Evaluating Oxidative Stress Using Blood Chemistry

Dr. Bryan Walsh

Biogenetix

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Oxidative Stress

* Are you currently evaluating oxidative stress in patients?
* Would you like to know which patients need antioxidants and which don't?
* Would you like to know which patients might benefit from glutathione?

Overview

1. Oxidative Stress – What is it and why is it important
2. Markers of oxidative stress on a blood chemistry
3. What to do

Oxidative Stress

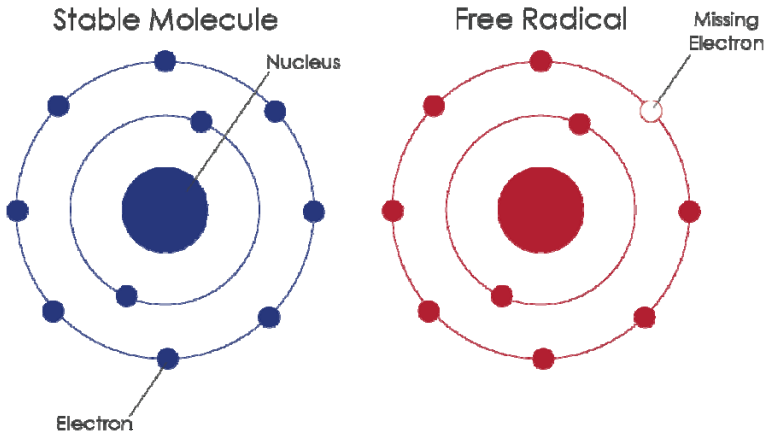
* Oxidative stress has been linked to heart disease, cancer, rheumatoid arthritis, hypertension, Alzheimer's disease, and Parkinson's disease, and much more

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

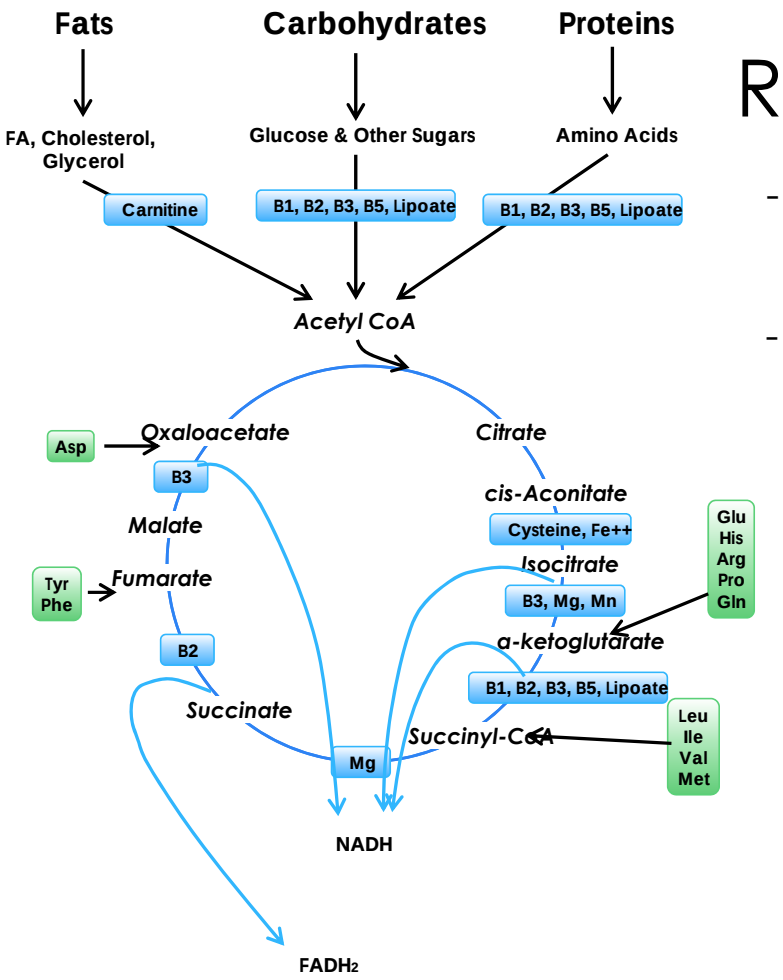


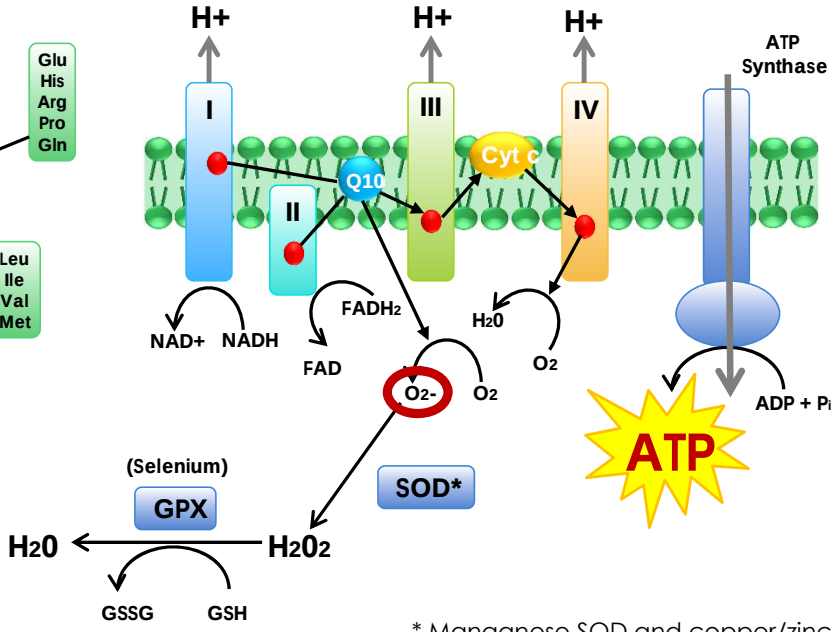
Free Radicals

* Examples include:
  * Hydroxyl radical (OH)
  * Peroxyl radical (ROO)
  * Superoxide radical (O2-)
  * Nitric oxide (NO), peroxynitrite (ONOO-), and NO2

Reactive oxygen species

- 0.4–4.0% of oxygen is converted to the superoxide (O2-) radical.
- Mitochondrial damage leads to more ROS, causing damage to mitochondrial DNA

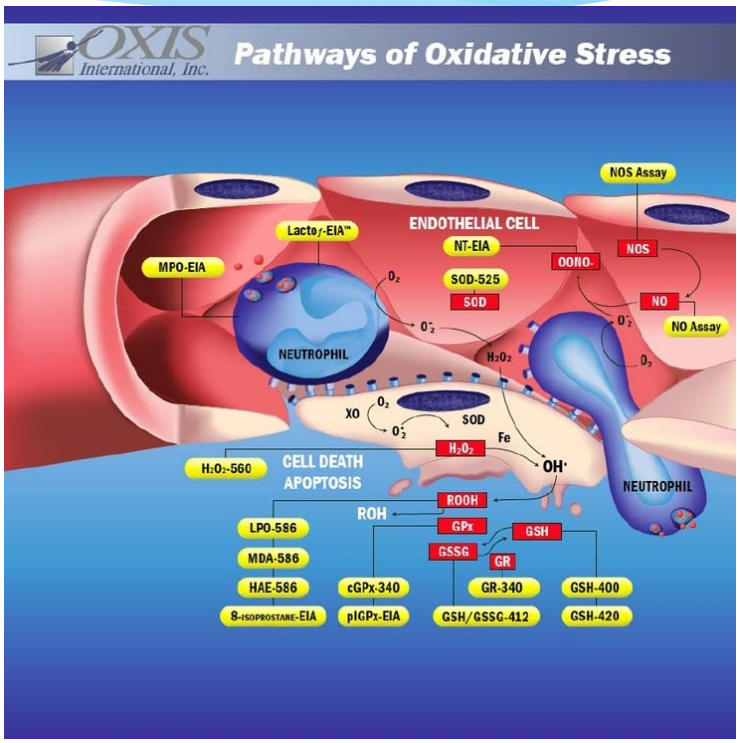




* Manganese SOD and copper/zinc SOD

Causes of Free Radicals

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

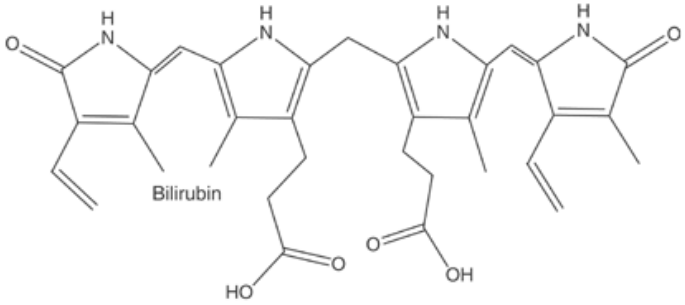


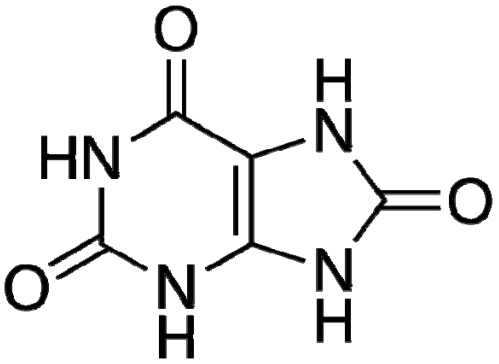
Conventional Markers of Oxidative Stress

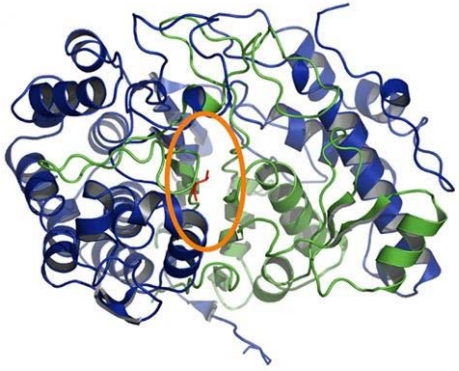
Organelle	Biomarker activity
Nucleus	8-hydroxy deoxyguanosine
Mitochondria	Catalase, Cu/Zn SOD, Mn-SOD
Endoplasmic reticulum and golgi	PEG-SOD, F2-isoprostanes, HNE
Plasma	TBARS, CUPRAC, 8-iso-PGF(2a), LHP
Total cellular constituents	Cytokines, chaperones, teleomeres

Three Makers of Oxidative Stress

* Uric Acid
* GGT
* Bilirubin





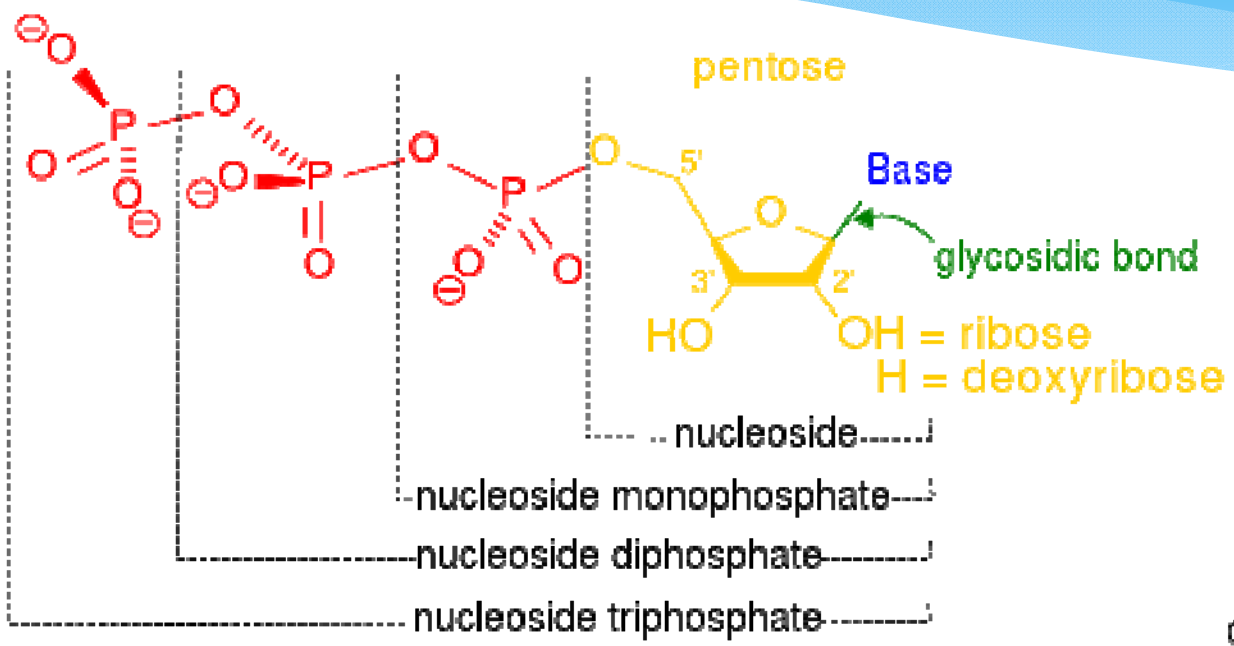


Uric Acid

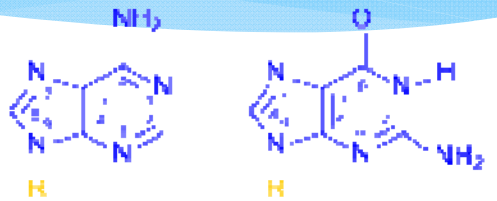
Uric Acid

* 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.

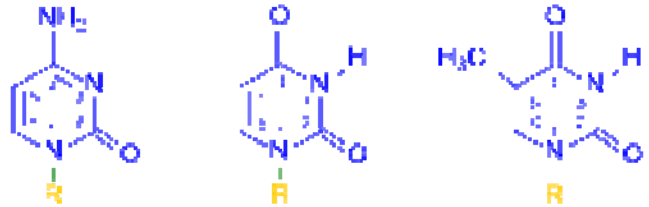


Purines



Adenine Guanine

Pyrimidines



Cytosine Uracil Thymine

Uric Acid

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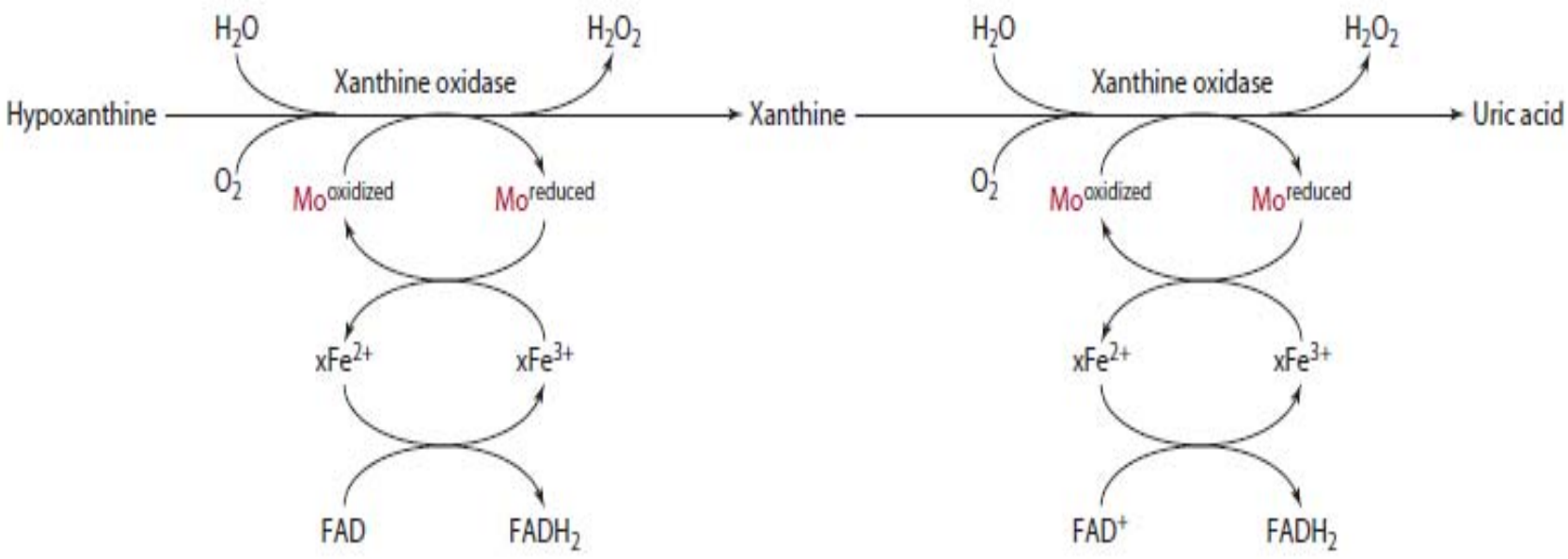
Uric Acid


H2O
H2O2
Xanthine oxidase
Hypoxanthine
Xanthine
O2
Mo oxidized
Mo reduced
xFe2+
xFe3+
FAD
FADH2
H2O
H2O2
Xanthine oxidase
Uric acid
O2
Mo oxidized
Mo reduced
xFe2+
xFe3+
FAD+
FADH2

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

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

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.

4.5 ± 0.2 mg/dL; *n* = 16) levels were studied; 13 subjects with HUA levels were studied again after 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 ± 25% to 156 ± 17%, *P* = NS). These results demonstrate that circulating UA is a major antioxidant and might help protect against free-radical oxidative damage.

culture systems have shown that products of oxidative stress impair insulin-mediated translocation of GLUT4 in 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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Competing Interests: The authors have declared that no competing interests exist.

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/Height^2, kg/m^2) 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.

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.**

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Introduction

Myasthenia gravis (MG), caused by autoantibodies against the acetylcholine receptor (AChR) on the postsynaptic 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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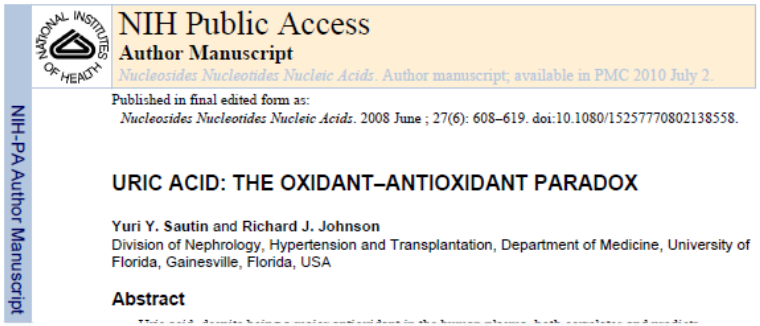
Uric Acid Summary

* 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 Summary

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



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URIC ACID: THE OXIDANT–ANTIOXIDANT PARADOX

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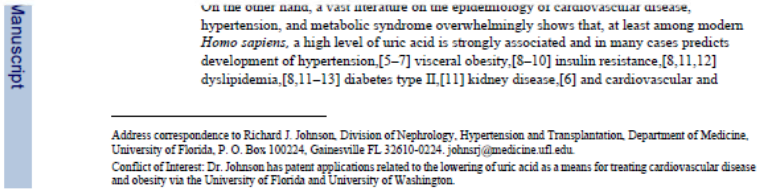
Abstract

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.

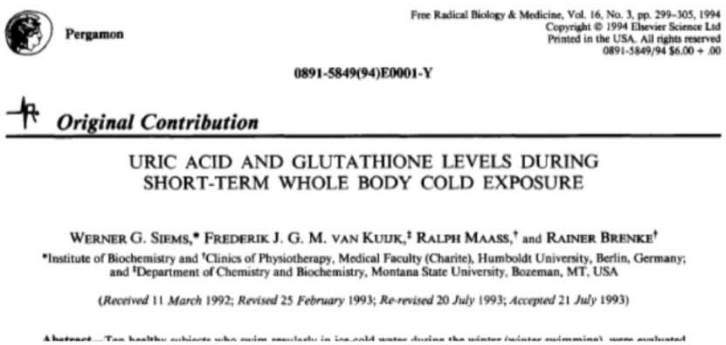
Uric Acid

Traditional Reference range:

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

Optimal Reference Range:

* 3.7-6.0 mg/dL (men)
* 3.2-5.5 mg/dL (women)



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Original Contribution

URIC ACID AND GLUTATHIONE LEVELS DURING SHORT-TERM WHOLE BODY COLD EXPOSURE

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Abstract—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.

eases, 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-

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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.

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
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
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

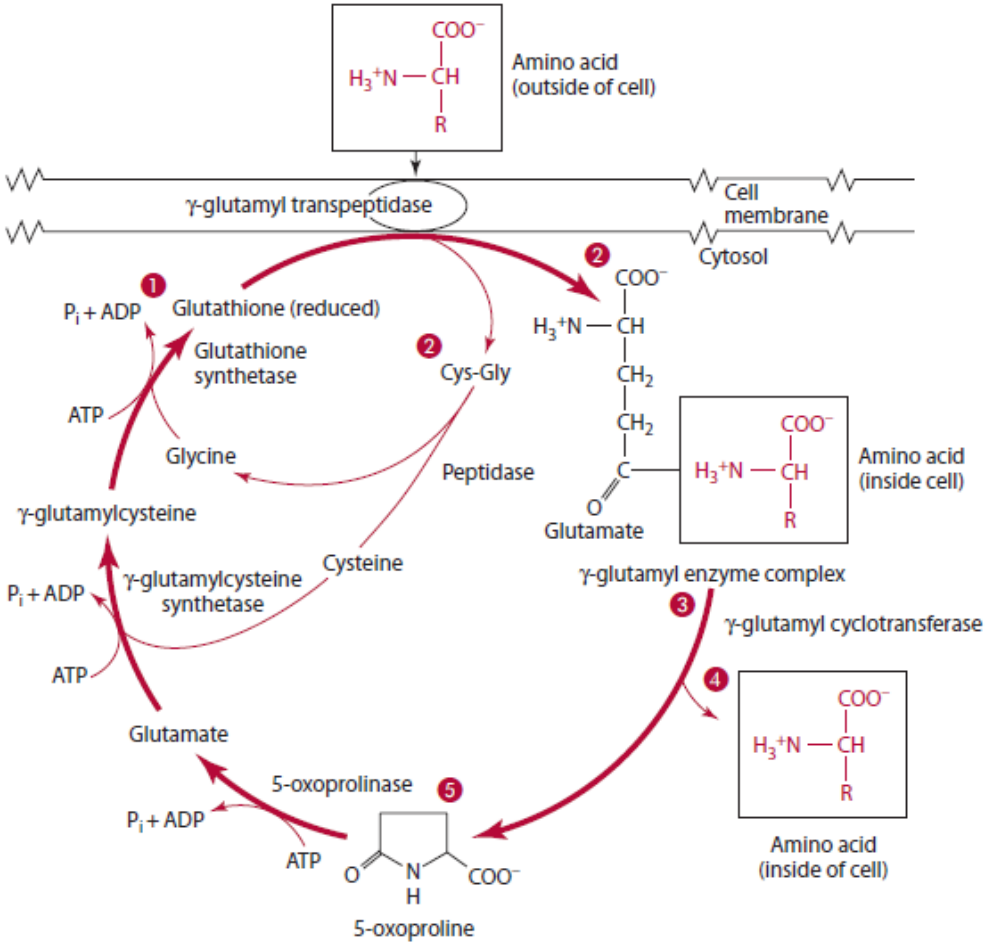
Gamma-Glutamyltransferase (GGT)

Gamma-Glutamyltransferase (GGT)

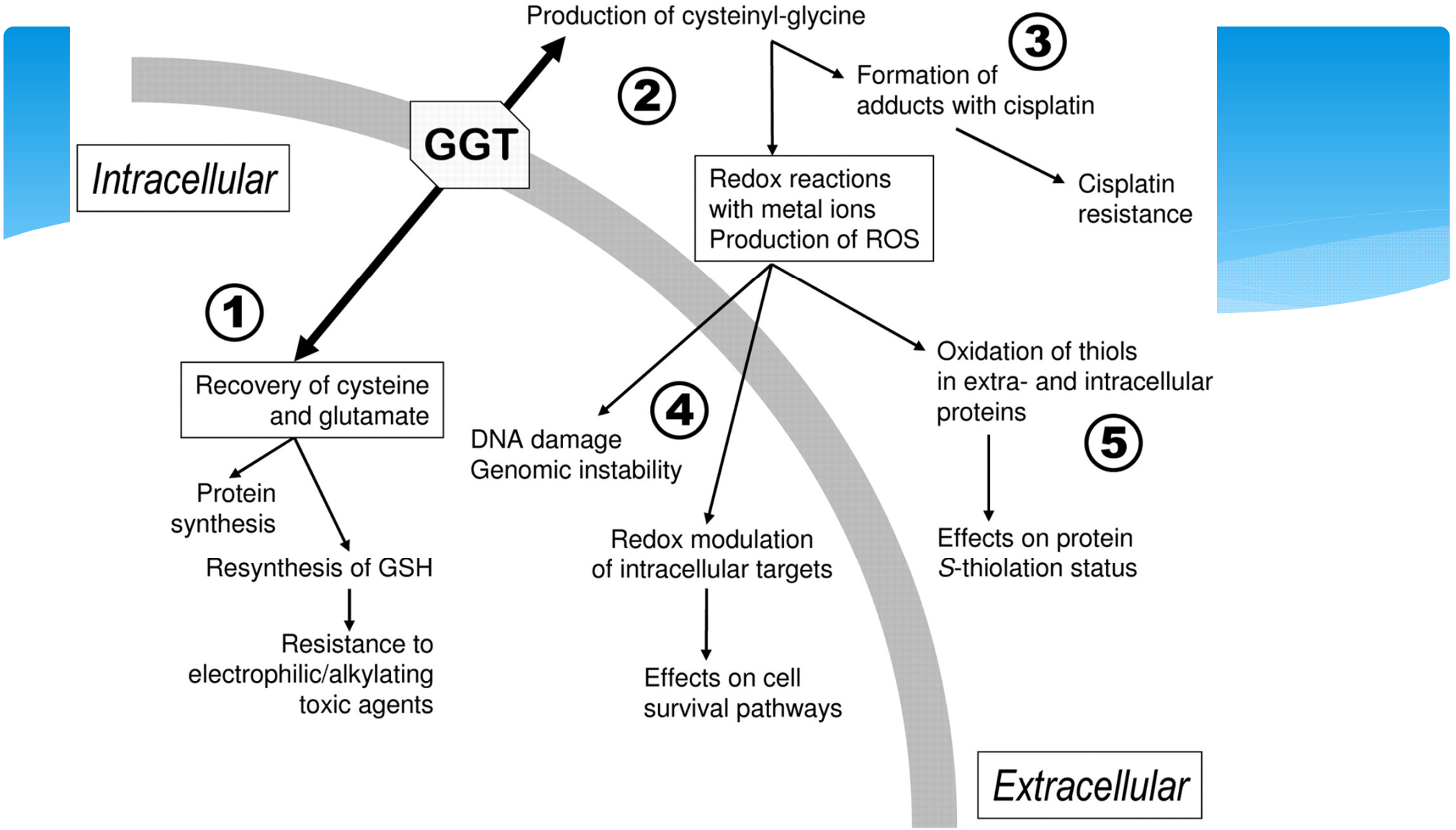
* Gamma-glutamyltransferase (GGT) is present primarily in the liver, kidney and pancreas.
* GGT is highly sensitive for biliary tree issues, and is often elevated in excess alcohol consumption
* GGT is present in cell membranes and is involved in the transfer of amino acids
* Plays key roles in catabolism and synthesis of glutathione

Gamma-Glutamyltransferase (GGT)

* Introduced to clinical laboratories a half century ago
* Recognized as biomarker of liver injury and alcohol consumption
* More recently, suggested to be a biomarker of transition across the disease spectrum (similar to cholesterol several decades ago)



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).


Production of cysteinyl-glycine
2
3
Formation of
adducts with cisplatin
GGT
Intracellular
Redox reactions
with metal ions
Production of ROS
Cisplatin
resistance
1
Recovery of cysteine
and glutamate
4
DNA damage
Genomic instability
Oxidation of thiols
in extra- and intracellular
proteins
5
Protein
synthesis
Resynthesis of GSH
Redox modulation
of intracellular targets
Effects on protein
S-thiolation status
Resistance to
electrophilic/alkylating
toxic agents
Effects on cell
survival pathways
Extracellular

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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].

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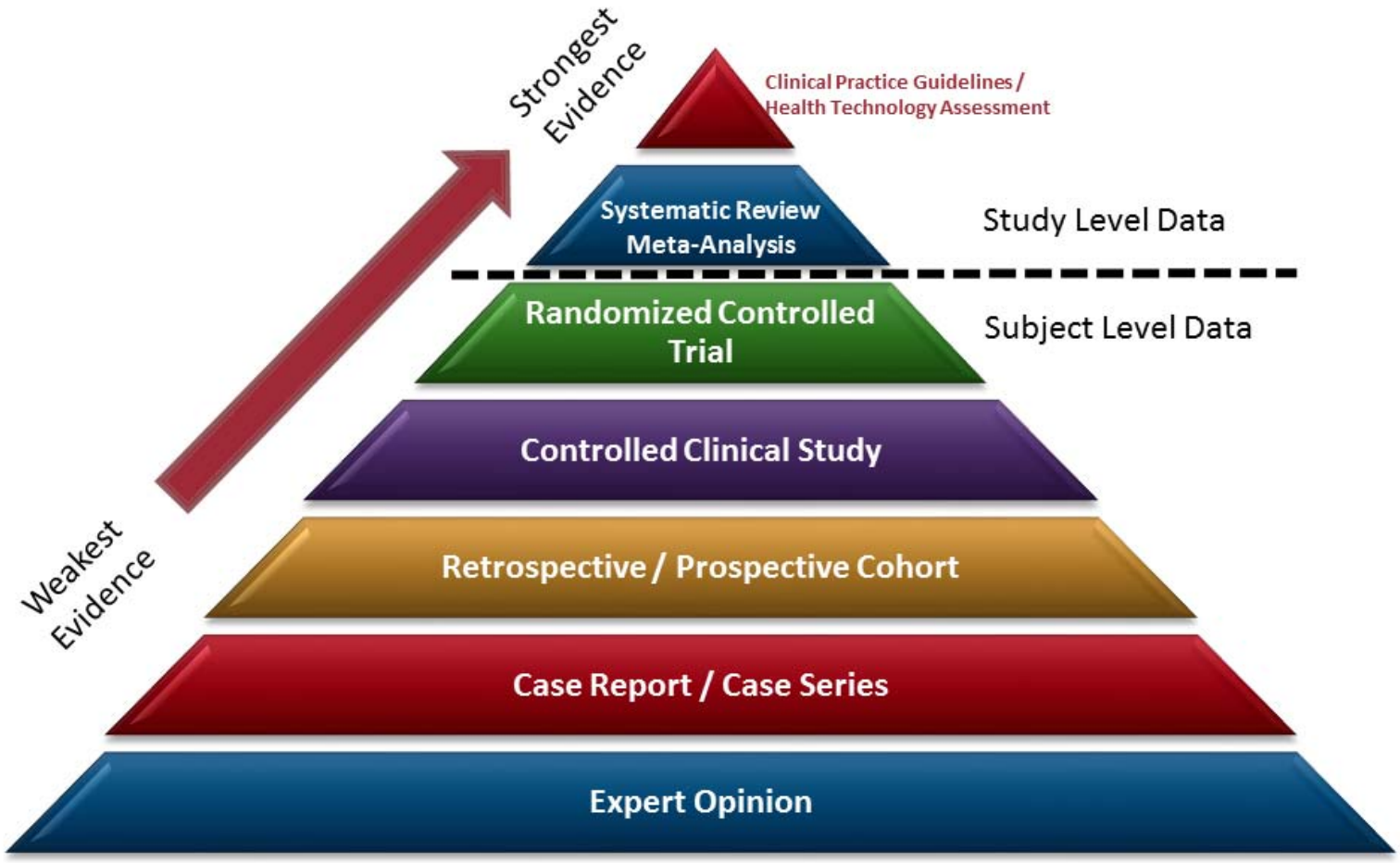
Strongest Evidence
Clinical Practice Guidelines / Health Technology Assessment
Systematic Review Meta-Analysis
Study Level Data
Randomized Controlled Trial
Subject Level Data
Controlled Clinical Study
Retrospective / Prospective Cohort
Case Report / Case Series
Expert Opinion
Weakest Evidence

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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

Giovanni Targher*
Section of Endocrinology, Department of Biomedical and Surgical Sciences, University of Verona, Verona, Italy

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

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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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γ-Glutamyltranspeptidase Stimulates Receptor Activator of Nuclear Factor-κB Ligand Expression Independent of Its Enzymatic Activity and Serves as a Pathological Bone-resorbing Factor*

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A novel bone-resorbing factor was cloned using an expression cloning technique, which involved a *Xenopus* oocyte expression system and an assay for osteoclast formation. A candidate clone was isolated from a BW5147 mouse T-lymphoma cell cDNA library. Sequence and protein from bone marrow stromal cells. This report is the first demonstration of a novel biological activity of GGT protein in a manner independent of its enzymatic activity.

Osteoclasts are potent bone resorbing cells that are derived from hematopoietic cells of the monocyte/macrophage lineage (1–3). Osteoclast differentiation is regulated by the simultaneous stimulation of colony stimulating factor-1 (CSF-1/M-CSF) and the receptor activator of nuclear factor-κB ligand (RANKL),[1] which are produced by osteoblasts/stromal cells (3–8). The expression of these essential factors is stimulated by systemic bone-resorbing factors such as 1α,25-dihydroxyvitamin D[3], resistant acid phosphatase-positive (TRAP$^+$) multinucleated osteoclast (MNC) formation in the mouse bone marrow culture. Because TRAP activity is expressed specifically in osteoclasts lineage (16–18), it has been suggested that the BW5147 cells produce an osteoclast-forming factor that may play a role in pathological bone resorption such as metastatic lesions.

To identify and characterize the factor that promotes pathological bone resorption, we cloned a cDNA for the osteoclast-forming factor(s) from a BW5147 cell cDNA library by expression cloning using a *Xenopus* oocyte translation system. Consequently, we identified γ-glutamyltranspeptidase (GGT) as the bone-resorbing factor. GGT is an ectoenzyme that plays an important role in regulating glutathione metabolism (19) and is well known as a clinical marker for a number of diseases. In this study, we showed a possible mechanism by which GGT induces osteoclast formation using a mouse bone marrow culture system.

EXPERIMENTAL PROCEDURES

Cell Culture—A mouse T-lymphoma cell line, BW5147 (CRL-1588), (20) was maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin.

Expression Cloning—Poly(A)$^+$ RNA was prepared from BW5147 cells and size-fractionated by means of sucrose density gradient centrifuga-

* This study was supported in part by a grant-in-aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan (to S. N. and N. T.) for scientific research, and the Program for Promotion of Fundamental Studies in Health Sciences of the Organization for Pharmaceutical Safety and Research of Japan (to K. I.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) E15738 and U30509.

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[1] The abbreviations used are: RANKL, receptor activator of nuclear factor-κB ligand; IL, interleukin; TRAP, tartrate-resistant acid phosphatase; MNC, multinucleated osteoclast; GGT, γ-glutamyltranspeptidase; 1,25(OH)$_2$D$_3$, 1α,25-dihydroxyvitamin D$_3$; I-GGT, inactive GGT; OPG, osteoprotegerin.

Furthermore, both native GGT and inactive GGT stimulated the expression of the receptor activator of nuclear factor-B ligand (RANKL) mRNA and protein from bone marrow stromal cells. This report is the first demonstration of a novel biological activity of GGT protein in a manner independent of its enzymatic activity.

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Hindawi

Review Article

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

Gerald Koenig[1,2] and Stephanie Seneff[3]

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[2]*Iron Disorders Institute, Greenville, SC 29615, USA*
[3]*Computer 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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[3]*Computer Science and Artificial Intelligence Laboratory, MIT, Cambridge, MA 02139, USA*

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.

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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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 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.

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Review Article

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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.

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 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.

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Gamma-Glutamyltransferase (GGT)

Traditional Reference Range

* 0-65 IU/L

Optimal Reference Range

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

Do you just give glutathione?

ELSEVIER

Biochemical Pharmacology 64 (2002) 1027–1035

Biochemical Pharmacology

Glutathione catabolism as a signaling mechanism

Aldo Paolicchi[a], Silvia Dominici[b], Lisa Pieri[a], Emilia Maellaro[b], Alfonso Pompella[a,*]

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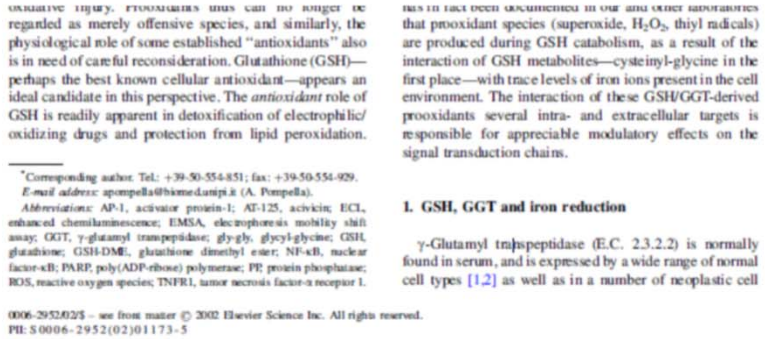
Received 31 January 2002; accepted 22 April 2002

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 decribed, 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 g-glutamyl transpeptidatse (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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E-mail address: apompella@biomed.unipi.it (A. Pompella).

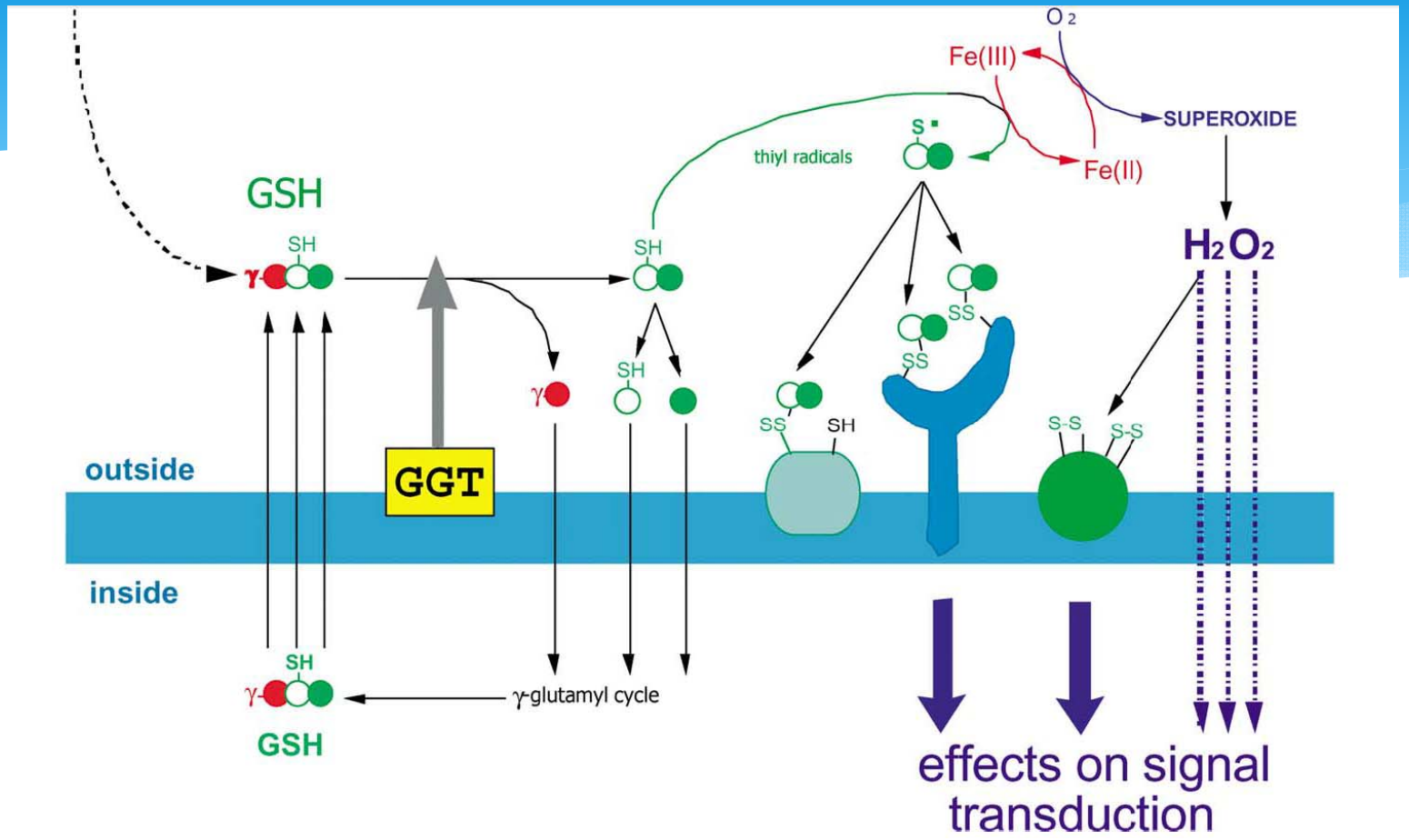
Abbreviations: AP-1, activator protein-1; AT-125, acivicin; ECL, enhanced chemiluminescence; EMSA, electrophoresis mobility shift assay; GGT, γ-glutamyl transpeptidase; gly-gly, glycyl-glycine; GSH, glutathione; GSH-DME, glutathione dimethyl ester; NF-κB, nuclear factor-κB; PARP, poly(ADP-ribose) polymerase;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

that prooxidant species (superoxide, H_2O_2, thiyl 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

PII: S0006-2952(02)01173-5


GSH
SH
γ-
GGT
outside
inside
γ
SH
SH
γ-glutamyl cycle
GSH
thiyl radicals
S ▪
O 2
Fe(III)
Fe(II)
SUPEROXIDE
H2O2
SS
SH
SS
SS
S-S
S-S
effects on signal transduction

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

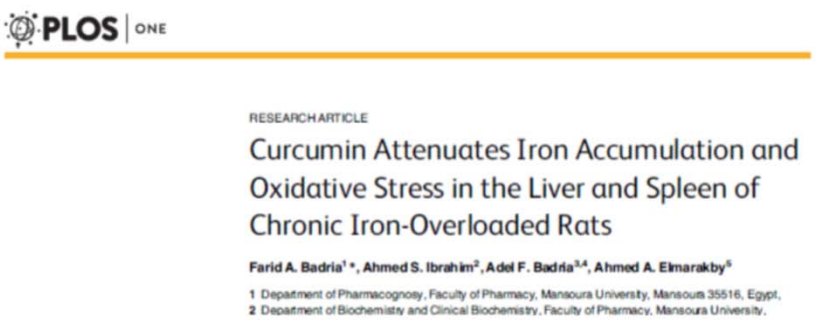
Supplement Facts

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



PLOS | ONE

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. Ibrahim[2], Adel F. Badria[3,4], Ahmed A. Elmarakby[5]

1 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 chelationation 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)

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.
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).

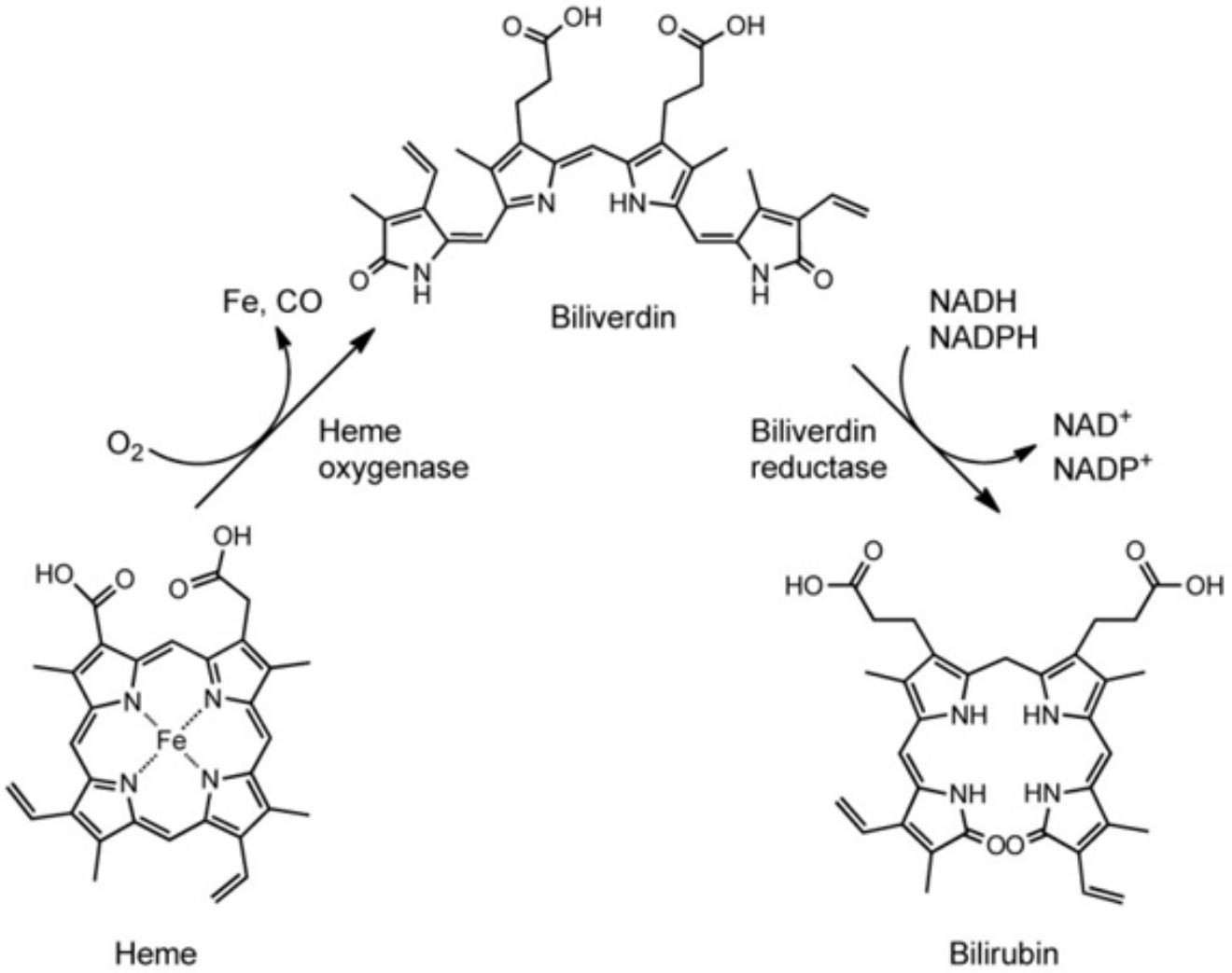
GGT - Decreased

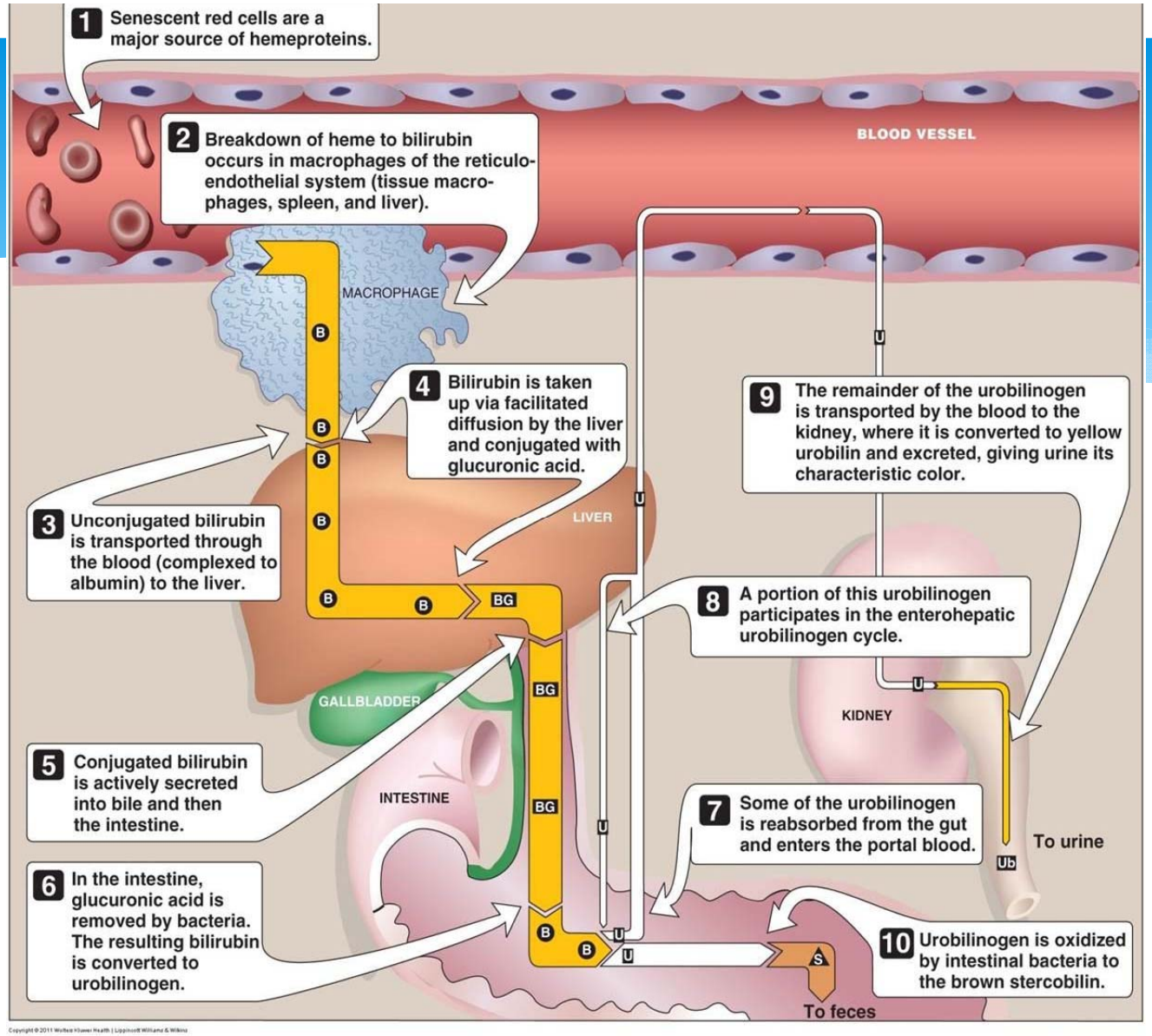
Cause	Reason	Additional Inquiry
Hypothyroidism		
Magnesium deficiency	Association. Inverse correlation between magnesium level and GGT levels.	Evaluate magnesium levels as well as signs and symptoms.

Bilirubin

Bilirubin

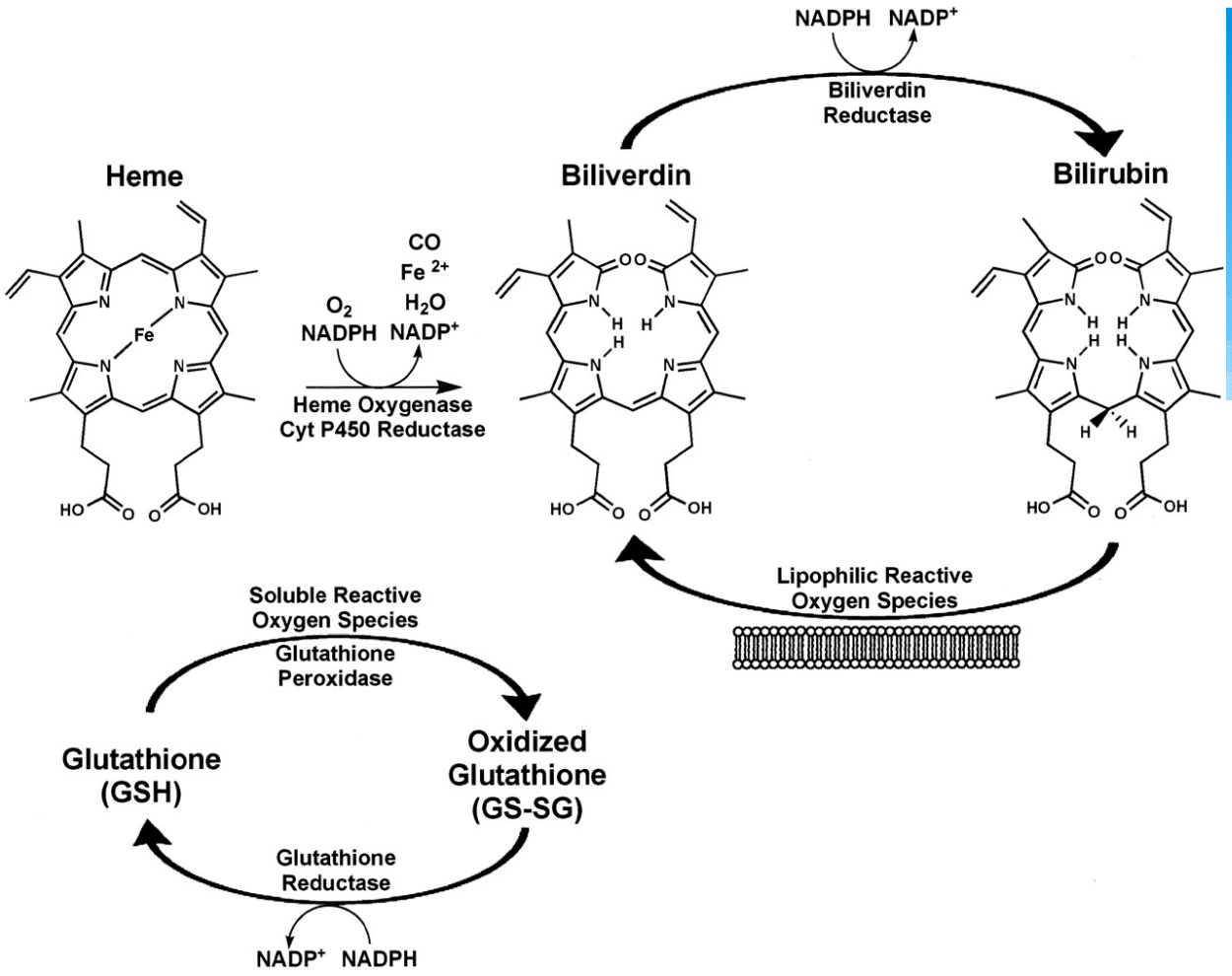
* Bilirubin is the byproduct of red blood cell breakdown.
* Serum bilirubin is a combination of *direct* (conjugated) and *indirect* (unconjugated) bilirubin
  * Normally, indirect bilirubin is approximately 70-85% of total bilirubin
  * If 50% or more is direct, hepatic/biliary obstruction is suspected
  * 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


Fe, CO
O_2
Heme oxygenase
Biliverdin
NADH
NADPH
Biliverdin reductase
NAD^+
$NADP^+$
Heme
Bilirubin


1 Senescent red cells are a major source of hemeproteins.
2 Breakdown of heme to bilirubin occurs in macrophages of the reticulo-endothelial system (tissue macrophages, spleen, and liver).
BLOOD VESSEL
MACROPHAGE
4 Bilirubin is taken up via facilitated diffusion by the liver and conjugated with glucuronic acid.
3 Unconjugated bilirubin is transported through the blood (complexed to albumin) to the liver.
LIVER
9 The remainder of the urobilinogen is transported by the blood to the kidney, where it is converted to yellow urobilin and excreted, giving urine its characteristic color.
8 A portion of this urobilinogen participates in the enterohepatic urobilinogen cycle.
GALLBLADDER
KIDNEY
5 Conjugated bilirubin is actively secreted into bile and then the intestine.
INTESTINE
7 Some of the urobilinogen is reabsorbed from the gut and enters the portal blood.
To urine
6 In the intestine, glucuronic acid is removed by bacteria. The resulting bilirubin is converted to urobilinogen.
10 Urobilinogen is oxidized by intestinal bacteria to the brown stercobilin.
To feces
B
BG
U
Ub
S

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



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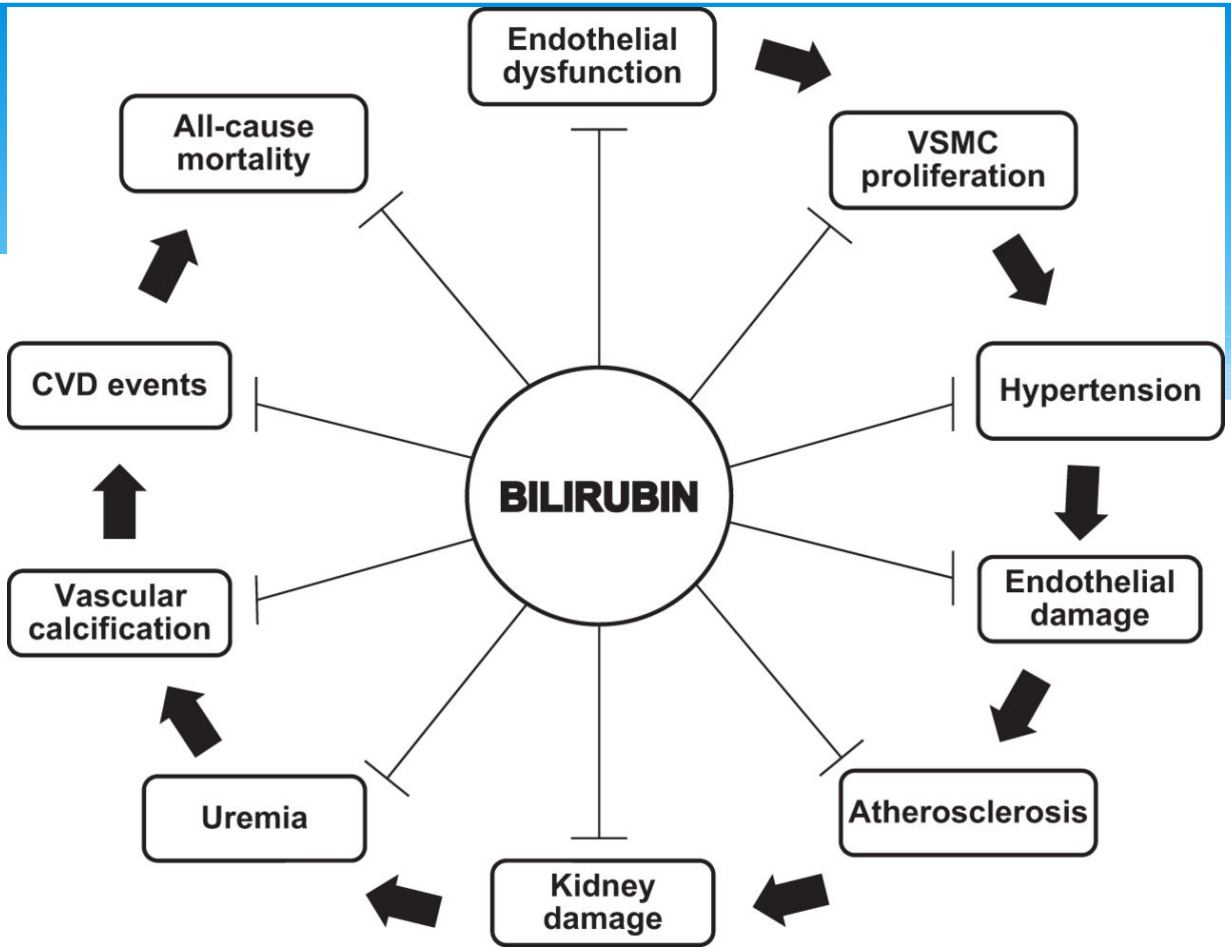

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ORIGINAL ARTICLE

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

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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%, 39.6%, 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.

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.

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2214-7624/

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The Relationship between Total Bilirubin Levels and Total Mortality in Older Adults: The United States

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.

[a]Adjusted for survey period, age, sex, and race/ethnicity (n = 4,303).

[b]Further adjusted for body mass index, education, smoking, and regular alcohol consumption (n = 3,928).

[c]Further 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).

[d]Further 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).

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Notably, bilirubin has anti-oxidant and anti-inflammatory effects, and can protect serum lipids from oxidation [1]. In this regard, previous studies have suggested bilirubin to be a protective

disease, myocardial infarction, and all-cause mortality in a non-linear and L-shaped relationship [12]. However, it is not known

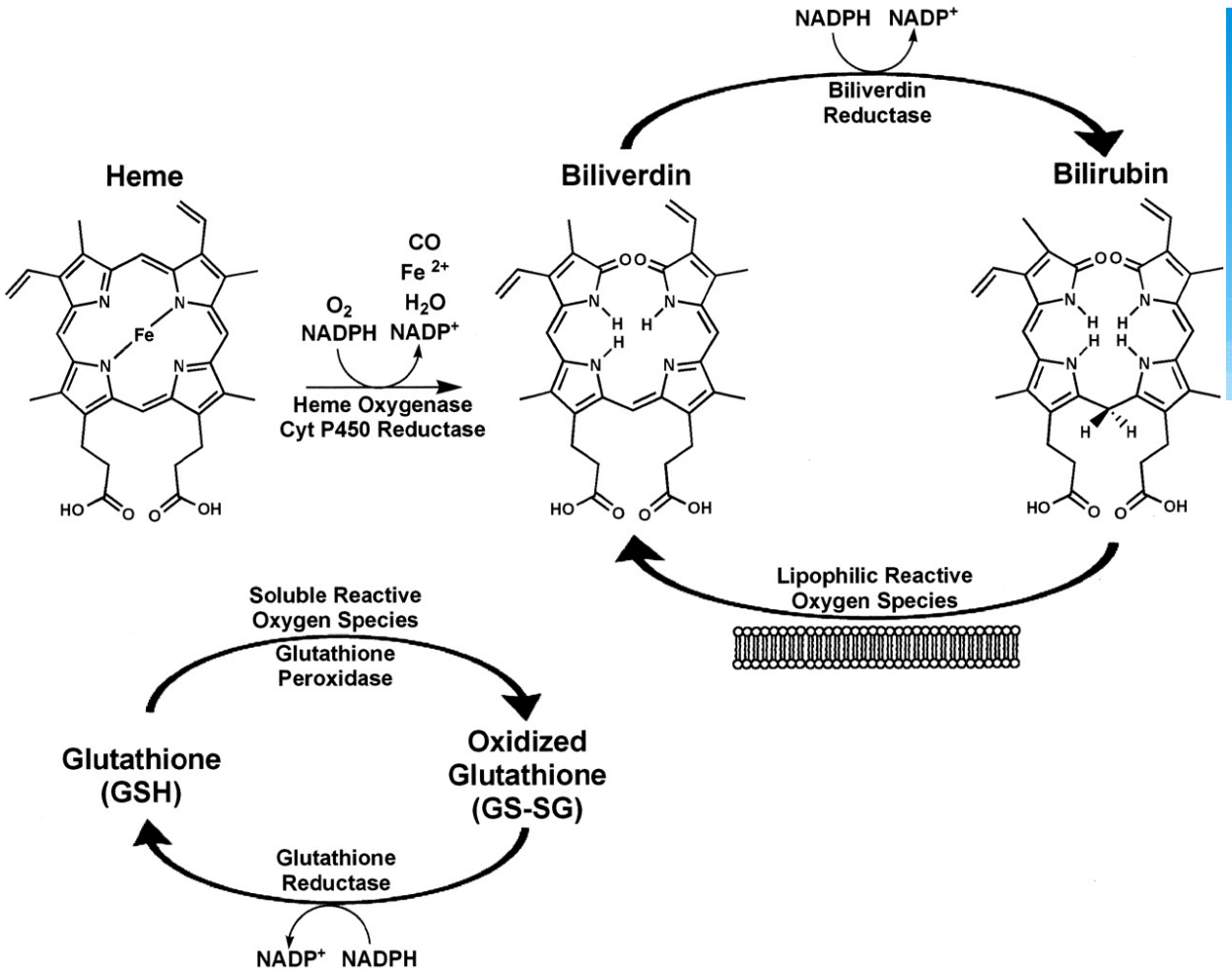
Bilirubin

Traditional Reference Range

* 0.1-1.2 mg/dL

Optimal Reference Range

* 0.5 – 0.8 mg/dL



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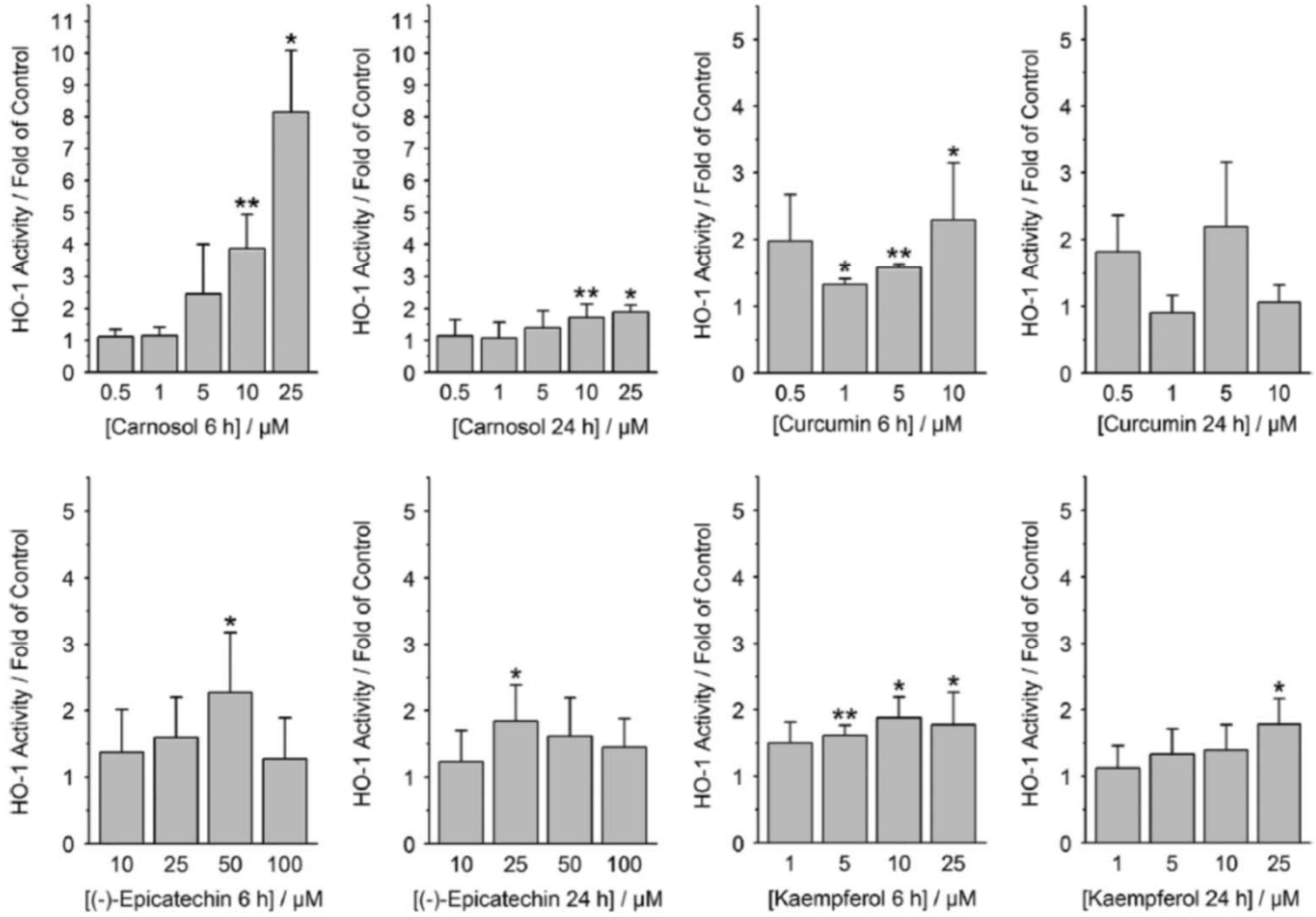

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Curcumin protects retinal pigment epithelial cells against oxidative stress via induction of heme oxygenase-1 expression and reduction of reactive oxygen

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At this concentration, curcumin also increased the cytoprotective effect against the oxidative stress of H_2O_2 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 H_2O_2 oxidative stress in cultured human retinal pigment epithelial cells.

METHODS

Materials: Curcumin, H_2O_2, 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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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.

Combined Markers

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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.

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 ≥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.

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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[1] Hyun Sun Cho and Sung Won Lee contributed equally to this project as co-first authors.

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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)	*P*-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: peroxyl radical scavenging activity. Bilirubin means total bilirubin concentration. Data expressed as median and 25–75%; * values represent micromoles of Trolox equivalents.

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as evidenced by lipid and protein oxidation in the vascular wall of affected subjects (Stocker and Keaney 2004). Oxidatively modified LDL (oxLDL), which is a surrogate marker of upregulated oxidative stress, promotes diverse potentially pro-atherogenic events and is a hallmark of atherosclerosis and cardiovascular disease (CVD) development (Stocker

Original Article

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) μmol/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, γGT, 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.[6,7,10] 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

Oxidative Stress

* Are you currently evaluating oxidative stress in patients?
* Would you like to know which patients need antioxidants and which don't?
* Would you like to know which patients might benefit from glutathione?

Summary

* Uric acid is a by-product of purine metabolism. It, too, is an antioxidant and when low, can indicate a number of possibilities, including oxidative stress
* γ-Glutamyltransferase (GGT) is an enzyme that metabolizes extracellular reduced glutathione, which allows for the amino acid precursors to be utilized for intracellular glutathione synthesis.
  * High-normal GGT is a marker of oxidative stress.
* Bilirubin is a by-product of red blood cell breakdown. It is also acts as an antioxidant.
  * Studies suggest that when low (< 0.5) can indicate excess oxidative stress

Summary

* Oxidative stress is associated with a number of chronic conditions
* You now have an easy and reliable way of evaluating oxidative stress, and thus recommending antioxidant therapy, to your patients/clients using a standard blood chemistry

Thank You

thank you!

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