

Applied Blood Chemistry

Fall Edition. Boston, MA

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CSCS CFMP Pn1

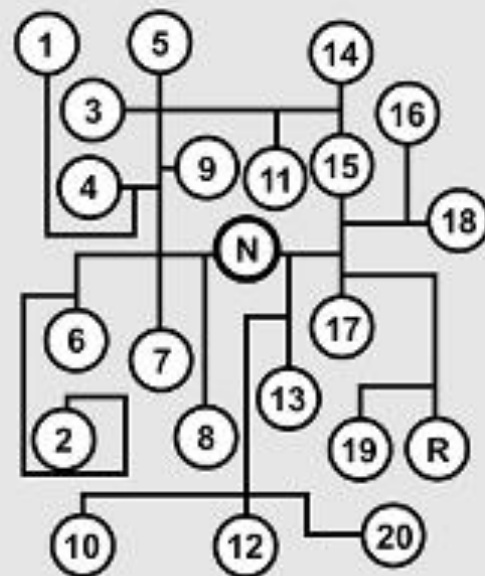


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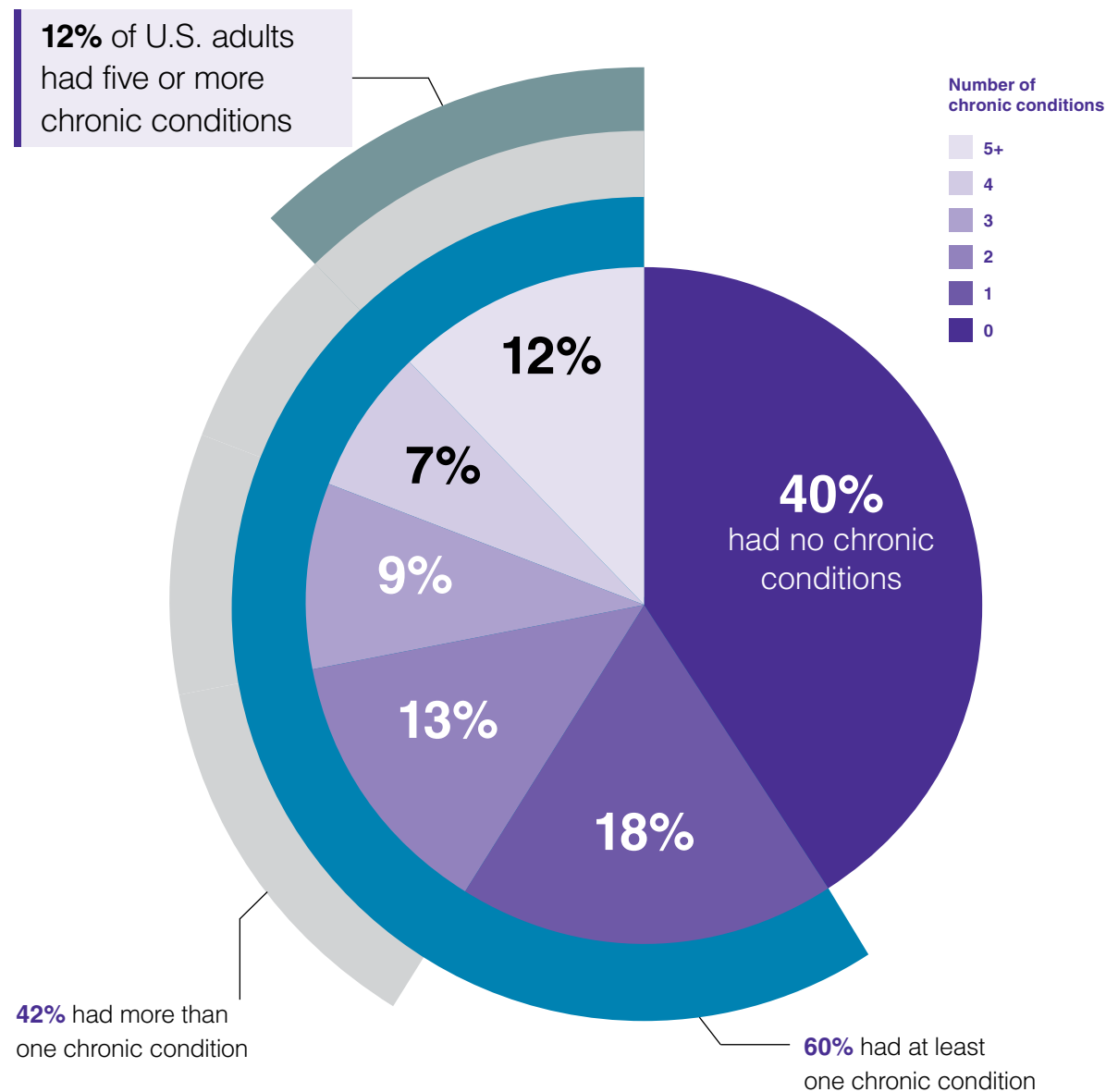
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Shift Pattern

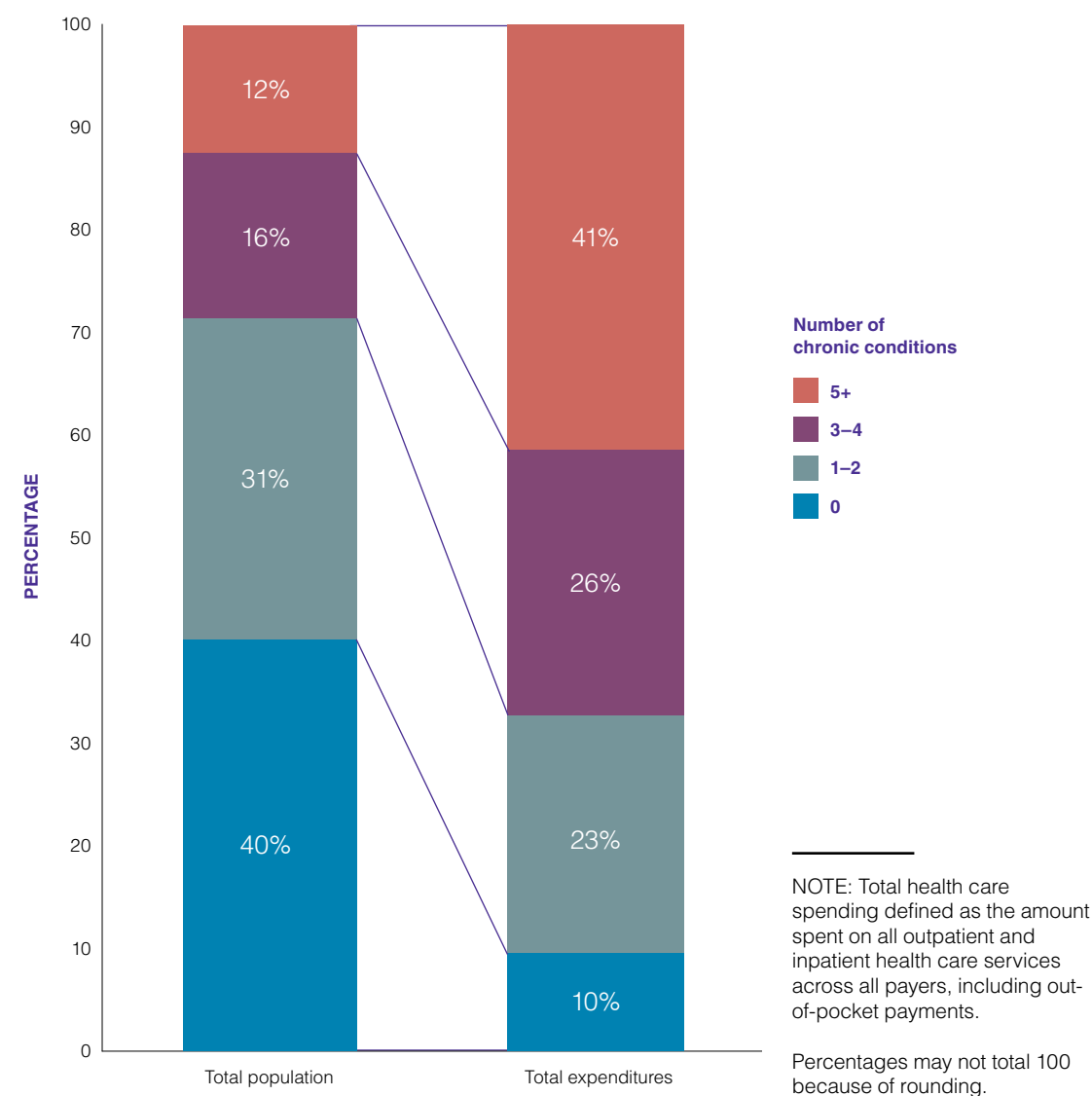


As of 2014, 60% of American adults had at least 1 chronic condition, 42% had more than 1 chronic condition.



NOTE: Percentages may not total 100 because of rounding.

Figure 2.2. Prevalence and Spending by Number of Chronic Conditions (2014)



Americans with 5 or more chronic conditions make up 12% of the population, but account for 41% of all health related expenses.

Patients with 5 or more chronic conditions spend 2x as much as those with 3-4, and **14x** as much as someone without any chronic condition at all.

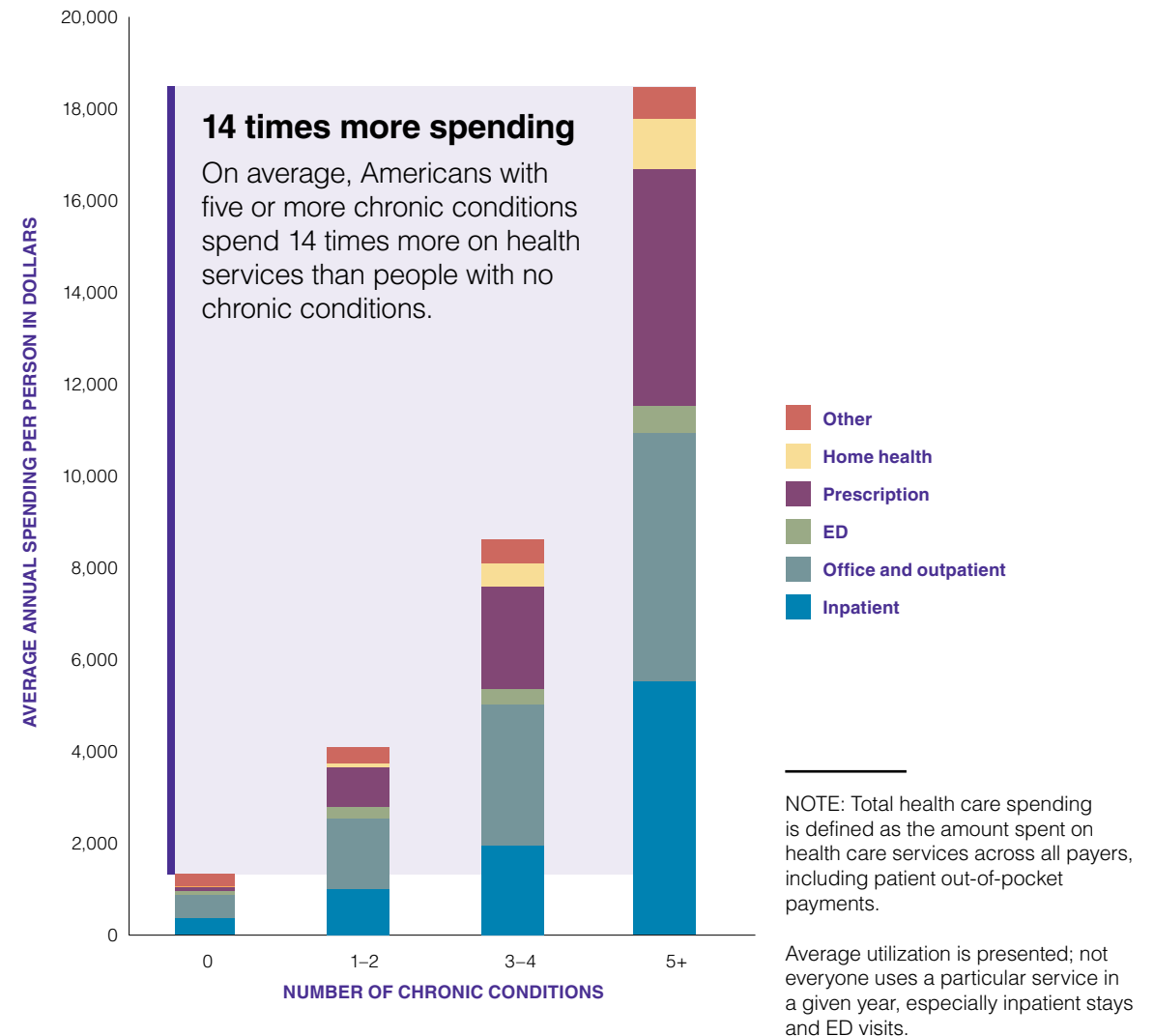
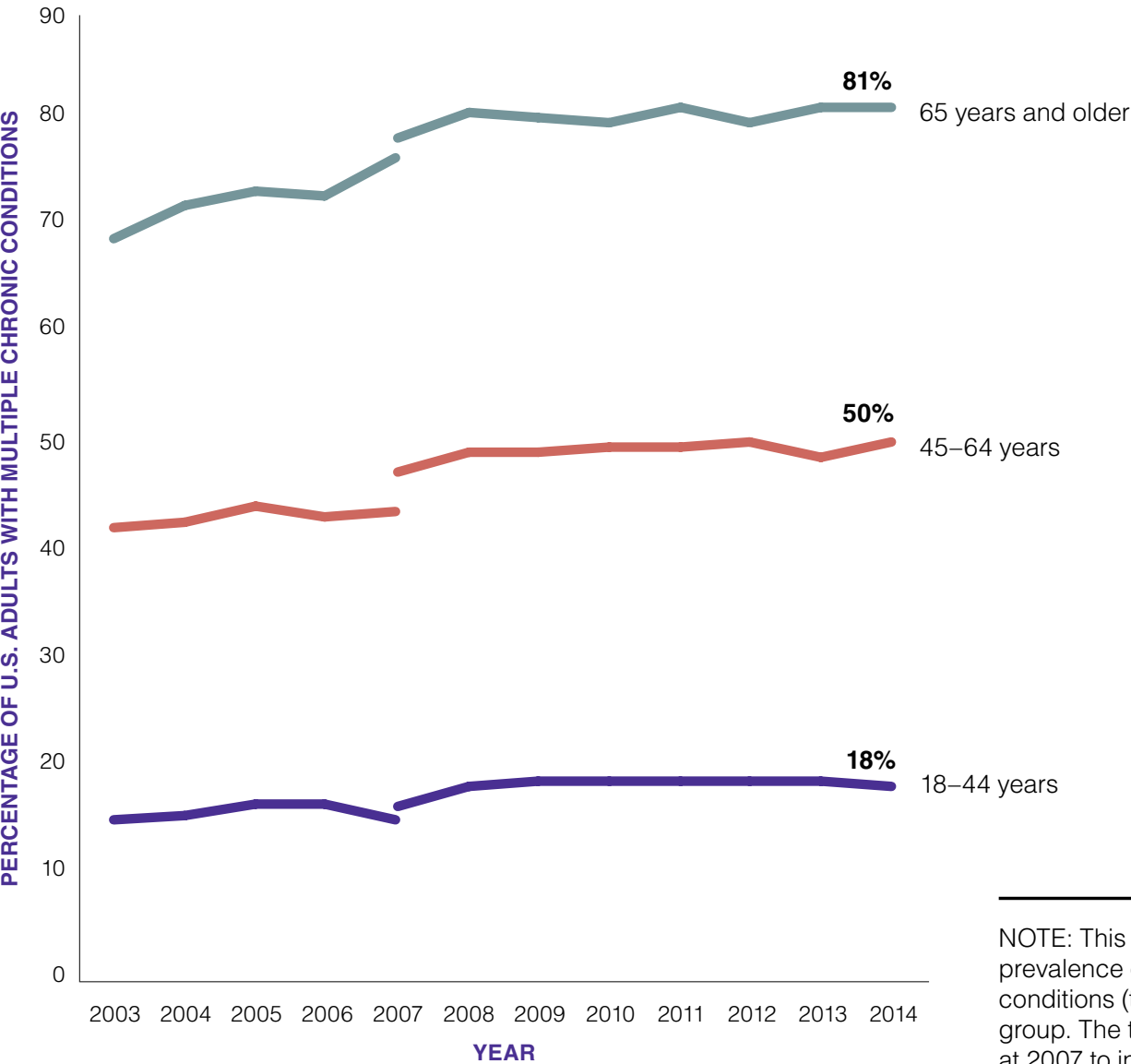


Figure A.1. Prevalence of Multiple Chronic Conditions (2003–2014)



NOTE: This figure documents the prevalence of multiple chronic conditions (two or more) by age group. The trend lines break at 2007 to indicate that MEPS changed its approach to the chronic disease questions.

Chronic Conditions and Lifespan

0 chronic conditions:	67+22.6 additional years
5 chronic conditions:	67+14.9 additional years
10 chronic conditions:	67+5 additional years



April 2018

Goldman Sachs asks in biotech research report: 'Is curing patients a sustainable business model?'

"...where the success of [its] hepatitis C franchise has gradually exhausted the available pool of treatable patients," the analyst wrote. "In the case of infectious diseases such as hepatitis C, curing existing patients also decreases the number of carriers able to transmit the virus to new patients, thus the incident pool also declines ... Where an incident pool remains stable (eg, in cancer) the potential for a cure poses less risk to the sustainability of a franchise."

People are not commodities...
unless THEY CHOOSE TO BE.

How do you transition them?

re·spon·si·bil·i·ty

/rəˌspänsəˈbilədē/ 

noun

the state or fact of having a duty to deal with something or of having control over someone.

synonyms: authority, control, power, leadership

"a job with greater responsibility"

- the state or fact of being accountable or to blame for something.

"the group has claimed responsibility for a string of murders"

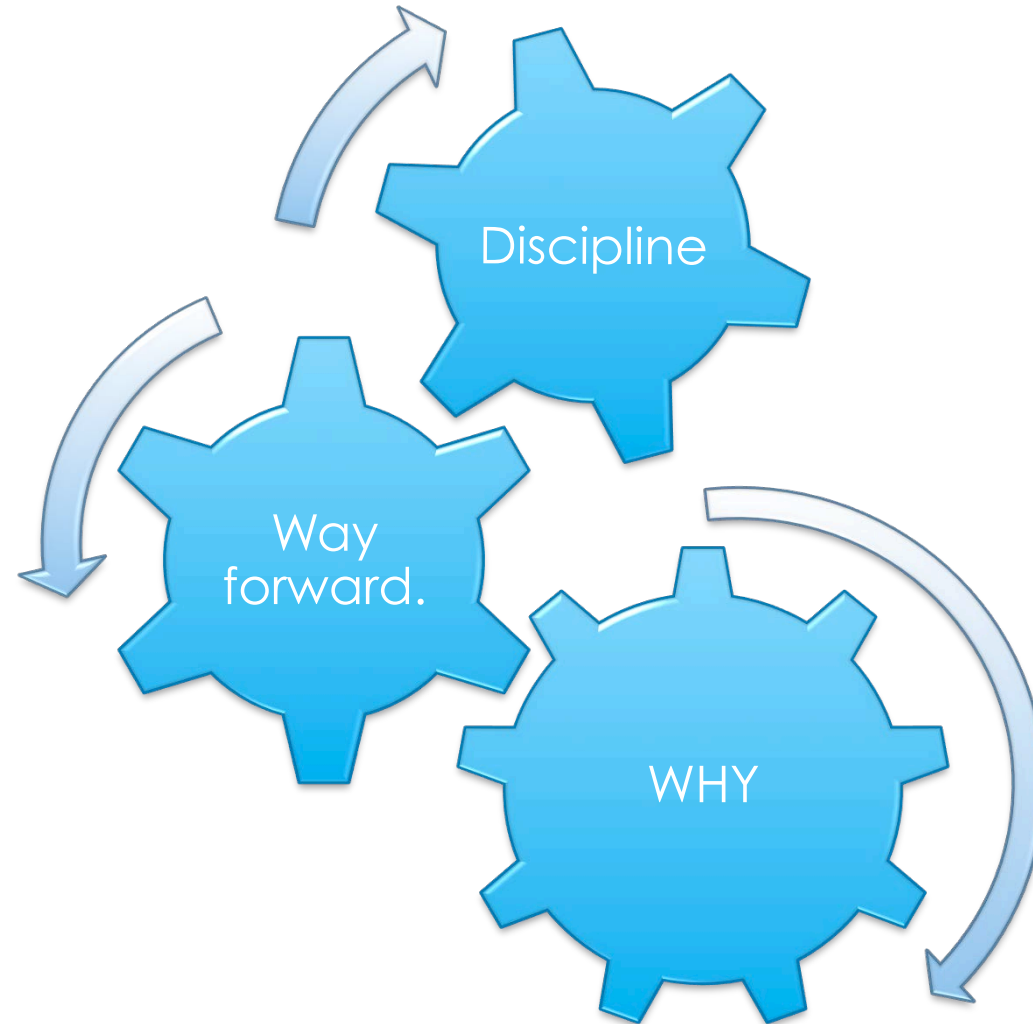
synonyms: blame, fault, guilt, culpability, liability

"they denied responsibility for the bomb attack"

- the opportunity or ability to act independently and make decisions without authorization.

"we would expect individuals lower down the organization to take on more responsibility"

Work the Responsibility Machine



Responsibility Machine



Functional Medicine Diagnostic Workup



Applied FM



Responsibility Machine

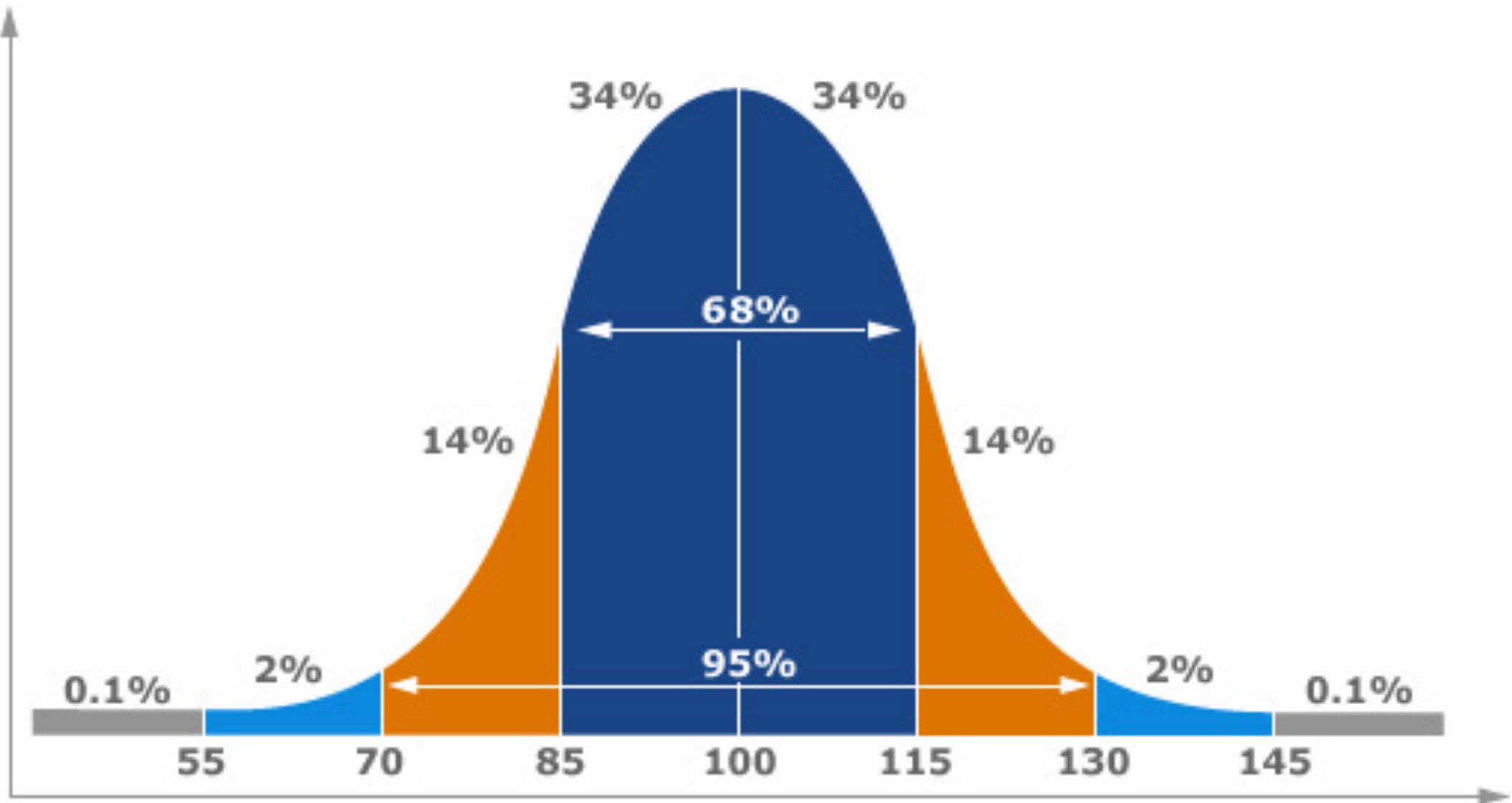


Functional Medicine Diagnostic Workup



Progress.

Raw Data



Optimal Range

A tighter set of ranges designed to help:

1. Identify underlying physiological issues that can help explain symptoms
2. Evaluate health rather than disease
 - * Catch potentially more serious disease processes earlier

Sodium

Laboratory ref range:

135 – 145 mmol/L

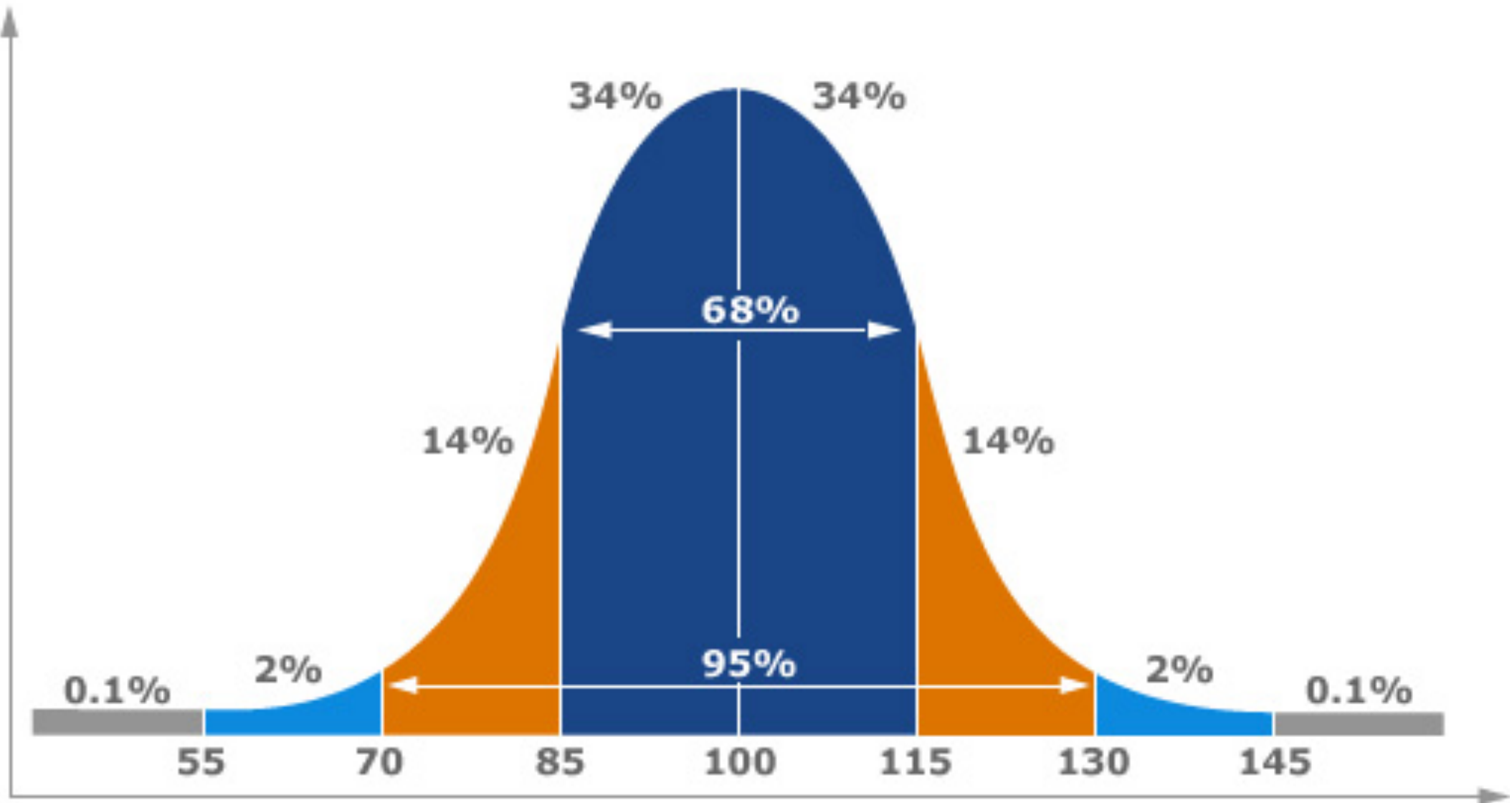
Optimal ref range:

139 – 142 mmol/L

Potential issues with normal ranges

1. Individuals with subclinical or undiagnosed disease are included in the data to establish normal values.
2. References calculated from non-random (non-Gaussian) requires calculations and therefore may be inaccurate
3. Reference ranges may change
4. At $\pm 2SD$ from the average means 5% of the normal population will fall above (2.5%) and below (2.5%) the range.
 - * If a wider range ($\pm 3SD$) was used, would increase specificity and decrease sensitivity (eg people with the disease but small fluctuations in test values might be missed)

Raw Data



Sensitivity and Specificity

Sensitivity

- Ability of a test to detect a positive test in every patient with a given disease
- A test with 100% sensitivity will be positive in every patient with a specific disease
- 90% sensitivity will not accurately identify 10% of individuals with a specific disease (false negative)
- The higher the sensitivity, the lower the chance of false negatives.
- PKU test is highly sensitive for infants with PKU. PSA test for prostate issues has a low sensitivity (~40%)

Sensitivity and Specificity

Specificity

- Percent of negative results in people without the disease
- Helps predict false positives
- Refers to how well a test successfully (specifically) identifies a given disease (only found in people with the disease)
- A test with 90% specificity may be positive in 10% of the people that *do not* have the disease
- The lower the specificity, the higher the chance for false positives

Sensitivity and Specificity - Example

“The Heart Attack Test” (THAT test)

Used on 200 people who have had a heart attack.

196 people tested positive (98% sensitivity)

- Four false negatives (people had it, but weren't identified)

Used on 200 people who did *not* have a heart attack

20 people tested positive (90% specificity)

- Identified the disease 10% of the time even though the disease wasn't present

Factors to Consider

- * Laboratory Error
 - * Equipment, human, calibration, sample prep, reagent discrepancies, drawing blood
 - * Tourniquet left on longer than one minute can increase protein, iron, AST, and cholesterol, and decrease potassium and creatinine
- * Laboratory reference range
- * Hydration status
- * Diurnal rhythms
 - * TSH, ALT, GGT, LDH, uric acid, phosphorus, calcium, hemoglobin, hematocrit, iron, certain WBCs
- * Altitude
- * Age, race, gender
- * Fasting (or starving?)
- * Medications
- * Nutritional supplements
- * Exercise
- * Diet

Medicine's Own Rules:

- * The majority of blood chemistry markers, when out of range, merely point to a list of possibilities
 - * Combining this information with history, intake, signs/symptoms, and other data can help narrow to a list of probabilities
- * **Rarely will a lab test dictate the course of treatment**
 - * Offers additional insights

1) Neutrophils, High

HEIRARCHICAL

- 1 Abscess pathophysiology
- 1 Abscess, lesser sac (567.2)
- 1 Bacterial infection (041.9 unspecified)
- 1 Epidural abscess (324.9)
- 1 Malignancy/cancer
- 1 Muscle abscess (728.89 unspecified)
- 1 Neoplasms/tumors
- 1 Periorbital cellulitis (376.01)
- 1 Right sided diverticulitis (562.00 unspecified)
- 1 Thigh cellulitis (682.6)

DRUGS

- 1 Corticosteroid administration
- 1 Fluphenazine (Prolixin) admin
- 1 Lithium (Lithane/Lithonate) admin
- 1 Lithium toxicity/overdose (985.8)
- 1 Niacin/Nicotinic acid admin

TRAUMA

- 1 Multiple trauma (959.8)

POISON AGENT SPECIFIC

- 1 Black widow spider bite (989.5 - E906.4)
- 1 Iron intoxication, acute (964.0)
- 1 Snakebite (rattlesnake/pit viper type) (989.5 - E906.2)

ELECTROMAGNETIC PHYSICS TRAUMA

- 1 Burns/severe/extensive (948.0 - 948.9)
- 1 Electrical injuries (994.8)

INFECTIOUS AGENT SPECIFIC

- 1 Amebiasis (006.9)
- 1 Bacterial tracheitis (464.10)
- 1 Eosinophilic meningoencephalitis (323.9)
- 1 Erysipelas (035)
- 1 Glanders (malleomyces mallei) (024)
- 1 Glanders abscess (024)
- 1 Lassa fever (078.89)
- 1 Legionaire's disease (482.84)
- 1 Leptospiiral disease (100.9 leptospirosis, unspecified)
- 1 Leptospiiral meningitis (100.81)
- 1 Leptospirosis/severe (Weils) type (100.0)
- 1 Meningococchemia/meningococcus (036.2 - 036.9)
- 1 Metastatic staphylococcus abscess synd (682.9)
- 1 Myocarditis, bacterial (422.92)
- 1 Primary bacterial peritonitis/ascites (567.2 - 789.5)
- 1 Rat bite fever, spirillary (026.0)
- 1 Respiratory syncytial virus infection (079.6)
- 1 Staphylococcus aureus infection (041.11)
- 1 Streptococcal pharyngitis, beta type A (034.0)
- 1 Tetanus
- 1 Trichinosis (124)
- 1 Tularemia (021.9)

INFECTED ORGAN ABSCESS

- 1 Abscess Bartholin gland cyst (616.2)
- 1 Abscess, abdominal, visceral (567.2)
- 1 Abscess, cervical gland (682.1)
- 1 Abscess, dentalalveolar (522.5)
- 1 Abscess, gallbladder/pericholecystic (575.0)

- 1 Abscess, intracranial (324.0)
- 1 Abscess, intraperitoneal, RL quadrant (567.2)
- 1 Abscess, ischiorectal (566)
- 1 Abscess, liver (572.0)
- 1 Abscess, myocardium (422.92)
- 1 Abscess, parapharyngeal (478.29)
- 1 Abscess, paraspinal (324.1)
- 1 Abscess, perianal/pararectal (566)
- 1 Abscess, periapical (522.5)
- 1 Abscess, renal (590.2)
- 1 Abscess, retroperitoneal (567.2)
- 1 Abscess, retropharyngeal (478.24)
- 1 Abscess, subarachnoid (324.9)
- 1 Abscess, subcutaneous (682.9 unspecified)
- 1 Abscess, submammary (611.0)
- 1 Abscess, submastoid/Bezold's abscess (383.01)
- 1 Abscess, subphrenic (998.59)
- 1 Anterior tibial compartment/fasciitis (729.4 unspecified)
- 1 Appendicitis (541)
- 1 Appendix abscess (540.1)
- 1 Areolar abscess/nipple (661.0)
- 1 Arthritis (716.90)
- 1 Bacterial endocarditis/acute (421.0)
- 1 Brain abscess (324.0)
- 1 Breast abscess (611.0)
- 1 Brodie's abscess (730.1)
- 1 Cellulitis (682.9)
- 1 Cellulitis, chronic pelvic (614.4)
- 1 Cellulitis, dissecting, of scalp (704.8)
- 1 Cellulitis, pelvic, acute (614.3)
- 1 Cellulitis, periurethral, male (597.0)
- 1 Cellulitis/lower extremities (682.6 - 682.7)
- 1 Cellulitis/pinna (380.10)
- 1 Cerebral abscess/congen. cardiac anomly (324.0)
- 1 Cervical adenitis/abscess (289.5 - 289.0)
- 1 Cholangitis, ascending (576.1)
- 1 Cholecystitis without gallstones (575.0)
- 1 Cholecystitis, acute (575.0)
- 1 Diverticulitis/phlegmon/abscess (562.11 - 682.9)
- 1 Emphysematous cholecystitis (575.0)
- 1 Endocarditis, infective (421.0)
- 1 Endometritis, acute (615.0)
- 1 Gingiva, abscess (523.3)
- 1 Hepatic abscess, pyogenic (572.0)
- 1 Intraperitoneal abscess (567.2 - 567.0)
- 1 Lacrimal gland infection/abscess (375.03)
- 1 Lung abscess (513.0)
- 1 Mastoiditis, zygomatic (383.9 unspecified)
- 1 Meckel's acute diverticulitis (751.0)
- 1 Mediastinal abscess (513.1)
- 1 Nasal septum abscess/hematoma (920)
- 1 Omentum abscess (567.2)
- 1 Osteomyelitis (730.2)
- 1 Osteomyelitis, vertebral (730.28)
- 1 Ovarian abscess/Tubo-ovarian abscess (614.2)
- 1 Pancreas abscess (577.0)
- 1 Pelvic abscess (614.4)
- 1 Pelvic inflammatory disease (614.9)
- 1 Penis cellulitis (607.2)
- 1 Perinephric abscess (590.2)
- 1 Peritonitis, acute (567.9)
- 1 Peritonsillar abscess (475)
- 1 Periurethral abscess (597.0)
- 1 Prostate abscess (601.2 - 098.12)
- 1 Prostatitis, acute (601.0)
- 1 Psoas abscess (728.89)
- 1 Puerperal pelvic cellulitis/sepsis (670.00)
- 1 Pyopneumothorax (510.9 - 510.0)
- 1 Scrotum abscess (608.4)
- 1 Septicemia (038.9)
- 1 Sialadenitis (527.2)
- 1 Spinal epidural abscess (324.1)
- 1 Splenic abscess (289.59)
- 1 Stomach abscess (535.0)

- 1 Subhepatic/gallbladder bed abscess (575.0)
- 1 Submaxillary gland infection/abscess (527.3)
- 1 Suppurative lymphangitis (682.9)
- 1 Testis, abscess (604.0)
- 1 Tibia osteomyelitis (015.7)
- 1 Tonsil, abscess (475)
- 1 Vitreous, abscess (360.04)

GRANULOMATOUS INFLAMMATORY

- 1 Pancreatitis, acute (577.0)

METABOLIC STORAGE DISORDER

- 1 Diabetic ketoacidosis/coma (250.3)

ALLERGIC COLLAGEN AUTO-IMMUNE

- 1 Anaphylaxis, generalized (995.0)
- 1 Dressler's syndrome (411.0)
- 1 Hypersensitivity pneumonitis (495.9)
- 1 Nephrotic syndrome (581.9)
- 1 Polyarteritis nodosa (446.0)
- 1 Purpura fulminans (286.6)
- 1 Reiter's syndrome (099.3)
- 1 Rheumatic fever (390)
- 1 Serum sickness (999.5 unspecified)
- 1 Temporal/cranial arteritis (446.5)
- 1 Thrombotic thrombocytopenic purpura (287.3)
- 1 Urticaria (708.9)

CONGENITAL DEVELOPMENTAL

- 1 Granulomatous dis/childhood, chronic (686.1)
- 1 Hemolytic disease of newborn (773.2)

HEREDITOFAMILIAL

- 1 Agranulocytosis, infantile hereditary (288.0)
- 1 Sickle cell anemia (282.60)
- 1 Sickle cell crisis (282.62)

RELATIONAL PSYCHIATRIC

- 1 Stress/Emotional/Physical (308.0)

STRUCTURAL ANATOMIC FOREIGN BODY

- 1 Acute gastric acid/aspiration syndrome (536.0 - 535.40)
- 1 Choledocholithiasis (574.50)
- 1 Gastroduodenal perforation (533.90 - 533.91)
- 1 Pulmonary embolism (415.11)
- 1 Thrombophlebitis, peripheral (451.2)
- 1 Thrombosis, arterial (444)

ARTERIOSCLEROTIC VASCULAR

- 1 Cerebral hemorrhage (431)
- 1 Gangrene, ischemic (785.4 - 440.24)
- 1 Infarction, bowel (557.0)
- 1 Myocardial infarction, acute (410.90)
- 1 Pulmonary infarction (415.10)

FUNCTIONAL PHYSIOLOGIC

- 1 Exercise/Aerobic exercise (V57.0)

VEGETATIVE AUTONOMIC

- 1 Cushing's disease (255.0)
- 1 Eclampsia of pregnancy (642.60)
- 1 Mastitis, puerperal (675.20)

REFERENCE TO ORGAN SYSTEM

- 1 Hemorrhage, acute (459.0)
- 1 Leukemoid reaction (288.8)
- 1 Renal failure, acute (586)

NEOPLASTIC

- 1 Adenocarcinoma, pancreatic (157.9)
- 1 Carcinoma, bronchogenic (162.9 primary - 212.3 benign)
- 1 Carcinoma, hepatocellular (155.0)
- 1 Hodgkins disease (201.90)
- 1 Metastatic malignancy (cancer)
- 1 Myelogenous leukemia, chronic (205.1)
- 1 Myelogenous/Blastic Leukemia, Acute (205.00)
- 1 Myeloproliferative disease (238.7)
- 1 Polycythemia vera (238.4)

HEIRARCHICAL

- ## DRUGS

1 Crush syndrome (muscle injury severe) (958

- ## POISON AGENT SPECIFIC

- ## POISONED ORGAN

- ## ELECTROMAGNETIC PHYSICS TRAUMA

- ## INFECTIOUS AGENT SPECIFIC

- 1 Hepatic leptospirosis (100.0)
- 1 Hepatitis A (070.1)
- 1 Hepatitis B (070.30)
- 1 Hepatitis B vasculitis syndrome (447.6 vasculitis - 070.30 Hep B)
- 1 Hepatitis C virus (non-A, non-B) (070.51)
- 1 Hepatitis E virus (070.53)
- 1 Hepatitis G virus (573.3 unspecified)
- 1 Hepatitis, amebic (006.3)
- 1 Hepatitis, cholestatic, viral type (573.8)
- 1 Hepatitis, chronic active/HBV (571.49)
- 1 Hepatitis, chronic C type (070.54)
- 1 Hepatitis, complicating (non-hep virus) (573.3)
- 1 Hepatitis, mononucleosis (075)
- 1 Hepatitis, persistent, chronic (571.41)
- 1 Hepatitis, toxoplasmosis (130.5)
- 1 Hepatitis, viral (070.9)
- 1 Hepatitis/chronic aggressive (571.49)
- 1 Infectious mononucleosis (075)
- 1 Kawasaki disease (446.1)
- 1 Legionaire's disease (482.84)
- 1 Leptospiral disease (100.9 leptospirosis, unspecified)
- 1 Leptospirosis/severe (Weils) type (100.0)
- 1 Listeria hepatitis, newborn (774.4 - 027.0)
- 1 Perihepatitis, gonococcal (098.86)
- 1 Plasmodium falciparum malaria (084.0)
- 1 Q fever (083.0)
- 1 Reye's syndrome (331.81)
- 1 Rubella (056.9)
- 1 Scrub typhus (081.2)
- 1 Toxic shock syndrome (040.89)
- 1 Typhoid fever (002.0)
- 1 Typhus, acute/epidemic (080)

INFECTED ORGAN ABSCESS

- 1 Cholecystitis without gallstones (575.0)
- 1 Cholecystitis, acute (575.0)
- 1 Drug-induced Hepatitis, idiosyncratic (573.3)
- 1 Hepatitis, bacterial (070.1)
- 1 Hepatitis, granulomatous (573.3 - 681.1 granuloma)

GRANULOMATOUS INFLAMMATORY

- ## METABOLIC STORAGE DISORDER

METABOLIC STORAGE DISORDER

- ## 1 Wilsons Disease (275.1)

ALLERGIC COLLAGEN AUTO-IMMUNE

- 1 Autoimmune hepatitis (Plasma cell) (571.49)
- 1 Cirrhosis, primary biliary (571.6)
- 1 Dermatomyositis (710.3)
- 1 Polymyositis (710.4)
- 1 Primary sclerosing cholangitis (571.6)
- 1 Transplanted liver rejection (996.82)
- 1 Ulcerative colitis (556.9)

CONGENITAL DEVELOPMENTAL

- 1 Cirrhosis (571.5)
1 Cirrhosis, congenital/neonatal (777.8)

USE DEGENERATIVE NECROSIS AGE

- 1 Hepatic necrosis, acute/subacute (570)
1 Rhabdomyolysis (728.89)

ARTERIOSCLEROTIC VASCULAR

- 1 Hepatic vein occlusion/thrombus (453.0)
- 1 Myocardial infarction, acute (410.90)
- 1 Veno-occlusive hepatic disease (459.9)

VEGETATIVE AUTONOMIC

- 1 Disseminated intravascular coagulation (286.6)
- 1 Eclampsia of pregnancy (642.60)
- 1 Hypothyroidism (myxedema) (244.9)
- 1 Myxedema coma (244.9)
- 1 Thyrotoxic crisis (242.90)

REFERENCE TO ORGAN SYSTEM

- 1 Chronic liver disease (571.9)
- 1 Cirrhosis, cardiac (571.5)
- 1 Cirrhosis, postnecrotic (571.5)
- 1 Cryoimmunoglobulinemic syndrome (289.9)
- 1 Fatty liver (571.8)
- 1 Fatty liver of pregnancy, acute (571.8)
- 1 Fulminant hepatic failure (570)
- 1 HELLP syndrome of pregnancy (V22.1)
- 1 Hepatic encephalopathy (572.2)
- 1 Hepatic failure (572.8)
- 1 Hepatic function impairment (573.8)
- 1 Jaundice of pregnancy, recurrent (782.4)
- 1 Peliosis hepaticus (573.9)
- 1 Right congestive heart failure/chronic (428.0)
- 7 Steatosis/Non-alcoholic fatty liver dis (571.8)

NEOPLASTIC

- 1 Adenocarcinoma, gastric (151.9)
- 1 Angiosarcoma (171.9)
- 1 Carcinoma, gallbladder (156.0)
- 1 Carcinoma, hepatocellular (155.0)
- 1 Cholangiocarcinoma, intrahepatic (155.0)
- 1 Lymphoblastic leukemia, acute (208.00)
- 1 Lymphoma/malignant, non-Hodgkins (201.9)
- 1 Metastatic liver disease (197.7)

Proteins

Proteins

- * Total protein
- * Albumin
- * Globulin
- * Albumin/Globulin ratio

Total Protein

- * Total protein levels reflect the albumin and globulin content of blood.
- * Therefore a shift in either albumin, globulin or both will alter total protein levels.
 - * Marker has limited clinical value as it is possible for albumin to drop, globulin to elevate, leading to a normal total protein
 - * Albumin/Globulin ratio helps to compensate for this however

Traditional Reference Range:

- * 60-85 g/dL

Optimal Reference Range

- * 6.5-7.5 g/dL

Total Protein

Elevated by:

- * Chronic liver disease, dehydration, immune system issues (increased globulins)

Decreased by:

- * Poor protein intake, digestion and absorption (lack of amino acids to synthesize proteins)?
 - * Crohn's and ulcerative colitis
- * Alcoholism and/or liver cirrhosis (poor liver function)
- * Hypothyroidism

Albumin

- * Albumin, **synthesized in the liver**, is the most abundant plasma protein in the serum.
 - * 60% of plasma protein is albumin
 - * **Small protein that can be lost into extravascular spaces** (eg kidney dysfunction)
- * It has two major functions
 - * Binding – free fatty acids, minerals (calcium, magnesium), some hormones, medications, wastes, etc
 - * Osmotic gradient – primary contributor to the plasma osmotic gradient, which **helps keep fluid in the circulatory system**
 - * Also acts as a buffer and has recently been found to have antioxidant properties as well
- * Life span of approximately 20 days

Albumin

- * Transcription of albumin gene is down-regulated by:
 - * Cytokines (TNF, interleukins, **transforming growth factor**)
 - * Vitamin (vitamin A, B6)
 - * Colloid-osmotic pressure
 - * Amino acid deficiency

Albumin - Elevated

Cause	Reason	Additional Inquiry
Dehydration	Hemoconcentration. Albumin appears elevated but is relatively elevated due to low plasma volume.	Evaluate other markers of dehydration.

Vitamin B₆ Suppresses Growth and Expression of Cyclin D1 Gene in a Human Hepatoma Cell Line

Ana Molina, Tatsuzo Oka, Saturnino M. Muñoz, Mika Chi
Masashi Kuwahata, and Yasuo Natori

Abstract: The effect of vitamin B₆ on the growth of a human hepatoma cell line HepG2 in culture was studied. The growth of HepG2 cells and protein synthesis were almost completely inhibited in medium supplemented with 5 mM pyridoxine. Pyridoxal was as effective as pyridoxine, but pyridoxamine showed no inhibitory action. The growth inhibition of HepG2 cells by pyridoxine was accompanied by a marked inhibition of secretion of plasma proteins, particularly albumin. Northern blot analysis of albumin mRNA showed that pyridoxine caused a rapid decrease in the expression of albumin gene. The electron-microscopic examination of pyridoxine-treated HepG2 cells revealed a smoothing of nuclear membrane, a decrease in the number of nucleoli, and an appearance of aggregated heterochromatin structures. These morphological features are compatible with the depressed transcriptional activity in the pyridoxine-treated cells. The mechanism by which vitamin B₆ exerts its inhibitory effect was discussed in terms of our recent finding that vitamin B₆ modulates expression of albumin gene by inactivating tissue-specific DNA-binding proteins. Binding of pyridoxal phosphate with tissue-specific transcription factors may reduce the capacity of these factors to interact with the regulatory region of albumin gene, resulting in the inhibition of the gene expression.

Introduction

Vitamin B₆ is an essential water-soluble vitamin required for normal growth and development. The physiologically active form of the vitamin, pyridoxal 5'-phosphate (PLP), is derived from inactive dietary precursors and functions as a cofactor in numerous enzyme reactions of amino acid metabolism (1).

Many earlier studies have shown that tumor growth is inhibited in animals maintained on a vitamin B₆-deficient diet (2-4). By contrast, recent studies by Disorbo and colleagues (5-7) have demonstrated that high levels of pyridoxine or pyridoxal may suppress growth or exhibit cytotoxicity against animal or human cancer cells *in vitro* and *in vivo*. A more

recent study by Gridley [1996] showed that a high dietary intake of vitamin A increases the number of T cells and enhances immune status. These findings indicate that supraphysiological concentrations of vitamin A have the potential as an antineoplastic agent. The mechanism by which vitamin A exerts its antineoplastic effect has remained unknown.

Recent work in our laboratory has shown that vitamin B₆ modulates expression of tissue-specific DABP. Binding of PLP with transcription factors such as HNF-1 and C/EBP factors to interact with the gene, resulting in the induction of

In the present study, we found that the levels of pyridoxine on the albumin gene in HepG2 human hepatoma differentiated cell line was selected. This cell secretes plasma proteins, including the inducible cytochrome *P*-450, and cytokines in the liver (10,11). We report here that the expression of albumin gene is severely inhibited by the presence of pyridoxine in culture medium, and this inhibition is accompanied by a marked decrease in albumin gene. We also found that chromatin undergoes a phase transition at a high concentration of pyridoxine.

Materi

Cell Culture

Human hepatocarcinoma Type Culture Collection (ATCC) medium, minimum essential medium (MEM) supplemented with fetal bovine serum, 1 mM amino acids, 100 IU/ml penicillin, and 100 IU/ml streptomycin. Cells were grown

All-trans retinoic acid down-regulates human albumin through the induction of C/EBP β -LIP

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ATRA (all-*trans* retinoic acid), which is a major bioactive metabolite of vitamin A and a potent regulator of development and differentiation, mediates down-regulation of the human albumin gene. However, the mechanism of ATRA-mediated down-regulation is not well understood. In the present study, deletion analysis and luciferase assays demonstrate that ATRA causes a marked decrease in the activity of the albumin promoter, the region between nt -367 and -167 from the transcription start site, where C/EBP (CCAAT/enhancer-binding protein)-binding sites are tightly packed, is indispensable for ATRA-mediated down-regulation. ChIP (chromatin immunoprecipitation) assays revealed that *in vivo* binding of C/EBP α to the region markedly decreases upon incubation with ATRA, whereas ATRA treatment marginally increases the recruitment of C/EBP β . We found that ATRA has the ability to differentially and directly induce expression of a truncated isoform of C/EBP β , which is an

INTRODUCTION

Serum albumin is the most abundant and characteristic protein that is produced by the mature liver; albumin functions as a transporter of various substances and is a prime regulator of colloid osmotic pressure [1]. Albumin is exclusively synthesized in the liver, approx. 200 mg/kg per day [2], leading to its high steady-state concentration in plasma (35–50 g/l in humans). It has been reported that the albumin level in plasma is decreased as a result of reduced albumin synthesis in clinical disorders such as liver disease [2], infectious disease [3], and cancer [4]. Serum albumin can be used as a reliable indicator for the prognosis and severity of these diseases [5,6]. Therefore it is probable that albumin synthesis is regulated accurately and dramatically in a variety of physiological and pathophysiological conditions.

Albumin synthesis is regulated mainly at the transcriptional level through tissue-specific transcription factors such as HNF (hepatocyte nuclear factor)-1 and C/EBP (CCAAT/enhancer-binding protein) [7,8]. The transcription of the albumin gene is down-regulated by a number of factors, including cytokines [9–11], vitamins [12–14], colloid osmotic pressure [15,16] and amino acid limitation [17]. ATRA (all-*trans* retinoic acid), a major bioactive metabolite of vitamin A, plays a crucial role in hepatocyte differentiation, proliferation and apoptosis [18,19]. ATRA has been shown to down-regulate albumin gene expression in rat hepatocytes [20] and human hepatoma cell lines [12,31].

LIP (liver-enriched transcr a transactivation domain, of C/EBP β -LIP to its re C/EBP β -LIP negatively r of albumin, as well as th induced by C/EBP transa length C/EBP β . In conclu down-regulation of the alb an increase in the translat C/EBP transactivators by i the albumin promoter.

Key words: all-*trans* retinoic acid, transcription factor, CCAAT, dominant-negative factor, transcriptional inhibitory protein (LAP)

In animal experiments, it is known that the serum albumin concentration of ATRA to rodents [21] and fenretinide (4-hydroxyphenylretinamide) of ATRA that possesses inhibitory activity on malignant cells [22–24], causes hypoalbuminemia as an adverse effect. It is known about the ATRA-induced hypoalbuminemia that the ATRA expression either in exper-

In the present study, we examined the mechanism by which ATRA directs differentiation of human HCC (hepatocellular carcinoma) cells to the transcription factor C/EBP β . ATRA preferentially induced expression of C/EBP β : 20 kDa LIP (lipocalin) protein. We also present evidence for the role of an antagonist of C/EBP transcription in the albumin gene.

EXPERIMENTAL

Plasmids

The promoter fragment of nt -1867 and +39 was of the genomic DNA of human

Abbreviations used: ATHA, all-trans retinoic acid; C/EBP, CCAAT/enhancer-binding protein; C/EBP β -L, C/EBP β long isoform; Co-IP, coimmunoprecipitation; CUG-BP1, CUG triplet-repeat binding protein 1; DR, direct repeat; eIF, eukaryotic translation initiation factor; HCC, hepatocellular carcinoma; HNF, hepatocyte nuclear factor; LAP, liver-enriched transcriptional inhibitory protein; RARE, retinoic acid response element; RT, reverse transcriptase.

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Functional activity of hepatocyte nuclear factor-1 is specifically decreased in amino acid-limited hepatoma cells

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Abstract

Limitation of cultured rat hepatoma cells for an essential amino acid results in a specific decrease in expression of several genes that are preferentially expressed in the liver, including the serum albumin and transthyretin genes. In the work presented here, we examined whether the coordinate repression of these genes is caused by decreased activity of one or more of the liver-enriched transcription factors, hepatocyte nuclear factor-1 (HNF-1), HNF-3, HNF-4 or C/EBP. To address this question, HepG2 human hepatoma cells were transiently transfected with luciferase reporter constructs containing multiple copies of individual transcription factor binding sites. Limitation for an essential amino acid resulted in specific repression of a construct in which luciferase expression was directed by HNF-1. A single HNF-1 binding site located adjacent to the TATA box plays a major role in transcription directed by the serum albumin promoter in transient transfection assays. Amino acid limitation of cells transfected with an albumin promoter/luciferase reporter construct resulted in specific repression of promoter activity. In addition, bacterial methylation or site-directed mutagenesis of the HNF-1 binding site in the albumin proximal promoter region eliminated the regulation of an albumin promoter-luciferase reporter construct under conditions of amino acid limitation. These results demonstrated that the HNF-1 binding site played a major role in regulation of the albumin promoter by amino acid availability. Deletion analysis of the albumin promoter confirmed regulation through the HNF-1 binding site and also identified a second amino acid regulatory element in the upstream region of the albumin promoter, which has been shown previously to contain a functional binding site for HNF-3. The repression of albumin promoter and HNF-1 reporter constructs in amino acid-limited cells occurred without a change in the DNA binding activity of HNF-1. Moreover, HNF-3 DNA binding activity was also not decreased in amino acid-limited cells. These results suggest that the regulation of transcription by amino acids occurs at the level of transcriptional activation by HNF-1 and HNF-3, rather than by alteration of the DNA binding activity of either factor. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Serum albumin; Amino acid; Hepatocyte nuclear factor-1

Abbreviations: TTR, transthyretin; HNF, hepatocyte nuclear factor; CPS-I, carbamyl phosphate synthetase-I; UAAARE, upstream amino acid regulatory element; HSV-TK, herpes simplex virus thymidine kinase; CMV, cytomegalovirus; EMSA, electrophoretic mobility shift assay; apoA1, apolipoprotein A1 gene; apoCIII, apolipoprotein CIII gene; DCoH, dimerization cofactor of HNF-I.

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Albumin - Decreased

Cause	Reason	Additional Inquiry
Infection and/or inflammation	Albumin is a <i>negative acute phase reactant</i> , which will decrease during some infections and inflammatory processes.	Evaluate other inflammatory makers. Client history. CIRS
Liver disease	Includes alcoholism and cirrhosis. Poor liver function can lead to poor synthesis of plasma proteins, such as albumin.	Evaluate other liver markers.
Kidney disease	Due to albumin's small size, if there are issues in the glomerular filtration membrane of the kidneys, albumin can be lost in the urine.	Evaluate kidney markers.
Poor protein intake, digestion and absorption	Poor protein intake or absorption limits the amount of amino acids present for protein synthesis.	Evaluate intake and digestive function.

Reassessment of Albumin as a Nutritional Marker in Kidney Disease

The decision by nephrologists, renal dietitians, federal agencies, health care payers, large dialysis organizations, and the research community to embrace serum albumin as an important index of nutrition and clinical performance is based on numerous misconceptions. Patients with analbuminemia are not malnourished and individuals with simple malnutrition are rarely hypoalbuminemic.

Viewed in this manner, hypoalbuminemia may offer an opportunity to improve patient well-being by identifying and treating the underlying disorder.

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Kidney disease is closely associated with protein–calorie malnutrition. The World Health Organization defines malnutrition as “bad nourishment” characterized by “inadequate or excess intake of protein, energy, and micronutrients such as vitamins, and the frequent infections and disorders that result.”¹ The definition implies that protein–calorie malnutrition (henceforth referred to as “malnutrition”) will improve when missing nutrients are provided.

Serum albumin is the principal nutritional marker used to identify malnutrition in patients with chronic kidney disease (CKD). Through endorsements by nephrologists, renal dietitians, the research community, federal agencies, health care payers, and large dialysis organizations, it has also become a *de facto* index of clinical performance. The use of serum albumin as a nutritional and quality care marker involves the following as-

sumptions: Serum albumin is a reliable index of malnutrition; because serum albumin is typically low in patients with CKD, these patients should be considered malnourished; replacing missing nutrients will raise low albumin levels; and, because hypoalbuminemia is strongly associated with mortality, replacing missing nutrients to raise albumin will also improve patient outcomes. This review expands on previous viewpoints² by critically examining these assumptions and offering an alternative vision to interpreting serum albumin.

DETERMINANTS OF SERUM ALBUMIN

General Population

Albumin is a negatively charged, water-soluble protein (molecular weight 65

kilodaltons) that is synthesized in the liver. Its production is regulated by nutritional intake—specifically protein consumption—and illness.⁵ Reduced protein consumption slows mRNA synthesis of albumin and results in lower serum levels,^{3,6–11} although only in the setting of negligible dietary protein intake. Protein restriction also slows albumin degradation, although to a lesser degree than reductions in the synthesis rate.^{3,12} Refeeding with amino acids or protein induces an immediate rise in albumin synthesis.^{7,10}

It is also well established that albumin levels fall in patients with inflammatory disorders and other illnesses. Possible contributory mechanisms include downregulated production of albumin mRNA by the liver, leading to re-

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MEN STARVE IN MINNESOTA

CONSCIENTIOUS OBJECTORS VOLUNTEER FOR STRICT HUNGER TESTS TO STUDY EUROPE'S FOOD PROBLEM

Above:

Conscientious objectors during starvation experiment. Life magazine - July 30, 1945. Volume 19, Number 5, p. 43. Credit: Wallace Kirkland/Time Life Pictures/Getty Images.

Left:

Dr Ansel Keys measures the chest width of James Plaugher.

Reassessment of Albumin as a Nutritional Marker in Kidney Disease

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ABSTRACT

The decision by nephrologists, renal dietitians, federal agencies, health care pay-

ers, and patients (CKD) that is synthesized in the liver. Its

Albumin's widespread use as a nutritional marker is understandable in light of the desire of clinicians for a convenient, widely applicable, easily interpretable, and accurate indicator of nutritional status. Unfortunately, no such indicator exists or probably will for the foreseeable future. This does not mean that serum albumin lacks utility. Although we have demonstrated that serum albumin is not a good nutritional index in the great majority of cases, it is a powerful way to detect underlying illness; that is, the higher the serum albumin, the more intact is overall health.

protein-calorie malnutrition (henceforth referred to as "malnutrition") will improve when missing nutrients are provided.

Serum albumin is the principal nutritional marker used to identify malnutrition in patients with chronic kidney disease (CKD). Through endorsements by nephrologists, renal dietitians, the research community, federal agencies, health care payers, and large dialysis organizations, it has also become a *de facto* index of clinical performance. The use of serum albumin as a nutritional and quality care marker involves the following as-

suming nutrients to raise albumin will also improve patient outcomes. This review expands on previous viewpoints² by critically examining these assumptions and offering an alternative vision to interpreting serum albumin.

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Climate Associated Variations in the Human Serum Albumin Level*

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University of Mainz

Received September 1, 1975

Summary. Quantitative determinations of the human serum albumin level showed significantly higher values in tropical (Negroes 46.98 mg/ml, Indians 54.30 mg/ml) than in non-tropical populations (Germans 44.41 mg/ml). These observations are in agreement with those of several other authors. It is assumed that these variations in the serum albumin level are related to climate, and that they may indicate some biological advantage of higher albumin levels under the climatic conditions of tropical biotops. This hypothesis is discussed considering several aspects.

Introduction

In the course of its evolution the human species has spread over nearly all parts of the world, so that the various human races and populations came to exist under very different environmental conditions, among which, without any doubt, climate plays an important role. That means that human populations had to adapt biologically to the various climatic conditions on the earth. From a great number of investigations it is known already that anthropometric as well as physiological and biochemical traits and properties are related to those adaptation processes, which enabled the human species as a whole to survive successfully and permanently e.g. under the life conditions of tropical heat resp. under those of arctic cold (Dill *et al.*, 1964; Weiner, 1971; Walter, 1974).

In this connection we examined the question how far the serum albumin level might be involved in the adaptability to heat. In our opinion this question is admissible for the following reasons:

1. Due to their large surfaces, albumin molecules can bind considerable amounts of water (and thus also in water dissolved mineral salts). It is known that 1 g albumin can bind about 18 cm³ water and keep it in the blood circulation. By this the dispersion of liquid from the bloodvessels into the surrounding tissues is normally restricted.

2. In addition, in the healthy state a large amount of albumin spends quite a long time outside the vessels in the surrounding tissues, by which the above mentioned water binding capacity of albumin can doubtless be still more effective (Wünsche, 1954; Schultze and Heremans, 1966).

* Supported by the DFG.



Serum albumin levels predict vascular dysfunction with paradoxical pathogenesis in healthy individuals

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A U-shaped relationship between serum albumin and PWV was statistically significant when albumin level was treated as a continuous variable in g/dl and centered at 4.4g/dl. **The highest tertile of albumin level (4.6–5.4g/dl) was associated with increased odds ratios for hyperglycemia** compared to the middle tertile (4.4–4.5g/dl), whereas the lowest tertile (3.3–4.3g/dl) was associated with reduced odds ratios for hyperglycemia. The highest tertile was also associated with increased odds ratios for metabolic syndrome compared to the middle tertile, whereas the lowest tertile was associated with reduced odds ratios. Furthermore, **the lowest tertile was associated with increased prevalence of inflammation.**

death [4–6]. Among end-stage renal disease patients in particular, it is speculated the presence of the “malnutrition-inflammation complex syndrome” (MICS) may partly explain these paradoxical

risk factors for atherosclerosis such as obesity, insulin resistance, and metabolic syndrome, all indicators of overnutrition [10–13]. Our findings suggest that serum albumin is linked to atherogenesis through two paradoxical mechanisms, i.e. overnutrition and malnutrition-inflammation, not only in patients with renal dysfunction, but in healthy individuals. Several studies including the ARIC Study, the NHLBI Family Heart Study, and the Framingham Offspring Study, have looked at the relationship between serum

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Serum albumin levels predict vascular dysfunction with paradoxical pathogenesis in healthy individuals

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Table 3

Frequencies and odds ratios (95% CI) for hyperglycemia, metabolic syndrome, and inflammatory state according to tertiles of serum albumin.

	Albumin range (g/dl)			P-value
	Low (3.4–4.3)	Middle (4.4–4.5)	High (4.6–5.4)	
Hyperglycemia ^a				
Hyperglycemia (%)	38.9	42.7	48.9	0.013
Adjusted odd ratios (95% CI) [*]	0.80 (0.65–0.99)	1	1.35 (1.07–1.70)	<0.0001
Metabolic syndrome ^b				
Metabolic syndrome (%)	11.6	15.2	18.4	0.0018
Adjusted odd ratios (95% CI) [*]	0.70 (0.51–0.95)	1	1.30 (0.96–1.76)	0.0005
Inflammatory state ^c				
Inflammatory state (%)	6.8	4.1	3.9	0.016
Adjusted odd ratios (95% CI) [#]	1.85 (1.15–2.97)	1	0.92 (0.52–1.63)	0.0064

1. Introduction

Indicators of overnutrition such as obesity, hyperlipidemia, and the metabolic syndrome, are well-established risk factors for atherosclerosis [1–3]. In contrast, indicators of undernutrition, such as a low body mass index (BMI) or a reduced serum cholesterol concentration, are also strongly correlated with increased morbidity and mortality, including a high risk of cardiovascular events and death [4–6]. Among end-stage renal disease patients in particular, it is speculated the presence of the “malnutrition-inflammation complex syndrome” (MICS) may partly explain these paradoxical

observations which have been referred to as “reverse epidemiology” or the “risk factor paradox” [7].

Serum albumin level is affected by both nutrition and inflammation, and albumin is a negative acute phase protein. Low serum albumin concentrations have been shown to be associated with a higher risk of morbidity and mortality from cardiovascular disease, particularly in end-stage renal disease patients [8,9]. However, high serum albumin levels appear to be linked to conventional risk factors for atherosclerosis such as obesity, insulin resistance, and metabolic syndrome, all indicators of overnutrition [10–13]. Our findings suggest that serum albumin is linked to atherogenesis through two paradoxical mechanisms, i.e. overnutrition and malnutrition-inflammation, not only in patients with renal dysfunction, but in healthy individuals. Several studies including the ARIC Study, the NHLBI Family Heart Study, and the Framingham Offspring Study, have looked at the relationship between serum

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Albumin

Traditional Reference Range:

- * 3.5-5.5 g/dL

Optimal Reference Range:

- * 4.3-4.7 g/dL

Globulin

- * Serum globulin refers to a number of different plasma proteins synthesized in different locations of the body:
 - * α -Globulin
 - * 1 – antitrypsin, alpha 1-lipoprotein
 - * 2 – ceruloplasmin, haptoglobin, **thyroid binding globulin**, angiotensinogen, Protein C
 - * β -Globulin
 - * Plaminogen, **sex hormone binding globulin**, transferrin
 - * γ -Globulin
 - * **Immunoglobulins** (antibodies)
- * Thus an increase or decrease in serum globulin levels can indicate a variety of changes within these subtypes
- * Protein electrophoresis identifies these subtypes on a blood chemistry

Globulin

- * IgG >
- * beta-globulin (i.e. SHBG, plasminogen, complement) >
- * alpha-2-globulin (i.e. TBG, ceruloplasmin, angiotensinogen) >
- * alpha-1-globulin (i.e. antitrypsin) >
- * IgA
- * IgM
- * IgE
- * IgD

Globulin

Traditional Reference Range:

- * 1.5-4.5 g/L

Optimal Reference Range

- * 2.3-2.7 g/L

Globulin - Elevated

In general:

- * Cancer
- * Autoimmunity
- * Elevated estrogen
 - * Increase in SHBG and thyroid binding globulin

Globulin - Elevated

Alpha globulins

- * Acute phase reactant response – infection, inflammation
- * **Gall bladder dysfunction** (biliary cirrhosis, obstructive jaundice – check bilirubin levels)
- * Ulcerative Colitis
 - * “Digestive inflammation” used by other practitioners

Beta globulins

- * **Gall bladder dysfunction** (biliary cirrhosis, obstructive jaundice – check bilirubin levels)

Gamma globulins

- * **Autoimmune diseases, chronic infections**, some liver diseases

Globulin - Decreased

Alpha globulins

- * Acute hemolytic anemia
- * Nephrosis

Beta globulins

- * Nephrosis

Gamma globulins

- * Immune system compromise

Globulin – Quick Thought

- * Contain 10x as much tryptophan as albumin
- * If increased globulin is observed, ask patients about depressive tendencies
 - * As globulin synthesis increases, serum tryptophan may decrease, thus limiting the availability for serotonin synthesis

Lower Serum L-Tryptophan Availability in Depression as a Marker of a More Generalized Disorder in Protein Metabolism

Michael Maes, M.D., Ph.D., Annick Wauters, M.Sc., Robert Verkerk, R.T., Ing., Paul Demedts, M.Sc., Hugo Neels, Ph.D., Ann Van Gastel, M.D., Paul Cosyns, M.D., Simon Scharpé, Ph.D., and Roger Desnyder, M.D.

Recently, it has been reported that major and melancholic depression are accompanied by a lower availability of total L-tryptophan (L-TRP) to the brain and by significant changes in electrophoretically separated protein fractions, such as albumin and α_2 -globulin. The aim of this study was to examine the relationships between serum L-TRP availability and total serum protein, albumin, and α_2 -globulin in 42 depressed and 24 normal subjects. In depressed and normal subjects, alone and together, there were significant and positive correlations between serum L-TRP and total serum protein or albumin concentrations. In the depressed subjects, but not in normal controls, there were significant inverse relationships between the L-TRP/competing amino acid ratio and the α_2 -globulin fraction.

Serum L-TRP and albumin were significantly lower in melancholic subjects than in normal and minor depressed subjects. Depressed subjects had a significantly lower L-TRP/competing amino acid ratio and significantly higher serum α_2 -globulin than normal controls. Total serum protein was significantly lower in major depressed subjects than in normal controls. The results suggest that lower L-TRP availability to the brain in depression is related to lower serum albumin and to increased α_2 -globulin fraction, which are both hallmarks of the acute phase response in depression. The results further corroborate the hypothesis that lowered L-TRP availability in depression is related to the acute phase response in that illness.

[*Neuropsychopharmacology* 15:243–251, 1996]

KEY WORDS: Acute phase proteins; Electrophoresis; Serotonin; L-tryptophan; Albumin

There is now evidence that major depression is accompanied by disorders in the peripheral and central me-

tabolism of serotonin (5-HT) and by an acute phase response. One of the most consistently reported abnormal findings in serotonergic metabolism in major depression is a reduced availability of L-tryptophan (L-TRP) to the brain (DeMyer et al. 1981; Maes et al. 1990a; Cowen et al. 1989). Thus, serum L-TRP as well as the ratio of L-TRP to the sum of amino acids known to compete for the same cerebral uptake mechanisms (competing amino acids, e.g., tyrosine, valine, leucine, isoleucine, phenylalanine) is lower in major depressed subjects than in normal controls (review: Maes and Meltzer 1995). The relevance of these findings for the pathophysiology of major depression is underscored by recent reports that in normal and depressed subjects, acute lowering of plasma L-TRP may cause an acute lowering of mood or rapid clinically significant return of depressive symp-

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Albumin/Globulin Ratio

Optimal Range: 1.8-2.0

* Example: Albumin of 5.0 and Globulin of 2.5 is a 2:1 ratio or 2.0

Albumin/Globulin Ratio - Elevated

High albumin/globulin ratio is either:

- * Increased albumin - dehydration
- * Decreased globulin - (blank food tests)
- * Both

Albumin/Globulin Ratio - Decreased

Low albumin/globulin ratio is either:

- * Decreased albumin
- * Increased globulin
- * Both

Possibly caused by liver dysfunction, chronic inflammation/infection, loss of albumin via the kidneys, autoimmunity

Table 1. Probabilities of Assigning Samples to Either the Liver or Bone Group

Range	Probability, %	
	Liver	Bone
γ GT, U/L		
10-16	12	88
16-25	19	81
25-40	31	69
40-63	49	51
63-100	70	30
100-158	87	13
158-251	95	5
251-398	99	1
over 398	100	—
Albumin/globulin		
0.6-1.0	80	20
1.0-1.2	71	29
1.2-1.4	56	44
1.4-1.6	35	65
1.6-1.8	14	86
1.8-2.0	6	94
over 2.0	—	100

liter. This sample was then run by the proposed method excepting that incubation times were from 0 to 40 min in a 37 °C water bath. The experiment was then repeated with use of half the amount of glutaminase called for in the proposed method. Results are shown in Figure 1. Although a 30-min incubation was used throughout this study, it can be seen that the deamination reaction is rapid and incubation time can be considerably shortened. (Zero incubation time at 37 °C refers to incubation external to the aca.) The aca requires 7 min at 37 °C to complete the ammonia assay, during which time the glutaminase reaction may still proceed. With sufficient glutaminase incorporated into a test pack, complete automation of this procedure would appear to be feasible.

Linearity. L-Glutamine standards from 0.68 to 6.80 mmol/liter, run by the

Leijne, B., Evaluation of the DuPont aca ammonia procedure. *Clin. Chem.* 24, 489 (1978).

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Economical Single Cellulose Acetate/Plastic Sandwich for Developing and Scanning CPK/LDH Isoenzymes

To the Editor:

I have slightly modified isoenzyme electrophoresis by replacing one of the

in a small bin, then gently blotted before scanning in the plastic casing.

CPK. Peel off the plastic cover, blot, and air dry for a few minutes or until some protein fraction becomes visible. Place between blotters on a clean glass plate and dry in a 55 °C oven for 10 min or until dry (3). Cool, then observe under a longwave ultraviolet light, in a dark room.

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According to the data obtained here, if the albumin/globulin ratio is less than 1.1, there is an almost 80% chance that the liver is the predominant source of the increased alkaline phosphatase. Equally, if the albumin/globulin ratio exceeds 1.6, there is a better than 80% chance that bone is the course.

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- Ljpm, S. T., Blijenberg, B. G., and

with another plastic sheet 1 cm away from the narrow edge of the plastic base (this helps draining). Now squeeze out excess substrate by gently pressing a squeegee across the sandwich. Repeat this procedure in the opposite direction (avoid scratching the plastic cover). Blot, trim away extra plastic, place the preparation between two blotters, then between two glass plates, and incubate, supported in a 37 °C water bath, for 30 min for CPK or 20 min for LDH. The "sandwiches" can be stacked between the glass plates. A flat weight of about 0.5 kg is placed on the top of the stack to ensure contact.

LDH. Scan immediately, while wet and inside the plastic (2). I use a Beckman R110 Densitometer 580-mm filter (2), slit length 0.3 mm and filter holder 0.4 cal, or visually examine the bands. If not scanned immediately, and to avoid extensive diffusion of the bands, the cellulose strip is rinsed and kept in water

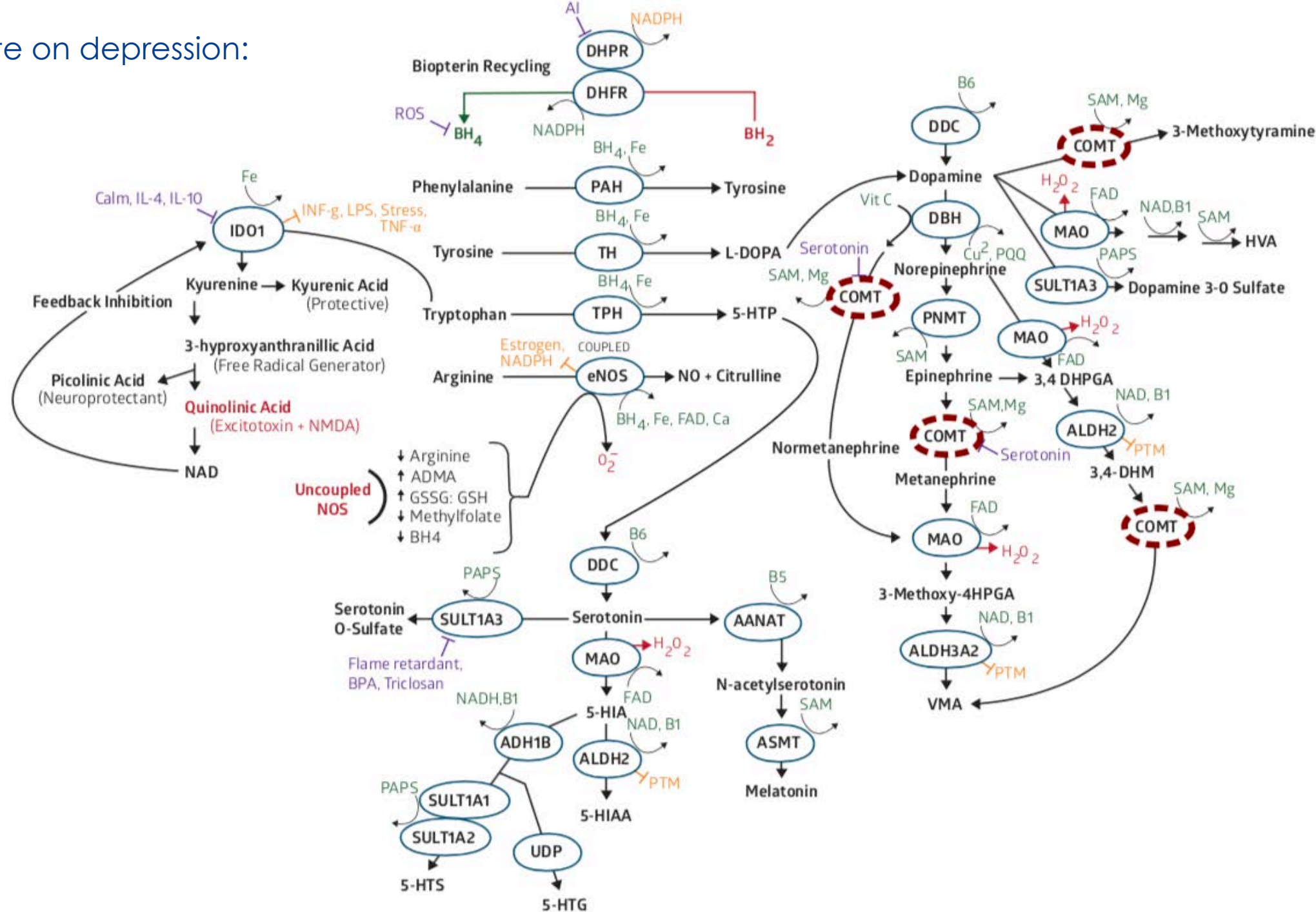
γ -glutamyltranspeptidase (γ GT; EC 2.3.2.1) (1, 2), 5'-nucleotidase (EC 3.1.3.5) (3, 4), leucine aminopeptidase (EC 3.4.1.1), and alanine and aspartate aminotransferases (EC 2.6.1.2 and EC 2.6.1.1) (2). The ideal situation is that they should be normal when only the bone isoenzyme is increased in concentration and above normal when the liver isoenzyme is present in increased amounts.

On each of a series of 96 routine specimens sent to the laboratory for "alkaline phosphatase isoenzymes," the following assays were done: (a) polyacrylamide disc electrophoresis of alkaline phosphatase by the method of Smith et al. (5), (b) γ GT at 37 °C with 3-carboxy-4-nitroanilide as substrate (6), and (c) total alkaline phosphatase by continuous-flow analysis (SMA 12/60), also at 37 °C, with phenyl phosphate as substrate.

Of the 96 samples, electrophoresis

Break Time.

Side note on depression:



Enzymes

Enzymes to note:

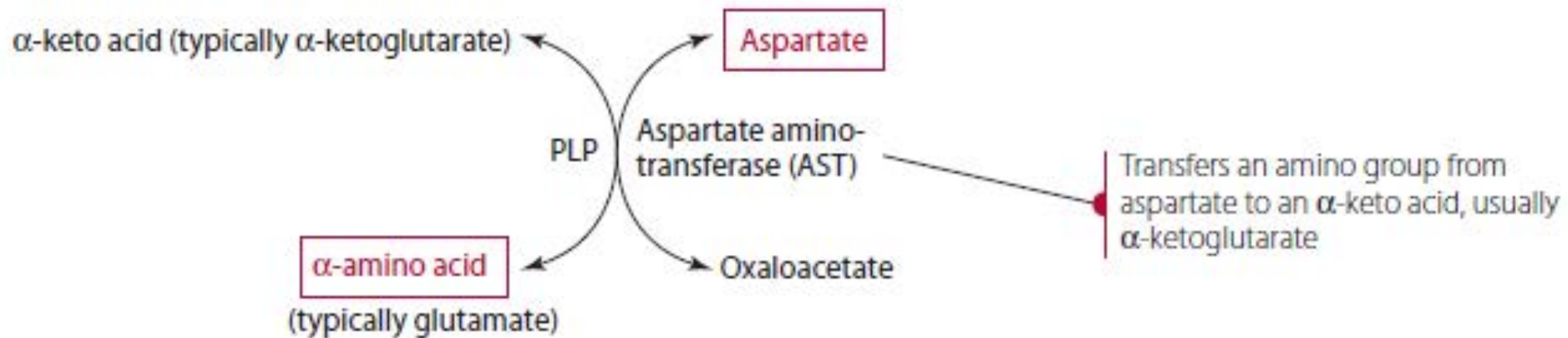
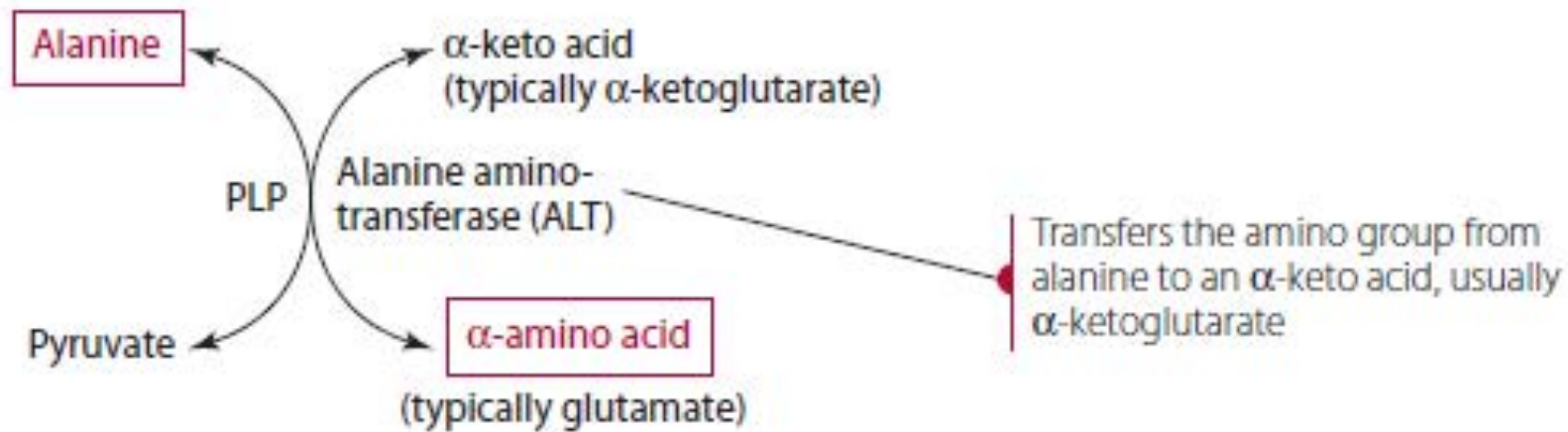
- * Aspartate Aminotransferase
- * Alanine Aminotransferase
- * Lactate Dehydrogenase
- * Gamma-glutamyl transferase
- * Alkaline phosphatase

Aspartate transferase (AST)

- * Aspartate transferase (AST), previously known as Serum Glutamic-Oxalacetic Transaminase (SGOT), is an enzyme primarily found in the liver and heart, but is also found in skeletal muscle, the pancreas, kidneys and gonads.
- * It is found in primarily in the cytosol of red blood cells and cardiac muscle, and in the **mitochondria of hepatocytes**.
- * As with other transaminases, it transfers an amine group and thus is involved with amino acid synthesis and metabolism. **All transaminase enzymes require vitamin B6 (pyridoxine) to carry out this task.**

Aspartate transferase (AST)

- * As an intracellular enzyme, AST will likely be elevated in blood during active tissue and cellular destruction.
- * In most types of liver diseases, ALT will be higher than AST and the AST/ALT ratio will be less than 1.
- * Caveats to this are in alcoholic liver damage and cirrhosis, where AST will generally be higher.
- * Has a half-life of 17 hours. (active damage)



Aspartate Transferase (AST)

Traditional Reference Range:

- * 0-40 IU/L

Optimal Reference Range:

- * Men: 12-25 IU/L
- * Women: 10-22 IU/L

AST - Elevated

Cause	Reason	Additional Inquiry
Hepatitis	AST can be elevated in liver inflammation, though ALT will be higher. Will likely be over 100.	Evaluate ALT.
Liver cirrhosis or alcoholism	AST will be elevated and higher than ALT.	Client history. Evaluate ALT.
Hypothyroidism	Possibly due to hepatocellular injury secondary to hypoxia created by low thyroid hormone.	Evaluate thyroid markers.

AST - Decreased

Cause	Reason	Additional Inquiry
Vitamin B6 deficiency	AST is a transaminase enzyme, which requires vitamin B6 for proper function.	Diet journal and client symptoms.
Azotemia (high nitrogen in the serum)	Nitrogen-containing compounds include urea, uric acid, creatinine, etc. If these are elevated, it may cause low AST levels.	Evaluate nitrogen containing compounds in the blood.

Alanine Aminotransferase (ALT)

- * Alanine Aminotransferase, previously known as Serum Glutamic-Pyruvic Transaminase (SGPT), is found in highest concentration in the liver. It is also found in the heart, muscle and kidney but in lower concentrations.
- * Has a half-life of 47 hours.
- * Interesting to note:
 - * One study showed a decrease in ALT in magnesium deficient individuals when magnesium supplementation was given
 - * High-normal (optimal range) ALT is associated with metabolic obesity and **atherosclerosis**.
 - * Mild ALT elevations can indicate early fatty liver disease

Normal Limit for Serum Alanine Aminotransferase Level and Distribution of Metabolic Factors in Old Population of Kalaleh, Iran

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Backgrounds: Normal or elevated values of serum alanine aminotransferase level (ALT) vary in different studies mostly related to characteristics of reference population including age, gender, body mass index, nonalcoholic fatty liver disease (NAFLD), and metabolic syndrome prevalence.

Objectives: To measure upper normal limit (UNL) for serum ALT in an apparently healthy Iranian old population (which we had not sufficient data before this study), and its modulating factors.

Patients and Methods: All inhabitants (> 50 years old) of Kalaleh, Golestan, Iran (N = 1986) were invited to the study. ALT measurements were performed for all subjects using the same laboratory method. Upper limit of normal (ULN) ALT was calculated based on its 95th percentile in normal weight subjects. Modulating factors of ALT were determined by multivariate analysis.

Results: A total of 1309 subjects, with the mean age of 61.5 ± 7.5 years were included. UNL of ALT was 18.8 U/L and 21.4 U/L in women and

A total of 1309 subjects, with the mean age of 61.5 ± 7.5 years were included.

AVG of ALT was 18.8 U/L and 21.4 U/L in women and men, respectively.

and sensitive indicator of liver-cell damage (1, 2). ALT is an important parameter for screening, diagnosis and follow-up of liver diseases (1, 2). Upper Limit of Normal (ULN) of serum ALT level should be able to differentiate between a healthy person and asymptomatic patient with a liver disease. ULN of ALT has been set at 40 U/L since 1950s, when serum ALT levels were used as a surrogate marker for non-A, non-B hepatitis among blood donors before identifying hepatitis C virus (HCV) (3). At that time, it was assumed that reference populations for measuring ULN of ALT included many asymptomatic patients with non-alcoholic fatty liver disease (NAFLD), so calculating ALT levels has not been performed in healthy populations. Nowadays, NAFLD is the most common cause of asymptomatic ALT elevation (4). ULN of ALT should be able to be used for screening NAFLD, too. ULN of ALT varies in different studies mostly related to characteristics of reference population including age, gender, body mass index,

The aim of this study was to investigate ULN for serum ALT and its modulating factors in an Iranian old population. This study also focuses on metabolic syndrome (MS) and its component and its association with ALT in this healthy appearing population.

3. Patients and Methods

The institutional review board (IRB) of the Digestive Disease Research Center (DDRC) of Tehran University of Medical Sciences reviewed and approved the study protocol and the informed consent forms. The DDRC Medical Ethics Committee has been registered in the Office for Human Research Protection (The US Department of Health and Human Services) under the registration number of IRB1641. Between July 2006 and January 2007, all men aged 50 to 79 and women aged 55 to 79 years old resident in Kalaleh, Golestan; Northern Iran, (N = 1986) were

Implication for health policy/practice/research/medical education:

This manuscript has a wide usage specifically for further research in the field of liver diseases and other related diseases affecting liver enzyme levels. It is specifically of value for determining an Iranian cutoff point for detecting normal or elevated liver enzyme values in subjects.

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Updated thresholds for serum alanine aminotransferase level in a large-scale population study composed of 34 346 subjects

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SUMMARY

Background

The sensitivity of current upper limit of normal (ULN) of serum alanine aminotransferase (ALT) levels for detecting chronic liver disease has been challenged recently.

A group of 21 282 subjects were used as a training set to define an ULN with the highest sensitivity; afterwards, this ULN was validated in another set of 13 064 subjects.

A reference healthy population was selected from the training set after excluding subjects with any abnormalities in independent risk factors associated with elevated serum ALT level (>40 IU/L) by multivariate analysis like body mass index, waist circumference, glucose, cholesterol, high-density lipoprotein-cholesterol, triglyceride, hepatitis B virus surface antigen, anti-hepatitis C virus antibody and fatty liver.

The new ULN of serum ALT level defined as the 95% percentile in the healthy population were 21 IU/L and 17 IU/L for men and women respectively.

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drome and fatty liver are the major risk factors of elevated serum alanine aminotransferase levels.

Aliment Pharmacol Ther



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Upper Limits of Normal for Alanine Aminotransferase Activity in the United States Population

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Abstract

Background & Rationale—Alanine aminotransferase (ALT) is an important test for liver disease, yet there is no generally accepted upper limit of normal (ULN) in the United States. Furthermore, the ability of ALT to differentiate persons with and without liver disease is

The cut-off for 95% sensitivity was an ALT=24 IU/L (70% specificity) for men and 18 (63% specificity) for women.

centrally.

Results—Maximum correct classification was achieved at ALT=29 IU/L for men (88% sensitivity, 83% specificity) and 22 (89% sensitivity, 82% specificity) for women. The cut-off for 95% sensitivity was an ALT=24 IU/L (70% specificity) for men and 18 (63% specificity) for women. The cut-off for 95% specificity was an ALT=44 IU/L (64% sensitivity) for men and 32 (59% sensitivity) for women. The area under the curve was 0.929 for men and 0.915 for women. If the cut-offs with the best correct classification were applied to the entire population, 36.4% of men and 28.3% of women would have had abnormal ALT.

Conclusion—ALT discriminates persons infected with HCV from those at low risk of liver disease, but would be considered elevated in a large proportion of the U.S. population.

Keywords

liver enzymes; reference range; National Health and Nutrition Examination Survey; epidemiology; population-based

Alanine aminotransferase (ALT) activity is an important screening, diagnostic, and monitoring test for liver disease.(1) As such, there is a need to have uniform measurement and definitions of normal range. However, there is no generally accepted upper limit of normal (ULN) in the United States (U.S.) and reference standards vary 2-fold.(2, 3) While

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The authors have no conflicts of interest.

Characteristic	Decile of ALT Level ^a									
	1		2		3		4–9		10	
	Mean (SD)	%	Mean (SD)	%	Mean (SD)	%	Mean (SD)	%	Mean (SD)	%
ALT level, IU/L	6.7 (1.4)		9.4 (1.3)		10.8 (1.8)		17.0 (5.4)		44.1 (23.4)	

Mortality Outcome and Decile of ALT Level ^a	No. of Deaths	Unadjusted Cumulative Mortality ^b	Age-adjusted Hazard Ratio ^c	95% Confidence Interval	P Value
All causes					
1	816	34.6	1.61	1.41, 1.83	<0.001
2	393	29.4	1.42	1.20, 1.68	<0.001
3	494	26.6	1.28	1.08, 1.53	0.006
4–9	1,760	17.5	1.00		
10	211	17.4	1.33	1.06, 1.66	0.014
Cardiovascular disease					
1	329	16.2	1.64	1.35, 1.98	<0.001
2	159	9.3	1.32	1.00, 1.75	0.047
3	187	11.6	1.26	0.90, 1.75	0.17
4–9	626	6.6	1.00		
10	63	4.2	1.23	0.83, 1.81	0.30
Cancer					
1	173	8.6	1.73	1.35, 2.21	<0.001
2	89	11.0	1.64	1.21, 2.22	0.002
3	114	7.1	1.46	1.02, 2.08	0.038
4–9	398	4.4	1.00		
10	44	5.2	0.95	0.61, 1.48	0.82
Diabetes mellitus					
1	19	0.63	1.06	0.44, 2.55	0.89
2	11	0.76	1.86	0.77, 4.52	0.16
3	12	0.18	0.41	0.20, 0.85	0.017
4–9	63	0.54	1.00		
10	11	1.7	2.74	0.97, 7.73	0.056
Liver disease ^d					
1	2	0.004	0.02	0.003, 0.12	<0.001
2	1	0.046	0.25	0.036, 1.71	0.15
3	3	0.028	0.15	0.031, 0.73	0.020
4–9	15	0.20	1.00		
10	16	0.70	5.27	1.59, 17.46	0.008

Alanine Aminotransferase (ALT)

Traditional Reference Range:

- * 0-40 IU/L

Optimal Reference Range:

- * Women: 10-19 IU/L
- * Men: 13-22 IU/L

ALT - Elevated

Cause	Reason	Additional Inquiry
Hepatocellular disease	Relatively high levels of ALT due to hepatocellular injury.	
Biliary issues	May be a mild increase in ALT. Other markers can help identify if it is related to biliary function.	Evaluate alkaline phosphatase and GGT.
Pancreatitis	Mild elevations in ALT may occur.	Evaluate symptoms. Serum amylase and lipase could be of value.

AST - Decreased

Cause	Reason	Additional Inquiry
Vitamin B6 deficiency	AST is a transaminase enzyme, which requires vitamin B6 for proper function.	Diet journal and client symptoms.
Azotemia (high nitrogen in the serum)	Nitrogen-containing compounds include urea, uric acid, creatinine, etc. If these are elevated, it may cause low AST levels. (grass?)	Evaluate nitrogen containing compounds in the blood.

Association Between High-Normal Levels of Alanine Aminotransferase and Risk Factors for Atherogenesis

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Low-normal ALT: less than Less than 19 IU/L in women and less than 31 IU/L in men
 High normal ALT: Between 19–40 IU/L in women and 31–40 IU/L in men
 Elevated ALT: Greater than 40 IU/L in either men or women

Level of ALT was associated with level of ApoB, concentration and particle size of very low-density lipoproteins (VLDL), concentration of LDL particles (LDL-P), and percentages of small dense LDL (sdLDL) and sdLDL-cholesterol (SdLDL-C) ($P < .0001$ for all). **High-normal level of ALT was associated with higher levels of LDL-cholesterol, LDL-P, sdLDL-C, and sdLDL particles ($P < .001$ for all).**

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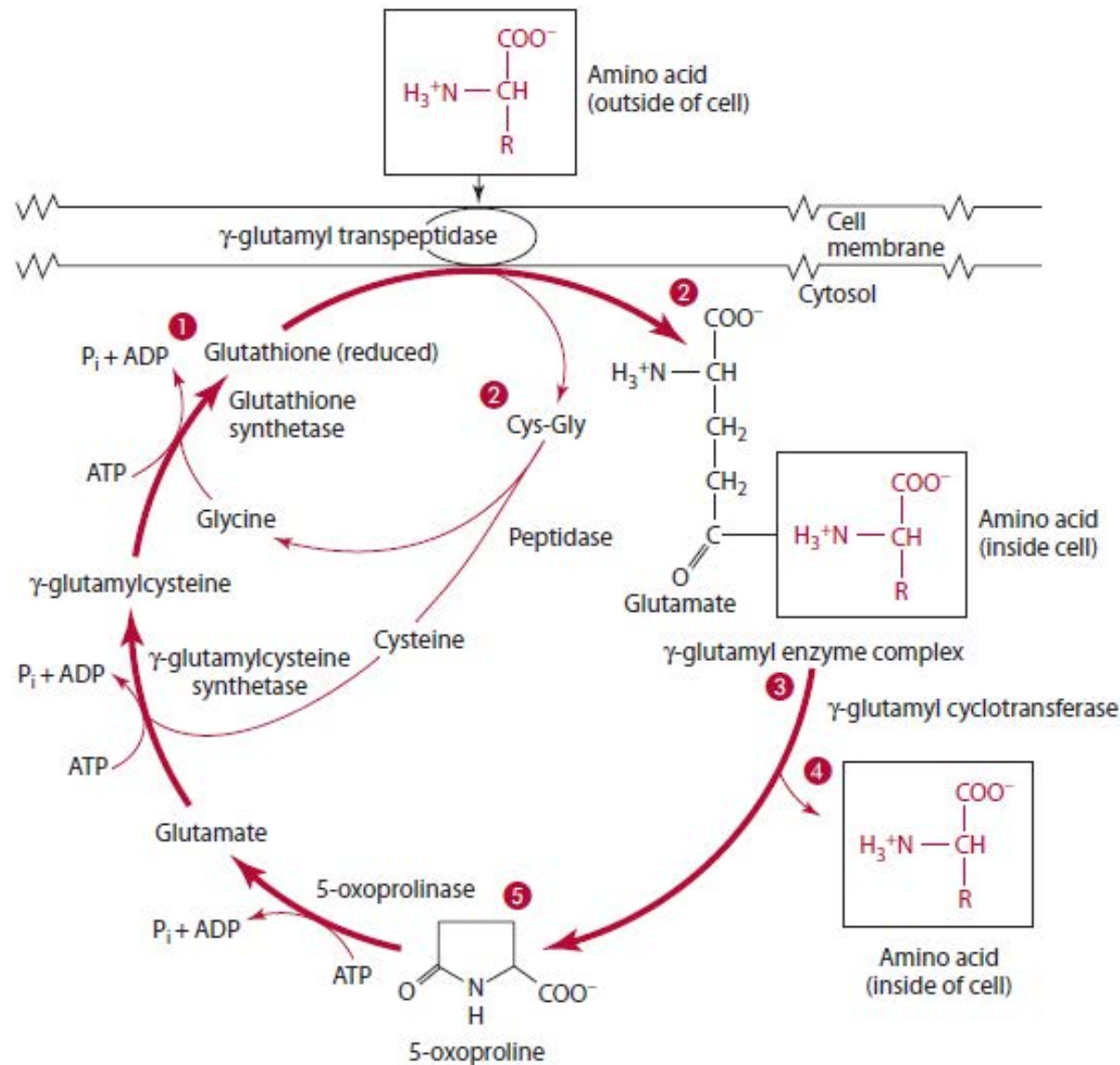
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AST and ALT

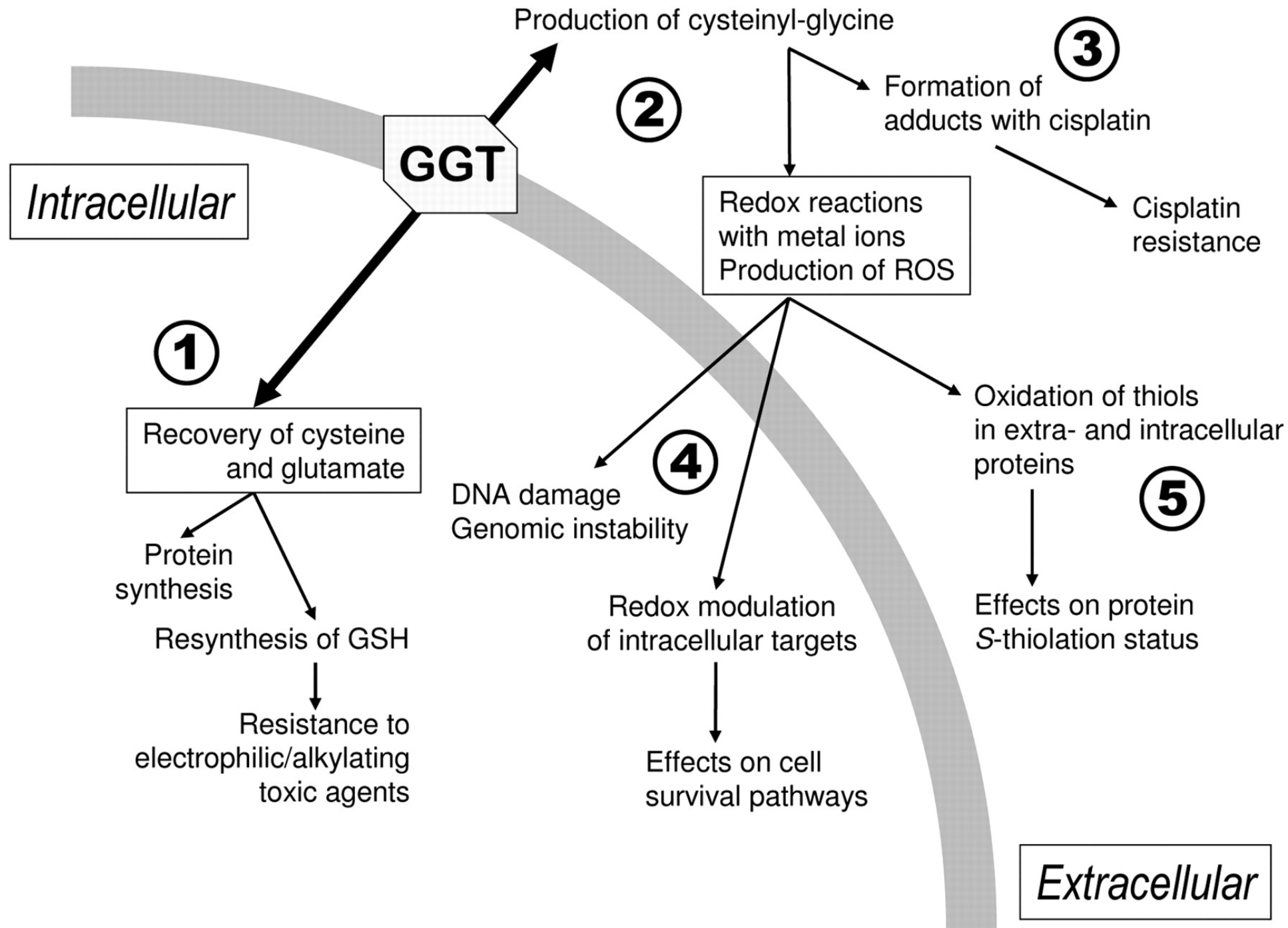
- * If of hepatic origin, reflect active hepatocellular damage, not damage that occurred weeks or months ago.
- * AST elevations without ALT elevations suggest damage outside of the liver.
- * Severe liver disease can cause a decrease in AST and ALT.
- * Elevated levels but less than 100 are likely not hepatitis, but can indicate fatty liver.
- * A vast number of medications can increase AST and ALT. Thus if elevated be sure to check a client's medication history.

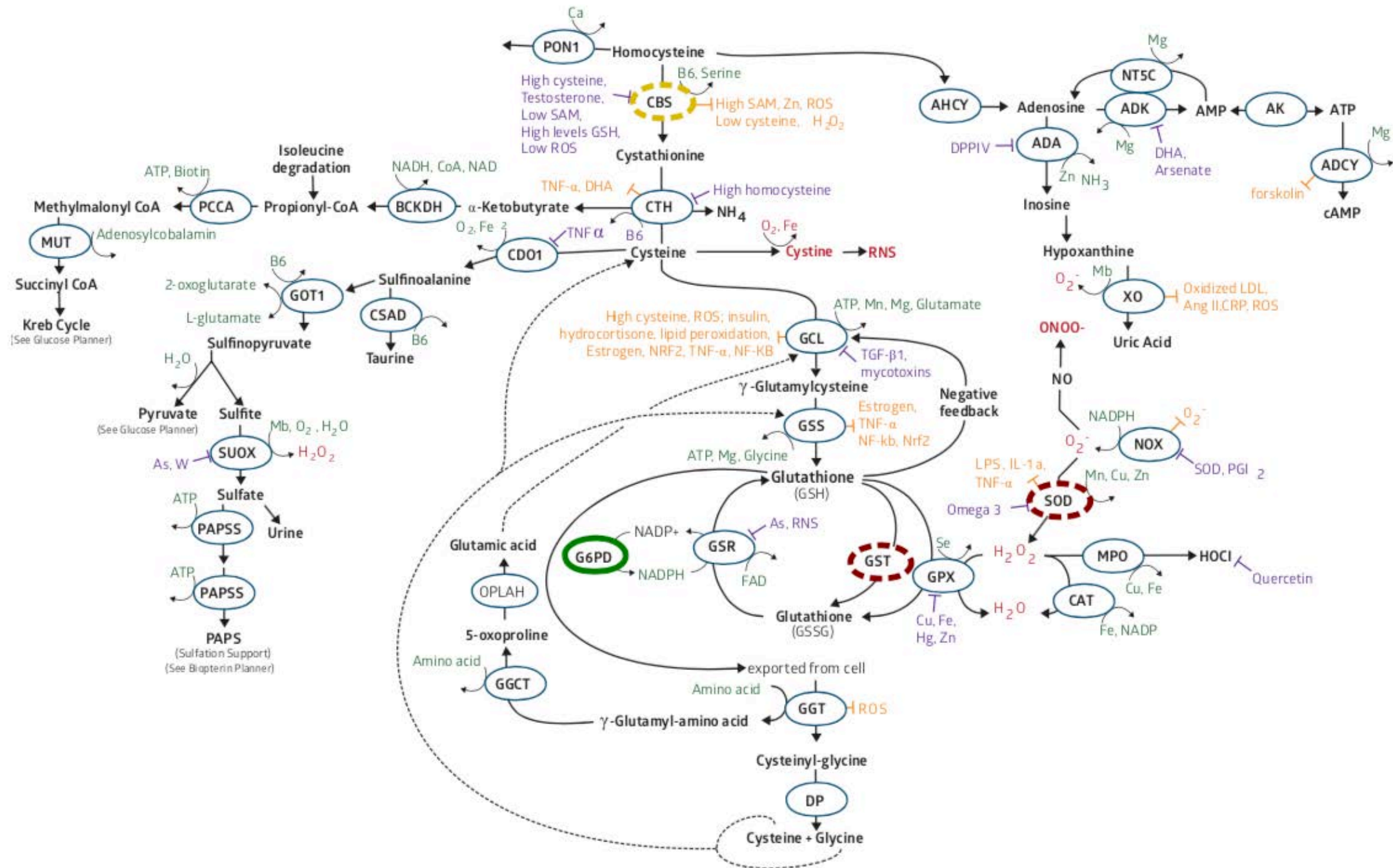
Gamma-Glutamyltransferase (GGT)

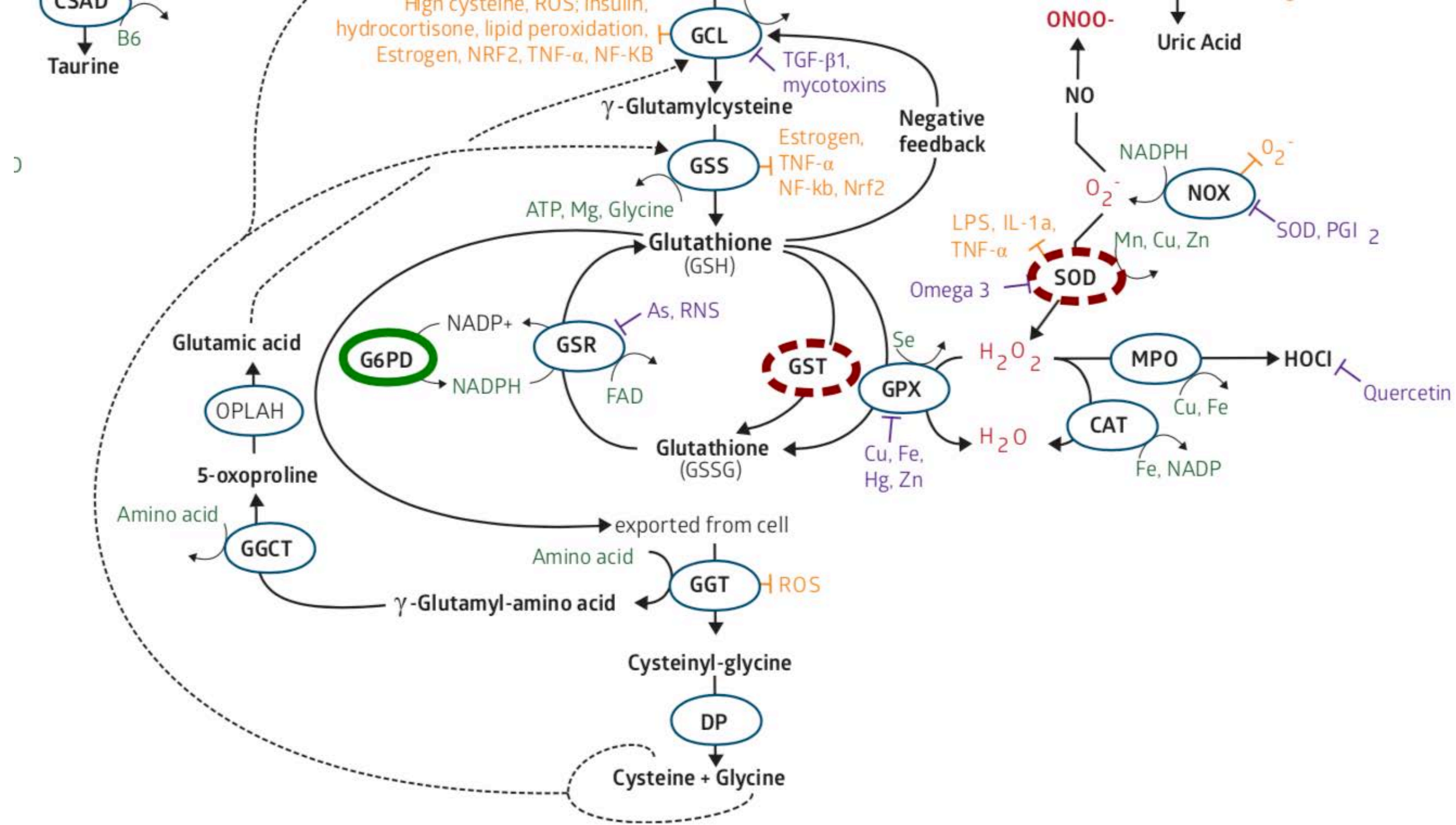
- * Gamma-glutamyltransferase (GGT) is present primarily in the liver, kidney and pancreas.
- * GGT is highly sensitive for biliary tree issues, and is often elevated in excess alcohol consumption
- * GGT is present in cell membranes and is involved in the transfer of amino acids
- * Historically used as an enzyme for liver and biliary dysfunction, and alcohol consumption, recently GGT has been identified as a marker of oxidative stress



- 1 Glutathione reacts with γ -glutamyl transpeptidase to form a γ -glutamyl enzyme complex.
- 2 The glutamate portion of glutathione remains attached to the enzyme complex while cysteinylglycine is released and an amino acid binds to the glutamate enzyme complex.
- 3 γ -glutamyl cyclotransferase cleaves the peptide bond between the amino acid and the γ -carbon of the glutamate enzyme complex.
- 4 The free amino acid can be used within the cell.
- 5 5-oxoproline generated from step 3 is used to reform glutamate and via several steps glutathione (step 1).







Gamma-Glutamyltransferase (GGT)

Traditional Reference Range

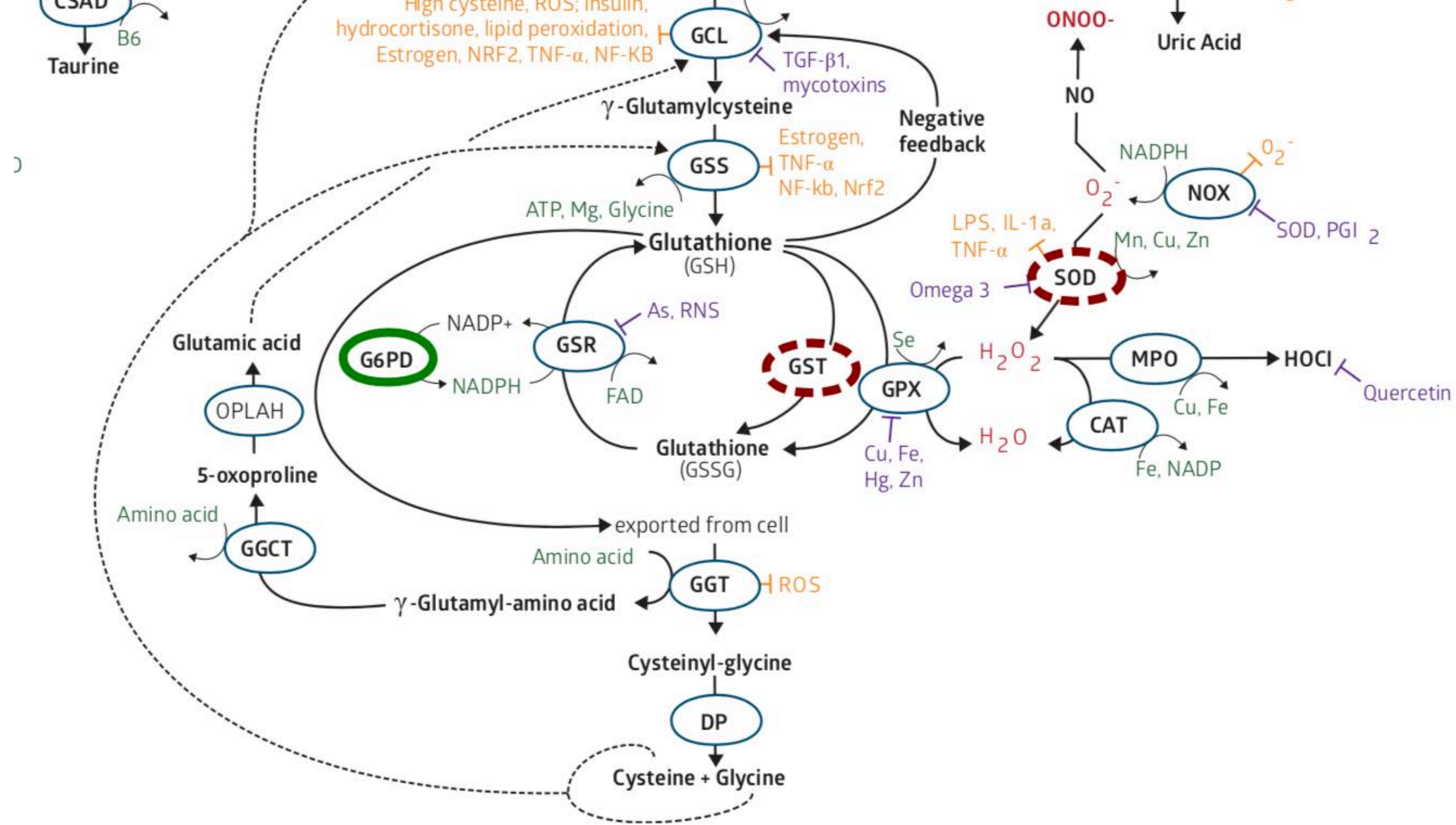
- * 0-65 IU/L

Optimal Reference Range

- * 12-24 IU/L (Men)
- * 10-22 IU/L (Women)

GGT - Elevated

Cause	Reason	Additional Inquiry
Biliary tree dysfunction (cholestasis)	GGT is found in high amounts in biliary epithelium. Thus in biliary tree dysfunction can increase GGT levels.	Gall bladder symptoms, alkaline phosphatase, bile acids.
Alcoholism	Alcohol seems to increase the activity of GGT, though the exact mechanism is unclear.	Client history.
Pancreatitis	Pancreatic inflammation can release enzymes normally found in that tissue. GGT is found in the pancreas and thus, can be elevated during pancreatic inflammation.	Consider running amylase and lipase.
Oxidative Stress	GGT, even when high-normal, may be an early and sensitive enzyme for oxidative stress.	Evaluate bilirubin (low) and uric acid (elevated).



GGT - Decreased

Cause	Reason	Additional Inquiry
Hypothyroidism	Observation	
Magnesium deficiency	Association. Inverse correlation between magnesium level and GGT levels.	Evaluate magnesium levels as well as signs and symptoms.

Jaundice



Jaundice



Lactate Dehydrogenase (LDH)

- * Lactate dehydrogenase (LDH) is found in all cells, including anucleated red blood cells.
- * Therefore when levels are elevated, it is difficult to ascertain what tissue is breaking down and is a non-specific marker for cellular damage.
 - * If elevated beyond the laboratory range, LDH isoenzymes can be run, which can better help identify the tissue.

Lactate Dehydrogenase (LDH)

Traditional Reference Range

- * 100-250 IU/L

Optimal Reference Range

- * 140-180 IU/L

LDH - Elevated

- * Liver diseases (cirrhosis, certain types of hepatitis)
- * Hypothyroidism - (AI mechanism of cellular damage)
- * Skeletal muscular damage, including intense exercise
- * Certain types of anemia (hemolytic, pernicious)
- * Fractures

LDH - Decreased

- * Poor carbohydrate entry into cells
 - * **Reactive hypoglycemia** (evaluate symptoms and glucose levels)
 - * Insulin resistance
 - * Ketosis

Alkaline Phosphatase

- * An enzyme primarily found in the liver (biliary tree), bone, kidneys, small intestines and placenta
- * As a phosphatase, it's responsible for removing phosphate from molecules and works well in an alkaline environment
- * Elevated in bone and liver disease
- * Intestinal alkaline phosphatase (IAP), has the ability to detoxify lipopolysaccharide and prevent bacterial invasion across the gut mucosal barrier

Alkaline Phosphatase

Traditional Reference Range

- * 25-150 IU/L

Optimal Reference Range

- * 65-90 IU/L

Alkaline Phosphatase - Increased

Cause	Reason	Additional Inquiry
Liver obstruction	Can cause damage to biliary cells releasing alkaline phosphatase into the blood.	Evaluate with bilirubin levels.
Liver or biliary cirrhosis	Same as above.	Evaluate with bilirubin levels.
Gastrointestinal issues (ulcerative colitis, peptic ulcer)	Damage to intestinal mucosa can release alkaline phosphatase into the blood stream.	Client history.
Hyperphosphatemia or high parathyroid hormone.	Association.	Evaluate phosphate and calcium level.

Phosphorus Content in Commonly Consumed Beverages

Erica Wickham, MS, RD, CDN

Patients with chronic kidney disease are likely to experience certain symptoms when their phosphorus gets too high. Individuals with diabetes may suffer from severe itching all over their body.¹ In the dialysis population, elevated serum phosphorus levels are a constant struggle for the clinical team and the patient alike. As a dietitian, there is a constant search for a solution to help renal patients control their serum phosphorus and in many cases to remember to take their phosphate binders.

phosphorus and are at a higher risk of developing hyperphosphatemia.¹ This may ultimately result in elevated parathyroid hormone secretion, progressive deterioration of kidney function, renal bone disease, vascular calcification, increased risk of cardiovascular accidents, and higher mortality rates.^{2,4} Because elevated phosphorus affects bone and cardiovascular risk, the Kidney Disease Improving Global Outcomes guidelines refer to this condition as mineral bone disorder of chronic kidney disease.⁵

Patients with chronic kidney disease are likely to experience certain symptoms when their phosphorus gets too high. Individuals with diabetes may suffer from severe itching all over their body.¹ In the dialysis population, elevated

much higher (>90%) than that of natural sources, such as animal and plant proteins (40–60%).⁴ Several preservative and additive salts containing phosphorus are found in processed, convenience foods. These additives are useful in prolonging shelf life, enhancing flavor, retaining moisture, and improving the color of the food item.^{4,6} The form of phosphorus in additives is not bound to protein, but rather it is a salt that is readily absorbed in the intestinal tract. Table 1 illustrates the nutritional content, including the calorie, potassium, and phosphorus content found in commonly consumed beverages. It is of interest that most clear sodas are lower in phosphorus, but many still contain natural and added potassium (Gordon D. per-

Table 1. Phosphorus and Potassium Content of Commonly Consumed Beverages

Product	Calories	Potassium (mg)	Phosphorus (mg)
Aquafina Flavor Splash Grape	<1	<1	85
Lemon	<1	57	67
Peach Mango	<1	57	82
Raspberry	<1	-	62
Strawberry Kiwi	<1	57	82
Wild Berry	<1	<1	62
A&W Root Beer	174	<1	<1
Diet Root Beer	<1	<1	<1
Cream Soda	174	<1	<1
Diet Cream Soda	<1	<1	<1
Barq's Root Beer	166	<1	<1
Barq's Diet Root Beer	<1	12	<1
Barq's Red Cream	172	<1	<1
Barq's Diet Red Cream	6	<1	<1
French Vanilla Cream	168	<1	<1
Diet French Vanilla Cream	2	<1	<1
Canada Dry Ginger Ale	140	<1	<1
Diet Ginger Ale	<1	<1	<1
Cranberry Lime Seltzer	<1	<1	<1
Mandarin Orange Seltzer	<1	<1	<1
Raspberry Seltzer	<1	<1	<1
Lemon Lime Seltzer	<1	<1	<1
Tonic Water	135	<1	<1
Club Soda	<1	<1	<1
Coca-Cola	145	<1	58
Coca-Cola with Lime	146	51	56
Coke Zero	2	47	54
Diet Coke	2	18	27
Diet Coke with Lime	3	29	27
Cherry Coke	156	<1	56
Diet Cherry Coke	2	29	35
Cherry Coke Zero	2	50	56
Vanilla Coke	200	0	56
Vanilla Coke Zero	2	47	56
Crush			
Orange	162	<1	<1
Diet Orange	<1	<1	<1
Grape	162	5	5
Cherry	162	5	5
Dr. Pepper	150	20	54
Dr. Pepper 10	10	<1	28
Diet Dr. Pepper	<1	<1	50
Dr. Pepper Cherry	150	20	54
Cherry Vanilla	144	28	60
Fanta Apple	182	<1	<1
Berry	176	26	2
Citrus	136	2	<1
Grape	183	<1	<1
Lemon	168	<1	<1
Orange	167	53	<1
Fresca Original Citrus	<1	<1	<1
Black Cherry	<1	<1	<1
Peach Citrus	<1	<1	<1
Fruit Works Apple Raspberry	150	35	100
Fruit Punch	165	60	123
Guava Berry	165	56	117
Passion Orange	165	60	123
Peach Papaya	165	60	123
Pink Lemonade	165	80	63
Strawberry Melon	165	110	120
Tangerine Citrus	165	35	90

Table 1. Phosphorus and Potassium Content of Commonly Consumed Beverages (Continued)

Product	Calories	Potassium (mg)	Phosphorus (mg)
Tropical Berry	165	60	140
Hawaiian Punch	170	50	175
Fruit Juicy	170	45	173
Green Berry Rush	170	43	173
Lipton Iced Green Tea with Citrus	70	10	65
Iced Tea Sweetened	70	15	19
Iced Tea Unsweetened	<1	10	18
Mello Yellow	170	10	<1
Diet	<1	<1	<1
Mountain Dew	110	<1	<1
Diet Mountain Dew	<1	63	<1
Mountain Dew Coe Red	170	10	53
Mountain Dew Live Wire	170	5	<1
Mug Root Beer	170	15	<1
Diet Mug Root Beer	<1	10	<1
Mug Cream Soda	180	<1	<1
Diet Mug Cream Soda	<1	<1	<1
Nestea Lemon Sweet	129	<1	<1
Honey Lemon Green	120	35	<1
Raspberry	135	<1	<1
Sweetened	125	<1	32
Unsweetened	<1	<1	32
Orange Slice	180	<1	35
Cherry Slice	165	<1	35
Dr. Slice	180	<1	35
Pepsi	150	10	53
Diet Pepsi	<1	25	33
Pepsi Max	<1	20	42
Pepsi Next	35	15	33
Wild Cherry	160	10	53
Diet Pepsi Lime	<1	40	89
Diet Pepsi Vanilla	<1	45	89
Pibb Xtra	145	21	44
Pibb Zero	2	33	44
Schweppes Ginger Ale	180	<1	<1
Diet Ginger Ale	<1	<1	<1
Tonic Water	135	<1	<1
Club Soda	<1	<1	<1
Sierra Mist	90	35	<1
Diet Sierra Mist	<1	71	<1
Sierra Mist Cranberry Splash	160	50	<1
Diet Cranberry Splash	<1	37	<1
Sierra Mist Strawberry Kiwi	140	50	<1
Diet Strawberry Kiwi	5	137	<1
Sprite	144	<1	<1
Sprite Zero	3.6	110	<1
Tropicana Fruit Punch	130	90	93
Lemonade	120	40	38
Pink Lemonade	170	39	37
Peach Papaya	120	41	93
Strawberry Melon	150	76	93
Tropicana Twister Orange	195	53	<1
Diet Orange	0	53	<1
Strawberry	165	30	<1
Grape	180	53	<1

Alkaline Phosphatase - Decreased

Cause	Reason	Additional Inquiry
Zinc deficiency	Alk Phos is a zinc dependent enzyme. If zinc is low, alkaline phosphatase may be decreased.	Diet journal. Zinc deficiency symptoms.
Vitamin C deficiency.	Alk phos is low in individuals with scurvy.	Diet journal. Evaluate vitamin C deficiency symptoms.
Magnesium deficiency	Alk Phos also requires magnesium for proper function.	Evaluate GGT and serum magnesium.

Isoenzymes

LDH Isoenzymes

- * Isoenzyme #1: heart, red blood cells
- * Isoenzyme #2: heart, lymph, red blood cells
- * Isoenzyme #3: pulmonary, spleen, adrenal, kidney
- * Isoenzyme #4: hepatic, skeletal muscle, prostate/uterus
- * Isoenzyme #5: hepatic, skeletal muscle

Alkaline Phosphatase Isoenzymes

- * Liver Isoenzyme a2
- * Liver Isoenzyme a1
- * Bone Isoenzyme
- * Intestinal Isoenzyme
- * Placental Isoenzyme

Break Time.

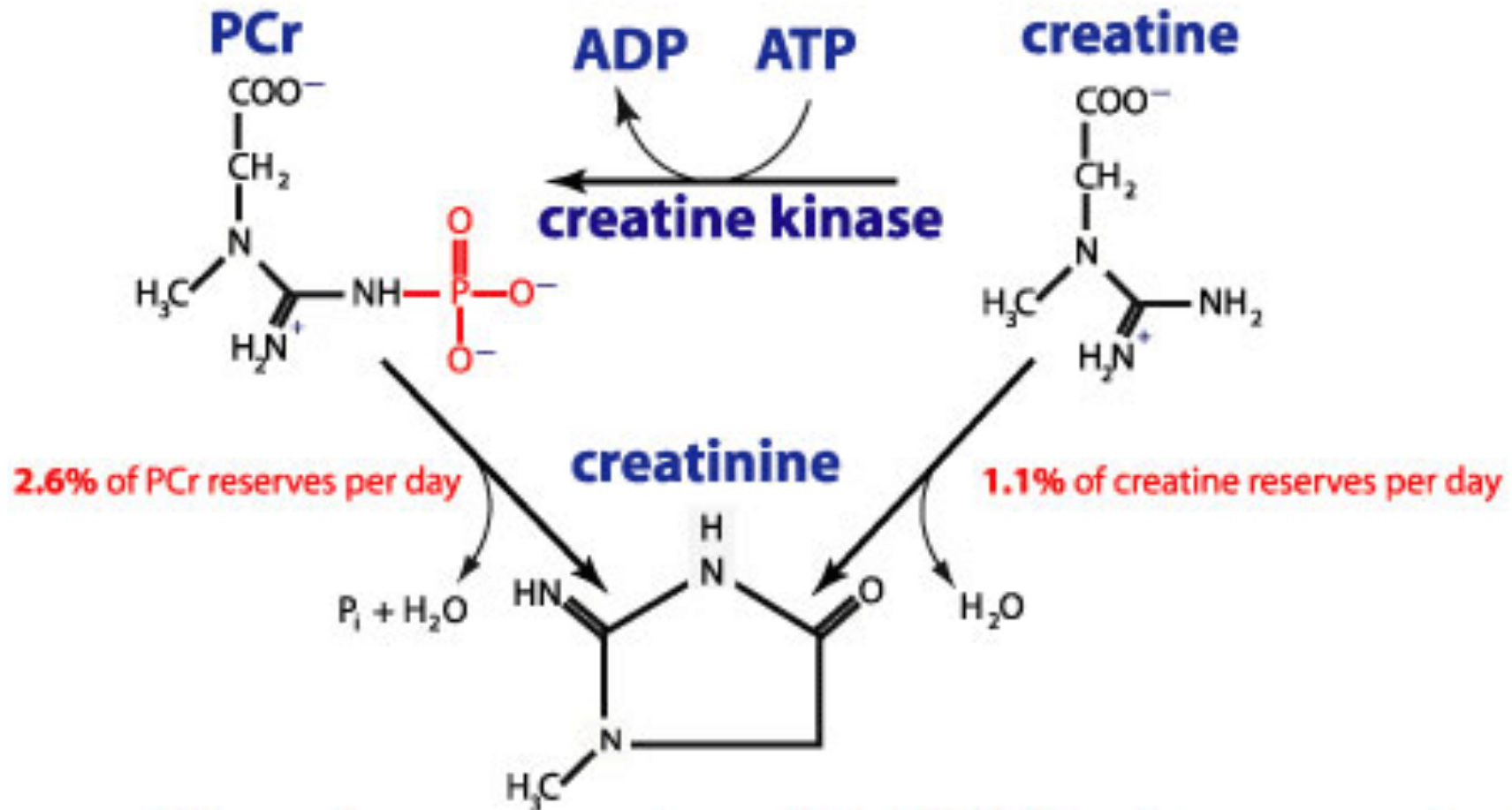
Wastes

- 
- * Creatinine
 - * Uric acid
 - * Bilirubin
 - * Blood urea nitrogen (BUN)

Creatinine

- * Byproduct of creatine breakdown
- * Majority of creatine is found in skeletal muscle, therefore serum creatinine is seen as a byproduct of skeletal muscle metabolism
- * Creatinine is cleared in the kidneys and thus, is viewed as one of the “kidney markers”
- * Creatinine production is considered to be constant as long as muscle mass is constant

Creatinine



Creatinine

Traditional Reference Range

- * 0.5-1.5 mg/dL

Optimal Reference Range

- * Men: 0.8-1.1 mg/dL
- * Women: .7-1.0 mg/dL

Creatinine - Increased

Cause	Reason	Additional Inquiry
Kidney dysfunction	Poor kidney function leads to decreased filtration and excretion of creatinine, thus elevating serum creatinine levels.	Evaluate other kidney markers, eg BUN.
Dehydration	Hemoconcentration.	Evaluate other dehydration markers.
Increased muscle mass	More muscle means more creatine and thus, more creatinine.	
Hyperthyroidism	Increased muscle breakdown	Evaluate thyroid markers.
High meat intake	Meat contains creatine and thus can lead to increased creatinine levels.	Diet history.
Ketones	Increases creatinine levels considerably by competing for filtration in the liver.	Client history.

Creatinine - Decreased

Cause	Reason	Additional Inquiry
Decreased muscle mass	Less muscle means less creatine.	
Poor dietary protein intake or absorption	Diet contains creatine. Poor protein digestion or absorption can lead to low creatinine levels.	Diet history. GI symptoms.

Creatinine

Interfering Factors	
Elevated	Decreased
High ascorbic acid intake	Increased bilirubin and glucose levels.

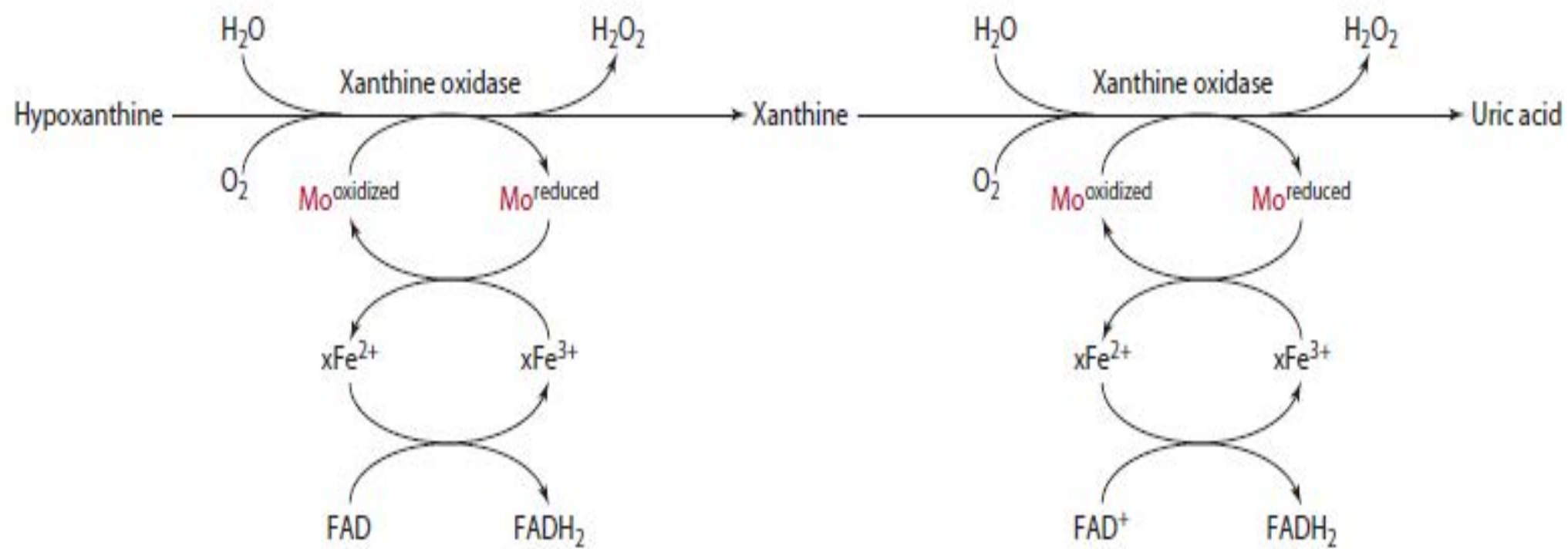
Uric Acid

- * Uric acid is the major end product of DNA purine base metabolism, specifically purine nucleosides adenosine and guanosine.

- * Adenosine → inosine → hypoxanthine → xanthine → uric acid*

- * Guanosine → guanine → xanthine → uric acid*

*Xanthine oxidase is the final enzyme that converts xanthine to uric acid.



Uric Acid

- * Uric acid synthesis primarily occurs in liver and intestinal mucosa due to high *xanthine oxidase* enzyme activity in those two tissues.
- * Daily synthesis of uric acid is approximately 700mg with dietary sources of nucleoprotein contributing approximately 300mg to the daily total uric acid production, though this will vary considerably depending on dietary intake.

Uric Acid

- * Uric acid is excreted via the kidneys.
- * Approximately 400-800mg of uric acid is excreted daily.
- * There may be day-to-day and seasonal variations to uric acid with levels being slightly higher in the summer than in winter months.
- * The more alkaline the urine, the more uric acid is excreted. Conversely the more acidic the urine the more uric acid is reabsorbed and less is excreted.
- * Increases in exercise, stress, weight, hypertension, diabetes and type A personalities are associated with higher levels of uric acid.

Uric Acid

- * Newer research suggests that uric acid can be a marker of oxidative stress and **act as an antioxidant or reducing agent**.
- * And similar to other reducing agents (eg vitamin C), uric acid can also act as a pro-oxidant.
- * Elevated uric acid levels are associated with cardiovascular disease, hypertension and diabetes, though there is some debate as to whether high uric acid are causative or correlative with these.

Uric Acid

Traditional Reference range:

- * 3.4-7.0 mg/dL (men)
- * 2.4-6.0 mg/dL (women)

A. Mazza • S. Zamboni • E. Rizzato • A.C. Pessina • V. Tikhonoff • L. Schiavon • E. Casiglia

Serum uric acid shows a J-shaped trend with coronary mortality in

Tertiles

<0.29, 0.30–0.36, .>37 mmol/L

4.88, 5.04–6.05, 6.22 mg/dL

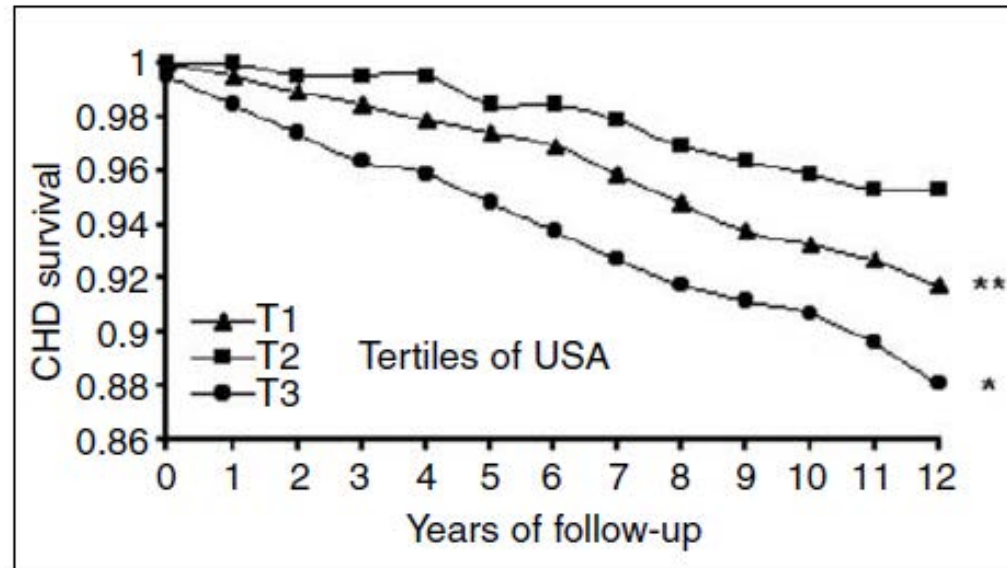
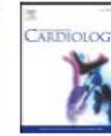


Fig. 1 Coronary (CHD) cumulative survival in the three tertiles (T1, T2, T3) of SUA. * $p < 0.001$ vs. 2nd tertile, ** $p < 0.05$ vs. 2nd tertile



Uric acid levels, even in the normal range, are associated with increased

Men: 48–320, 321–370, 371–419 (.81–5.38, 5.4–6.22, 6.24–7.04)

Women: 43–269, 270–310, 311–359 (.72–4.52, 4.54–5.21, 5.23–6.04)

In both men and women with normouricemia (UA < 420 μ mol/l in men and < 360 μ mol/l in women), tertiles of UA levels were adversely associated with body mass index, waist circumference, waist-to-hip ratio, total- and HDL-cholesterol, apolipoprotein A1, systolic and diastolic blood pressures, pulse pressure, fasting plasma glucose and white blood cell count.

with various diseases [3,4]. Patients with hyperuricemia have a 1.5- to 3-fold increased risk of cardiovascular disease than those without hyperuricemia [5–7]. However, there is an increasing evidence that higher uric acid (UA), even in those without the diagnosis of hyperuricemia but with a UA level considered to be in the high-normal (310–330 μ mol/l) range, was positively associated with higher cardiovascular risk or mortality [6,8,9].

The effect of UA on cardiovascular disease (CVD) or all-cause mortality may vary by diabetes status, but results from earlier studies were heterogeneous [10–12]. A recent longitudinal study of 1268 patients with type 2 diabetes reported no association between baseline UA level and all-cause or CVD mortality after a follow-up of more than 10 years [11]. The authors suggested that serum UA level may not be

an independently predictive factor for mortality in patients with and without reported diabetes, but not in those without diabetes or for CVD events [10]. A more recent study from the Casale Monferrato Study also showed that baseline serum UA level predicted all-cause, but not CVD mortality in 1540 older subjects with diabetes after a follow-up of 15 years [12].

There are limited data describing the cardiovascular risk from serum UA that may begin below the current diagnostic level of hyperuricemia in people without diabetes [6,8,9]. In the present study using data from Phase 1 of the Guangzhou Biobank Cohort Study (GBCS), we examined the association between increasing level of serum UA and a wide range of cardiovascular risk factors in an older Chinese sample in Guangzhou, China.

2. Methods

GBCS is a three-way collaboration among the Guangzhou 12th Hospital, China, the University of Hong Kong, and the University of Birmingham, UK. The study aims to examine environmental and occupational factors, genetic and lifestyle determinants

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Men
4.72
4.72-5.36
5.36-6.09
6.09

Women
3.5
3.5-4.07
4.07-5.5
5.5

Table 1 Baseline characteristics [mean (SD)] in AMORIS population free of AMI, stroke or CHF by gender and quartiles (Q) of uric acid

	Q1	Q2	Q3	Q4	All
Female					
No. of patient	49 882	49 551	48 613	48 500	196 556
No. of strokes (%)	927 (1.9)	1177 (2.4)	1646 (3.4)	3202 (6.6)	6952 (3.5)
No. of IS-strokes (%)	588 (1.2)	801 (1.6)	1167 (2.4)	2362 (4.9)	4918 (2.5)
No. of HS-strokes (%)	156 (0.3)	148 (0.3)	227 (0.5)	388 (0.8)	909 (0.5)
No of unspecified strokes (%)	183 (0.4)	228 (0.5)	252 (0.5)	452 (0.9)	1125 (0.6)
No. of AMI (%)	568 (1.1)	752 (1.5)	1127 (2.3)	2441 (5.0)	4888 (2.5)
No. of CHF (%)	477 (1.0)	528 (1.1)	914 (1.9)	2239 (4.6)	4158 (2.1)
Age (years)	45.1 (10.6)	46.8 (11.3)	49.4 (12.1)	54.8 (13.1)	49.0 (12.4)
Uric acid ($\mu\text{mol L}^{-1}$)	181.7 (22.0)	225.7 (9.7)	261.0 (11.4)	330.9 (47.6)	249.2 (61.0)
Total cholesterol (mmol L^{-1})	5.4 (1.0)	5.6 (1.1)	5.8 (1.1)	6.1 (1.2)	5.7 (1.2)
Triglycerides (mmol L^{-1})	0.9 (0.5)	1.0 (0.5)	1.1 (0.6)	1.5 (0.8)	1.1 (0.6)
Hypertension (%)	130 (0.3)	141 (0.3)	251 (0.5)	840 (1.7)	1362 (0.7)
DM (%)	992 (2.0)	777 (1.6)	924 (1.9)	2273 (4.7)	4966 (2.5)
Male					
No. of patient	55 976	55 383	55 105	54 714	221 178
No. of strokes (%)	1999 (3.6)	2009 (3.6)	2261 (4.1)	3055 (5.6)	9324 (4.2)
No. of IS-strokes (%)	1408 (2.5)	1483 (2.7)	1634 (3.0)	2282 (4.2)	6807 (3.1)
No. of HS-strokes (%)	385 (0.7)	308 (0.6)	356 (0.6)	473 (0.9)	1522 (0.7)
No of unspecified strokes (%)	206 (0.4)	218 (0.4)	271 (0.5)	300 (0.5)	995 (0.4)
No. of AMI (%)	2555 (4.7)	2678 (4.8)	3077 (5.6)	3976 (7.3)	12286 (5.6)
No. of CHF (%)	925 (1.7)	970 (1.8)	1267 (2.3)	2013 (3.7)	5175 (2.3)
Age (years)	46.8 (10.8)	46.7 (10.8)	47.3 (11.0)	48.9 (11.6)	47.4 (11.1)
Uric acid ($\mu\text{mol L}^{-1}$)	249.5 (27.2)	300.8 (10.8)	339.7 (12.3)	409.5 (43.7)	324.4 (64.3)
Total cholesterol (mmol L^{-1})	5.5 (1.0)	5.7 (1.1)	5.8 (1.1)	6.0 (1.1)	5.7 (1.1)
Triglycerides (mmol L^{-1})	1.2 (0.7)	1.3 (0.7)	1.5 (0.8)	1.8 (0.9)	1.4 (0.8)
Hypertension (%)	213 (0.4)	212 (0.4)	307 (0.6)	710 (1.3)	1442 (0.7)
DM (%)	2883 (5.2)	1621 (2.9)	1585 (2.9)	2281 (4.2)	8370 (3.8)

SD, standard deviation; AMI, acute myocardial infarction; CHF, congestive heart failure; DM, diabetes mellitus.

Uric Acid

Traditional Reference range:

- * 3.4-7.0 mg/dL (men)
- * 2.4-6.0 mg/dL (women)

Optimal Reference Range:

- * 3.7-5.5 mg/dL (men)
- * 3.2-4.4 mg/dL (women)

Uric Acid - Elevated

Elevated - If uric acid is elevated above either the optimal or laboratory reference range, it is either increased production, decreased excretion, or a combination of both.

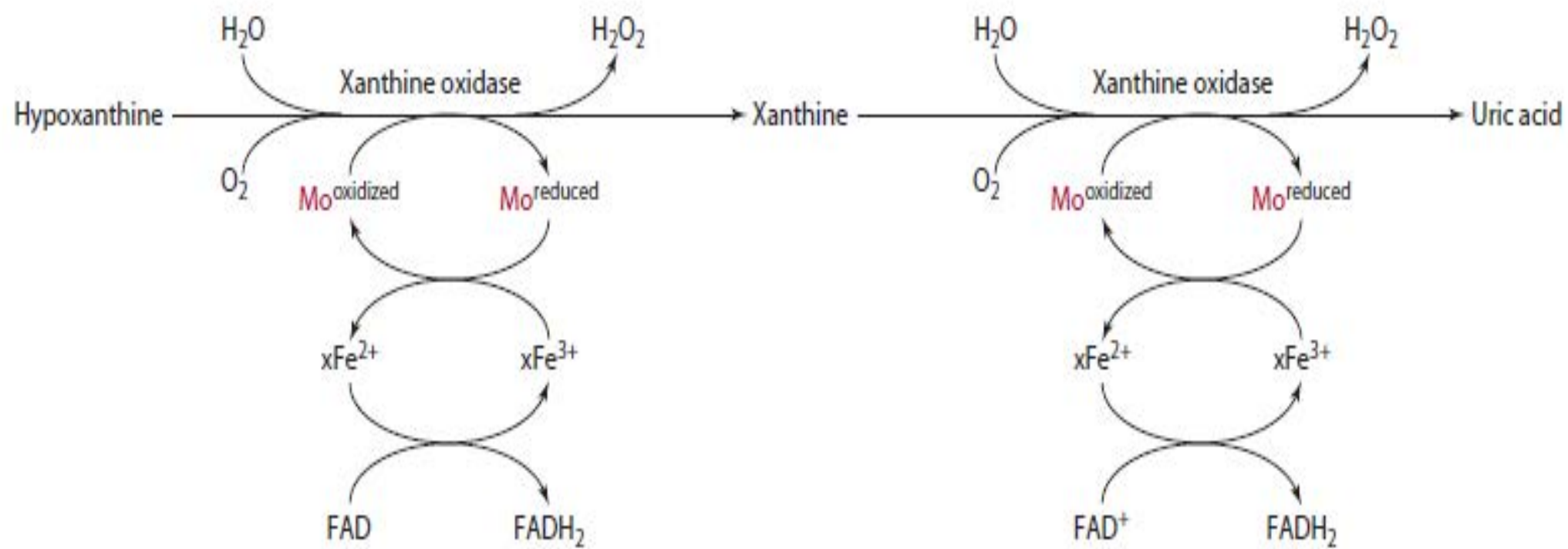
Cause	Reason	Additional Inquiry
Gout	Excessive breakdown of purines	Ask about history of gout and/or systemic joint pain
Kidney dysfunction	Poor filtration and excretion of uric acid, thus keeping serum levels elevated	Evaluate BUN, creatinine, phosphorus; urinalysis
Excess alcohol intake	Hepatocellular destruction	Ask about alcohol intake
Starvation and/or extreme calorie restriction	Catabolism of proteins and thus purine	Diet history
Hypothyroidism	Association	Evaluate TSH
Hyperlipidemia	Association	Evaluate cholesterol

Uric Acid - Elevated

Liver dysfunction	Excess destruction of hepatic cells	Evaluate AST, ALT, GGT, Alk Phos, LDH
Hemolytic anemia	Excess destruction of cells	Evaluate CBC markers
Excess consumption of fructose	Excess fructose increases conversion of ATP to inosine; increases synthesis of purines via the pentose phosphate pathway; fructose may also decrease uric acid excretion	Diet journal - this is huge in diabetes cases!
Chronically elevated serum glucose	Association	Evaluate glucose, hemoglobin A1C
Fungal infection	Some researchers consider uric acid to be a mycotoxin produced by yeast and fungus	
Ketogenic diet	May impair ability of kidneys to excrete uric acid due to competition with ketones. Alternatively, acidic urine increases uric acid reabsorption.	Inquire about diet; ketones in urine; CD cases
High supplemental niacin intake		Supplement history
High protein diet	More protein typically means more purine intake and thus uric acid production	Diet journal
Excess acidity	Excess acidity can lead to acidity of the urine, which tends to reabsorb uric acid leading to higher serum levels.	Acidity is often correlated with blood sugar dysregulation; low CO2 levels can indicate hyperacidity

Uric Acid - Decreased

Cause	Reason	
Molybdenum deficiency	Xanthine oxidase is a molybdenum dependent enzyme. Low levels of molybdenum may lead to decreased uric acid production.	Ask about increased sensitivity to smells and/or consumed sulfites/nitrites (molybdenum also used in sulfite oxidase)
Zinc deficiency	May increase urinary uric acid excretion; low zinc can also lead to high copper, which can negatively impact iron (See below)	Evaluate alk phos; skin issues; taste acuity issues
Iron deficiency	May cause relative increase in copper, which may displace iron with uric acid production	Evaluate ferritin, TIBC, CBC markers
Low purine intake (eg vegetarian)		Diet journal
Oxidative stress	Uric acid is an abundant serum antioxidant. If oxidative stress is high, uric acid levels may be decreased.	Evaluate bilirubin and GGT. Low bilirubin and elevated GGT may further indicate oxidative stress.
Excess alkalinity	Generally the more alkaline the blood, the more alkaline the urine, which is associated with higher levels of excretion of uric acid and thus lower serum levels.	High CO2 levels can indicate hyperalkalinity



Uric Acid – Interfering factors

- * Prolonged fasting can raise uric acid, as can nicotinic acid (vitamin B3)

Uric Acid

- * *Clinical Note: As with a number of other markers, the possibility of decreased production but also decreased excretion could lead to a normal value on a blood chemistry, making thorough history taking of the utmost importance.*

Food Sources of Molybdenum

- * Animal products: pork, lamb, beef liver
- * Nuts/seeds – sunflower seeds
- * Vegetables: lentils, peas, lima beans
- * Grains: oats, buckwheat, sorghum

Bilirubin

- * Bilirubin is the byproduct of red blood cell breakdown.
- * Serum bilirubin is a combination of *direct* (conjugated) and *indirect* (unconjugated) bilirubin
 - * Normally, indirect bilirubin is approximately 70-85% of total bilirubin
 - * If 50% or more is direct, hepatic/biliary obstruction is suspected
 - * If less than 20% is direct, accelerated hemolysis (RBC breakdown) or liver dysfunction is suspected
- * Bilirubin is a major component of bile and gives it its green pigmentation

Bilirubin

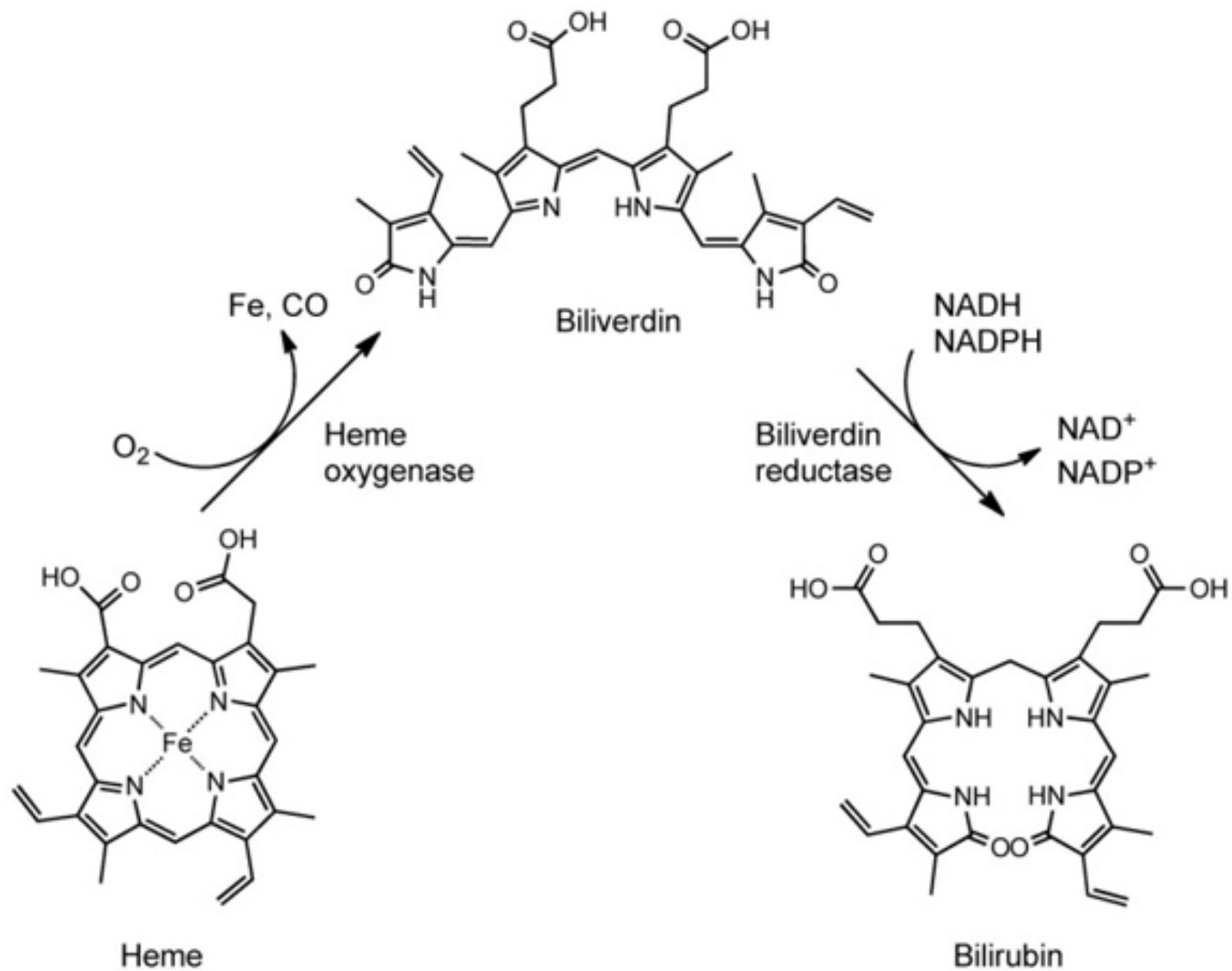
- * Bilirubin also functions as an antioxidant and thus may be decreased during oxidative stress
- * Because of this, low bilirubin has also been associated with increased risk of cardiovascular disease and all cause mortality (death from all causes). Elevated levels may increase mortality as well.
- * Bilirubin may also increase insulin sensitivity and protect against future diabetes

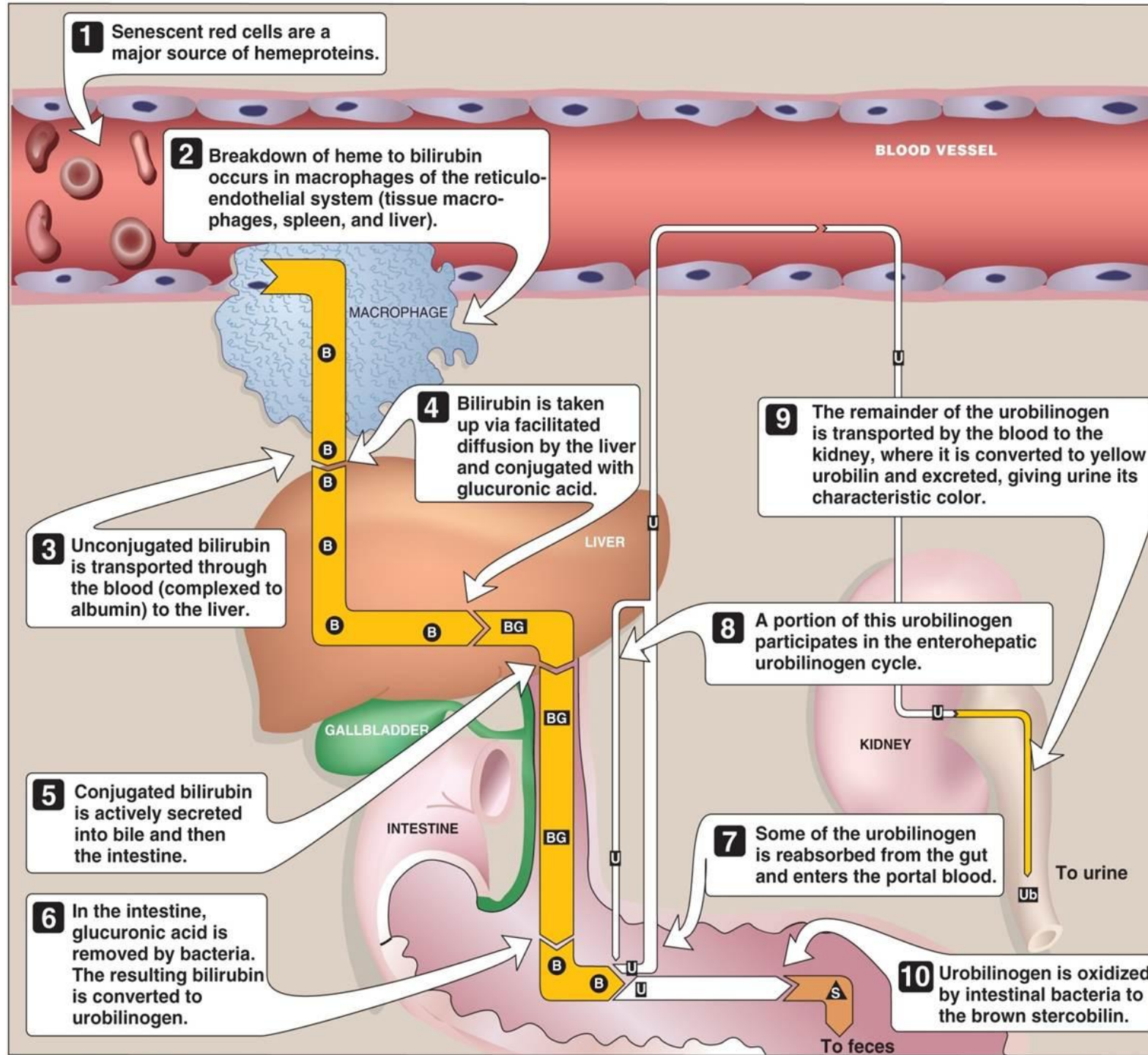
Traditional Reference Range

- * 0.1-1.2 mg/dL

Optimal Reference Range

- * 0.5 – 0.8 mg/dL





Bilirubin - Elevated

Cause	Reason	Additional Inquiry
Excess hemolysis	Excess red blood cell breakdown increases bilirubin (indirect/unconjugated).	
Liver dysfunction	The liver conjugates bilirubin. If the liver is not functioning properly, indirect/unconjugated bilirubin will be elevated.	Evaluate liver markers.
Bile duct obstruction	Bilirubin is cleared from the liver via the biliary ducts into the intestines. Thus if the biliary ducts are obstructed, conjugated/direct bilirubin will enter into circulation.	Evaluate alkaline phosphatase and GGT.
Gilbret's Syndrome	Genetic cause of elevated bilirubin.	Ask client if they have a history of elevated bilirubin. If so, likely Gilbret's

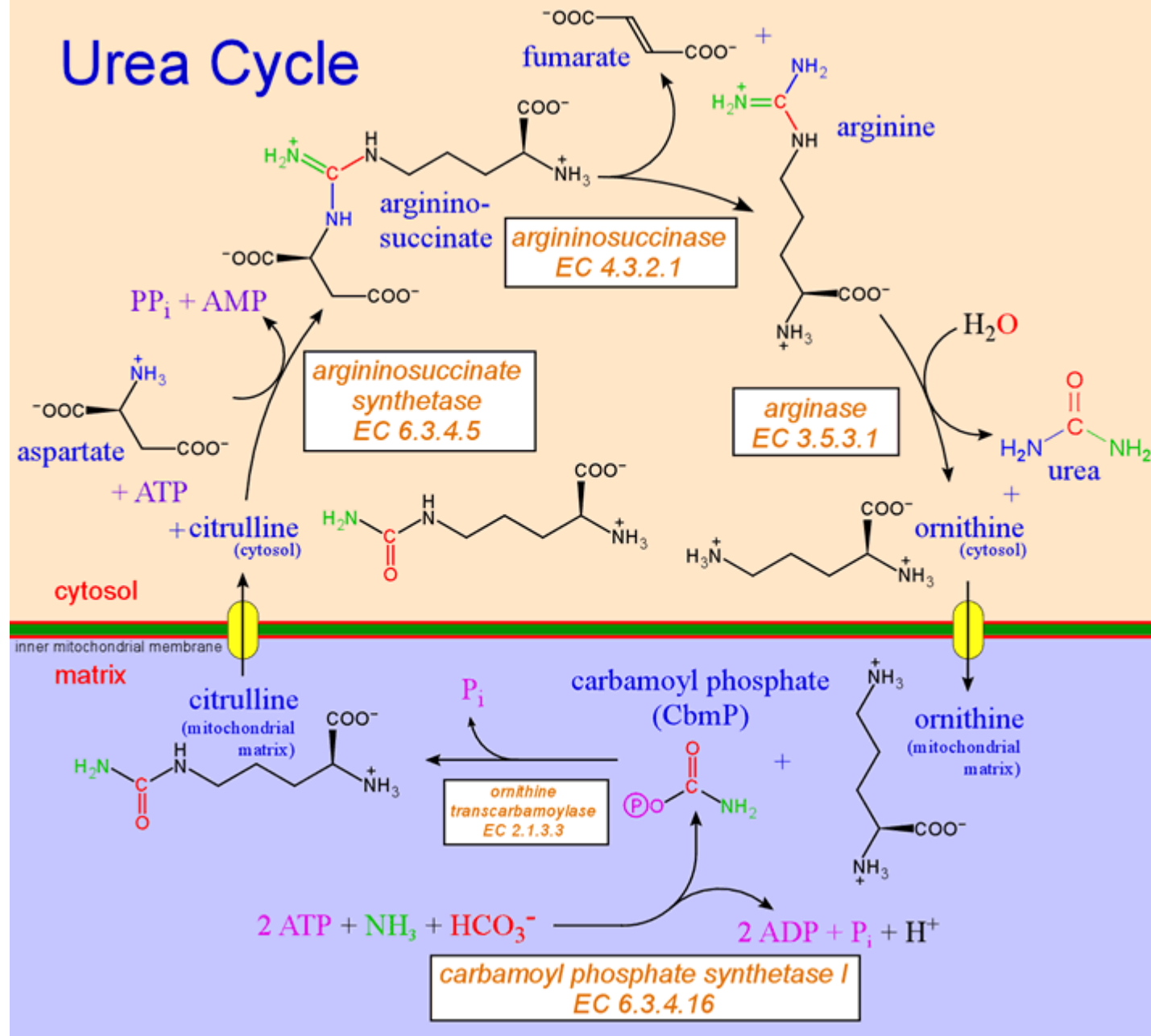
Bilirubin - Decreased

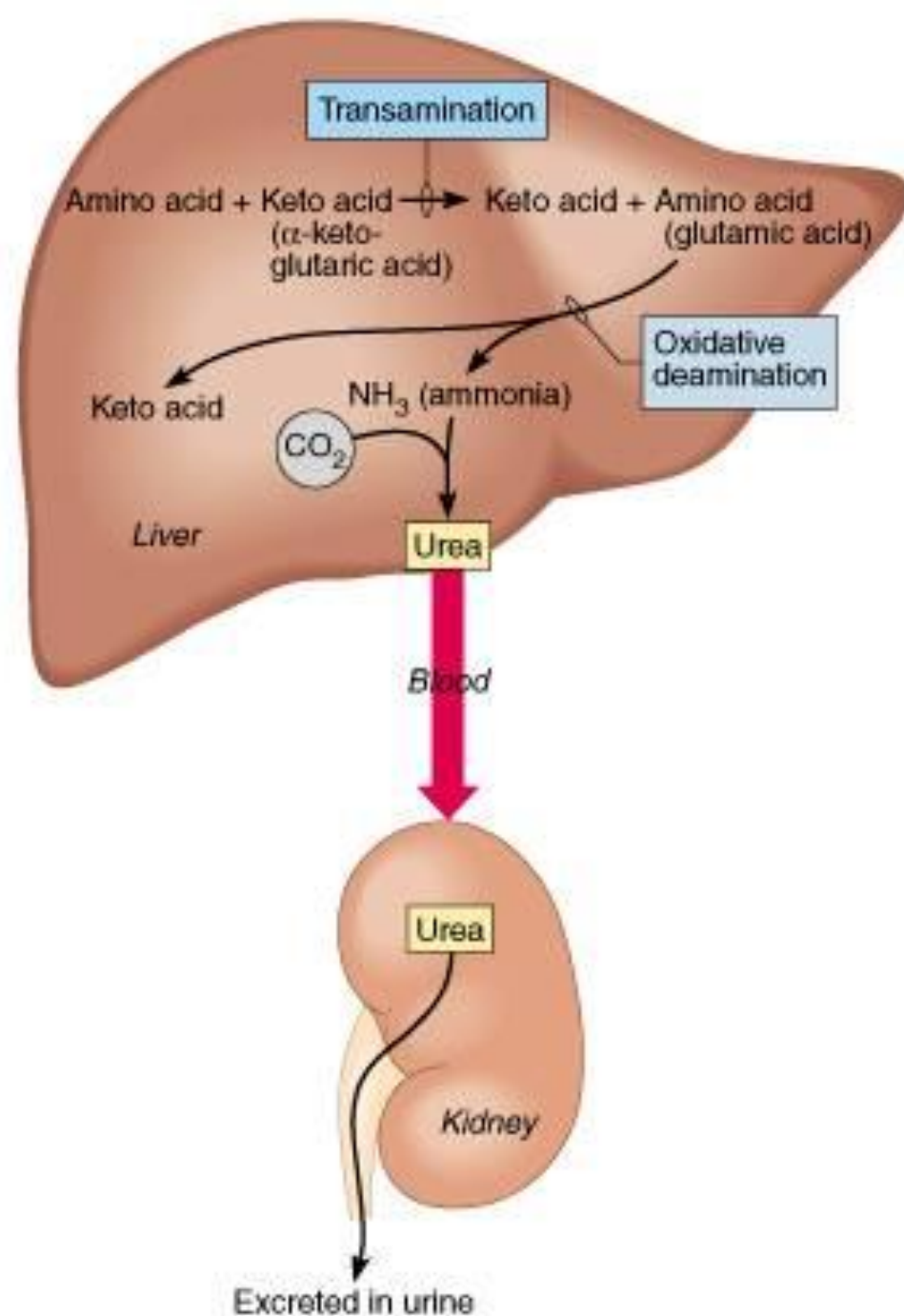
Cause	Reason	
Oxidative stress	Bilirubin can act as an antioxidant and thus, oxidative stress may lower levels.	Evaluate uric acid and GGT as well.
Zinc deficiency	Biliverdin reductase is a zinc dependent enzyme and converts biliverdin to bilirubin, thus leading to low bilirubin levels.	Evaluate alkaline phosphatase.

Blood Urea Nitrogen (BUN)

- * Blood Urea Nitrogen (BUN) is the excretory component of protein and amino acid metabolism from the liver.
- * Specifically, the liver removes amine groups (NH_3) from amino acids, which can create ammonia, which is toxic to the body. Thus the liver synthesizes urea as a means of disposing excess ammonia from the body.
- * Urea enters circulation and is excreted via the kidneys.

Urea Cycle





Blood Urea Nitrogen (BUN)

Traditional Reference Range

- * 5-26 mg/dL

Optimal Reference Range

- * 12-17 mg/dL

Blood Urea Nitrogen - Elevated

Cause	Reason	Additional Inquiry
High protein diet	High protein diet leads to more amino acids, which leads to excess urea production.	Client history and diet journal.
Dehydration	Hemoconcentration. Could be secondary to hypoadrenal function.	Evaluate other dehydration markers.
Kidney dysfunction	Because the kidney clears urea, if the kidneys are dysfunctional, urea will build up in circulation.	Evaluate other kidney markers.
Fatty Liver	Mechanism unknown. One study demonstrated that individuals with non-alcoholic fatty liver had elevated BUN.	Evaluate other liver markers. Client history.
Catabolic states	Due to increased amino acid turnover, catabolic states, such as extreme dieting, can cause elevations in BUN.	Client history.

Blood Urea Nitrogen - Decreased

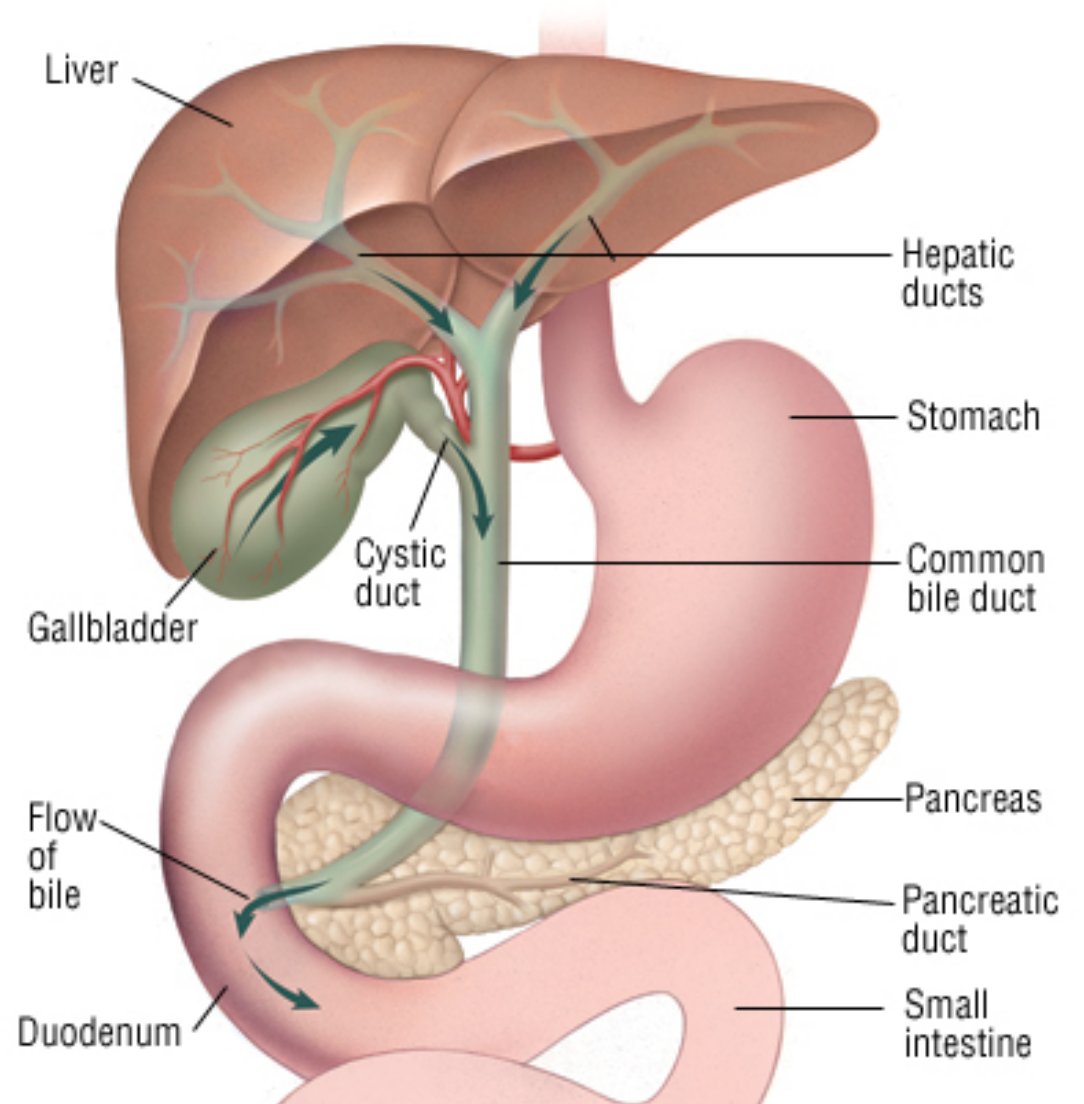
Cause	Reason	Additional Inquiry
Poor protein intake, digestion or absorption	Urea is a byproduct of amino acid metabolism and as such, if protein is low due to intake, digestion or absorption, BUN can be low.	Client history. GI symptoms and function.
Severe liver disease	The liver is responsible for the urea cycle. If the liver is dysfunctional, it will not adequately produce urea.	Evaluate other liver markers.
Possible B6 deficiency	Vitamin B6 (pyridoxine) is responsible for transamination reactions, which is necessary for non-essential amino acid synthesis. Thus if B6 is low, urea may be low as well.	Evaluate AST, ALT and B6 deficiency signs and symptoms.
Excess hydration	Excessive hydration is difficult to attain due to the body's regulatory mechanisms. However hormonal influences can impact this, such as the adrenals and pituitary glands.	

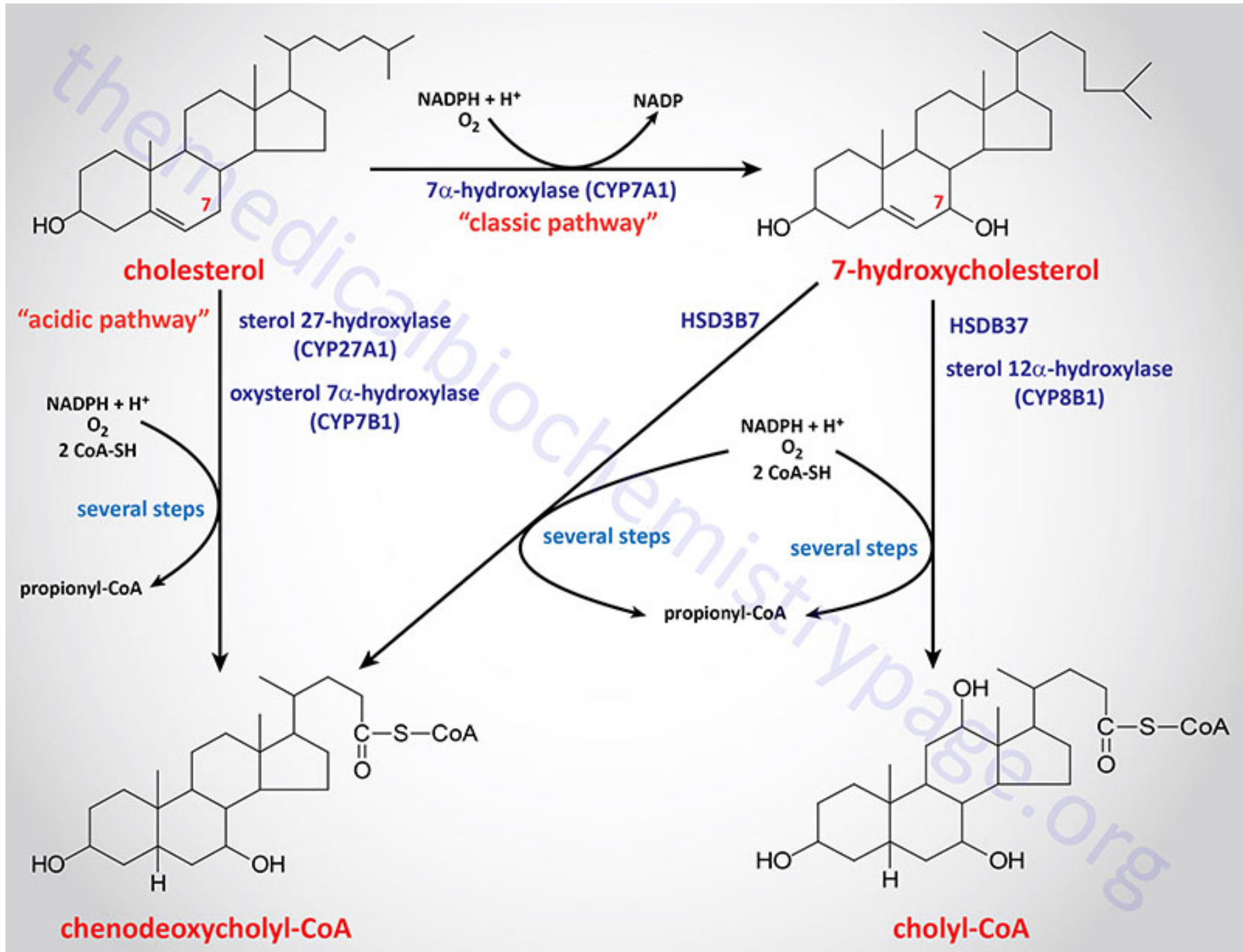
BUN/Albumin Ratio

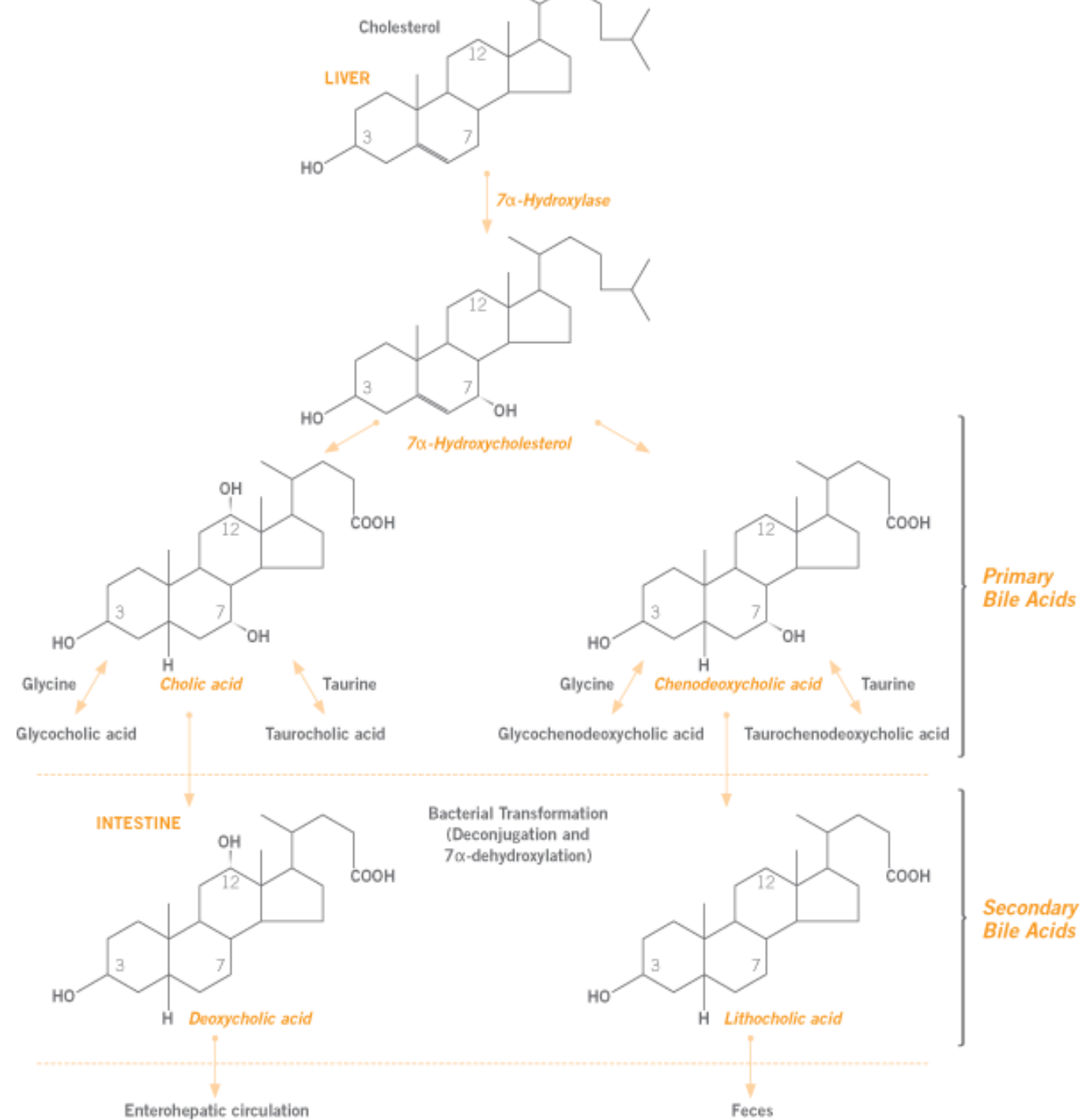
- * The BUN/Albumin ratio has been used clinically to evaluate risk in certain diseased population, such as those with pulmonary disease.
- * However it may have clinical utility in a nutritional practice as BUN can be elevated in dehydration and albumin can be decreased in infection, inflammation, and liver dysfunction, thus increasing the BUN/albumin ratio.
- * No studies have been conducted using the BUN/albumin ratio in a nutritional setting but using optimal values for each marker, it would seem a value of <4.0 is desirable.

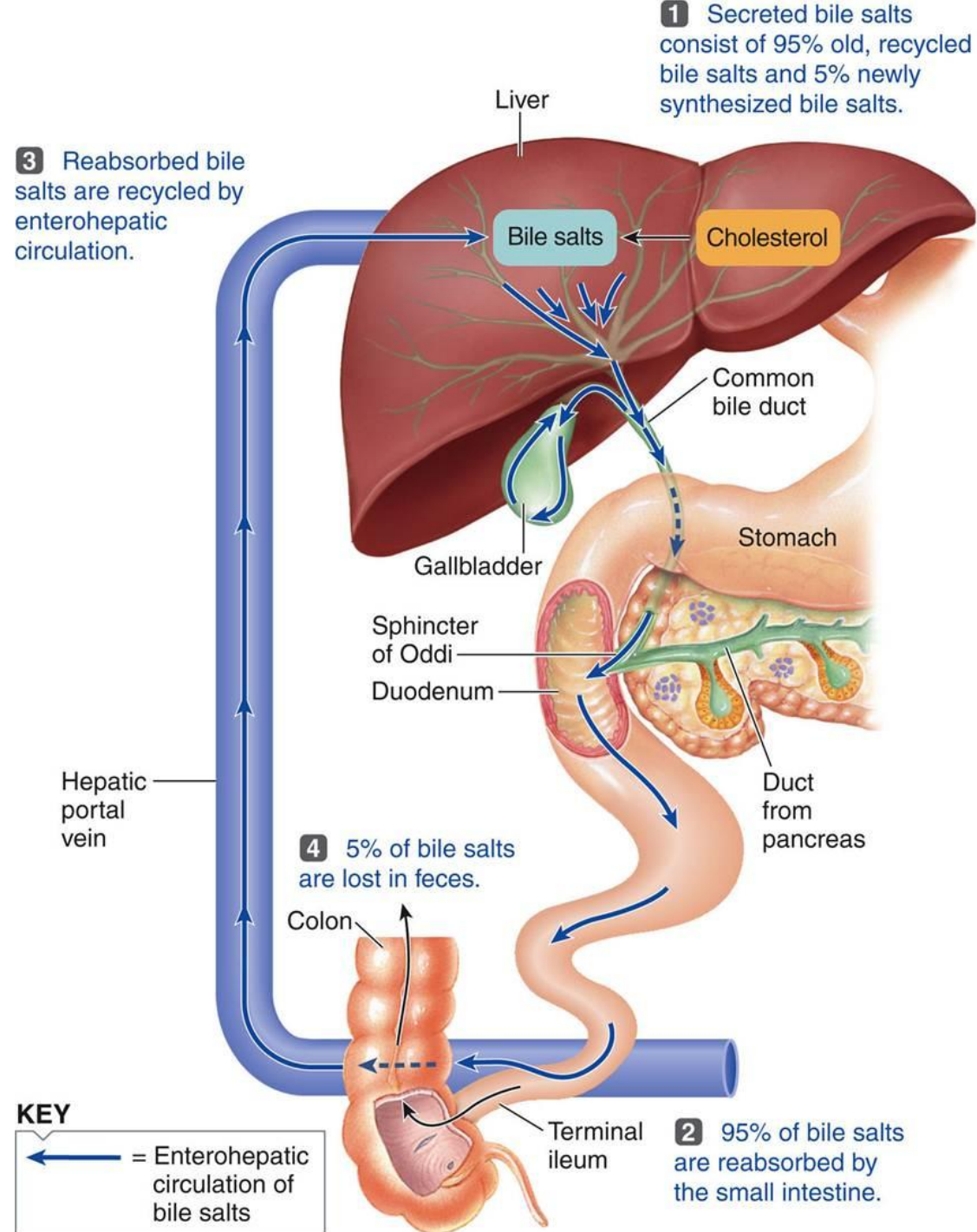
Bile Acids

- * Traditionally understood to be involved in emulsification of ingested fat, allowing pancreatic enzymes (lipase, co-lipase) to breakdown triacylglycerols for absorption in the small intestine
- * Involved in the removal of cholesterol, hormones, and toxins









Serum Bile Acids

- * A decrease in hepatic blood flow, and/or hepato-cellular damage, or any compromise in liver function will increase serum bile acids
- * Test for liver function, where as AST and ALT reflect liver tissue destruction
- * Will likely show liver dysfunction well before elevations in liver-based enzymes
- * Has highest sensitivity for early stage liver dysfunction

Serum Bile Acids

- * May be of benefit in identifying hepatic dysfunction as a result of chemical or environmental injury
 - * 73% of patients exposed to organic solvents had increased TBA (total bile acids), whereas GGT, ALT, AST, and bilirubin were only elevated 8, 3, 2 and 1% respectively
- * Useful in minor hepatic derangements

Bile Acids

- * Traditional reference range:
 - * 4.7-24.5 $\mu\text{mol/L}$ (LabCorp)
 - * <19 nmol/mL (Mayo)
 - * <10 $\mu\text{mol/L}$ (obstetric cholestasis)
- * Optimal reference range:
 - * 4.7-10 $\mu\text{mol/L}$

Bile Acid - Elevated

Cause	Reason	Additional Inquiry
Biliary tree dysfunction	Poor elimination of bile from the liver, through the bile ducts, to the gall bladder and intestines.	Evaluate bilirubin, alkaline phosphatase, and/or GGT. If elevated, may be biliary tree dysfunction.
Liver dysfunction	Hepatic cholestasis, or blockages or liver ducts responsible for the transport of bile.	Evaluate AST, ALT, albumin, BUN.
Decreased GI motility		Patient symptoms; slow transit time

Bile Acid - Decreased

Cause	Reason	Additional Inquiry
Decreased bile synthesis	Poor bile synthesis due to low cholesterol, low taurine/glycine, low reducing agents (NADPH), or liver dysfunction.	Evaluate cholesterol, digestion, and other liver markers.
Bile acid malabsorption	Significant digestive (ileal) dysfunction	Patient history of digestive symptoms.
Increased GI motility		Patient symptoms; increased transit time test

Break Time.

Lipids

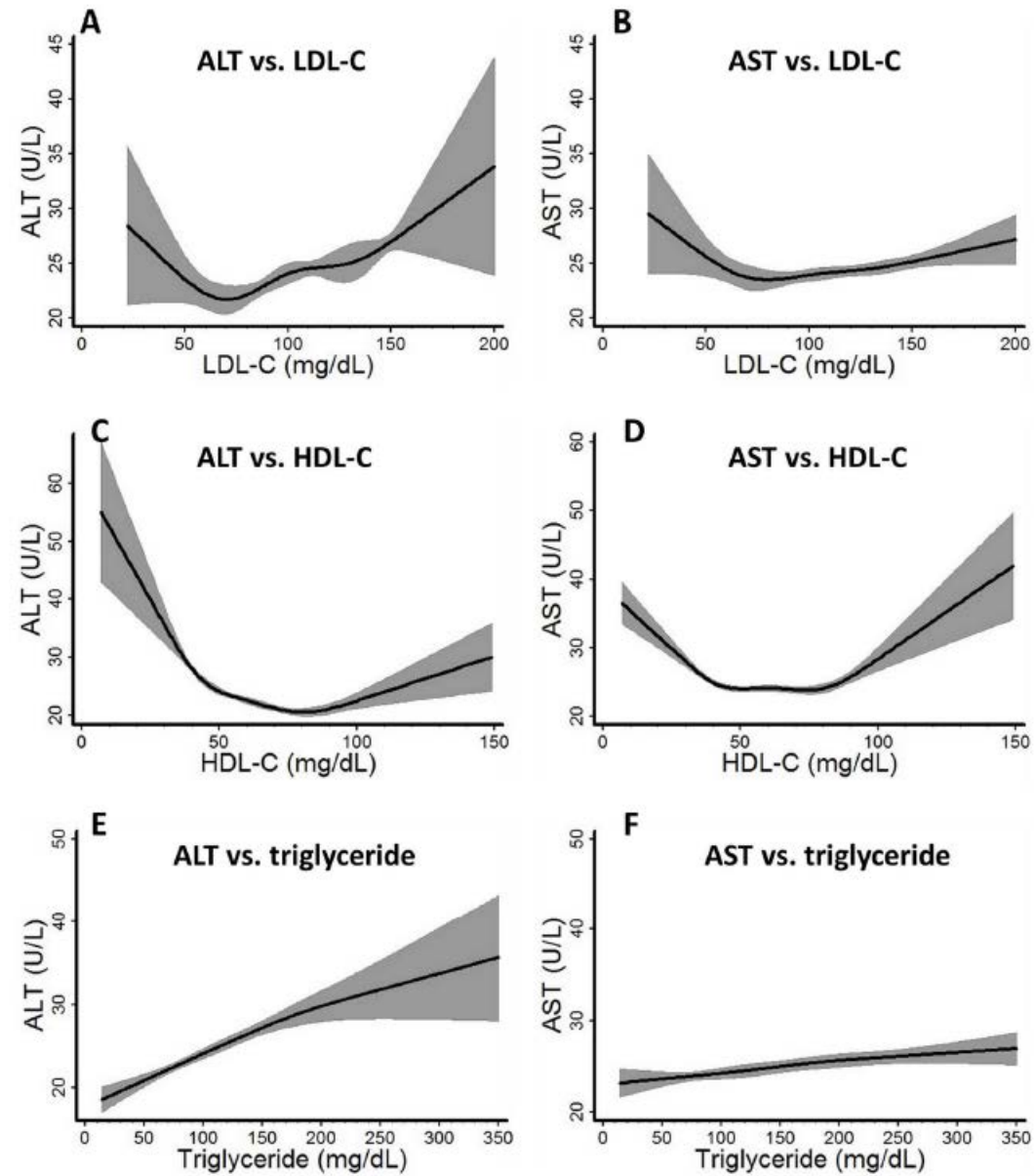
Lipids

- * Cholesterol, HDL-C, LDL-C, triglycerides
- * Not valuable for identifying cardiovascular risk
- * Low cholesterol is not associated with sex hormone levels
- * Elevated cholesterol
 - * Insulin, glucose dysregulation, low thyroid hormone

Lipids

Ratio	Range	Consideration
Cholesterol/ Triglyceride	>2	Insulin resistance
Cholesterol/HDL	< 3.0	Cardiovascular disease risk
Triglyceride/HDL	< 3.8	LDL Particle size
LDL/HDL	> 2.0	Inflammation, liver dysfunction

Low LDL-C and High HDL-C Levels Are Associated with Elevated Serum Transaminases amongst Adults in the United States: A Cross-sectional Study



High Density Lipoprotein-Lipopolysaccharide Binding Lowers HDL's Basal Antioxidant Capacity

- * It has been established that HDL has a role in the innate immune system through its ability to sequester lipopolysaccharide (LPS) or lipoteichoic acid (LTA) in the blood. This sequestering prevents the endotoxins from binding to their respective toll-like receptors and subsequently stimulating the transcription of pro-inflammatory cytokines. Elevated HDL levels are seen to attenuate sepsis by rapidly removing LPS molecules from the blood.

High Density Lipoprotein-Lipopolysaccharide Binding Lowers HDL's Basal Antioxidant Capacity

- * The anti-oxidative capacity of HDL was not observed in the presence of LPS and elicited greater oxidative stress, possibly due to increased LPS-HDL binding. This data suggest that the anti-inflammatory effect of HDL may come at the cost of its other biological functions. It also suggests that chronic low-grade endotoxemia may counter the clinical significance of HDL as a negative risk factor for cardiovascular and metabolic disease.

Science is moving toward particle number and size, rather than cholesterol numbers for heart disease and insulin resistance risk assessments.

(Heads will explode)

LDL-P is the measurement of lipoprotein particles. (carriers)

LDL-C is the measurement of cholesterol mass within the LDL particles.

Cholesterol is transported by lipoproteins in the blood
Cholesterol carried by low density lipoprotein (LDL) is associated
with increased risk of heart disease



The trucks represent LDL particles.

The yellow sand represents cholesterol carried by LDL particles

Each truck (LDL) carries one ton of sand (cholesterol)

LDL cholesterol (LDL-C) is the number of tons of cholesterol carried
by all the trucks

LDL particle number (LDL-P) is the number of trucks

LDL-C is 5 (tons of sand) and LDL-P is 5 (number of trucks)



Here are bigger trucks (larger LDL particles)
Each truck is carrying more sand (LDL cholesterol)

Each truck is carrying 5 tons of cholesterol
The total amount of cholesterol carried by the three trucks is 15
LDL-P (number of trucks) is 3

This is an example of discordance
LDL-C is high but LDL-P is low



This patient has 10 small trucks (small LDL-particles)

Each truck only carries 0.2 tons of sand (LDL-cholesterol)

The 10 trucks together carry 2 tons of cholesterol

LDL-C is 2

LDL-P is 10

This is another type of discordance where LDL-C is low but
LDL-P is high



Moderate LDL-C and moderate LDL-P



High LDL-C and low LDL-P



Low LDL-C and high LDL-P

NMR LipoProf wSubCls+Graph

LDL Particle Number					01
LDL-P	1200	High	nmol/L	<1000	01
		Low		< 1000	
		Moderate		1000 - 1299	
		Borderline-High		1300 - 1599	
		High		1600 - 2000	
		Very High		> 2000	
Lipids					01
LDL-C	92		mg/dL	0 - 99	
		Optimal		< 100	
		Above optimal		100 - 129	
		Borderline		130 - 159	
		High		160 - 189	
		Very high		> 189	
Comment:					01
LDL-C is inaccurate if patient is non-fasting.					
HDL-C	57		mg/dL	>39	01
Triglycerides	51		mg/dL	0 - 149	01
Cholesterol, Total	159		mg/dL	100 - 199	01
LDL and HDL Particles					01
HDL-P (Total)	36.6		umol/L	>=30.5	01

Small LDL-P	362	nmol/L	<=527	01
LDL Size	20.8	nm	>20.5	01

```

-----
                ** INTERPRETATIVE INFORMATION**
                PARTICLE CONCENTRATION AND SIZE
                <--Lower CVD Risk   Higher CVD Risk-->
LDL AND HDL PARTICLES   Percentile in Reference Population
HDL-P (total)           High       75th    50th    25th    Low
                        >34.9     34.9    30.5    26.7    <26.7
Small LDL-P             Low        25th    50th    75th    High
                        <117      117     527     839    >839
LDL Size   <-Large (Pattern A)->   <-Small (Pattern B)->
                        23.0    20.6                20.5    19.0
-----

```

Comment: 01

Small LDL-P and LDL Size are associated with CVD risk, but not after LDL-P is taken into account. These assays were developed and their performance characteristics determined by LipoScience. These assays have not been cleared by the US Food and Drug Administration. The clinical utility of these laboratory values have not been fully established.

Insulin Resistance/Diab. Risk				01
Large VLDL-P	1.0	nmol/L	<=2.7	01
Small LDL-P	362	nmol/L	<=527	01
Large HDL-P	8.1	umol/L	>=4.8	01
VLDL Size	40.8	nm	<=46.6	01
LDL Size	20.8	nm	>=20.8	01
HDL Size	9.4	nm	>=9.2	01
Insulin Resistance Score				01
LP-IR Score	<25		<=45	01

INSULIN RESISTANCE / DIABETES RISK MARKERS					
<--Insulin Sensitive			Insulin Resistant-->		
	Percentile in Reference Population				
Large VLDL-P	Low	25th	50th	75th	High
	<0.9	0.9	2.7	6.9	>6.9
Small LDL-P	Low	25th	50th	75th	High
	<117	117	527	839	>839
Large HDL-P	High	75th	50th	25th	Low
	>7.3	7.3	4.8	3.1	<3.1
VLDL Size	Small	25th	50th	75th	Large
	<42.4	42.4	46.6	52.5	>52.5
LDL Size	Large	75th	50th	25th	Small
	>21.2	21.2	20.8	20.4	<20.4
HDL Size	Large	75th	50th	25th	Small
	>9.6	9.6	9.2	8.9	<8.9
Insulin Resistance Score					
LP-IR SCORE	Low	25th	50th	75th	High
	<27	27	45	63	>63

Lipid Lab Ranges

	Range	
Cholesterol	145-199	Be Smart.
HDL	45-59	What happens when its too high?
Trigs	50-99	Particle size will skew.
LDL	50-99	

Quick word on dehydration

Dehydration

- * Hemoconcentration: albumin, BUN, Na, K, RBC/Hgb/Hct
- * Check specific gravity on a urinalysis:
 - * Reference range: 1.005-1.030
 - * If low-normal = dilute urine → losing water → might be dehydrated despite drinking adequate amounts of water.
 - * If high-normal = concentrated urine → not drinking enough water
- * Also calculate serum osmolarity

Oxidative Stress

Oxidative Stress

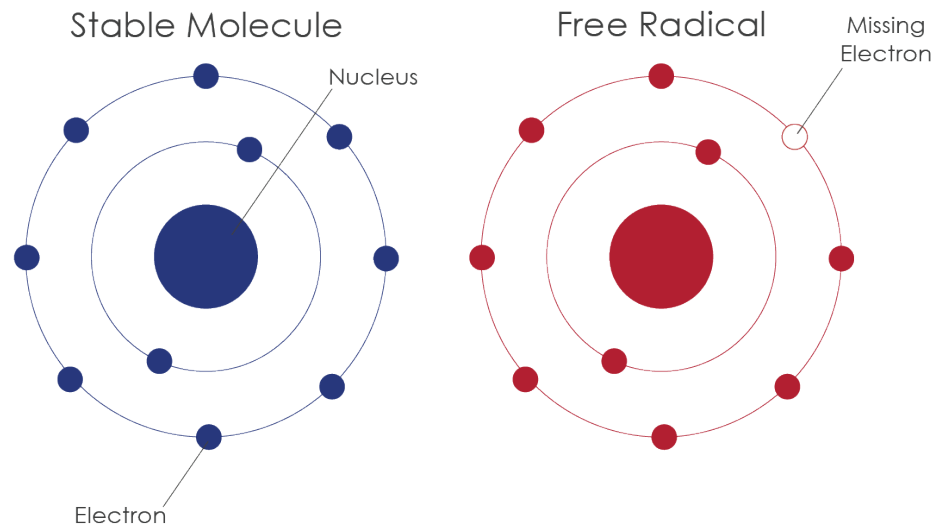
- * Oxidative stress has been linked to heart disease, cancer, rheumatoid arthritis, hypertension, Alzheimer's disease, and Parkinson's disease, and much more

Oxidative Stress

- * Linked to inflammation and production of proinflammatory cytokines
 - * Cytokines linked to diseases as well (osteoporosis, diabetes)

Free Radicals

- * Oxidative stress is caused by excessive production of free radicals from oxygen (ROS) and nitrogen (RNS)
- * Atoms, molecules or ions with unpaired electrons
- * Highly reactive



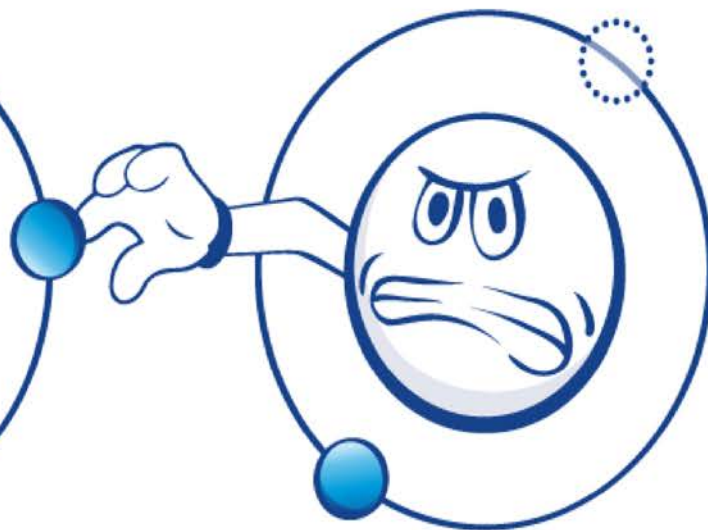
Free Radicals

- * Examples include:
 - * Hydroxyl radical (OH)
 - * Peroxyl radical (ROO)
 - * Superoxide radical (O_2^-)
 - * Nitric oxide (NO), peroxynitrite (ONOO^-), and NO_2





**HEALTHY
ATOMS**



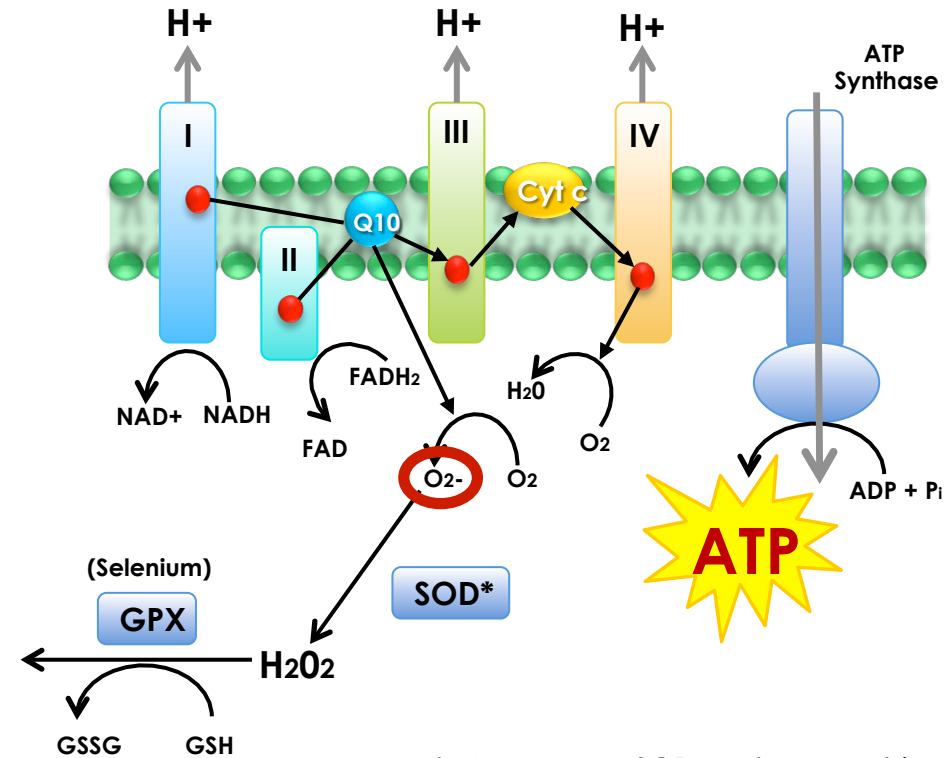
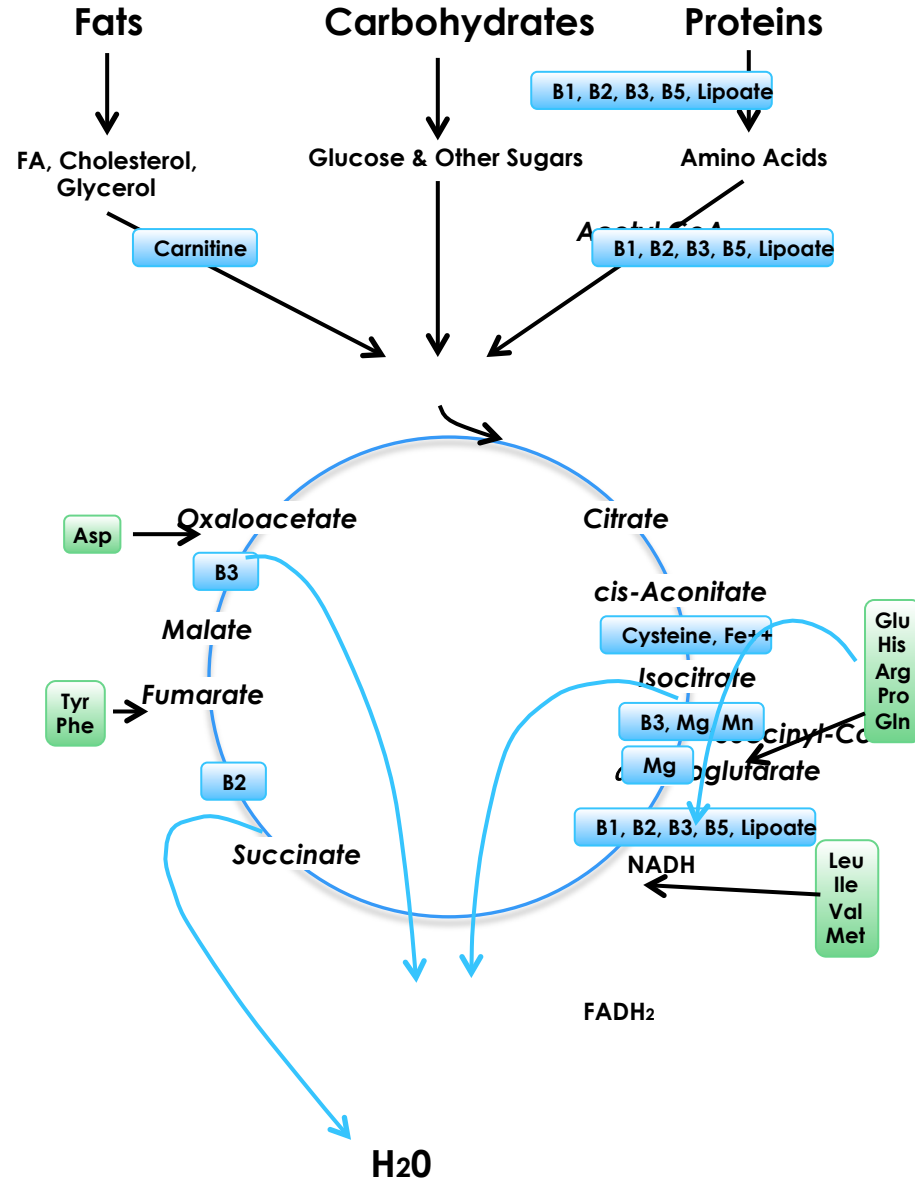
**FREE
RADICALS**



ANTIOXIDANTS

Reactive oxygen species

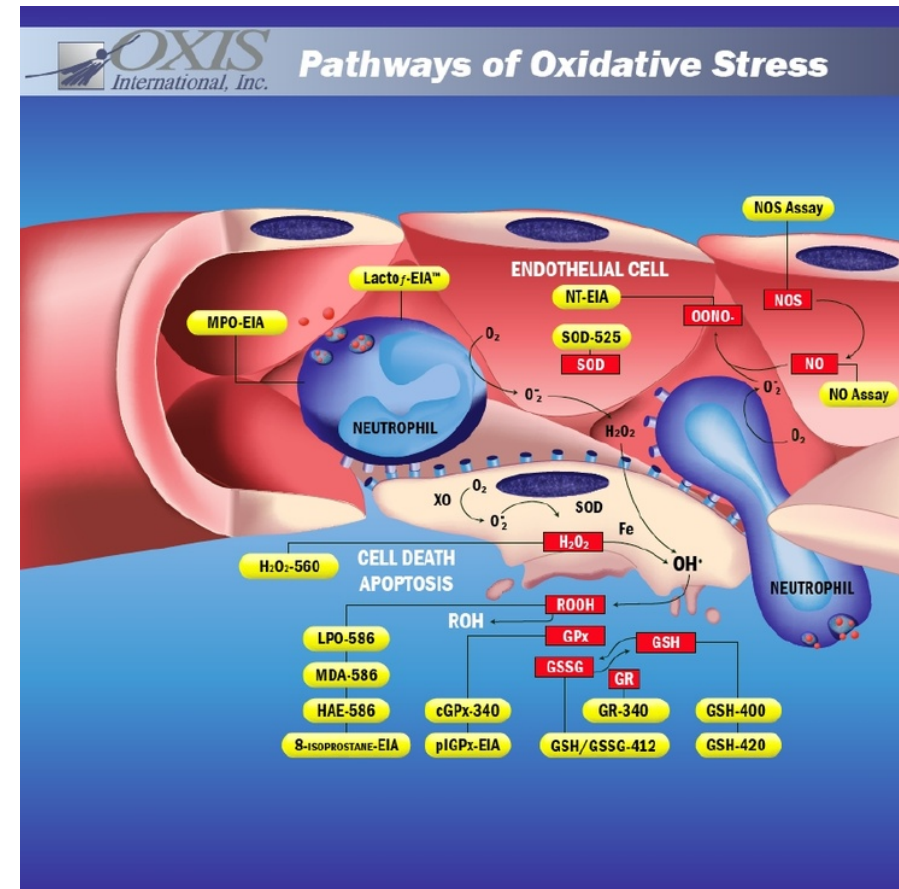
- 0.4–4.0% of oxygen is converted to the superoxide (O_2^-) radical.
- Mitochondrial damage leads to more ROS, causing damage to mitochondrial DNA



* Manganese SOD and copper/zinc SOD

Causes of Free Radicals

- * Ionizing radiation
- * UV radiation
- * Metabolism (mitochondria)
- * Xenobiotics (pollution, toxin)
- * Inflammation
- * Medications
- * Diet
- * Stress
- * Exercise



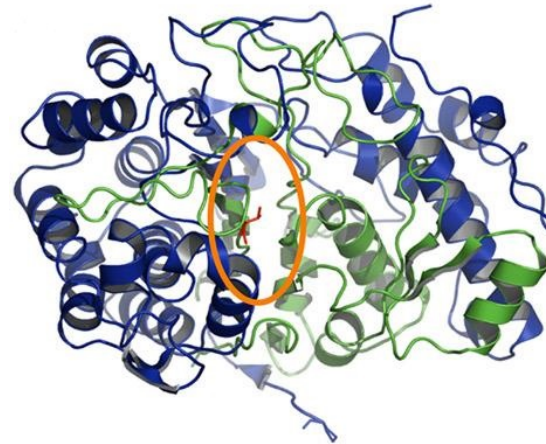
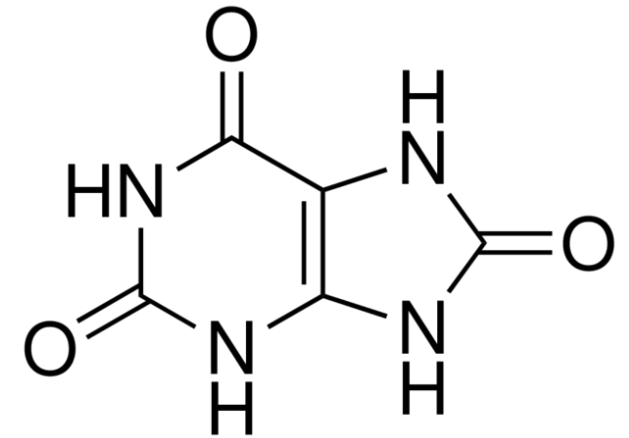
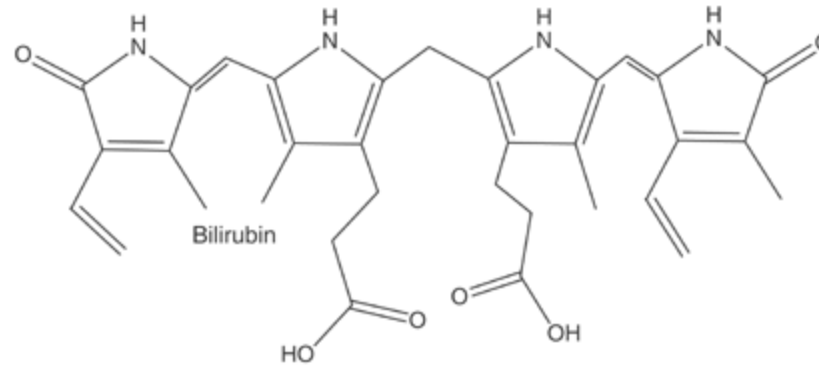
Conventional Markers of Oxidative Stress

Organelle	Biomarker activity
Nucleus	8-hydroxy deoxyguanosine
Mitochondria	Catalase, Cu/Zn SOD, Mn-SOD
Endoplasmic reticulum and golgi	PEG-SOD, F2-isoprostanes, HNE
Plasma	TBARS, CUPRAC, 8-iso-PGF(2α), LHP
Total cellular constituents	Cytokines, chaperones, telomeres



Three Makers of Oxidative Stress

- * Uric Acid
- * GGT
- * Bilirubin



Elisa Fabbrini,¹ Mauro Serafini,² Irena Colic Baric,² Stanley L. Hazen,³ and Samuel Klein¹

Effect of Plasma Uric Acid on Antioxidant Capacity, Oxidative Stress, and Insulin Sensitivity in Obese Subjects

We found that a marked decrease in serum UA levels caused a decrease in serum and saliva antioxidant capacity, assessed by TRAP and FRAP assays, and an increase in oxidative stress, assessed by measuring urinary isoprostanes and skeletal muscle protein carbonylation.

It is possible that an increase in UA concentration is a protective mechanism to attenuate the adverse effects of an increase in oxidative stress.

reduction of serum UA levels to 0 by infusing a recombinant urate oxidase. HUA subjects had 20–90% greater NEAC, but lower insulin sensitivity (40%) and levels of markers of oxidative stress (30%) than subjects in the NUA group (all $P < 0.05$). Acute UA reduction caused a 45–95% decrease in NEAC and a 25–40% increase in levels of systemic and muscle markers of oxidative stress (all $P < 0.05$), but did not affect insulin sensitivity (from $168 \pm 25\%$ to $156 \pm 17\%$, $P = \text{NS}$). These results demonstrate that circulating UA is a major antioxidant and might help protect against free-radical oxidative damage.

myotubes and adipocytes (2,3), and suppress gene transcription of insulin in β -cells (5) and adiponectin in adipocytes (4).

Uric acid (UA) is a powerful scavenger of free radicals and provides ~60% of free-radical scavenging capacity in plasma (6). Although the antioxidant effect of UA suggests that it might have therapeutic effects, high serum UA concentration is associated with obesity and insulin resistance (7,8), and hyperuricemia has even been proposed as a component of the metabolic syndrome (9). However, it is possible that this increase in circulating levels of UA represents an adaptive response to protect

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RESEARCH ARTICLE

Elevated Serum Uric Acid Is Associated with Greater Bone Mineral Density and Skeletal Muscle Mass in Middle-Aged and Older Adults

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* These authors contributed equally to this work.
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Abstract

Background and objective

Previous studies have suggested a positive link between serum uric acid (UA) and bone mineral density (BMD). In this study, we re-examined the association between UA and BMD and further explored whether this was mediated by skeletal muscle mass in a general Chinese population.

Method

This community-based cross-sectional study was conducted among 3079 (963 men and 2116 women) Chinese adults aged 40–75 years. Face-to-face interviews and laboratory analyses were performed to determine serum UA and various covariates. Dual-energy X-ray absorptiometry was used to assess the BMD and appendicular skeletal muscle mass. The skeletal muscle mass index (SMI = ASM/Height², kg/m²) for the total limbs, arms, and legs was then calculated.

Results

The serum UA was graded and, in general, was significantly and positively associated with the BMD and muscle mass, after adjustment for multiple covariates in the total sample. Compared with participants in lowest quartile of UA, those participants in highest quartile showed a 2.3% (whole body), 4.1% (lumbar spine), 2.4% (total hip), and 2.0% (femoral neck) greater BMDs. The mean SMIs in the highest (vs. lowest) quartile increased by 2.7% (total), 2.5% (arm), 2.7% (leg) respectively. In addition, path analysis suggested that the favorable association between UA and BMD might be mediated by increasing SMI.

OPEN

Serum uric acid levels in patients with myasthenia gravis are inversely correlated with disability

Dehao Yang^{a,*}, Yiyun Weng^{a,*}, Haihua Lin^b, Feiyan Xie^c, Fang Yin^e, Kangliang Lou^e, Xuan Zhou^e, Yixiang Han^d, Xiang Li^a and Xu Zhang^a

Uric acid (UA), the final product of purine metabolism, has been reported to be reduced in patients with various neurological disorders and is considered to be a possible indicator for monitoring the disability and progression of multiple sclerosis. However, it remains unclear whether there is a close relationship between UA and myasthenia gravis (MG), or whether UA is primarily deficient or secondarily reduced because of its peroxynitrite scavenging activity. We investigated the correlation between serum UA levels and the clinical characteristics of MG. We assessed 338 serum UA levels obtained in 135 patients with MG, 47 patients with multiple sclerosis, and 156 healthy controls. In addition, we compared serum UA levels when MG patients were stratified according to disease activity and classifications performed by the Myasthenia Gravis Foundation of America, age of onset, duration, and thymus histology (by means of MRI or computed tomography). MG patients had significantly lower serum UA levels than the controls ($P < 0.001$). Moreover, UA levels in patients with MG were inversely correlated with disease activity and disease

progression ($P = 0.013$). However, UA levels did not correlate significantly with disease duration, age of onset, and thymus histology. Our findings suggest that serum level of UA was reduced in patients with MG and serum UA might be considered a surrogate biomarker of MG disability and progression. *NeuroReport* 27:301–305 Copyright © 2016 Wolters Kluwer Health, Inc. All rights reserved.

NeuroReport 2016, 27:301–305

Keywords: disability, myasthenia gravis, uric acid

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*Dehao Yang and Yiyun Weng are co-first authors.

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Introduction

Myasthenia gravis (MG), caused by autoantibodies against the acetylcholine receptor (AChR) on the post-synaptic membrane at the neuromuscular junction, is an acquired autoimmune disease characterized by a defective transmission of nerve impulses to muscles [1]. Accumulating data have implicated oxidative stress in the immunopathogenesis of neuromuscular diseases [2,3].

As the final product of the common pathway of purine metabolism, uric acid (UA) is a naturally occurring antioxidant, with metal-chelating properties [4]. Previous studies have reported that UA can scavenge nitrogen radicals and superoxide, thus helping to block the generation of the strong oxidant peroxynitrite [5]. Peroxynitrite exerts toxic effects and irreversibly jeopardizes cellular metabolism and cell structures, including lipids, carbohydrates, protein, and DNAs [6]. Several studies have identified a therapeutic role of UA in experimental allergic encephalomyelitis and a beneficial function for increasing serum UA levels in multiple

sclerosis (MS) patients [7,8]. Furthermore, UA might be a surrogate marker for monitoring MS activity [9].

Therefore, the aim of this study was to investigate whether the serum UA levels were decreased in MG patients and whether the decrease was associated with disease disability and progression.

Patients and methods

Serum samples were collected from 338 individuals: 135 patients with MG, 47 patients with MS, and 156 healthy controls (CTL). Venous blood was drawn from an antecubital vein in the morning after an overnight fast to measure the concentration of serum UA using a Clinical Analyzer Beckman Coulter AU5831 (Beckman Coulter, Brea, California, USA). In our hospital, the normal range of serum UA values is 208–428 μM for men and 155–357 μM for women. Simultaneously, concentrations of glutamate-pyruvate transaminase (normal range: 9–50 μM for men, 7–40 μM for women), glutamic-oxaloacetic transaminase (normal range: 15–40 μM, 13–35 μM for women), blood fasting sugar, and blood urea nitrogen were also measured using an enzymatic method on the same analyzer.

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Competing Interests: The authors have declared that no competing interests exist.

Uric Acid ROS Summary

- * Elevated uric acid is associated with a number of conditions (i.e. hypertension, cardiovascular disease)
- * Elevated uric acid is also associated with increased Total Antioxidant Capacity (TAC)
- * Elevated uric acid can be due to fructose and alcohol consumption, dietary purine consumption, hyperinsulinemia, or poor renal clearance of UA
- * Elevated uric acid *may* indicate oxidative stress, and an attempt to attenuate oxidative stress by the body

Uric Acid ROS Summary

- * Decreased uric acid, on the other hand, may indicate unchecked oxidative stress

URIC ACID: THE OXIDANT–ANTIOXIDANT PARADOX

Yuri Y. Sautin and Richard J. Johnson

Division of Nephrology, Hypertension and Transplantation, Department of Medicine, University of Florida, Gainesville, Florida, USA

Abstract

Uric acid, despite being a major antioxidant in the human plasma, both correlates and predicts development of obesity, hypertension, and cardiovascular disease, conditions associated with oxidative stress. While one explanation for this paradox could be that a rise in uric acid represents an attempted protective response by the host, we review the evidence that uric acid may function either as an antioxidant (primarily in plasma) or pro-oxidant (primarily within the cell).

The presence of ascorbic acid in the plasma is required for the antioxidant effect of uric acid. (lipid peroxidation)

Uric acid cannot scavenge all radicals, with superoxide as an example.

On the other hand, a vast literature on the epidemiology of cardiovascular disease, hypertension, and metabolic syndrome overwhelmingly shows that, at least among modern *Homo sapiens*, a high level of uric acid is strongly associated and in many cases predicts development of hypertension,[5–7] visceral obesity,[8–10] insulin resistance,[8,11,12] dyslipidemia,[8,11–13] diabetes type II,[11] kidney disease,[6] and cardiovascular and

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Conflict of Interest: Dr. Johnson has patent applications related to the lowering of uric acid as a means for treating cardiovascular disease and obesity via the University of Florida and University of Washington.

Uric Acid + ROS

Traditional Reference range:

- * 3.4-7.0 mg/dL (men)
- * 2.4-6.0 mg/dL (women)

Optimal Reference Range:

- * 3.7-5.5 mg/dL (men)
- * 3.2-4.4 mg/dL (women)

*Original Contribution***URIC ACID AND GLUTATHIONE LEVELS DURING
SHORT-TERM WHOLE BODY COLD EXPOSURE****WERNER G. SIEMS,* FREDERIK J. G. M. VAN KUIJK,[†] RALPH MAASS,[†] and RAINER BRENKE[†]**

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(Received 11 March 1992; Revised 25 February 1993; Re-revised 20 July 1993; Accepted 21 July 1993)

Abstract—Ten healthy subjects who swim regularly in ice-cold water during the winter (winter swimming), were evaluated

Ten healthy subjects who swim regularly in ice-cold water during the winter (winter swimming), were evaluated before and after this short-term whole body exposure. A drastic decrease in plasma uric acid concentration was observed during and following the exposure to the cold stimulus. We hypothesize that the uric acid decrease can be caused by its consumption after formation of oxygen radicals. In addition, the erythrocytic level of oxidized glutathione and the ratio of oxidized glutathione/total glutathione also increased following cold exposure, which supports this hypothesis.

cases, and infectious diseases, but also to lability of the central nervous system.¹ The negative results of natural stimulator deficiency can be partly prevented by exercise and “hardening,” which play an important role in physiotherapy. The exposure to an intensive short-term cold stimulus like swimming in cold water of lakes or rivers, especially during winter, has been used as one form of body hardening for many years.^{2–4} Hardening means exposure against a natural, e.g., thermic stimulus, resulting in an increased resis-

duced glutathione (GSH), and oxidized glutathione (GSSG) in red blood cells of winter swimmers were measured before and after cold stimulus.

MATERIALS AND METHODS

In Berlin, one winter swimmer club has been flourishing for several years. The members are not selected in any way and all swim regularly outdoors in ice-cold water. Their activities start in the fall, and members swim at least once per week for about 5 min in ice-cold water. Sometimes, ice on the lake has to be removed to allow them to swim.

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GGT + ROS

ORIGINAL ARTICLE

Gamma-glutamyltransferase predicts increased risk of mortality: A systematic review and meta-analysis of prospective observational studies

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Abstract

The aim of this study was to evaluate the association between gamma-glutamyltransferase (GGT) and mortality through a comprehensive analysis of existing evidence. PubMed, Embase, Chinese Biomedical Literature, and Science Citation Index databases were electronically searched. Studies were included if the study design was prospective and included reference and at-risk levels of GGT at baseline and mortality as a separate outcome. The quality of the studies included was assessed on the basis of Newcastle–Ottawa scale. Data from selected qualified studies were systematically reviewed, pooled, and analyzed according to the MOOSE guidelines and PRISMA statement. The results included the following: 1. 35 studies including 571 511 participants and 72 196 cases of mortality; 2. GGT, even at physiologic

The results included the following:

1. 35 studies including 571 511 participants and 72 196 cases of mortality;
2. GGT, even at physiologic levels, was associated with increased all-cause mortality and cardiovascular mortality, and might also be associated with cancer-related mortality in the general population

Gamma-glutamyltransferase (GGT) is most abundant in kidney and liver and exists in serum and nearly all epithelial tissues [1]. GGT plays key roles in catabolism and synthesis of glutathione (GSH), the most important non-protein antioxidant outside the cell [1]. Changes in GGT are accompanied by an altered status of oxidative stress, a suggested common soil of many modern chronic diseases, such as metabolic syndrome [2], type 2 diabetes mellitus [3], hypertension [4], cancer [5], and cardiovascular diseases [6].

GGT was introduced to clinical laboratories nearly half a century ago and has become recognized as a biomarker of liver injury and alcohol consumption [1]. It was not until the 1990s that published studies began to suggest that GGT predicted death not only from alcohol-related illness but also from myocardial infarction, pointing out that GGT was an independent predictor of death and not merely a marker of alcohol consumption [1]. Lately, GGT has even been suggested to be a biomarker of transition across

However, previous studies, especially with prospective design, have been few, mostly confined to special populations (especially alcoholics), and have considered GGT as a marker of alcohol consumption [1], which limited the understanding of GGT and mortality. In recent decades, plenty of prospective observational studies have been published. Most of these studies recruited more representable populations (e.g., free-living residence, patients with cardiovascular diseases or type 2 diabetes mellitus), some of which investigated the association between GGT and cause-specific death (e.g., cardiovascular death, cancer-related death). An increasing body of evidence has made it possible to perform a systemic analysis of the relationship between GGT and mortality. To this end, this study searched published data with prospective design on GGT and mortality and comprehensively analyzed their potential association following the guidelines of the Meta-Analysis of Observational Studies in Epidemiology (MOOSE) [8].

*Graduated.

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Review

Elevated serum γ -glutamyltransferase activity is associated with increased risk of mortality, incident type 2 diabetes, cardiovascular events, chronic kidney disease and cancer – a narrative review

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that are within the high-normal range, in risk prediction of incident type 2 diabetes, cardiovascular events, chronic kidney disease (CKD) or cancer, independent of alcohol consumption and other prognostic factors.

Several population based cohort studies have consistently shown that increased serum GGT activity, even high normal values within the reference range, is associated with an increased risk of major vascular and non-vascular outcomes (especially incident type 2 diabetes and CVD morbidity and mortality) in both men and women, in different ethnic groups and among self-reported non-drinkers.

Keywords: cancer; cardiovascular disease; chronic kidney disease; diabetes; epidemiology; γ -glutamyltransferase.

Introduction

Serum γ -glutamyltransferase (GGT) enzyme activity has long been used as a reliable marker of liver dysfunction and excessive alcohol intake (1). However, in recent years our knowledge of the physiological functions of this enzyme has expanded and several important epidemiological associations have been reported.

This review critically appraises studies examining the prognostic value of serum GGT enzyme activity, even values

heavy chain in vitro. Presumably, in vivo the heavy chain not only secures the light chain to the cell membrane but also modifies its catalytic activity. There are up to eight potential sites for glycosylation, and the protein is heavily glycosylated with considerable heterogeneity (1–4). GGT does not have isoenzymes in the sense of proteins with different amino acid sequence but with the same catalytic function. However, there are a variety of GGT isoforms that differ in their carbohydrate content or structure (2–4). These isoforms may be separated by electrophoresis, isoelectric focusing, or by lectin-affinity chromatography. Much of the variation in electrophoretic mobility is due to the association of the isoforms with lipoproteins or immunoglobulin A (1). Indeed, several multiple forms of GGT have been described in serum. Most of these are large complexes between the enzyme and circulating lipoproteins or immunoglobulin A. Approximately 60%–80% of the total GGT in sera from patients with hepatobiliary diseases is complexed with lipoproteins. One dominant form of GGT is complexed with high-density lipoproteins (HDLs); a small, hydrophilic, form is also present in minor amounts (1).

GGT activity varies considerably between normal tissues and during stages of embryonic development. Hanigan and

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Review Article

Gamma-Glutamyltransferase: A Predictive Biomarker of Cellular Antioxidant Inadequacy and Disease Risk

Gerald Koenig^{1,2} and Stephanie Seneff³

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³Computer Science and Artificial Intelligence Laboratory, MIT, Cambridge, MA 02139, USA

Here, we examine the relationship of GGT to other serum markers such as serum ferritin (SF) levels, and we **suggest a link to exposure to environmental and endogenous toxins, resulting in oxidative and nitrosative stress.**

GGT is an early predictive marker for atherosclerosis, heart failure, arterial stiffness and plaque, gestational diabetes, and various liver diseases, including viral hepatitis, other infectious diseases, and several life-threatening cancers.

1. Introduction

A comprehensive review by Whitfield in 2001 [1] described GGT in its traditional role as a marker of liver dysfunction, bile duct conditions, and alcohol consumption. Some generalized or summary medical and scientific literature still describe GGT in those terms [2]. However, Whitfield had already extended that description to include elevated GGT in association with risk of coronary heart disease, type-II diabetes (T2D), and stroke [1]. Although gamma-glutamyl compounds include antioxidants, inflammatory molecules, drug metabolites, and neuroreactive compounds [3], the major function of GGT is enabling metabolism of glutathione and glutathionylated xenobiotics. However, elevated GGT levels, as noted by Whitfield and others, contribute to prooxidant activity, particularly in the presence of iron or copper [4, 5]. When GGT levels are elevated, damage to red blood cell membranes can occur causing the release of

these potentially toxic transition metals, which can further result in chain, prooxidant reactions [6]. Increased levels of prooxidation can lead to downstream cell, tissue, and DNA damage caused by oxidative and nitrosative stress and the generation of deleterious reactive oxygen species or nitric oxide (ROS or NO) [7]. This combination of factors is observed with increasing frequency in many chronic diseases. Other investigators have added many newly identified GGT-related diseases and conditions to a rapidly growing list that very recently was modified by Sreeram et al. [8] to even include GGT as a marker for oxidative stress in periodontal disease.

In this paper, we review an extensive research literature on GGT as a marker for disease. Not only do we demonstrate strong potential for GGT to predict later disease risk, but we also show variations in GGT levels among different population groups (gender, ethnic, and regional) and show evidence of temporal upward trends in population level GGT

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We hypothesize that **GGT is a marker for glutathione depletion in the liver** and that elevations in GGT reflect increased exposure to organic xenobiotics that are metabolized in the liver through glutathionylation.

We note that the population-wide level of GGT has been steadily increasing over time in the last three decades in the US and two decades in Korea. Several studies indicate this upward trend has affected other populations, including Europe, as well.

We suspect **this may be indicative of increased exposure to environmental xenobiotics, especially POPs**, as well as increased body iron burden.

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The prevalence of several other clinical symptoms are correlated with GGT, including hypertension, insulin resistance, artery calcification, and albuminuria, as well as biological markers including lipids, creatine, triglycerides, uric acid, HbA1c, and hs-CRP. In many cases, GGT is a stronger predictor of disease risk than these other symptoms and markers.

Its related disorders have risen in virtual lockstep. GGT is an early predictive marker for atherosclerosis, heart failure, arterial stiffness and plaque, gestational diabetes, and various liver diseases, including viral hepatitis, other infectious diseases, and several life-threatening cancers. We review literature both from the medical sciences and from life insurance industries demonstrating that serum GGT is a superior marker for future disease risk, when compared against multiple other known mortality risk factors.

1. Introduction

A comprehensive review by Whitfield in 2001 [1] described GGT in its traditional role as a marker of liver dysfunction, bile duct conditions, and alcohol consumption. Some generalized or summary medical and scientific literature still describe GGT in those terms [2]. However, Whitfield had already extended that description to include elevated GGT in association with risk of coronary heart disease, type-II diabetes (T2D), and stroke [1]. Although gamma-glutamyl compounds include antioxidants, inflammatory molecules, drug metabolites, and neuroreactive compounds [3], the major function of GGT is enabling metabolism of glutathione and glutathionylated xenobiotics. However, elevated GGT levels, as noted by Whitfield and others, contribute to prooxidant activity, particularly in the presence of iron or copper [4, 5]. When GGT levels are elevated, damage to red blood cell membranes can occur causing the release of

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Low antioxidant defenses are also correlated with elevated GGT, particularly reduced levels of glutathione. GGT is needed to metabolize glutathionylated xenobiotics in the liver and multiple other tissue sites including the lungs, and this is a simple explanation for its elevation in association with increased exposure to xenobiotics. GGT induces oxidative stress in the artery wall in the presence of free iron, and GGT also likely is an indicator of depleted supply of glutathione, especially in the liver, which leads to a cascade of problems related to increased oxidative stress.

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GGT + ROS

Traditional Reference Range

- * 0-65 IU/L

Optimal Reference Range

- * 12-24 IU/L (Men)
- * 10-22 IU/L (Women)

Do you Just Give them
Glutathione?

Glutathione catabolism as a signaling mechanism

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Received 31 January 2002; accepted 22 April 2002

Abstract

Glutathione (GSH) is the main intracellular thiol antioxidant, and as such participates in a number of cellular antitoxic and defensive functions. Nevertheless, *non-antioxidant* functions of GSH have also been described, e.g. in modulation of cell proliferation and immune response. Recent studies from our and other laboratories have provided evidence for a third functional aspect of GSH, i.e. the *prooxidant*

Recent studies from our and other laboratories have provided evidence for a **third functional aspect of GSH, i.e. the *prooxidant* roles played by molecular species originating during its catabolism by the membrane ectoenzyme γ -glutamyl transpeptidase (GGT)**

The prooxidant reactions induced by GSH catabolism appear to represent a novel, as yet unrecognized mechanism for modulation of cellular signal transduction.

oxidative injury. Prooxidants thus can no longer be regarded as merely offensive species, and similarly, the physiological role of some established “antioxidants” also is in need of careful reconsideration. Glutathione (GSH)—perhaps the best known cellular antioxidant—appears an ideal candidate in this perspective. The *antioxidant* role of GSH is readily apparent in detoxification of electrophilic/oxidizing drugs and protection from lipid peroxidation.

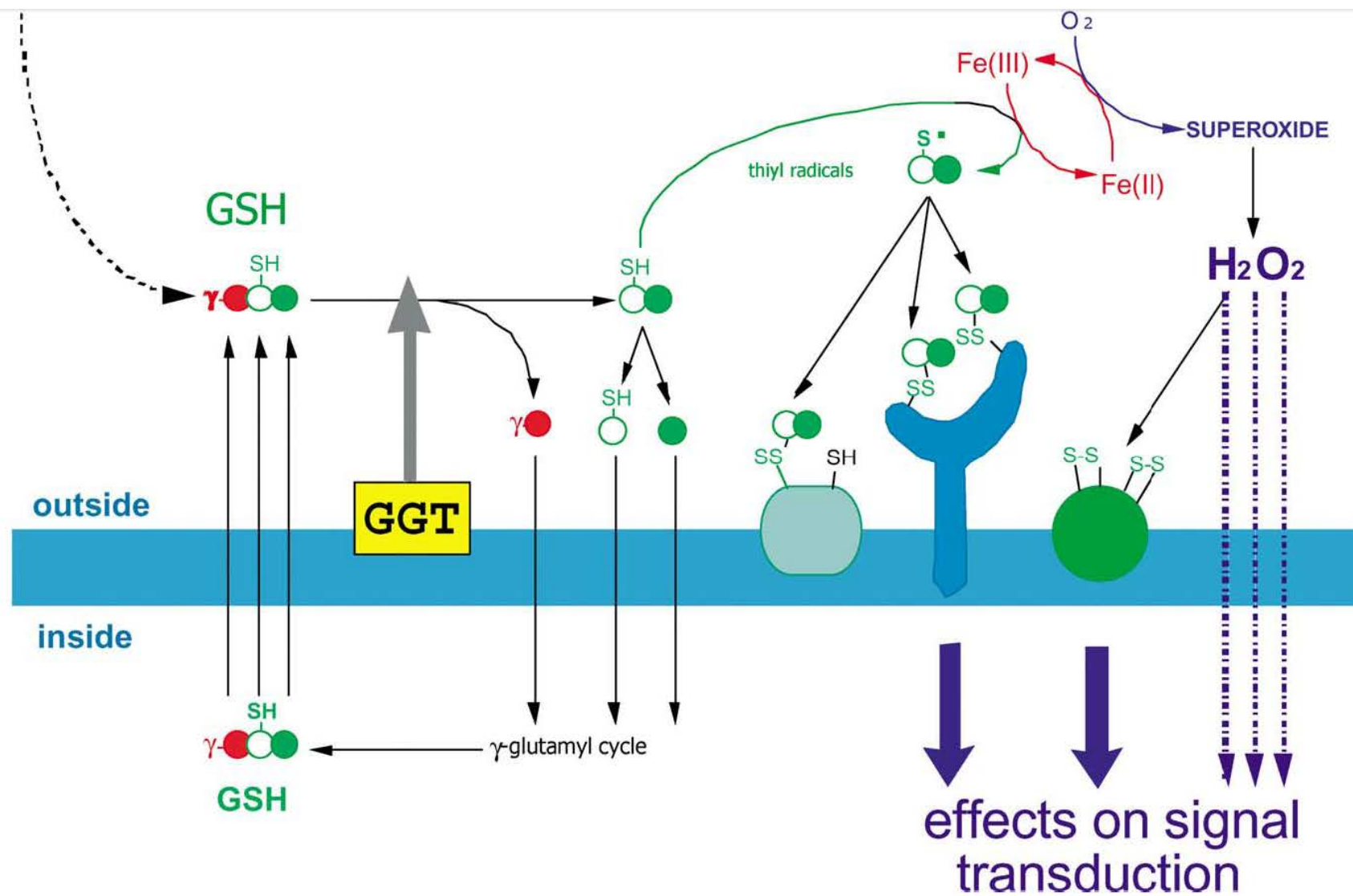
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Abbreviations: AP-1, activator protein-1; AT-125, acivicin; ECL, enhanced chemiluminescence; EMSA, electrophoresis mobility shift assay; GGT, γ -glutamyl transpeptidase; gly-gly, glycyl-glycine; GSH, glutathione; GSH-DME, glutathione dimethyl ester; NF- κ B, nuclear factor- κ B; PARP, poly(ADP-ribose) polymerase; PE, protein phosphatase; ROS, reactive oxygen species; TNFR1, tumor necrosis factor- α receptor 1.

has in fact been documented in our and other laboratories that prooxidant species (superoxide, H_2O_2 , thiol radicals) are produced during GSH catabolism, as a result of the interaction of GSH metabolites—cysteinyl-glycine in the first place—with trace levels of iron ions present in the cell environment. The interaction of these GSH/GGT-derived prooxidants several intra- and extracellular targets is responsible for appreciable modulatory effects on the signal transduction chains.

1. GSH, GGT and iron reduction

γ -Glutamyl transpeptidase (E.C. 2.3.2.2) is normally found in serum, and is expressed by a wide range of normal cell types [1,2] as well as in a number of neoplastic cell



Glutathione

- * GGT produces cysteinyl glycine as a product of the decomposition of glutathione, and this dipeptide reacts with free iron to induce the Fenton reaction and subsequent production of superoxide, a well-established reactive oxygen species (ROS).

Glutathione

- * Elevated GGT levels, contribute to prooxidant activity, particularly in the presence of iron or copper.
- * When GGT levels are elevated, damage to red blood cell membranes can occur causing the release of these potentially toxic transition metals, which can further result in chain, prooxidant reactions.

Glutathione and Curcumin

Supplement Facts

Serving Size: 2 pumps (1mL)
Servings Per Container: 50

	Amount per Serving	% Daily Value
Sodium	7mg	0.3%
Reduced Glutathione	100mg	†
Phosphatidylcholine (from purified sunflower lecithin)	84mg	†

† Daily Value not established.

Other Ingredients: Water, Glycerin, Ethanol, Vitamin E (as d-alpha tocopheryl polyethylene glycol 1000 succinate), essential oils



RESEARCH ARTICLE

Curcumin Attenuates Iron Accumulation and Oxidative Stress in the Liver and Spleen of Chronic Iron-Overloaded Rats

Farid A. Badria^{1*}, Ahmed S. Ibrahim², Adel F. Badria^{3,4}, Ahmed A. Elmarakby⁵¹ Department of Pharmacognosy, Faculty of Pharmacy, Mansoura University, Mansoura 35516, Egypt,² Department of Biochemistry and Clinical Biochemistry, Faculty of Pharmacy, Mansoura University,

Our study suggests that **curcumin may represent a new horizon in managing iron overload-induced toxicity** as well as in pathological diseases characterized by hepatic iron accumulation such as thalassemia, sickle cell anemia, and myelodysplastic syndromes, **reduced oxidative stress derived lipid peroxide, possibly via iron chelation** and improving the body endogenous antioxidant defense mechanism.

Although Zn^{2+} showed little binding affinity to curcumin, Cu^{2+} and Fe^{2+} appeared to bind at least two curcumin molecules.

This raises the possibility that curcumin could provide beneficial antioxidant effects during chronic iron overload beyond its role as an iron-chelating agent, such as the possession of antioxidant property.

Funding: The authors received no specific funding for this work.

Competing Interests: The authors have declared that no competing interests exist.

cantly decreased in chronic iron overload and administration of curcumin restored the decrease in the hepatic and splenic antioxidant activities/levels.

Glutathione and Curcumin

Supplement Facts

Serving Size: 2 pumps (1mL)
Servings Per Container: 50

	Amount per Serving	% Daily Value
Sodium	7mg	0.3%
Reduced Glutathione	100mg	†
Phosphatidylcholine (from purified sunflower lecithin)	84mg	†

† Daily Value not established.

Other Ingredients: Water, Glycerin, Ethanol, Vitamin E (as d-alpha tocopheryl polyethylene glycol 1000 succinate), essential oils



Gamma-Glutamyltransferase (GGT)

Traditional Reference Range

- * 0-65 IU/L

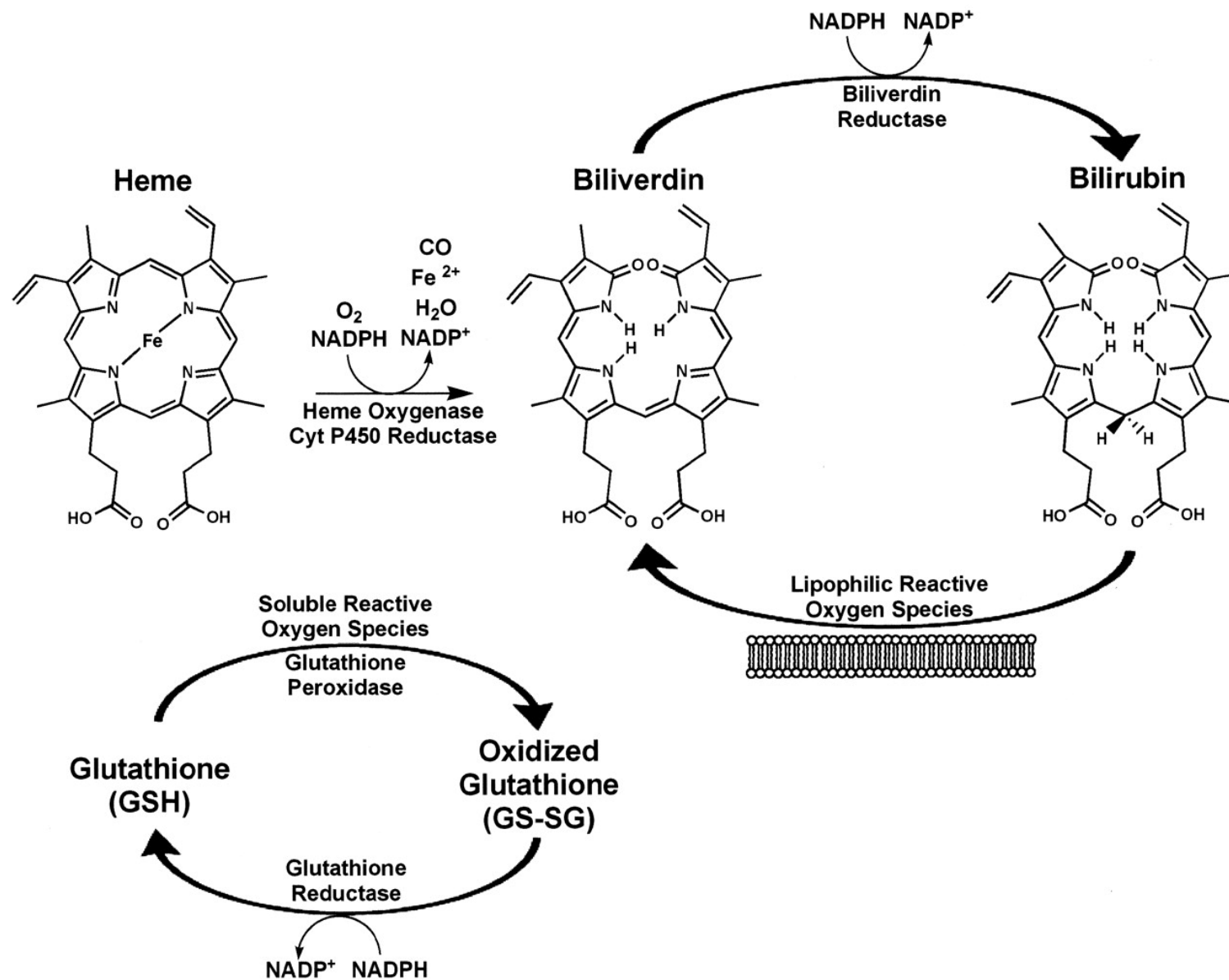
Optimal Reference Range

- * 12-24 IU/L (Men)
- * 10-22 IU/L (Women)

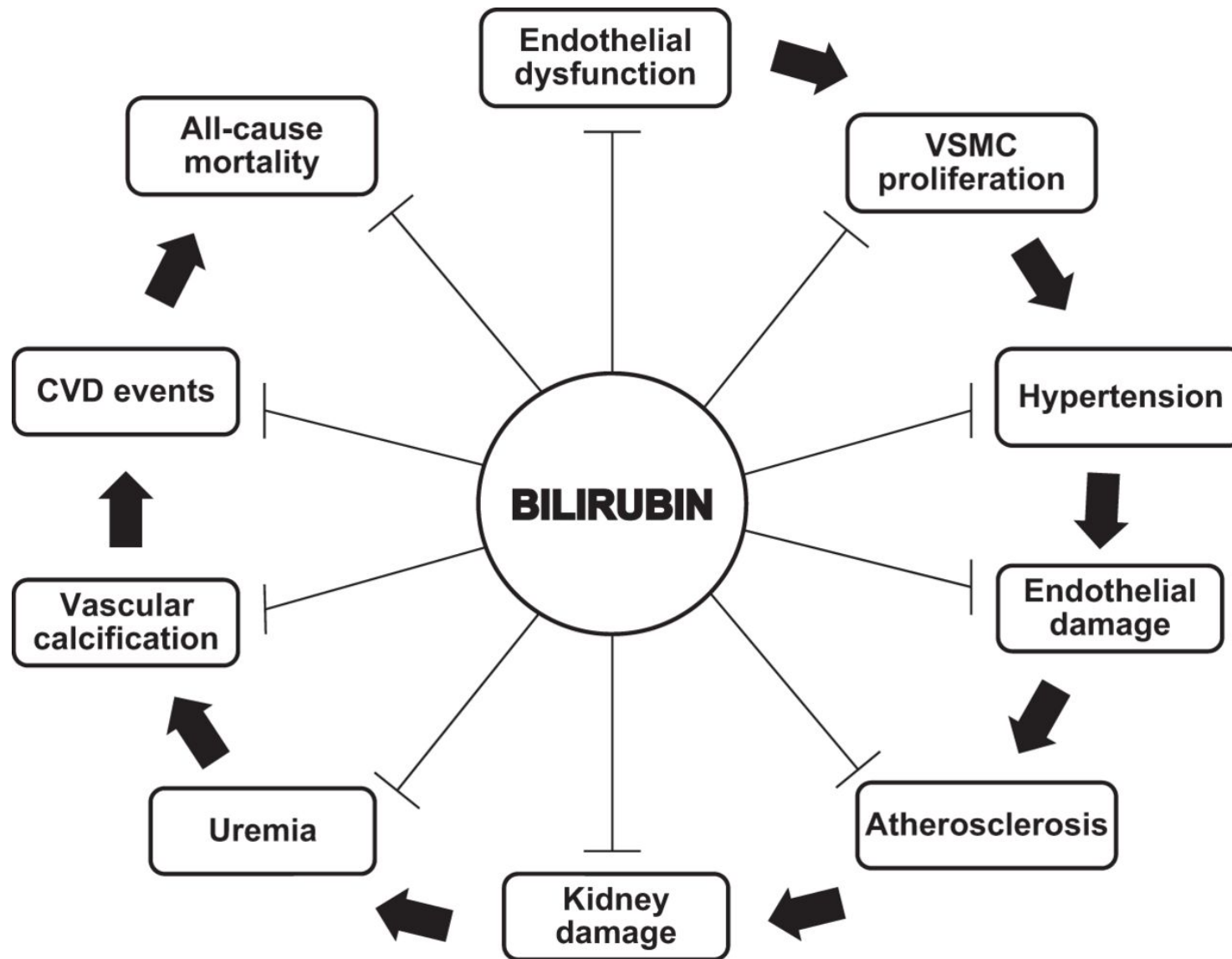
Bilirubin

Bilirubin

- * Bilirubin also functions as an antioxidant and thus may be decreased during oxidative stress
- * Bilirubin appears to be the most potent antioxidant against lipid peroxides
 - * Glutathione is hydrophilic
- * Because of this, low bilirubin has also been associated with increased risk of cardiovascular disease and all cause mortality (death from all causes). Elevated levels may increase mortality as well.
- * Bilirubin may also increase insulin sensitivity and protect against future diabetes



Sedlak, Thomas W., and Solomon H. Snyder. 2004. "Bilirubin Benefits: Cellular Protection by a Biliverdin Reductase Antioxidant Cycle." *Pediatrics* 113 (6): 1776–82.



Boon, Ai-Ching, Andrew C. Bulmer, Jeff S. Coombes, and Robert G. Fasset. 2014. "Circulating Bilirubin and Defense against Kidney Disease and Cardiovascular Mortality: Mechanisms Contributing to Protection in Clinical Investigations." *American Journal of Physiology - Renal Physiology* 307 (2): F123–36. doi:10.1152/ajprenal.00039.2014.

Serum total bilirubin is inversely associated with brachial-ankle pulse wave velocity in men with hypertension

Zheng-Yun Zhang · Lu-Qin Bian · Sae-Young Jae · Ji-Dong Sung · Yoon-Ho Choi

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Abstract Serum total bilirubin has been suggested to have the potential anti-inflammatory and antioxidant effects on the vasculature. This study was designed to investigate the association of bilirubin with brachial-ankle pulse wave velocity (baPWV), a marker of arterial stiffness and cardiovascular disease. Hypertensive male subjects ($n = 2,361$) were classified into groups according to the 50th, 75th, and 95th percentiles of baPWV value. Correlation and regression analysis were used to assess the relationship between baPWV and other variables. Hypertensive subjects with baPWV above the 50th, 75th, and 95th percentiles had a significantly lower bilirubin level than those with baPWV under them (0.97 ± 0.40 vs. 1.00 ± 0.41 mg/dL, $P < 0.001$; 0.95 ± 0.39 vs. 0.99 ± 0.41 mg/dL, $P = 0.001$; 0.92 ± 0.36 vs. 0.99 ± 0.42 mg/dL, $P = 0.048$, respectively). Bilirubin is inversely related to

baPWV ($R^2 = 0.0032$, $P = 0.003$) and C-reactive protein (CRP) (correlation coefficient = -0.13 , $P < 0.001$). A 0.1 mg/dL increase in bilirubin was associated with a 19, 20, and 34 % reduced odds ratio for baPWV above the 50th, 75th, and 95th percentiles, respectively [odds ratio (OR) 0.77 (95 % confidence interval (CI) 0.62–0.95), $P = 0.015$; OR 0.80 (95 % CI 0.64–0.99), $P = 0.044$; OR 0.68 (95 % CI 0.45–1.00), $P = 0.048$, respectively] after adjustment for several variables. This study demonstrates an independent inverse association between bilirubin and baPWV in hypertensive men. Additionally, reduced CRP may be one of mediators on the mechanisms how bilirubin reduces baPWV.

Keywords Serum total bilirubin · C-reactive protein (CRP) · Brachial-ankle pulse wave velocity (baPWV) · Arterial stiffness · Hypertension

Z.-Y. Zhang and L.-Q. Bian contributed equally to the work.

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Impact of serum bilirubin levels on carotid atherosclerosis in patients with coronary artery disease

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ABSTRACT

Background/objectives: Bilirubin protects against oxidative stress-mediated diseases, especially atherosclerotic diseases. On the other hand, subjects with carotid atherosclerosis have a high incidence of adverse cardiovascular events. The aim of this study was to evaluate the possible relationship between serum bilirubin levels and carotid atherosclerosis in patients with coronary artery disease (CAD).
Methods: We evaluated a total of 394 patients with chronic CAD, defined as stable angina pectoris or a previous myocardial infarction. They were divided into four groups according to serum bilirubin level. Carotid intima-media thickness and plaque score (PS) in the common carotid artery were measured using an ultrasound system. Severe carotid atherosclerosis was defined as PS > 10.
Results: With increasing quartiles of serum bilirubin levels, the prevalence of severe carotid atherosclerosis significantly decreased (48.2%, 39.8%, 30.3%, and 27.0%, respectively, p for trend = 0.007). After adjusting for other risk factors, low serum bilirubin levels were independently correlated with severe carotid atherosclerosis in CAD patients (odds ratio 0.89, 95% confidence interval, 0.81–0.99, $p = 0.027$).
Conclusion: We demonstrated that low serum bilirubin levels were associated with severe carotid atherosclerosis in CAD patients. Our data suggest that serum bilirubin levels might be an independent, useful, and cost-effective tool for evaluating atherosclerotic status in CAD patients.
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1. Introduction

It is commonly accepted that bilirubin, the major product of heme catabolism, has strong antioxidant properties that enable it to scavenge peroxyl radicals and to inhibit oxidation of low-density lipoprotein-derived lipids [1]. Several recent reports have demonstrated that elevated serum bilirubin levels provide important protective effects against oxidative stress-mediated diseases, especially atherosclerotic diseases [2–4].

Increased carotid intima-media thickness (IMT) is a well-established index of subclinical atherosclerosis and a risk factor for subsequent cardiovascular disease (CVD) events [5–7]. It was recently reported that greater carotid atherosclerosis is also associated with future CVD events in high-risk patients [8,9]. Therefore, evaluation of carotid

atherosclerosis could be helpful in predicting future CVD events in patients with chronic ischemic heart disease. However, few studies have investigated the relationship between carotid atherosclerosis and serum bilirubin levels in CAD patients. In the present study, we examined the association between serum bilirubin levels and carotid atherosclerosis in CAD patients.

2. Methods

2.1. Study subjects

This observational study included a total of 394 patients who were treated for chronic CAD at Nagoya University Hospital between October 2011 and December 2013. Chronic CAD was defined as stable angina pectoris or a previous myocardial infarction. Patients were divided into four groups according to quartiles of total bilirubin levels. The exclusion criteria were as follows: acute cardiovascular events within 6 months before screening, previous carotid endarterectomy or carotid artery stenting, chronic liver disease, acute heart failure, and hemodialysis. Institutional ethics committee approval was obtained, and all patients

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¹ All authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

The Relationship between Total Bilirubin Levels and Total Mortality in Older Adults: The United States National Health and Nutrition Examination Survey (NHANES) 1999-2004



Kwok-Leung Ong^{1,2*}, Matthew A. Allison³, Bernard M. Y. Cheung⁴, Ben J. Wu^{1,2}, Philip J. Barter^{1,2}

Total bilirubin, mg/dl	Model 1 ^a		Model 2 ^b		Model 3 ^c		Model 4 ^d	
	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
Multicategory model A								
0.1–0.4	1.57 (1.14–2.16)	0.007	1.53 (1.06–2.20)	0.024	1.68 (1.21–2.32)	0.002	1.51 (1.09–2.09)	0.014
0.5	1.33 (0.98–1.79)	0.063	1.13 (0.81–1.59)	0.45	1.18 (0.83–1.68)	0.34	1.19 (0.85–1.68)	0.31
0.6	1.00 (referent)		1.00 (referent)		1.00 (referent)		1.00 (referent)	
0.7	1.03 (0.80–1.33)	0.81	1.05 (0.79–1.39)	0.73	1.06 (0.76–1.49)	0.73	1.18 (0.86–1.61)	0.30
0.8–0.9	1.09 (0.86–1.38)	0.46	1.16 (0.90–1.50)	0.25	1.18 (0.87–1.59)	0.27	1.35 (1.00–1.83)	0.051
≥1.0	0.98 (0.69–1.39)	0.90	1.20 (0.84–1.73)	0.31	1.18 (0.80–1.73)	0.40	1.38 (0.98–1.94)	0.061
Overall P		0.10		0.14		0.013		0.022
Multicategory model B								
0.1–0.4	1.39 (1.09–1.78)	0.010	1.44 (1.10–1.88)	0.009	1.55 (1.23–1.96)	<0.001	1.36 (1.07–1.72)	0.012
0.5–0.7	1.00 (referent)		1.00 (referent)		1.00 (referent)		1.00 (referent)	
≥0.8	0.96 (0.79–1.18)	0.70	1.12 (0.91–1.39)	0.28	1.11 (0.89–1.37)	0.36	1.24 (0.98–1.56)	0.072
Overall P		0.030		0.031		0.002		0.008

HR = hazard ratio; CI = confidence interval.

^aAdjusted for survey period, age, sex, and race/ethnicity (n = 4,303).

^bFurther adjusted for body mass index, education, smoking, and regular alcohol consumption (n = 3,928).

^cFurther adjusted for history of cardiovascular disease, diabetes, albuminuria, cancer, fibrates, angiotensin-converting enzyme inhibitors/angiotensin receptor blockers, diuretics, and calcium channel blockers (n = 3,764).

^dFurther adjusted for high-density lipoprotein cholesterol, serum albumin, blood urea nitrogen, estimated glomerular filtration rate, C-reactive protein, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, γ -glutamyltransferase, uric acid, white blood cell count, and hemoglobin (n = 3,758).

doi:10.1371/journal.pone.0094479.t003

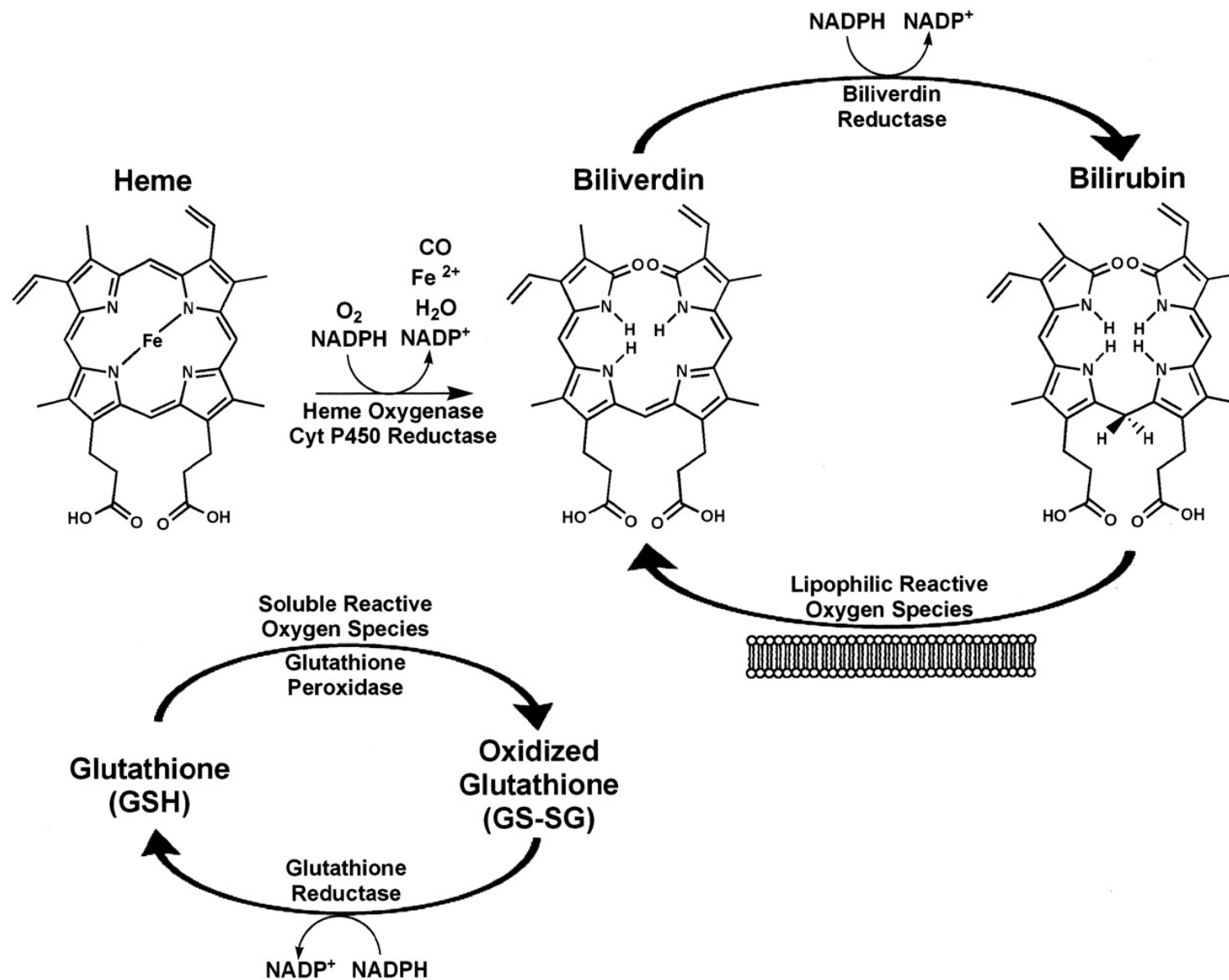
Bilirubin

Traditional Reference Range

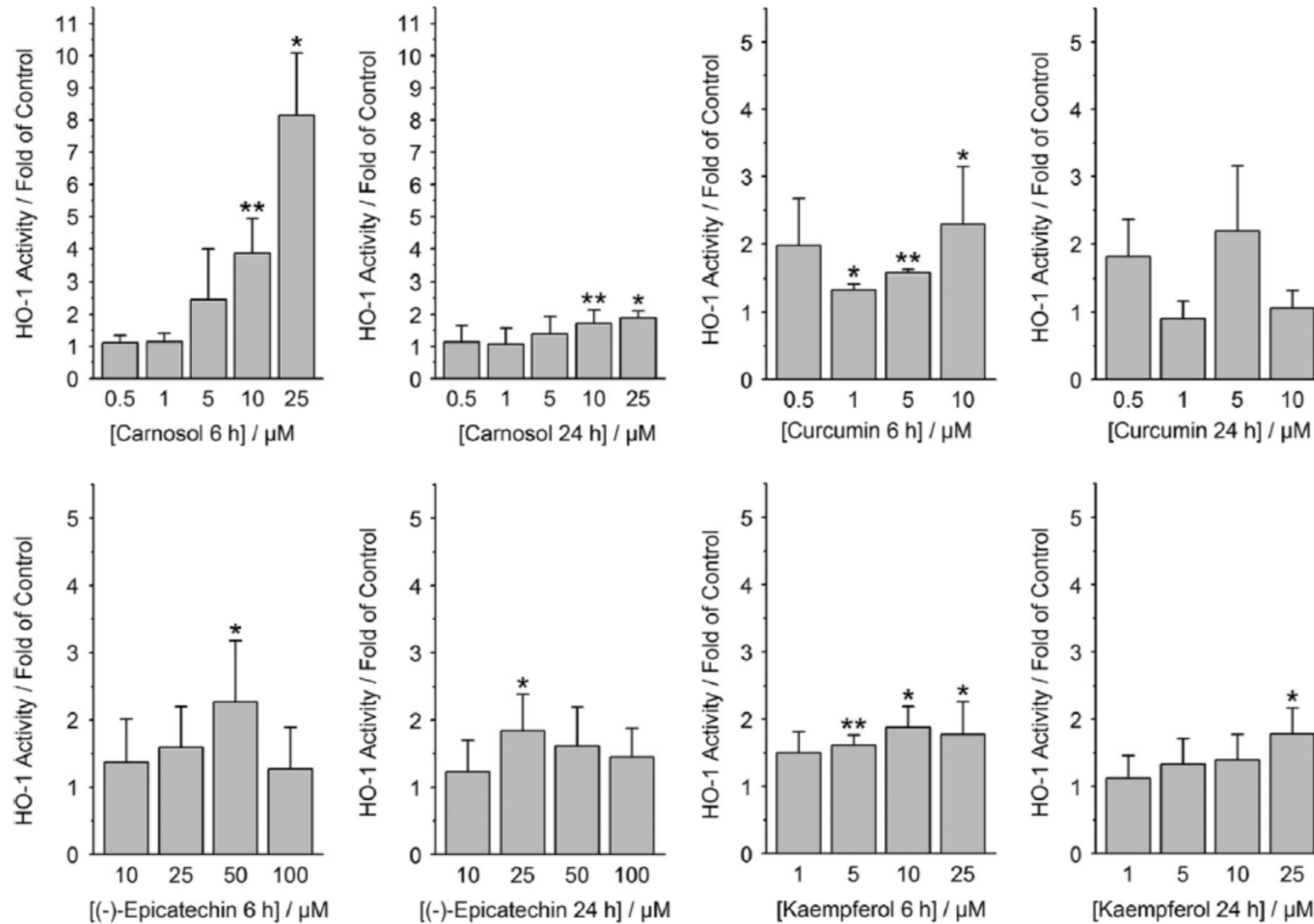
- * 0.1-1.2 mg/dL

Optimal Reference Range

- * 0.5 – 0.8 mg/dL



Sedlak, Thomas W., and Solomon H. Snyder. 2004. "Bilirubin Benefits: Cellular Protection by a Biliverdin Reductase Antioxidant Cycle." *Pediatrics* 113 (6): 1776–82.



Curcumin protects retinal pigment epithelial cells against oxidative stress via induction of heme oxygenase-1 expression and reduction of reactive oxygen

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At this concentration, curcumin also increased the cytoprotective effect against the oxidative stress of H₂O₂ through the reduction of ROS levels in human retinal pigment epithelial cells. **Curcumin's effect on the reduction of ROS was mediated by the increase in HO-1 expression.**

of ROS was mediated by the increase in HO-1 expression.

Conclusions: Curcumin upregulated the oxidative stress defense enzyme HO-1 and may protect human retinal pigment epithelial cells against oxidative stress by reducing ROS levels.

Age-related macular degeneration (AMD) is the most common cause of blindness in patients aged 65 or over in the Western world [1], and incidence continues to rise as a result of the increasing percentage of older adults in the general population. Pathologically, AMD results from retinal pigment epithelium (RPE) dysfunction or loss associated with photoreceptor fallout, Bruch's membrane thickening, and choriocapillary hypoperfusion [2]. The RPE is a monolayer of pigmented cells forming part of the blood retina barrier and is particularly susceptible to oxidative stress because of the layer's high consumption of oxygen. Thus, chronic oxidative stress induces RPE damage that is responsible for the aging process and may therefore play an important role in the pathogenesis of AMD [3,4]. Human RPE has many antioxidative enzymes such as superoxide dismutase, heme oxygenase, and enzymes involved in glutathione synthesis [5,6]. Heme oxygenase-1 (HO-1) is a ubiquitous and redox-sensitive inducible stress protein known to protect cells against various types of stress. The importance of this protein in physiologic and pathological states is underlined by the

versatility of HO-1 inducers and the protective effects attributed to heme oxygenase byproducts in conditions associated with moderate or severe cellular stress [7,8].

Curcumin, a biologically active component of turmeric, which has been used in India for medical purposes for centuries, has a variety of pharmacological activities, including antioxidant, anti-inflammatory, and antiproliferative effects. Curcumin is an effective scavenger of reactive oxygen species in vitro and indirectly enhances the synthesis of antioxidative enzymes [9,10]. In this study, we hypothesized that curcumin has cytoprotective effects with HO-1 expression against H₂O₂ oxidative stress in cultured human retinal pigment epithelial cells.

METHODS

Materials: Curcumin, H₂O₂, zinc protoporphyrin (ZnPP; HO-1 inhibitor), cobalt protoporphyrin (CoPP; HO-1 stimulator), and SB 203580 were purchased from Sigma Aldrich (St. Louis, MO). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and 2',7'-dichlorodihydro-fluorescein diacetate (H2DCFDA) were obtained from Invitrogen Molecular Probes, Inc. (Carlsbad, CA).

Cell culture: ARPE-19 cells originated from human retinal pigment epithelial cells. The ARPE cells were purchased from

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Combined Markers



Clinical significance of serum bilirubin and gamma-glutamyltransferase levels on coronary atherosclerosis assessed by multidetector computed tomography

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Low TB and high GGT levels were concomitantly associated with coronary atherosclerosis in Korean men.

Keywords:
Atherosclerosis;
Coronary artery
calcification score;
Coronary artery
stenosis

Methods and results: A cross-sectional analysis was performed on 1520 subjects who underwent multidetector computed tomography scans. Coronary atherosclerosis was assessed by coronary artery calcium score (CACS) and obstructive coronary artery disease (OCAD), was defined as the presence of coronary artery stenosis of $\geq 50\%$. Total bilirubin (TB) level was negatively correlated with CACS and coronary stenosis whereas GGT level was positively correlated with CACS in men. However, there was no correlation between TB, GGT levels and either CACS or coronary artery stenosis in women. In a multivariate-adjusted model, TB level was inversely associated with a CACS > 100 [odds ratio (OR) per log standard deviation (SD), 0.67; 95% confidence interval (CI), 0.52–0.87], and OCAD (OR per log SD, 0.77; 95% CI, 0.62–0.95) in men. By contrast, GGT level was positively associated with a CACS > 100 (OR per log SD, 1.35; 95% CI, 1.05–1.73) but not with OCAD. Adding TB and GGT to the conventional risk factors increased predictive accuracy for CACS > 100 (net reclassification improvement index [NRI] = 13.1%, $P = 0.026$; integrated discrimination index [IDI] = 0.024, $P = 0.001$) and for OCAD (NRI = 12.6%, $P = 0.026$; IDI = 0.010, $P = 0.013$).

Conclusions: Low TB and high GGT levels were concomitantly associated with coronary atherosclerosis in Korean men. Future studies are needed to elucidate the causal associations of TB and GGT with CVD.

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Introduction

Cardiovascular disease (CVD) is the leading cause of death worldwide and has become a major health issue due to its

increasing prevalence. Interestingly, the prevalence of CVD in Asia has been reported to be similar to or even higher than that in Europe despite of comparatively lower average body mass index (BMI) [1]. The reason for this may

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ORIGINAL ARTICLE

Table 2. Plasma oxysterols and some serum antioxidants in the healthy Italian and Czech subjects.

	Italian (n=138)	Czech (n=84)	<i>P</i> -value
7OxCH (ng/ml)	7.3 5.3–11	4.0 3.3–5.2	<10 ⁻⁶
7OxCH/total cholesterol	1.59 1.3–2.6	0.76 0.59–0.94	<10 ⁻⁶
7BCH (ng/ml)	10.5 7.7–19.7	6.0 5.3–7.4	<10 ⁻⁶
7BCH/total cholesterol	2.28 1.6–4.3	1.15 0.99–1.3	<10 ⁻⁶
PERSA*	5.75 4.8–6.90	6.95 5.7–8.5	<10 ⁻⁶
Bilirubin (μmol/l)	7.2 5.1–10.2	11.3 8.6–15.6	<10 ⁻⁶
Uric acid (μmol/l)	271 215–329	324 252–364	<10 ⁻⁶
Vitamin E/total cholesterol	0.13 0.11–0.17	0.14 0.1–0.19	0.612

7OxCH: 7-oxo-cholesterol; 7BCH: 7β-hydroxycholesterol; PERSA: peroxy radical scavenging activity. Bilirubin means total bilirubin concentration. Data expressed as median and 25–75%; * values represent micromoles of Trolox equivalents.

The Relationship Between Gamma-Glutamyltransferase (GGT), Bilirubin (Bil) and Small Dense Low-Density Lipoprotein (sdLDL) in Asymptomatic Subjects Attending a Clinic for Screening Dyslipidaemias

Kazuhiko Kotani,^{1,2} MD, PhD, Kokoro Tsuzaki,¹ BSc, Naoki Sakane,¹ MD, PhD

Abstract

Introduction: Gamma-glutamyltransferase (GGT), bilirubin (Bil) and small dense low-density lipoprotein (sdLDL) particles are each known to be risk markers for cardiometabolic diseases which are characterised by oxidative stress conditions. These markers are connected with the oxidative milieu; however, the association between GGT,

The mean (standard deviation) levels of GGT, Bil, and the mean LDL particle size were found to be 21.7 (8.3) IU/L, 14.0 (4.3) μ mol/L, and 26.7 (0.6) nm, respectively. An univariate correlation test showed both a significant inverse correlation between the mean LDL particle size and GGT and a significant positive correlation between the mean LDL particle size and Bil.

Ann Acad Med Singapore 2014;43:216-9

Key words: Atherosclerosis, LDL particle size, Oxidative stress, γ GT, Total bilirubin

Introduction

Oxidative stress, which is caused by an oxidant-antioxidant imbalance, is an emerging risk factor for the development of cardiovascular disease (CVD), a crucial health problem in Western countries, Asia and other developing countries.¹ Therefore, understanding oxidative stress conditions and developing a therapeutic approach to limit the effects of oxidative stress are crucial for the prevention of CVD.^{2,3} Recently, 2 serum markers, gamma-glutamyltransferase (GGT) and bilirubin (Bil), which are reflective of hepatic function in general, have been recognised as oxidative stress and antioxidative markers.⁴⁻¹⁰ In fact, GGT and Bil levels are shown to be associated with the development of cardiometabolic diseases,⁴⁻¹⁰ while the underlying mechanisms of the association between GGT and CVD,

as well as the underlying mechanisms of the association between Bil and CVD, are not yet sufficiently established.

The presence of small, dense low-density lipoprotein (sdLDL) particles, which exhibit both a small LDL particle size and a greater susceptibility to oxidation, in the blood is considered to be a risk marker of CVD.^{11,12} We have noted that subjects with insulin resistance and metabolic syndrome (oxidative stress conditions that are often concomitantly accompanied by hepatic dysfunction) display an atherogenic lipoprotein profile that includes the presence of sdLDL particles.¹³⁻¹⁵ Subjects with metabolic syndrome can have high GGT levels and low Bil levels.^{6,7,16} Circulating GGT is also thought to bind to lipoproteins, including LDL particles.⁷ In addition, a few studies indicate that the blood

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Bottom Line - What To Do

- * Uric acid < 3.7 mg/dL (men) or 3.2 mg/dL (women)
 - * Consider deficiencies (molybdenum, zinc, iron)
 - * Glutathione (BioG Max – GSH) and/or antioxidant therapy
- * GGT > 12-24 IU/L (Men) or 10-22 IU/L (Women)
 - * Glutathione and curcumin
- * Bilirubin < 0.5 mg/dL
 - * Curcumin, ECGC, rosemary, quercitin

Thyroid



Thyroid

- * “Shield” gland
- * Normally 6-20 gram in weight (non-goitrous)
 - * Depends on body size and iodine supply
- * Abundant blood supply with approximately 5ml/g/minute
 - * Entire blood supply travels through the thyroid per hour
 - * Even at only .4% of body weight, it receives approximately 2% of blood flow

Thyroid Hormone Function

- * Basal metabolic rate
 - * Thermogenesis (calorigenesis), or O₂ consumption and energy production
 - * Encourages synthesis of certain enzymes within the CAC
- * Essential to normal growth, development and metabolism

Thyroid

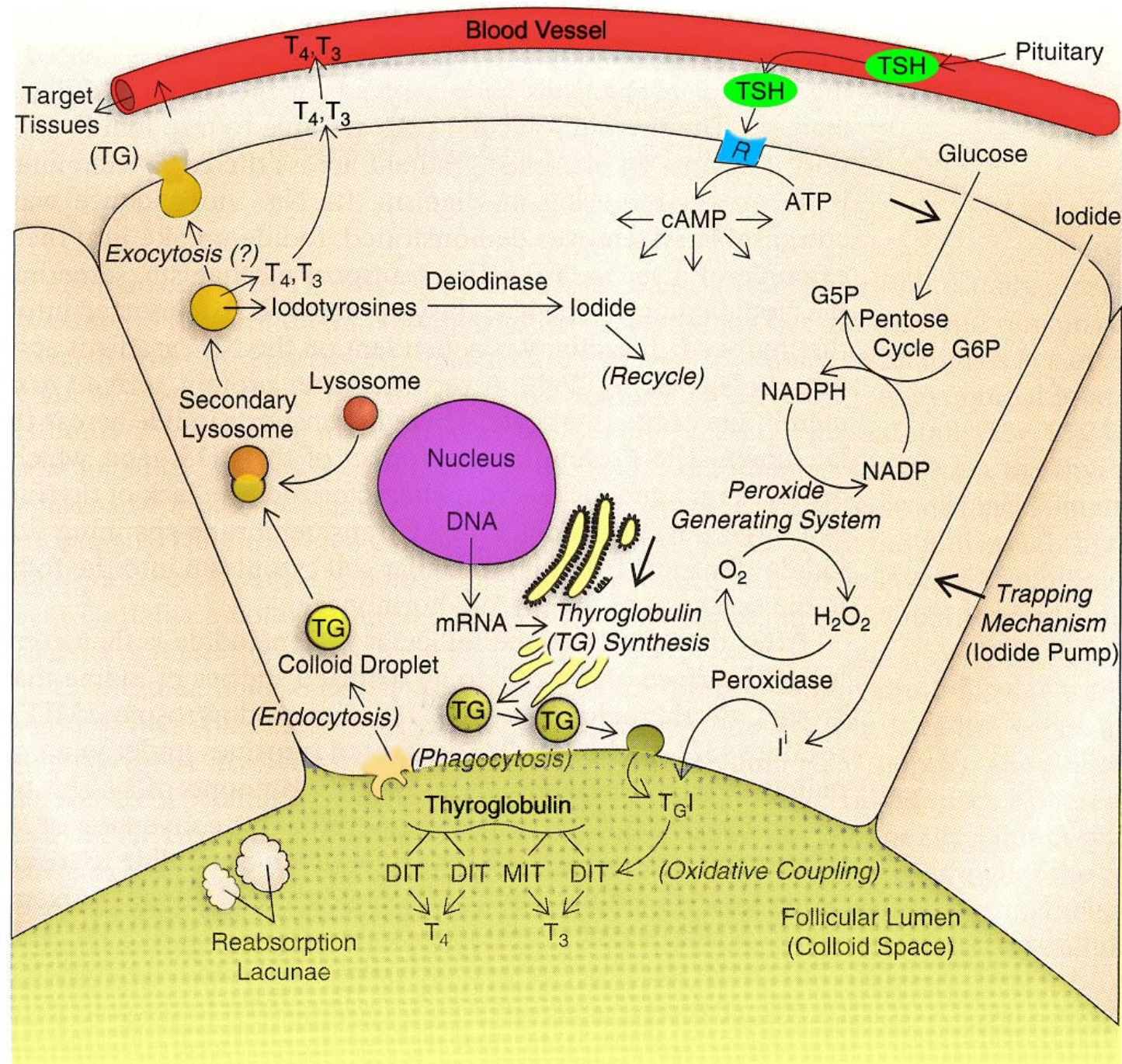
- * Consists of *follicles* and “C cells”
 - * C cells are responsible for calcitonin production, which aids in lowering plasma calcium levels when elevated
- * Follicles
 - * Simple cuboidal cells (thyrocytes) surrounding colloid (“glue like”)
 - * Primary secretory cells of the thyroid gland
 - * Colloid is largely a mixture of proteins suspended in a gelatinous solution
 - * Free fatty acids are the primary fuel source for follicular cells
 - * Does use glucose for the pentose phosphate pathway
 - * Needs reducing agents (NADPH) for H_2O_2 , glutathione peroxidase, thyroid hormone synthesis, organification of iodide, etc

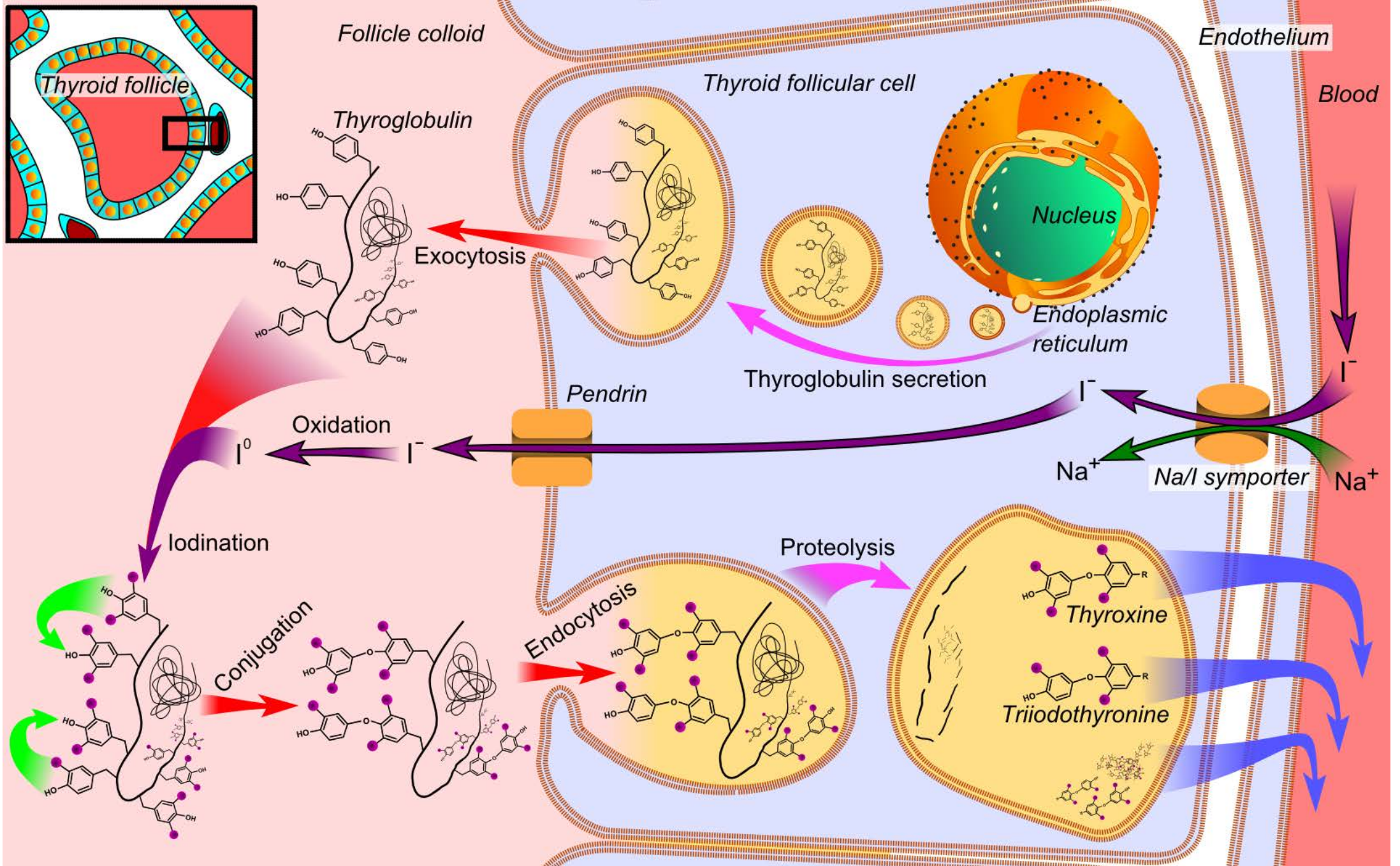
Thyroid Hormone Synthesis

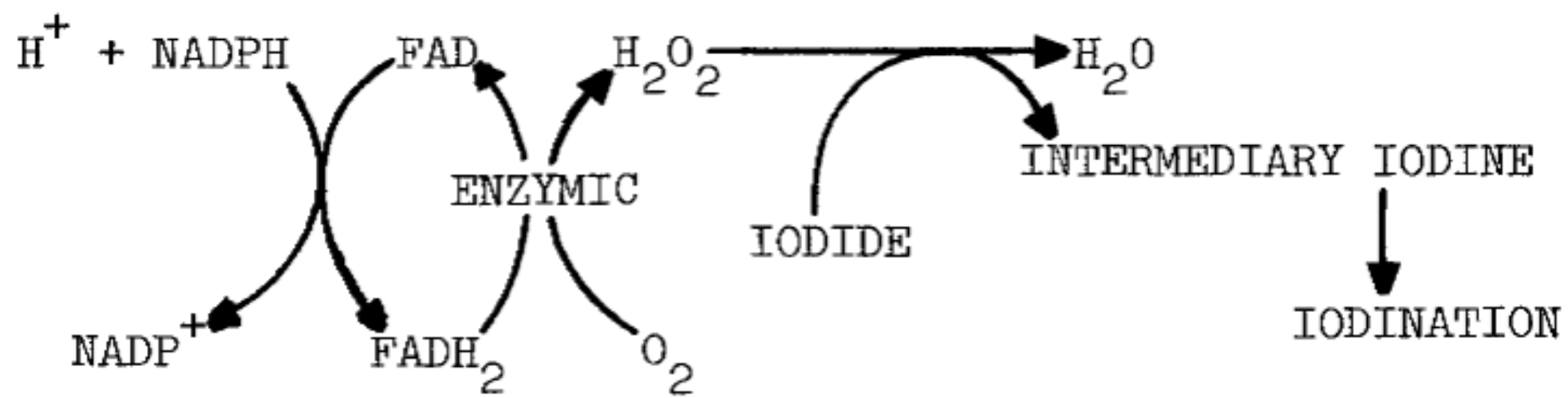
1. Iodine trapping
 - * Dietary iodine is converted to iodide in intestines
 - * Iodide (anion) is transported into follicular cells via Na/I symport (co-transport). This is increased under influence of TSH
2. Thyroglobulin
 - * Large polypeptide (5,000 amino acids) with 3% tyrosine
 - * Synthesized on ribosomes in response to TSH
3. Thyroid peroxidase oxidizes iodide (oxidation) and attaches iodine to tyrosine residues on thyroglobulin (iodination)
 - * Uses H_2O_2 to oxidize iodine
 - * Forms MIT (one iodine) and DIT (two iodines)

Thyroid Hormone Synthesis

4. MIT and DIT still on thyroglobulin, return back into follicular cell via endocytosis
5. MIT and DIT are joined together
 - * $\text{MIT} + \text{DIT} = \text{T3}$ (triiodothyronine)
 - * $\text{DIT} + \text{DIT} = \text{T4}$ (tetraiodothyronine or thyroxine)
6. Lysosome (proteolytic enzymes) cleaves off free T3 and T4, which leaves follicular cell via exocytosis
7. Free thyroid hormone is bound primarily to plasma proteins for transport in the circulatory system







Thyroid Hormone Transport

Functions

1. Protect body from excessive hormone fluctuations
 - * Eg. Bound thyroid hormone allows a significant drop in hormone secretion without significantly impacting serum hormone levels
2. Iodine binding to minimize loss in humans
 - * Limited iodine supply in diet
3. Targeting hormone delivery via site specific enzymatic modification of thyroid binding globulin

Thyroid hormone transport

1. Thyroid Binding Globulin (highest affinity)
 - * 5 day half-life
 - * 75% of T4 and 75% of T3
 - * Salicylates competitively bind
 - * Estrogen increases levels, androgens (eg testosterone) decrease levels
2. Transthyretin
 - * 2 day half-life
 - * 20% of T4 and <5% of T3
 - * Flavonoids may competitively bind
3. Albumin (lowest affinity)
 - * 15 day half-life
 - * 5% of T4 and 20% of T3
 - * Fatty acids and chloride ions may competitively bind
 - * Fluctuations are said to have little effect on serum thyroid hormone levels

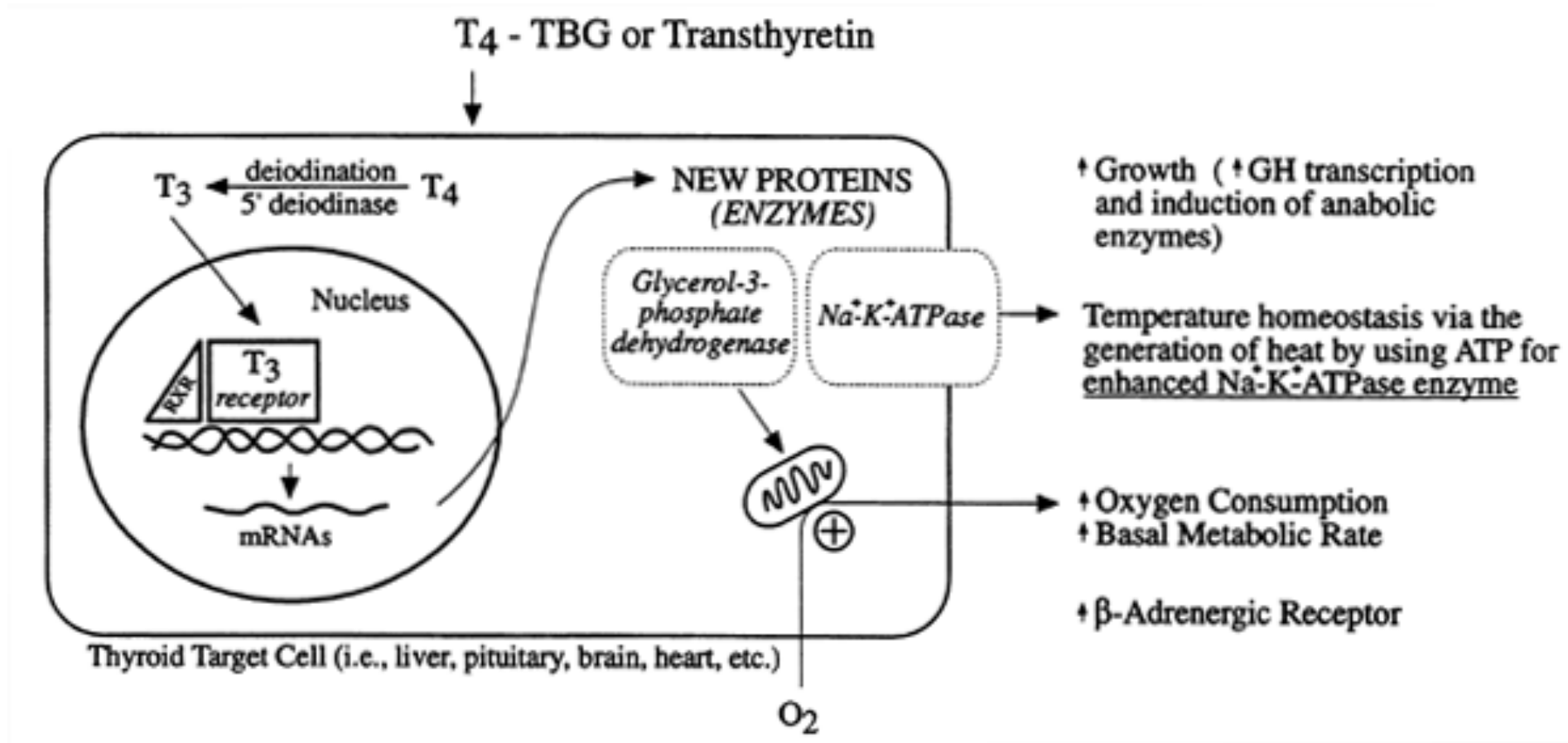
Thyroid Hormone Transport

In humans, .03% of T4 and .3% of T3 are found in the free (unbound) form.

Thyroid Hormone Conversion

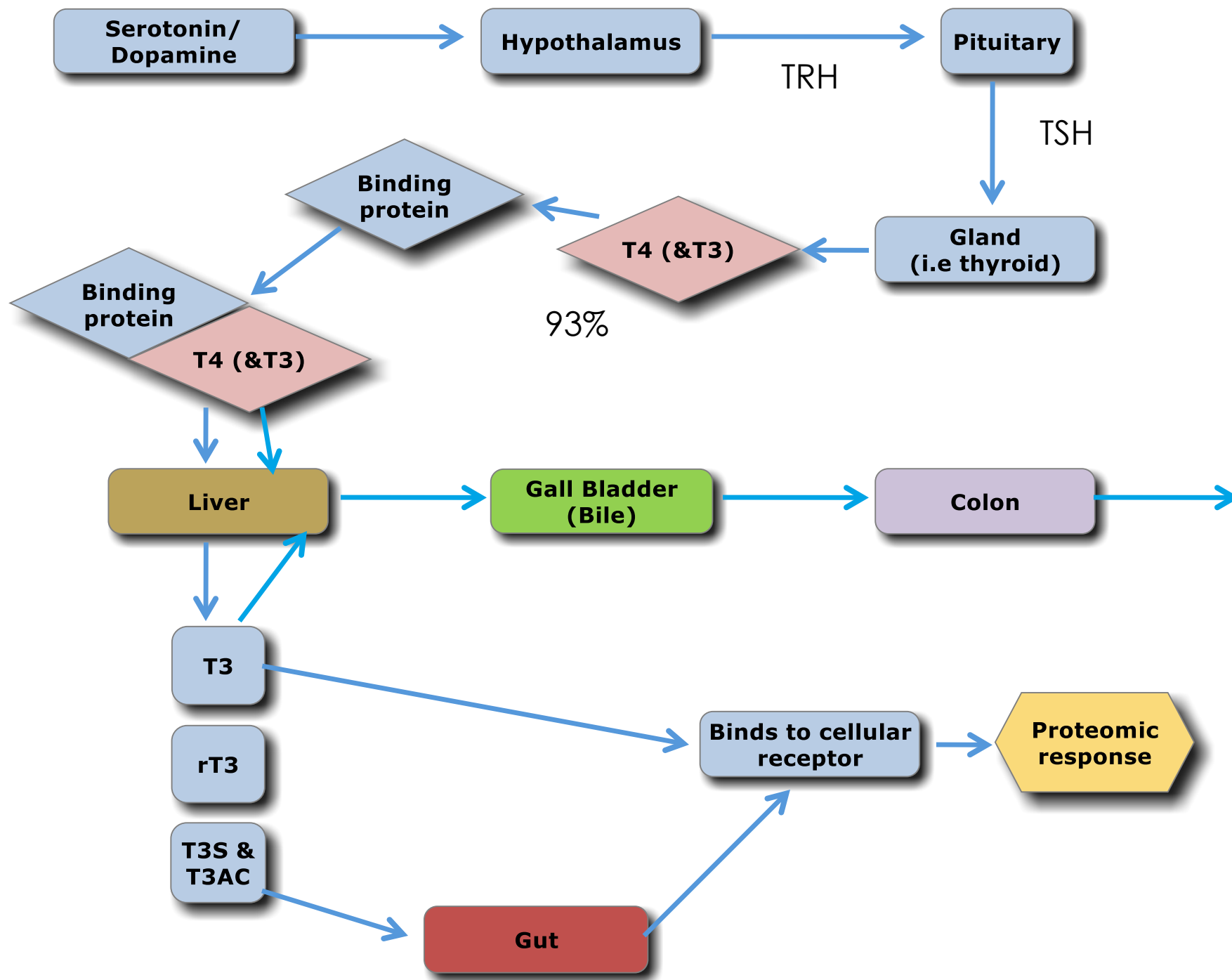
- * Approximately 1/3 of T4 is converted to T3, 1/3 is converted to rT3 and the remainder is metabolized in different pathways.
- * Deiodinases
 - * D1 – liver, kidney, muscle and thyroid
 - * Also used in the degradation of thyroid hormone
 - * D2 – CNS, pituitary, gonads and brown adipose tissue
 - * Maintains local T3 levels, but not plasma levels
 - * D3 – brain
 - * Degradation activities

Thyroid Hormone Mechanism



Low Thyroid Symptoms

- * Cold extremities
- * Difficulty losing weight
- * Depression; slow mental function
- * Dry skin, hair and brittle nails
- * Hair loss
- * Constipation
- * Poor/slow wound healing



Thyroid Markers

- * TSH
 - 1.5-2.0
- * Total Thyroxine (TT4)
 - 6.0-12.0
- * Free Thyroxine (FT4)
 - 1.0-1.5
- * Free Thyroxine Index (FTI)
 - Not accurate (calculation)
- * Resin T3 Uptake (T3U)
 - 28-38% (Men: 32-38; Women: 28-34)
- * Total Triiodothyronine (TT3)
 - 100-180
- * Free Triiodothyronine (FT3)
 - 3.0-4.0
- * Reverse T3 (rT3)
 - Lab range
- * Thyroid Antibodies (TGB, TPO)
 - If positive, likely Hashimoto's

What Pattern Is This?

Thyroid

TSH

1.460

uIU/mL

0.450 - 4.500

***Effective January 25, 2010, TSH reference interval for**

11 - 19 years will be changing to: 0.450 - 4.500 uIU/mL

Reference interval for all other ages will NOT be affected.

Thyroxine (T4)

6.7

ug/dL

4.5 - 12.0

T3 Uptake

33

%

24 - 39

Free Thyroxine Index

2.2

1.2 - 4.9

Triiodothyronine (T3)

99

ng/dL

83 - 200

Effective January 25, 2010, Total T3 reference interval for

11 years and greater will be changing to: 71 - 180 ng/dL

Reference interval for all other ages will NOT be affected.

What Pattern Is This?

TSH	1.846
Thyroxine (T4)	5.6
T3 Uptake	32
Free Thyroxine Index	1.8

Low TSH and Low T4

- * Could be poor hypothalamic function
 - * Serotonin, dopamine, heavy metals, inflammation
- * Could be pituitary suppression
 - * Cortisol, inflammation, dysbiosis (lipopolysaccharides), heavy metals

What Pattern is This?

Thyroid

TSH	3.820	uIU/mL	0.450 - 4.500
Thyroxine (T4)	5.9	ug/dL	4.5 - 12.0
T3 Uptake	31	%	24 - 39
Free Thyroxine Index	1.8		1.2 - 4.9

What Pattern is This?

Thyroid

TSH	3.970	uIU/mL	0.450 - 4.500
Thyroxine (T4)	7.7	ug/dL	4.5 - 12.0
T3 Uptake	33	%	24 - 39
Free Thyroxine Index	2.5		1.2 - 4.9
Triiodothyronine (T3)	113	ng/dL	71 - 180

What Pattern is This?

TSH	32.070	HIGH	uIU/mL	0.450-4.500
Thyroxine (T4)	4.8		ug/dL	4.5-12.0
T3 Uptake	33		%	24-39
Free Thyroxine Index	1.6			1.2-4.9

High TSH, Low T4

1. Autoimmune (Hashimoto's)
 - * Run thyroid antibodies
 - * Anti-thyroglobulin antibodies
 - * Thyroid peroxidase antibodies
2. Iodine deficiency
 - * Iodine intake
 - * Goitrogen
 - * Fluoride, bromide
 - * Arsenic
 - * Brassica family (cabbage, broccoli, kale, etc)
3. Tyrosine deficiency
 - * Not likely. Must be a fairly significant amino acid deficiency to cause this.

What Pattern is This?

TESTS	RESULT	FLAG	UNITS	REFERENCE INTERVAL
TSH	1.473		uIU/mL	0.450-4.500
Thyroxine (T4)	4.9		ug/dL	4.5-12.0
T3 Uptake	28		%	24-39
Free Thyroxine Index	1.4			1.2-4.9
T4,Free(Direct)	0.66		ng/dL	0.61-1.76
Triiodothyronine,Free,Serum	1.9	LOW	pg/mL	2.3-4.2
Reverse T3	185		pg/mL	90-350
Antithyroglobulin Ab Siemens (DPC) ICMA Methodology	<20		IU/mL	0-40
Thyroid Peroxidase (TPO) Ab	21		IU/mL	0-34

Low T3 Uptake, Normal T4/T3, Low free T4/T3

1. Likely excess thyroid binding globulin
 - * Elevated estrogen is most common reason for excess thyroid binding globulin

What Pattern is This?

Thyroid

TSH	1.780	uIU/mL	0.450 - 4.500
Thyroxine (T4)	7.5	ug/dL	4.5 - 12.0
T3 Uptake	37	%	24 - 39
Free Thyroxine Index	2.8		1.2 - 4.9
Triiodothyronine (T3)	90	ng/dL	83 - 200

High T3 Uptake, Normal T4/T3, high free T4/T3

1. Likely due to elevated testosterone
 - * In women, consider blood sugar dysregulation and Polycystic Ovary Syndrome
 - * High correlation between Hashimoto's and PCOS

What Pattern is This?

Thyroid Panel With TSH

TSH	2.918		uIU/mL	0.370 - 6.000
Thyroxine (T4)	8.7		ug/dL	4.5 - 12.0
T3 Uptake	33		%	24 - 38
Free Thyroxine Index	2.9			1.2 - 4.9
Reverse T3	421	High	pg/mL	90 - 350

Normal T4, Low T3

1. Underconversion of T4 to T3
 - * May also be overconverting to rT3
 - * Selenium deficiency
 - * Inflammation, cortisol, heavy metals, arsenic

What Pattern is This?

- TSH 1.8 mIU/ml
- Total Thyroxine (TT4) 8.0 μ g/dL
- Free Thyroxine (FT4) 1.2ng/dL
- Free Thyroxine Index (FTI) 2.8
- Resin T3 Uptake (T3U) 30%
- Total Triiodothyronine (TT3) 150ng/dl
- Free Triiodothyronine (FT3) 350pg/dL
- Reverse T3 (rT3) 200pg/ml
- TPO- 7 IU/ml
- Anti Thyroid Globulin AntiBody- <20IU/ml

Normal thyroid panel, hypothyroid symptoms

1. Consider receptor function

- * Inflammation, cortisol
- * Vitamin A deficiency
 - * Consider night blindness
 - * Also could be zinc deficiency causing vitamin A deficiency
 - * Skin issues, decrease in taste, “zinc spots” on fingernails

What Pattern is This?

Thyroid

TSH	3.600	uIU/mL	0.450 - 4.500
Thyroxine (T4)	7.6	ug/dL	4.5 - 12.0
T3 Uptake	33	%	24 - 39
Free Thyroxine Index	2.5		1.2 - 4.9
Triiodothyronine (T3)	92	ng/dL	71 - 180

T4F+ThyAbs+T3Free

Triiodothyronine, Free, Serum	2.8	pg/mL	2.0 - 4.4
T4, Free (Direct)	1.31	ng/dL	0.82 - 1.77
Thyroid Peroxidase (TPO) Ab	37	High IU/mL	0 - 34
Antithyroglobulin Ab	20	IU/mL	0 - 40
Siemens (DPC) ICMA Methodology			

What Pattern is This?

Thyroid

TSH	4.550	High	uIU/mL	0.450 - 4.500
Thyroxine (T4)	7.2		ug/dL	4.5 - 12.0
T3 Uptake	33		%	24 - 39
Free Thyroxine Index	2.4			1.2 - 4.9

T4F+ThyAbs+T3Free

Triiodothyronine, Free, Serum	2.5		pg/mL	2.0 - 4.4
T4, Free (Direct)	1.28		ng/dL	0.82 - 1.77
Thyroid Peroxidase (TPO) Ab	539	High	IU/mL	0 - 34
Antithyroglobulin Ab	94	High	IU/mL	0 - 40

Siemens (DDC) TCMA Methodology

Chemicals and the Thyroid

1. Iodine uptake – phthalates, perchlorate
2. Thyroid enzymes – pesticides and fungicides
3. Deactivating thyroid hormones – lead, chromium, methoxychlor (pesticide)
 - * PBDEs – inhibit thyroid hormone binding to transthyretin
 - * PCBs – binds to thyroid binding globulin
 - * Pentachlorophenol – inhibits thyroid hormone production
 - * Cadmium, lead, methyl-mercury, Bisphenol A, dioxin, pesticides
 - * Isoflavones, catechin?

Impacts on TSH

STIMULATORY

Thyrotropin-releasing hormone (TRH)

Prostaglandins (?)

α -adrenergic agonists (? Via TRH)

Opioids (humans)

Vasopressin (AVP) (long term)

Glucagon-like peptide 1 (GLP-1)

Leptin

Glucocorticoids (in vitro)

INHIBITORY

Thyroid hormones and analogues

Dopamine

Gastrin

Opioids (rat)

Glucocorticoids (in vivo)

Serotonin

Cholecystokinin (CCK)

Gastrin-releasing peptide (GRP)

Vasopressin (AVP) (short-term)

Neuropeptide Y (NPY)

Interleukin 1 β and 6

Tumor necrosis factor α

Regulators of TSH

- * Stimulatory – TRH, prostaglandins, opioids, arginine vasopressin, GLP-1, Leptin
- * Inhibitory – Thyroid hormone, dopamine, gastrin, glucocorticoids (high dose), serotonin, CCK, gastrin-releasing peptide, arginine vasopressin, Neuropeptide Y, IL-1b, IL-6, Somatostatin

Thyroid Interactions

- * Interactions with other hormones

- * Cortisol

- * Increased – decreases TSH and TBG (high dose), decreases T4/T3 ratio (increases T4/rT3 ratio)
 - * Decreased – increased TSH
 - * Increases in thyroid hormone, increase cortisol clearance in liver, and thus leads to more ACTH production

- * Estrogen

- * Increased TBG, TSH (postmenopausal women), increased T4 requirement in hypothyroid patients

- * Testosterone

- * Decreased TBG, decreased T4 turnover in women, reduced T4 requirement in hypothyroid patients

- * Growth hormone

- * Decreased D3 activity

Goiter



Goiter



Goiter



Source: Usatine RP, Smith MA, Mayeaux EJ, Chumley HS: *The Color Atlas of Family Medicine, Second Edition*: www.accessmedicine.com
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Goiter

A goiter can occur in a gland that is producing too much hormone (hyperthyroidism), too little hormone (hypothyroidism), or the correct amount of hormone (euthyroidism). A goiter indicates there is a condition present which is causing the thyroid to grow abnormally.

Goiter

Hashimoto's thyroiditis is a more common cause of goiter formation in the US. This is an autoimmune condition in which there is destruction of the thyroid gland by one's own immune system. As the gland becomes more damaged, it is less able to make adequate supplies of thyroid hormone. The pituitary gland senses a low thyroid hormone level and secretes more TSH to stimulate the thyroid. This stimulation causes the thyroid to grow, which may produce a goiter.

Goiter

Another common cause of goiter is Graves' disease. In this case, one's immune system produces a protein, called thyroid stimulating immunoglobulin (TSI). As with TSH, TSI stimulates the thyroid gland to enlarge producing a goiter. However, TSI also stimulates the thyroid to make too much thyroid hormone (causes hyperthyroidism). Since the pituitary senses too much thyroid hormone, it stops secreting TSH. In spite of this the thyroid gland continues to grow and make thyroid hormone. Therefore, Graves' disease produces a goiter and hyperthyroidism.

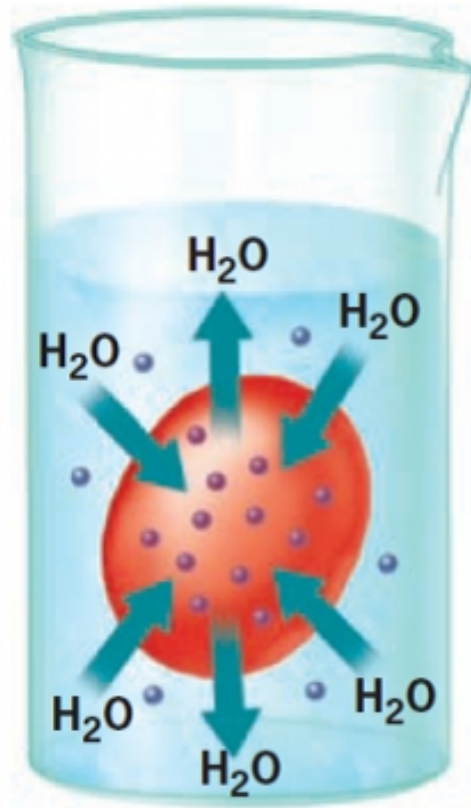
Goiter

Multinodular goiters are another common cause of goiters. Individuals with this disorder have one or more nodules within the gland which cause thyroid enlargement. This is often detected as a nodular feeling gland on physical exam. Patients can present with a single large nodule or with multiple smaller nodules in the gland when first detected.

Calculations

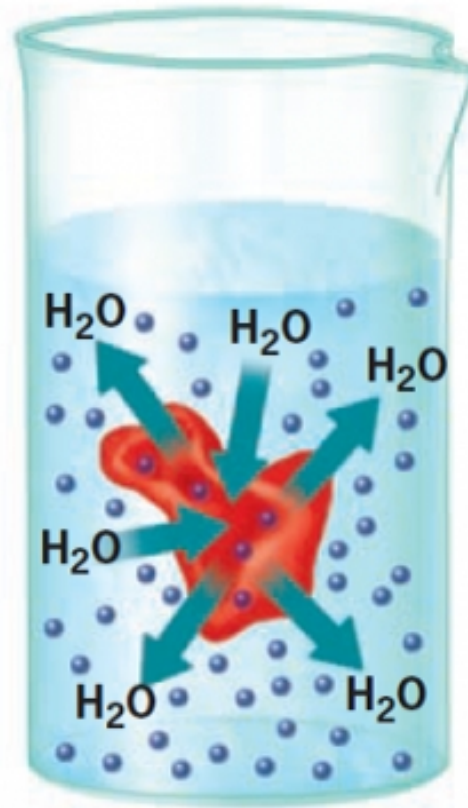
Osmolarity

(a) Hypotonic solution



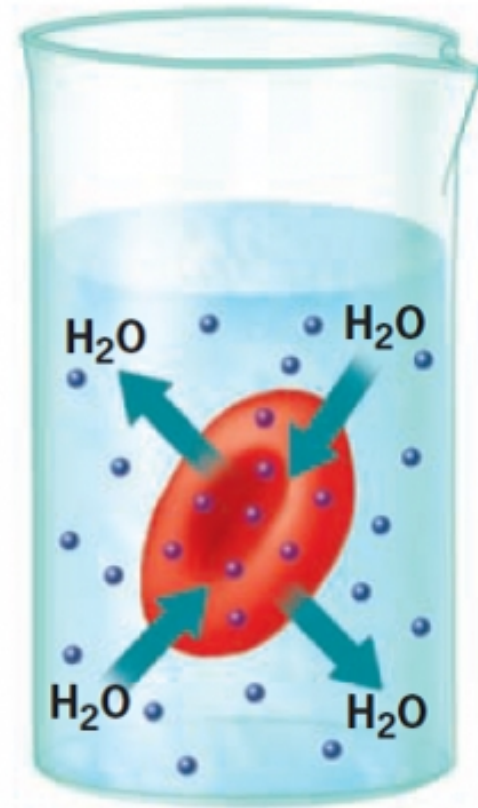
Net water gain
Cell swells

(b) Hypertonic solution



Net water loss
Cell shrinks

(c) Isotonic solution



No net loss or gain

Osmolarity

- * Suggested that serum osmolarity (extracellular fluid) reflects intracellular osmolarity

Osmolarity

Increased Osmolarity	Decreased Osmolarity
Hyperglycemia Hypernatremia (dehydration)	Hyponatremia (hypothyroidism, adrenal insufficiency)



Physiologic basis for understanding quantitative dehydration assessment¹⁻⁴

Samuel N Cheuvront, Robert W Kenefick, Nisha Charkoudian, and Michael N Sawka

ABSTRACT

Dehydration (body water deficit) is a physiologic state that can have profound implications for human health and performance. Unfortunately, dehydration can be difficult to assess, and there is no single, universal gold standard for decision making. In this article, we review the physiologic basis for understanding quantitative dehydration assessment. We highlight how phenomenologic interpretations of dehydration depend critically on the type (dehydration compared with volume depletion) and magnitude (moderate compared with severe) of dehydration, which in turn influence the osmotic (plasma osmolality) and blood volume-dependent compensatory thresholds for antidiuretic and thirst responses. In particular, we review new findings regarding the biological variation in osmotic responses to dehydration and discuss how this variation can help provide a quantitative and clinically relevant link between the physiology and phenomenology of dehydration. Practical measures with empirical thresholds are provided as a starting point for improving the practice of dehydration assessment. *Am J Clin Nutr* 2013;97:455-62.

INTRODUCTION

Dehydration (body water deficit) is a common physiologic state that can have profound implications for human health (1-7) and performance (8). Although mild dehydration can be easily corrected and is principally associated with impaired physical performance (8), it may be linked with common public health disorders if left chronically untreated (9, 10). A greater severity of dehydration can result in significant medical costs, morbidity, and mortality across the life span (11, 12). Although the physiology of osmotic and vascular volume responses to dehydration in humans have been well described (13, 14), the phenomenology of dehydration assessment has not. For example, there is no single, universal gold standard method of dehydration assessment for clinical decision making (7, 15, 16), which contributes greatly to the difficulty that clinicians encounter when trying to accurately assess dehydration in practice (17-25). This discordance between the physiology and phenomenology of dehydration is a recognized source of clinical confusion (17) for which clarity is needed to improve the practice of dehydration assessment.

In this review, we highlight how phenomenologic interpretations of dehydration depend critically on the type (dehydration

compared with volume depletion) and magnitude (moderate compared with severe) of dehydration, which, in turn, influence the plasma osmolality (Posm)⁵ and blood volume (BV)-dependent compensatory thresholds for antidiuretic and thirst responses. We also discuss the recent application of biological variation analysis to osmotic responses during dehydration for its novel potential as an adjunct (17) to clinical decision making. Posm is the primary focus of this review because it is the key regulated variable in fluid balance (13, 14, 26-28), and it is commonly used to screen for dehydration and complement more quantitative differential diagnoses of dysnatremias and other diseases (3, 5, 28-30). The osmolality of other body fluids commonly used to assess dehydration (ie, urine and saliva) are also mentioned as is the practical assessment of volume depletion. Descriptions of other potential methods of dehydration and volume-depletion assessment have been provided by other authors (7, 16, 19, 31, 32). Complementary reviews (33) are similarly suggested for detailed information related to sodium (natriuresis and appetite) and nonosmotic contributors (eg, baroreceptors) to osmotic homeostasis.

FUNDAMENTALS OF OSMOTIC RESPONSES TO DEHYDRATION IN HUMANS

In its simplest form, the net body water balance is generally the zero sum of food (water and solute) and fluid intake minus insensible and obligatory renal water losses (7). Fluid intakes,

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² The opinions or assertions contained herein are the private views of the authors and should not be construed as official or reflecting the views of the Army or the Department of Defense.

³ Supported by the United States Army Medical Research and Materiel Command.

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⁵ Abbreviations used: AVP, arginine vasopressin; Bm, body mass; BV, blood volume; Posm, plasma osmolality; PV, plasma volume; Sosm, saliva osmolality; TBW, total body water; Uosm, urine osmolality.

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Osmolality Calculation

Osmolarity	$2 \times \text{Na} + .9 \times \text{glucose} + .93 \times .5 \times \text{urea} + 8$ $1.897 \times \text{Na} + \text{glucose} + \text{urea} \times .5 + 13.5$ $1.9 \times (\text{Na} + \text{K}) + \text{glucose} + \text{urea} \times .5 + 5$	288-292 mOsm/kg
Example	$1.9 \times (\text{Na} + \text{K}) + \text{glucose} + \text{urea} \times .5 + 5$ $1.9 \times (141 + 4.1) + 5.8 + 5.36 \times .5 + 5 =$ 289.17	
	Glucose (mg/dl)/18 = mmol/L • $104/18 = 5.7777$ BUN x .357 = mmol/L • $15 \times .357 = 5.355$	

(MVMM) supplementation had no significant effect on the risk of all-cause mortality, mortality due to cancer, or mortality due to cardiovascular disease.

Despite our overall finding, Hemilä asserts that some vitamins and minerals may be beneficial for specific subpopulations. We concur with his suggestion that variables such as age, sex, and lifestyle factors might modify the effects of some vitamins, such that differential effects may emerge in different subpopulations. However, as pointed out by Hemilä, we were unable to perform subanalyses to examine the modifying effect of these different variables given that only trial-level data were available.

If individual-level data were accessible we could have performed any number of subanalyses. A limitation of this approach is that each subanalysis involves an additional statistical comparison and thus a greater risk of a type I error. Furthermore, subgroup analysis based on post hoc examination of data can lead to erroneous conclusions (2). The findings discussed by Hemilä, relating to vitamin E mortality risk across different age groups, still require replication for this reason. To avoid these issues, we used a limited number of prespecified analyses to determine the overall effects of MVMM supplementation in the general population, rather than in specific subpopulations.

Our results were strengthened by the large number of trials included in our analyses, generating a large pooled sample size. Although there are several advantages to undertaking an individual-level data meta-analysis, such an analysis is not always feasible. For example, we excluded 7 relevant trials from our analysis simply because trial-level data were unobtainable. Given the difficulty in obtaining raw data from chief investigators (especially when many of the trials included in our analysis were more than a decade old), undertaking a patient-level meta-analysis would have further diminished the number of trials included in our analysis.

Hemilä states that our meta-analysis is "important in discouraging ordinary middle-aged people from taking MVMMs." We are not sure how this conclusion was derived from our work given that our meta-analysis did not specifically focus on middle-aged adults. Moreover, whereas we found no effect of MVMMs on mortality across adults of all ages, this does not rule out other possible benefits to health or well-being.

Before our investigation, information on the association of MVMM use and mortality had frequently been obtained from observational studies (3). Our meta-analysis showed that, across randomized controlled trials, MVMM supplementation had no effect on mortality (1). Although we acknowledge that vitamins may have different effects in different subpopulations, it was first necessary to investigate the overall effects of MVMM supplementation in the general population. Identifying a harmful effect of MVMM use across all adults would have shown greater implications than identifying a harmful effect in one of many narrow subgroups. As discussed in our meta-analysis, we call for further research into the effects of MVMM use on all aspects of human health (1). This includes examination of MVMM use in specific subpopulations.

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doi: 10.3945/ajcn.113.064709

Limitations to the use of plasma osmolality as a hydration biomarker

Dear Sir:

In some laboratories, plasma osmolality (P_{osm}) is used as the gold standard for detecting dehydration (1), without consideration of its limitations; however, published data dispute this technique (2, 3), which prompts us to write in response to the recent article by Cheuvront et al (4) with regard to quantitative dehydration assessment. This article correctly states that P_{osm} is the key regulated variable in fluid balance, which means that P_{osm} is constantly regulated toward a central set point as the kidneys modify urine concentration and water excretion in response to diet and daily activities. We believe that this controlled regulation limits the efficacy of P_{osm} as an index of hydration change in many experimental designs. This article (4) also states that the "criticisms for adopting P_{osm} as a gold standard for dehydration assessment are minimal" (p 460). We disagree and write to describe several limitations to the use of P_{osm} as a gold standard for dehydration.

First, individuals who lose a large amount of body water (reported as % body mass loss relative to a beginning euhydrated state) may exhibit a decreased P_{osm} , contrary to anticipated hemoconcentration. For example, a summary of 2 studies (5) reported that the P_{osm} of 6 individuals (out of 39) decreased after they lost 3-8% of body mass. In a different study, men and women who consumed a 500-mL bolus of fluid acutely exhibited an increased P_{osm} , contrary to anticipated hemodilution (1); that is, after 90 min of rest, 4 of 30 P_{osm} measurements increased. These values show that P_{osm} may not reflect widely accepted physiologic principles, and that variance of P_{osm} measurements may be large.

Second, evidence suggests that P_{osm} changes are time- and protocol-specific. Unpublished observations (CX Muñoz, EC Johnson, JK DeMartini, et al, 2012) show that dehydration equivalent to 2% of body mass resulted in P_{osm} changes that were twice as large during mild cycling exercise (2.3 h; ΔP_{osm} of 9 mOsm/kg) compared with a passive exposure (5.0 h; ΔP_{osm} of 4 mOsm/kg); participants consumed no water during either trial in



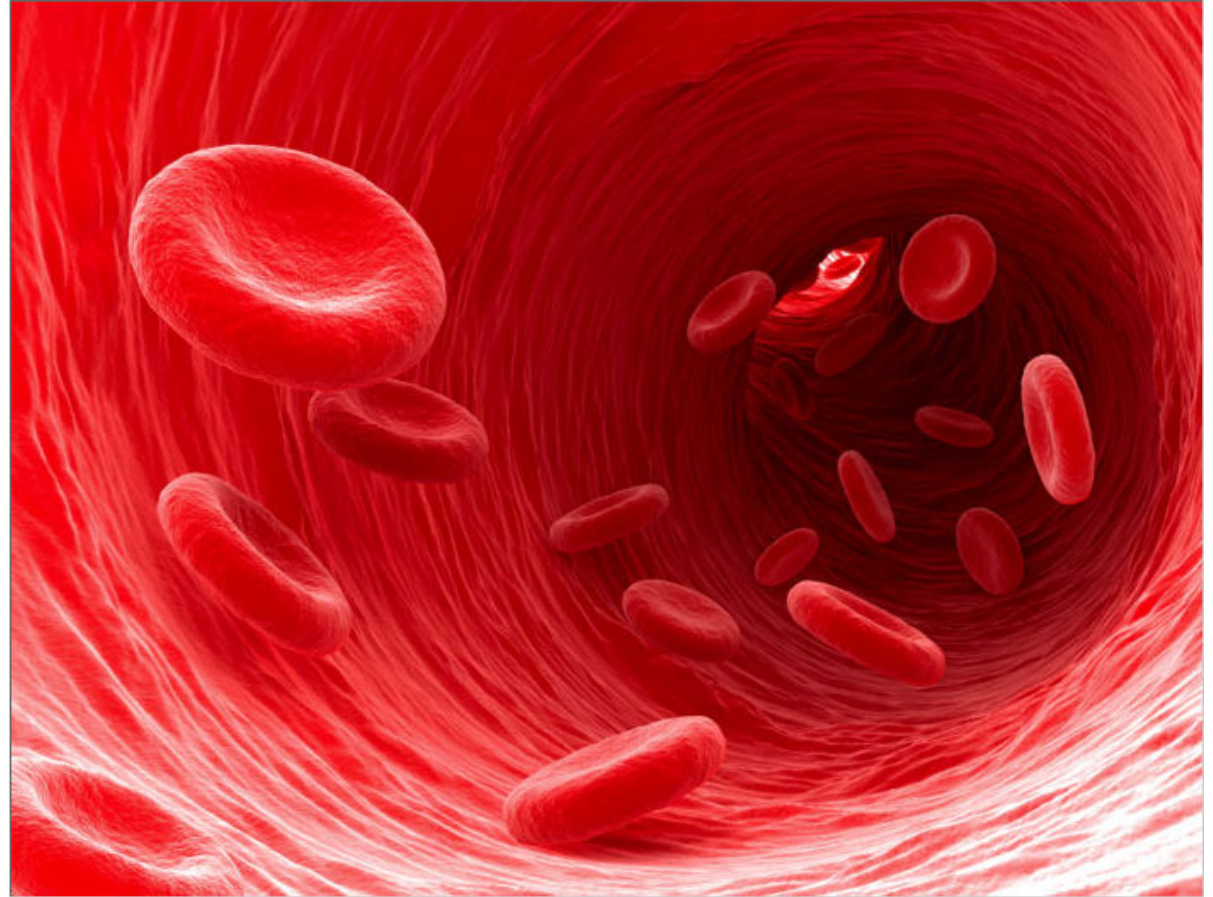
Dehydration

- * Increased osmolarity
- * Increased RBC, hemoglobin, hematocrit
- * Increased albumin (4.9 or above)
- * Urine specific gravity
 - * If high, likely not drinking enough water
 - * If low, likely losing water

Viscosity

Viscosity

- * Thickness of blood
- * How easily blood flows through vessels



Effect of Hydration on Whole Blood Viscosity in Firefighters

Ralph E. Holsworth Jr, DO; Young I. Cho, PhD; Joseph Weidman, BS, PharmDc

ABSTRACT

Context • Cardiovascular disease (CVD) is the leading cause of on-duty death among firefighters, totaling 45% of

Participants • Participants were 9 healthy, nonsmoking firefighters who were volunteers.

Outcome Measure(s) • Vital signs, traditional medical

Dehydration during the mock fire drill resulted in elevated WBV at both low- and high-shear rates. HCT and Hb increased due to dehydration and hemoconcentration. Hb and HCT returned to baseline values after exercise and rehydration, and while WBV improved, baseline values were not restored. After exercise but before rehydration, WBV changes were significantly larger than HCT and Hb changes, suggesting the profound influence of hydration states on WBV.

WBV measurements were better determinants of hydration states than HCT or Hb and should be performed to monitor the cardiovascular health of at-risk firefighters.

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Cardiovascular disease (CVD) is the leading cause of on-duty death among firefighters—45% of on-duty fatalities—and a major cause of morbidity.¹ Proportionately, firefighters experience the highest rates of mortality while on duty due to CVD when compared to the rates of other occupational groups and other public safety

into acute and chronic stressors. Acute stressors include irregular physical exertion, smoke exposure, excessive heat and dehydration, and duty-specific hazards, whereas chronic stressors include long sedentary periods, shift work and partial sleep deprivation, firehouse dietary patterns, and occupational stress.¹

A significantly higher prevalence of cardiovascular risk factors, such as current smoking, hypertension, diabetes mellitus, and hypercholesterolemia, was found among

Increased whole blood viscosity is associated with silent cerebral infarction.

A cross-sectional study was conducted to evaluate the association between hemorheological parameters and SCI in 1487 subjects (868 men and 619 women) undergoing medical check-up.

The participants with SCI had higher whole blood viscosity (WBV) levels at low shear rate than those without SCI. Moreover, the subjects with a high WBV had a higher prevalence of SCI.

Whole blood viscosity at low shear rate is a novel indicator for SCI regardless of classical cardiovascular risk factors. Early measurement of whole blood viscosity may be helpful to assess the risk of stroke.

Li, Rui-yan, Zhi-gang Cao, Ying Li, and Rui-tao Wang. 2015. "Increased Whole Blood Viscosity Is Associated with Silent Cerebral Infarction." *Clinical Hemorheology and Microcirculation* 59 (4): 301–7. doi:10.3233/CH-131760.

Article Type: 3 Original Article - Australia, Japan, SE Asia

Elevated whole blood viscosity is associated with insulin resistance and nonalcoholic fatty liver disease-a population study

Short title: Elevated whole blood viscosity is associated with NAFLD

Authors: Hong-yan Zhao*, Jing Li*, Min Xu, Tian-ge Wang, Wan-wan Sun, Ying Chen, Yufang Bi, Wei-qing Wang+, Guang Ning

Key Laboratory for Endocrine and Metabolic Diseases of Ministry of Health, the National Clinical

Over 8,000 Chinese subjects were used. 30% had NAFLD.
Positively association between WBV and NAFLD.

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Keywords: whole blood viscosity, insulin resistance, nonalcoholic fatty liver disease

Disclosure statement: The authors have nothing to declare.

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Association between whole blood viscosity and arterial stiffness in patients with type 2 diabetes mellitus

Ying Li · Xiu-xia Tian · Tiemin Liu ·
Rui-tao Wang

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The findings showed that baPWV increased as WBV (3 s^{-1}) elevated in DM. Moreover, WBV (3 s^{-1}) is independently associated with baPWV even after adjusting other cardiovascular risk factors. **Early detection of abnormal WBV levels at low shear rate should warrant for early search of undetected arterial stiffness in patients with DM.**

metabolic parameters were compared across WBV (3 s^{-1}) quartiles. The mean values of baPWV gradually increased with WBV (3 s^{-1}) quartiles. In addition, there was a positive correlation between baPWV and WBV 3 s^{-1} in patients with DM after adjusting confounding factors ($r = 0.285$, $p = 0.039$). Stepwise multiple linear regression analysis further revealed that WBV (3 s^{-1}) is a

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Published online: 14 October 2014

Introduction

Type 2 diabetes mellitus (DM) is associated with an increased risk of stroke and coronary heart disease (CHD) and non-vascular mortality [1, 2]. The occurrence of insulin resistance accompanied with hyperviscosity worsens the state of atherosclerosis in patients with DM. Furthermore, both DM and hyperviscosity have the tendency to develop thrombosis [3]. Recent studies demonstrated that hyperviscosity in DM is strongly influenced by the excellence of glycemic control [4–6].

Arterial stiffness due to decreased arterial compliance is one of the major signs of vascular aging [7]. Elevated arterial stiffness, an indicator of subclinical atherosclerosis, is associated with myocardial infarction, heart failure, stroke, renal disease, and all-cause mortality [8]. Brachial-ankle pulse wave velocity (baPWV) measurement, a simple, non-invasive, and automated measurement method, reflects the stiffness of muscular arteries and is widely used

HEPATOLOGY

Increased whole blood viscosity associated with arterial stiffness in patients with non-alcoholic fatty liver diseaseKai-jiang Yu,* Mei-jie Zhang,[†] Ying Li^{*,‡} and Rui-tao Wang[†]

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Key words

arterial stiffness, brachial-ankle pulse wave velocity, non-alcoholic fatty liver disease, whole blood viscosity.

Abstract**Background and Aims:** Non-alcoholic fatty liver disease (NAFLD) is an independent risk factor for increased cardiovascular disease. The brachial-ankle pulse wave velocity

The present study showed that baPWV elevated as WBV (3/s) increased in NAFLD. Moreover, WBV(3/s) is independently associated with baPWV even after adjusting other cardiovascular risk factors. **Early detection of abnormal WBV levels at low shear rate should warrant for early search of undetected arterial stiffness in patients with NAFLD.**

chronic liver condition in the Western countries, affecting 20–40% of the general population. Recent studies showed that NAFLD is an independent risk factor for increased cardiovascular disease. Evidence also indicated a graded association between NAFLD severity and increased vascular risk.¹ However, there are no suitable noninvasive biomarkers of NAFLD severity to allow better risk stratification based on cardiovascular outcomes.

Elevated arterial stiffness, an index of subclinical atherosclerosis, is linked with myocardial infarction, heart failure, stroke, dementia, renal disease, and elevated total mortality.² Pulse wave velocity (PWV) reflects the stiffness of central and peripheral muscular arteries, and is widely used as an index of arterial stiffness and vascular damage. Brachial-ankle PWV (baPWV) measurement, a simple, noninvasive, and automated measurement method, is closely correlated with aortic PWV.³ Previous studies found that increased baPWV is associated with metabolic syndrome, cardiovascular diseases, stroke, and renal disease, as well as elevated total mortality.^{4–7}

are associated with changes in hemorheological parameters.^{8–10} Moreover, increased viscosity is observed in metabolic syndrome, hypertension, diabetes, ischemic heart disease, stroke, and cognition.^{11–14} A recent study confirmed that whole blood viscosity (WBV) is a predictor of cardiovascular events.

We speculated that WBV may be one of the major pathological mediators of cardiovascular disease in NAFLD. Therefore, the purpose of the study was to investigate if rheological parameters are independently associated with baPWV in patients with NAFLD.

Methods

Participants. The study included 2032 persons (1035 men and 997 women) who received general health examination in our hospital from January 2009 to December 2010. We obtained informed consent from all subjects. The study protocol was

Table 1 Clinical and biochemical characteristics of male subjects with non-alcoholic fatty liver disease

<i>n</i>	Quartiles of WBV (3/s)				<i>P</i> value
	Q1 259	Q2 259	Q3 259	Q4 258	
Age (years)	48.5 (5.5)	48.2 (6.2)	47.3 (5.9)	49.9 (6.7)	< 0.001
BMI (kg/m ²)	24.5 (3.3)	24.4 (3.2)	24.9 (3.4)	24.8 (2.6)	0.124
Smoker (%)	116 (44.8%)	104 (40.2%)	135 (52.1%)	122 (47.3%)	0.050
SBP (mmHg)	126.1 (9.9)	124.8 (9.8)	124.6 (9.9)	128.8 (11.3)	< 0.001
DBP (mmHg)	74.4 (7.9)	73.1 (7.9)	73.8 (8.1)	75.7 (9.3)	< 0.001
Heart rate (bpm)	68.8 (9.8)	68.4 (9.1)	68.4 (9.6)	67.1 (8.6)	0.197
FPG (mmol/L)	4.83 (4.54–5.20)	4.75 (4.50–5.15)	4.79 (4.48–5.13)	4.91 (4.64–5.25)	0.019
TC (mmol/L)	4.88 (0.82)	4.81 (0.82)	4.87 (0.80)	4.96 (0.90)	0.223
TG (mmol/L)	2.89 (2.36–3.33)	2.94 (2.53–3.44)	2.89 (2.42–3.33)	3.19 (2.86–3.54)	< 0.001
HDL (mmol/L)	1.64 (1.37–1.85)	1.66 (1.43–1.87)	1.60 (1.33–1.80)	1.65 (1.49–1.84)	0.155
LDL (mmol/L)	2.98 (0.72)	2.96 (0.68)	3.00 (0.64)	3.17 (0.78)	0.002
AST (U/L)	22.0 (19.0–29.0)	24.0 (19.0–29.0)	23.0 (18.0–29.0)	21.0 (17.0–28.0)	0.001
ALT (U/L)	24.0 (18.0–42.0)	27.0 (19.0–49.0)	27.0 (18.0–43.0)	24.0 (16.0–38.0)	0.004
GGT (U/L)	37.0 (19.0–70.0)	36.0 (22.0–60.0)	41.0 (20.0–69.0)	37.0 (20.0–76.0)	0.648
Hemoglobin (g/dL)	125.9 (10.8)	125.0 (10.3)	124.1 (10.2)	125.2 (10.7)	0.292
Hematocrit (%)	42.5 (5.0)	43.1 (4.0)	42.8 (4.9)	43.8 (3.6)	0.010
Fibrinogen (mg/dL)	329.1 (69.4)	333.2 (65.9)	334.9 (72.8)	351.0 (55.1)	0.001
WBV200s ⁻¹ (mPa.s)	4.46 (0.39)	4.39 (0.30)	4.57 (0.37)	4.48 (0.40)	< 0.001
PV (mPa.s)	1.60 (0.08)	1.59 (0.08)	1.60 (0.09)	1.58 (0.10)	0.008

Data are expressed as means (SD) or median (interquartile range) or percentage.

P value was calculated by one-way ANOVA test or Kruskal–Wallis *H* or chi-square test.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; DBP, diastolic blood pressure; FPG, fasting plasma glucose; GGT, gamma-glutamyl transpeptidase; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; PV, plasma viscosity; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride; WBV, whole blood viscosity.

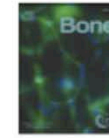
Table 2 Clinical and biochemical characteristics of female subjects with non-alcoholic fatty liver disease

<i>n</i>	Quartiles of WBV (3/s)				<i>P</i> value
	Q1 251	Q2 248	Q3 251	Q4 247	
Age (years)	47.5 (4.2)	48.9 (4.6)	48.2 (4.1)	50.8 (4.7)	< 0.001
BMI (kg/m ²)	24.1 (3.1)	24.1 (2.9)	24.3 (3.4)	24.6 (3.0)	0.346
Smoker (%)	5 (2.0%)	4 (1.6%)	6 (2.4%)	8 (3.2%)	0.659
SBP (mmHg)	123.8 (11.0)	122.2 (10.9)	124.9 (11.4)	127.7 (11.2)	< 0.001
DBP (mmHg)	78.3 (9.7)	76.3 (9.2)	78.9 (9.3)	79.3 (9.4)	0.002
Heart rate (bpm)	65.7 (7.4)	66.2 (10.1)	65.9 (9.0)	66.8 (9.5)	0.571
FPG (mmol/L)	5.11 (4.72–5.64)	5.00 (4.72–5.33)	5.01 (4.77–5.47)	5.29 (4.90–5.72)	< 0.001
TC (mmol/L)	5.06 (1.00)	4.94 (0.85)	5.08 (0.87)	5.27 (1.12)	0.002
TG (mmol/L)	2.10 (1.60–2.97)	2.01 (1.66–2.81)	2.22 (1.83–2.80)	2.31 (1.82–2.89)	0.110
HDL (mmol/L)	1.38 (1.20–1.56)	1.39 (1.20–1.54)	1.36 (1.17–1.55)	1.30 (1.10–1.55)	0.047
LDL (mmol/L)	3.00 (0.88)	2.79 (0.83)	2.93 (0.77)	2.98 (0.87)	0.033
AST (U/L)	21.0 (18.0–24.0)	21.0 (18.0–24.8)	19.0 (16.0–23.0)	20.0 (17.0–23.0)	0.002
ALT (U/L)	16.0 (14.0–20.0)	17.0 (13.3–20.0)	17.0 (14.0–19.0)	17.0 (12.0–21.0)	0.936
GGT (U/L)	16.0 (12.0–27.0)	17.0 (10.3–23.0)	16.0 (11.0–23.0)	20.0 (11.0–26.0)	0.201
Hemoglobin (g/dL)	132.6 (10.5)	133.2 (11.0)	132.3 (10.2)	133.3 (10.6)	0.684
Hematocrit (%)	42.8 (4.8)	43.3 (3.9)	42.6 (4.9)	43.6 (3.9)	0.045
Fibrinogen (mg/dL)	323.0 (66.2)	329.9 (73.5)	332.2 (72.9)	321.6 (71.0)	0.264
WBV200s ⁻¹ (mPa.s)	3.92 (0.40)	3.99 (0.36)	4.20 (0.33)	4.07 (0.43)	< 0.001
PV (mPa.s)	1.44 (0.08)	1.41 (0.08)	1.43 (0.09)	1.42 (0.08)	0.014

Data are expressed as means (SD) or median (interquartile range) or percentage.

P value was calculated by one-way ANOVA test or Kruskal–Wallis *H* or chi-square test.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; DBP, diastolic blood pressure; FPG, fasting plasma glucose; GGT, gamma-glutamyl transpeptidase; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; PV, plasma viscosity; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride; WBV, whole blood viscosity.



Original Full Length Article

Whole blood viscosity is negatively associated with bone mineral density in postmenopausal women with osteoporosis

Zong-yan Teng^a, Li-chun Pei^a, Ying Zhang^a, Ying Li^{a,b}, Rui-tao Wang^{a,*}^a Department of Geriatrics, The Second Affiliated Hospital, Harbin Medical University, Harbin, Heilongjiang, China^b International Physical Examination and Healthy Center, the Second Affiliated Hospital, Harbin Medical University, Harbin, Heilongjiang, China

Osteoporosis (OP) is associated with cardiovascular disease. Moreover, osteoporosis has been shown to be an independent predictor of cardiovascular mortality.

In conclusion, The findings show that WBV is elevated in osteoporosis and negatively correlated with BMD.

nant of bone mass, but exercise with loading of the bone also has a major impact on bone mass. The association between osteoporosis with carotid atherosclerosis, peripheral arterial disease, cardiovascular disease, and stroke has been well documented [1–4]. Furthermore, recent studies show that osteoporosis is an independent predictor of cardiovascular mortality [5,6].

Altered hemorheological parameters have also been shown to play a crucial role in atherogenesis. Many cardiovascular risk factors, including ageing, obesity, carotid intima-media thickness, are associated with changes in hemorheological parameters [7–9]. Moreover, increased viscosity is observed in metabolic syndrome, hypertension, diabetes, ischemic heart disease, and stroke [10–13]. A recent study confirmed that whole blood viscosity (WBV) is a predictor of cardiovascular events. Sedentary lifestyle can easily result in slowing down of blood flow and increasing plasma or whole blood viscosity. Recent studies

Materials and methods

Study population

The study enrolled 481 postmenopausal women with similar education background and income from the International Physical Examination and Healthy Center of our hospital between Jan. 2009 to Dec. 2010 [16]. This is a case-control study and there are 318 patients and 163 controls. They were matched for type 2 diabetes and hypertension. Informed consent was obtained from every subject. The study protocol was approved by the Ethics Committee of the Second Hospital of Harbin Medical University, China.

Clinical characteristics

Clinical data including medical history, lifestyle behaviors and medication use were recorded for each participant. Regular leisure-time physical activity was defined as participation in moderate or vigorous activity for 30 minutes or more per day at least 3 days a week. All the subjects underwent physical examination which included anthropometric and

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Blood Viscosity in Subjects With

Concetta Irace, Claudio Corallo,

Enrolled subjects were divided into three groups according to blood glucose: group A, blood glucose <90 mg/dL; group B, blood glucose ranging from 90 to 99 mg/dL; and group C, blood glucose ranging from 100 to 125 mg/dL.

Hematocrit ($P < 0.05$) and BV (P between 0.01 and 0.001) were significantly higher in groups B and C compared with group A.

The current study shows a direct relationship between BV and blood glucose in nondiabetic subjects. It also suggests that, even within glucose values considered completely normal, individuals with higher blood glucose levels have increased BV comparable with that observed in subjects with prediabetes.

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Elevated Whole-Blood Viscosity is Associated with Gallstones

Authors' Contribution:

Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation EBCEF 1 Ji-bin Yin
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Among 849 enrolled patients, 421 (49.6%) had gallstone disease. Compared with control subjects, whole-blood viscosity (WBV) levels were increased in patients with gallstones.

We found that whole-blood viscosity at low shear rate was independently associated with gallstones. Whether control of hyperviscosity would reduce the risk of developing gallstones deserves further investigation.



ORIGINAL ARTICLE

**Blood viscosity as a sensitive indicator
for paclitaxel induced oxidative stress
in human whole blood**



Whole-blood samples were collected from healthy volunteers and co-incubated with PTX, CrEL or their combination then compared with control blood samples. After a 24 h incubation time, the whole-blood viscosity (WBV), erythrocyte sedimentation rate (ESR), levels of whole-blood malondialdehyde (MDA), protein carbonyl content (PCC) and reduced glutathione (GSH) were determined. Moreover, plasma nitrite and plasma sialic acid (SA) values were measured.

The present study demonstrates that PTX-induced oxidative stress is associated with an increase of WBV.



Serum bilirubin and lipoprotein-a: How are these associated with whole blood viscosity?

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Background: It has been demonstrated that oxidative stress can induce red blood cell rigidity and haemolysis, which in turn can cause hyperviscosity and hyperbilirubinaemia, respectively. However, haemolysis may be associated with a low level of haemoglobin, which reduces whole blood viscosity (WBV). Bilirubin can behave as antioxidant or oxidant, and one uncharted course for diagnostic pathology

This pilot study suggests that hyperbilirubinaemia and hyperviscosity are associated and positively correlated. Consideration of whether serum bilirubin (as an indirect index of oxidative stress) can be used in combination with WBV (as index of macrovascular effect of oxidative stress) to assess oxidative damage is recommended

Introduction

In clinical diagnostic practice, bilirubin measurement is an important component of the test panel for liver functions. Total bilirubin is commonly used for monitoring of patients with anaemia who are suspected of disorders associated with haemolysis. Hyperbilirubinaemia has been associated with circadian rhythm and oxidative stress.¹ Circadian rhythm has been linked to several physiological processes including antioxidant activities *vis-à-vis* oxidative stress and blood viscosity.²⁻⁴ Interestingly, and typical of paradoxical properties of antioxidants, bilirubin can behave as an antioxidant or oxidant depending on its concentration.⁵ Particularly, bilirubin at low concentration is an antioxidant in neonatal jaundice,⁵ but during haemolysis it is possible

that bilirubin free radicals such as lumirubin may be generated;^{6,7} thereby conferring oxidant properties on bilirubin.

The concept of bilirubin being associated with oxidative stress is interesting with potential for its use in laboratory medicine diagnosis, because it is an established routine laboratory index. Given that oxidative stress may be associated with serum bilirubin level on one hand, and exacerbates whole blood viscosity (WBV) on the other, it is worth investigating whether bilirubinaemia could be associated with WBV. Further, evidence of oxidative stress and a concomitant demonstration of any of the effective vascular events including increase in WBV is necessary to establish oxidative damage.⁸ If bilirubinaemia is associated with WBV; it would be worth investigating how or whether the two parameters are correlated, given the antioxidant and pro-oxidant properties of

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Plasma viscosity, ADMA and oxLDL values were significantly higher in subjects with dyslipidemia than in subjects with normolipidemia. Plasma NOx concentration was decreased in dyslipidemic subjects compared to the normo-lipidemic subjects.

Our results demonstrated that the rheological impairment of dyslipidemic patients was related with endothelial dysfunction and this was a possible cause of both micro and macrovascular complications. **Therefore, as plasma viscosity is also a sensitive parameter, it can add useful information about the diagnosis and treatment of various disorders, and it should be utilized more frequently in clinical medicine.**

develop further understanding of CVD process to pinpoint new areas named as hemorheology for targeted

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Viscosity Calculation

Viscosity	High shear rate: WBV (208 Sec ⁻¹) [0.12 X h] + [0.17 X (p - 2.07)]	16-18 (males) 15.5 – 17.5 (females)
Example	[0.12 X 147] + [0.17 X (7.3 - 2.07)] = 18.5	

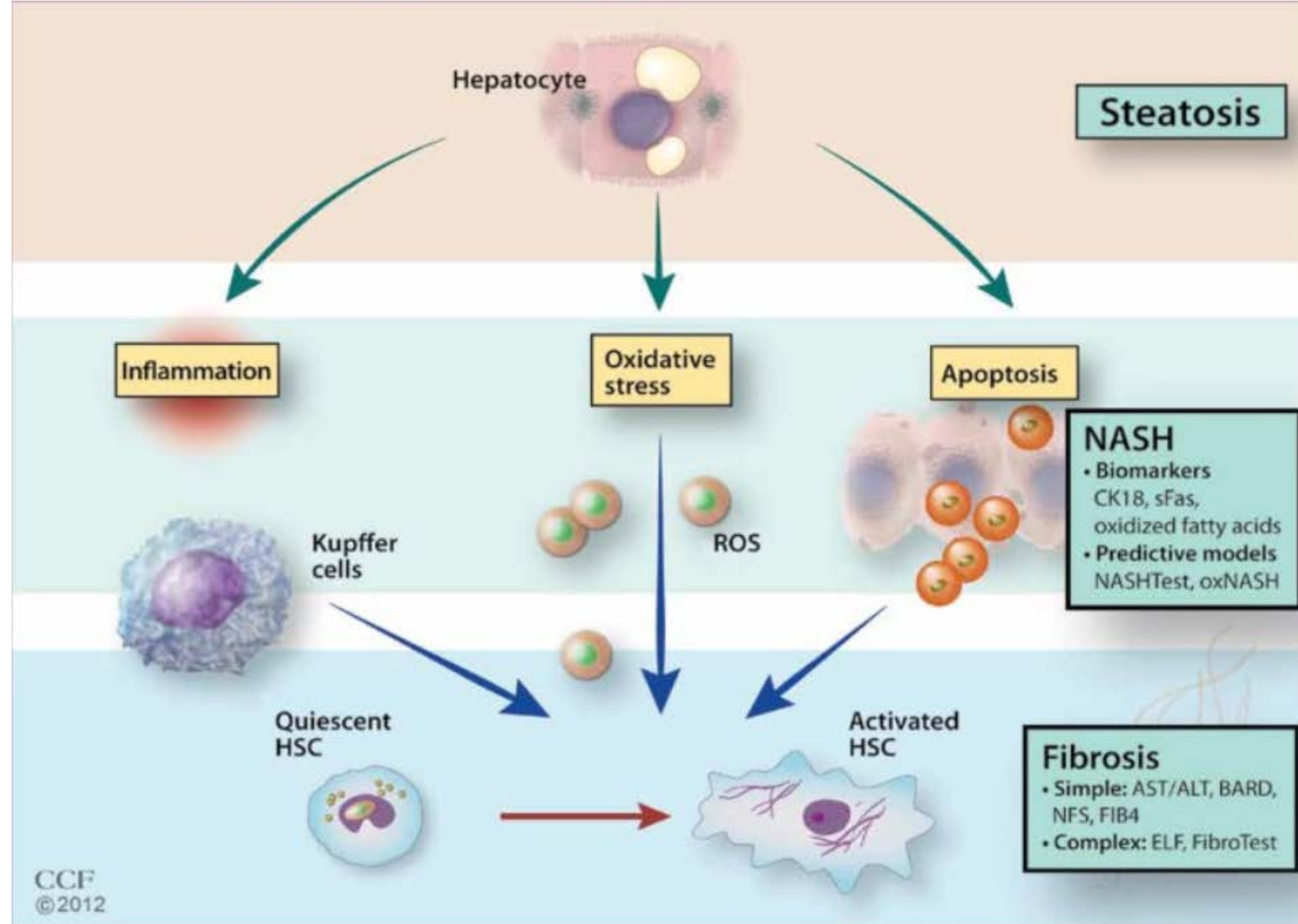
Liver Disease

Why be concerned about the liver?

- * Associated with obesity, insulin resistance and diabetes
- * Also associated with sub-clinical depression and sub-clinical anxiety
- * Associated with cognitive impairment and even loss of self-esteem
- * Also associated with chronic pain

Why use blood chemistry calculations?

- * 30% of the population has fatty liver
- * 90% of the obese population has fatty liver
- * Liver enzymes (AST, ALT) fail to detect fatty liver most of the time
 - * Up to 60% of patients with NAFLD and normal ALT can have NASH or advanced fibrosis
 - * 53% of patients with NAFLD with increased ALT do not have NASH or advanced fibrosis.
- * Liver biopsy only evaluates 1/50,000th of total liver mass
 - * Prone to error due to “patchy” histological changes
 - * Expensive, invasive



Research article

Open Access

The Fatty Liver Index: a simple and accurate predictor of hepatic steatosis in the general populationGiorgio Bedogni¹, Stefano Bellentani², Lucia Miglioli², Flora Masutti¹, Marilena Passalacqua², Anna Castiglione¹ and Claudio Tiribelli^{*1}

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216 subjects with and 280 without suspected liver disease were studied. FL was diagnosed by ultrasonography

An algorithm based on BMI, waist circumference, triglycerides and GGT had an accuracy of 0.84 (95%CI 0.81-0.87) in detecting FL. We used this algorithm to develop the "fatty liver index" (FLI), which varies between 0 and 100. A FLI < 30 (negative likelihood ratio = 0.2) rules out and a FLI ≥ 60 (positive likelihood ratio = 4.3) rules in fatty liver.

epidemiologic studies. Validation of FLI in external populations is needed before it can be employed for these purposes.

Fatty Liver Index

- * Uses triglycerides, BMI, GGT and waist circumference

Fatty Liver Index

$$FLI = \left(e^{0.953 \cdot \log_e(\text{triglycerides}) + 0.139 \cdot \text{BMI} + 0.718 \cdot \log_e(\text{GGT}) + 0.053 \cdot \text{waist circumference} - 15.745} / (1 + e^{0.953 \cdot \log_e(\text{triglycerides}) + 0.139 \cdot \text{BMI} + 0.718 \cdot \log_e(\text{GGT}) + 0.053 \cdot \text{waist circumference} - 15.745}) \right) * 100$$

- * <30 rules out fatty liver
- * >60 rules in hepatosteatosis

flicalc.xls [Protected View] - Excel

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PROTECTED VIEW Be careful—files from the Internet can contain viruses. Unless you need to edit, it's safer to stay in Protected View. [Enable Editing](#)

	A	B	C	D	E	F	G
1			predictors	logits			
2							
3	Triglycerides (mg / dL)		150	4.775			
4	BMI (kg / m ²)		30	4.170			
5	GGT (U / L)		30	2.442			
6	Waist circumference (cm)		120	6.360			
7	Constant		*****	-15.745			
8	Sum		*****	2.002			
9							
10	The fatty liver index (FLI) is		88				
11							
12	Use this table to interpret the FLI						
13	The FLI was developed at the Liver Research Center - Italy						
14							
15							
16							
17							

www.giorgiobedogni.it/varie/flicalc.xls

Sheet1 Sheet2 Sheet3

Ready 150%

Fatty Liver Index (FLI) For Hepatic Steatosis Calculator

Search

This **fatty liver index (FLI)** for **hepatic steatosis calculator** helps diagnosis FL based on patient BMI, triglycerides, GGT and waist circumference for referral to ultrasonography. There are instructions on the calculation method used and more information on the original study in the text below the form.

BMI ([Calculate BMI separately](#)):*

kg/m²

Waist Circumference:*

cm

Serum Triglycerides:*

mg/dL

Serum GGT:*

IU/L

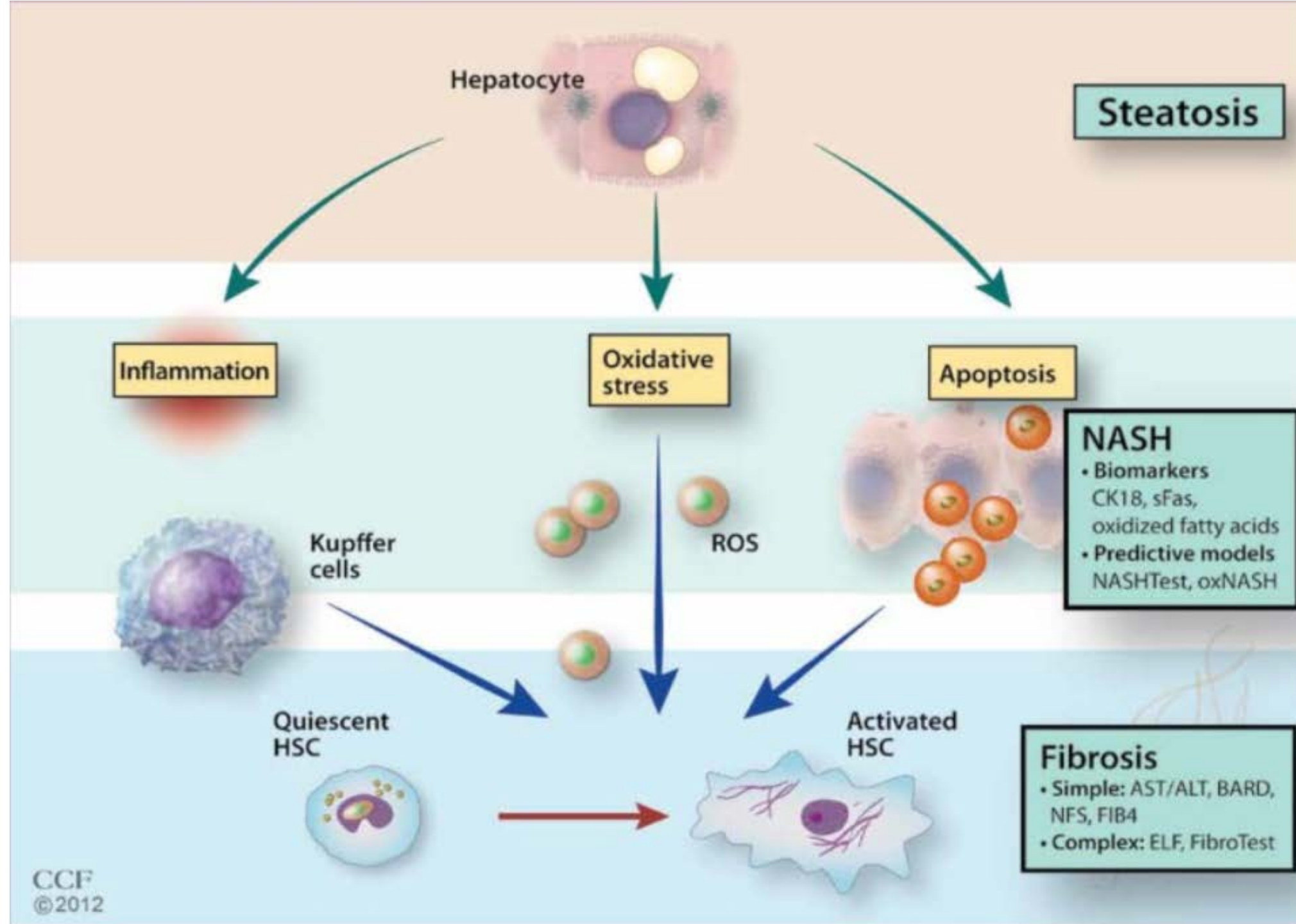
CALCULATE

RESET

* [https://www.thecalculator.co/health/Fatty-Liver-Index-\(FLI\)-For-Hepatic-Steatosis-Calculator-1109.html](https://www.thecalculator.co/health/Fatty-Liver-Index-(FLI)-For-Hepatic-Steatosis-Calculator-1109.html)

Table 1 Clinical and metabolic characteristics of subjects according to the presence of FLI ≥ 40

Variables	All subjects (<i>n</i> = 448)	Group A (<i>n</i> = 208) (FLI <40)	Group B (<i>n</i> = 240) (FLI ≥ 40)	<i>p</i> value
Age (years), median (IQR)	62.54 (56.77–70.15)	63.92 (54.80–68.65)	68.37 (59.22–70.70)	0.07
LUTS, <i>n</i> (%)				<0.01
Mild	144 (32.1 %)	88 (42.3)	56 (23.3)	
Moderate	216 (48.2 %)	104 (50.0)	112 (46.7)	
Severe	88 (19.6 %)	16 (7.7)	72 (30.0)	
MetS, <i>n</i> (%)	216 (48.2 %)	40 (19.2)	176 (73.3)	<0.01
IPSS, median (IQR)	14.50 (6.00–19.75)	17.00 (6.00–18.00)	19.00 (11.00–23.00)	<0.01
IPSS-storage, median (IQR)	5.00 (2.00–8.75)	5.00 (2.00–8.00)	7.0 (4.00–10.00)	<0.01
IPSS-voiding, median (IQR)	9.00 (4.25–11.009)	9.00 (5.00–11.75)	10.00 (7.00–12.00)	<0.01
IIEF-5, median (IQR)	19.00 (10.00–23.00)	18.00 (8.50–23.00)	16.00 (11.00–21.00)	<0.01
ED (IIEF-5 <22), <i>n</i> (%)	334 (76.8)	144 (69.2)	200 (83.3)	<0.01
PSA (ng/ml), median (IQR)	2.03 (0.74–4.75)	3.31 (0.88–7.20)	3.10 (1.38–4.70)	0.56
Total prostate volume (cc), median (IQR)	36.25 (30.00–58.50)	34.00 (25.00–50.00)	50.00 (30.00–68.00)	<0.01
Waist circumference (cm), median (IQR)	99.00 (92.00–104.75)	91.00 (86.25–98.75)	102.00 (98.00–105.00)	<0.01
Hip circumference (cm), median (IQR)	98.50 (94.00–103.00)	95.00 (91.00–97.00)	101.00 (96.00–103.00)	<0.01
BMI (kg/m ²), median (IQR)	26.51 (24.54–29.23)	24.00 (23.04–25.71)	27.43 (26.64–30.12)	<0.01
Glucose (mg/dl), median (IQR)	93.00 (86.00–102.75)	93.00 (85.75–101.75)	95.00 (89.00–104.00)	<0.01
Cholesterol (mg/dl), median (IQR)	193.50 (169.25–221.50)	203.00 (174.00–242.75)	190.00 (169.00–215.00)	0.22
HDL-C (mg/dl), median (IQR)	40.50 (34.25–49.75)	47.00 (33.00–56.00)	42.00 (39.25–55.00)	<0.01
LDL-C (mg/dl), median (IQR)	127.20 (102.85–153.15)	135.40 (112.00–176.40)	118.40 (97.20–141.20)	0.31
Tryglicerides (mg/dl), median (IQR)	118.50 (75.25–163.00)	70.00 (50.25–120.00)	142.00 (101.00–164.00)	<0.01
SBP (mmHg), median (range)	125.00 (120.00–140.00)	125.00 (120.00–140.00)	125.00 (120.00–140.00)	0.25
DPB (mmHg), median (IQR)	80.00 (70.00–85.00)	80.00 (66.25–83.75)	75.00 (70.00–85.00)	0.16
Insulin (mUI), median (IQR)	6.95 (4.62–9.85)	6.40 (4.60–7.90)	8.60 (4.60–10.80)	<0.01
HOMA index, median (IQR)	1.63 (1.08–2.30)	1.50 (1.06–1.75)	1.98 (1.08–2.44)	<0.01
HOMA ≥ 3 , <i>n</i> (%)	56 (12.5 %)	8 (3.8)	48 (20.0)	0.03
Total testosterone (mg/dl), median (IQR)	4.31 (3.56–5.80)	4.69 (4.20–6.00)	4.26 (3.31–5.74)	<0.05
Estradiol (mg/dl), median (IQR)	16.90 (11.40–24.10)	20.30 (7.10–22.70)	16.10 (11.40–21.80)	0.56
AST (U/l), median (IQR)	21.00 (18.00–28.00)	21.00 (18.00–27.00)	24.00 (18.00–28.00)	<0.01
ALT(U/l), median (IQR)	25.00 (17.25–30.00)	23.00 (22.00–27.00)	26.00 (16.00–40.00)	<0.01
GGT (U/l), median (IQR)	20.00 (13.00–28.00)	14.00 (11.00–26.00)	21.00 (16.00–28.00)	<0.01
NAFLD, <i>n</i> (%)	206 (45.98 %)	58 (27.88)	148 (61.67)	<0.01
Fatty liver grade, <i>n</i> (%)				<0.01
Grade 0	242 (52.02)	150 (72.12)	92 (38.33)	
Grade 1	152 (33.93)	46 (22.12)	91 (37.92)	
Grade 2	30 (6.70)	10 (4.81)	42 (17.50)	
Grade 3	24 (5.36)	2 (0.96)	15 (6.25)	



NAFLD fibrosis score

- * Validated calculation to identify level of fibrosis in NAFLD
- * Age, BMI, hyperglycemia (fasting glucose levels ≥ 110 mg/dL or previously diagnosed T2DM), platelet count, albumin and AST/ALT ratio
- * NAFLD
 - * Simple steatosis or non-alcoholic fatty liver
 - * NASH (steatosis, inflammation, hepatocellular ballooning)
 - * With or without fibrosis
 - * Cirrhosis
 - * Hepatocellular carcinoma

NAFLD fibrosis score calculation

Non-Alcoholic Fatty Liver Disease	$-1.675 + 0.0373 \times \text{age} + 0.0943 \times \text{BMI} + 1.13 \times \text{IFG or diabetes (yes = 1, no = 0)} + 0.99 \times (\text{AST/ALT}) \text{ ratio} - 0.013 \times \text{platelet} - 0.66 \times \text{albumin}$	Low risk of fibrosis: < -1.455 Intermediate risk of fibrosis: -1.455-0.676 High risk of fibrosis: >0.676
Example	$-1.675 + 0.0373 \times 38 + 0.0943 \times 26 + 1.13 \times \text{IFG or diabetes (yes = 1, no = 0)} + 0.99 \times (28/21) \text{ ratio} - 0.013 \times 163 - 0.66 \times 4.6 = -1.6408 \text{ (If "yes" = -0.5108)}$	

Fibrosis-4 (FIB-4)

- * Age, AST, ALT and platelet count
- * FIB-4 outperformed the following calculations for the diagnosis of advanced fibrosis :
 - * NAFLD fibrosis score
 - * APRI
 - * age/platelet index
 - * AST/ALT ratio
 - * BARD score
 - * Nippon score
- * Thought to distinguish NASH from simple steatosis

Fibrosis-4 (FIB-4)

$$* \text{ (Age[years] } \times \text{ AST[U/L])} / (\text{platelet } \times \sqrt{\text{ALT[U/L]}})$$

NASH

- * Fib4 score < 1.30 = F0-F1 (80% positive predictive value)
- * Fib4 score > 2.67 = F3-F4 (90% negative predictive value)

Hep C

- * FIB-4 < 1.45: absence of cirrhosis
- * FIB-4 between 1.45 - 3.25: inconclusive
- * FIB-4 > 3.25: cirrhosis

Predictive Values of FIB-4 Index Scores for Advanced Fibrosis (stage 3–4)*

	Low cutoff point (<1.30)	Indeterminate ($1.30\text{--}2.67$)	High cutoff point (>2.67)	Total
Total	327	163	51	541
No advanced fibrosis	294	112	10	416
Advanced fibrosis	33	51	41	125
Sensitivity	74%		33%	
Specificity	71%		98%	
Positive predictive value	43%		80%	
Negative predictive value	90%		83%	
Interpretation	Absence of advanced fibrosis		Presence of advanced fibrosis	

- * Shah, Amy G, Alison Lydecker, Karen Murray, Brent N. Tetri, Melissa J. Contos, and Arun J. Sanyal. 2009. "USE OF THE FIB4 INDEX FOR NON-INVASIVE EVALUATION OF FIBROSIS IN NONALCOHOLIC FATTY LIVER DISEASE." *Clinical Gastroenterology and Hepatology: The Official Clinical Practice Journal of the American Gastroenterological Association* 7 (10): 1104–12. doi:10.1016/j.cgh.2009.05.033.

Fibrosis-4 (FIB-4)

NASH	$(\text{Age}[\text{years}] \times \text{AST}[\text{U/L}]) / (\text{platelet} \times \sqrt{\text{ALT}[\text{U/L}]})$	Fib4 score < 1.30 = F0-F1 Fib4 score > 2.67 = F3-F4
Example	$(45 \times 36) / (250 \times \sqrt{36}) = 1.08$	

A novel model using mean platelet volume and neutrophil to lymphocyte ratio as a marker of nonalcoholic steatohepatitis in NAFLD patients: multicentric study

NASH patients had an increased N/L ratio compared with non-NASH cases (2.6 ± 1.1 and 1.9 ± 0.7 fl, respectively, $P < 0.001$).

The N/L ratio correlated positively with NAFLD activity score, proinflammatory cytokines, and CRP ($P < 0.001$).

In addition, patients with advanced fibrosis (F3-4) had an N/L ratio (2.5 ± 1.1) comparable with that of patients with early fibrosis (F1-2) (1.8 ± 0.9) ($P < 0.001$).

N/L Ratio statistically significant at a cut-off of 2.4

- Neutrophils of 65 and Lymphocytes of 27 ($65/27 = 2.4$)

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N/L ratio was used to estimate prognosis in hepatocellular carcinoma patients [10]. This ratio incorporates data on two different immune pathways – the lymphocytes that expound the regulatory pathway and the neutrophils that are responsible for continuous inflammation [11].

Functional Medicine Diagnostic Workup



The Plan.

Cook vs. Chef





“Dysfunction” as an adaptation.

A conversation.

Insulin resistance is a cellular antioxidant defense mechanism

Kyle L. Hoehn^{a,1,2,3}, Adam B. Salmon^{b,1}, Cordula Hohnen-Behrens^a, Nigel Turner^a, Andrew J. Hoy^a, Ghassan J. Maghazal^c, Roland Stocker^c, Holly Van Remmen^b, Edward W. Kraegen^a, Greg J. Cooney^a, Arlan R. Richardson^b, and David E. James^{a,2}

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Edited by Michael Karin, University of California, San Diego School of Medicine, La Jolla, CA, and approved August 28, 2009 (received for review March 4, 2009)

In the present study, we took a comprehensive approach to identify factor(s) that might unify multiple models of IR. Initially, we compared four diverse models of IR including chronic treatment with insulin, corticosteroids, proinflammatory cytokines, or lipid in both muscle and adipose cell lines.

We have now identified a direct correlation between mitochondrial oxidative stress in all models.

Many corticosteroids, but also insulin, converge at a common intermediate in the insulin action pathway or does IR represent a collection of distinct cellular disorders? For example, endoplasmic reticulum (ER) stress, proinflammatory responses, oxidative stress, intracellular ceramide accumulation, or the activation of JNK, IKK, or PKC are all currently implicated in the development of IR in overnourished or obese rodents (2, 3). In such models, correcting any one of these intracellular stresses is sufficient to improve IR leading to the possibility that these factors are somehow interconnected. One view is that insulin receptor substrate 1 (IRS1) represents a common convergence point for many defects contributing to IR (4). However, this view has been challenged in that the ability of IRS1-independent receptor tyrosine kinases to activate

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The authors declare no conflict of interest.

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¹K.L.H. and A.B.S. contributed equal data.

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This article contains supporting information online at www.pnas.org/cgi/content/full/0902380106/DCSupplemental.

Insulin resistance is a cellular antioxidant defense mechanism

Kyle L. Hoehn^{a,1,2,3}, Adam B. Salmon^{b,1}, Cordula Hohnen-Behrens^a, Nigel Turner^a, Andrew J. Hoy^a, Ghassan J. Maghazal^c, Roland Stocker^c, Holly Van Remmen^b, Edward W. Kraegen^a, Greg J. Cooney^a, Arlan R. Richardson^b, and David E. James^{a,2}

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Edited by Michael Karin, University of California, San Diego School of Medicine, La Jolla, CA, and approved August 28, 2009 (received for review March 4, 2009)

Mitochondrial $O_2^{\bullet-}$ has previously been linked to hyperglycemia-induced metabolic dysfunction in endothelial cell systems and in inflammation in adipocytes.

A major advance of the present study is the observation that mitochondrial $O_2^{\bullet-}$ is upstream of IR in skeletal muscle and adipose tissue.

The origin of IR has been difficult to elucidate in part due to the diverse set of risk factors linked to this condition including overnutrition, physical inactivity, pregnancy, Hepatitis C, polycystic ovarian syndrome, HIV protease inhibitor therapy, and antiinflammatory corticosteroids. Do such factors converge at a common intermediate in the insulin action pathway or does IR represent a collection of distinct cellular disorders? For example, endoplasmic reticulum (ER) stress, proinflammatory responses, oxidative stress, intracellular ceramide accumulation, or the activation of JNK, IKK, or PKC are all currently implicated in the development of IR in overnourished or obese rodents (2, 3). In such models, correcting any one of these intracellular stresses is sufficient to improve IR leading to the possibility that these factors are somehow interconnected. One view is that insulin receptor substrate 1 (IRS1) represents a common convergence point for many defects contributing to IR (4). However, this view has been challenged in that the ability of IRS1-independent receptor tyrosine kinases to activate

described a reproducible system for studying IR in myotubes and adipocytes in culture relying on the translocation of the facilitative glucose transporter GLUT4 to the plasma membrane (5). This

Author contributions: K.L.H., A.B.S., and D.E.J. designed research; K.L.H., A.B.S., C.H.B., N.T., A.J.H., and G.J.M. performed research; K.L.H., A.B.S., R.S., H.V.R., E.W.K., G.J.C., and A.R.R. contributed new reagents/analytic tools; K.L.H., A.B.S., and C.H.B. analyzed data; and K.L.H. and D.E.J. wrote the paper.

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Insulin resistance is a cellular antioxidant defense mechanism

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This hypothesis is exciting for a number of reasons. For example, it suggests that IR may be a protective mechanism, in which case we should perhaps reconsider using therapeutic strategies to overcome unless they also eliminate the primary defect. Moreover, it suggests that cells have evolved sophisticated mechanisms to not only guard against nutrient lack, such as the AMPK pathway, but also nutrient excess.

The origin of IR has been difficult to elucidate in part due to the diverse set of risk factors linked to this condition including over-nutrition, physical inactivity, pregnancy, Hepatitis C, polycystic ovarian syndrome, HIV protease inhibitor therapy, and antiinflammatory corticosteroids. Do such factors converge at a common intermediate in the insulin action pathway or does IR represent a collection of distinct cellular disorders? For example, endoplasmic reticulum (ER) stress, proinflammatory responses, oxidative stress, intracellular ceramide accumulation, or the activation of JNK, IKK, or PKC are all currently implicated in the development of IR in overnourished or obese rodents (2, 3). In such models, correcting any one of these intracellular stresses is sufficient to improve IR leading to the possibility that these factors are somehow interconnected. One view is that insulin receptor substrate 1 (IRS1) represents a common convergence point for many defects contributing to IR (4). However, this view has been challenged in that the ability of IRS1-independent receptor tyrosine kinases to activate

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Insulin Resistance as a Physiological Defense Against Metabolic Stress: Implications for the Management of Subsets of Type 2 Diabetes

The β -cell is particularly vulnerable to glucolipotoxicity. Other tissues, such as heart and skeletal muscle, that express the insulin-regulated glucose transporter GLUT4 have the capacity to protect themselves from glucolipotoxicity by developing IR, which restrains glucose entry into cells and therefore the glucose arm of this potentially damaging process.

T2D. Potential molecular mechanisms underlying these concepts; their clinical implications for stratification of T2D management, particularly in overweight and obese patients with difficult glycemic control; and future research requirements are discussed.

high-dose insulin therapy) will cause them harm. We believe that the concept of "insulin-induced metabolic stress" provides a plausible explanation for many of the unexpected outcomes of major T2D clinical trials. The important implications of this concept for ongoing

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Functional Medicine Diagnostic Workup



✓ Cause
✓ Participant
✓ Result

Thyroid Hormones, Oxidative Stress, and Inflammation

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OS has been shown to be associated with both hyperthyroidism and hypothyroidism [42]. However, the mechanisms by which OS is generated in these two clinical conditions are different: increased ROS production in hyperthyroidism and low availability of antioxidants in hypothyroidism.

OS in animals and humans. In this context, the nonthyroidal illness syndrome (NTIS) that typically manifests as reduced conversion of thyroxine (T₄) to triiodothyronine (T₃) in different acute and chronic systemic conditions is still a debated topic. The pathophysiological mechanisms of this syndrome are reviewed, together with the roles of deiodinases, the enzymes responsible for the conversion of T₄ to T₃, in both physiological and pathological situations. The presence of OS indexes in NTIS supports the hypothesis that it represents a condition of hypothyroidism at the tissue level and not only an adaptive mechanism to diseases.

1. Introduction

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Oxidative stress (OS) is defined as an unbalance between the production of prooxidant substances and antioxidant defenses. The most important prooxidants are the reactive oxygen species (ROS) and reactive nitrogen species (RNS) [1]. The ROS family includes superoxide anion, hydroxyl radical, hydrogen peroxide, and hypochlorous acid. The first three substances are produced *in vivo* mainly by the mitochondrial respiratory chain during the oxidative metabolism of energetic substrates [2, 3]. They are regulators of redox-sensitive pathways involved in cellular homeostasis [4] and influence some transcription factors, in addition to the endogenous antioxidant pool [4–7]. RNS are peroxynitrite, produced by the reaction of nitric oxide (NO) with superoxide, and nitrosoperoxycarbonate, formed by the reaction of peroxynitrite with carbon dioxide. ROS and RNS are considered important pathogenetic factors

Thyroid Hormones, Oxidative Stress, and Inflammation

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...on hypothyroidism and OS in humans are conflicting. In a group of patients with primary hypothyroidism, Baskol et al. [64] found high plasma levels of malondialdehyde (MDA), an OS marker that is formed by lipid peroxidation, and NO, low activity of paraoxonase- (PON-) 1, an enzyme synthesized in the liver with antioxidant properties, and SOD levels not significantly different from those of controls. Interestingly, the treatment with thyroid hormones decreased MDA levels and increased PON-1 activity, even though values similar to those observed in controls were not reached [64]. They hypothesized that in patients with hypothyroidism the prooxidant environment could play a role in the development of atherosclerosis.

Oxidative stress (OS) is defined as an imbalance between the production of prooxidant substances and antioxidant defenses. The most important prooxidants are the reactive oxygen species (ROS) and reactive nitrogen species (RNS) [1]. The ROS family includes superoxide anion, hydroxyl radical, hydrogen peroxide, and hypochlorous acid. The first three substances are produced *in vivo* mainly by the mitochondrial respiratory chain during the oxidative metabolism of energetic substrates [2, 3]. They are regulators of redox-sensitive pathways involved in cellular homeostasis [4] and influence some transcription factors, in addition to the endogenous antioxidant pool [4–7]. RNS are peroxynitrite, produced by the reaction of nitric oxide (NO) with superoxide, and nitrosoperoxycarbonate, formed by the reaction of peroxynitrite with carbon dioxide. ROS and RNS are considered important pathogenetic factors

Functional Medicine of the next 10 years is going to stop asking “What’s wrong” and start asking:

WHAT IS YOUR INNATE INTELLIGENCE UP TO?

When you ask “What’s Wrong,” you end up chasing sickness and disease.

When you SHIFT and ask “What’s Innate Intelligence up to,” you end up chasing health and wellness.