Testing for Hidden Causes of Disease: Detoxification and Optimization

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Overview

1. Types and sources of toxin exposure
2. How toxins cause damage
3. Clinical consequences of toxin exposure
4. Detoxification overview
5. Evaluation of exposure and detoxification
6. Treatment recommendations

Detoxification

- Integral part of many natural healing paradigms, especially:
  - Ayurvedic medicine from India
  - Naturopathic medicine from USA
  - Traditional health spa
- Old naturopathic adages:
  - “When in doubt, detoxify the liver”
  - “Disease begins in the colon”

Types of Toxins

Environmental

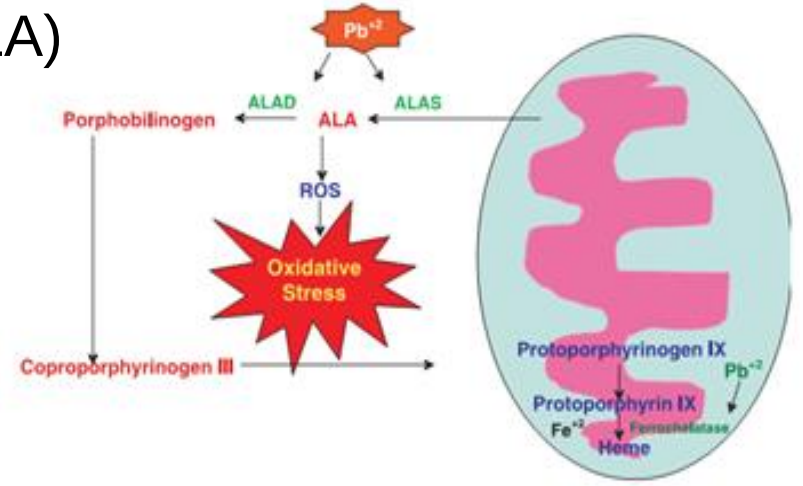
- Metals
- Chemicals
  - Inorganic
  - Organic
  - Persistent
  - Drugs
    - Recreational
    - Prescription
- Microbial
- Radiation

Endogenous

- Non-end product metabolites
- Non-optimal detoxification of hormones
- Gut-derived

Heavy Metals

- Lead, mercury, arsenic, and cadmium among most toxic and prevalent
- Multiple & overlapping mechanisms of toxicity
  - Increase free radical production
  - Poison enzymes (i.e. lead and ALA)
  - Direct DNA damage
  - Endocrine disruption
  - Mitochondrial or cell wall damage



Flora 2008

Flora SJ Heavy metal induced oxidative stress & its reversal by chelation therapy. Indian J Med Res. 2008

Lead Prevalence

- No threshold for safety –
  - Children who had **whole blood lead concentrations of <5 µg/dL (but > zero) associated with decreased IQ**
  - 2.4 million children at levels between 5 and 9. ug/dL
  - NHANES III: In 1999-2002, **91.7% of childre** in US had detectable levels of lead in the blood
  - July 2012: CDC changed recommended level to intervene in children from 10 ug/dL to 5.0. Also eliminated term “level of concern”, to avoid giving false sense of safety

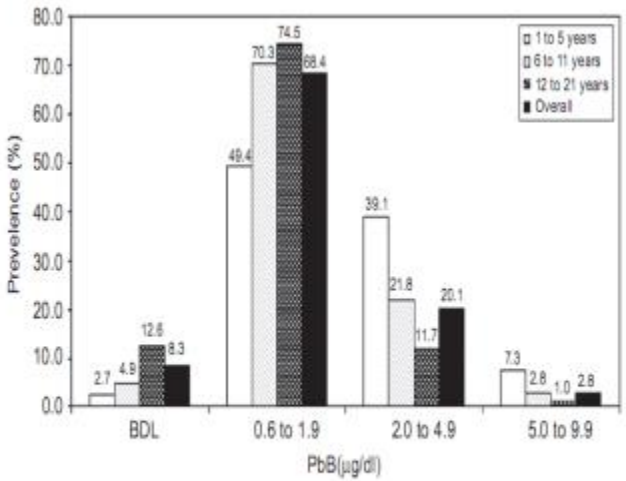


Fig. 1. Blood lead levels below 10µg/dl among 1–21 years old US population 1999–2002.

Iqbal 2008

Iqbal S, et al. Estimated burden of blood lead levels 5 microg/dl in 1999-2002 and declines from 1988 to 1994. Environ Res. 2008
http://www.cdc.gov/nceh/lead/acclpp/cdc_response_lead_exposure_recs.pdf

Lead in Adults

- 100% of Canadian population had detectable blood lead in national study
- Even among adults, **a BLL of 5-9 ug/dL has been associated with an increased risk of death from all causes, cardiovascular disease, and cancer**

Bushnik T, et al. Lead and bisphenol A concentrations in the Canadian population. Health Rep. 2010 Sep;21(3):7-18.
Schober SE, Mirel LB, Graubard BI, et al. Blood lead levels and death from all causes, cardiovascular disease, and cancer: results from the NHANES III mortality study. Environ Health Perspect. 2006 Oct;114(10):1538-41.

Mercury Toxicity

- Enzyme poisoning
- Oxidative stress
- Tissue damage
  - **Neurological especially susceptible**
  - Inhibits formation of myelin
  - Significant lag time (weeks to months) before symptoms occur
- Organ dysfunction
  - Brain, kidneys especially susceptible
  - **Fetus and infants especially susceptible**

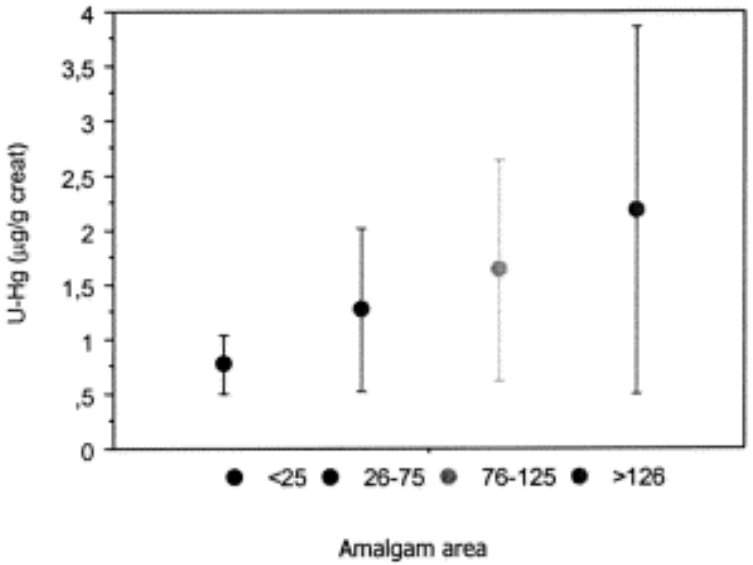
Sources of Mercury

- Average exposure in non-industrial populations
  - Amalgams: 10 ug/d
  - Fish: 2.3 ug/d
  - Water: 0.3 ug/d
  - Air
  - Vaccinations
- Industrial

Vimy, M.J., and Lorscheider, F.L (1990) Dental amalgam mercury daily dose estimated from intra-oral vapor measurements: A predictor of mercury accumulation in human tissues.]. Trace Elem. Exp. Med 3, 111-123

Amalgams

- Typical amalgam is 55% Hg
  - = **400 mg/filling**
- Release 10 ug/d
- Hg excretion proportional to surface area (poorer correlation with count)
- Release elemental Hg which is methylated by bacteria in gut and absorbed
- **Amalgam area, not # of amalgams**

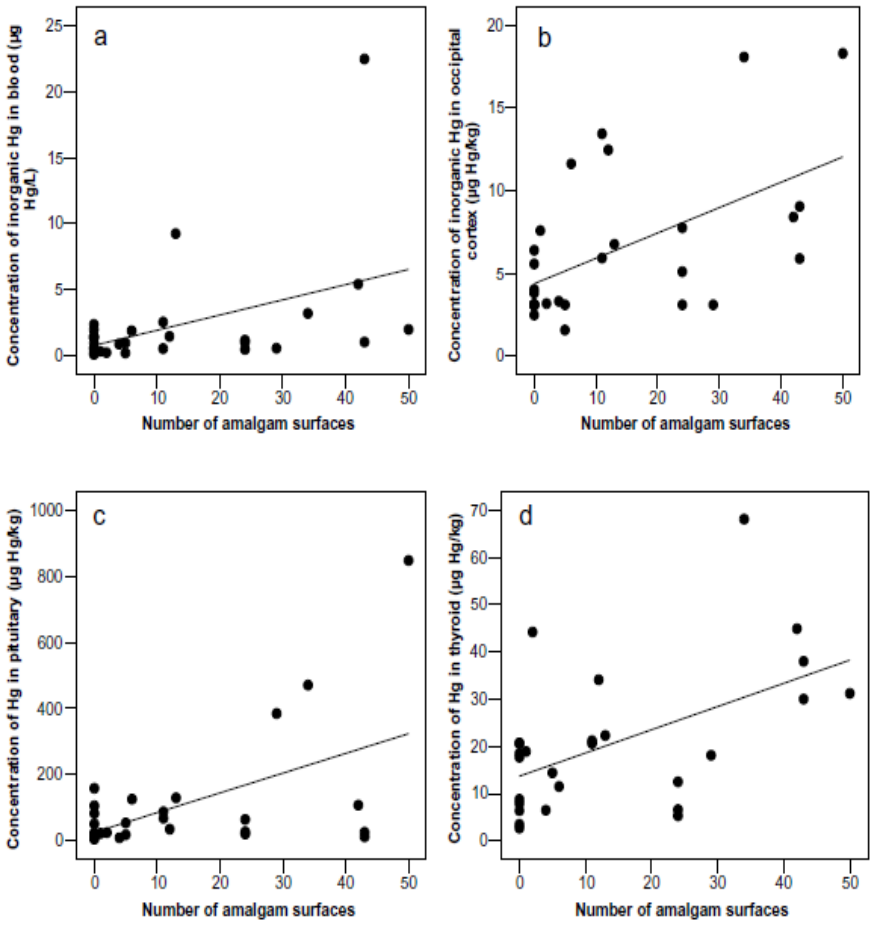


Lorscheider FL, Vimy MJ, Summer O. Mercury exposure from “silver” tooth fillings: emerging evidence questions a traditional dental paradigm. FASEBJ. 9,504-508(1995)
Apostoli P, ICortesi I, Mangili A, et al. Assessment of reference values for mercury in urine: the results of an Italian polycentric study. The Science of the Total Environment 289 (2002)13-24

What Autopsies Tell Us

- **Mercury accumulates in the brain in proportion to surface area**

- Study of 18 cadavers
  - Hg in brain, thyroid and kidneys proportional to the number of amalgam surfaces
  - For those with more than 12, Hg in brain disproportionately higher
  - Suggests that at higher levels of exposure the brain's mercury excretion pathways become overloaded.



J.W. Reinhardt. Side-Effects: Mercury Contribution to Body Burden From Dental Amalgam. Adv. Dent. Res. 1992; 6; 110
Guzzi G, et al. Dental amalgam and mercury levels in autopsy tissues. Am J Forensic Med Pathol. 2006 Mar;27(1):42-5

What Live Tissues Tell Us

- Donated kidneys for transplant
- R = 0.62 correlation with number of amalgam surfaces
- **6% increase in kidney Hg per amalgam surface**

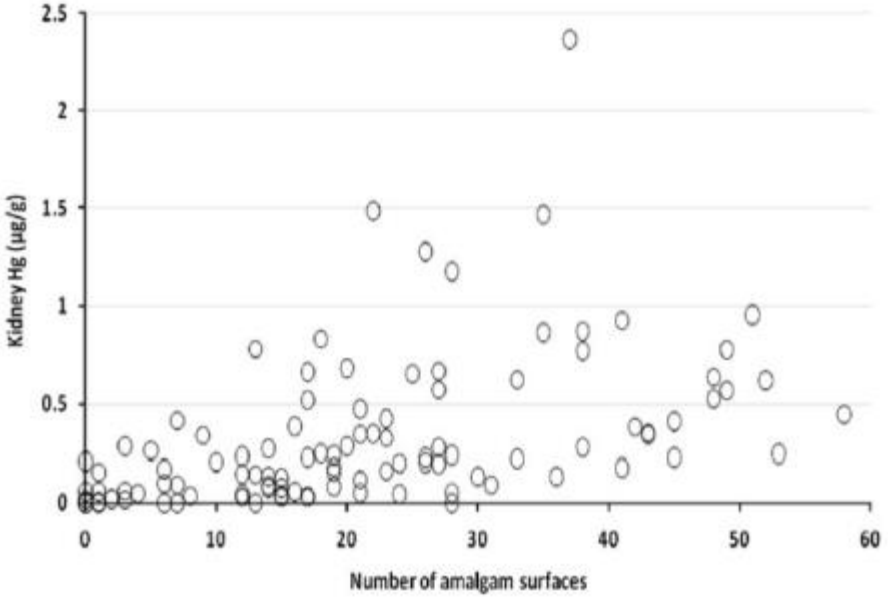


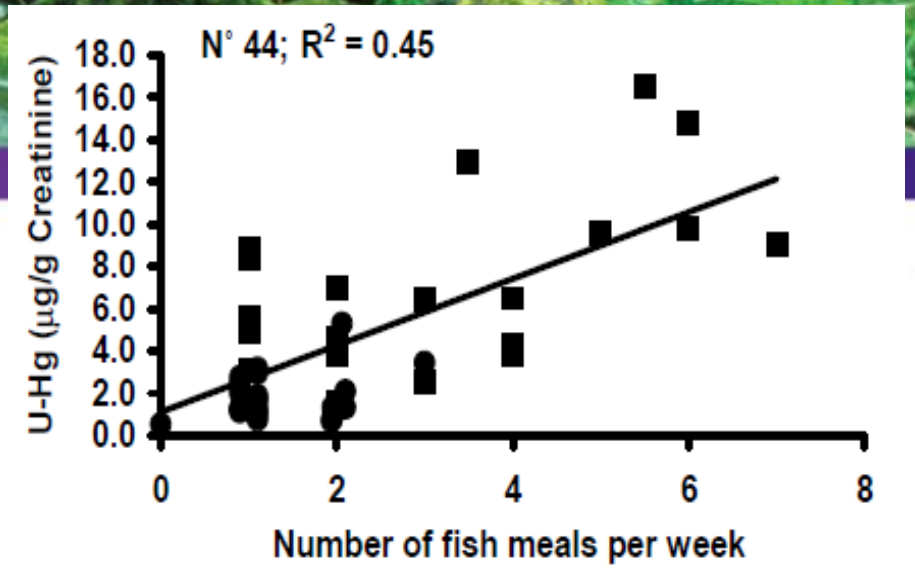
Fig. 3. Kidney mercury levels (µg/g wet weight) in living kidney donors vs. number of amalgam surfaces.

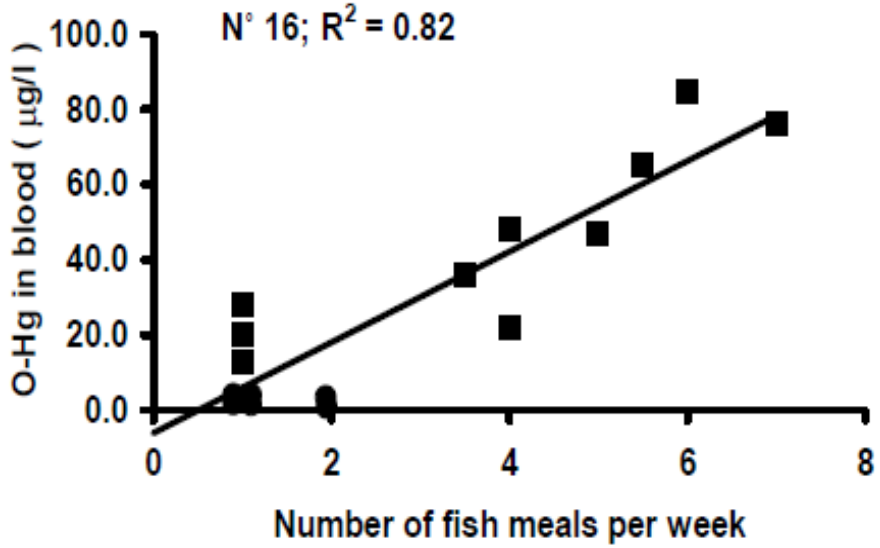
Barregard 2010

Barregard L, et al. Cadmium, mercury, and lead in kidney cortex of living kidney donors: Impact of different exposure sources. Environ Res. 2010 Jan;110(1):47-54

Hg From Fish

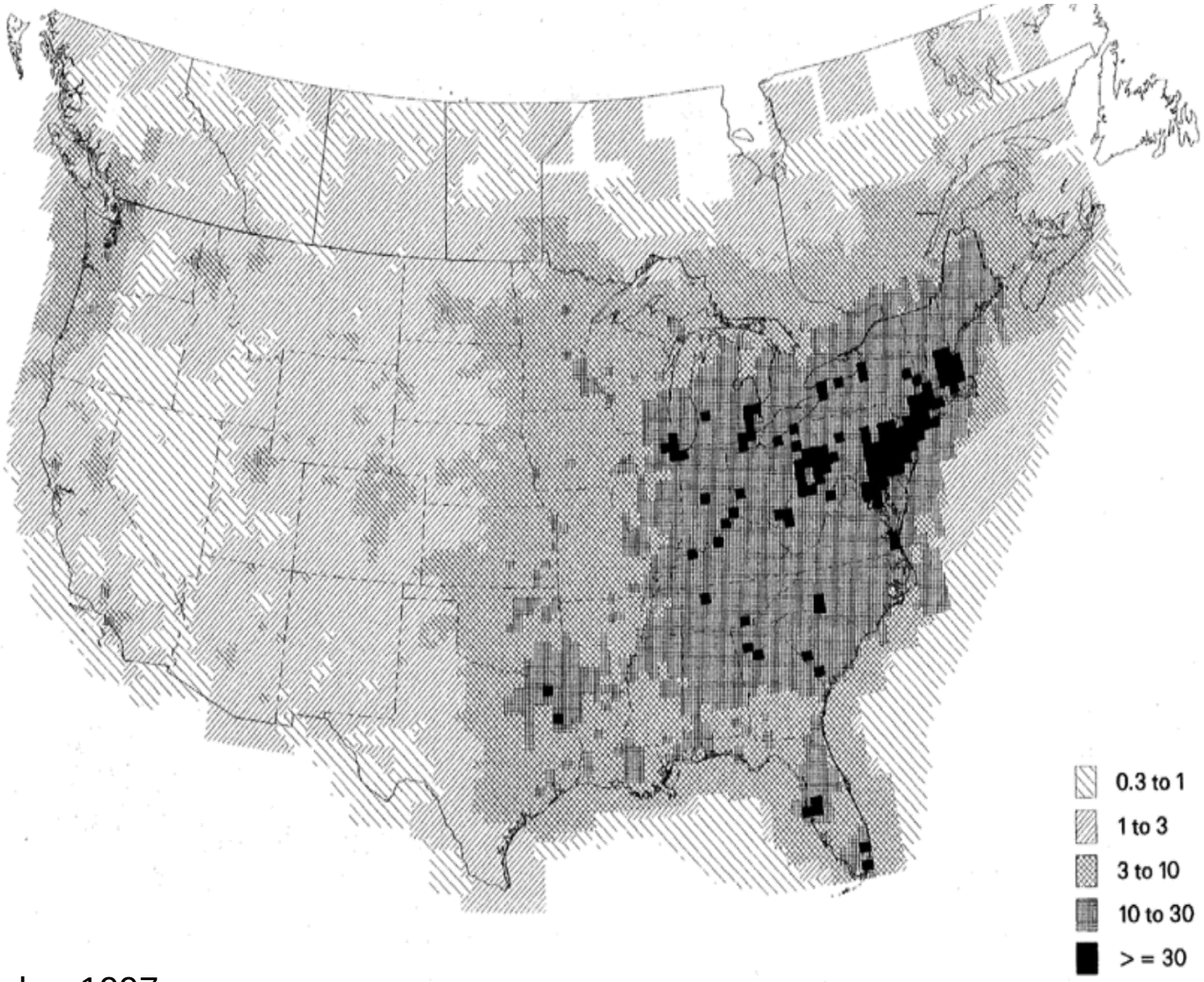
- **Total Hg urinary excretion proportional to amount of fish eaten**
- Impaired psychomotor performance
  - R = 0.38 blood
  - R = 0.77 urine
- Huge variation in amount of Hg in fish





Apostoli P, ICortesi I, Mangili A, et al. Assessment of reference values for mercury in urine: the results of an Italian polycentric study. The Science of the Total Environment 289 (2002)13-24
Carta P, et al. Sub-clinical neurobehavioral abnormalities associated with low level of mercury exposure through fish consumption. NeuroToxicology 24 (2003) 617–623

Mercury in the Air



EPA-452/R-97-003 December 1997

Hg Concentrates in Fetus

- Both MeHg and elemental Hg
- MeHg 100% higher in cord blood
- Cord blood Hg correlates with # of maternal amalgams, r = 0.46
- **Fetal brain 40% higher than maternal brain AND more sensitive to damage**

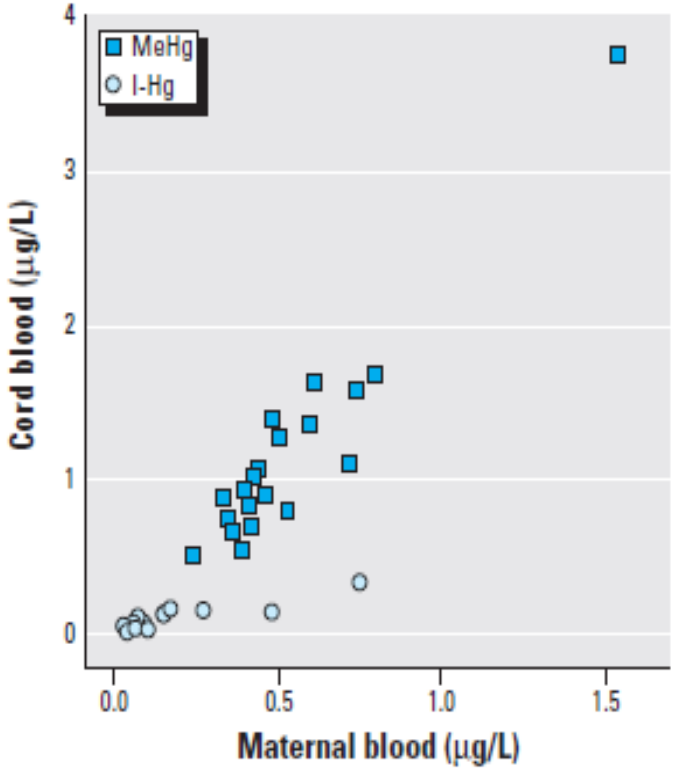


Figure 2. The associations between concentrations in cord blood and maternal blood for MeHg ($r = 0.95$; $p < 0.001$) and I-Hg ($r_S = 0.77$; $p < 0.001$).

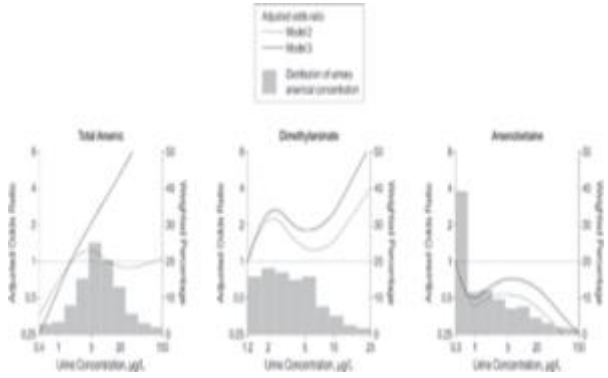
Björnberg 2005

Palkovicova L, et al. Maternal amalgam dental fillings as the source of mercury exposure in developing fetus and newborn. J Expo Sci Environ Epidemiol. 2008 May;18(3):326-31

Björnberg KA, et al. Transport of methylmercury and inorganic mercury to the fetus and breast-fed infant. Environ Health Perspect. 2005 Oct;113(10):1381-5

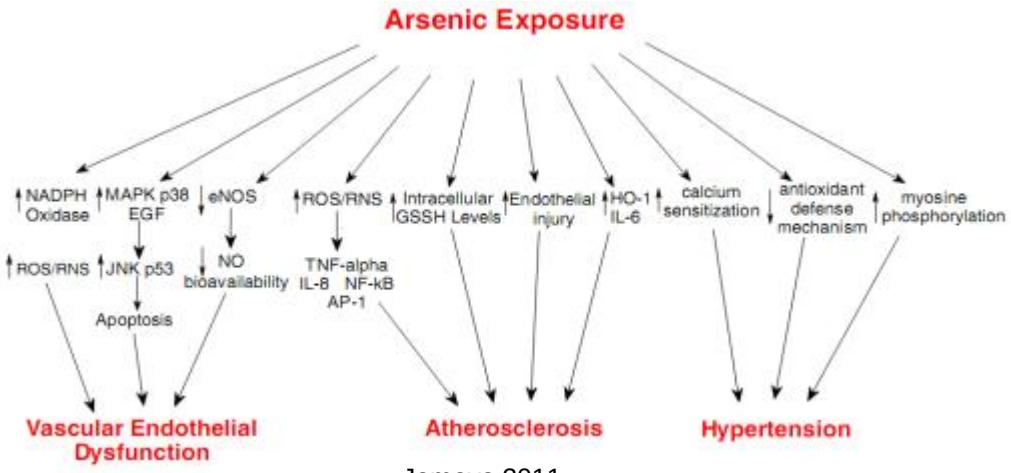
Arsenic Clinical Consequences

- **Inorganic organic associated with risk for diabetes, with no obvious threshold for safety**



Navas-Acien 2008

- Arsenic linked to increased **CVD, cancer, dermal disease, and gastrointestinal disease**



Jomova 2011

Navas-Acien A, et al. Arsenic exposure and prevalence of type 2 diabetes in US adults. JAMA. 2008

Jomova K, et al. Arsenic: toxicity, oxidative stress and human disease. J Appl Toxicol. 2011 Mar;31(2):95-107.

Arsenic and Cancer

- Associated with multiple cancer types
  - Population with low/moderate exposure
    - ~**3-5 fold risk for lung cancer** (small cell & squamous carcinoma) in those with higher levels of toenail arsenic
  - Also evidence for bladder, renal, and liver cancer
- Trivalent arsenicals appear to be much more toxic than pentavalent
  - Detoxification routes not only methylate arsenic, but in the process create trivalent forms – potential problem

Heck JE, et al. Lung cancer in a U.S. population with low to moderate arsenic exposure. Environ Health Perspect. 2009 Nov;117(11):1718-23.

Arsenic Exposure

- Arsenic – main exposure through **diet & water**
- **13 million in US exposed to public water which exceeds EPA limit of 10ug/L**
- **Seafood, rice, mushrooms and poultry** are main food sources, with seafood primarily arsenobetaine (organic form, considered less toxic)
  - Organic rice recently found to be risk for inorganic exposure
- Inorganic considered much more toxic than organic
- Toxic to the majority of organ systems, the most sensitive target organs being the kidney & liver

Jomova K, et al. Arsenic: toxicity, oxidative stress and human disease. J Appl Toxicol. 2011 Mar;31(2):95-107.
Holtcamp W 2012. Suspect Sweetener: Arsenic Detected in Organic Brown Rice Syrup. Environ Health Perspect 120:a204-a204.

Cadmium—Multiple Paths to Toxicity

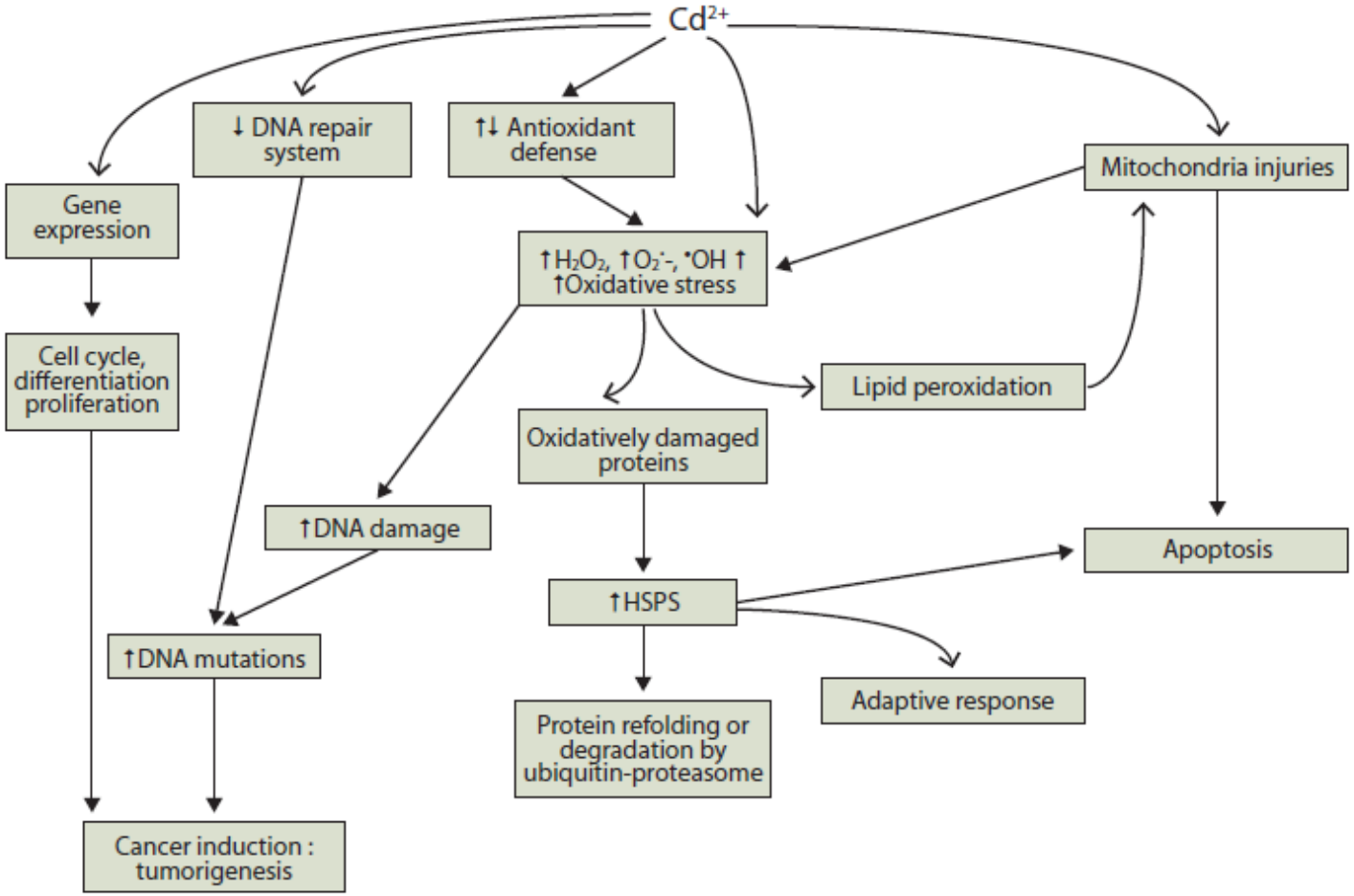


Figure 3.2 Biological consequences of cadmium intoxication in cells. From: Bertin G, Averbeck D. Cadmium: cellular effects, modifications of biomolecules, modulation of DNA repair and genotoxic consequences (a review). Biochimie. 2006 Nov;88(11):1549-59. Used with permission from Elsevier.

Cadmium – Displaces Critical

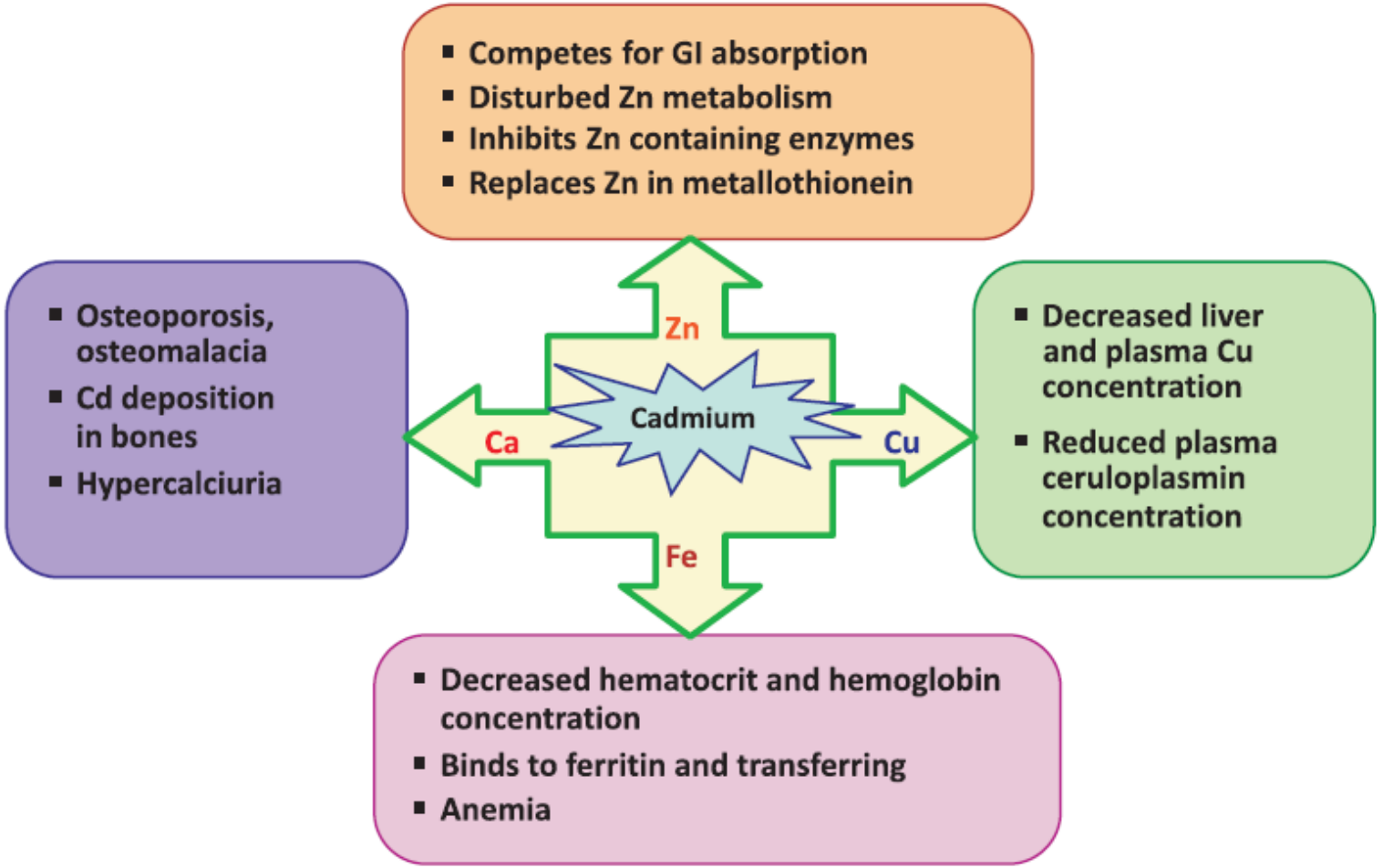
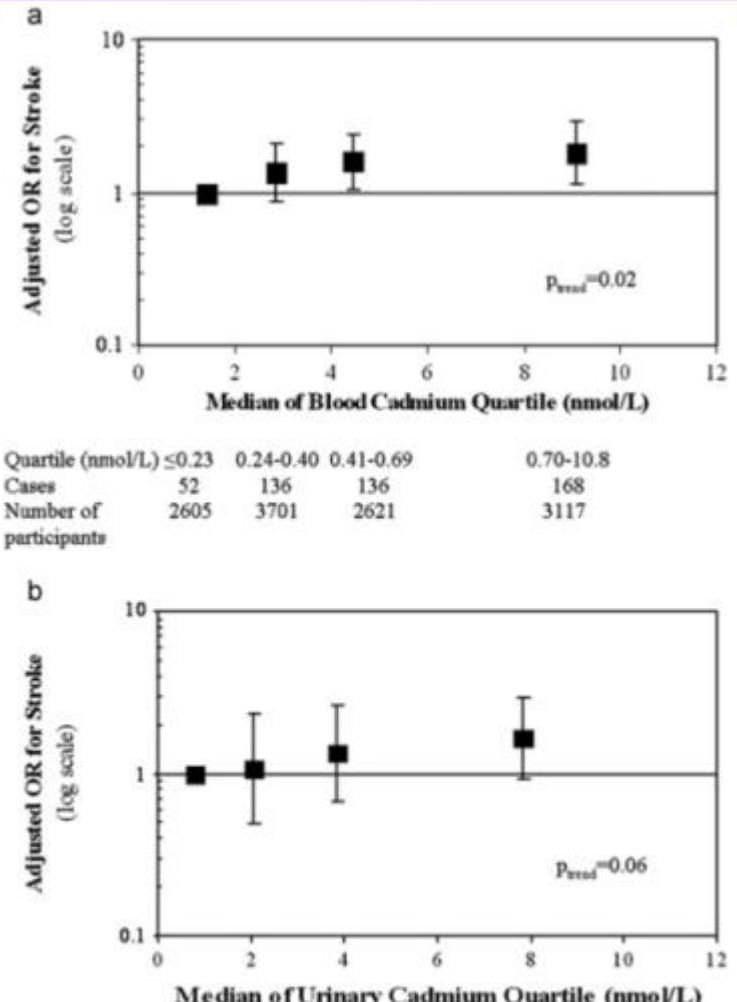


Figure 3.1 Interaction of cadmium with essential nutrients by which it causes toxicity. From: Flora SJ, Mittal M, Mehta A. Heavy metal induced oxidative stress and its possible reversal by chelation therapy. *Indian J Med Res.* 2008 Oct;128(4):501-23. Reprinted with permission from the *Indian Journal of Medical Research (IJMR).*

Cadmium and CVD

- Higher blood & urinary cadmium recently linked to a higher stroke & heart failure prevalence
  - **A 50% increase in blood cadmium was associated with a 35% increase in stroke prevalence and a 48% increase in HF prevalence**
  - A 50% increase in urinary cadmium was associated with a 9% and 12% increase in prevalent stroke and HF



Peters 2010

Peters JL, et al. Cadmium exposure in association with history of stroke and heart failure. Environ Res. 2010 Feb;110(2):199-206.

Cadmium

- **Half from cigarette smoke, half from diet**
- 73% of U.S. women age 50+ have levels of cadmium > 0.50 ug/g creatinine, a level associated with increased risk for **osteoporosis** (43% greater if 0.5-1.0 than if <0.50)
- Same level of cadmium exposure which increases osteoporosis risk also increases **MI** risk 80% (in women only)
- Dietary cadmium (not cadmium from smoking) is the source of Cd related osteoporosis
- Recent analysis by Fred Hutchinson found **tofu/soy primary dietary source among premenopausal women**
- Crustaceans, mollusks and cephalopods also high sources

Gallagher CM, et al. Urinary cadmium and osteoporosis in U.S. Women >or= 50 years of age: NHANES 1988-1994 and 1999-2004. Environ Health Perspect. 2008
Adams SV, et al. Sources of cadmium exposure among healthy premenopausal women. Sci Total Environ. 2011 Apr 1;409(9):1632-7.
Everett CJ, et al. Association of urinary cadmium and myocardial infarction. Environ Res. 2008 Feb;106(2):284-6.

POPs - Definition

- **Organic compounds resistant to environmental degradation**: chemical, biological, and photolytic
- **Bioaccumulate** in human and animal tissue, bio-magnify in food chains
- Pesticides, solvents, industrial chemicals, plasticizers, etc.
- Original 1995 “dirty dozen”:
  - Aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, hexachlorobenzene, mirex, polychlorinated biphenyls, polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans, and toxaphene
- Since added:
  - Carcinogenic polycyclic aromatic hydrocarbons (PAHs), brominated flame-retardants, some organometallic compounds such as tributyltin

POPs – Incidence and Exposure

- 49 POPs measured by NHANES study – at least **20 found in >60% of US pop**, some found in >80%
  - Hexachlorobenzene found in 0.6%; DDE found in nearly all
- Canadian study found 46 of 69 POPs measured
- Thresholds unclear—large genetic variation
- **Collectively, dose-dependent association**:
  - Cardiovascular disease
  - Hypertension prevalence, 2-5 fold increased risk (varies by POP type & gender)
  - Insulin resistance and diabetes
  - Obesity

Ha MH, et al. Association between serum concentrations of persistent organic pollutants and self-reported cardiovascular disease prevalence: results from the National Health and Nutrition Examination Survey, 1999-2002. Environ Health Perspect. 2007 Aug;115(8):1204-9.
Ha MH, et al. Association between serum concentrations of persistent organic pollutants and prevalence of newly diagnosed hypertension: results from the National Health and Nutrition Examination Survey 1999-2002. J Hum Hypertens. 2009 Apr;23(4):274-86.

Physiological Effects of POPs

- **Diverse mechanisms**, due to various chemical structures of POPs:
  - Endocrine disruption (thyroid & sex hormones)
  - Blood sugar regulation disruption (interference/mimic insulin)
  - Mitochondrial damage
  - Inflammatory cytokines
  - Methylation disruption
  - Alterations in aryl hydrocarbon nuclear receptor translocator
  - Peroxisome proliferator activated receptor (PPAR) agonist
  - Stimulation of tumor necrosis factor-α expression
  - **Intrauterine and/or epigenetic and trans-generational effects**.
    - Ex: higher cord blood levels of hexachlorobenzene associated with 2x greater risk for obesity in children
    - Ex: Maternal levels of BPA while pregnant impairs behaviour/executive function in children, esp. girls (e.g. depression, anxiety, emotional regulation)

Smink A, et al. (2008) Exposure to hexachlorobenzene during pregnancy increases the risk of overweight in children aged 6 years. Acta Paediatr 97, 1465–1469.
Braun JM, et al. Impact of early-life bisphenol A exposure on behavior and executive function in children. Pediatrics. 2011 Nov;128(5):873-82. doi: 10.1542/peds.2011-1335.

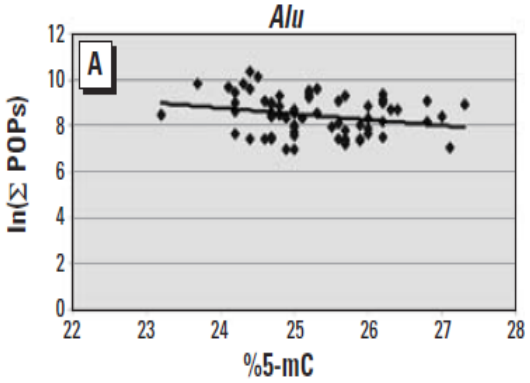
POPs and Disease Risk

- Diabetes:
  - **With undetectable POPs, no association between obesity & diabetes**
  - Metabolic syndrome & insulin resistance, especially organochlorine pesticides, OR 5.3 for MetS
- Hypertension:
  - Exposure to POPs in non-diabetics associated with increased risk hypertension
- Obesity
  - Many POPs found to be "**obesogens**" BUT… Effects of weight loss has inverse relationship (i.e. lose weight, levels go up)

Lee DH, et al. Low dose of some persistent organic pollutants predicts type 2 diabetes: a nested case-control study. Environ Health Perspect. 2010 Sep;118(9):1235-42.
Ha MH, et al. Association between serum concentrations of persistent organic pollutants and prevalence of newly diagnosed hypertension: results from the National Health and Nutrition Examination Survey 1999-2002. J Hum Hypertens. 2009 Apr;23(4):274-86.
Lee DH, et al. Relationship between serum concentrations of persistent organic pollutants and the prevalence of metabolic syndrome among non-diabetic adults: results from the National Health and Nutrition Examination Survey 1999-2002. Diabetologia. 2007 Sep;50(9):1841-51.
Lim JS, et al. Inverse associations between long-term weight change and serum concentrations of persistent organic pollutants. Int J Obes (Lond). 2010 Sep 7.

POPs - Global Hypomethylation

- Greenland Inuit Adults
  - Consistent inverse correlation and linear relationship between POP levels & percent DNA methylation
- Korean Adults
  - Inverse relationship, especially OC pesticides
  - Observed mostly at background exposure levels vs. high exposure
- Global DNA hypomethylation is associated with chronic disease risk, including cancer and atherosclerosis, as well as epigenetic dysregulation

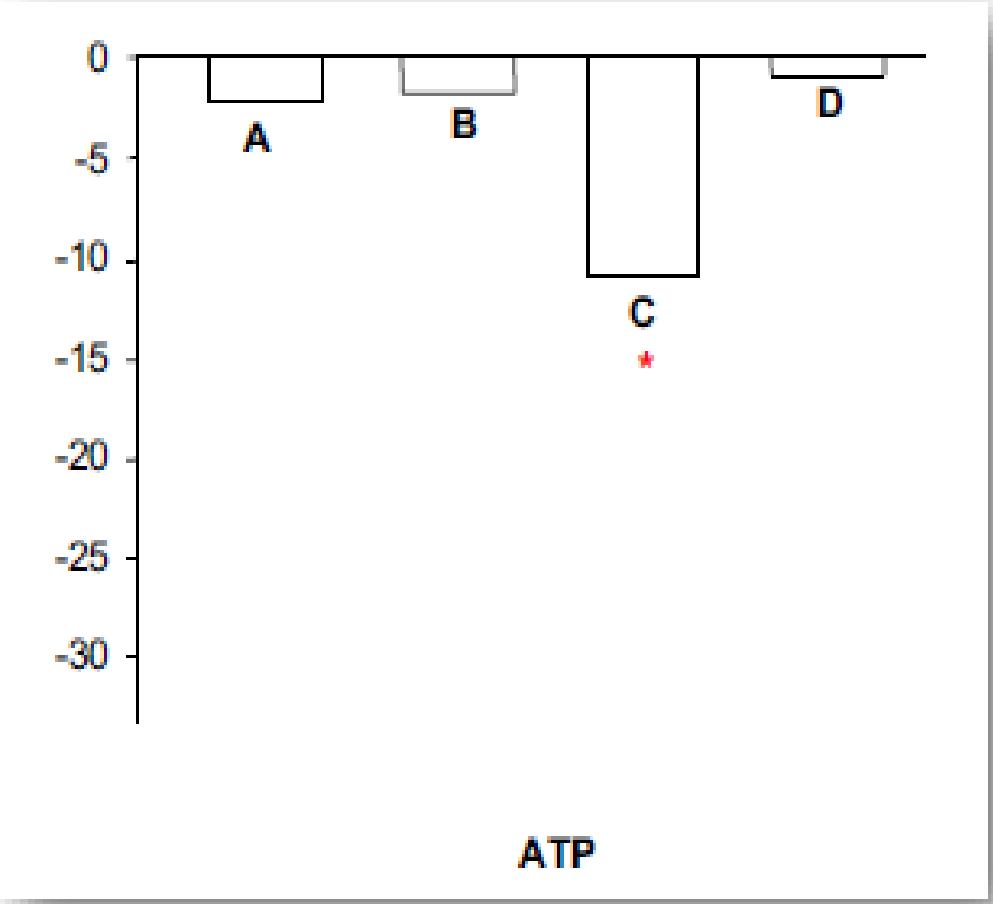


Rusiecki 2008

Rusiecki JA, et al. Global DNA hypomethylation is associated with high serum-persistent organic pollutants in Greenlandic Inuit. Environ Health Perspect. 2008 Nov;116(11):1547-52.
Kim KY, et al. Association of low-dose exposure to persistent organic pollutants with global DNA hypomethylation in healthy Koreans. Environ Health Perspect. 2010 Mar;118(3):370-4.

Alcohol Decreases ATP Production

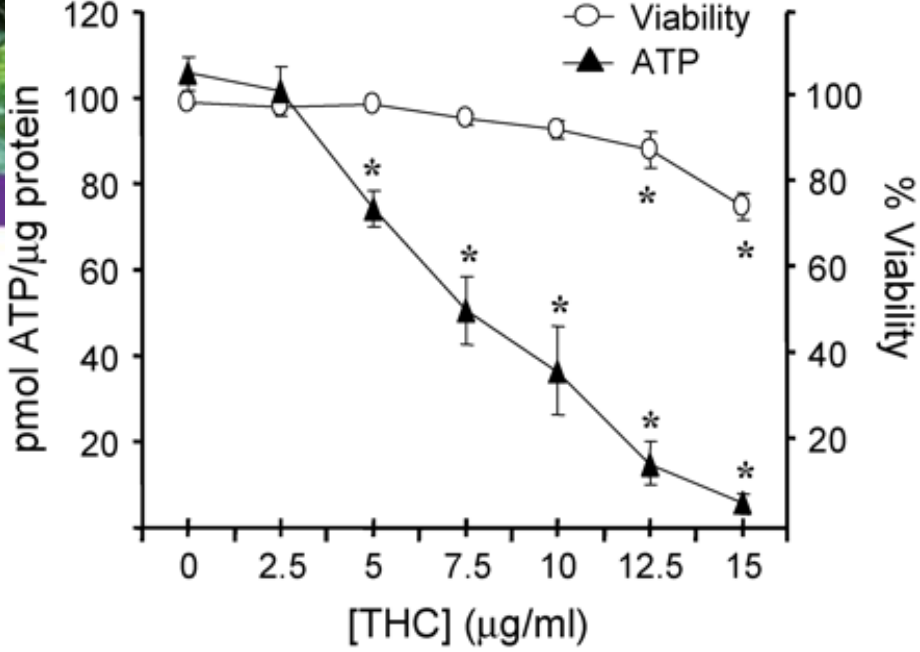
- 40 g/d for 30d
- Type of EtOH
  - A = beer
  - B = wine
  - C = spirits
  - D= control



Addoloratoa G, et al. Effects of short-term moderate alcohol administration on oxidative stress and nutritional status in healthy males. Appetite 2008;50:50–56

Marijuana Poisons Mitochondria

- Does-dependent decline
- Lung levels of THC 10x higher in lungs
- A single joint will affect mitochondrial function in mouth and lungs



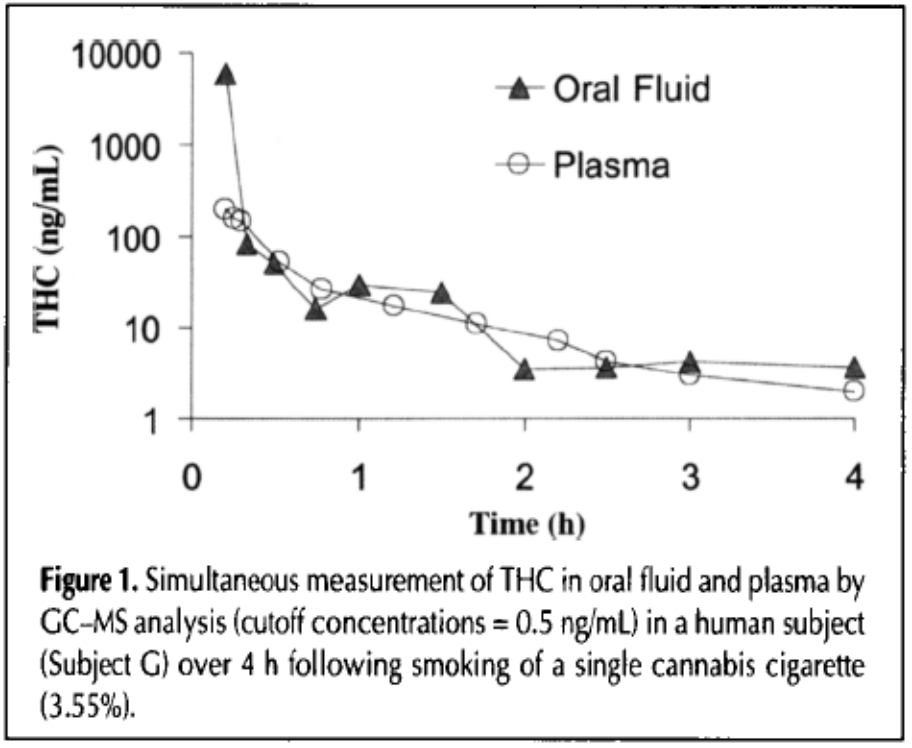


Figure 1. Simultaneous measurement of THC in oral fluid and plasma by GC–MS analysis (cutoff concentrations = 0.5 ng/mL) in a human subject (Subject G) over 4 h following smoking of a single cannabis cigarette (3.55%).

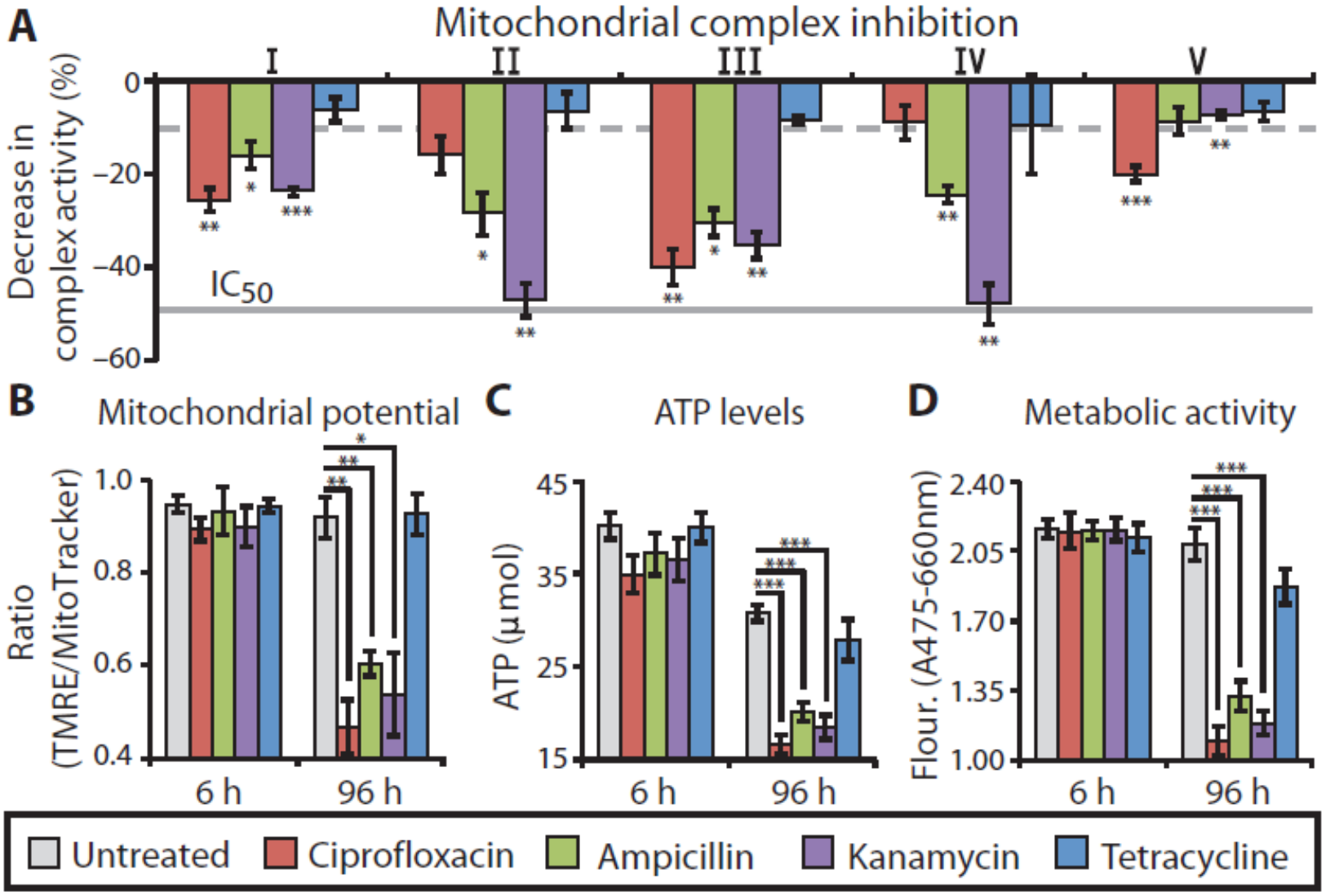
Sarafian TA, et al. Delta 9-tetrahydrocannabinol disrupts mitochondrial function and cell energetics. Am J Physiol Lung Cell Mol Physiol. 2003 Feb;284(2):L298-306

Statins Decrease CoQ_{10}

- HMG-CoA Reductase Inhibitors
- Adverse effects appear to be related to mitochondrial dysfunction, including cognitive loss, neuropathy, pancreatic and hepatic dysfunction, myalgia, cancer, and sexual dysfunction
  - Meta-analysis did not find significant mortality statin benefit for treatment of high risk primary prevention (i.e. those without established CHD).
- Most trials show benefit of CoQ10 for myalgia from statins
- Statins dose-dependent reduction in blood and lymphocyte CoQ10
- Plasma CoQ10 predictor of mortality in patients with heart failure

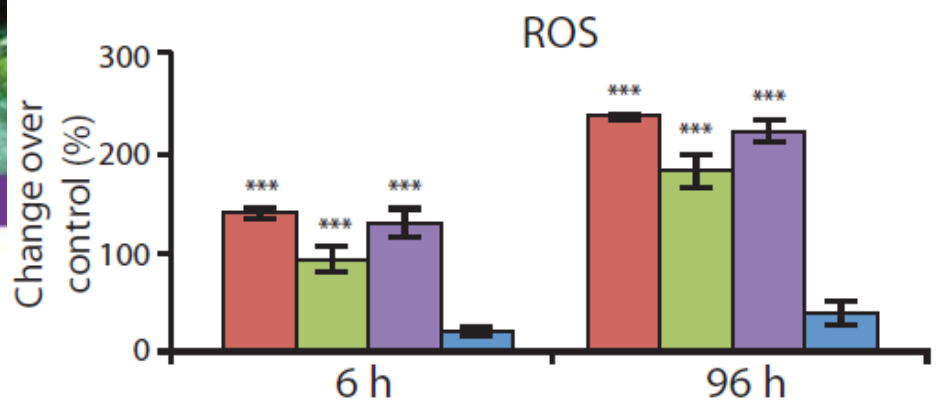
Ray KK, et al. Statins and all-cause mortality in high-risk primary prevention: a meta-analysis of 11 randomized controlled trials involving 65,229 participants. Arch Intern Med. 2010 Jun 28;170(12):1024-31.
Golomb BA, Evans MA. Statin adverse effects : a review of the literature and evidence for a mitochondrial mechanism. Am J Cardiovasc Drugs. 2008;8(6):373-418.
Mortensen SA, et al. Dose-related decrease of serum coenzyme Q10 during treatment with HMG-CoA reductase inhibitors. Mol Aspects Med 1997;18:137-44
Molyneux SL, Florkowski CM, George PM, et al. Coenzyme Q10: an independent predictor of mortality in chronic heart failure. J Am Coll Cardiol. 2008 Oct 28;52(18):1435-41.
Caso G, et al. Effect of coenzyme q10 on myopathic symptoms in patients treated with statins. Am J Cardiol. 2007 May 15;99(10):1409-12.

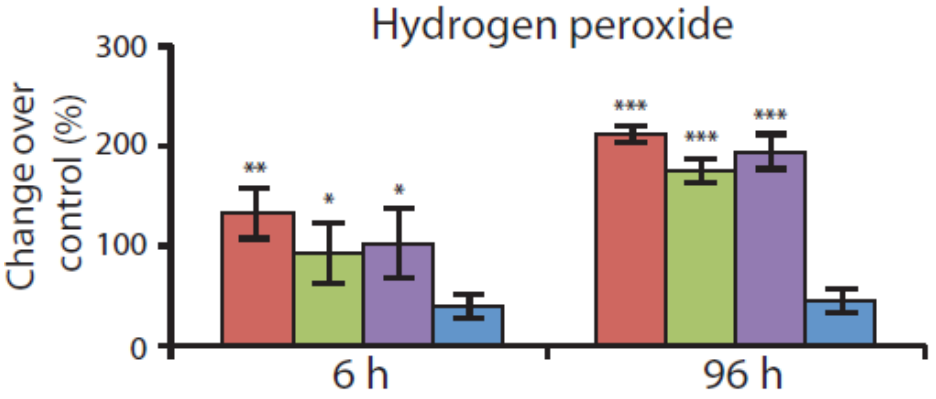
Antibiotics Impair Mitochondrial ATP Production

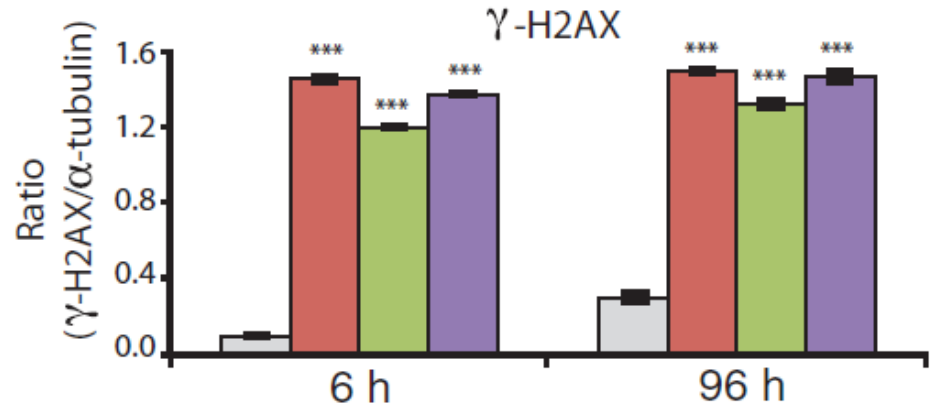


Kalghatgi S, Spina CS, Costello JC, et al. Bactericidal antibiotics induce mitochondrial dysfunction and oxidative damage in Mammalian cells. Sci Transl Med. 2013 Jul 3;5(192):192

Antibiotics Increase Mitochondrial Oxidants







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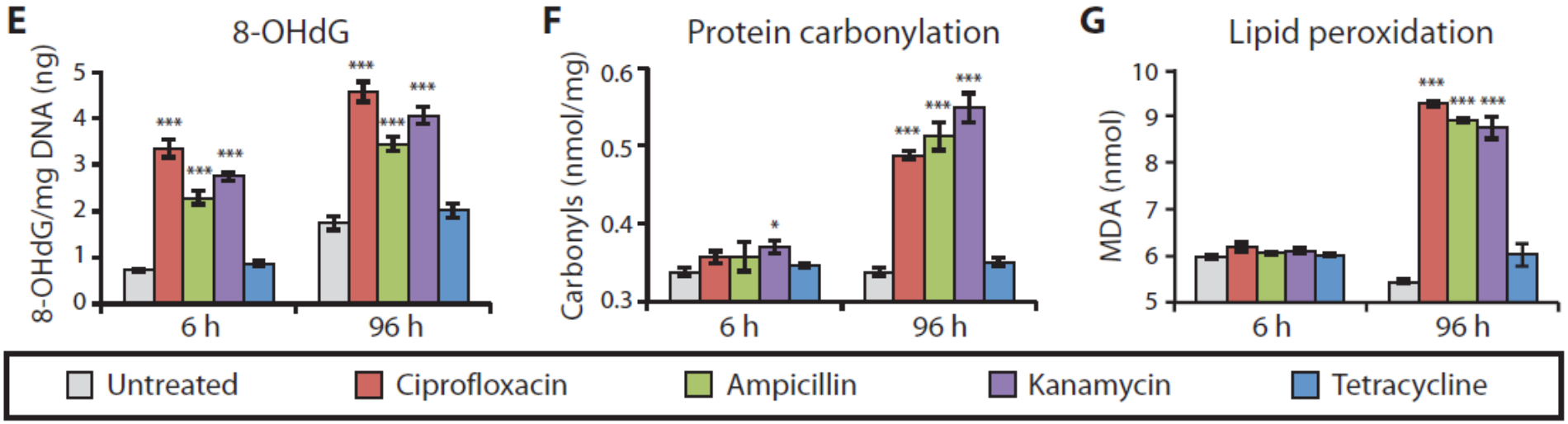
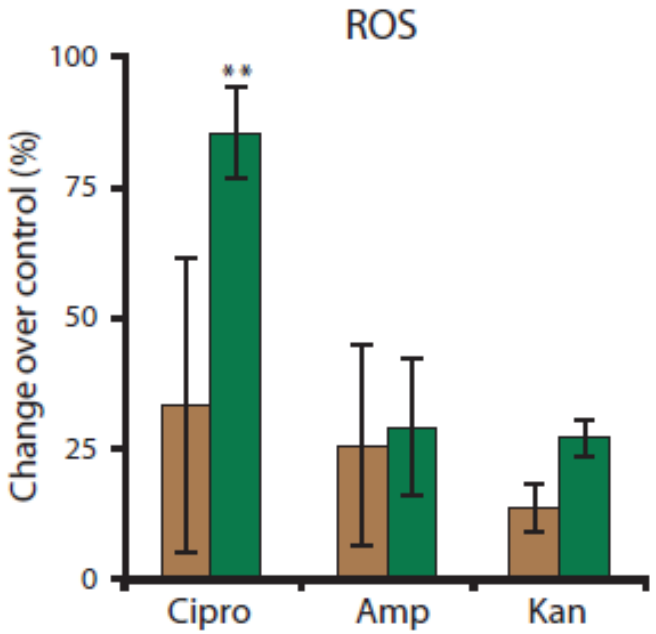
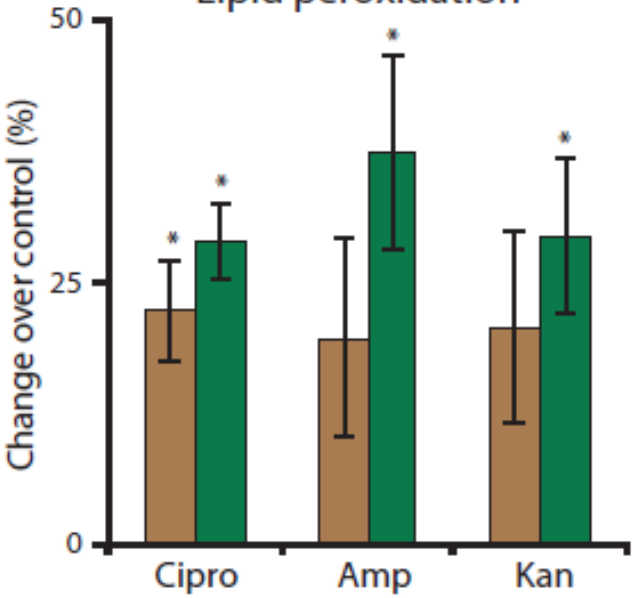
Antibiotics Damage Mitochondria

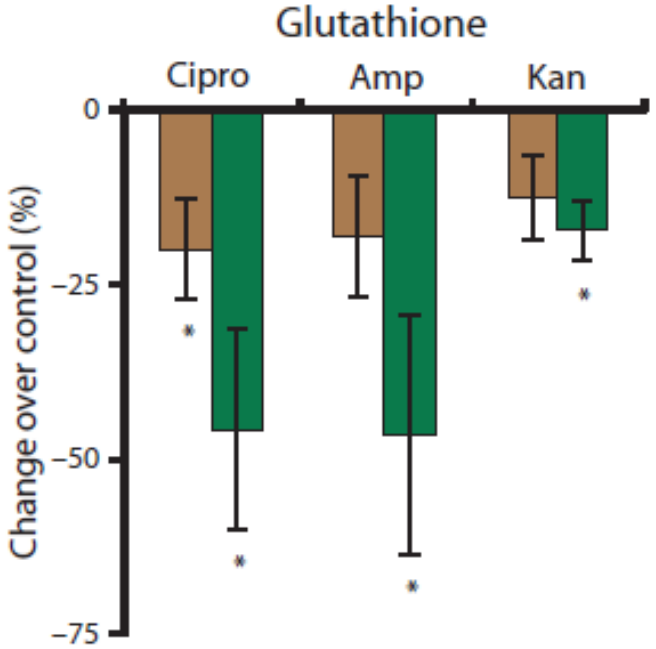


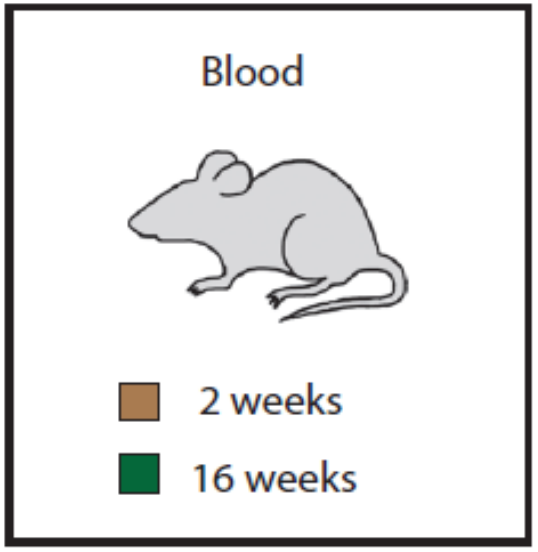
Kalghatgi S, Spina CS, Costello JC, et al. Bactericidal antibiotics induce mitochondrial dysfunction and oxidative damage in Mammalian cells. Sci Transl Med. 2013 Jul 3;5(192):192

Antibiotics Damage Mitochondria – Animal Study









Kalghatgi S, Spina CS, Costello JC, et al. Bactericidal antibiotics induce mitochondrial dysfunction and oxidative damage in Mammalian cells. Sci Transl Med. 2013 Jul 3;5(192):192

Sources of Toxins - Endogenous

- Bowel Toxins
- Normal metabolites not detoxified properly
  - Estrogen derivatives – catechol estrogens and estrogen quinones are both associated with reproductive tissue cancers and oxidative damage
  - Homocysteine
    - Risk factor for ischemic heart disease and stroke, neural tube defects, and neurodegenerative diseases
    - Depends on a number of cofactors, including B6, B12, and folic acid
  - Methylmalonic acid
    - Directly toxic to neurological and renal tissues, due to DNA or B12 deficiency

Crooke PS, et al. Estrogens, enzyme variants, and breast cancer: a risk model. Cancer Epidemiol Biomarkers Prev. 2006
Dawling S, et al. In vitro model of mammary estrogen metabolism: structural and kinetic differences between catechol estrogens 2- and 4-hydroxyestradiol. Chem Res Toxicol. 2004
Perła-Kaján J,et al. Mechanisms of homocysteine toxicity in humans. Amino Acids. 2007
Kölker S, et al. Methylmalonic acid--an endogenous toxin? Cell Mol Life Sci. 2005

Effects of Toxic Bowel Bacteria

Organism	Toxic Reaction	Diseases
Bacteroides	Separate B12	Anemia
Campylobacter	Cross reacts with collagen	Reiter's syndrome
Candida albicans	Damage intestines Immune suppression	IBS Leaky gut
Klebsiella pneumoniae	Cross reacts with nerve acetylcholine receptors and joints	Myasthenia gravis Rheumatoid arthritis
Nisseria meningitidis	Cross reacts with nerve membranes	Meningitis
Yersinia enterocolitica	Cross reacts with thyroid plasma membrane	Arthritis, erythema nodosum, Graves' disease, Hashimoto's

Detoxification of Estrogen (&Testosterone!)

CYP1B1 vs CYP1A2

- 1B1 metabolizes estradiol at C4, making it more genotoxic
- 1A2 metabolizes at C2, reducing carcinogenesis
- Breast and prostate cancer association with genotype
- **Quercetin and cruciferous vegetables increase 1A2 activity relative to 1B1, leading to less toxic estrogen metabolites**

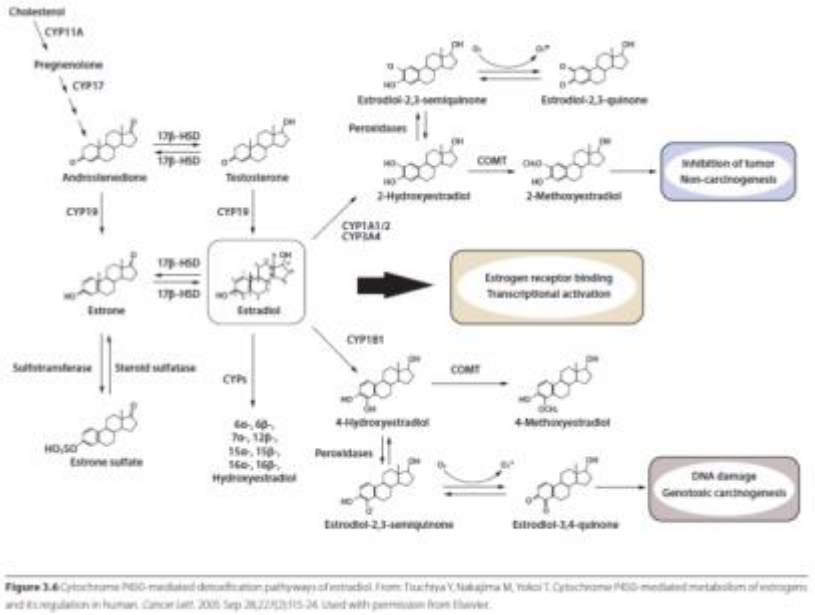


Figure 3.6 Cytochrome P450-mediated detoxification pathways of estradiol. From: Tsuchiya Y, Nakajima M, Yokoi T. Cytochrome P450-mediated metabolism of estrogens and its regulation in human. Cancer Lett. 2005 Sep 28;227(2):115-24. Used with permission from Elsevier.

Tsuchiya Y, Nakajima M, Yokoi T. Cytochrome P450-mediated metabolism of estrogens and its regulation in human. Cancer Lett. 2005 Sep 28;227(2):115-24. Reproduced with permission from Elsevier.

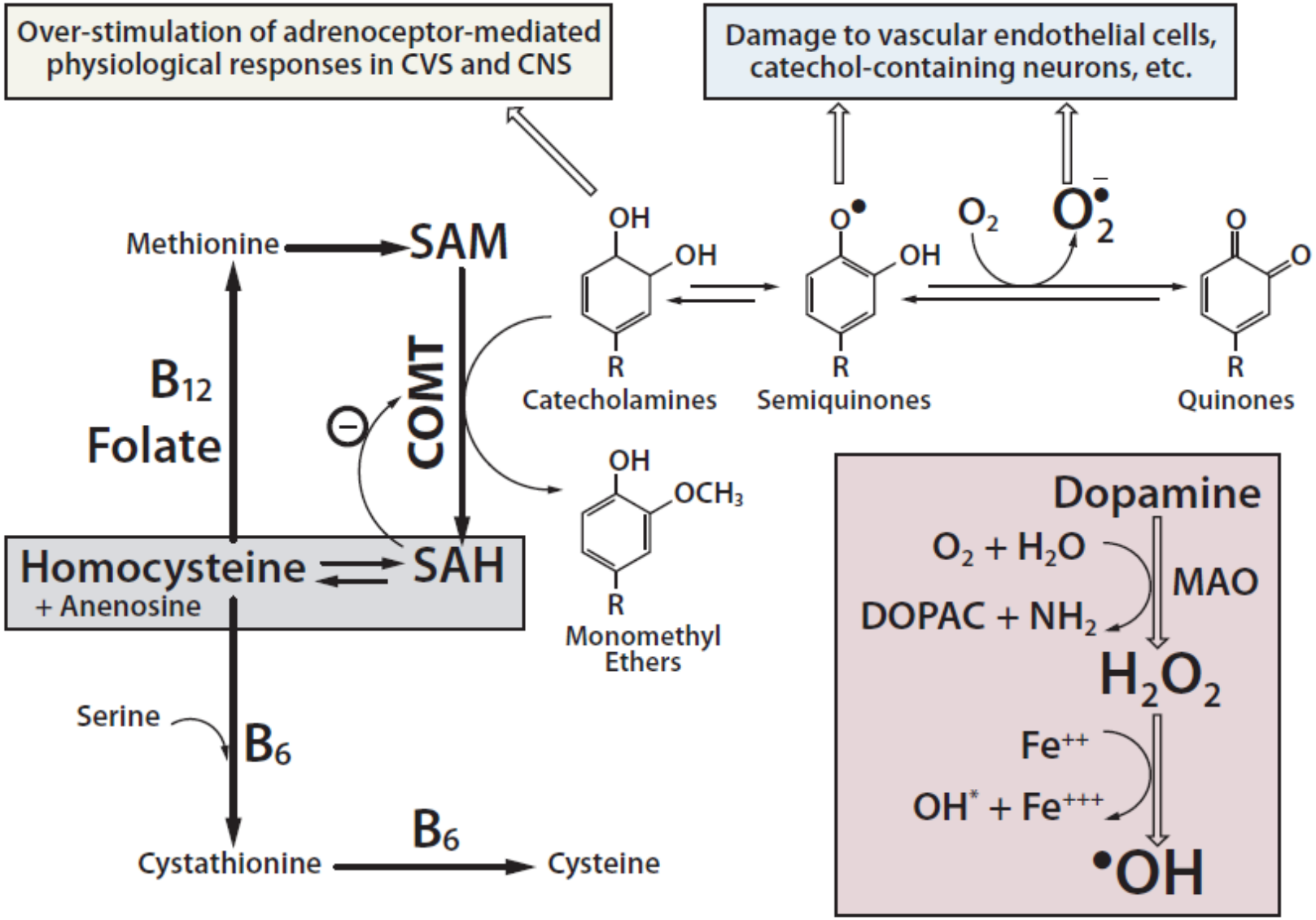


Figure 3.7 Proposed mechanism of homocysteine pathophysiology and pathogenesis based on accumulation of intracellular SAH. From: Zhu BT. Catechol-O-Methyltransferase (COMT)-mediated methylation metabolism of endogenous bioactive catechols and modulation by endobiotics and xenobiotics: importance in pathophysiology and pathogenesis. *Curr Drug Metab.* 2002 Jun;3(3):321-49. Used with permission from Bentham Science Publishers Ltd.

How Toxins Cause Damage

- Increase free radical production
  - Cd induces production of ROS
  - Malathion inhibits catalase and glutathione peroxidase
  - Homocysteine oxidizes LDL cholesterol
- Poison enzyme systems (compete with nutritional cofactors for binding sites)
  - Pb poisons delta aminolevulinic acid dehydratase
  - Cd inhibits catalase, glutathione reductase, and lowers glutathione levels
- Replace structural minerals
  - Pb displaces Ca in bone

How Toxins Cause Damage

- Damage DNA
  - Benzene - causes chromosomal damage
  - Pesticides – fruit growers have more DNA damage
  - Phthalates and insecticides – damage sperm DNA
  - 4-hydroxy catechol estrogen (4-OHE) damages breast DNA
- Epigenetic dysmodulation
  - Higher cord blood levels of hexachlorobenzene associated with 2x greater risk for obesity in children
- Damage cell membranes
  - Common mechanism for heavy metals, inactivates membrane enzymes, ion channels and pumps

How Toxins Cause Damage

- Imbalance hormones, many exogenous toxin examples
  - Arsenic
    - Disrupts thyroid hormone and retinoic acid receptors
  - Phthalates
    - Act as anti-androgens
    - More than 75% of the U.S. population has measurable levels of several phthalate metabolites in the urine
  - PCBs
    - Lowers testosterone levels in men
    - Highest levels associated with a 4-fold greater diabetes risk

Toxin Exposure and Disease Risk

- Diabetes
  - Arsenic (OR 3.58)
  - Persistent organic pollutants (38-fold risk for highest exposure)
- Osteoporosis
  - Cadmium (21% of all osteoporosis in women age 50+)
- Arthritis
  - RA: OR 8.5 for PCBs, OR 3.5 for organochlorine pesticides
  - Osteoarthritis: OR 2.9 for some types of PCBs

Navas-Acien A, et al. Arsenic exposure and prevalence of type 2 diabetes in US adults. JAMA. 2008

Lee DH A strong dose-response relation between serum concentrations of persistent organic pollutants and diabetes: results from the National Health and Examination Survey 1999-2002. Diabetes Care. 2006

Lee DH, Positive associations of serum concentration of polychlorinated biphenyls or organochlorine pesticides with self-reported arthritis, especially rheumatoid type, in women. Environ Health Perspect. 2007

Gallagher CM, et al. Urinary cadmium and osteoporosis in U.S. Women >or= 50 years of age: NHANES 1988-1994 and 1999-2004. Environ Health Perspect. 2008

Toxin Exposure and Disease Risk

- Cardiovascular disease
  - Persistent organic pollutants (OR 5.0, women especially)
- Myocardial infarction
  - Cd (at level found in 70% of US women 50+ increases risk 80%)
  - PCBs (individually and as a group) associated with atherosclerotic plaques and echogenicity of the intima-media complex (independent of CV risk factors)
- Neurodegenerative disease
  - Pesticides and mitochondrial poisons (OR 2.16)
- Learning disorders and neurodevelopmental damage
  - >300,000 born every year in US at increased risk due to Hg
  - 92% of children in US have blood lead levels with some risk

Ha MH, et al. Association between serum concentrations of persistent organic pollutants and self-reported cardiovascular disease prevalence: results from the National Health and Nutrition Examination Survey, 1999-2002. Environ Health Perspect. 2007

Priyadarshi A et al. Environmental risk factors and Parkinson's disease: a metaanalysis. Environ Res. 2001

Everett CJ, et al. Association of urinary cadmium and myocardial infarction. Environ Res. 2008

Lind PM, et al. Circulating levels of persistent organic pollutants (POPs) and carotid atherosclerosis in the elderly. Environ Health Perspect. 2012 Jan;120(1):38-43.

Mahaffey KR, et al. Blood organic mercury and dietary mercury intake: National Health and Nutrition Examination Survey, 1999 and 2000. Environ Health Perspect. 2004

Iqbal S, et al. Estimated burden of blood lead levels 5 microg/dl in 1999-2002 and declines from 1988 to 1994. Environ Res. 2008

High Level of Exposure

- 60,000 different chemicals now in use in USA
- 6.5 billion pounds of chemicals released into the air in USA / yr
- Exposure sources include diet, air, water and skin. Examples:
  - Ch_3Hg: Fish is main source
  - Pb: Older paint is a major source in US
  - PBDE: Diet primary source, particularly poultry and red meat consumption
  - Phthalates: HABAs

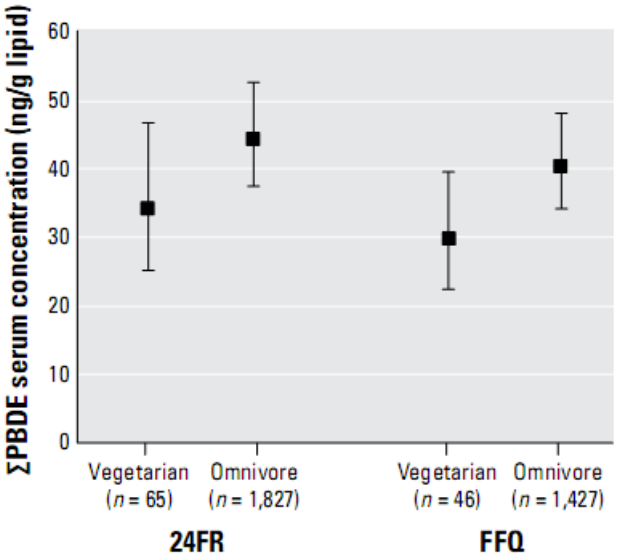


Figure 1. GM ΣPBDE concentrations in vegetarians and omnivores based on two distinct dietary assessments: 24FR and 1-year FFQ. Means are adjusted for race/ethnicity, sex, age, age^2, PIR, and BMI. Error bars represent 95% CIs. For 24FR, $p = 0.006$; for FFQ, $p = 0.009$.

Vegetarian body load lower than omnivore

Fraser AJ Diet contributes significantly to the body burden of PBDEs in the general U.S. population. Environ Health Perspect. 2009

Dórea JG. Studies of fish consumption as source of methylmercury should consider fish-meal-fed farmed fish and other animal foods. Environ Res. 2009

Toxin Synergy

- Effects of low-levels of multiple chemicals unknown
- Synergism between toxins often **greater than additive effects**
  - Parkinson's disease – synergistic effect of metals and pesticides. "The total brain load of pesticides and metals, rather than individual levels, is a very important contributor to the potential effect on α-synuclein fibrillation"

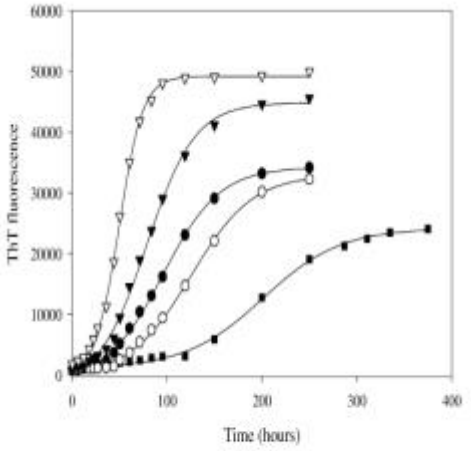
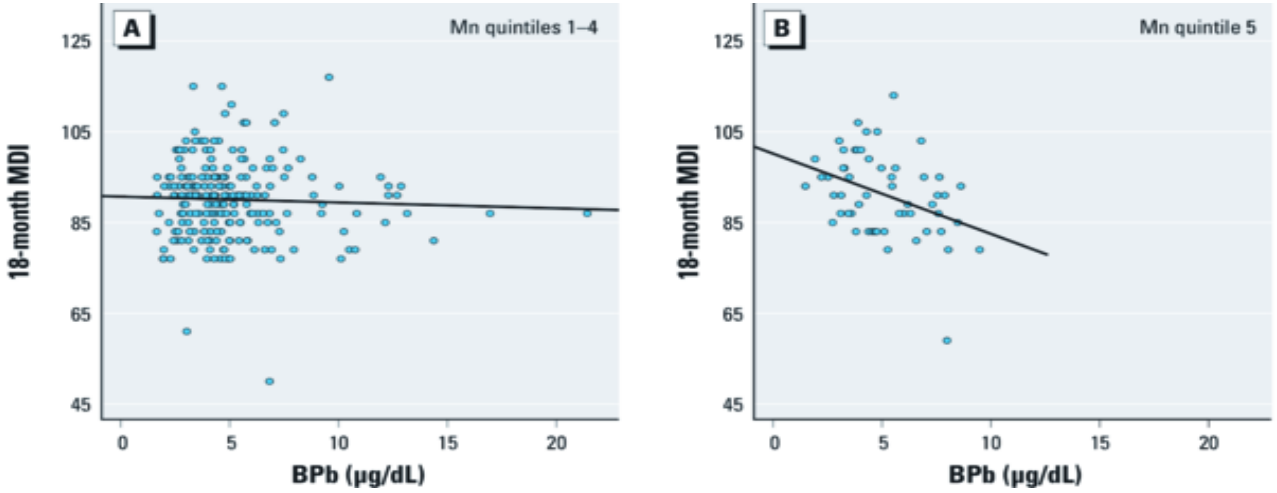


Fig. 5. Synergistic interactions between pesticide and metal in the stimulation of α-synuclein fibril formation. The kinetics of fibril formation were monitored as in the legend to Fig. 1. α-Synuclein (28 μM) alone (■); with 100 μM DDC (○); with 4 mM $AlCl_3$ (●); with DDC (100 μM) and $AlCl_3$ (4 mM) added in that order (▼); and with $AlCl_3$ (4 mM) and DDC (100 μM) added in that order (▽).

Uversky VN Synergistic effects of pesticides and metals on the fibrillation of alpha-synuclein: implications for Parkinson's disease. Neurotoxicology. 2002

Metal Synergy

- Prospective study in children (n=455)
- Evaluated effect of manganese & lead on mental and psychomotor development
- **Greater harm of Pb with high Mn exposure than Pb alone**
- Synergism highlights risk of mixed exposures



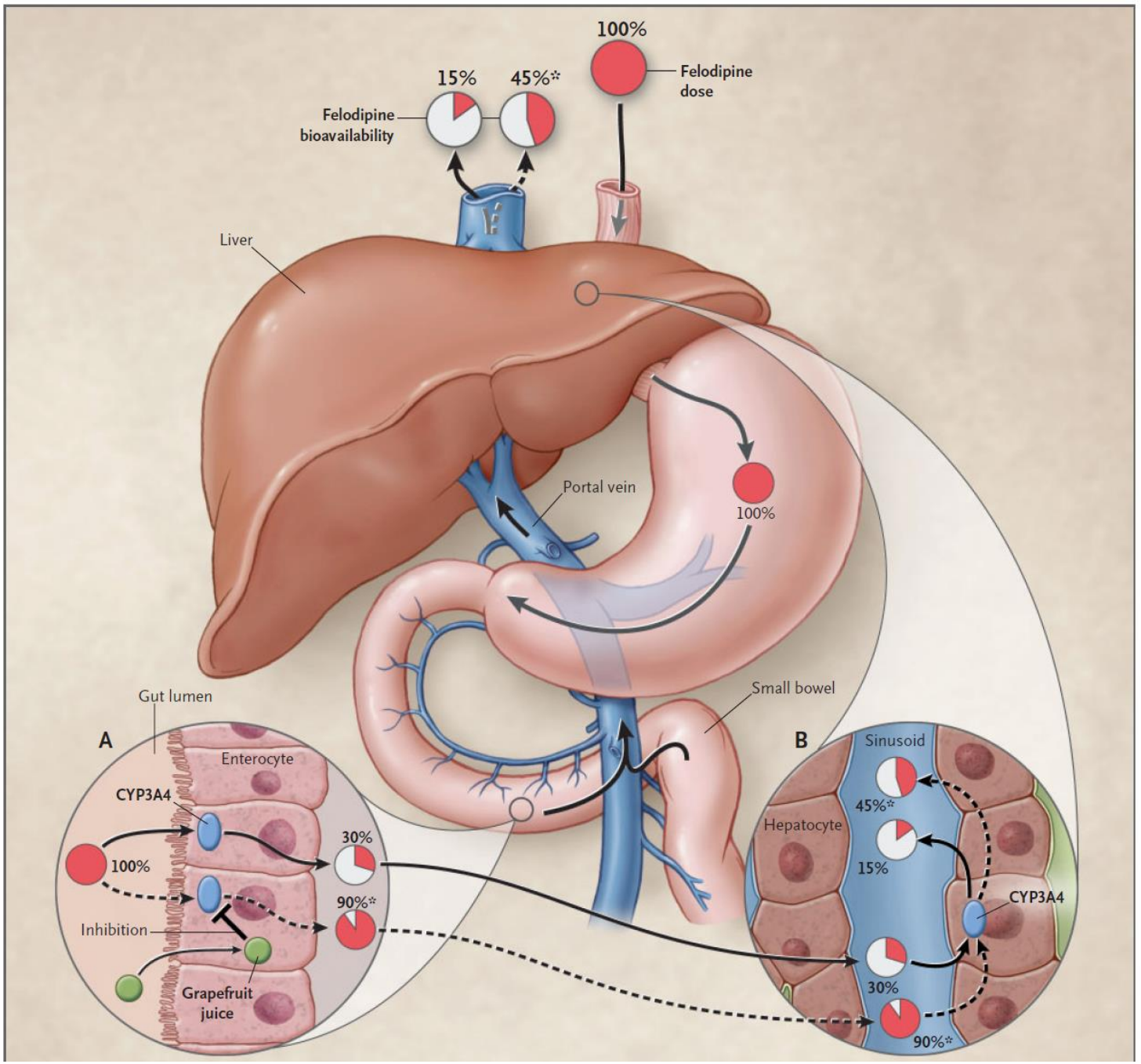
Claus Henn B, et al. Associations of early childhood manganese and lead coexposure with neurodevelopment. Environ Health Perspect. 2012 Jan;120(1):126-31.

Detoxification Overview

- Most tissues have detoxification enzymes
- Especially high in:
  - Intestines
  - Lung
  - Nasal epithelium (To destroy compounds so can smell again, easily saturated)
  - Kidney
  - Reproductive system
  - Liver (not higher concentration, just more of it)

Humans have 1,000 fold variation in detoxification ability

Liver Detoxification of Bowel Content



Yang J, et al. Prediction of intestinal first-pass drug metabolism. Curr Drug Metab. 2007 Oct;8(7):676-84.

Liver Detoxification

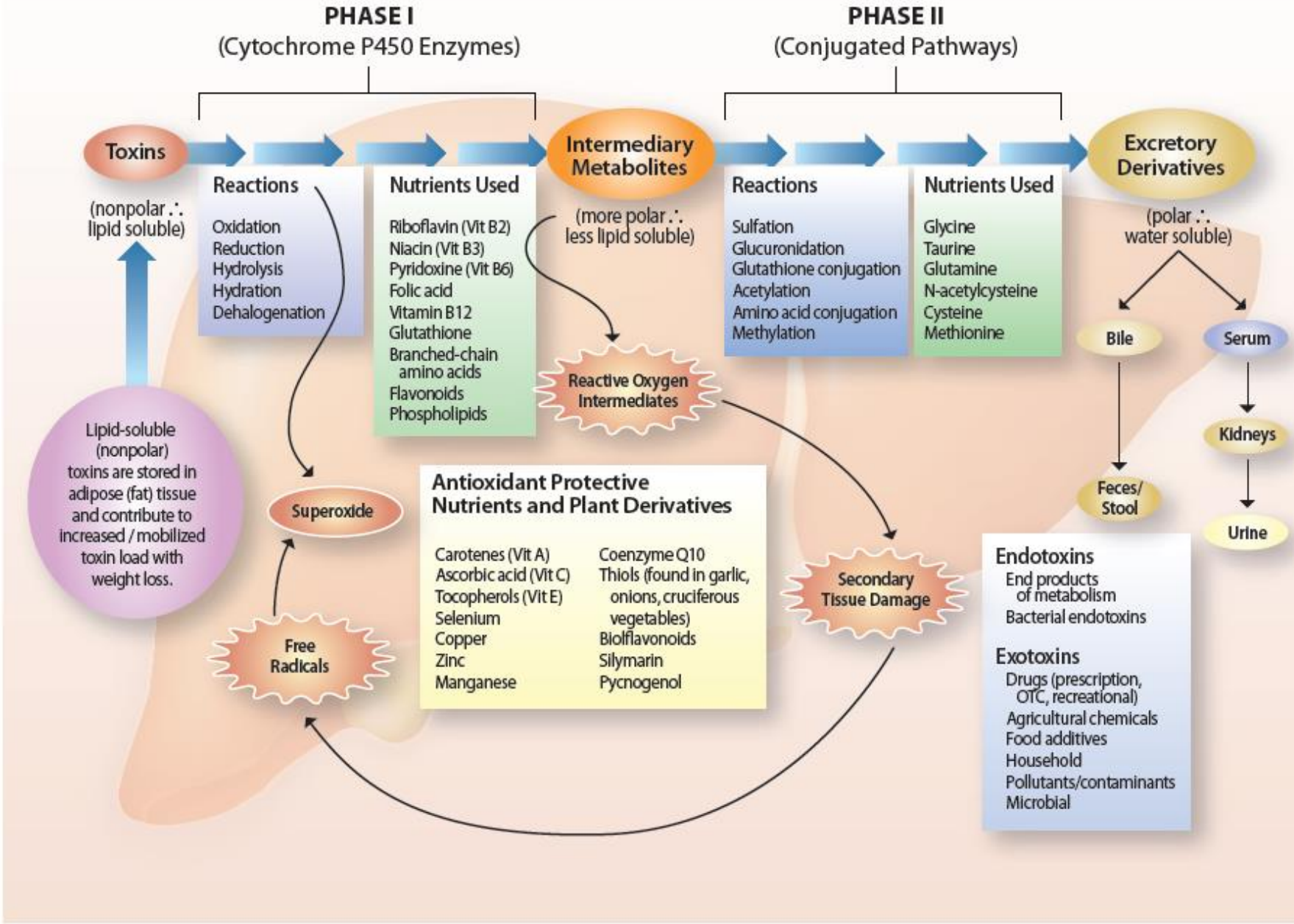


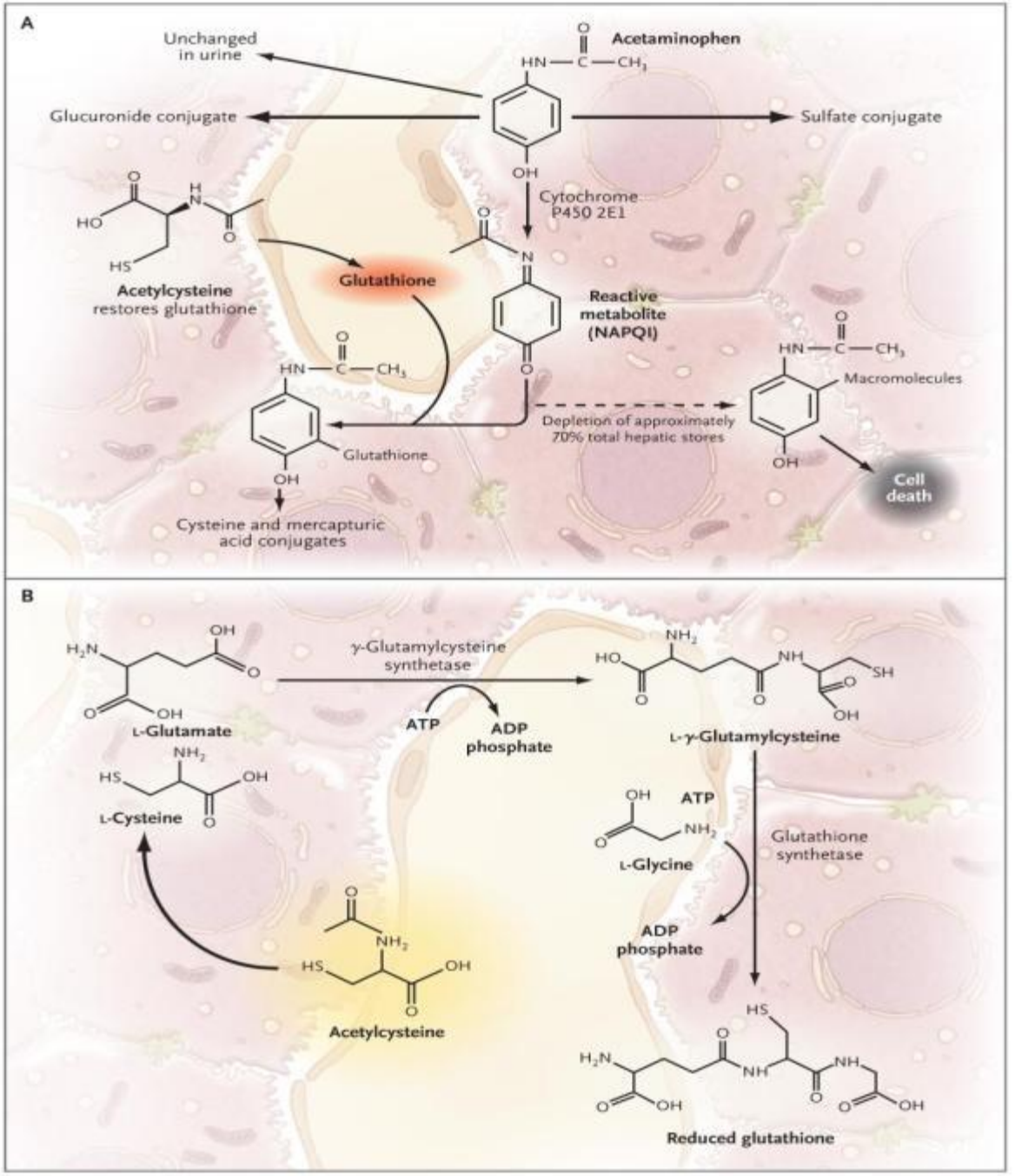
Figure 3.5 Liver detoxification pathways, activities, influences, and effects. (c) 2005 The Institute for Functional Medicine. Used with permission granted by The Institute for Functional Medicine, www.functionalmedicine.org. No part of this content may be reproduced or transmitted in any form or by any means without the express written consent of The Institute for Functional Medicine, except as permitted by applicable law.

Phase I

- Oxidation
  - C-oxidation – aliphatic/aromatic/epoxide
  - N-oxidation – hydroxylation
  - S-oxidation – oxidation/desulfuration
  - Alcohol and aldehyde oxidation
  - Purine oxidation
  - Monoamine/diamine oxidase
- Reduction
  - Azo and nitro reduction
- Hydrolysis
  - Ester and amine hydrolysis
  - Peptide bond hydrolysis
  - Epoxide hydration
- Operate in low oxygen environment
- Higher activity not always better
  - Ex: High CYP1A1 combined with GSTM1 null increases lung cancer risk, due to bottleneck of more reactive compounds
- **Broad substrate specificity ⇒ can be overloaded**

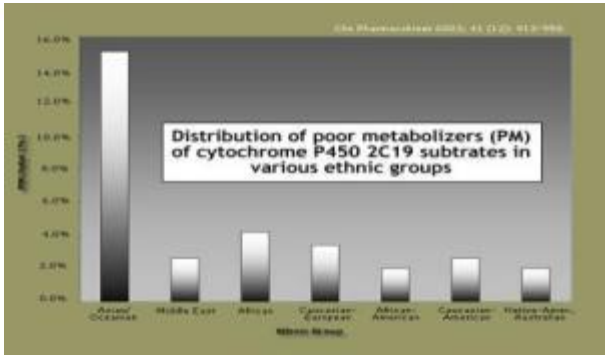
Hung RJ, et al. CYP1A1 and GSTM1 genetic polymorphisms and lung cancer risk in Caucasian non-smokers: a pooled analysis. Carcinogenesis. 2003 May;24(5):875-82

Detoxification of Acetaminophen



Huge Genomic Variability

- CYP2C19
  - Proton pump inhibitors and some antidepressants.
  - 5-fold difference in activity between poor metabolizers and extensive metabolizers, more so if CYP3A4 is also inhibited
  - Ultrarapid allele in 18% of both Swedes and Ethiopians
- CYP2D6
  - Metabolizes a large number of drugs (~25%)
  - 7% of Caucasians poor metabolizers
  - ~30% ultra rapid metabolizers in Arabian and Eastern African populations
  - **More adverse effects in poor metabolizers, and ineffective dosing in ultrarapid metabolizers**
  - Inhibited by ginger



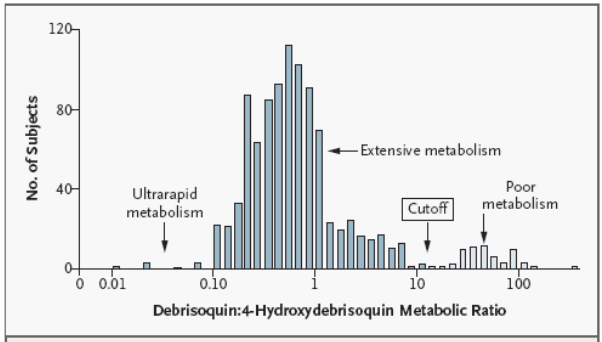


Figure 3. Pharmacogenetics of CYP2D6.
Urinary metabolic ratios of debrisoquin to its metabolite, 4-hydroxydebrisoquin, are shown for 1011 Swedish subjects. The Cutoff box indicates the cut-off point between subjects with poor metabolism as a result of decreased or absent CYP2D6 activity and subjects with extensive metabolism. Modified from Bertilsson et al.[17] with the permission of the publisher.

Desta Z, et al. Clinical significance of the cytochrome P450 2C19 genetic polymorphism. Clin Pharmaco 2002
Sim SC, et al. A common novel CYP2C19 gene variant causes ultrarapid drug metabolism relevant for the drug response to proton pump inhibitors and antidepressants. Clin Pharmacol Ther. 2006
Wilkinson GR. Drug metabolism and variability among patients in drug response. N Engl J Med. 2005
Kirchheiner J, et al. Pharmacokinetics of codeine and its metabolite morphine in ultra-rapid metabolizers due to CYP2D6 duplication. Pharmacogenomics J. 2007

Not just Detoxification!

	Physiological role	Major organ/tissue
Sterols		
1B1	Androgen metabolism, retinoic acid metabolism	Adrenal gland, ovary, testis, lung, prostate
7A1	Bile acid synthesis	Liver
7B1	Bile acid synthesis	Brain, testis, ovary, prostate, liver, colon, kidney, and small intestine
8B1	Bile acid synthesis	Liver
11A1	Steroid hormone synthesis—first step	Steroidogenic tissues
11B1	Steroid hormone synthesis	Adrenal cortex
11B2	Steroid hormone synthesis	Adrenal cortex
17	Steroid hormone synthesis	Adrenal cortex
19	Steroid hormone synthesis (Estrogen)	Brain, placenta and gonads
21A2	Steroid hormone synthesis	Adrenal cortex
27A1	Bile acid synthesis (also hydroxylation of vit. D_3)	Liver+many other tissues
39	Bile acid synthesis	Liver
46	Cholesterol homeostasis in the brain	Brain
51	Cholesterol synthesis	Ubiquitously expressed, highest levels in testis, ovary, adrenal, prostrate, liver, kidney and lung
Fatty acids		
2J2	Arachidonic acid epoxidation	Heart, kidney+other tissues
2U1	Hydroxylation of long chain fatty acids	Thymus, brain
4A11	ω- and (ω-1)-hydroxylation of saturated fatty acids	Kidney and liver
4B1	ω- and (ω-1)-hydroxylation of saturated fatty acids	Lung
Eicosanoids		
4F12	Leukotriene metabolism	Small intestine, liver, colon and heart
4F2	Leukotriene metabolism	Liver
4F3	Leukotriene metabolism	Leukocytes
4F8	Production of 19R-hydroxyprostaglandins	Seminal vesicle, prostate, liver
5A1	Thromboxane-A_2 synthase	Platelets, lung, kidney, spleen, macrophages and lung fibroblasts
8A1	Prostaglandin-I_2 synthase	Heart, vascular endothelial cells, ovary, skeletal muscle, lung, prostate...
Vitamins		
2R1	25-hydroxylation of vit. D_3	Liver
24	24-hydroxylation of 1,25-dihydroxyvitamin D_3	Kidney
26A1	Retinoic acid metabolism	Highest levels in adult liver, heart, pituitary gland, adrenal gland, placenta and regions of the brain
26B1	Retinoic acid metabolism	Highly expressed in brain, particularly in the cerebellum and pons
26C1	Retinoic acid metabolism	Low levels in most tissues
27B1	25-hydroxyvitamin D_3 1-alpha-hydroxylase	Kidney

Seliskar M, Rozman D. Mammalian cytochromes P450--importance of tissue specificity. Biochim Biophys Acta. 2007;1770(3):458-66

Phase II

- **Increase water solubility**
- Energy dependent
- Categories
  - Glucuronidation (UGT)
  - Sulfate conjugation (ST)
  - Mercapturic acid transformation (GST)
  - Glutathione S-transferase)
  - Acetylation (NAT)
  - Quinone recductase
  - Epoxide hydrolase
  - N-, O-, S-methylation
- Sulfation often first, but limited by S pool
- Glucuronidation has huge capacity
  - But used up with high toxin exposure (e.g., alcohol)

- **Every molecule going through Phase I produces one molecule of free radical ⇒ uses up glutathione**

Influence of Diet & Genetics on Phase II

- Glutathione conjugation
  - **Polymorphisms in glutathione transferase increase risk of cancer and smoking related heart disease**
  - Breast cancer – increased risk with low activity, > high cruciferous intake
  - Cruciferous intake decreases kidney, bladder, and colorectal cancers
  - Cruciferous upregulation may also protect against diesel exhaust
- Acetylation
  - Breast cancer- increased risk in smokers (OR of 1.49 for slow genotypes and smoking)
  - Bladder - approximately 40% increased risk with low activity genotype for NAT2, particularly if a smoker

Palma S et al. Influence of glutathione S-transferase polymorphisms on genotoxic effects induced by tobacco smoke. Mutat Res. 2007

Manfredi S, et al. GSTM1, GSTT1 and CYP1A1 detoxification gene polymorphisms and susceptibility to smoking-related coronary artery disease: a case-only study. Mutat Res. 2007

Lee SA, et al. Cruciferous vegetables, the GSTP1 Ile105Val genetic polymorphism, and breast cancer risk. Am J Clin Nutr. 2008

Ambrosone CB, et al. Cigarette smoking, N-acetyltransferase 2 genotypes, and breast cancer risk: pooled analysis and meta-analysis. Cancer Epidemiol Biomarkers Prev. 2008

Phase II Genomic Variation

Acetylation

- Factor of 4 variation in rate (group means)
- Slow acetylator phenotype is found in 52–68% of Caucasians and only 10-15% of Japanese

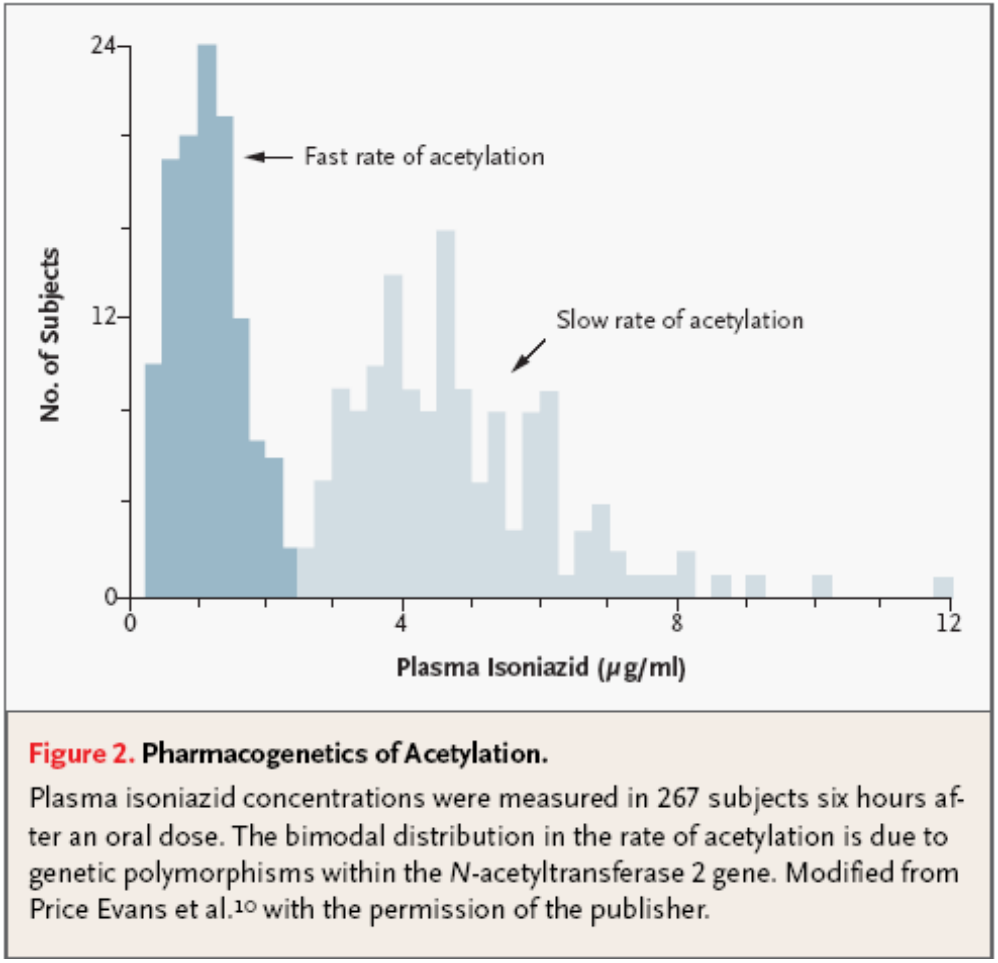


Figure 2. Pharmacogenetics of Acetylation.

Plasma isoniazid concentrations were measured in 267 subjects six hours after an oral dose. The bimodal distribution in the rate of acetylation is due to genetic polymorphisms within the *N*-acetyltransferase 2 gene. Modified from Price Evans et al.[10] with the permission of the publisher.

Weinshilboum, R. Inheritance and drug response. NEJM 2003;348:529-37

Boukouvala S, et al. Arylamine N-acetyltransferases: what we learn from genes and genomes. Drug Metab Rev. 2005

Other Phase II Genomic Variations

Table 2. Pharmacogenetics of Phase II Drug Metabolism.*

Drug-Metabolizing Enzyme	Frequency of Variant Poor-Metabolism Phenotype	Representative Drugs Metabolized	Effect of Polymorphism
N-Acetyltransferase 2	52% among white Americans[10] 17% of Japanese[58]	Isoniazid[10] Hydralazine[11] Procainamide[12]	Enhanced drug effect[13]
Uridine diphosphate–glucuronosyltransferase 1A1 (TATA-box polymorphism)	10.9% among whites[59] 4% of Chinese[60] 1% of Japanese[60]	Irinotecan[61] Bilirubin[62]	Enhanced drug effect[63] Gilbert's syndrome[62]
Thiopurine *S*-methyltransferase	Approximately 1 in 300 whites[50,57] Approximately 1 in 2500 Asians[57]	Mercaptopurine[51] Azathioprine	Enhanced drug effect (toxicity)[51-53]
Catechol *O*-methyltransferase	Approximately 25% of whites[51,64]	Levodopa[51,65]	Enhanced drug effect[51,65]

* Examples of genetically polymorphic phase II (conjugating) enzymes are listed that catalyze drug metabolism, including selected examples of drugs that have clinically relevant variations in their effects.

Weinshilboum, R. Inheritance and drug response. NEJM 2003;348:529-37

Glutathione Conjugation: Main Route of POP Detox

- **POPs are eliminated by phase I biotransformation, followed by phase II conjugation to glutathione (GSH)**
- GSH plays a crucial role against endogenously generated reactive oxygen/nitrogen species
- GSH levels decline as conjugation reactions exceed cells' ability to regenerate GSH
- PCBs and organochlorine pesticides increase oxidative damage and deplete glutathione levels

Awasthi YC, et al. Physiological and pharmacological significance of glutathione-conjugate transport. J Toxicol Environ Health B Crit Rev. 2009 Aug;12(7):540-51.

Ludewig G et al. Mechanisms of toxicity of PCB metabolites: generation of reactive oxygen species and glutathione depletion. Cent Eur J Public Health. 2000 Jul;8 Suppl:15-7.

Ahmed T, Endosulfan-induced apoptosis and glutathione depletion in human peripheral blood mononuclear cells: Attenuation by N-acetylcysteine. J Biochem Mol Toxicol. 2008 Sep;22(5):299-304.

Toxic Metal Assessment

- Both clinical and laboratory
- Often no gold standard for testing
- **Must differentiate between acute exposure and body load**
- Serum, whole blood, hair, urine, feces, nails, and adipose
- In general, serum levels represent acute exposure:
  - E.g. Lead, mercury - blood levels and urine (Hg) represent acute exposure, not chronic or body burden
- **Testing reveals exposure, not necessarily toxicity**
- Challenge testing can reveal body load

DeVito MJ, Comparisons of estimated human body burdens of dioxinlike chemicals and TCDD body burdens in experimentally exposed animals. Environ Health Perspect. 1995
Rooney JP. The role of thiols, dithiols, nutritional factors and interacting ligands in the toxicology of mercury. Toxicology. 2007

Sample Questions for Toxic Metal Exposure

1. Has the patient knowingly been exposed to metals?
2. What is patient's occupation (dentist, welder, ship builder, etc.)?
3. How frequently does the patient eat tuna, swordfish or shark?
4. Does the patient have mercury amalgam fillings?
5. If the patient is taking any dietary supplements, do they have certificates of analysis that they are free of contaminants?
6. Is the patient taking any Ayurvedic or traditional Chinese medicine dietary supplements?
7. Does the patient experience a metallic taste in their mouth *and* have not recently been taking medications documented to cause metallic taste?
8. Does the patient have a history of smoking (particularly high in cadmium)?

Neustadt J, Pieczenik S. Mercury—an example of heavy metal toxicity. IMCJ 2007;6:1

Evaluation of Metal Exposure – Provocation

- Provocation – the use of a chelating agent – before urine collection often done clinically, but several limitations
  - **No "official" reference range for provoked urine**
  - Most chelating agents do not extract metals from all tissues, thus does not necessarily represent total body burden
    - Example: Brain is one of the main target organs for both elemental and organic mercury, yet agents do not chelate brain mercury
- Despite limitations, widely used and advocated by clinicians, in part to see efficacy of chelating agent as a guide to treatment, and based on empirical evidence

Rooney JP. The role of thiols, dithiols, nutritional factors and interacting ligands in the toxicology of mercury. Toxicology. 2007

Laboratory Evaluation of Lead

- **Blood levels widely accepted to represent acute exposure**
- Bone lead represents chronic exposure and better predictor of disease - >90% of body lead stored in bones
- Prospective trial of 1,000 men found bone lead to be associated with all cause (HR 2.52) and cardiovascular mortality (HR 5.63) in a population with supposedly low blood lead levels
- **Provoked lead reflects toxicologically active fraction of the total lead body burden**
- In individuals with previous lead exposure but low recent exposure, blood lead may be only slightly increased whereas the mobilizable pool is significantly increased
- DMSA 4hr found to be highly correlated to 24hr urine collection

Weisskopf MG, A prospective study of bone lead concentration and death from all causes, cardiovascular diseases, and cancer in the Department of Veterans Affairs Normative Aging Study. Circulation. 2009

Risher et al. Mercury exposure: evaluation and intervention the inappropriate use of chelating agents in the diagnosis and treatment of putative mercury poisoning, *Neurotoxicology* 2005

Hoet P, Clinical evaluation of a lead mobilization test using the chelating agent dimercaptosuccinic acid. Clin Chem. 2006

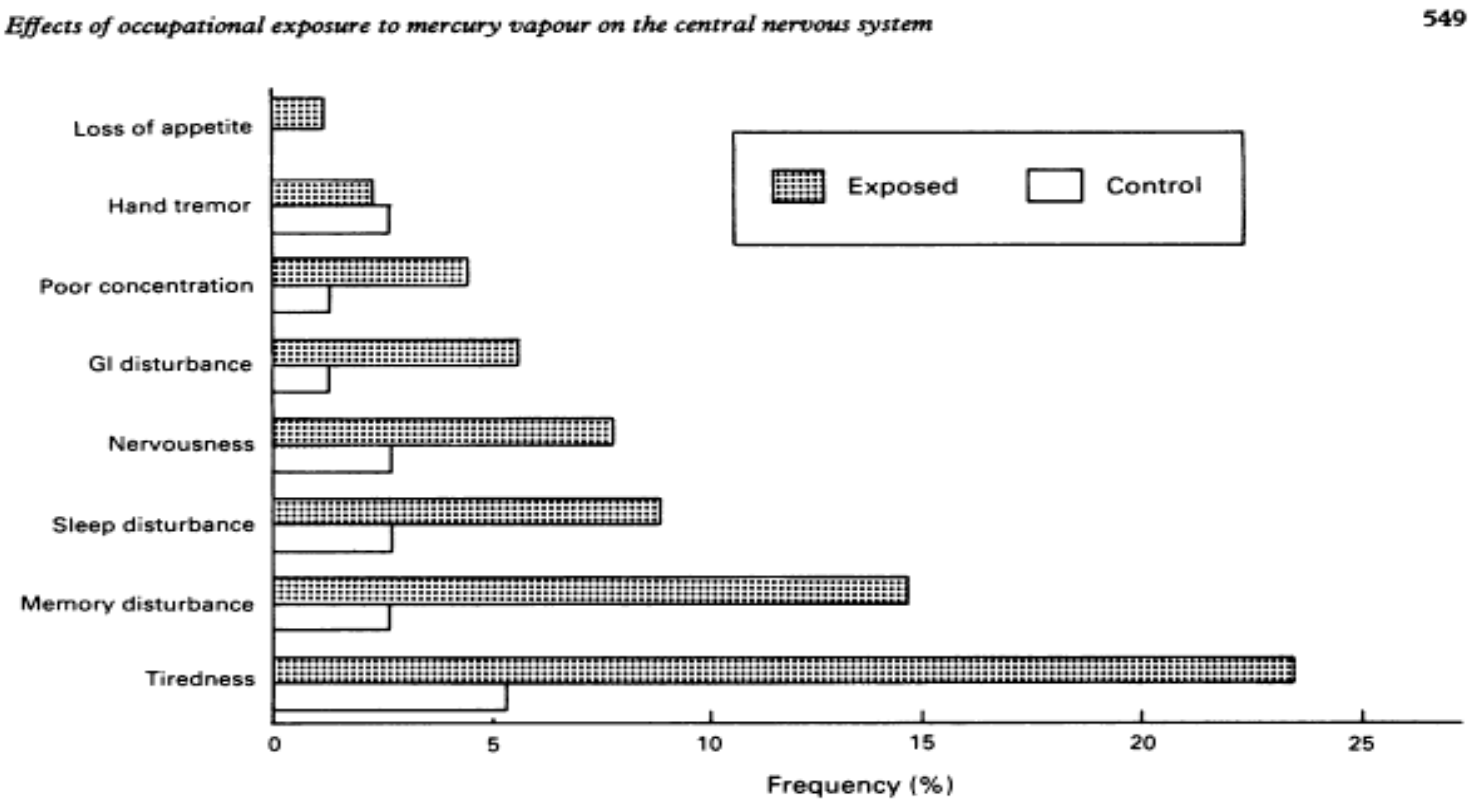
Lead Assessment

- Blood levels limited to acute toxicity, but symptoms are related to degree of elevation

Gracia 2007, 17189579

Level of Toxicity	Blood Lead Concentration (μg/dL)	Clinical Presentation[a]	
		Children	Adults
Asymptomatic or impaired abilities	<10	Decreased learning and memory, decreased verbal ability, impaired fine motor coordination, signs of ADHD or hyperactivity, lower IQ, impaired speech and hearing	. . .[b]
Mild	10–39	Myalgia or parasthesia, irritability, mild fatigue/lethargy, occasional abdominal discomfort	. . .
Moderate	>40–50	Arthralgia, difficulty concentrating, general fatigue, headache, muscular exhaustibility, tremor, weight loss, vomiting, constipation, diffuse abdominal pain	Fatigue, somnolence, moodiness, lessened leisure interest, impaired psychometrics, chronic hypertensive effects, reproductive effects
Severe	>70–80	Lead lines (blueish black appearance on gingival tissue), colic (intermittent, severe cramps), parasthesia or paralysis, encephalopathy	Headache, memory loss, decreased libido, insomnia, metallic taste, abdominal pain, constipation, myalgia/arthralgia, nephropathy
Severe, acute	>100–150	Encephalopathy, seizures, anemia, nephropathy	Encephalopathy, various CNS effects, anemia, nephropathy

Mercury Neurological Symptoms



S Langworth, O Almkvist, E Söderman, and B O Wikström. Effects of occupational exposure to mercury vapour on the central nervous system. Br J Ind Med. 1992 August; 49(8): 545–555

Most Common Symptoms of Hg

Symptom	Frequency
Depression	73%
Memory loss	70%
Anxiety	69%
Unintentionally dropping things	60%
Headache	56%
Moody	45%
Shakiness in hands	44%
Stomach problems	43%
Fatigue	39%
Confusion	35%
Change in sense smell or taste	29%
Parasthesia	26%
Sleep disturbance	25%
Coordination problems	20%
Muscle weakness	16%

Pizzorno J. The mercury epidemic. IMCJ 2009; 8:1

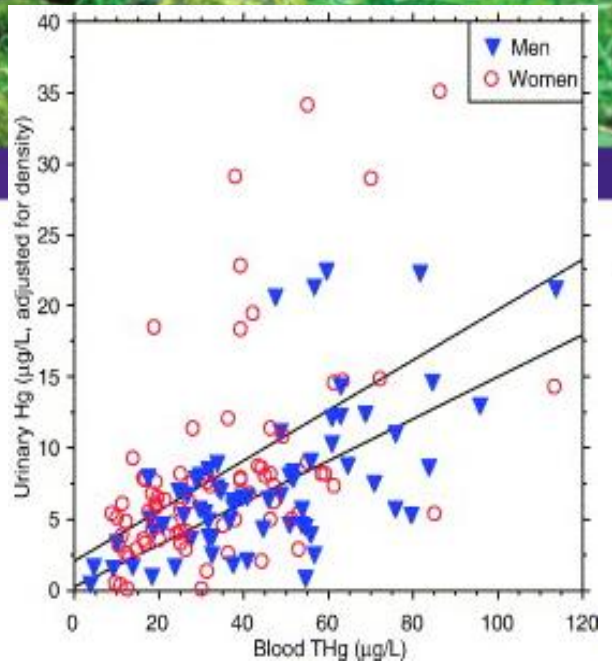
Laboratory Evaluation of Mercury

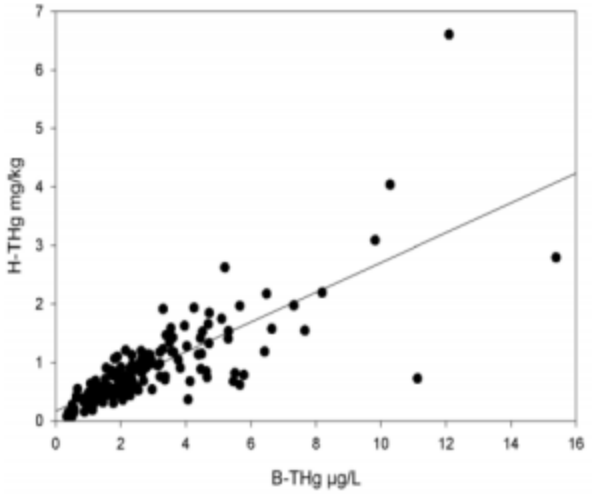
- No gold standard
- Metallic Hg has a short ½ life (24-hour urine preferred)
- Ethyl/methyl mercury (whole blood is primary indicator of acute exposure)
- Hair Hg >5 ppm indicative of methyl mercury intoxication
  - However, poor detoxifiers will have false negative
  - Not good estimate of inorganic mercury
  - Decrease in child IQ of 0.18 points/ppm increase in maternal hair Hg
- Fecal Hg
  - > 90% of Hg is excreted via bile (enterohepatic recirculation)
  - Fecal Hg correlates with # amalgams
- Urine
  - Unprovoked urine represents acute exposure
  - **Provoked urine probably better measure of body load**

Katz SA, et al. Use of hair analysis for evaluating mercury intoxication of the human body: a review. J Appl Toxicol. 1992
Axelrad DA, et al. Dose-response relationship of prenatal mercury exposure and IQ: an integrative analysis of epidemiologic data. Environ Health Perspect. 2007
Marques RC, et al. Hair mercury in breast-fed infants exposed to thimerosal-preserved vaccines. Eur J Pediatr. 2007
Berglund M, Lind B, Björnberg KA, et al. Inter-individual variations of human mercury exposure biomarkers: a cross-sectional assessment. Environ Health. 2005
Risher JF, De Rosa CT. Inorganic: the other mercury. J Environ Health. 2007

Poor Hg Inter-Test Correlation

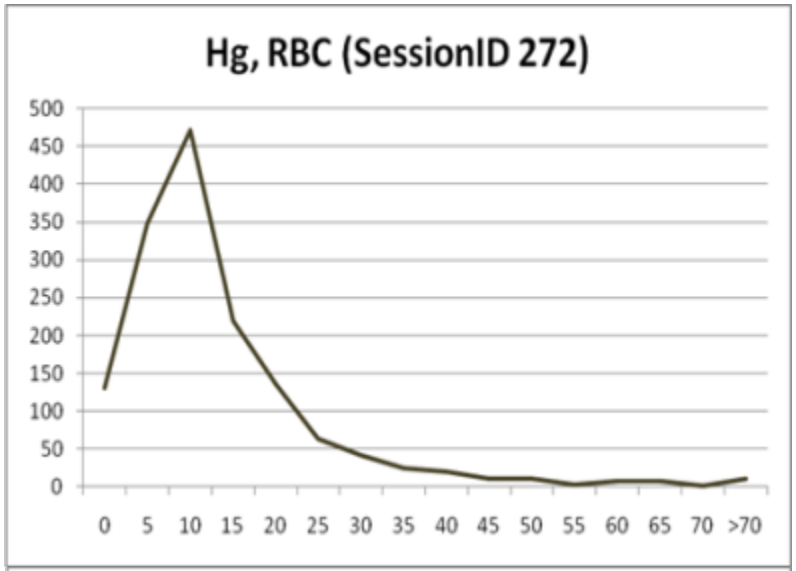
- **Poor correlation between blood and urine** , r = 0.30
- Better correlation between blood and hair, r = 0.56

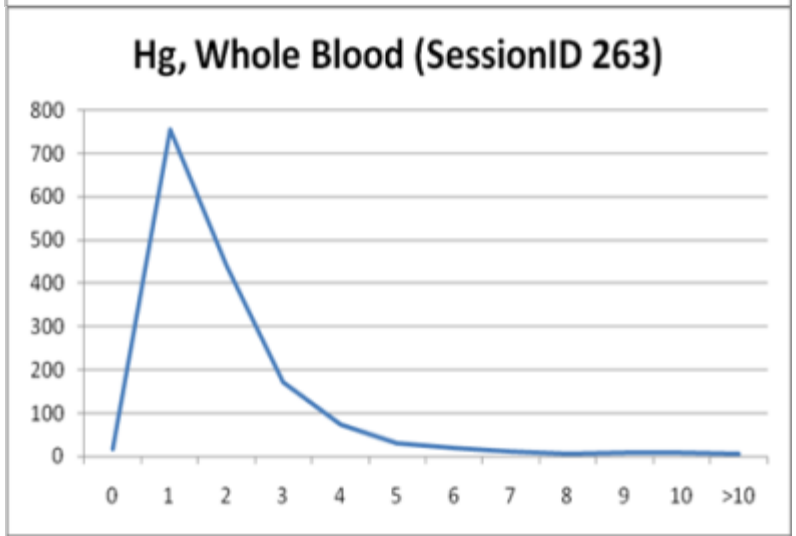


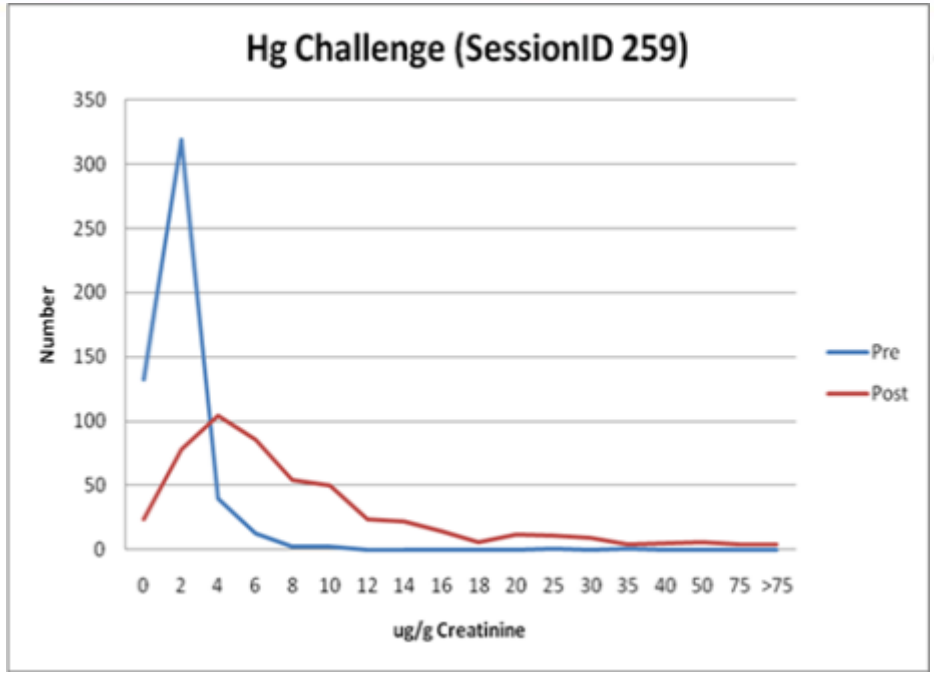


Zimmera H, et al. Determination of mercury in blood, urine and saliva for the biological monitoring of an exposure from amalgam fillings in a group with self-reported adverse health effects. Int. J. Hyg. Environ. Health 2002;205(3):205-211
Berglund M, et al. Inter-individual variations of human mercury exposure biomarkers: a cross-sectional assessment. Environ Health. 2005 Oct 3;4:20

What We Found In Canada



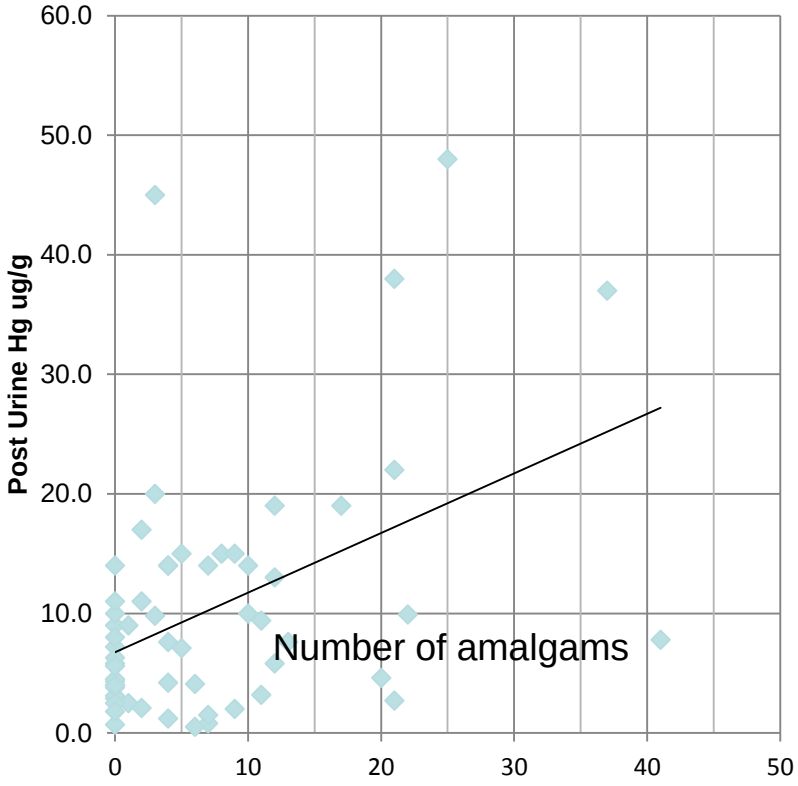




- Deviations from the mean of 14%, 29% and 91% respectively
- DMPS is spreading distribution, suggesting that it is better at differentiating mercury body load
- Some VERY high

Hg Assessment Correlations

- Extensive measurements in 65
  - Whole blood Hg
  - Oral DMPS challenge
  - Amalgam surfaces
- Correlations
  - Whole blood w pre urine: r = 0.40
  - Whole blood w post urine: r = 0.57
  - Pre urine w post urine: r = 0.68
  - Amalgams w pre urine: r = 0.26
  - Amalgams w whole blood: r = 0.36
  - **Amalgams with post urine: 0.44**
- Clear documentation that challenge testing is better



Cadmium

- Blood cadmium - a marker of current exposure but may also reflect body burden from long-term retention of cadmium in the liver and kidney
  - Assessed as whole blood
- **Urinary cadmium is thought to more specifically be a marker of cumulative exposure**

Järup L, Akesson A. et al. Current status of cadmium as an environmental health problem. Toxicol Appl Pharmacol. 2009 Aug 1;238(3):201-8.

Cadmium - Urinary

- Urinary cadmium is mainly influenced by the body burden of cadmium and is proportional to the concentration in the kidney
- Cadmium is efficiently retained mainly in the kidney with a biological half-time of around 10–30 years
- Cd excretion in 24-h urine
- 0.5 µg Cd /g creatinine or greater may be reasonable cut-off

Järup L, Akesson A. et al. Current status of cadmium as an environmental health problem. Toxicol Appl Pharmacol. 2009 Aug 1;238(3):201-8.

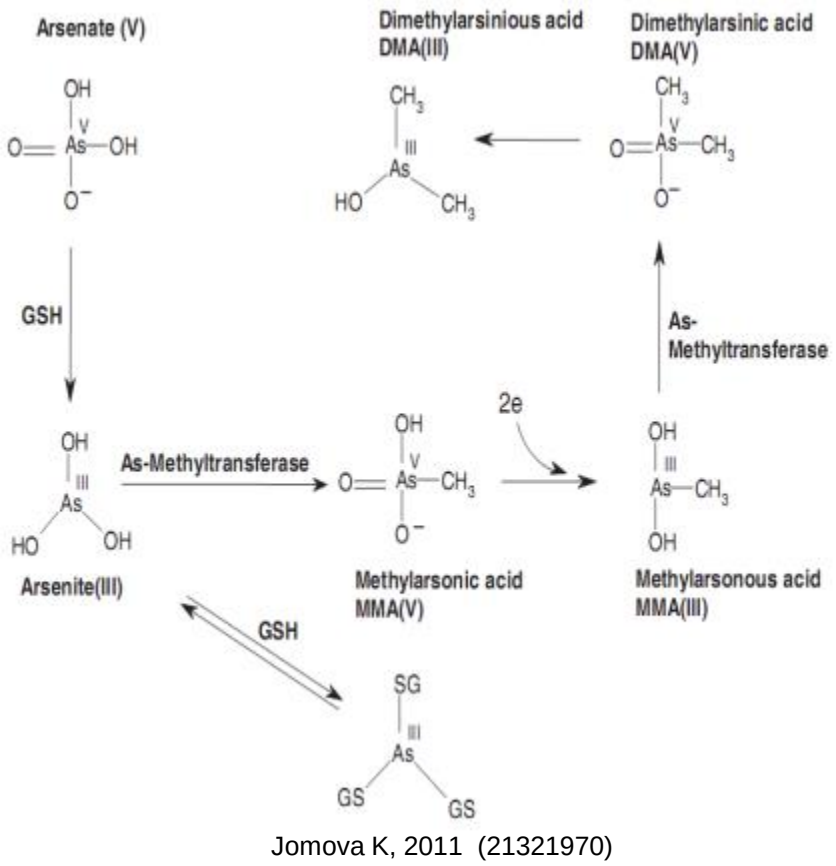
Arsenic Evaluation

- Blood arsenic not a good marker for long term exposure
- May not be a sensitive marker for acute exposure
- Urinary arsenic used as a marker for acute exposure
  - Variety of arsenic compounds in urine, may reflect toxicity
- Urinary levels also used for chronic exposure, but may only be relevant if exposure has stayed constant (and still present)
- Other tissues (hair, nails) may reflect chronic exposure
  - Hair & toenails do reflect past exposure, but susceptible to external contamination and lack standard ranges

Orloff K, et al. Biomonitoring for environmental exposures to arsenic. J Toxicol Environ Health B Crit Rev. 2009 Aug;12(7):509-24.

Arsenic Methylation

- Arsenic methylation was previously believed to be a simple arsenic-detoxification reaction.
- However, it is actually a complex metabolic process which may determine arsenic toxicity, because the toxicological profiles of arsenic metabolites vary
- Intermediate MMAIII and DMAIII have consistently been reported to be more toxic than any other As metabolite
- Variation in methylation capacity may determine individual susceptibility to arsenic – many contributing factors, such as cystathionine-β-synthase SNP



Jomova K, 2011 (21321970)

Gomez-Rubio P et al. Association between body mass index and arsenic methylation efficiency in adult women from southwest U.S. and northwest Mexico. Toxicol Appl Pharmacol. 2011 Apr 15;252(2):176-82.

Toxic Metal Assessment Recommendation

- Acute exposure
  - First morning urine
- Body load
  - 300 mg DMPS + 500 mg of DMSA
  - 6 hour collection
  - DMPS for Hg and DMSA for Pb and Cd

POPs – Laboratory Tests

- Urine, blood, adipose tissue and breath
- Labs:
  - Metametrix – www.metametrix.com
  - Pacific Toxicology – www.pactox.com
  - NMS reference labs – www.nmslab.com
  - Rocky Mountain Analytical – www.rmalab.com

GGT: Indirect Measure of POPs

- Glutathione is key intracellular defense against oxidative stress
- Cellular GGT metabolizes extracellular GSH, allowing precursor amino acids to be reutilized for intracellular GSH.
- **Exposure to POPs induces GGT as a defensive mechanism.**
- Within normal range predicts type 2 diabetes, coronary heart disease, hypertension, stroke, dyslipidemia, chronic kidney disease and cancer.
- **Men with GGT >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 (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.

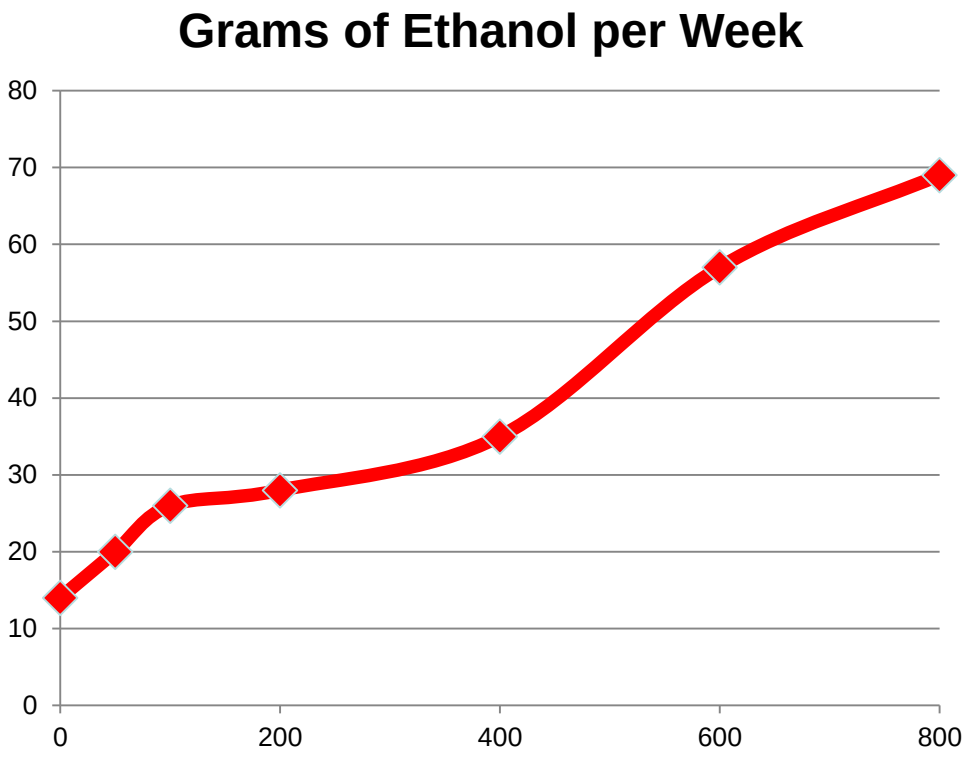
Lee DH, et al.Serum gamma-glutamyltransferase predicts non-fatal myocardial infarction and fatal coronary heart disease among 28,838 middle-aged men and women. Eur Heart J 2006;27:2170–6

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.

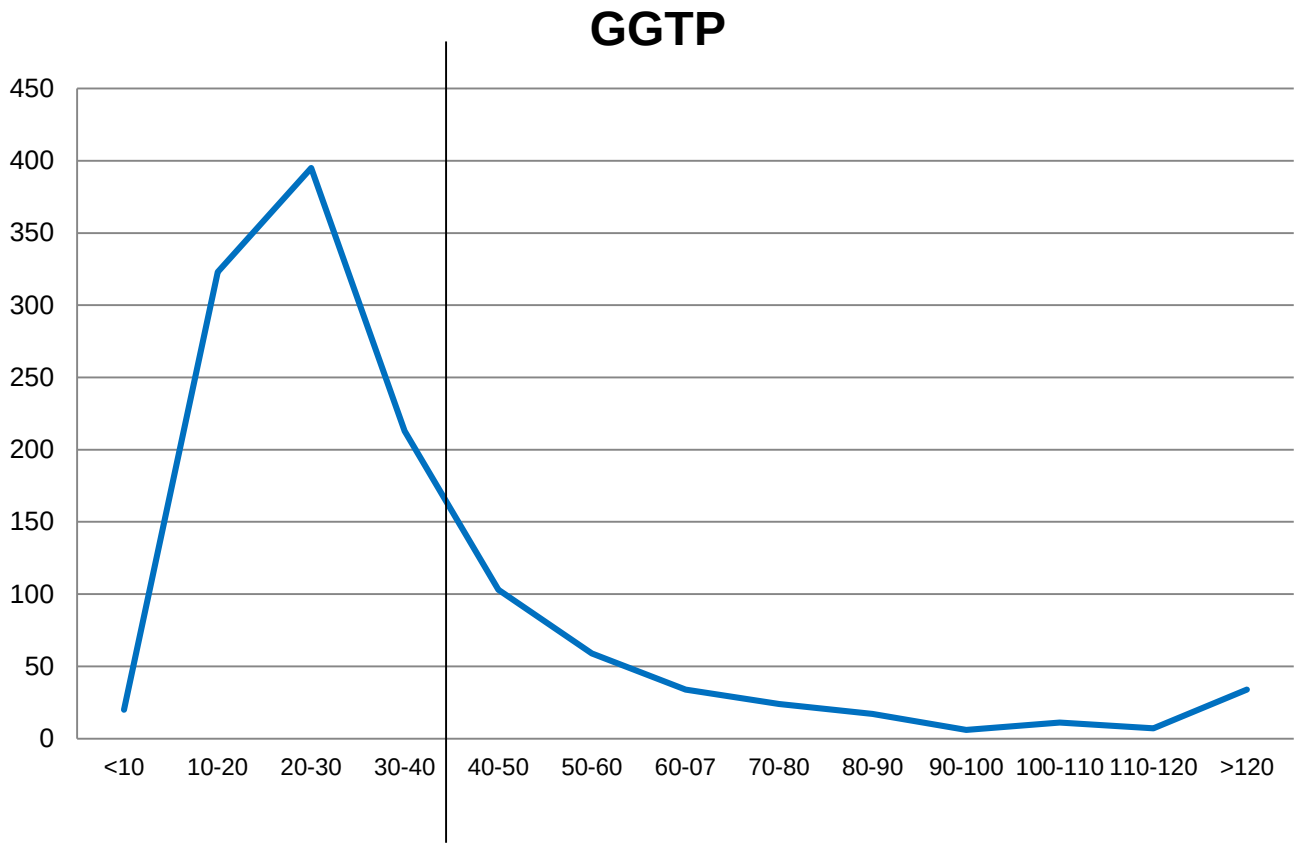
GGT and Alcohol Consumption

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

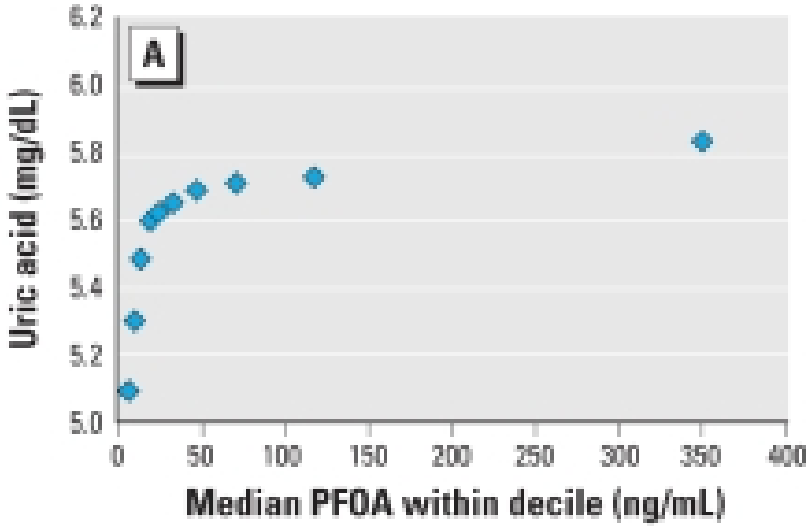
GGT Data from Canadian Oil Field Workers

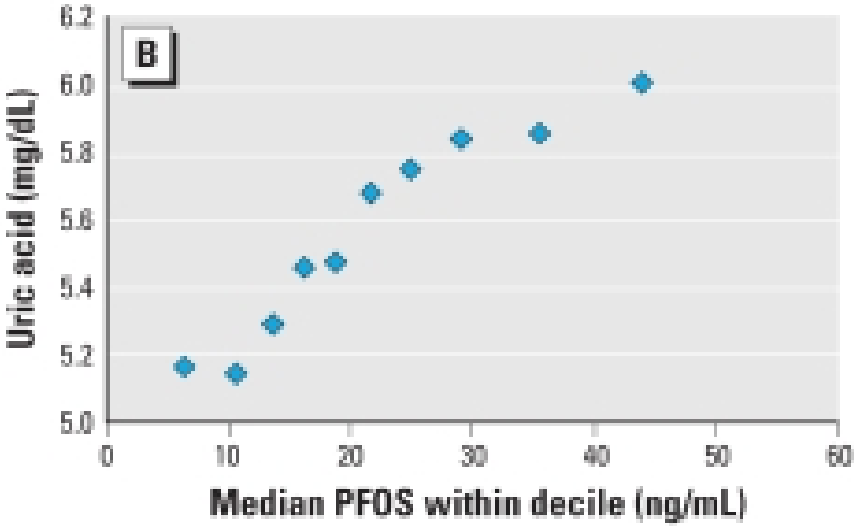


20-fold increased risk of diabetes

Uric Acid: Indirect Measure of POPs

- Poly-fluorinated hydrocarbons (PFOA and PFOS) associated with **increased serum uric acid**





Lin CY, et al. Association among serum perfluoroalkyl chemicals, glucose homeostasis, and metabolic syndrome in adolescents and adults. Diabetes Care. 2009 Apr;32(4):702-7.
Steenland K et al. Association of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) with uric acid among adults with elevated community exposure to PFOA. Environ Health Perspect. 2010 Feb;118(2):229-33.

ALT: Indirect Measure of POPs

- ALT (proxy marker) elevation in 10.4% (not including viral hepatitis, hemochromatosis, or alcoholic liver disease) of NHANES 03-04 subset
- **Risk of elevated ALT increased dose-dependently with lead, mercury, *and* PCB exposure**
- 100% of individuals had detectable PCBs, 92.5% mercury, and 99.6% had detectable lead
- In 2005-08, prevalence of NAFLD in US was 11%, a growing cause of chronic liver disease.

Cave M, et al. Polychlorinated biphenyls, lead, and mercury are associated with liver disease in American adults: NHANES 2003-2004. Environ Health Perspect. 2010 Dec;118(12):1735-42.
Younossi ZM et al. Changes in the prevalence of the most common causes of chronic liver diseases in the United States from 1988 to 2008. Clin Gastroenterol Hepatol. 2011 Jun;9(6):524-530.e1;

Bowel Toxicity Evaluation

- Obermeyer Test (Indican test)
  - Indoles and scatoles (putresceine, cadaverine)
- CDSA
- Intestinal permeability tests

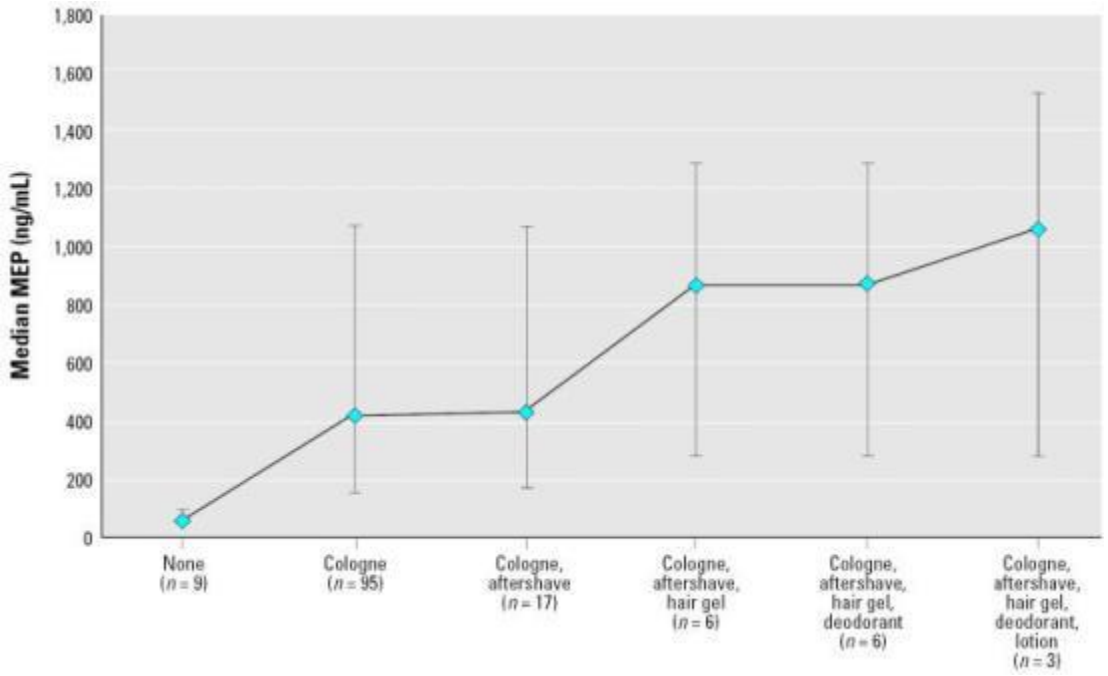
Evaluation of Detoxification

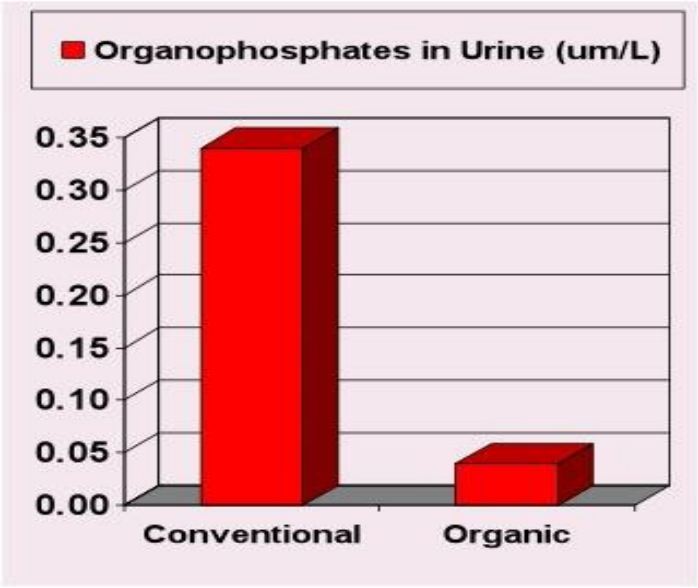
- Many genomic tests available
  - CYP2C9 (*2 and *3 alleles low activity)
  - CYP2C19 (ultra-rapid alleles and those with low activity)
  - CYP2D6 (poor and extensive metabolizer genotype)
  - Acetylation, methylation, and glucuronidation genotype
- Substrate metabolism also used to predict phenotype
  - Buproprion clearance for CYP2B6
  - Caffeine clearance for CYP1A2
  - Glutathione conjugation, glucuronidation, and sulfation predicted by acetaminophen clearance
  - Amino acid conjugation predicted by Acetylsalicylic acid clearance
  - Homocysteine for methylation

Treatment

- Decrease Exposure!!
- Organic, mostly plant-based diet
- Heavy metal chelation if indicated
- Increase glutathione production
- Supportive therapies
  - Antioxidant support
  - Detoxification support
- Bowel detoxification
- Systemic detoxification
  - Sauna
  - Fasting
  - Hydrotherapy

Decrease Exposure!





10-fold increase in pesticides
→ Doubling of ADHD

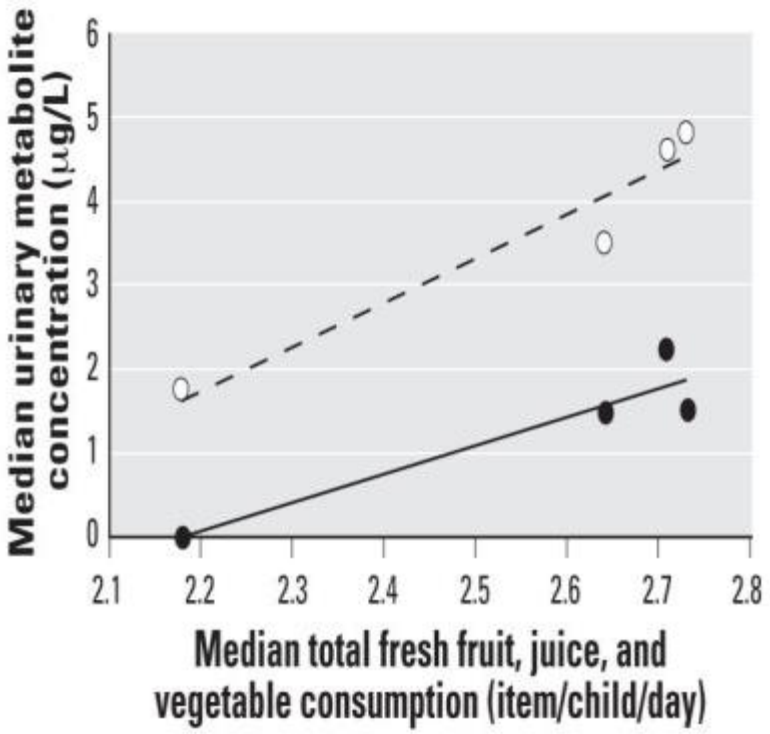
Duty SM, et al. Personal care product use predicts urinary concentrations of some phthalate monoesters. Environ Health Perspect. 2005 Nov;113(11):1530-5

Curl CL, et al. Organophosphorus pesticide exposure of urban and suburban preschool children with organic and conventional diets. Env Health Perspect. 2003;111:377-82

Bouchard MF, et al. Attention-deficit/hyperactivity disorder and urinary metabolites of organophosphate pesticides. Pediatrics. 2010 Jun;125(6):e1270-7

OP Pesticide Exposure in Children Largely Diet

- Longitudinal study: Children's Pesticide Exposure Study (CPES), in Seattle WA
  - Quantitatively assess exposures to OP pesticides using repeated biological sample collection
  - Children switched to organic diet for 5 consecutive days in summer & fall
  - Undetectable malathion (MDA) and chlorpyrifos (TCPy) metabolites following organic
  - Otherwise, levels parallel fresh produce consumption



Solid line, MDA Dashed line TCPy

Lu C, et al. Dietary intake and its contribution to longitudinal organophosphorus pesticide exposure in urban/suburban children. Environ Health Perspect. 2008 Apr;116(4):537-42.

POP Elimination

- Little research for elimination/removal
- Bile sequestrants (eg., colestimide)
- Glutathione support
- Antioxidant support, increased sweating, and reducing intake all likely to benefit
  - Vegan diet associated with lower PCB serum content, but vegetarian diet associated with higher pesticide intake
  - Organic diet eliminated OP pesticide metabolites in children's urine
- Detoxification support – cruciferous vegetables
- Systemic (sauna, fasting, hydrotherapy)

Arguin H, et al. Impact of adopting a vegan diet or an olestra supplementation on plasma organochlorine concentrations: results from two pilot studies. Br J Nutr. 2010 May;103(10):1433-41.
Van Audenhaege M, et al. Impact of food consumption habits on the pesticide dietary intake: comparison between a French vegetarian and the general population. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 2009 Oct;26(10):1372-88.

POPs - Increase Excretion

- Fibre
  - Rice bran (polycyclic biphenyl (PCB), polychlorinated dibenzofurans (PCDFs), polychlorinated-p-dioxines (PCDDs)
  - Wheat bran (PCBs)
  - Slow!!
- Bile sequestrants
  - Cholestyramine and colestimide
- Chlorella/chlorophyll containing foods?
- Green tea polyphenols?

Sera N, et al. Binding effect of polychlorinated compounds and environmental carcinogens on rice bran fiber. J Nutr Biochem. 2005 Jan;16(1):50-8

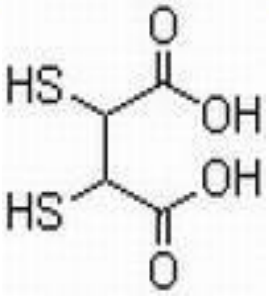
Intervention For Mercury

- Avoidance
  - Choose lower Hg fish
  - Amalgam removal
- Decrease damage
- Provide competing minerals
- Facilitate body's excretion processes
- Directly remove

Interventions We Used In Canada

- Removal of amalgams—must use ecological dentist!!
- IV DMPS
  - Stopped because of excessive adverse events “Brain fog”
- DMSA
  - 250 mg x 3 days, 11 days off
- NAC
  - 600 mg bid
- Fibre
  - PGX: 2.25 g tid
- Supportive nutrients
  - Multivitamin designed to promote glutathione production
  - Ca/Mg/Zn until custom multivitamin available

DMSA



- 2,3-Dimercaptosuccinic acid
- SH-containing, water-soluble, low-toxicity, oral (IV toxic)
- Developed in 1950s as alternative to more toxic chelating agents
- 10-20% of oral dose absorbed
- Chelates all forms of mercury (more effective for Pb)
- Half through urine, half through bile
- Amount of Hg bound: ~7.5 ug/g of oral DMSA
- Increases glutathione production
- Half life in blood 2-3 hours

Ruha AM, Curry SC, Gerkin RD, et al. Urine mercury excretion following meso-dimercaptosuccinic acid challenge in fish eaters. Arch Pathol Lab Med. 2009 Jan;133(1):87-92

Roels HA, Boeckx M, Ceulemans E, Lauwerys RR. Urinary excretion of mercury after occupational exposure to mercury vapour and influence of the chelating agent meso-2,3-dimercaptosuccinic acid (DMSA). Br J Ind Med. 1991 Apr;48(4):247-53

DMSA

- Nutrients to improve efficacy
  - Alpha lipoic acid
  - NAC
  - Probiotics
  - Fibre
- Research studies use 30 mg/kg/day
  - 7 days on, 7 off
  - Not recommended
- Protocol we used:
  - 50 mg trial dose; if no reaction within 2 hours:
    - 250 mg qd for 3 days then off for 11 days, or
    - 250 mg every 3rd day before bed

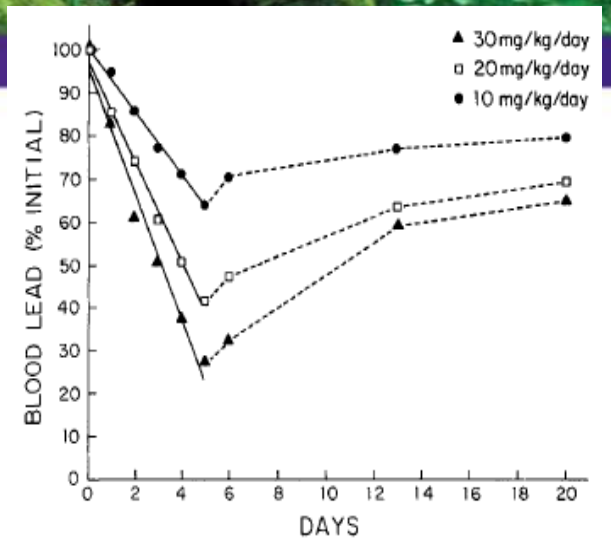
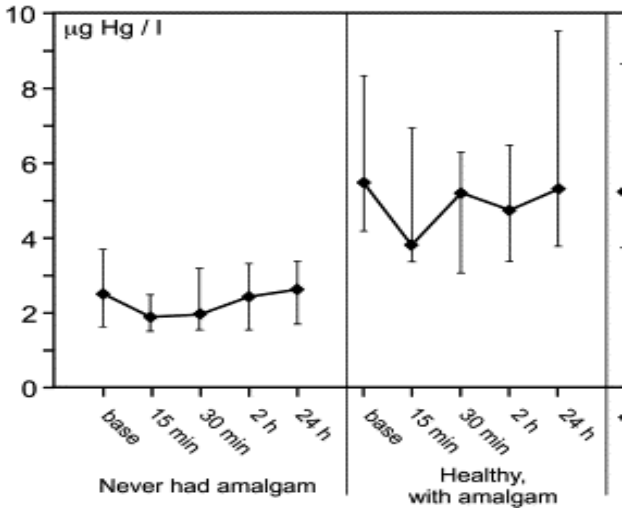


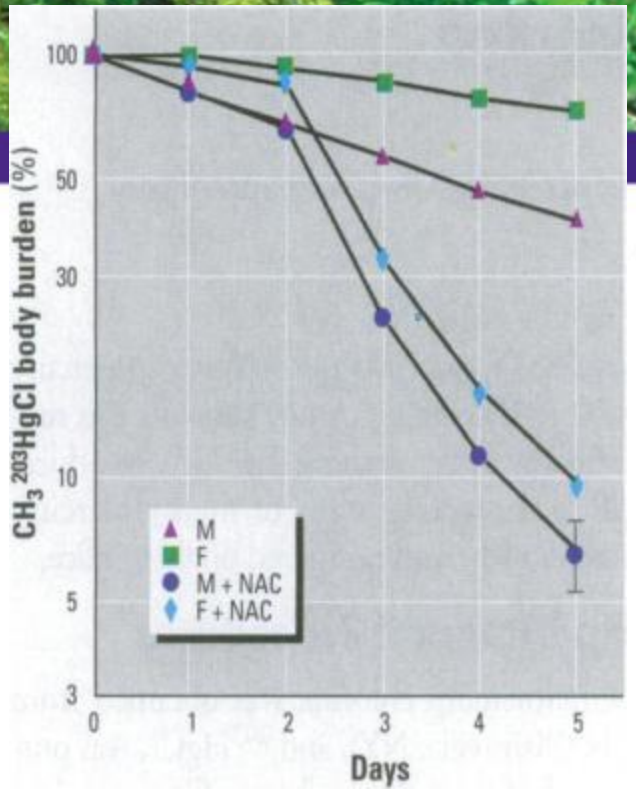
Fig. 1. Mean BPb concentration (expressed as percentage of pretreatment value) after 5 days of DMSA therapy.



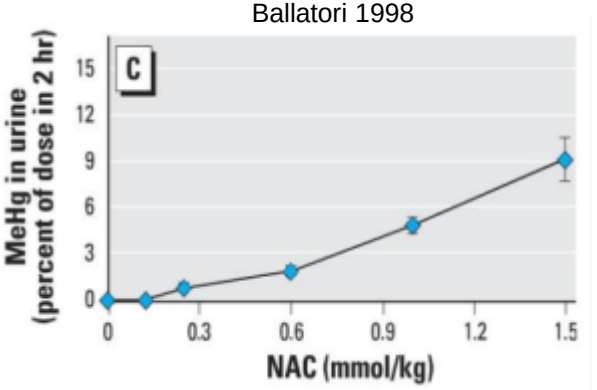
Graziano JH, et al. 2,3-Dimercaptosuccinic acid as an antidote for lead intoxication. Clin Pharmacol Ther. 1985;37(4):431-8

NAC and 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

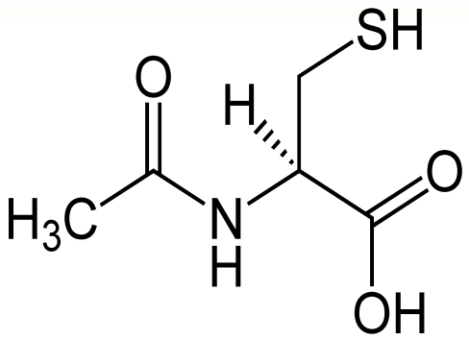


Aremu 2008

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



- IV
  - Treatment of acute acetaminophen poisoning
  - 150 mg/kg body weight given over 15-60 minutes
  - Effective but high incidence of adverse events—ER only!!
- Oral
  - Safe: No serious adverse events in review of 4,000 patients
  - 500 mg: 1-2 times a day

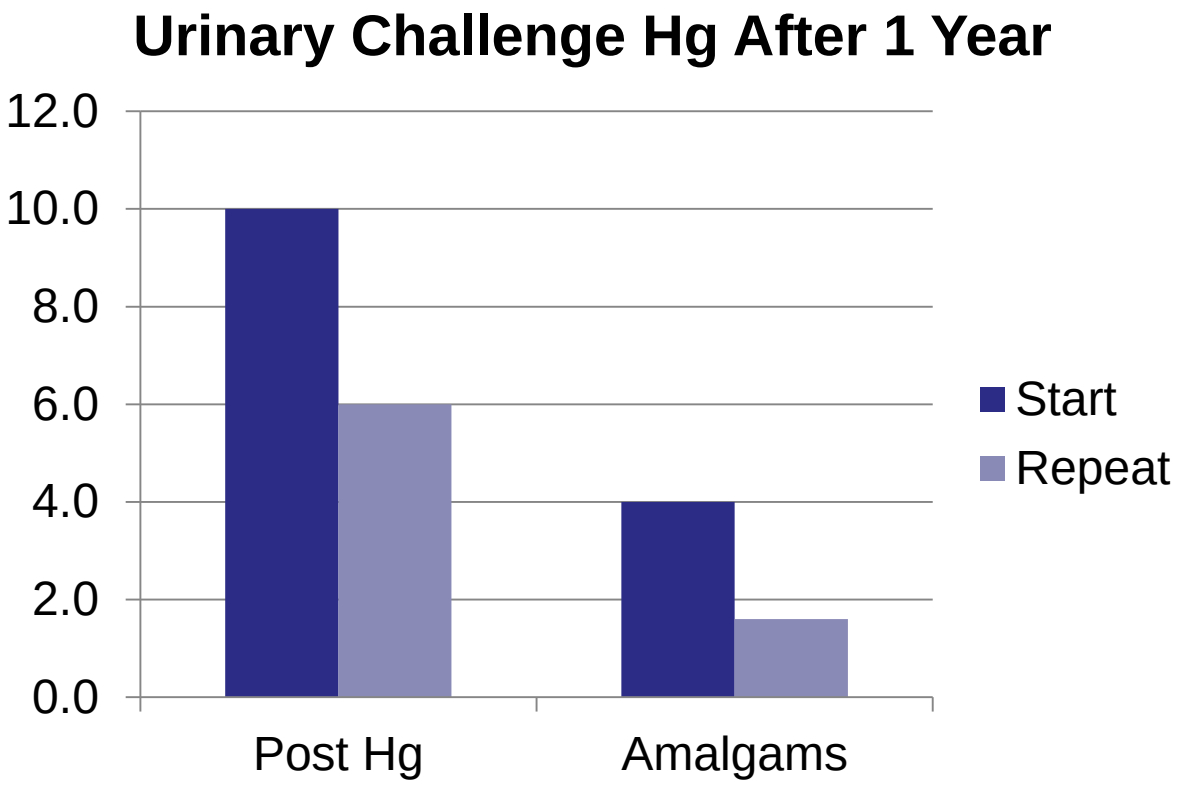
Atkuri KR, et al. N-Acetylcysteine--a safe antidote for cysteine/glutathione deficiency. Curr Opin Pharmacol. 2007 Aug;7(4):355-9

Fibre

- Decreases enterohepatic recirculation
  - Useful for more than just Hg!
- No significant research on fibre
- PGX
  - Research support for improving insulin sensitivity and weight loss
  - 2.25 g tid

Results of Intervention

- DMPS challenge test, ~ 1 year apart



40% reduction!

Lead Chelation

- Chelators: DMPS, DMSA, EDTA, d-penicillamine (DPA)
  - Long history of EDTA use, most often used IV
  - EDTA also depletes Zn, Cu, Fe, Co, and Mn
  - **Oral DMSA as effective as IV EDTA**
  - Not clear if DMSA removes lead from bone, but does reduce hippocampal lead
  - Combination of EDTA and DMSA increase amount excreted

Bradberry, S et al. A comparison of sodium calcium edetate (edetate calcium disodium) and succimer (DMSA) in the treatment of inorganic lead poisoning. Clinical Toxicology 2009
Bradberry Use of oral DMSA in adult patients with inorganic lead poisoning. QJM. 2009
Lee BK, Provocative chelation with DMSA and EDTA: evidence for differential access to lead storage sites. Occup Environ Med. 1995

Cadmium Chelation

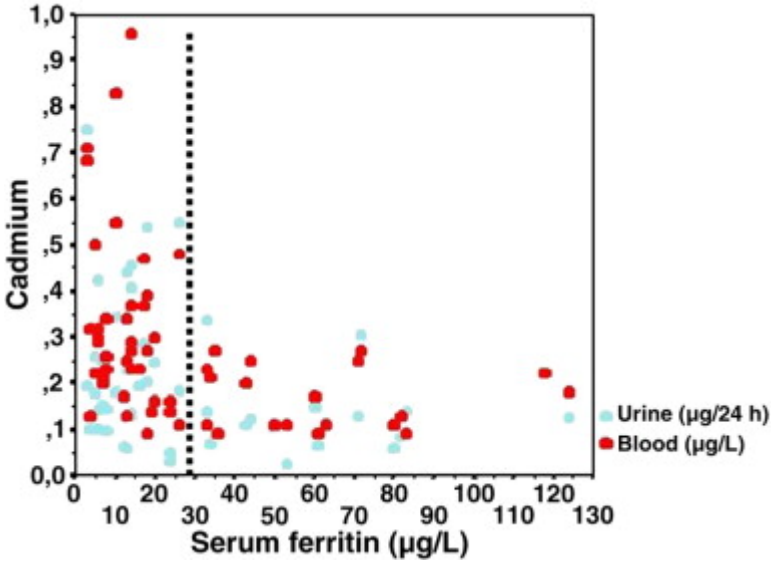
- Little data for chelation, but EDTA primarily used
- DMSA will chelate some
- **May redistribute cadmium and increase its toxicity**
- In a case study, glutathione was used with EDTA, and appeared to increase both blood cadmium and renal excretion of cadmium
- In workers with high exposure, a 14 year study found if urinary levels were initially > 10 microg/g Cr, renal damage was irreversible with EDTA
- Extreme care as easily concentrates in kidneys

Gil HW, et al. Effect of glutathione on the cadmium chelation of EDTA in a patient with cadmium intoxication. Hum Exp Toxicol. 2011 Jan;30(1):79-83.

Wu X, et al. Lack of reversal effect of EDTA treatment on cadmium induced renal dysfunction: a fourteen-year follow-up. Biometals. 2004 Aug;17(4):435-41.

Cadmium Absorption and Iron

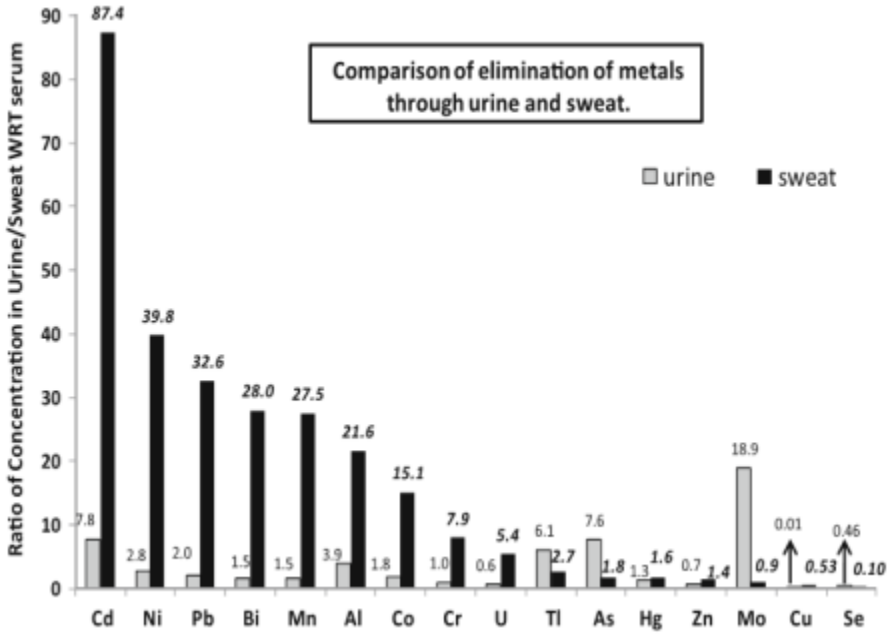
- Iron supplementation if low ferritin
- **Cadmium absorbed much more efficiently if low in iron**



Järup 2009 (19409405)

Cadmium – Sweat it Out

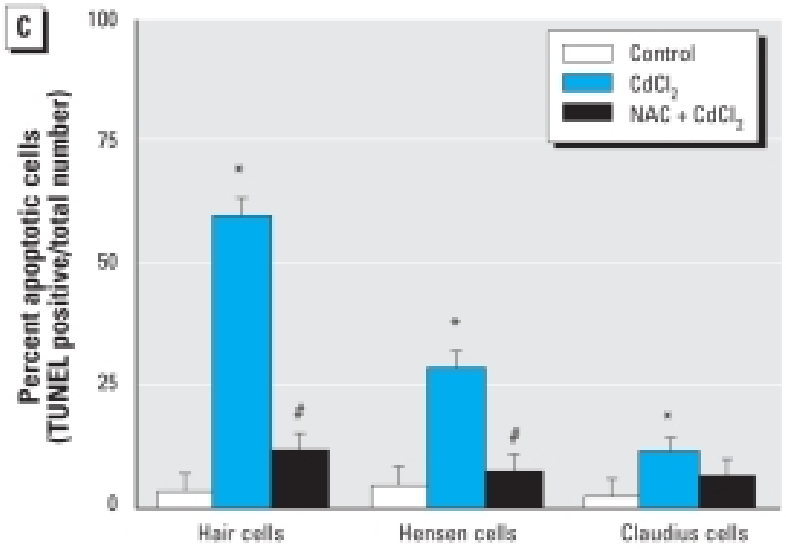
- Cadmium **eliminated efficiently through sweat**
- 20 individuals sweat via exercise, steam or infrared sauna
- Cadmium found in sweat in those with undetectable serum levels, suggesting it could be used for assessment of burden
- Elimination of other minerals (Cu, Mn) suggests need replenishmen during induced sweat



Genuis SJ, et al. Blood, Urine, and Sweat (BUS) Study: Monitoring and Elimination of Bioaccumulated Toxic Elements. Arch Environ Contam Toxicol. 2011 Aug;61(2):344-57

Cadmium - Support

- Antioxidants have been shown to reduce cadmium toxicity
  - NAC reduced ototoxicity in animal studies near to control level, and prevented apoptosis
  - Quercetin reduced cadmium induced oxidant damage



Kim 2008

Kim SJ, et al. The protective mechanism of antioxidants in cadmium-induced ototoxicity in vitro and in vivo. Environ Health Perspect. 2008 Jul;116(7):854-62.
Morales AI, Protective effect of quercetin on experimental chronic cadmium nephrotoxicity in rats is based on its antioxidant properties. Food Chem Toxicol. 2006 Dec;44(12):2092-100.

Arsenic Chelation

- Randomized trial of IV DMPS for chronic arsenicosis found significant reduction in symptoms compared to placebo
  - Dose: 100mg capsules orally, qid x 1 week. Repeat on weeks 3, 5, and 7.
- Monoisoamyl dimercaptosuccinic acid (MiADMSA) removes from blood and soft tissue when given with DMSA, animal studies only
- Spirulina and zinc given together also had significant benefit compared to placebo

Guha Mazumder DN, et al. Randomized placebo-controlled trial of 2,3-dimercapto-1-propanesulfonate (DMPS) in therapy of chronic arsenicosis due to drinking arsenic-contaminated water. J Toxicol Clin Toxicol. 2001;39(7):665-74.

Misbahuddin M, et al. Efficacy of spirulina extract plus zinc in patients of chronic arsenic poisoning: a randomized placebo-controlled study. Clin Toxicol (Phila). 2006;44(2):135-41.

Flora SJ et al. Arsenic and lead induced free radical generation and their reversibility following chelation. Cell Mol Biol (Noisy-le-grand). 2007

Bhadauria S, et al. Response of arsenic-induced oxidative stress, DNA damage, and metal imbalance to combined administration of DMSA and monoisoamyl-DMSA during chronic arsenic poisoning in rats. Cell Biol Toxicol. 2007

Arsenic Methylation

- **Supporting methylation pathways** seems logical
- Increased urinary MMAV in arsenic-exposed subjects suggests an inefficient methylation and probably an increased concentration of the highly toxic MMAIII at cellular level
- Dietary intake and serum concentration of cysteine, methionine, choline, selenium, zinc, folate, niacin, vitamin B12, ferritin, may all modify the metabolism, retention, and toxicity of arsenic
- Arsenic related skin conditions are more common in those with low folate status
- 400ug folate per day in those with low plasma folate increased urinary DMA, and lowered blood MMA, which would seem likely to reduce disease risk

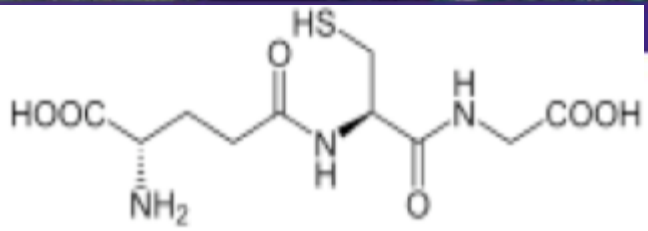
Gamble MV, et al. Folic acid supplementation lowers blood arsenic. Am J Clin Nutr. 2007 Oct;86(4):1202-9.

Arsenic & B12?

- Somewhat surprisingly, B12 alone may not be desirable
- Study of ~800 individuals in Bangladesh, in which low B12 oversampled
  - Plasma cobalamin was inversely associated with %iAs and positively associated with %MMA in urine
  - It did not influence %DMA – thus it may increase the speed of the 1st step to MMA (more toxic), but not the next step to DMA (less toxic).
  - Awaits further evaluation

Hall MN, et al. Influence of cobalamin on arsenic metabolism in Bangladesh. Environ Health Perspect. 2009 Nov;117(11):1724-9.

Glutathione: Critical

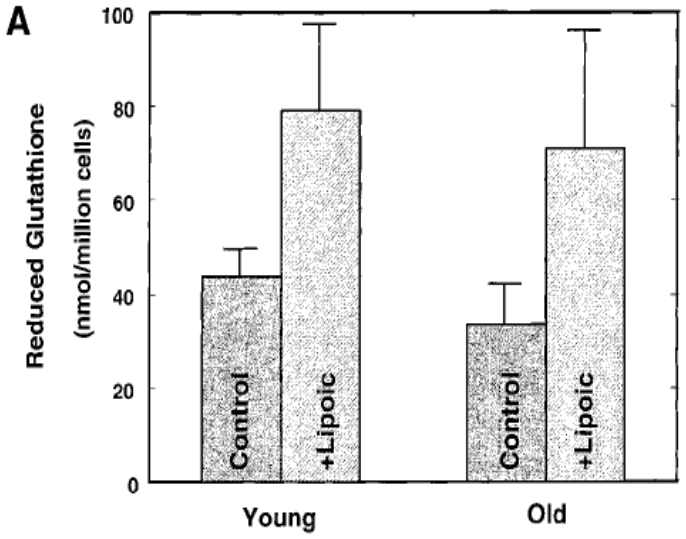


- **Difficult to overstate its importance**
- Most important intracellular and intra-mitochondrial antioxidant
- Binds and transports mercury out of cells and brain
- Irreversibly(?) binds to mercury in the brain
- Neutralizes oxidative damage from mercury and other oxidative toxins
- Facilitates detoxification of POPs
- Depleted by oxidative stress, metals, alcohol
- Even predictor of healthy aging!

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.

Glutathione: Decrease Depletion

- Decrease utilization
  - Decrease toxic exposure, esp alcohol
- Decrease oxidative stress
  - Decrease oxidative markers, increase GSH
  - Alpha lipoic acid for mitochondria
  - Vitamin D for brain
  - Melatonin for brain



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

Glutathione: Direct Administration

- Oral glutathione
  - Inconsistent research; 3 g did not work in humans
- Oral liposomal glutathione
  - Early promising research
- Topical glutathione
- IV glutathione
  - Very effective, but may increase Hg transport into brain
- Intranasal glutathione
  - Intriguing, does not transport Hg from blood
  - Only lung absorption documented (very effective)

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

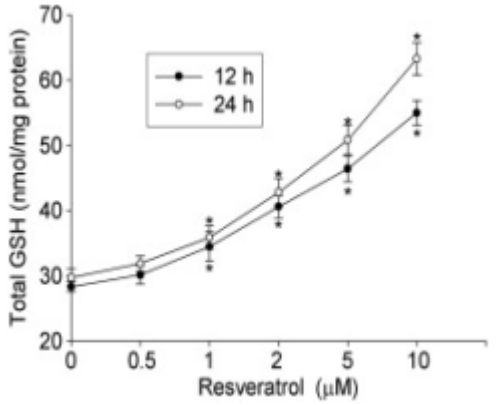
Glutathione – Oral Research

- Recent trial at Bastyr:
  - 500mg 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)
  - GSH used was analyzed and contained no less than 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 at Penn State:
  - 6 months long, using 250-1000mg GSH
  - Found GSH levels were increased 30–35% in RBC, plasma, and lymphocytes, and 260% in buccal cells at higher dosage…NK cytotoxicity up 2x
  - Awaiting full publication

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)

Glutathione: Support Production

- Supply cysteine – primary rate limiting step
- Whey protein – 15 g bid
- NAC
  - 3-500 mg bid
- SAMe
  - Not methionine (increases homocysteine)
- Resveratrol



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

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;26(9):697-703

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

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

Glutathione: Lifestyle Interventions

- Hours/week of moderate exercise positively associated with blood glutathione levels (not excessive exercise!)
- Aerobic and weight training combined more effective than either alone
- Cruciferous vegetables with intact glucosinolates likely to boost glutathione levels and detoxifying enzyme activity

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.

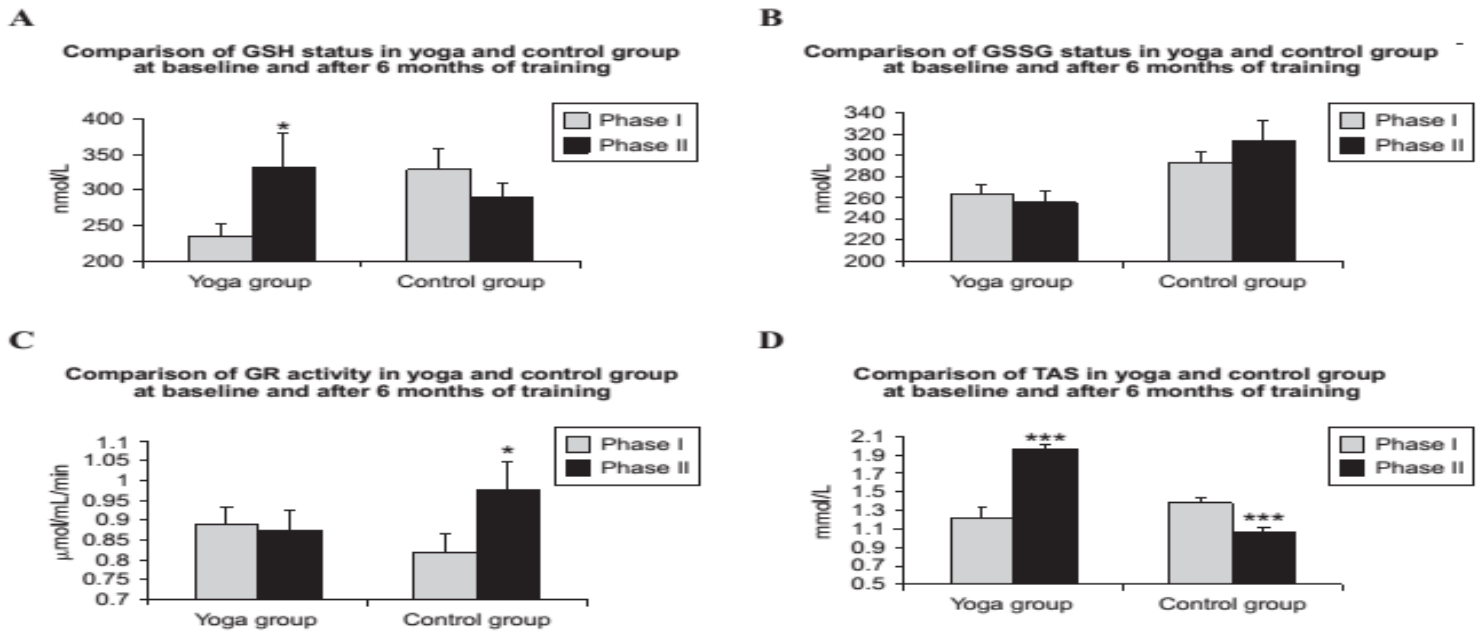
Glutathione & Meditation

- 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

Supportive Therapies: Antioxidants

- N-acetyl-cysteine
  - Animal studies support use as antioxidant for both lead, arsenic, and cadmium toxicity, and may assist in Hg removal
- Lipoic acid
  - Inhibits neuronal Hg damage and increases excretion, and has shown protection against cadmium induced hepatotoxicity
- Vitamins C&E
  - Protective against oxidative damage, and human study showed small reduction in lead retention

Wang L et al. Protective effect of N-acetylcysteine on experimental chronic cadmium nephrotoxicity in immature female rats. Hum Exp Toxicol. 2009

Wang L Protective effect of N-acetylcysteine on experimental chronic lead nephrotoxicity in immature female rats. Hum Exp Toxicol. 2010

Kim SJ, et al. The protective mechanism of antioxidants in cadmium-induced ototoxicity in vitro and in vivo. Environ Health Perspect. 2008

Flora SJ Heavy metal induced oxidative stress & its reversal by chelation therapy. Indian J Med Res. 2008

Supportive Therapies: Antioxidants

- Melatonin
  - Excellent antioxidant, several animal studies demonstrated protective effect for lead, cadmium and arsenic toxicity
  - Also induces antioxidant and detoxification enzymes
- Beta-carotene
  - Significant dose-response relationship was observed for arsenic-related ischemic heart disease and serum level of alpha- and beta-carotene

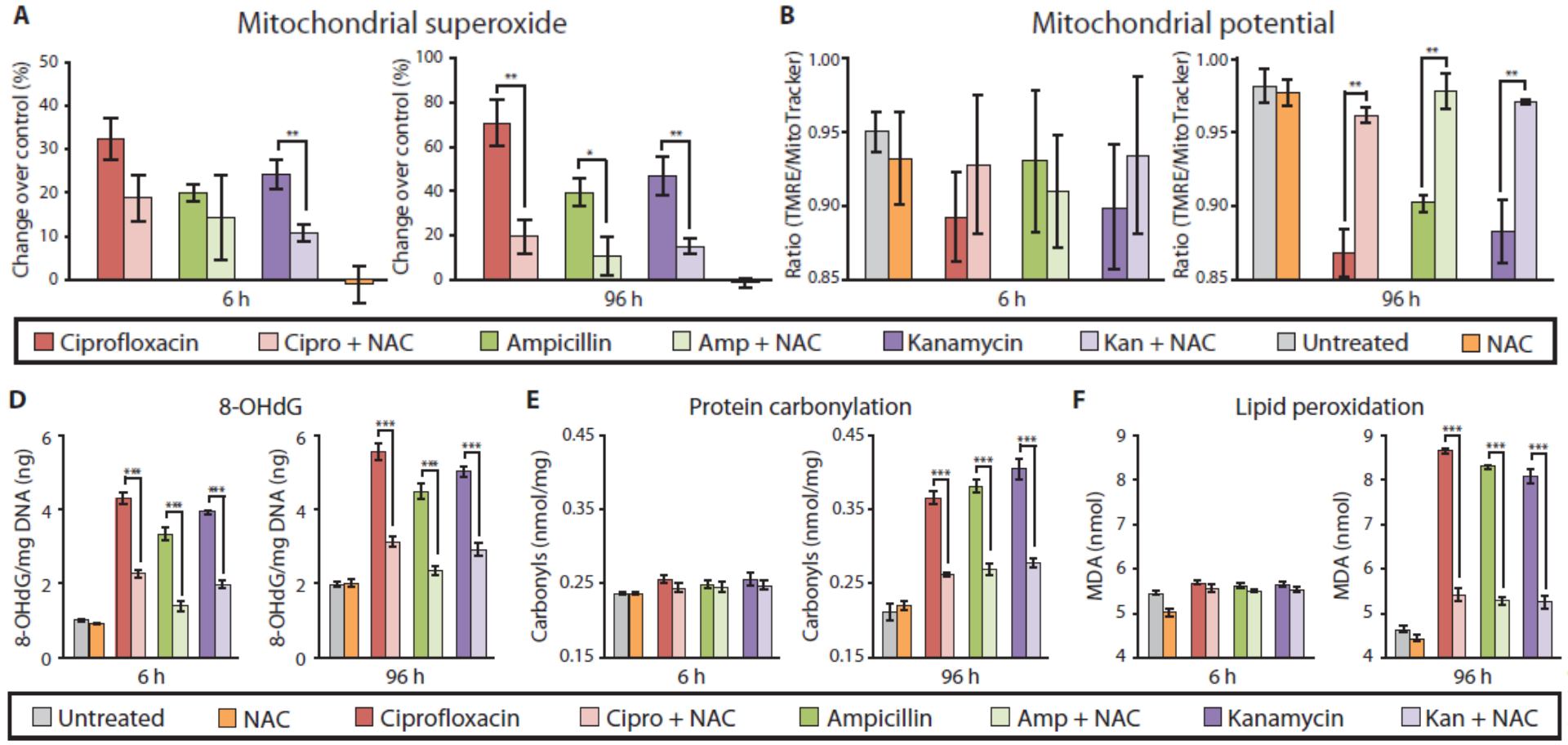
Pal S, et al. Possible Benefcial Effects of Melatonin Supplementation on Arsenic-Induced Oxidative Stress in Wistar Rats. Drug Chem Toxicol 2006
Hsueh YM, Low serum carotene level and increased risk of ischemic heart disease related to long-term arsenic exposure. Atherosclerosis. 1998
Kim YO, et al. Infuence of melatonin on immunotoxicity of lead. Int J Immunopharmacol 2000
Kotler M, et al. Melatonin increases gene expression for antioxidant enzymes in rat brain cortex. J Pineal Res 1998

Supportive Therapy: Detoxification Support

- Phase 2 support
  - **Cruciferous vegetables**
  - Milk thistle
    - Increases liver glutathione by 35% in normals, and stimulates liver regeneration
- Binders of phase 1 intermediates
  - Ellagic acid (**berries**)
    - Prevents estrogen induced mammary tumors in animals; preventing oxidative damage to DNA
  - Catechins (green tea)
    - Protects liver from CYP2E1-dependent alcoholic liver damage
    - Inhibits proliferation of breast cancer cells in vitro and in vivo
- Botanicals to increase bile secretion
  - Cynara scolymus (**artichoke**)
  - Curcuma longa (turmeric) upregulates GST and NAD(P)H:quinone oxidoreductase-1
  - Taraxacum officinalis (dandelion)

Barch DH, et al. Ellagic acid induces NAD(P)H:quinone reductase through activation of the antioxidant responsive element of the rat NAD(P)H:quinone reductase gene. Carcinogenesis. 1994
Nikaidou S, et al. Effect of components of green tea extracts, caffeine and catechins on hepatic drug metabolizing enzyme activities and mutagenic transformation of carcinogens. Jpn J Vet Res. 2005

NAC – Protects Against Toxins



Kalghatgi S, Spina CS, Costello JC, et al. Bactericidal antibiotics induce mitochondrial dysfunction and oxidative damage in Mammalian cells. Sci Transl Med. 2013 Jul 3;5(192):192

Bowel Detoxification

- Large portion of liver's load comes via portal vein from GI
- Toxins include bacteria, undigested proteins, immune complexes, metabolic products
- Although total CYP3A in small intestine is only 1% of liver, intestinal extraction of 3A substrates equals or exceeds liver for some substances (likely due to slower flow in enterocytes)
- Increased intestinal permeability & enterocyte inflammation increases load on liver
- Healthy flora lowers load
  - L. acidophilus increases first pass metabolism, protects liver & stomach from injury
  - Bifidobacterium, by reducing endotoxin, lowers rate of diabetes and obesity
  - Gut flora detoxes many chemicals, such as heterocyclic aromatic amines

Qing L, et al. Lactic acid bacteria prevent alcohol-induced steatohepatitis in rats by acting on the pathways of alcohol metabolism. Clin Exp Med. 2008

Cani PD, et al. Selective increases of bifidobacteria in gut microflora improve high-fat-diet-induced diabetes in mice through a mechanism associated with endotoxaemia. Diabetologia. 2007

Knasmüller S, et al. Impact of bacteria in dairy products and of the intestinal microflora on the genotoxic and carcinogenic effects of heterocyclic aromatic amines. Mutat Res. 2001

Stidl R, et al. Binding of heterocyclic aromatic amines by lactic acid bacteria: results of a comprehensive screening trial. Mol Nutr Food Res. 2008

Bowel Detoxification

- Eliminate toxic bacteria
  - Hydrastis and garlic
- Remove toxins
  - Fibre - animal studies suggest fiber may help reduce toxins from C. difficile
  - Charcoal - large single doses within 1 hour of toxin consumption are effective for acute toxicity, multiple smaller doses more effective for ongoing toxicity

Scazzocchio F, et al. Antibacterial activity of Hydrastis canadensis extract and its major isolated alkaloids. Planta Med. 2001
Ruddock PS, et al. Garlic natural health products exhibit variable constituent levels and antimicrobial activity against Neisseria gonorrhoeae, Staphylococcus aureus and Enterococcus faecalis. Phytother Res. 2005
Bond GR. The role of activated charcoal and gastric emptying in gastrointestinal decontamination: a state-of-the-art review. Ann Emerg Med. 2002
Neuvonen PJ, et al. Oral activated charcoal in the treatment of intoxications. Role of single and repeated doses. Med Toxicol Adverse Drug Exp. 1988

Bowel Detoxification

- Reseed with healthy bacteria
- Support growth of healthy bacteria with prebiotics (yogurt, oats, legumes, beets, artichoke, etc.)
- Repair damaged intestines
  - Gottschall diet
  - Glutamine: 250 mg bid
  - Quercetin: 500 mg/d
  - Omega-3 fatty acids: 2 g/d

TABLE 1. Recommendations for Probiotic Use

Clinical Condition	Effectiveness	Organism	References
Diarrhea			
Infectious adult—treatment	A	Saccharomyces boulardii, LGG	2–4
Infectious childhood—treatment	A	LGG, *Lactobacillus reuteri*	4–7
Prevention of infection	B	*S. boulardii*, LGG	3
Prevention of AAD	A	*S. boulardii*, LGG, *L. casei*, *L. bulgaricus*, *S. thermophilus*	8, 9
Treatment of recurrent CDAD	B	*S. boulardii*, LGG	8, 9
Prevention of CDAD	B	LGG, *S. boulardii*	8–12
IBD			
Pouchitis			
Preventing and maintaining remission	A	VSL#3	13–15
Induce remission	C	VSL#3	16
Ulcerative colitis			
Inducing remission	C	*Escherichia coli* Nissle, VSL#3	17–19
Maintenance	C	*E. coli* Nissle, VSL#3	20–22
Crohn's	C	*E. coli* Nissle, *S. boulardii*, LGG	23–26
IBS	B	*Bifidobacterium infantis*	27–29
	C	*Bifidobacterium animalis*, VSL#3, *Lactobacillus plantarum*	30–34
Immune response	A	LGG, *Lactobacillus acidophilus*, *L. plantarum*, *Bifidobacterium lactis*, *Lactobacillus johnsonii*	35, 36
Allergy			
Atopic eczema associated with cow's milk allergy			
Treatment	A	LGG, *B. lactis*	37–43
Prevention	A	LGG, *B. lactis*	44–48
Radiation enteritis	C	VSL#3, *L. acidophilus*	49–52
Vaginosis and vaginitis	C	*L. acidophilus*, LGG, *L. reuteri*	53–55

AAD indicates antibiotic-associated diarrhea; IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; CDAD, *Clostridium difficile*-associated diarrhea; LGG, *Lactobacillus* GG.

Rosella O, et al: Polyunsaturated fatty acids reduce non-receptor-mediated transcellular permeation of protein across a model of intestinal epithelium in vitro. J Gastroenterol Hepatol 15:626-631, 2000

Kim H, et al. Metabolic and pharmacological properties of rutin, a dietary quercetin glycoside, for treatment of inflammatory bowel disease. Pharm Res. 2005

Systemic Detoxification

- Sauna
- Fasting
- Hydrotherapy

Sauna
(Heat Chamber Depuration)

- Extended time: 1-2 hours
- Modest temperature: 150-170^{o}F
- Increases excretion of:
  - Heavy metals: arsenic, **cadmium**, lead, mercury
  - Chemicals: phthalates, PCBs, PBBs, and HCBs
  - Essential trace minerals
- Increases:
  - Lipolysis
  - Growth hormone

Sears ME, et al. Arsenic, cadmium, lead, and mercury in sweat: a systematic review. J Environ Public Health. 2012;2012:184745
Schnare DW, et al: Body burden reductions of PCBs, PBBs, and chlorinated pesticides in human subjects. Ambio, 1984
Tretjak Z, et al: PCB reduction & clinical improvement by detoxification: and unexploited approach? Hum Exp Toxicol, 1990
Cohn JR, et al: The excretion of trace metals in human sweat. Ann Clin Lab Sci 1978
Lammintausta T, et al: Change in hormones reflecting sympathetic activity in Finnish sauna. Ann Clin Res, 1976

Sauna – Clinical Results

- Improved neurobehavioral function in firement exposed to PCBs
- Increased excretion of phthalates in sweat
- Increased excretion of BPA in sweat

Schnare DW, et al: Evaluation of detoxification for fat stored xenobiotics. Med Hyp 1982

Kilburn KH, et al. Neurobehavioral dysfunction in firemen exposed to polycholorinated biphenyls (PCBs): possible improvement after detoxification. Arch Environ Health. 1989 Nov-Dec;44(6):345-50

Genuis SJ, et al. Human Elimination of Phthalate Compounds: Blood, Urine, and Sweat (BUS) Study. ScientificWorldJournal. 2012;2012:615068

Genuis SJ, et al. Human excretion of bisphenol A: blood, urine, and sweat (BUS) study. J Environ Public Health. 2012;2012:185731

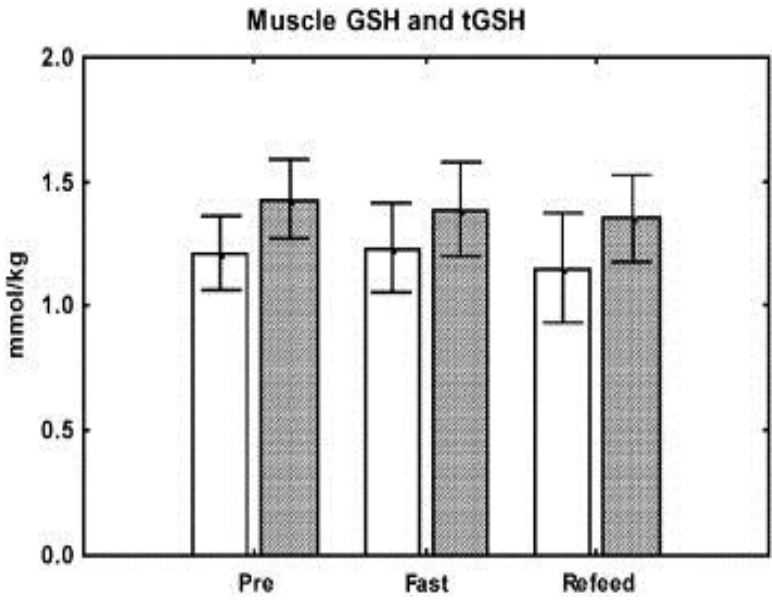
Fasting

Increases detoxification of:

- PCBs, DDT
- Circulating immune complexes
- Food allergies

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

Hydrotherapy

- Bath General Hospital
  - Lead poisoning (colica pictonum)
  - 120 years of records analyzed
  - 3,377 patients with lead poisoning
  - 45.4% cured; 93% improved
- Treatment
  - 1+ hour full body (standing) immersion at 35^{o}C
  - 3 times per week; average stay 150 days
- Physiological research
  - Standing full immersion:
    - Increases cardiac output 50%
    - Increases excretion of lead 250%
    - Peak excretion at 2.5 hours

Heywood A: A trial of Bath waters: The treatment of Pb poisoning. Med Hist Supl 10:82-101, 1990

Summary Assessment

- Total body chemical load:
  - GGT: > 30 U/l
  - ALT:
  - Uric acid: > 5.0
- Metal body load:
  - DMPS: 300 mg
  - DMSA: 500 mg
  - Collect urine for 6 hours
- Gut toxin load:
  - Obermeyer: light blue or darker

Summary Intervention

- Find and eliminate source of toxins
- Eat organically grown foods, esp. dirty dozen
- Use low POP HABAs (Health and Beauty Aids)
- Facilitate detoxification:
  - High fibre diet
  - Multivitamin and mineral
  - Long saunas
- Protect from damage by promoting glutathione
  - NAC: 500 mg/d

Summary

- Body load of exogenous and endogenous toxins substantial
- Most of the population has body loads at levels associated with increased incidence of a wide range of diseases
- Avoidance works, but exposure not avoidable
- Nutrients, botanicals, hydrotherapy and drugs work to decrease body load