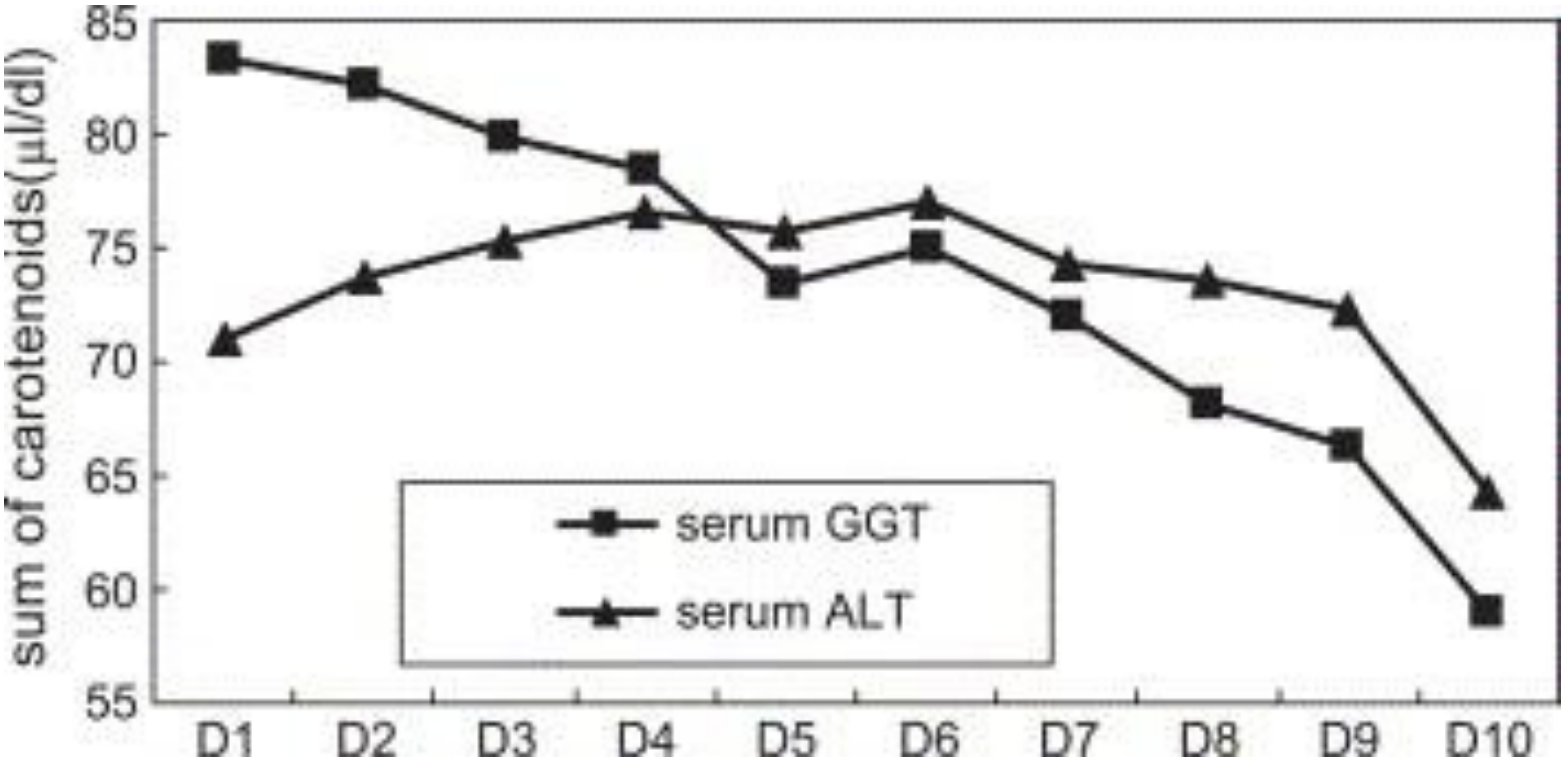
Lee DH, Gross MD, Jacobs DR Jr. Association of serum carotenoids and tocopherols with gamma-glutamyltransferase: the Cardiovascular Risk Development in Young Adults (CARDIA) Study. Cardiovascular Risk Development in Young Adults Study. Clin Chem. 2004;50(3):582-8

Dixon JB, et al. Weight loss and non-alcoholic fatty liver disease: falls in gamma-glutamyl transferase concentrations are associated with histologic improvement. Obes Surg. 2006;16(10):1278-86

Seifert CF, Anderson DC. Acetaminophen usage patterns and concentrations of glutathione and gamma-glutamyl transferase in alcoholic subjects. Pharmacotherapy. 2007;27(11):1473-82

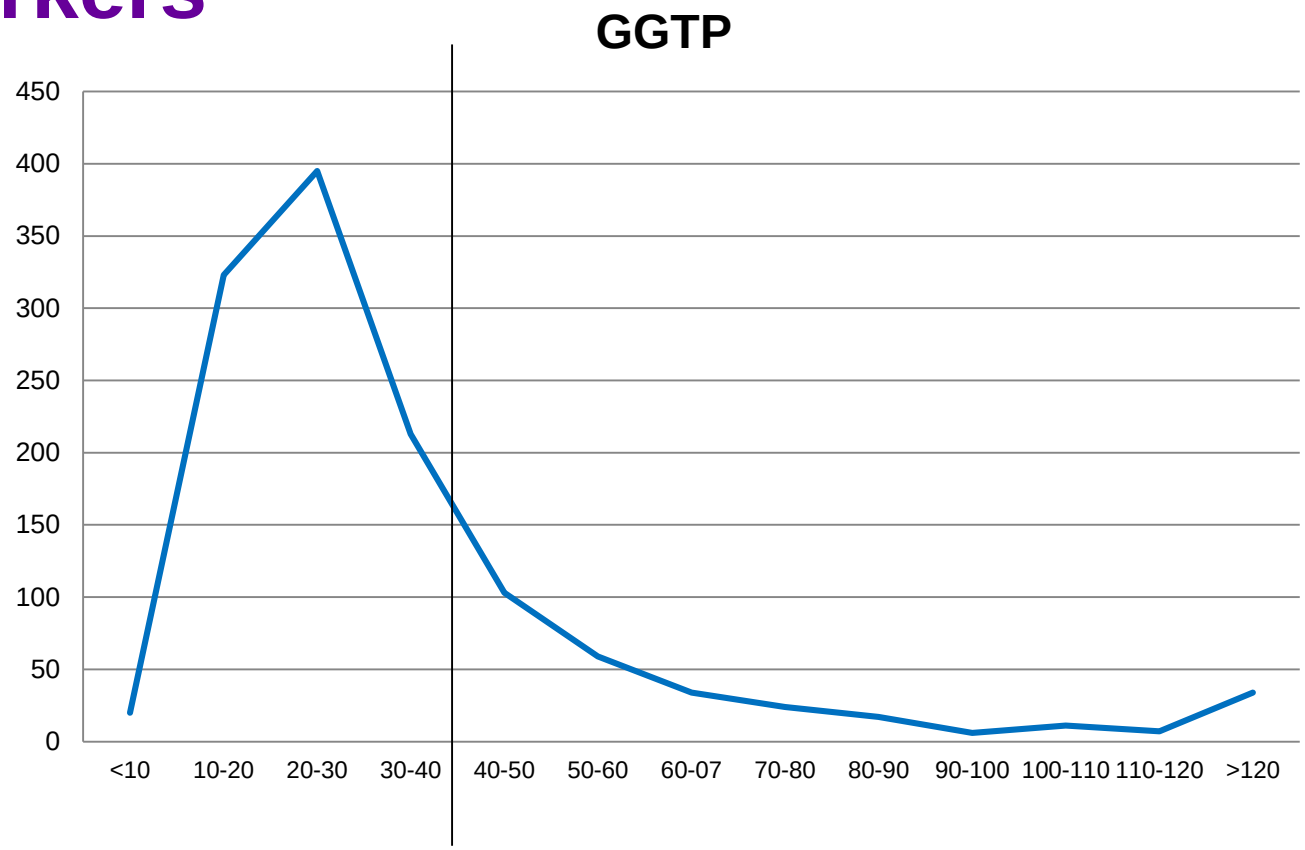
Lee DH, et al. Is serum gamma glutamyltransferase a marker of oxidative stress? Free Radic Res. 2004;38(6):535-9

Dietary Carotenoids and GGT



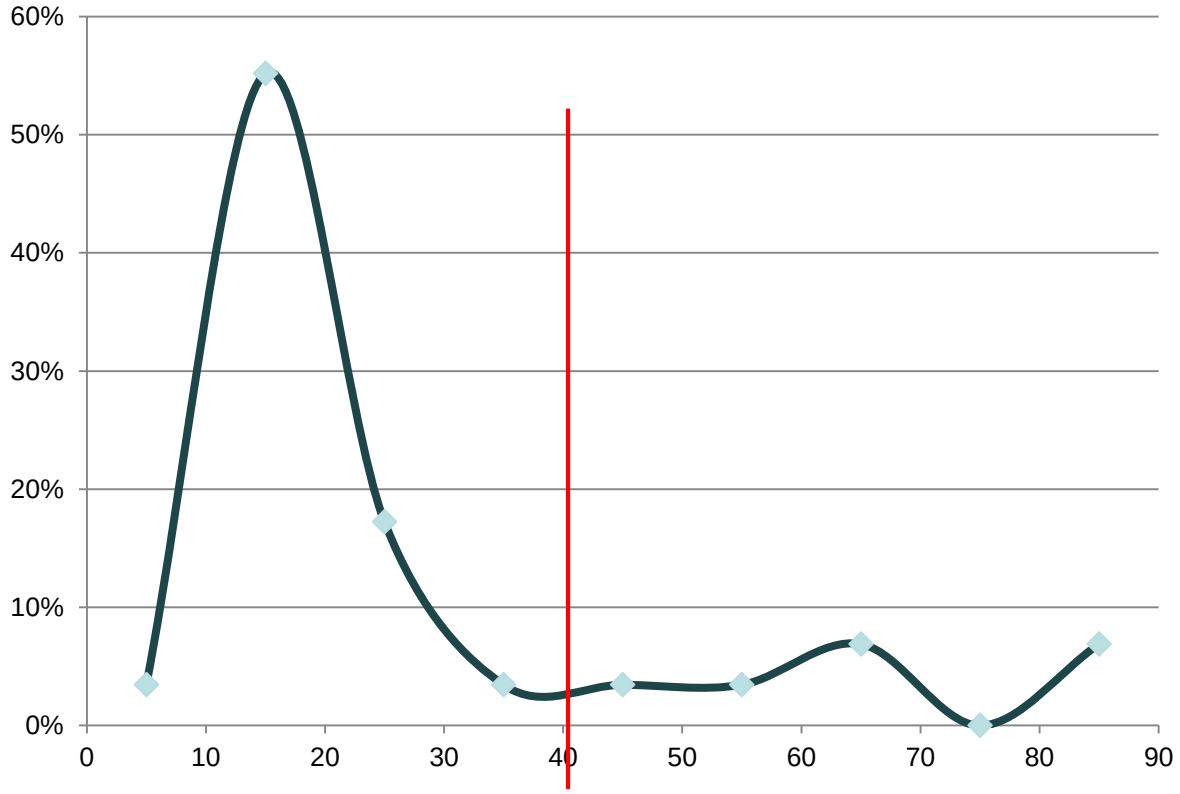
Lim JS, et al. Is serum gamma-glutamyltransferase inversely associated with serum antioxidants as a marker of oxidative stress? Free Radic Biol Med. 2004;37(7):1018-23

GGT Data from Canadian Oil Field Workers



20-fold increased risk of diabetes

GGT From Small US Company with Young Workers

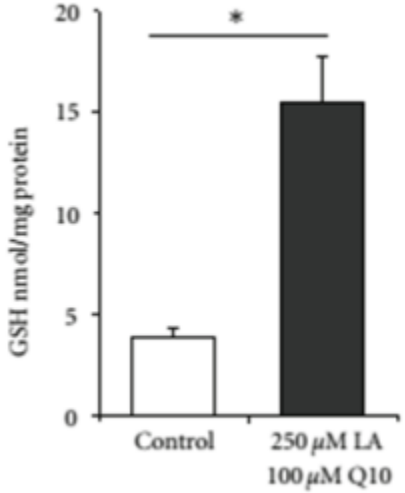


Glutathione Strategies

- Decrease depletion
- Directly administer
- Promote production
- Lifestyle

Glutathione: Decrease Depletion

- Decrease utilization
  - Decrease toxic exposure, esp alcohol
- Decrease oxidative stress
  - Decrease oxidative markers, increase GSH
  - Alpha-lipoic acid for mitochondria
    - Increases GSH in skeletal muscle cells with CoQ10
  - Vitamin D for brain
  - Melatonin for brain



Wagner 2012; Creative commons

Addolorato G, et al. Effects of short-term moderate alcohol administration on oxidative stress and nutritional status in healthy males. Appetite. 2008 Jan;50(1):50-6

Liu J. The effects and mechanisms of mitochondrial nutrient alpha-lipoic acid on improving age-associated mitochondrial and cognitive dysfunction: overview. Neurochem Res 2008;33:194-203

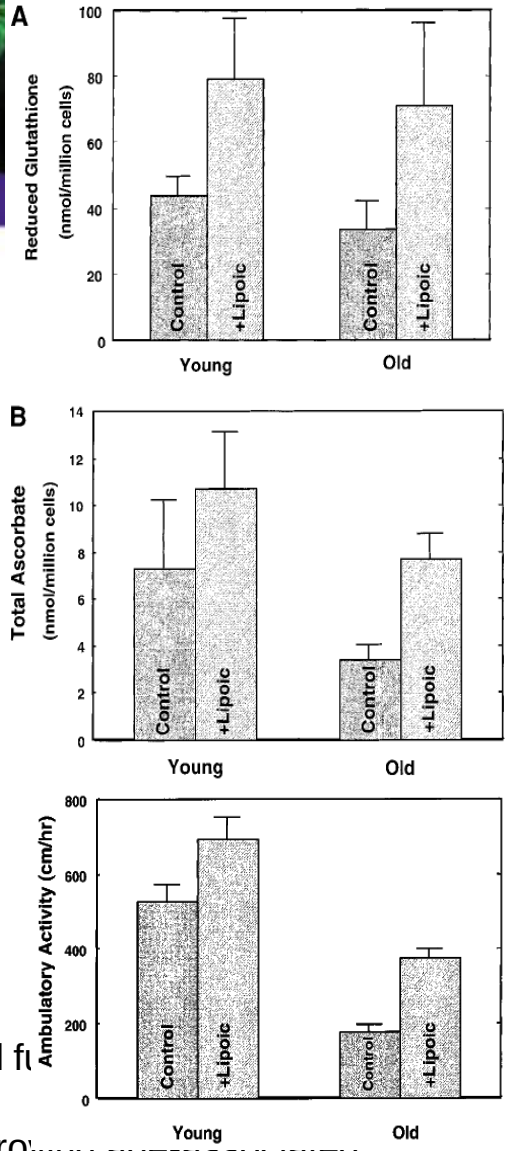
Garcion E, et al. New clues about vitamin D functions in the nervous system. Trends Endocrinol Metab. 2002 Apr;13(3):100-5

Herrera J,. Melatonin prevents oxidative stress resulting from iron and erythropoietin administration. Am J Kidney Dis. 2001 Apr;37(4):750-7

Wagner AE, et al. A combination of lipoic acid plus coenzyme Q10 induces PGC1α, a master switch of energy metabolism, improves stress response, and increases cellular glutathione levels in cultured C2C12 skeletal muscle cells. Oxid Med Cell Longev. 2012;2012:835970

Alpha Lipoic Acid

- Increases mitochondrial GSH
- Protects mitochondria from oxidants
- Improves mitochondrial function
- Up-regulates Phase II enzymes that provide additional support against oxidative damage
- Decreases age-associated decline of memory
- Benefit for migraine prophylaxis



Hagen TM, et al. (R)-Lipoic acid-supplemented old rats have improved mitochondrial fu
damage, and increased metabolic rate. FASEB J 1999;13:411-8

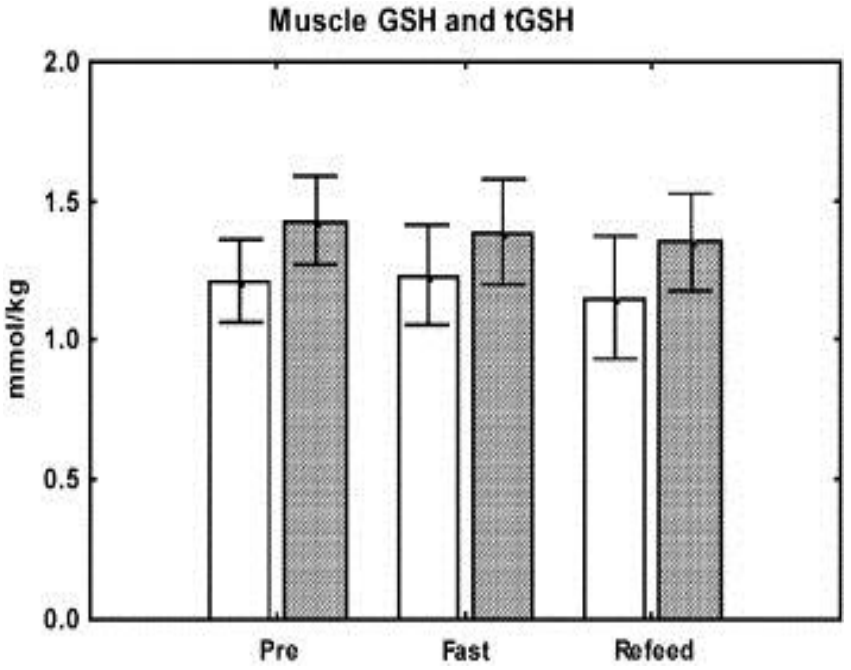
Liu J. The effects and mechanisms of mitochondrial nutrient alpha-lipoic acid on improving age-associated mitochondrial and cognitive dysfunction: overview. Neurochem Res 2008;33:194-203

Magis D, et al. A randomized double-blind placebo-controlled trial of thioctic acid in migraine prophylaxis. Headache. 2007 Jan;47(1):52-7.

Wagner AE, et al. A combination of lipoic acid plus coenzyme Q10 induces PGC1α, a master switch of energy metabolism, improves stress response, and increases cellular glutathione levels in cultured C2C12 skeletal muscle cells. Oxid Med Cell Longev. 2012;2012:835970

Does Fasting Deplete Glutathione?

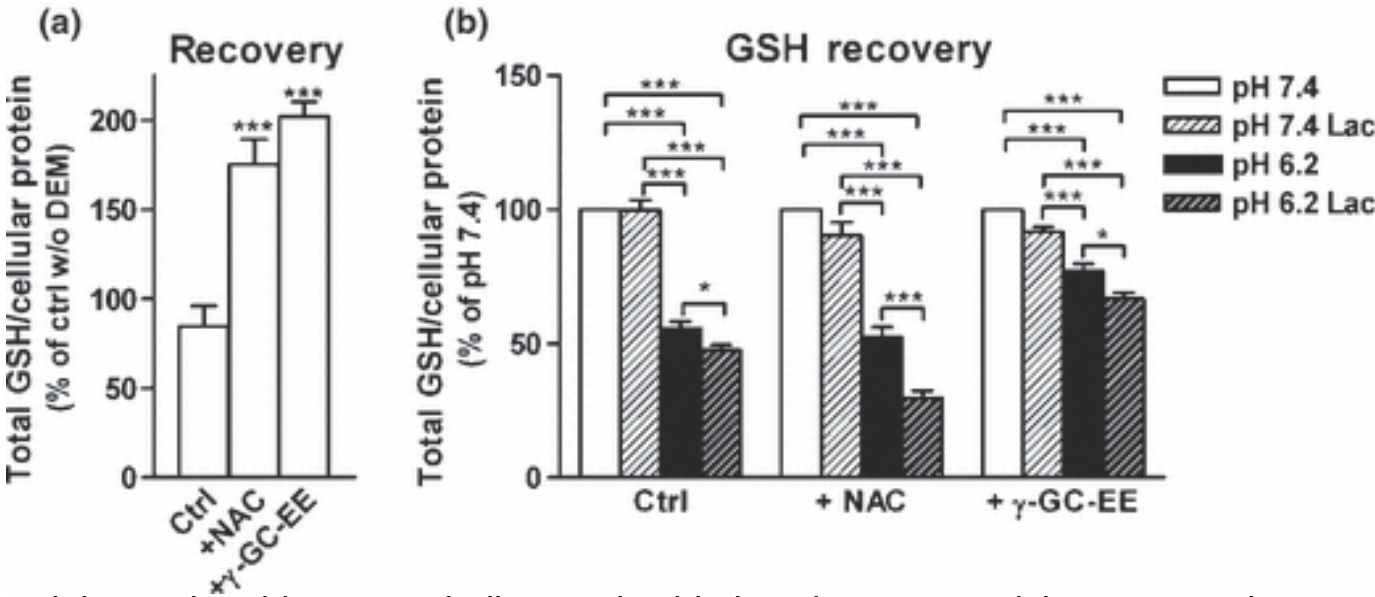
- Increases release of toxins, such as PCBs
- Depletes glutathione?
  - Not short term



Imamura M, Tung T: A trial of fasting cure for PCB poisoned patients in Taiwan. Am J Ind Med 5:147-53, 1984
Shakman RA: Nutritional influences on the toxicity of environmental pollutants: A review. Arch Env Health 28:105-33, 1974
Hammarqvist F, et al. Free amino acid and glutathione concentrations in muscle during short-term starvation and refeeding. Clin Nutr. 2005;24:236-43

Dietary Acidosis Impairs Glutathione

- Cell-based study
- Acidosis inhibits glutathione synthesis on multiple levels
- Inhibits cystine import (rate limiting AA)
- Also inhibits GSH synthesis by impairing GCL (pH optimum of 8.2–8.6)
- Gamma-glutamylcysteine ethyl ester (GCEE) looks promising for acidosis



Lewerenz J, et al. Lactacidosis modulates glutathione metabolism and oxidative glutamate toxicity. J Neurochem. 2010 Apr;113(2):502-14.

Dietary Acidosis & GSH

- Perhaps just as importantly, acidosis also impairs glutathione conjugation, i.e. binding of GSH to various organic substrates, and via the non-enzymatic coupling of GSH to organic molecules
- Acidosis may exacerbate GSH depletion during cell stress, depending on several factors, including depending on the relative rates of de novo-synthesis, recycling, export and consumption of GSH
- Thus, **acidosis impairs both synthesis & utilization of GSH!**

Lewerenz J, et al. Lactacidosis modulates glutathione metabolism and oxidative glutamate toxicity. J Neurochem. 2010 Apr;113(2):502-14.

Glutathione: Direct Administration

- IV glutathione
- Nebulized glutathione
- Oral glutathione
- Oral liposomal glutathione
- Topical glutathione
- Intranasal glutathione

Witschi A, et al. The systemic availability of oral glutathione. Eur. J. Clin. Pharmacol 1992;43(6): 667–9

Cooke RW, Drury JA. Reduction of oxidative stress marker in lung fluid of preterm infants after administration of intra-tracheal liposomal glutathione. Biol Neonate. 2005;87(3):178-80

Buhl R, et al. Augmentation of glutathione in the fluid lining the epithelium of the lower respiratory tract by directly administering glutathione aerosol. Proc Natl Acad Sci USA 1990;87:4063–7

Kern JK, et al. A clinical trial of glutathione supplementation in autism spectrum disorders. Med Sci Monit. 2011 Dec;17(12):CR677-82.

Glutathione – IV

- Extremely short half-life in the plasma
- High blood levels may carry mercury from blood into brain (animal research)
- Parkinson’s disease (symptomatic improvement)
- Peripheral artery disease (improved pain-free walking distance and several markers of circulation)
- Study comparing IV glutathione to IV N-acetylcysteine found the former to be more effective in preventing contrast-induced nephropathy

Sechi G, et al. Reduced intravenous glutathione in the treatment of early Parkinson's disease. Prog Neuropsychopharmacol Biol Psychiatry. 1996 Oct;20(7):1159-70.
Hauser RA, et al. Randomized, double-blind, pilot evaluation of intravenous glutathione in Parkinson's disease. Mov Disord. 2009 May 15;24(7):979-83.
Arosio E, et al. Effect of glutathione infusion on leg arterial circulation, cutaneous microcirculation, and pain-free walking distance in patients with peripheral obstructive arterial disease: a randomized, double-blind, placebo-controlled trial. Mayo Clin Proc. 2002 Aug;77(8):754-9.
Saitoh T, et al. Intravenous glutathione prevents renal oxidative stress after coronary angiography more effectively than oral N-acetylcysteine. Heart Vessels. 2011 Sep;26(5):465-72

Glutathione - Nebulized

- Elevates local and systemic levels
- In cystic fibrosis found to improve several clinical indicators, such as peak flow.
- Case reports of success in emphysema

Bishop C, Hudson VM, Hilton SC, Wilde C. A pilot study of the effect of inhaled buffered reduced glutathione on the clinical status of patients with cystic fibrosis. Chest. 2005 Jan;127(1):308-17.
Lamson DW, Brignall MS. The use of nebulized glutathione in the treatment of emphysema: a case report. Altern Med Rev. 2000 Oct;5(5):429-31

Glutathione – Oral

- 3 g oral showed no change in plasma glutathione
- Recent trial @ Bastyr:
  - 500 mg bid to 40 healthy volunteers, randomized & double-blinded, placebo-controlled – 4 weeks long
  - Measured RBC GSH & GSSG (and ratio), as well as urinary markers of oxidative stress (F2-isoprostanes and 8-hydroxy-2′-deoxyguanosine)
  - Analyzed and contained >98% reduced glutathione, <1.4% oxidized glutathione, and was free of microbial contamination and heavy metals.
  - **No significant changes in any parameter measured**
- Recent trial @ Penn State:
  - 6 months long, using 250-1000 mg GSH
  - GSH levels were increased 30–35% in RBC, plasma, and lymphocytes, and 260% in buccal cells at higher dosage NK cytotoxicity up 2x
  - Recently published

Witschi A, et al. The systemic availability of oral glutathione. Eur. J. Clin. Pharmacol 1992;43(6): 667–9
Allen J, Bradley RD. Effects of oral glutathione supplementation on systemic oxidative stress biomarkers in human volunteers. J Altern Complement Med. 2011 Sep;17(9):827-33.
Richie, et al. Enhanced Glutathione Levels in Blood and Buccal Cells by Oral Glutathione Supplementation. (The FASEB Journal. 2013;27:862.32)

Lipoceutical & Transdermal - Glutathione

- A small study of children with autism spectrum disorders found that both oral lipoceutical and transdermal glutathione had some efficacy in improving plasma reduced glutathione levels.

Kern JK, et al. A clinical trial of glutathione supplementation in autism spectrum disorders. Med Sci Monit. 2011 Dec;17(12):CR677-82

Glutathione - Intranasal

- Intriguing as does not transport Hg from blood
- Lung absorption documented (very effective)
- Children with chronic otitis media with effusion given glutathione as a nasal aerosol had improvement in 67% versus 8% of controls
- Uncontrolled survey found 78.8% success for multiple chemical sensitivity, allergies/sinusitis, Parkinson disease, Lyme disease, fatigue and “other.” 12.1% reported ADRs.
- MRI showed 53% increase in brain after 20 mg intranasal (unpublished)

Testa B, et al. Management of chronic otitis media with effusion: the role of glutathione. Laryngoscope. 2001 Aug;111(8):1486-9
Mischley LK, et al. Safety survey of intranasal glutathione. J Altern Complement Med. 2013 May;19(5):459-63

Glutathione: Increase Production

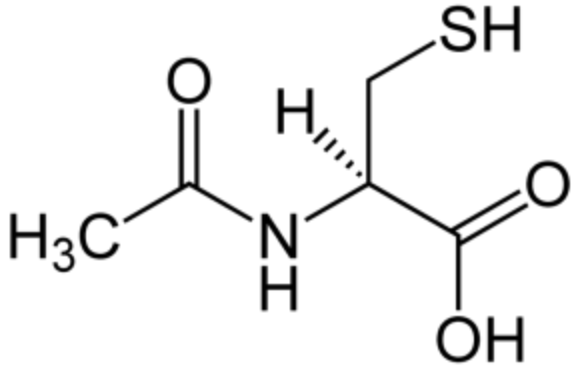
- Silymarin
  - Standardized extract, 100 mg tid
- NAC (also directly binds methyl-Hg)
  - 300-1000 mg bid
- Whey powder
  - 15 g bid
- SAMe
  - Not methionine as it also increases homocysteine

Soltan-Sharifi MS, et al. Improvement by N-acetylcysteine of acute respiratory distress syndrome through increasing intracellular glutathione. Hum Exp Toxicol. 2007;26(9):697-703

Micke P, et al. Oral supplementation with whey proteins increases plasma glutathione levels of HIV-infected patients. Eur J Clin Invest. 2001;31(2):171-8

Jariwalla RJ, et al. Restoration of blood total glutathione status and lymphocyte function following alpha-lipoic acid supplementation in patients with HIV infection. J Alt Comp Med. 2008;14(2):139-46

Liber CS, Packer L (November 2002). "S-Adenosylmethionine: molecular, biological, and clinical aspects—an introduction". Am J Clin Nutr. 76 (5): 1148S–50S



NAC Elevates Glutathione

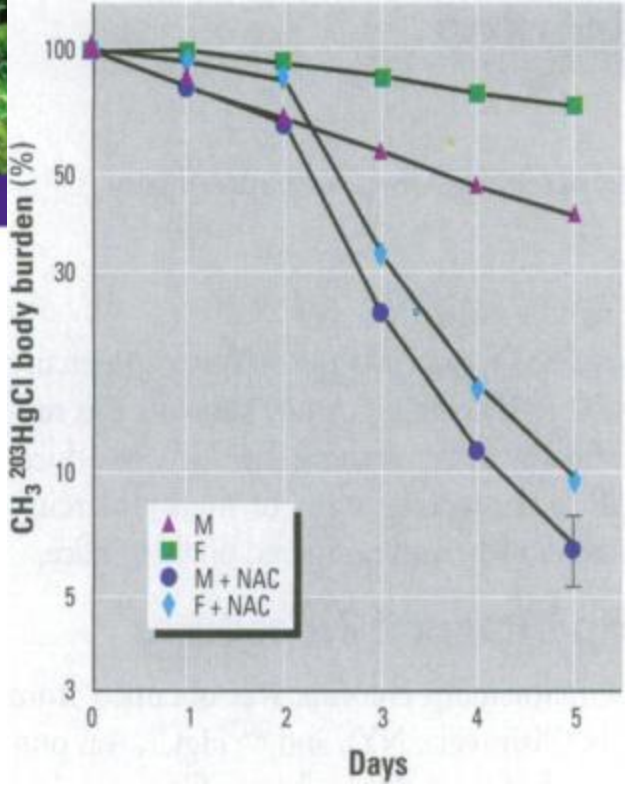
- N-acetylcysteine
- Amino acid cysteine is a rate-limiting factor for GSH synthesis
- Variety of both clinical trials and in-vitro/in-vivo data suggest that supplying cysteine as NAC is an effective strategy for enhancing GSH production and intracellular cysteine.
- Increases intracellular glutathione

Dodd S, Dean O, Copolov DL, Malhi GS, Berk M. N-acetylcysteine for antioxidant therapy: pharmacology and clinical utility. Expert Opin Biol Ther. 2008 Dec;8(12):1955-62

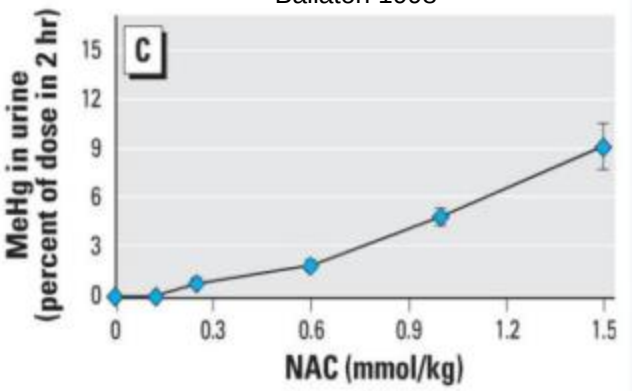
Soltan-Sharifi MS, et al. Improvement by N-acetylcysteine of acute respiratory distress syndrome through increasing intracellular glutathione, and extracellular thiol molecules and anti-oxidant power: evidence for underlying toxicological mechanisms. *Hum Exp Toxicol. 2007 Sep;26(9):697-703.*

NAC Also Excretes Hg

- Most research animal and human cell lines
- Multiple benefits:
  - Increases production of glutathione
  - Protects human neurological cells from Hg toxicity
  - Reverses damage to human pancreatic cells from Hg
  - Directly binds to Hg, esp. MeHg, and excrete through kidneys



Ballatori 1998



Aremu 2008

Aremu DA, et al. N-acetylcysteine as a potential antidote and biomonitoring agent of methylmercury exposure. Environ Health Perspect. 2008 Jan;116:26-31

Ballatori N, et al. N-acetylcysteine as an antidote in methylmercury poisoning. Environ Health Perspect. 1998 May;106:267-71

NAC Decreases GGT

- 600 mg/day for 4 weeks reduced GGT from 62.7 to 46.3 U/L.

Pamuk GE, Sonsuz A. N-acetylcysteine in the treatment of non-alcoholic steatohepatitis. J Gastroenterol Hepatol. 2003;18(10):1220-1

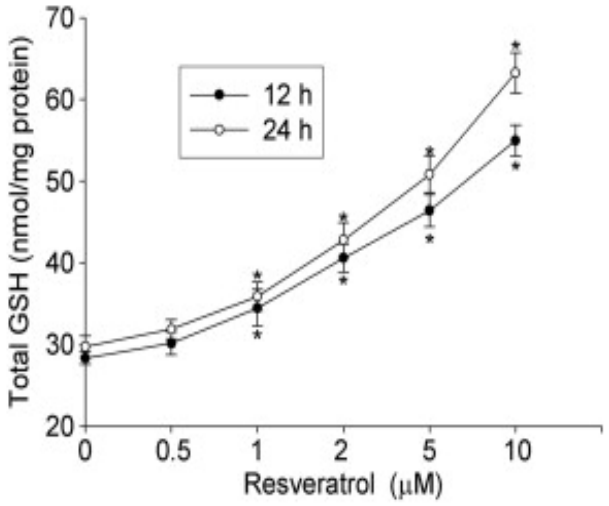
How About Beer (Alcohol-Free)?

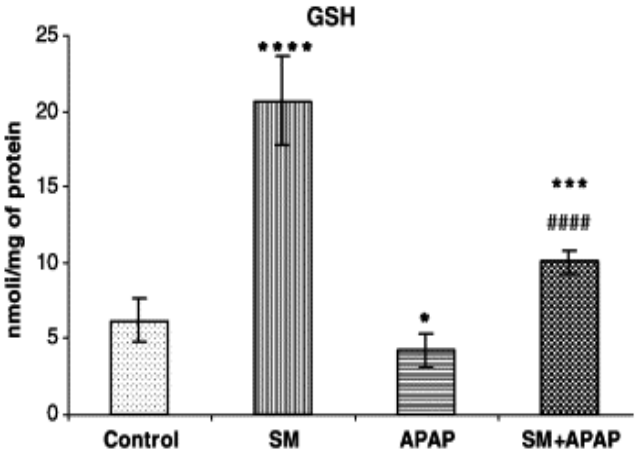
- 29 nuns, 58 to 73 y old, live in a convent with a disciplined, regular, and homogeneous lifestyle
- 500 mL/d of alcohol-free beer (0.0%) divided into two doses over 45-days
- 29% increase in RBC glutathione!

Martínez Alvarez JR, et al. Effects of alcohol-free beer on lipid profile and parameters of oxidative stress and inflammation in elderly women. Nutrition. 2009 Feb;25(2):182-7

Glutathione: Stimulate Production

- Resveratrol
  - 1 g/d (human study)
- Milk thistle/Silymarin
  - 166 mg bid in hepatitis C patients treated with antiviral drugs





Kode A, et al. Resveratrol induces glutathione synthesis by activation of Nrf2 and protects against cigarette smoke-mediated oxidative stress in human lung epithelial cells. Am J Physiol Lung Cell Mol Physiol. 2008 Mar;294(3):L478-88

Chow HH, et al. Resveratrol modulates drug- and carcinogen-metabolizing enzymes in a healthy volunteer study. Cancer Prev Res (Phila). 2010 Sep;3(9):1168-75

Nencini C, et al. Protective effect of silymarin on oxidative stress in rat brain. Phytomedicine. 2007 Feb;14(2-3):129-35

Purple Sweet Potato Leaves

- One reason a diet rich in colourful fruits and vegetables has been found to be protective against many diseases is its high content of polyphenols.
- Phenols strongly antioxidant and anti-inflammatory.
- Purple sweet potato leaves (PSPL), commonly consumed in Asia, are especially rich in polyphenols.
- Placebo-controlled study found that 200 g/d PSPL (containing 902 mg polyphenols) for 2 wks increased urinary total phenol excretion by 25%, increased RBC glutathione by 33% and decreased urinary 8-OHdG excretion by 37%.

Chen CM, et al. Consumption of purple sweet potato leaves decreases lipid peroxidation and DNA damage in humans. Asia Pac J Clin Nutr. 2008;17(3):408-14.

Almonds

- Study looked at the effect of almonds on DNA damage in young smokers.
- Found that at baseline the smokers had 3 times as much urinary 8-OHdG as the non-smokers (18.0 versus 6.3 umol/mol creatinine).
- Eating 83 g/d of almonds for 4 weeks resulted in an increase in the smokers' glutathione of 16% and a decrease in urinary 8-OHdG of 29%.
- Not enough to bring them to normal, but an important improvement.

Li N, Jia X, et al. Almond consumption reduces oxidative DNA damage and lipid peroxidation in male smokers. J Nutr. 2007 Dec;137(12):2717-22

Cruciferous Vegetables

- Cruciferous vegetables with intact glucosinolates boost glutathione levels and detoxifying enzyme activity
- Cooking the cabbage before enzyme activation from damaged cells eliminates most of benefit

Tarozzi A, et al. Sulforaphane as an inducer of glutathione prevents oxidative stress-induced cell death in a dopaminergic-like neuroblastoma cell line. Neurochem. 2009 Dec;111(5):1161-71

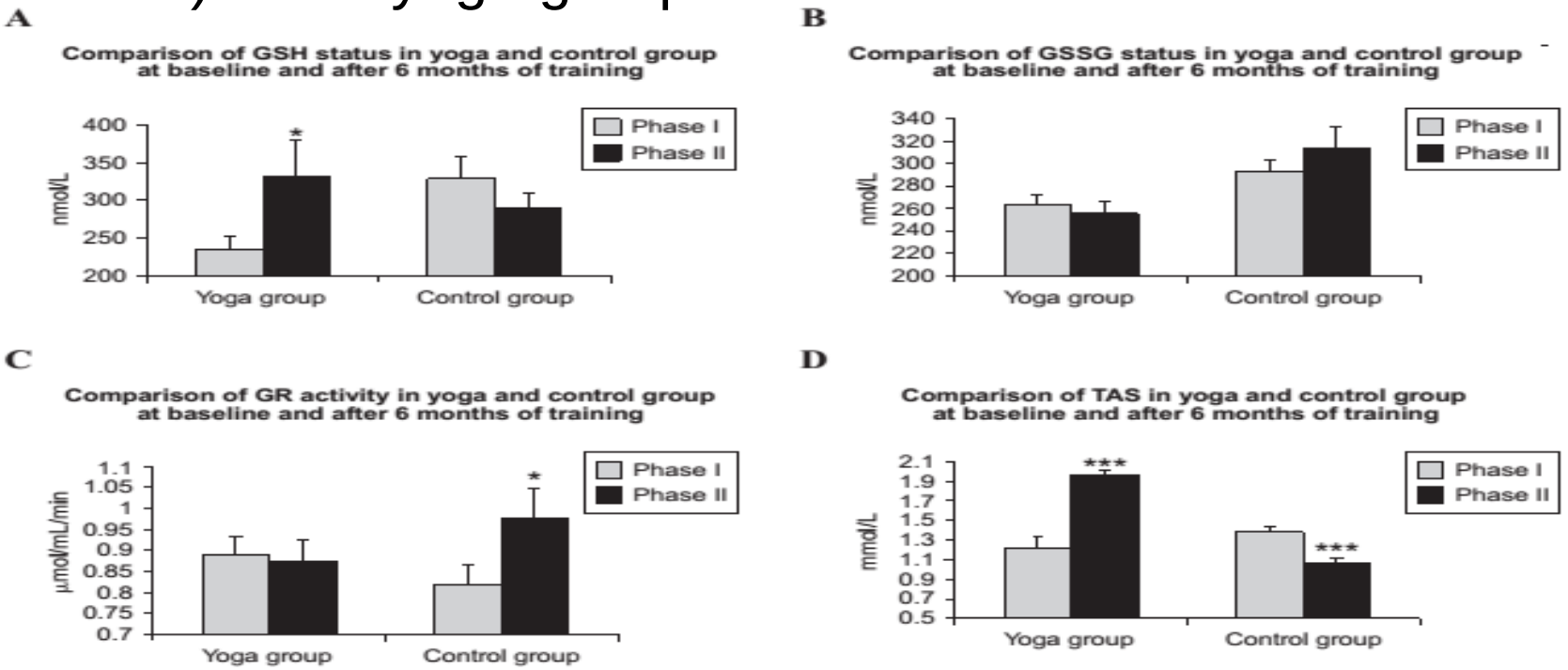
Glutathione & Meditation

- Forty-two Sudarshan Kriya practitioners (practiced at least 1 year) and 42 normal healthy controls – cross sectional study
- Controls and practitioners had the same socioeconomic status, comparable BMI, were vegetarians, and were nonsmokers
- Practitioners had higher glutathione levels – 76.7±4.06 nmol/ml in controls, and 96.5±4.41 in practitioners
- Also had higher antioxidant enzyme activities, and transcriptional level for glutathione peroxidase, catalase, and higher GST-P1 levels

Sharma H, et al. Gene expression profiling in practitioners of Sudarshan Kriya. J Psychosom Res. 2008 Feb;64(2):213-8

Glutathione & Yoga

- Healthy male volunteers, randomized to either 6 months yoga or routine physical training
- Reduced glutathione level increased significantly ($p < 0.05$) in the yoga group.



Sinha S, et al. Improvement of glutathione and total antioxidant status with yoga. J Altern Complement Med. 2007 Dec;13(10):1085-90

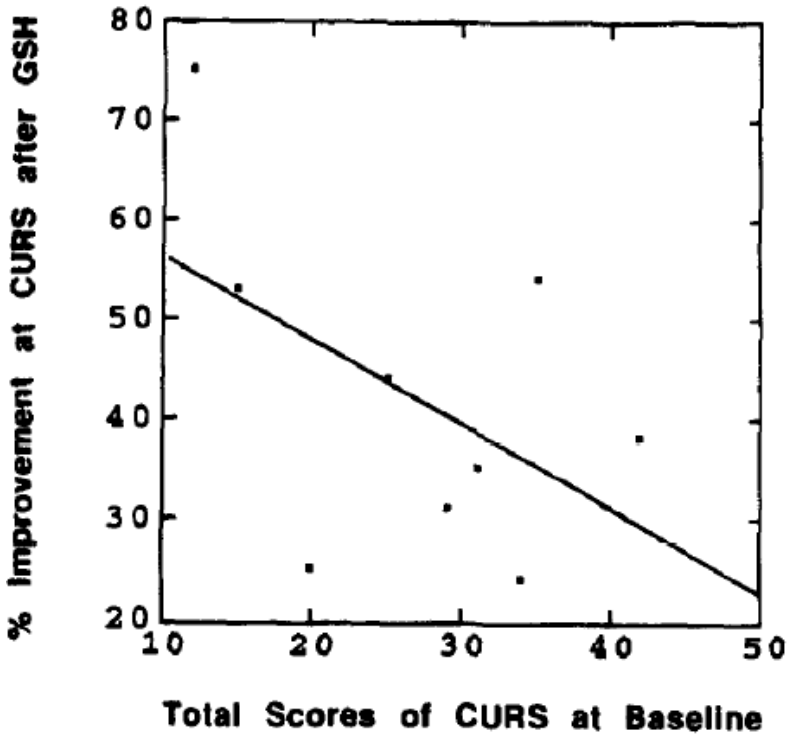
Glutathione & Exercise

- Hours/week of moderate exercise positively associated with blood glutathione levels (not excessive exercise!)
- **Aerobic and weight training combined more effective than either alone**

Rundle AG, et al. Preliminary studies on the effect of moderate physical activity on blood levels of glutathione. Biomarkers. 2005 Sep-Oct;10(5):390-400.
Elokda et al. Effects of exercise training on the glutathione antioxidant system. Eur J Cardiovasc Prev Rehabil. 2007 Oct;14(5):630-7.
Abdull Razis AF, et al. Intact glucosinolates modulate hepatic cytochrome P450 and phase II conjugation activities and may contribute directly to the chemopreventive activity of cruciferous vegetables. Toxicology. 2010 Nov 9;277(1-3):74-85.

IV Glutathione Protects Neurons

- 50% less glutathione (GSH) in the substantia nigra of Parkinson's patients
- But not in other parts of brain => used up in neutralization of local toxins
- GSH 600 mg IV bid x 30 days
  - 42% decline in disability
  - Lasted 2-4 months after stopped
- Protects both telomeres and mtDNA



Adapted from Johnson 2012; Creative Commons Attribution license

Perry TL, et al. Idiopathic Parkinson's disease: A disorder due to nigral glutathione deficiency. Neuroscience Letter 1986;67:269-74

Sechi G, et al. Reduced intravenous glutathione in the treatment of early Parkinson's disease. Prog Neuropsychopharmacol Biol Psychiatry 1996;20:1159-70

Johnson WM, et al. Dysregulation of glutathione homeostasis in neurodegenerative diseases. Nutrients. 2012 Oct 9;4(10):1399-440. doi: 10.3390/nu4101399.

NAC – Clinical Results

- Patients undergoing coronary artery bypass and/or valvular surgery, intravenous NAC reduced the incidence of post-operative atrial fibrillation
- Attenuates fatigue in endurance athletes in a small placebo-controlled trial
- Randomized double-blinded trial, NAC (600 mg twice per day) given with L-arginine (1200 mg per day) to diabetic men with hypertension lowered blood pressure, and improved many markers of endothelial function and inflammation, including C-reactive protein, fibrinogen, and LDL-cholesterol .
- 1 g twice per day significantly improve depression in patients with bipolar disorder

Ozaydin M, et al. N-acetylcysteine for the prevention of postoperative atrial fibrillation: a prospective, randomized, placebo-controlled pilot study. Eur Heart J. 2008 Mar;29(5):625-31.
Medved I, et al. N-acetylcysteine enhances muscle cysteine and glutathione availability and attenuates fatigue during prolonged exercise in endurance-trained individuals. J Appl Physiol. 2004 Oct;97(4):1477-85.
Martina V, et al. Long-term N-acetylcysteine and L-arginine administration reduces endothelial activation and systolic blood pressure in hypertensive patients with type 2 diabetes. Diabetes Care. 2008 May;31(5):940-4.
Berk M, et al. The efficacy of N-acetylcysteine as an adjunctive treatment in bipolar depression: an open label trial. J Affect Disord. 2011 Dec;135(1-3):389-94.

NAC – Clinical Results

- 1.2 g per day improving physical performance among COPD patients in a randomized trial.
- Meta-analysis of use in COPD found that it reduced occurrence of exacerbations by half over the treatment period, an effect somewhat attenuated by simultaneous steroid use, but not active smoking.
- Men with idiopathic infertility given 600 mg per day had improvement in several semen parameters as well as plasma antioxidant status.
- A small pilot study of women within 5 years of menopause given 2 g NAC per day suggests it may reduce bone resorption.

Stav D, Raz M. Effect of N-acetylcysteine on air trapping in COPD: a randomized placebo-controlled study. Chest. 2009 Aug;136(2):381-6.

Sutherland ER, et al. N-acetylcysteine and exacerbations of chronic obstructive pulmonary disease. COPD. 2006 Dec;3(4):195-202.

Ciftci H, et al. Effects of N-acetylcysteine on semen parameters and oxidative/antioxidant status. Urology. 2009 Jul;74(1):73-6.

Sanders KM, et al. Potential role of the antioxidant N-acetylcysteine in slowing bone resorption in early post-menopausal women: a pilot study. Transl Res. 2007 Oct;150(4):215.

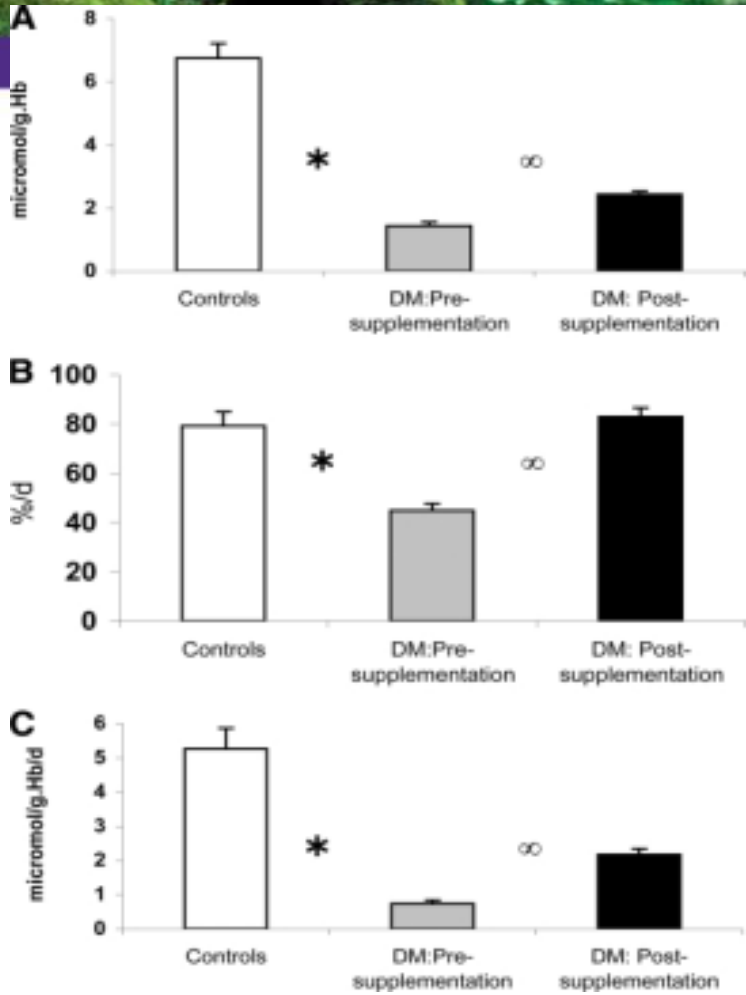
Clinical Outcomes of GSH-based Therapy

- Diabetic hypertensive men (randomized placebo-controlled)
  - NAC (600 mg bid) given with L-arginine (1200 mg qd)
  - Lowered blood pressure, and improved many markers of endothelial function and inflammation, including C-reactive protein, fibrinogen, and LDL-cholesterol
- Bipolar disorder
  - NAC 1 g bid significantly improved depression
- COPD
  - NAC 1.2 g qd improved physical performance

Martina V et al. Long-term N-acetylcysteine and L-arginine administration reduces endothelial activation and systolic blood pressure in hypertensive patients with type 2 diabetes. Diabetes Care. 2008 May;31(5):940-4.
Berk M, et al. The efficacy of N-acetylcysteine as an adjunctive treatment in bipolar depression: an open label trial. J Affect Disord. 2011 Dec;135(1-3):389-94.
Stav D et al. Effect of N-acetylcysteine on air trapping in COPD: a randomized placebo-controlled study. Chest. 2009 Aug;136(2):381-6.

Clinical Outcomes of GSH-based Therapy

- NAC and glycine
  - Poorly controlled type 2 diabetes
  - Markers of glutathione synthesis and plasma oxidative stress were both improved
  - 0.81 mmol/kg/day of cysteine (given as n-acetylcysteine) and 1.33 mmol/kg/day of glycine
- NAC and glycine
  - Completely restored GSH synthesis and concentration in elderly
  - Same dose as above



A. Erythrocyte GSH concentrations B: GSH fractional synthesis rate C: GSH absolute synthesis rate

Sekhar et al. Glutathione synthesis is diminished in patients with uncontrolled diabetes and restored by dietary supplementation with cysteine and glycine. Diabetes Care. 2011 Jan;34(1):162-7.

Sekhar et al. Deficient synthesis of glutathione underlies oxidative stress in aging and can be corrected by dietary cysteine and glycine supplementation. Am J Clin Nutr. 2011 Sep;94(3):847-53.

In Conclusion

1. Hard to overstated the critical role of glutathione in promoting and maintaining health
2. Depletion of glutathione associated with most chronic disease
3. Inflammation and toxin exposure deplete glutathione causing up regulation of production and regeneration
4. GGT is a useful measure of toxin exposure and glutathione need
5. Promoting glutathione production and regeneration yields many health benefits.
6. NAC especially effective in increasing glutathione with documented clinical benefit