Intestinal Permeability, Vitamin D and Autoimmune Disease

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Overview

Gastrointestinal Health

- Physiology of intestinal epithelial barrier function
- Causes of increased permeability (i.e. leaky gut)
- Consequences
- Assessment
- Treatment

Detoxification

- Toxic metals
- Persistent organic pollutants (POPs)

Information resources

Product resources

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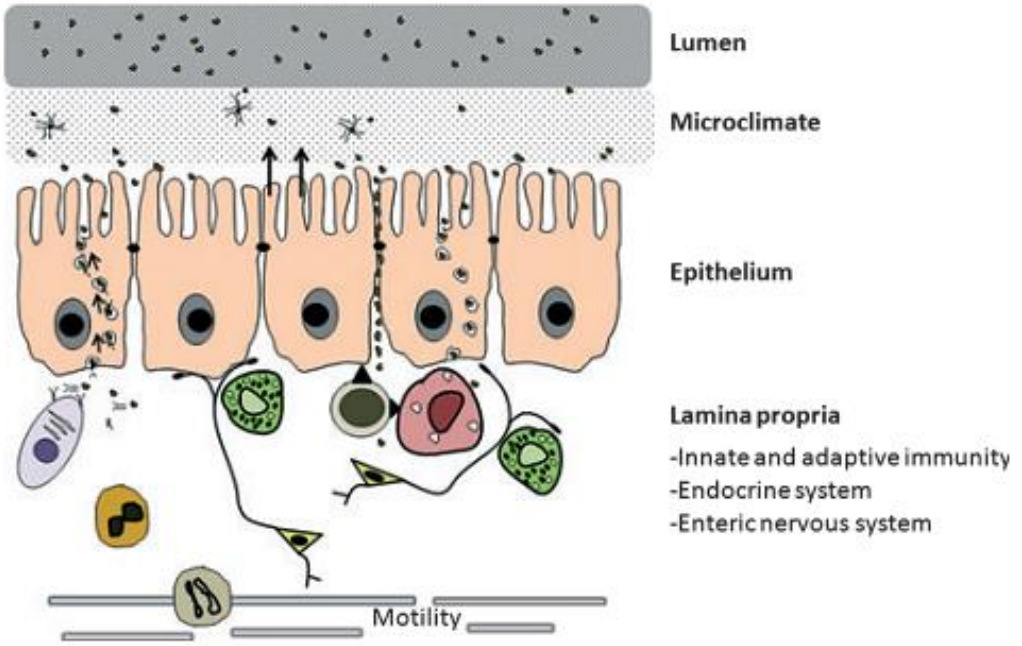
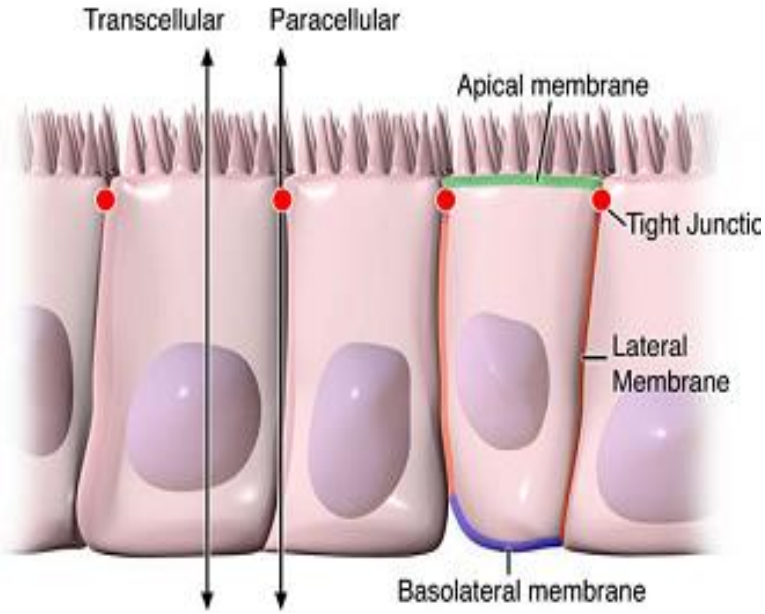
Physiology of Intestinal Barrier

- Largest mucosal surface in the body
- Coordinates digestive, absorptive, motility, neuroendocrine, and immunological/protective functions
- Barrier composed of luminal factors (pH, bacteriocins produced by commensal bacteria), microclimate (glycocalyx, IgA), epithelial cells (tight junctions), and lamina propria (innate and adaptive immune cells)



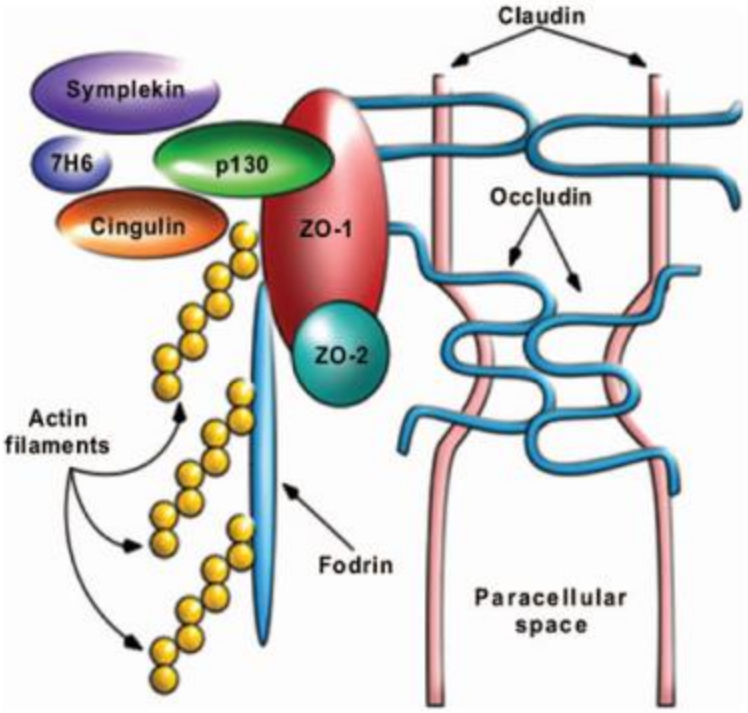
Dilemma of Opposing Functions

- Intestinal barrier function must balance the need for a barrier against a hostile environment with the necessity of active and passive transport of nutrients
- Barrier function comprised of immune cells, molecular pattern recognition receptors (i.e. Toll-like receptor 4), toxins (defensins, mucins), immune cells, and epithelial cells
- Molecular trafficking between the intestinal lumen and the submucosa regulated via the paracellular space (between 10 and 15 Å)
- Regulation of paracellular permeability depends on the modulation of intercellular tight junctions (TJs)



Tight Junctions

- Tight junctions, aka zonula occludens, regulate paracellular permeability
- Once viewed as an extracellular cement forming an absolute barrier, it is now apparent that tight junctions are extremely dynamic structures made up by a complex network of proteins



Composition of tight junctions

Increased Intestinal Permeability *(aka leaky gut)*

- During the healthy state, small quantities of immunologically active antigens cross the gut barrier, contributing to antigen tolerance
- Abnormal permeability refers to a measurable increase in flux of small water-soluble compounds across the paracellular pathway of the small intestine.
- When the integrity of tight junctions are compromised, increased paracellular permeation of substances (such as toxic antigens) leads to deleterious immune responses

Fasano A. Physiological, pathological, and therapeutic implications of zonulin-mediated intestinal barrier modulation: living life on the edge of the wall. Am J Pathol. 2008 Nov;173(5):1243-52.

Causes of Increased Intestinal Permeability

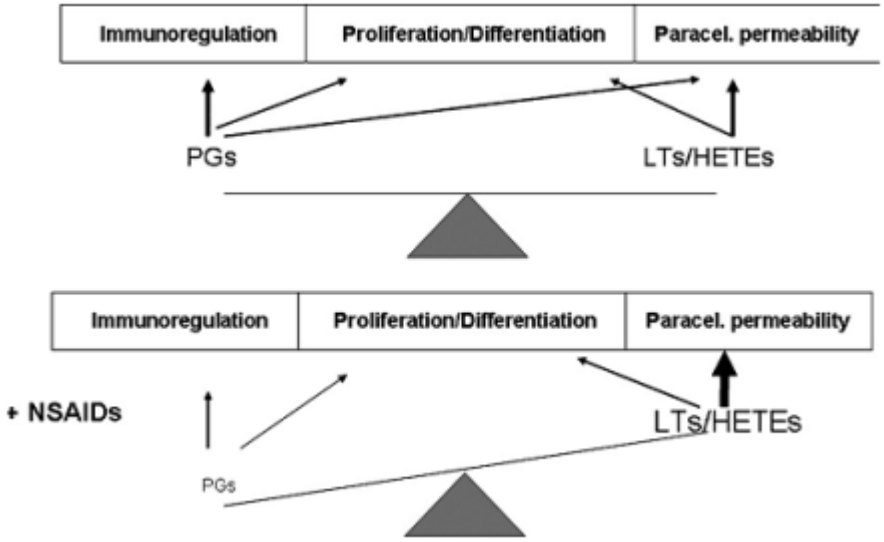
- Infection, inflammatory cytokines, dysbiosis, nutrient transporter activation, noxious environmental toxins
- Examples:
  - Aspirin was recently shown to increase the susceptibility to “gut leakiness” in patients with NASH, demonstrated by urinary sucralose
  - PCBs found to disrupt tight junction protein expression

Farhadi A, et al. Susceptibility to gut leakiness: a possible mechanism for endotoxaemia in non-alcoholic steatohepatitis. Liver Int. 2008

Choi YJ Polychlorinated Biphenyls Disrupt Intestinal Integrity via NADPH Oxidase-Induced Alterations of Tight Junction Protein Expression. Environ Health Perspect. 2010 Mar 18

Intestinal Hyperpermeability—Eicosanoids

- Eicosanoids are mediators of inflammation.
- In IBD the levels of prostanoids such as PGE2, PGF2α and PGD2, as well as 12-HETE, 15-HETE and LTB4 are higher in inflamed compared to normal mucosa
- Also, an increase in PGE2 levels is correlated with disease activity while PGE2 and LTB4 levels return normal during remission
- Treatment with NSAIDs reduces PG levels and increases the production of LTs/HETEs. This up-regulates the immune response and the disruption of epithelial barrier function



Ferrer R, Moreno JJ. Role of eicosanoids on intestinal epithelial homeostasis. Biochem Pharmacol. 2010 May 10.

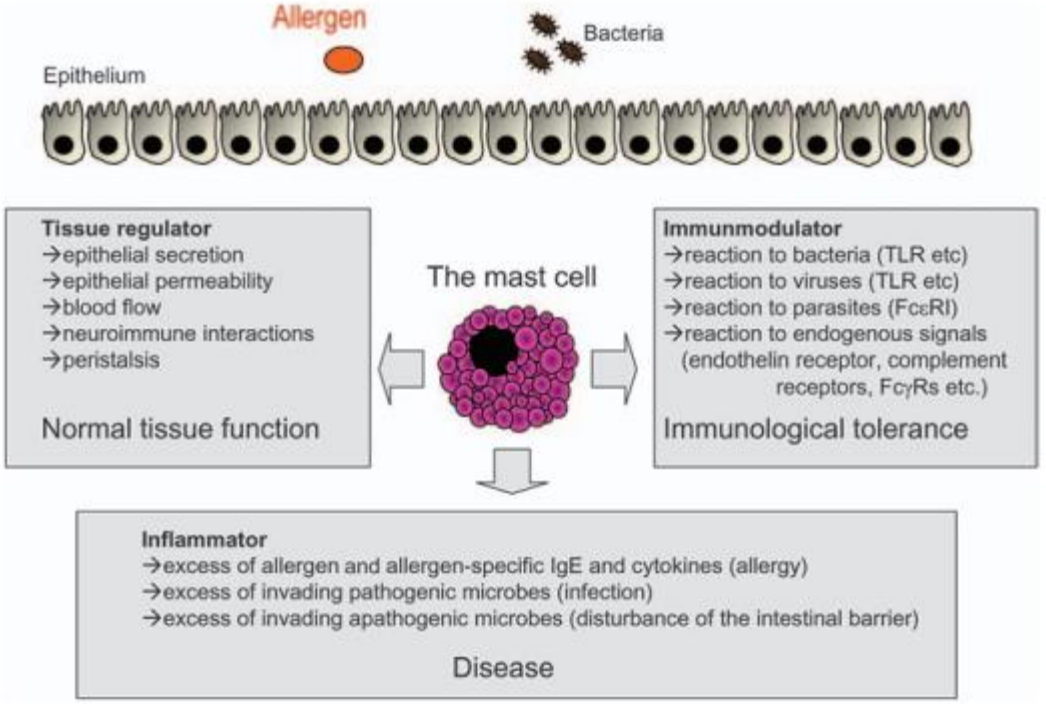
Intestinal Hyperpermeability—Dysbiosis

Intestinal Dysbiosis

- Intestinal microbes modulate intestinal permeability, at least partly by reducing inflammation
- Commensal bacteria seem to be able to minimize epithelial cell NF- B activation, and actively dampen intestinal inflammation

Intestinal Hyperpermeability—Mast Cells

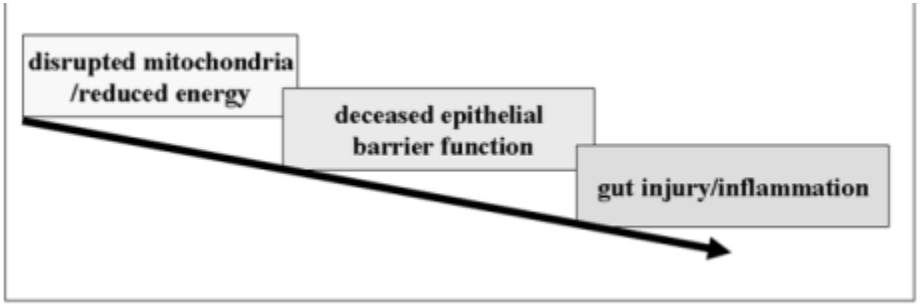
- Mast cells exert multiple roles in mucosal immunity
- Mast cells may become dysregulated because of excess allergens, allergen-specific IgE and cytokines, or invading microbes
- Abnormal mast cell activation causes impairment of the intestinal barrier



Bischoff SC, Krämer S. Human mast cells, bacteria, and intestinal immunity. Immunol Rev. 2007 Jun;217:329-37.

Intestinal Hyperpermeability—Metabolic Stress

Recent in-vitro and in-vivo data also suggests that metabolic stress, i.e. reduced ATP production via mitochondrial dysfunction, may also increase intestinal permeability and contribute to intestinal inflammation



Lewis K, McKay DM. Metabolic stress evokes decreases in epithelial barrier function. Ann N Y Acad Sci. 2009 May;1165:327-37

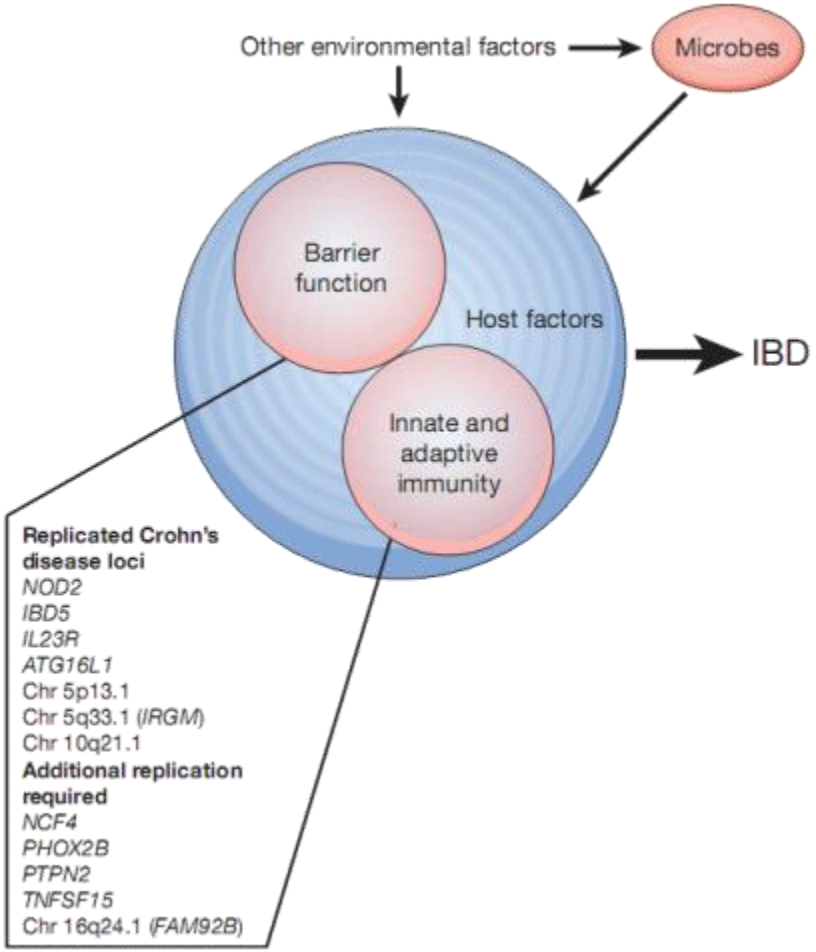
Intestinal Hyperpermeability—Dietary Factors

- A broken tolerance to dietary antigens may lead to immune activation and intestinal inflammation
  - E.g. Type 1 diabetes:
    - Wheat proteins have been suggested to be specific triggers of subclinical inflammation in patients with type 1 diabetes (NOTE: this is not in patients with celiac disease)
    - Dietary bovine insulin has been hypothesized to be a trigger of the [beta]-cell autoimmunity in the context of the dysregulated intestinal immune system

Vaarala O. Leaking gut in type 1 diabetes. Curr Opin Gastroenterol. 2008 Nov;24(6):701-6

Consequences

- Inflammatory bowel disease (IBD)
- Asthma
- Atopic dermatitis
- Cardiac failure
- Fibromyalgia
- Chronic regional pain syndrome
- Celiac disease
- Intestinal ischemia
- Food intolerance and allergy
- Malnutrition
- Rheumatoid arthritis
- Diabetes type 1
  - At least in the case of type 1 diabetes, the intestinal dysfunction appears to precede the clinical onset of diabetes



Keita AV, Söderholm JD. The intestinal barrier and its regulation by neuroimmune factors. Neurogastroenterol Motil. 2010 Apr 9

Bosi E, et al. Increased intestinal permeability precedes clinical onset of type 1 diabetes. Diabetologia. 2006

Consequences—Heart Failure

Heart failure

- It is not currently clear which comes first… if an increase in intestinal permeability is the primary event which causes systemic inflammation leading to heart disease, or if myocardial dysfunction can yield microcirculatory injuries which disrupt the intestinal barrier
- However, “lack of mucosal integrity with consecutive local and systemic inflammation and dysfunction of transport proteins may worsen the clinical symptoms of CHF”

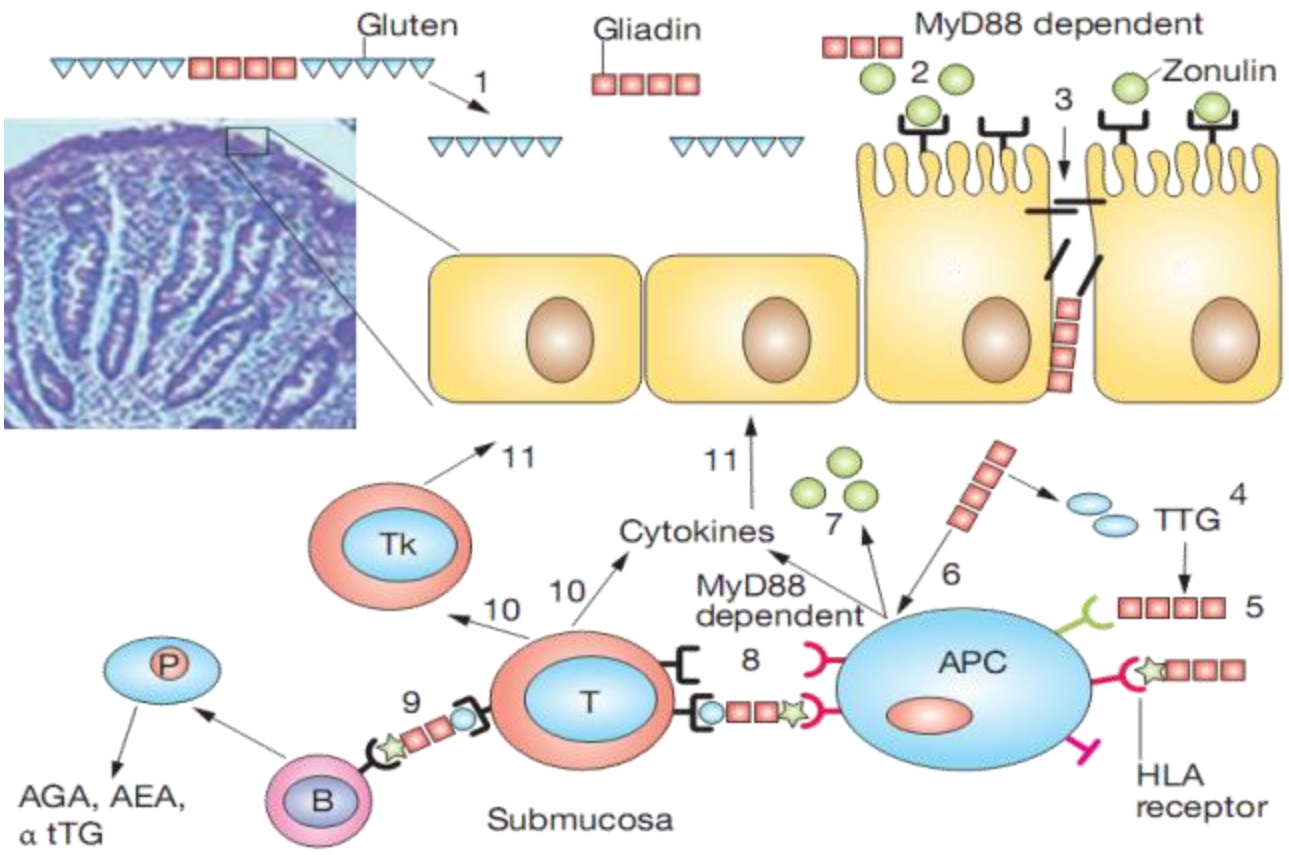
Sandek A, Rauchhaus M, Anker SD, et al. The emerging role of the gut in chronic heart failure. Curr Opin Clin Nutr Metab Care. 2008 Sep;11(5):632-9.

Sandek A, Bauditz J, Swidsinski A, et al. Altered intestinal function in patients with chronic heart failure. J Am Coll Cardiol. 2007 Oct 16;50(16):1561-9.

Consequences—Autoimmune Disease

Proposed role of abnormal intestinal permeability in pathogenesis of celiac disease

Gliadin up-regulates zonulin synthesis. Zonulin increases gut permeability—BBB as well!



Nature Clinical Practice Gastroenterology & Hepatology. September 2005, Vol.2.No.9

Fasano A. Regulation of intercellular tight junctions by zonula occludens toxin and its eukaryotic analogue zonulin. Ann N Y Acad Sci. 2000;915:214-22

Assessment

Small intestinal permeability is primarily measured with the lactulose/mannitol test.

- After drinking a premeasured amount of these two sugars, the amount recovered in the urine indicates the degree of absorption of each, and is an index of permeability.
- Monosaccharides, such as mannitol (or L-rhamnose), are absorbed through the transcellular pathway and reflect the degree of absorption of small molecules. Disaccharides, such as lactulose (or cellobiose), are absorbed through the paracellular junction complex, which corresponds to the permeability of larger molecules. The efficacy of treatment may also be monitored with this same test.

Dastych M, Dastych M Jr, Novotná H, et al. Lactulose/mannitol test and specificity, sensitivity, and area under curve of intestinal permeability parameters in patients with liver cirrhosis and Crohn's disease. Dig Dis Sci. 2008 Oct;53(10):2789-92.

Assessment

Additional considerations

- Screening for celiac disease
  - Anti-transglutaminase IgA has highest sensitivity and specificity.
  - Total serum IgA should be ordered simultaneously to rule out false negative
- Screening for dysbiosis
  - DNA analysis superior to culture-based methods

Mikesh LM, Crowe SE, Bullock GC, et al. Celiac disease refractory to a gluten-free diet? Clin Chem. 2008 Feb;54(2):441-4;

Frank DN, Pace NR. Gastrointestinal microbiology enters the metagenomics era. Curr Opin Gastroenterol. 2008 Jan;24(1):4-10.

Treatment

General principles of GI detoxification

- Eliminate toxic bacteria
- Remove toxins
- Reseed with healthy bacteria
  - Probiotics
  - Prebiotics
- Stimulate intestinal repair

Eliminate Toxic Bacteria

Growth of bacteria in small intestine

- Metabolism of carbohydrates to gas
- Putrefaction of proteins to vasoactive amines and carcinogens
- Increase bowel permeability

Clostridia and other anaerobes

Hydrastis canadensis: 1 tsp 3x/d

- Toxic to anaerobes
- Gentle to lactobacilli

Garlic: 1 clove 2x/d

Scazzocchio F, et al. Antibacterial activity of *Hydrastis canadensis* extract and its major isolated alkaloids. Planta Med. 2001;67:561-4

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;19:327-34

Remove Toxins

Fiber: 3 tbl/d
Charcoal: 2 tsp between meals
Colon irrigation

Ward PB, Young GP. Dynamics of *Clostridium difficile* infection. Control using diet. Adv Exp Med Biol. 1997;412:63-75
Bond GR. The role of activated charcoal and gastric emptying in gastrointestinal decontamination: a state-of-the-art review. Ann Emerg Med. 2002;39:273-86
Neuvonen PJ, Olkkola KT. Oral activated charcoal in the treatment of intoxications. Role of single and repeated doses. Med Toxicol Adverse Drug Exp. 1988;3:33-58
Korshunov VM, et al. [Effect of the lavage of the digestive tract on microflora in patients with polyps in the large intestine] [Russian] Zh Mikrobiol Epidemiol Immunobiol. 2001;3:76-80

Reseed with Healthy Bacteria

Ex: Bifidobacterium longum

- This strain prevented damage to intestinal cells and increased production of tight junction cell proteins
- Clinical success:
  - In a trial of patients with UC, BB536 was shown to not only induce remission, but it also upregulated gene expression of tight junction molecules (claudin-1 and ZO-1), known to be key to selective permeability
- Bifidobacteria, by reducing the level of endotoxin, may be able to reduce the rate of diabetes and obesity

Takeda Y, et al. Upregulation of T-bet and tight junction molecules by Bifidobactrium longum improves colonic inflammation of ulcerative colitis. Inflamm Bowel Dis. 2009 Nov;15(11):1617-8.

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 Nov;50(11): 2374-83.

Reseed with Healthy Bacteria

- The combination of a probiotic with a prebiotic (symbiotic) was recently shown to improve symptoms in patients with ulcerative colitis significantly compared to either therapy alone. Synbiotic therapy also reduced C-reactive protein, an effect not seen in the other groups

Fujimori S, Gudis K, Mitsui K, Seo T, et al. A randomized controlled trial on the efficacy of synbiotic versus probiotic or prebiotic treatment to improve the quality of life in patients with ulcerative colitis. Nutrition. 2009 Feb 6.

Reseed with Healthy Bacteria

Therapeutic Application	Most Appropriate Probiotic Strain(s)
Food allergies	L. rhamnosus GG, B. lactis Bb12, L. paracasei Shirota
NSADI use/Erosive gastritis	L. rhamnosus GG
Antibiotic use (during and after)	L. rhamnosus GG, Saccharomyces cerevisiae (Hansen CBS 5926), L. acidophilus LA5, L. plantarum 299v
Intestinal hyperpermeability	L. rhamnosus GG, S. cerevisiae (Hansen CBS 5926)
Giardia infection	L. rhamnosus GG, L. johnsonii La1, L. plantarum 299v,
Intestinal dysbiosis	L. rhamnosus GG, L. johnsonii La1, L. plantarum 299v, L. paracasei Shirota, L. acidophilus LA5
Irritable bowel syndrome	L. plantarum 299v, VSL# 3
Lactose intolerance	L. acidophilus NCFM, L. johnsonii La1, L. acidophilus LA5
Peptic ulcer/Nonerosive gastritis	L. rhamnosus GG, L. johnsonii La1, L. acidophilus LB, L. acidophilus strain NAS, L. acidophilus DDS-1

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Hawrelak J. Probiotics. In *Textbook of Natural Medicine*. Elsevier, 2006

Repair Intestinal Integrity

Avoid allergenic foods

- Screen for celiac disease
- A vegan diet free of gluten improves the signs and symptoms of rheumatoid arthritis: effects correlate with a reduction in antibodies to food antigens. 40.5% vs. 4% met criteria for improvement in vegan/gluten-free vs. healthy non-vegan

Some foods may be ok after restoration of normal permeability

- 86 Children with dairy intolerance (diagnosed with elimination double blinded reintroduction and IgE tests) avoided dairy and were re-challenged at 1, 2 and 3 years
    - After 1 year: 30% were tolerant
    - After 2 years: 55 % were tolerant
    - After 3 years: 70 % were tolerant

Consider elimination/challenge test

Hafström I, et al. A vegan diet free of gluten improves the signs and symptoms of rheumatoid arthritis: the effects on arthritis correlate with a reduction in antibodies to food antigens.

Carroccio A, et al. Evidence of very delayed clinical reactions to cow's milk in cow's milk-intolerant patients. Allergy. 2000 Jun;55(6):574-9

Repair Intestinal Integrity

- Limit damaging exogenous chemicals (Alcohol, NSAIDS, food additives, cytotoxic drugs)
- Specific carbohydrate diet
  - Elaine Gottschall's *Breaking the Vicious Cycle*
- Raw cabbage juice: 1 qt/d
  - Glutamine
  - CYP450
- Glutamine
  - Preferred energy substrate for intestinal cells
  - Increases thickness of intestinal membranes
  - Increases secretion of sIgA
  - Inhibits bacterial penetration of GI membranes
  - 500 mg 3x/d

Cheney G. Rapid healing of peptic ulcers in patients receiving fresh cabbage juice. Calif Med 1949;70:10–4

De-Souza DA, Greene LJ. Intestinal permeability and systemic infections in critically ill patients: effect of glutamine. Crit Care Med 2005;33:1125-35

Repair Intestinal Integrity

Quercetin

- Critical to intestinal integrity, and acts through a number of mechanisms. These have recently been shown to include the assembly of a number of tight junction proteins (zonula occludens (ZO)-2, occludin, claudin-1, and claudin-4).
- Additionally, quercetin has long been known to stabilize mast cells

Suzuki T, Hara H. Quercetin enhances intestinal barrier function through the assembly of zonula [corrected] occludens-2, occludin, and claudin-1 and the expression of claudin-4 in Caco-2 cells. J Nutr. 2009 May;139(5):965-74.
Amasheh M, Schlichter S, Amasheh S, et al. Quercetin enhances epithelial barrier function and increases claudin-4 expression in Caco-2 cells. J Nutr. 2008 Jun;138(6):1067-73.

Repair Intestinal Integrity

Omega-3 fatty acids

- Decreases intestinal permeability and reduce inflammation
- Fish oils, which contain the omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have been shown to improve the immune response in experimental models of ulcerative colitis, rheumatoid arthritis, and psoriasis
- 1-3 g/d

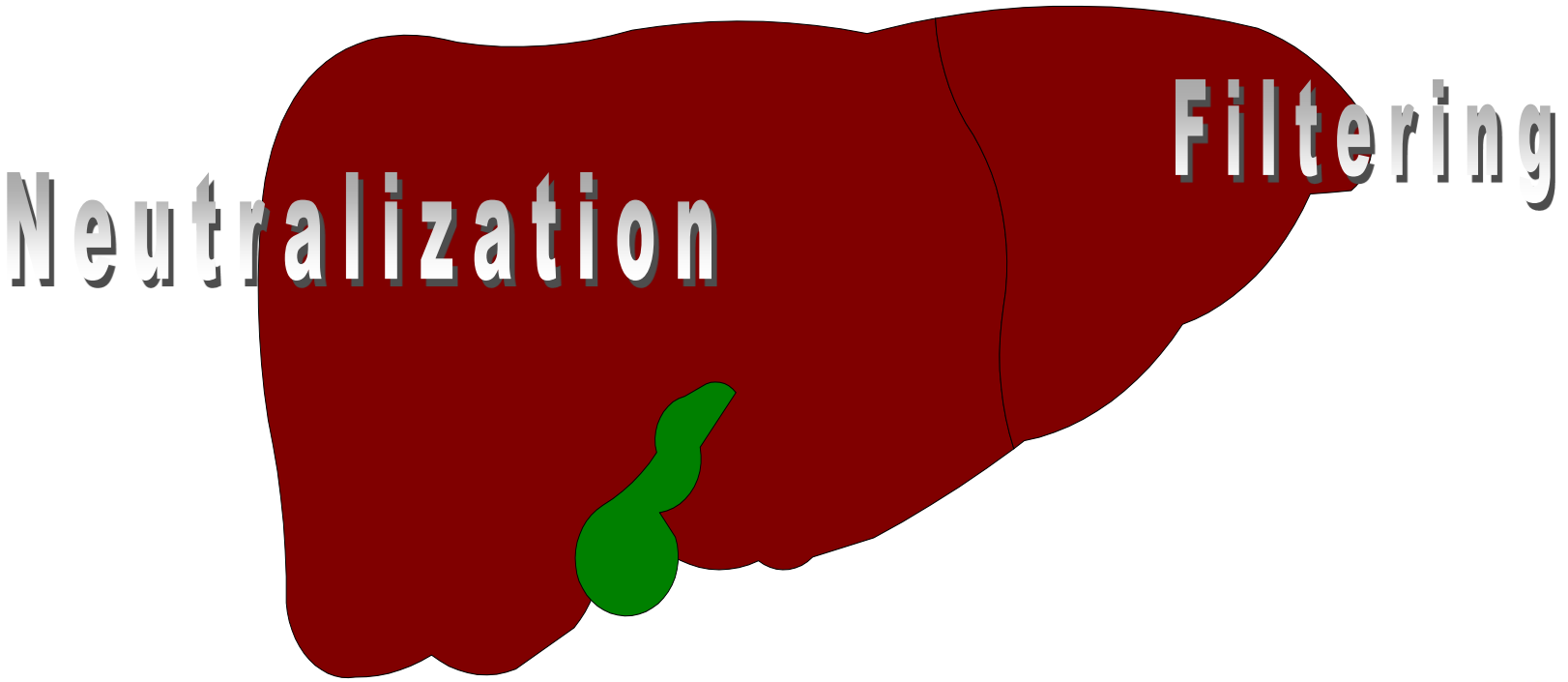
Rosella O, et al: Polyunsaturated fatty acids reduce non-receptor-mediated transcellular permeation of protein across a model of intestinal epithelium in vitro. J Gastroenterol Hepatol 15:626-631, 2000

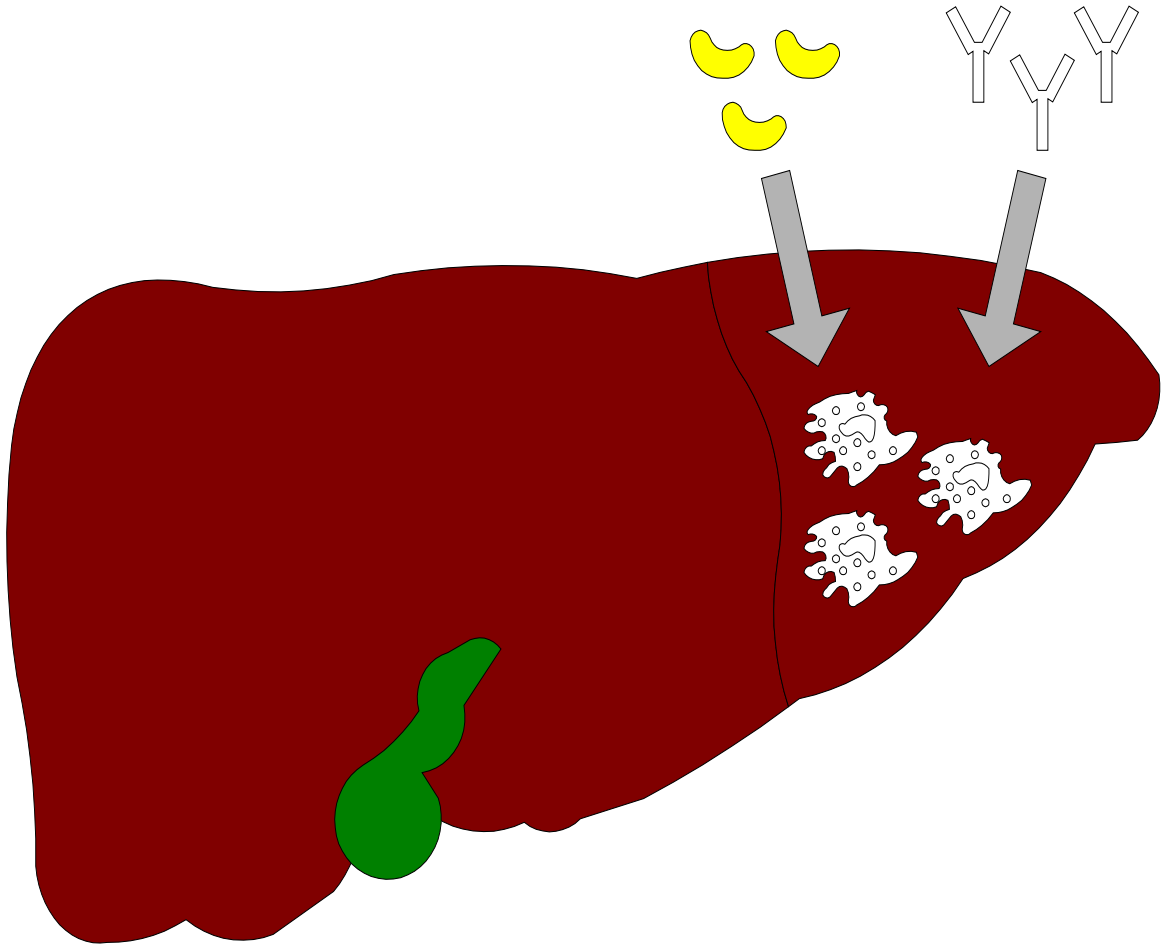
Repair Intestinal Integrity

Other nutrients/foods with restorative function

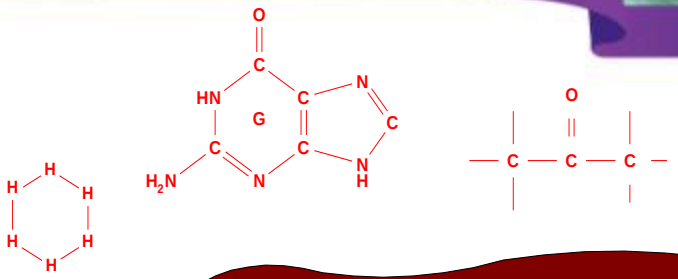
- Licorice root extract
  - Stimulates mucosal healing
  - 500-1,500 mg/d
- Kiwi fruit
  - Arabinogalactans support cell growth and gut function
- Aloe vera
  - Inhibits pathogens
  - 50-150mg per day
- Prebiotics:
  - Bifidobacterium spp.
    - Fructooligosaccharides (asparagus, onion, leek, garlic, artichoke, Jerusalem artichoke, chicory root)
    - Galactooligosaccharides (cow's milk, yoghurt, human milk)
    - Xylooligosaccharides (oats)
    - Galactosyl lactose (human milk)
  - Lactobacillus spp.
    - β-glucooligomers (oats)
    - Raffinose (legumes, beets)

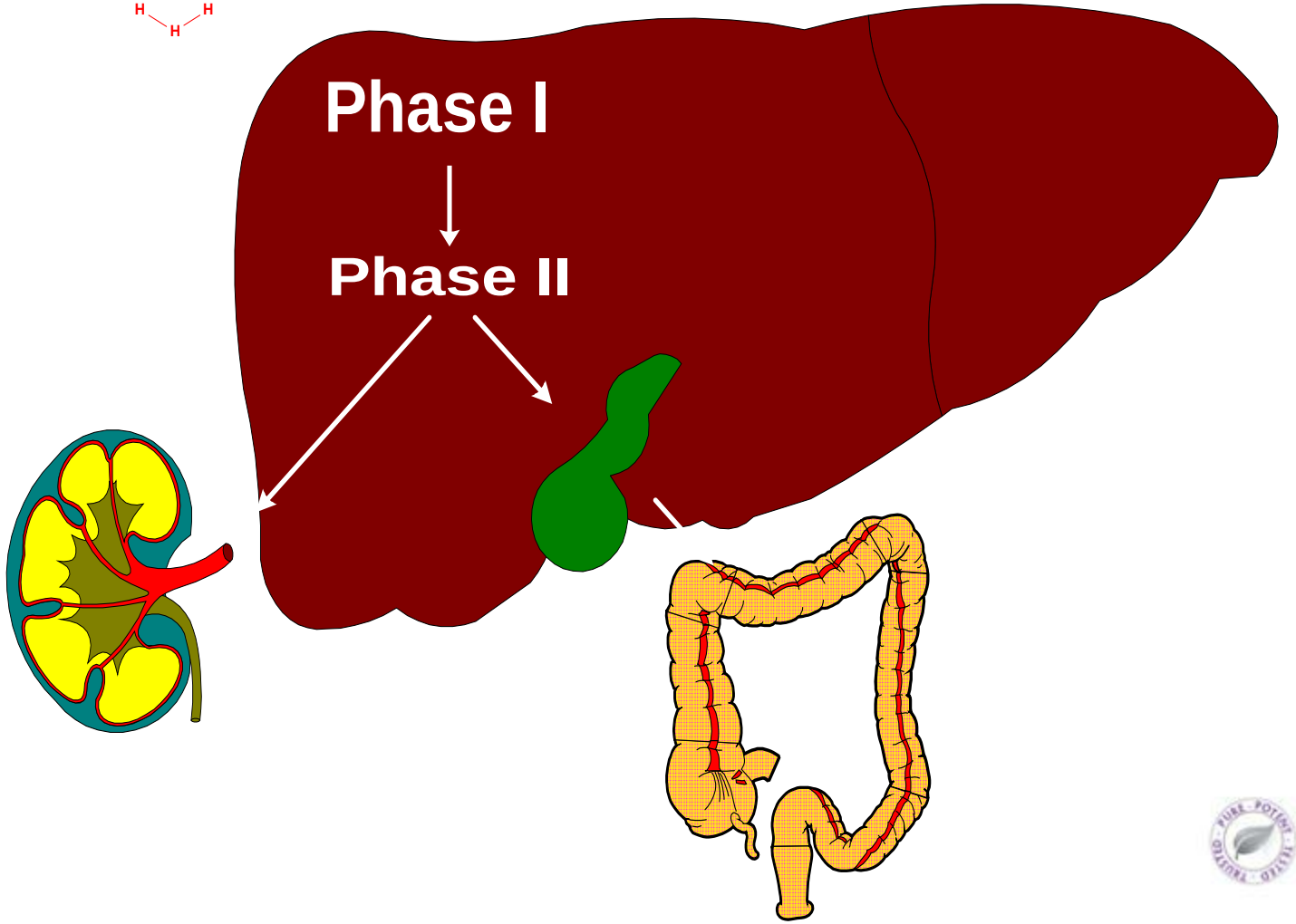
Liver's Detoxification Roles





Filtering





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Phase I Genomic Variation

- CYP_{2D6}
- 25% of drugs
- 5-10% of Caucasians greatly reduced clearance
  - → Many serious ADRs
- 29% of blacks greatly increased clearance
  - → Drugs ineffective
- Zingiber compounds inhibit up to 100%
  - → Induce ADRs

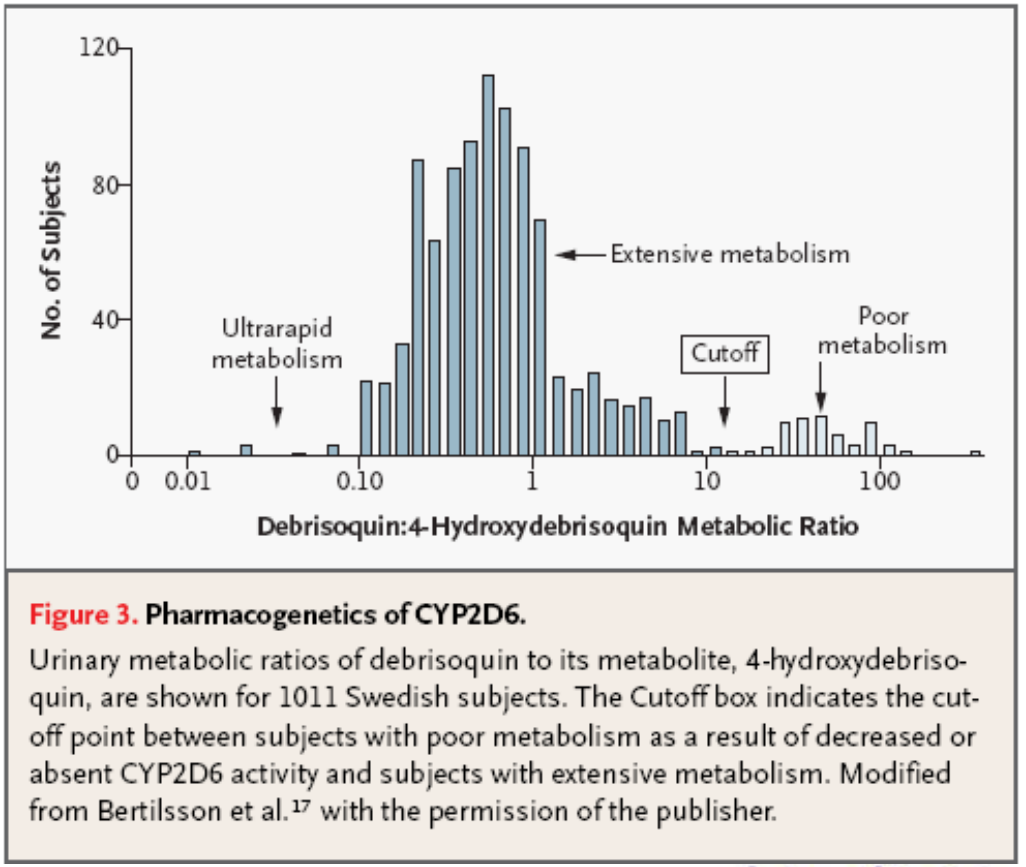


Figure 3. Pharmacogenetics of CYP2D6.

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.

Weinshilboum, R. Inheritance and drug response. NEJM 2003;348:529-37
Subehan, et al. Constituents of Zingiber aromaticum and Their CYP3A4 and CYP2D6 Inhibitory Activity. Chem Pharm Bull (Tokyo) 2005;53:333-5

Phase II Genomic Variation

Acetylation

Factor of 4 variation in rate (group means)

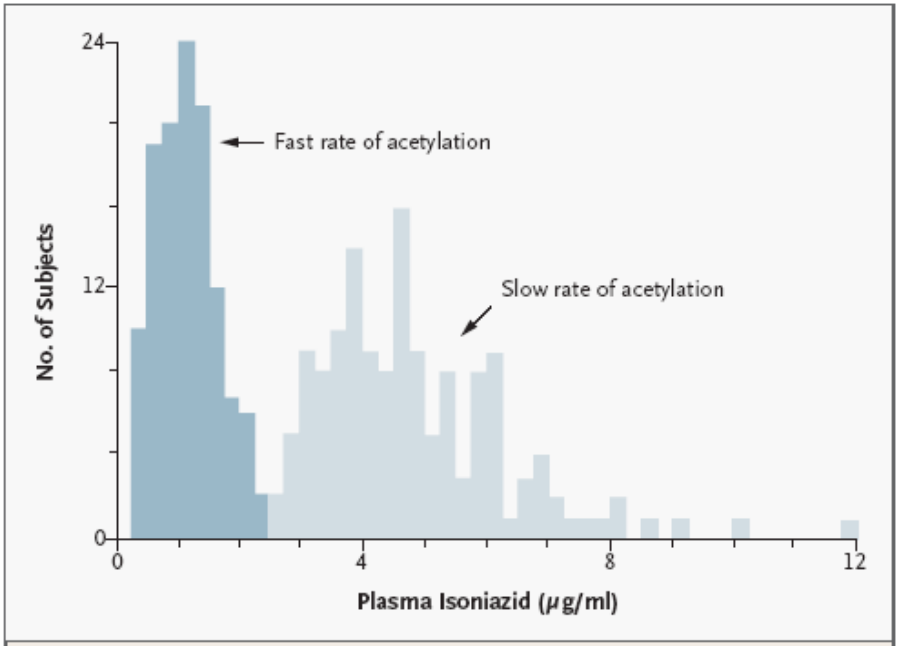


Figure 2. Pharmacogenetics of Acetylation.

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

Persistent Organic Pollutants

Persist in the environment:

- Polychlorinated dibenzo-p-dioxins (PCDDs)
- Polychlorinated dibenzofurans (PCDFs)
- Polychlorinated biphenyls (PCBs)
- Hexachlorobenzene (HCB)
- Organochlorines used as pesticides

Physiological Effects

Alter DNA receptor cite activity
Activate peroxisome proliferator receptors (PPAR)
Interfere with/mimic insulin, thyroid, & sex hormones
Damage mitochondria
Increase inflammatory cytokines
Decrease glucose transport in vitro and in vivo
Stimulate tumor necrosis factor-α expression

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.
Hatch EE et al. Association of endocrine disruptors and obesity: perspectives from epidemiological studies. Int J Androl. 2010 Apr;33(2):324-32.
Needham LL, et al. Concentrations of environmental chemicals associated with neurodevelopmental effects in U.S. population. Neurotoxicology. 2005 Aug;26(4):531-45
Lee DH, Association between serum concentrations of persistent organic pollutants and insulin resistance among nondiabetic adults: results from the National Health and Nutrition Examination Survey 1999-2002. Diabetes Care. 2007 Mar;30(3):622-8.

POPs—Disease Correlations

Diabetes:

- For 6 POPs from NHANES (all found in > 80% of US) - when summed the OR for highest levels was ~38.0
- In people with undetectable POPs, the typically robust association between obesity and diabetes is not observed

Metabolic syndrome & insulin resistance:

- Especially organochlorine pesticides, OR 5.3 for top quartile

Lee DH, et al. 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 Jul;29(7):1638-44.

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.

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.

Lee DH, et al. Association between serum concentrations of persistent organic pollutants and insulin resistance among nondiabetic adults: results from the National Health and Nutrition Examination Survey 1999-2002. Diabetes Care. 2007 Mar;30(3):622-8.

POPs—Disease Correlations

Hypertension:

- POPs in non-diabetic population associated with increased risk of hypertension

Cardiovascular disease:

- PCDDs in both genders, other POPS for women only

Obesity

- Many POPs found to be “obesogens”

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.

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.

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. [Epub ahead of print]

GGT as 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.

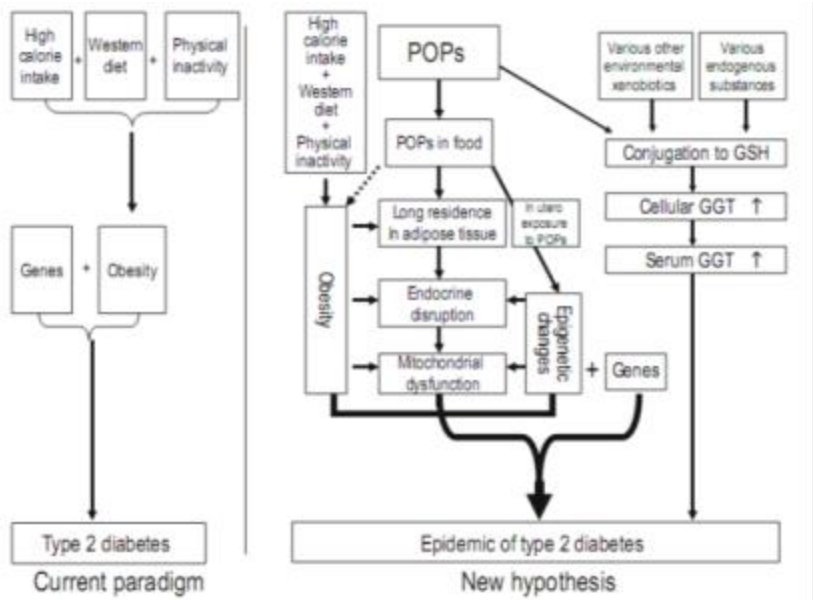
Exposure to POPs induce GGT as a defensive mechanism.

GGT **within its normal range** predicts type 2 diabetes, coronary heart disease, hypertension, stroke, dyslipidaemia, chronic kidney disease and cancer.

Men with GGT levels >50 U/l had ~26 fold risk for diabetes compared to those with <10. Those with 40-49 had a ~20 fold risk.

Higher levels of serum GGT *within its normal range* occur with obesity, excessive alcholo, cigarette smoking, physical inactivity, high meat and less 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

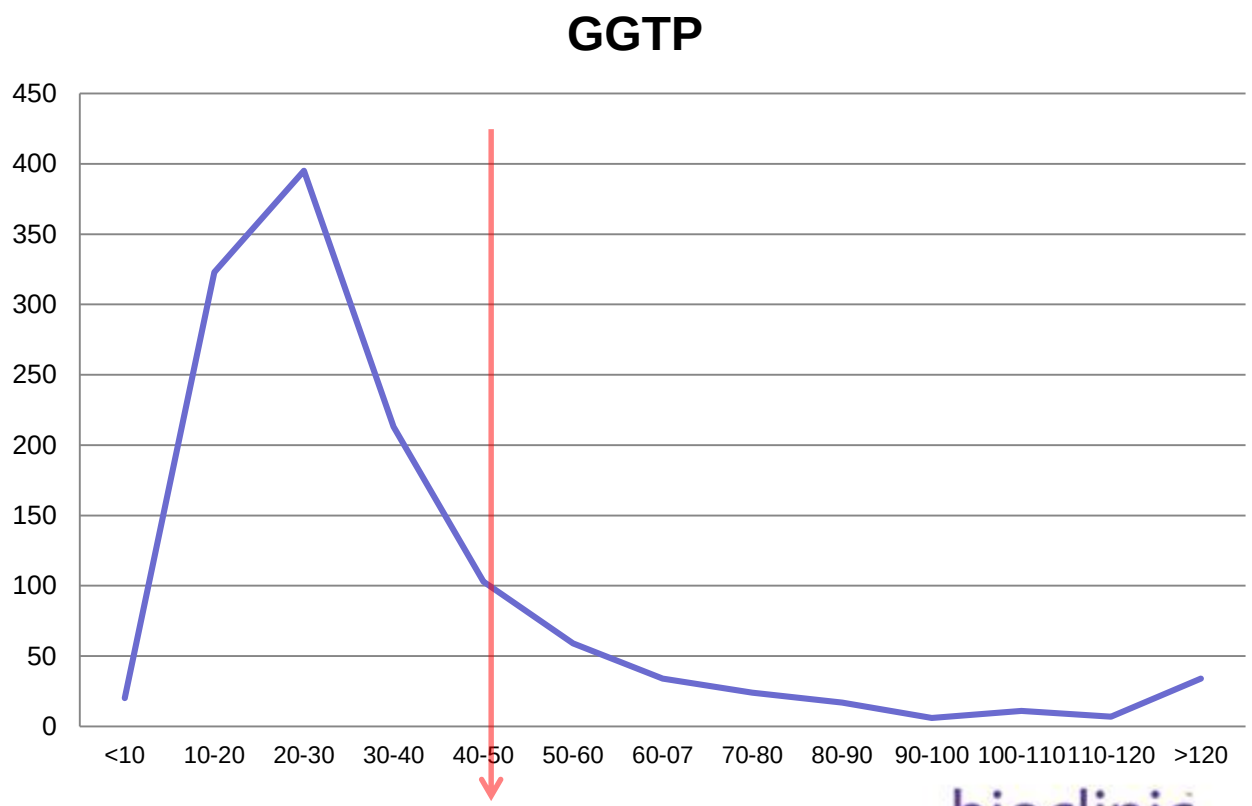
Lee DH, et al. Gamma-glutamyltransferase and diabetes--a 4 year follow-up study. Diabetologia. 2003 Mar;46(3):359-64.

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. (NOTE: Source of both figures)

GGT in Aberta

2,100 in Alberta

“Normal” <60



Diabetes risk 20x

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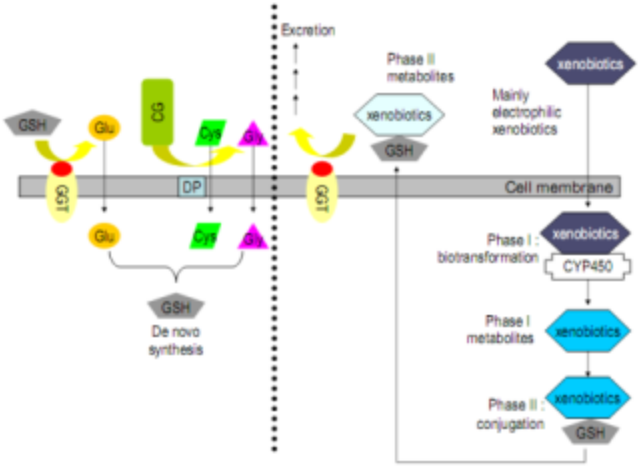
Glutathione Conjugation as Main Route of Detox

- POPs are eliminated by phase I biotransformation, followed by phase II conjugation to an anionic group, such as 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
  - Loss of GH prevented by NAC



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.

Increase Glutathione Production

Silymarin

- Standardized extract, 100 mg tid

NAC

- 500 mg bid, Also directly binds methyl-Hg

Alpha lipoic acid

- R form preferred. 250 mg bid

Whey powder

- 15 g bid

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

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

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

Sample Questions For Heavy 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. Do patients experience a metallic taste in their mouth *and* have not recently been taking medications documented to cause metallic taste?
8. Do 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

Heavy Metals Signs and Symptoms

- Learning deficits
- Reduced intelligence
- Behavioral changes
- Cognitive changes
- Tremor
- Memory loss
- Fatigue
- Irritability
- Depression
- Multiple sclerosis
- Alzheimer's disease
- Gingivitis Anemia
- Cancer
- Hypertension
- Headache
- Hyperuricemia
- Gout
- Chronic renal failure
- Male infertility
- Osteodystrophies

From Peter Bennet, ND: IM Conference Miami 2004

Treatment

AVOIDANCE!!

Organic, mostly plant-based diet

- Reduced toxins, nutrients for phase 2, and high antioxidant content

Facilitate metal excretion

Heavy metal chelation if indicated

- Supplementation with appropriate minerals depleted by chelation

Supportive therapies

- Antioxidant support
- Detoxification support
- Systemic detoxification
  - Sauna
  - Fasting
  - Hydrotherapy
- Bowel detoxification

Toxic Metal Chelation

Possible chelators: DMPS, DMSA, EDTA, BAL, d-penicillamine (DPA), newer esters of DMSA

- Mercury
  - Oral DMSA or IV DMPS
  - IV DMPS → many adverse events; depletes Cu and Zn
  - Oral DMSA → rare, primarily allergic, adverse events; small depletion of Zn
  - NAC inhibits neuronal Hg damage and increases excretion.
- Lead
  - Long history of IV EDTA
  - EDTA also depletes Zn, Cu, Fe, Co, and Mn
  - Oral DMSA equally effective, and may mobilize different sites.
  - Combination of EDTA and DMSA – early study in lead exposed workers found initial dose of EDTA increased amount excreted by DMSA
  - Oral EDTA does increase lead excretion. Slower than IV and all studies in 1950s and 60s

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 dimercaptosuccinic acid (succimer) 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

Toxic Metal Chelation

- Arsenic
  - DMSA useful for acute poisoning
  - DMSA derivative monoisoamyl dimercaptosuccinic acid MiADMSA removes from blood and soft tissue when given with DMSA, but animal studies only
  - NAC used in conjunction
- Cadmium
  - DMSA useful for acute poisoning and removes from kidneys
  - Must be careful in smokers as high kidney load

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
Jones MM, et al. Cadmium mobilization in vivo by intraperitoneal or oral administration of mono alkyl esters of meso 2, 3-dimercaptosuccinic acid. Pharmacol Toxicol 1992

Supportive Therapies: Antioxidants

N-acetyl-cysteine

- Antioxidant for As, Cd, Hg and Pb
- Removes methly Hg from fetus in animal studies

Lipoic acid

- Inhibits neuronal Hg damage and increases excretion
- 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

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: Detox Support

Phase 2 support

- Cruciferous vegetables
- Milk thistle

Binders of phase 1 intermediates

- Ellagic acid (berries)
- Catechins (green tea)

Botanicals to increase bile secretion

- Cynara scolymus (artichoke)
- Curcuma longa (turmeric)

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

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Resources to Help Your Patients: Educational

Clinical Guidebook

- Total Wellness for Physicians

Clinical Highlights

- Intestinal Rejuvenation
- Detoxification
- Insulin Resistance
- Weight Loss
- Diabetes II
- Insomnia and Sleep/Wake Disorders
- Stress Management

Patient handouts

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Resources to Help Your Patients: Products

RestorX

- Intestinal repair nutritional drink mix

DetoxiCleanse

- Detoxification nutritional drink mix

BioLivX

- Liver support formula

7-Day ReduceXS

- Total body cleansing program

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RestorX

Healing leaky gut syndrome

- Extremely low allergy potential
- High quality, hypoallergenic sprouted brown rice protein
- Enriched with glutamine and other “gut-critical” nutrients
- High in soluble fiber
- Rich in phytochemicals that reduce inflammation and promote healing

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RestorX

Supplement Facts

Serving Size 1 Scoop (approx. 32.4 g)
Servings Per Container 14

	Amount Per Serving	% Daily Value
Calories	120	
Calories from Fat	35	
Total Fat	4 g	6%‡
Saturated Fat	2.5 g	13%‡
Total Carbohydrate	10 g	3% ‡
Dietary Fiber	7 g	28%‡
Sugars	2 g	**
Protein	12 g	
Vitamin A (as beta carotene)	4,800 IU	96%
Vitamin C (ascorbic acid)	75 mg	125%
Vitamin D (as cholecalciferol) (lanolin)	100 IU	25%
Vitamin E (as *d*-alpha tocopheryl acetate)	25 IU	83%
Thiamin (as thiamin hydrochloride)	12.5 mg	833%
Riboflavin	10 mg	588%
Niacin (as niacinamide)	6 mg	30%
Vitamin B6 (as pyridoxine hydrochloride)	25 mg	1,250%
Folate (as folic acid)	250 mcg	63%
Vitamin B12 (as cyanocobalamin)	100 mcg	1,667%
Pantothenic Acid (as calcium pantothenate)	25 mg	250%
Calcium (as calcium citrate, dicalcium phosphate & calcium sulfate)	208 mg	21%
Phosphorus (as dicalcium phosphate)	44 mg	4%
Iodine (as potassium iodide)	50 mcg	33%
Magnesium (as magnesium citrate)	100 mg	25%
Zinc (as zinc citrate)	12.5 mg	83%
Selenium (as selenium chelate)	50 mcg	71%
Chromium (as chromium chelate)	50 mcg	42%
Molybdenum (as molybdenum citrate)	12.5 mcg	17%
Potassium (as potassium citrate)	100 mg	3%
Organic Sprouted Brown Rice Protein Concentrate	16 g	**
Cellulose	4,450 mg	**
Medium Chain Triglycerides	2,500 mg	**
Bifidobacterium longum (Patented Strain BB536) (milk)	5 billion§	**
Soy Lecithin	1,500 mg	**
Guar Gum	1,300 mg	**
L-Glutamine	1,000 mg	**
Fructooligosaccharides (FOS)	560 mg	**
L-Lysine Hydrochloride	119 mg	**
Stevia, powdered extract (leaf)	118 mg	**
L-Glycine	77 mg	**
N-Acetyl-L-Cysteine	42 mg	**
Bromelain (1,000 GDU/g)	37.5 mg	**
Quercetin	12.5 mg	**
N-Acetyl Glucosamine	12.5 mg	**
Grape Seed (*Vitis vinifera*), powdered extract Standardized to 87% Polyphenols	11 mg	**
Milk Thistle (*Silybum marianum*), powdered extract (seed) Standardized to 60% Silymarin	11 mg	**

‡ Percent Daily Values are based on a 2,000 calorie diet.
** Daily Value not established.
§ BB536 process stabilizes the most sensitive bifidobacteria and guarantees bioactivity even when not refrigerated (typically at room temperature).

Other ingredients: Organic cane sugar, natural flavors (apple, banana, blueberry, raspberry, strawberry), lemon oil.

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DetoxiCleanse

Broad support for liver regeneration and detoxification

Complete support for all liver functions
Nutrients, botanicals and food extracts to:

- Meet nutrient requirements of detoxification pathways
- Increase glutathione production
- Toxic metal excretion
- Protect the liver from oxidative stress

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DetoxiCleanse

Supplement Facts

Serving Size 2 Scoops (approx. 40 g)
Servings Per Container 15

	Amount Per Serving	% Daily Value
Calories	140	
Calories from Fat	35	
Total Fat	4 g	6%‡
Saturated Fat	3 g	15%‡
Total Carbohydrate	10 g	3%‡
Dietary Fiber	6 g	24%‡
Sugars	4 g	**
Protein	15 g	
Vitamin A (as beta carotene)	2,400 IU	48%
Vitamin C (ascorbic acid)	150 mg	250%
Vitamin D (as cholecalciferol) (lanolin)	100 IU	25%
Vitamin E (as *d*-alpha tocopheryl acetate)	25 IU	83%
Thiamin (as thiamin hydrochloride)	20 mg	1,333%
Riboflavin	10 mg	588%
Niacin (as niacinamide)	10 mg	50%
Vitamin B6 (as pyridoxine hydrochloride)	25 mg	1,250%
Folate (as folic acid)	200 mcg	50%
Vitamin B12 (as cyanocobalamin)	50 mcg	833%
Pantothenic Acid (as calcium pantothenate)	15 mg	150%
Calcium (as calcium citrate, dicalcium phosphate & calcium sulfate)	273 mg	27%
Phosphorus (as dicalcium phosphate)	37 mg	4%
Iodine (as potassium iodide)	15 mcg	10%
Magnesium (as magnesium citrate)	100 mg	25%
Zinc (as zinc citrate)	10 mg	67%
Selenium (as selenium chelate)	40 mcg	57%
Chromium (as chromium chelate)	75 mcg	63%
Molybdenum (as molybdenum citrate)	10 mcg	13%
Potassium (as potassium citrate)	150 mg	4%
Organic Sprouted Brown Rice Protein Concentrate	20 g	**
Cellulose	3,000 mg	**
Medium Chain Triglycerides	3,000 mg	**
Guar Gum	1,185 mg	**
Soy Lecithin	1,000 mg	**
L-Glutamine	500 mg	**
L-Glycine	500 mg	**
Sodium Alginate (extracted from *Laminaria japonica*)	400 mg	**
Chlorella	250 mg	**
L-Methionine	250 mg	**
L-Lysine Hydrochloride	165 mg	**
N-Acetyl-L-Cysteine	150 mg	**
Stevia, powdered extract (leaf)	150 mg	**
Methylsulfonylmethane (MSM)	100 mg	**
Apple Pectin	100 mg	**
Taurine	60 mg	**
Alpha-Lipoic Acid	50 mg	**
Sodium Copper Chlorophyllin	50 mg	**
Milk Thistle (*Silybum marianum*), powdered extract (seed) Standardized to 60% Silymarin	50 mg	**
Broccoli Sprout, powder	50 mg	**
Green Tea (caffeine free) (*Camellia Sinensis*), powdered extract (leaf) Standardized to 45% EGCg	50 mg	**
Bromelain (1,000 GDU/g)	45.5 mg	**
Calcium D-Glucarate	25 mg	**

‡ Percent Daily Values are based on a 2,000 calorie diet.
** Daily Value not established.

Other ingredients: Organic cane sugar, natural flavors (black cherry, lemon, lime), gum arabic.

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BioLivX

Focused support for liver Phase II detoxification

- Nutrients required for Phase II detoxification enzymes
- High in nutrients specifically required as conjugates for Phase II detoxification
- Nutrients and botanicals to protect the liver from the oxidative stress induced by detoxification

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BioLivX

Supplement Facts

Serving Size 2 Capsules
Servings Per Container 45

	Amount Per Serving	% Daily Value
Sodium (from Sodium Glucuronate)	40 mg	2%‡
Sodium Glucuronate	400 mg	**
Licorice (*Glycyrrhiza glabra*), powdered extract (root)	160 mg	**
Glycyrrhizin (12%)	19 mg	**
Silybin Phytosome™ (contains Silybin [from *Silybum marianum*] bound to Phosphatidylcholine [from soy lecithin])	100 mg	**
Schisandra (*Schisandra chinensis*), powdered extract (berry)	100 mg	**
Schizandrins (2%)	2 mg	**
Alpha-Lipoic Acid	100 mg	**
Curcumin	50 mg	**
Panax Ginseng Phytosome™ (contains Panax Ginseng bound to phosphatidylcholine [from soy lecithin])	50 mg	**

‡ Percent Daily Values are based on a 2,000 calorie diet.
** Daily Value not established.

Other ingredients: Vegetarian capsule (cellulose, purified water, silica), cellulose, silica, magnesium stearate (vegetable grade).

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7-Day ReduceXS

Comprehensive Program

Contains:

- RestorX: Intestinal repair nutritional drink mix for days 1-4
- DetoxiCleanse: Detoxification nutritional drink mix for days 5-7
- Colon and liver support packets taken throughout the week

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Indications For Use

- Virtually all patients suffer substantial toxic stress:
  - Toxic metals
  - POPS (Persistent organic pollutants)
  - Gut dysbiosis
  - Leaky gut
  - Food allergies
- Underlying cause or contributor to most chronic disease