Shawn M. Talbott, PhD

CNS, LDN, FACSM, FAIS, FACN

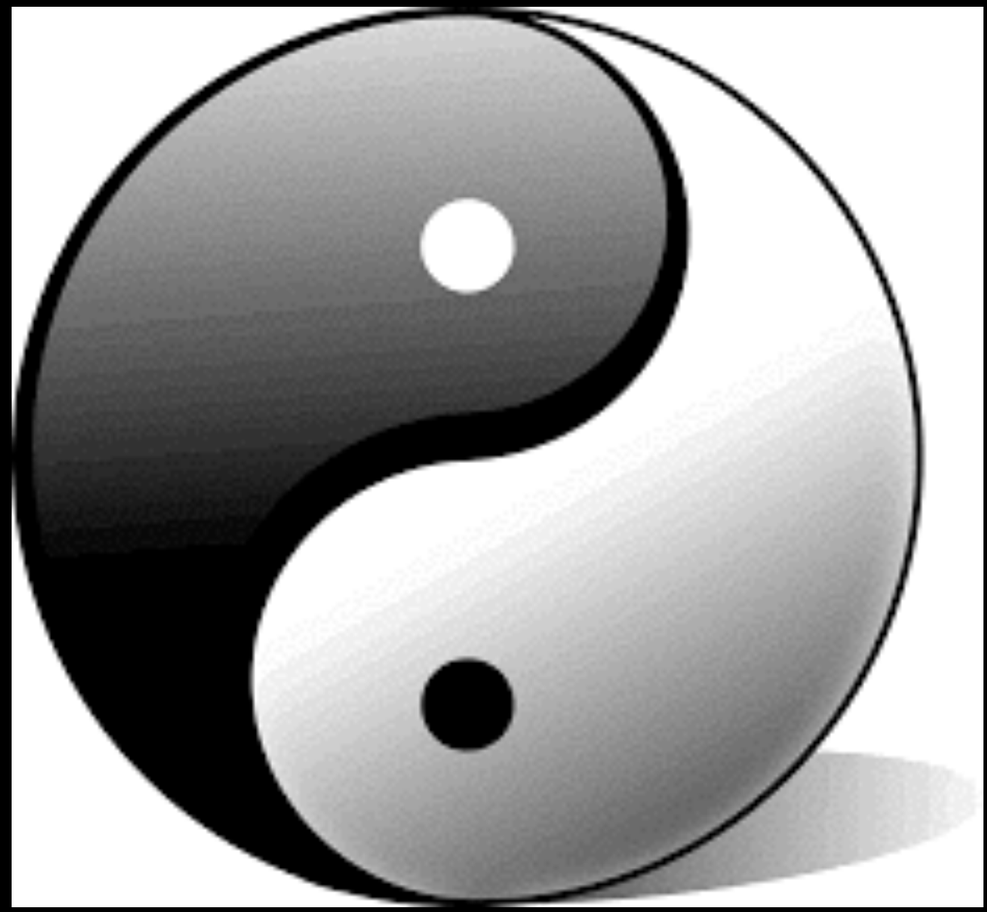
Chief Science Officer

LifeVantage FREEDOM


NRF2 REVOLUTION
What is Nrf2 and
Why should I care?
Mark Gordon, MD, FACC

WHAT IS OXIDATIVE STRESS?

- Oxidative stress, is a disturbance in the balance between the production of reactive oxygen species (free radicals) and antioxidant defenses,



Causes of Oxidative Stress

Smoking

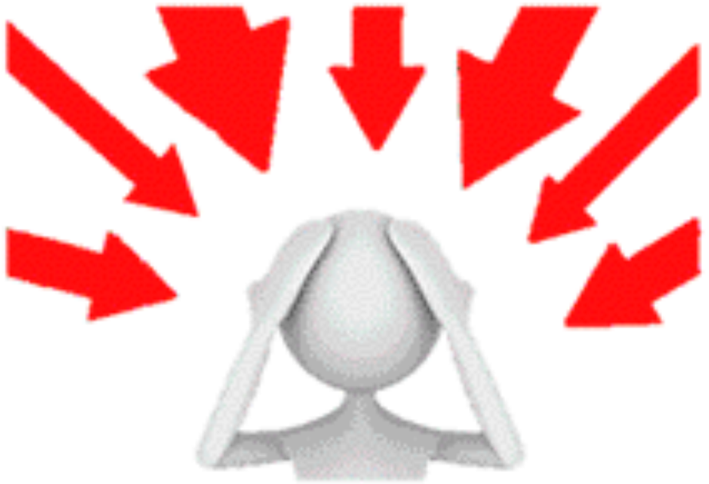
Diet

Alcohol

OXIDATIVE

Medication & Treatments

Pesticides



Exposure to Toxins

Air & Water Pollutants

Inadequate Intake of Fruits & Vegetables

Fast Foods [McDonald's]

Stress

STRESS!

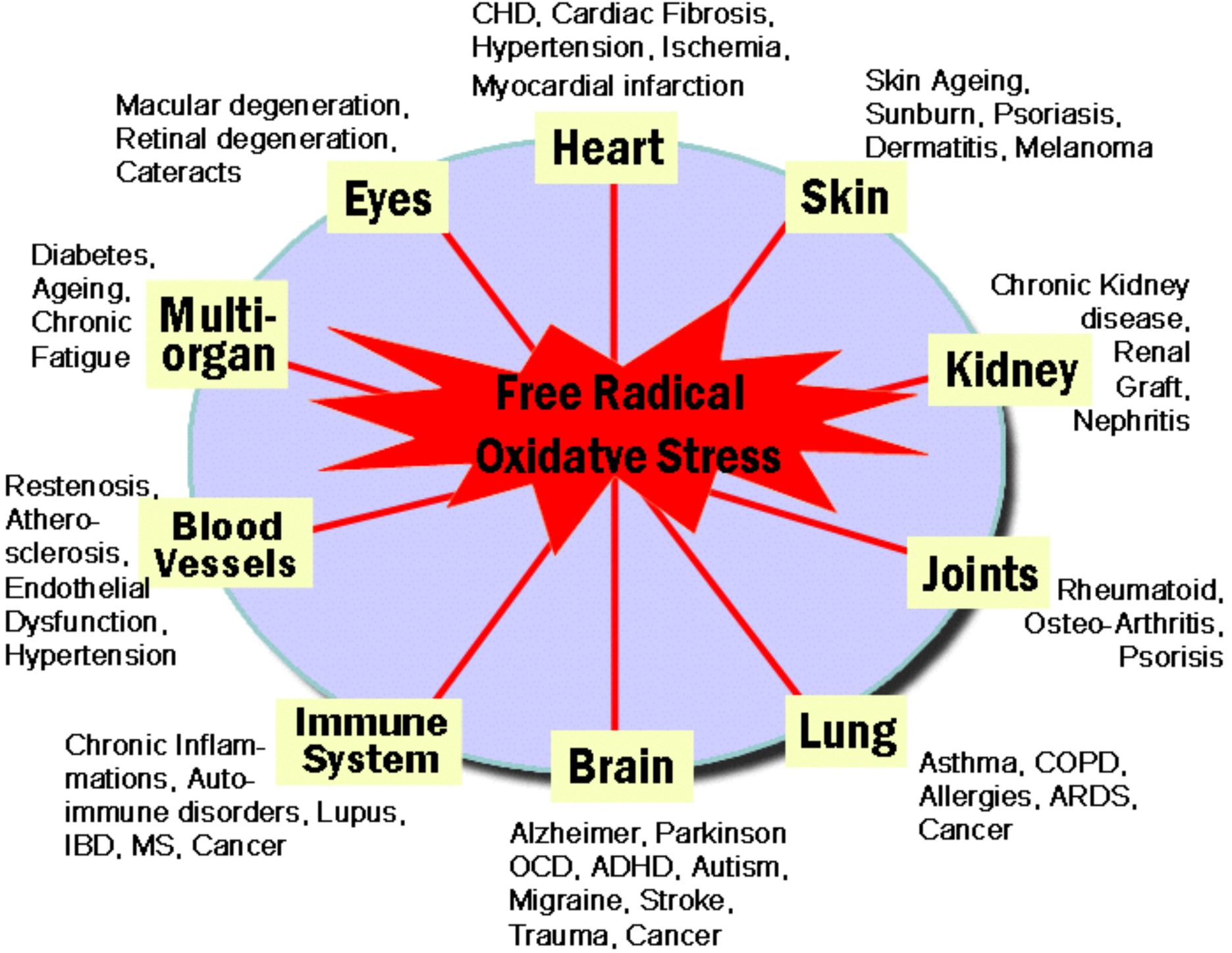
Contaminants

Lack of Good Nutrition

Inadequate amounts of physical activity

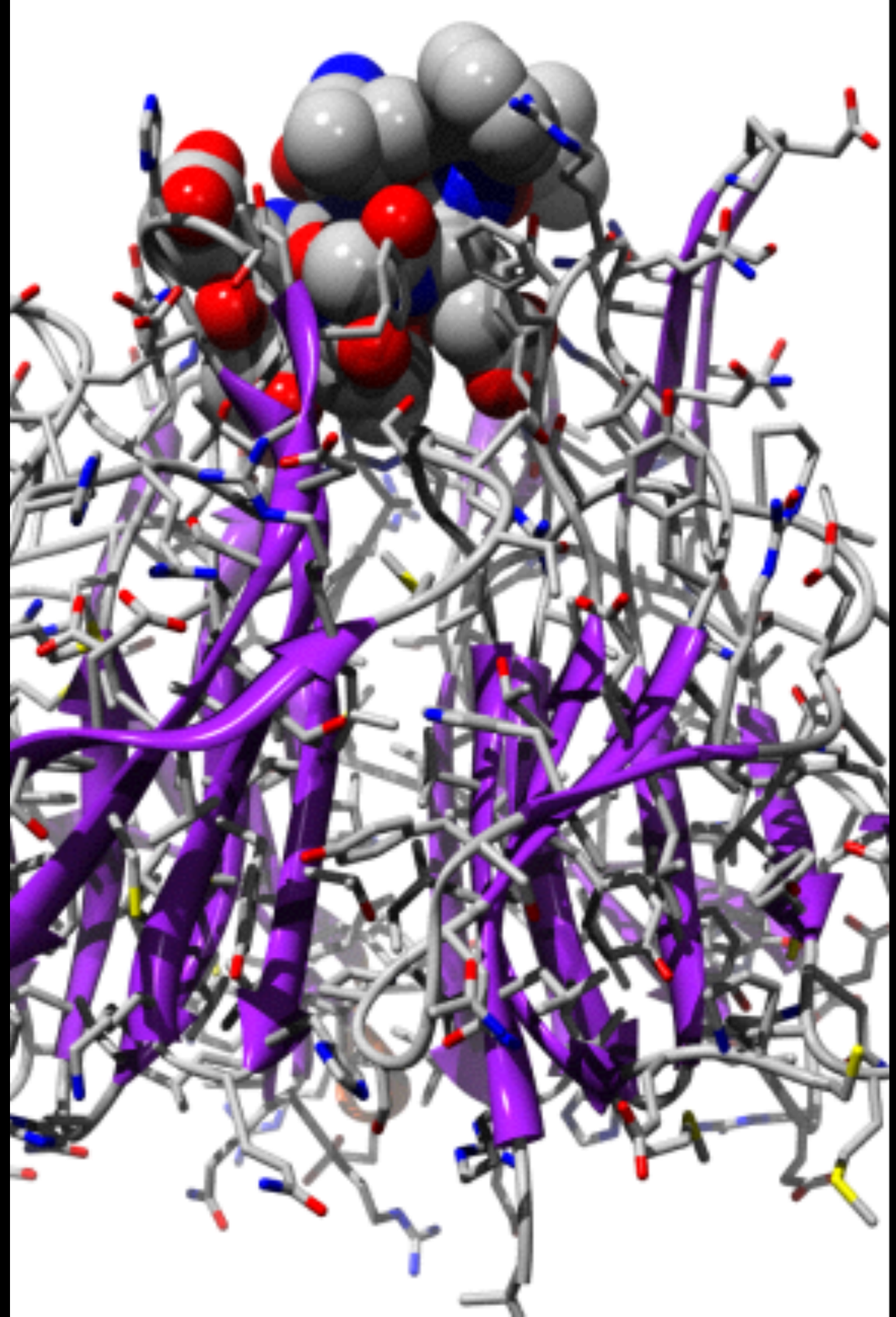
Excessive Exercise

Just about everything we do results in oxidation (or inflammation) producing potentially damaging free radicals!

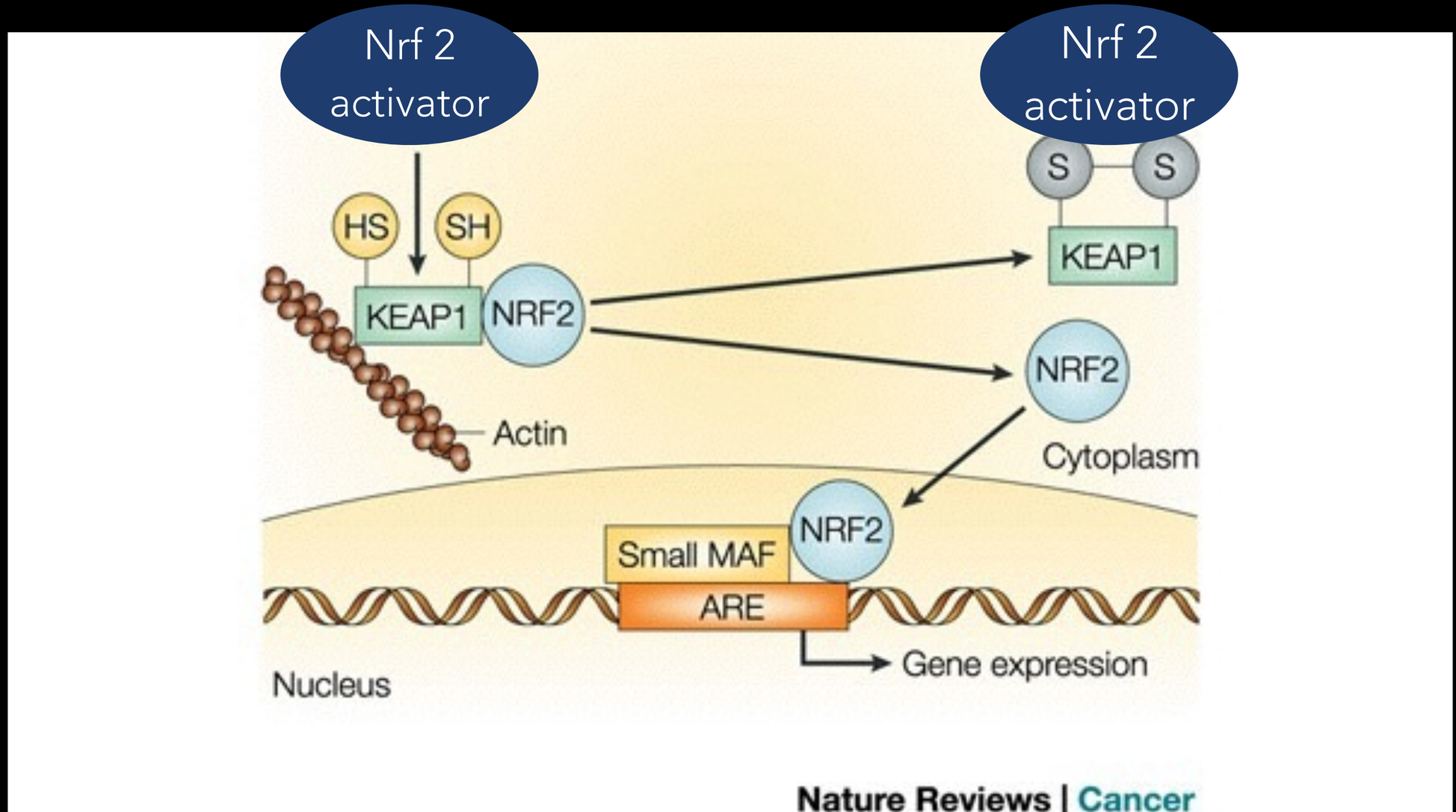

CHD, Cardiac Fibrosis,
Hypertension, Ischemia,
Myocardial infarction
Heart
Macular degeneration,
Retinal degeneration,
Cateracts
Eyes
Skin Ageing,
Sunburn, Psoriasis,
Dermatitis, Melanoma
Skin
Diabetes,
Ageing,
Chronic
Fatigue
Multi-
organ
Chronic Kidney
disease,
Renal
Graft,
Nephritis
Kidney
Free Radical
Oxidatve Stress
Restenosis,
Athero-
sclerosis,
Endothelial
Dysfunction,
Hypertension
Blood
Vessels
Joints
Rheumatoid,
Osteo-Arthritis,
Psorisis
Immune
System
Chronic Inflam-
mations, Auto-
immune disorders, Lupus,
IBD, MS, Cancer
Brain
Alzheimer, Parkinson
OCD, ADHD, Autism,
Migraine, Stroke,
Trauma, Cancer
Lung
Asthma, COPD,
Allergies, ARDS,
Cancer

NRF2

- Messenger Protein
- Activates genes
- Redox balance
- Delicate balance
- Master Regulator



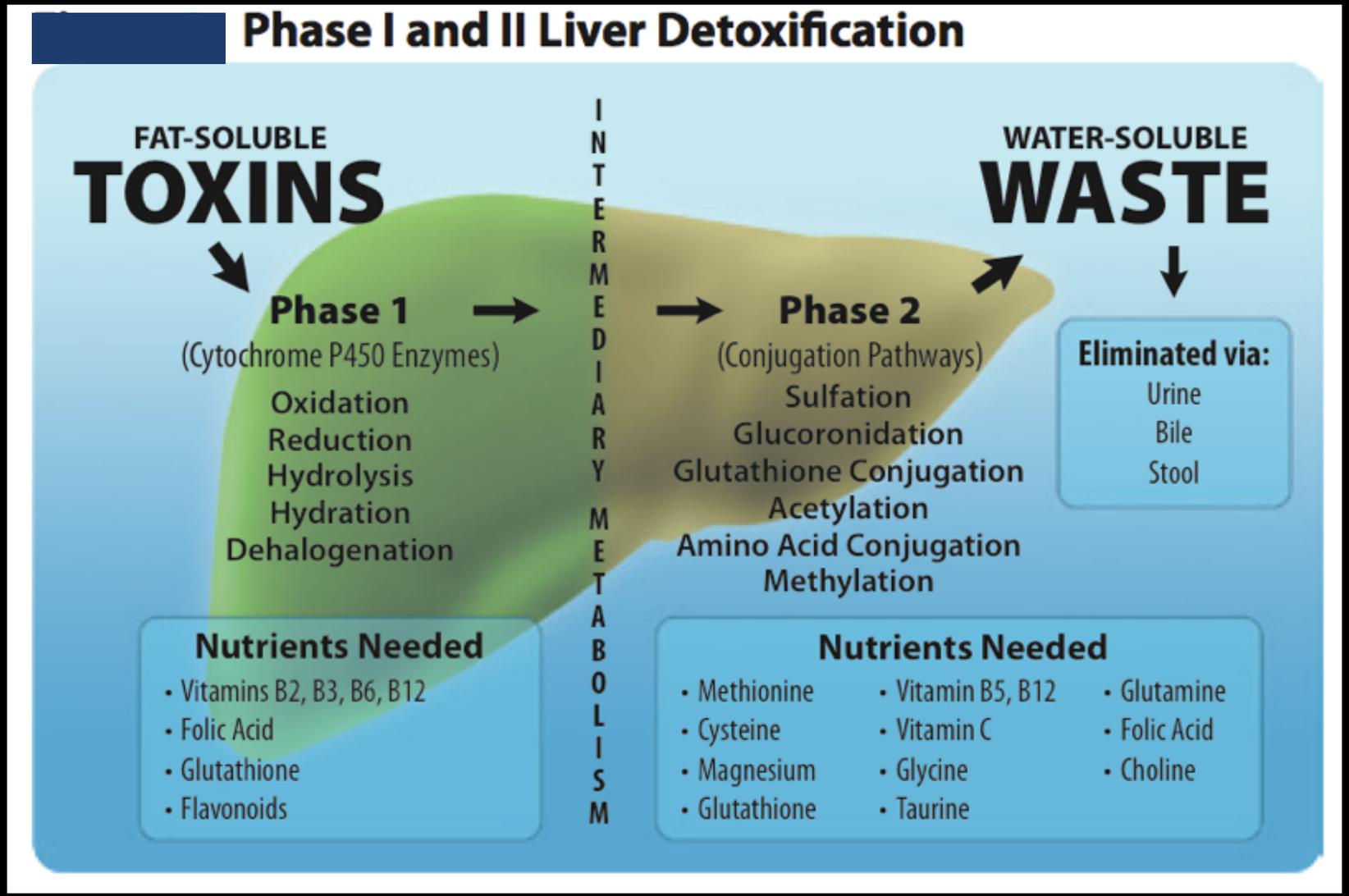
THE KEAP1/NRF2 PATHWAY



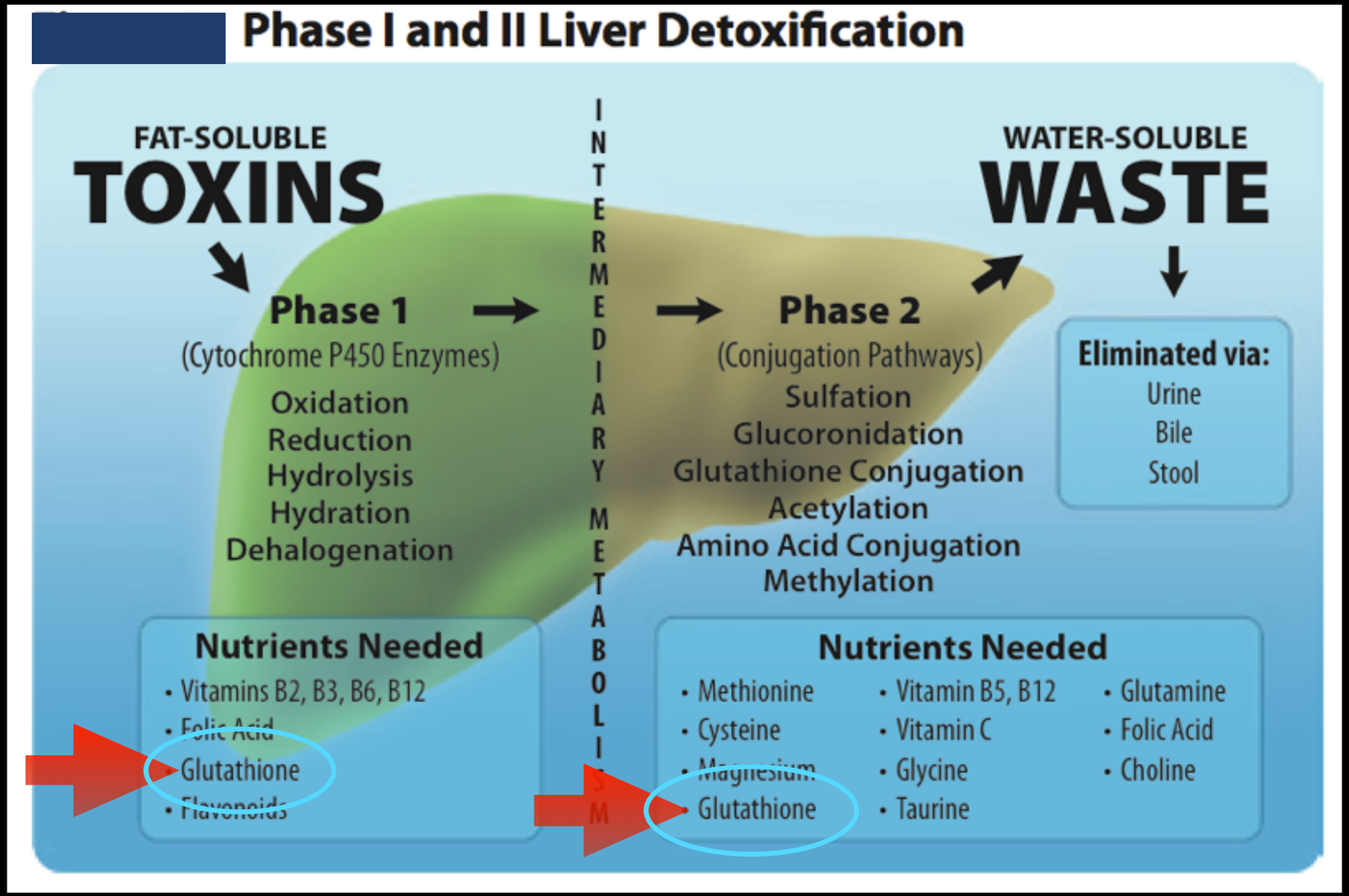
NRF2 ACTIONS:

- Increases the production of a variety of proteins that:
  - Reduce oxidative stress (anti-oxidant enzymes) (SOD, Catalase, Glutathione)
  - Have anti-inflammatory action (downregulates NFKB)
  - Detoxify toxic molecules

DETOXIFICATION:



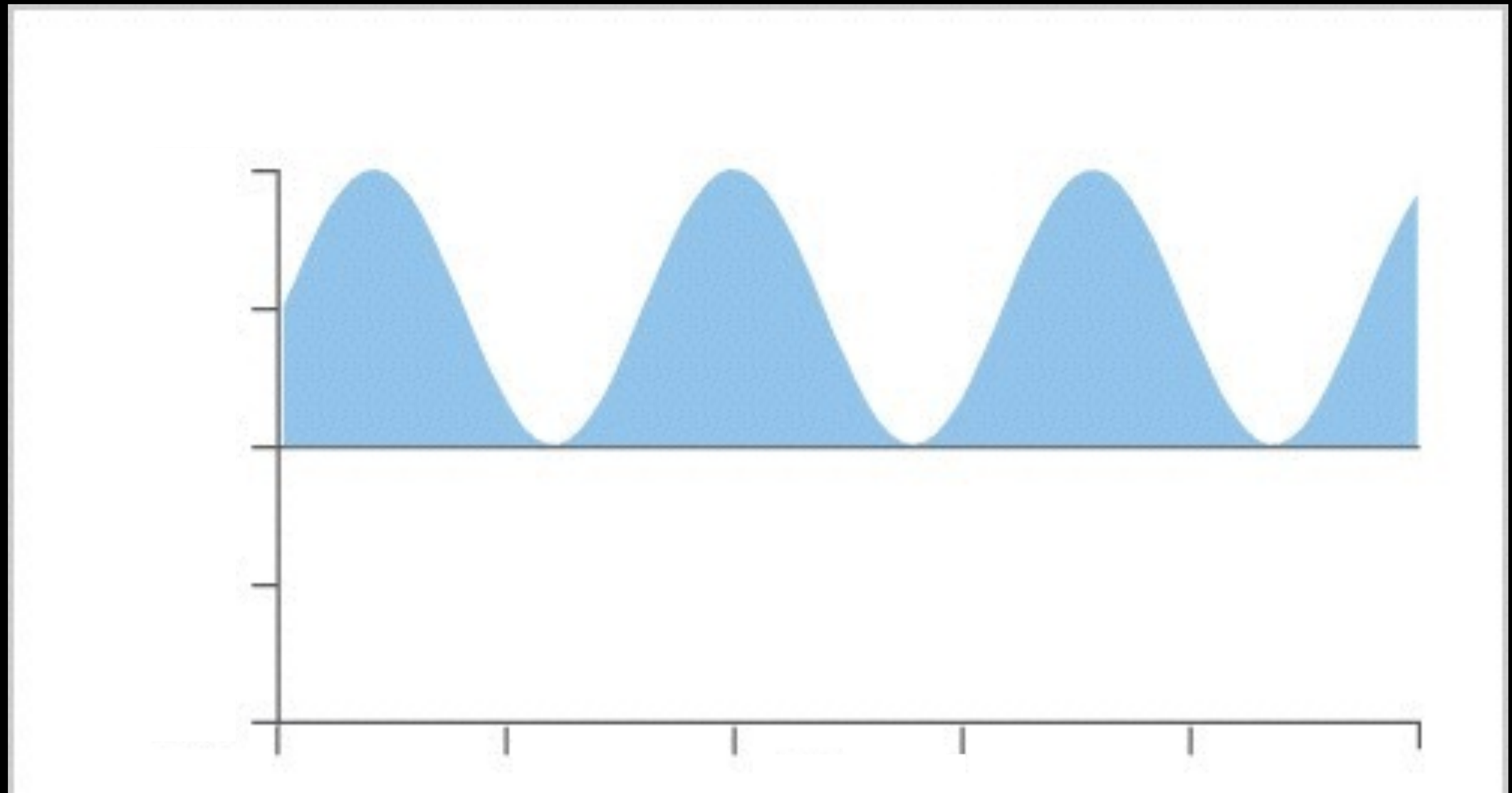
DETOXIFICATION:



GLUTATHIONE:

- Dramatically increased with Nrf 2 activation
- Single most important detoxification enzyme
- Acts as anti oxidant
- Regulates Nitric Oxide cycle - (blood vessel health)
- Involved in DNA repair, protein synthesis, enzyme activation
- Every organ system is affected by Glutathione

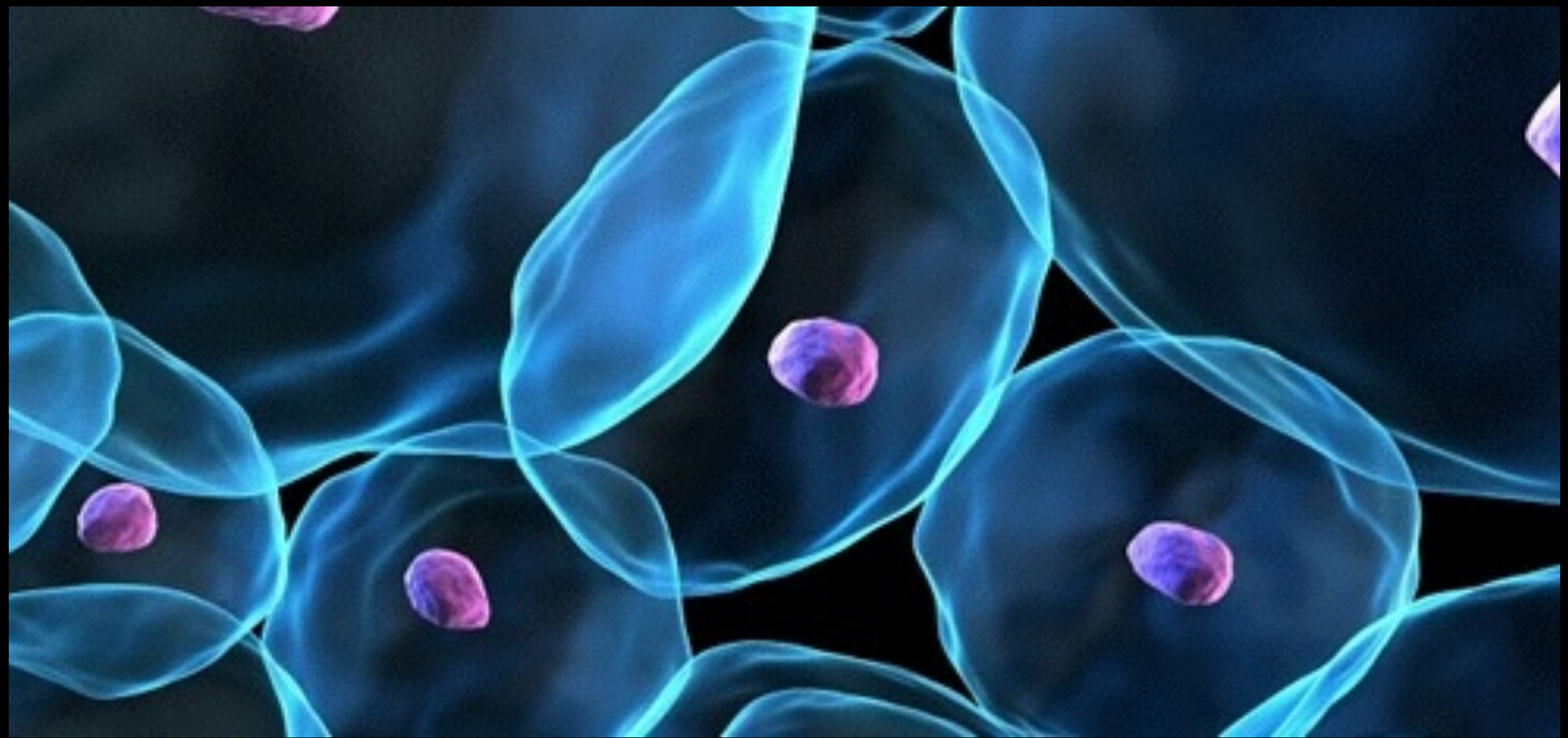
NRF2 IS PULSATILE IN THE NORMAL HEALTHY STATE



NOT...
PEDAL TO THE METAL!!!

"Nrf 2 is a revolution in science,…and is the most important anti-aging pathway in the human body."

– SPEAKER AT ANTI-AGING CONFERENCE, APRIL 2014




ACTIVATE YOUR NRF2 AND
EXPLORE YOUR LIMITS!
Jeep
RUBICON

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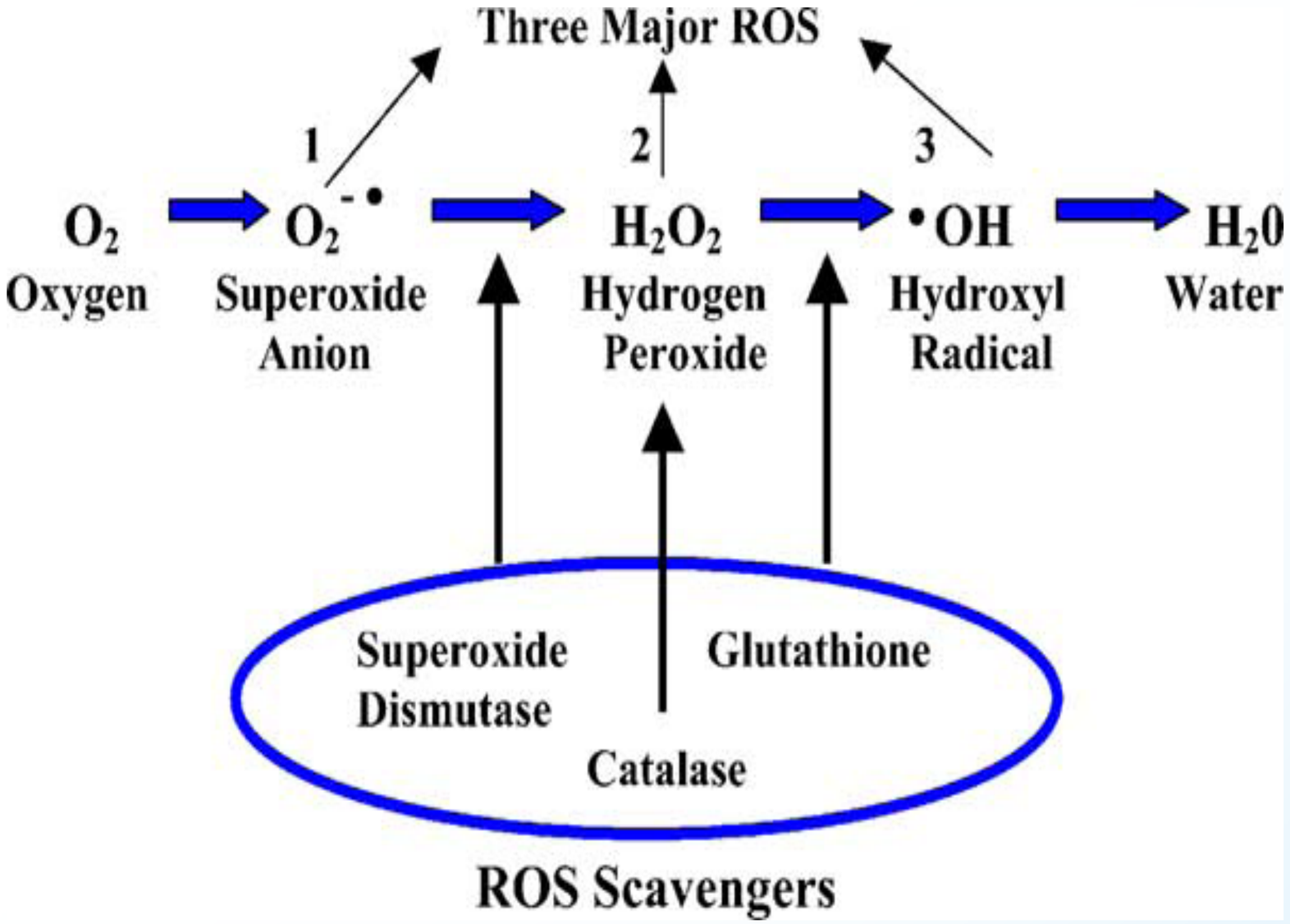
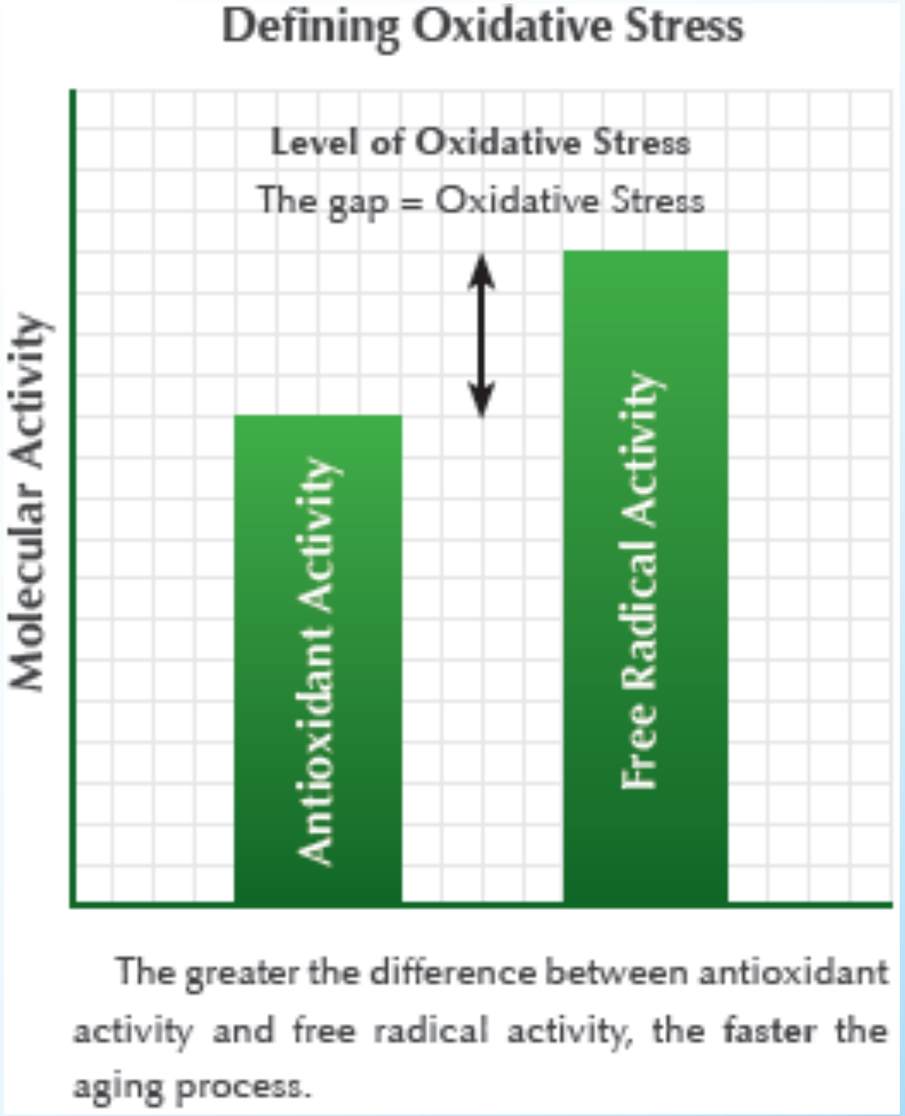
Chief Science Officer

LifeVantage FREEDOM


Three Major ROS
1
2
3
O_2
Oxygen
$O_2^{-\bullet}$
Superoxide Anion
H_2O_2
Hydrogen Peroxide
•OH
Hydroxyl Radical
H_20
Water
Superoxide Dismutase
Glutathione
Catalase
ROS Scavengers


Defining Oxidative Stress
Level of Oxidative Stress
The gap = Oxidative Stress
Molecular Activity
Antioxidant Activity
Free Radical Activity
The greater the difference between antioxidant activity and free radical activity, the faster the aging process.

Managing Cellular Stress

LifeVantage FREEDOM

ORIGINAL CONTRIBUTION

Vitamin E and the Risk of Prostate Cancer

The Selenium and Vitamin E Cancer Prevention Trial (SELECT)

Eric A. Klein, MD
Ian M. Thompson Jr, MD
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John J. Crowley, PhD
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Phyllis J. Goodman, MS
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Leslie G. Ford, MD
Howard L. Parnes, MD
J. Michael Gaziano, MD, MP
Daniel D. Karp, MD
Michael M. Lieber, MD
Philip J. Walther, MD, PhD
Laurence Klotz, MD
J. Kellogg Parsons, MD, MH
Joseph L. Chin, MD
Amy K. Darke, MS
Scott M. Lippman, MD
Gary E. Goodman, MD
Frank L. Meyskens Jr, MD
Laurence H. Baker, DO

LIFETIME RISK OF PROS
cer in the United Sta
rently estimated to be
though most cases ar
an early, curable stage, tre
costly and urinary, sexual, a
related adverse effects are c
Even men who choose activ
lance as an initial managem
egy face anxiety, uncertain
and a measurable risk of sepsi
low-up biopsies,[1] and more
third of those who initially del
are ultimately treated.[4,5] Wi

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EFFECTS OF A COMBINATION OF BETA CAROTENE AND VITAMIN A CARDIOVASCULAR DISEASE

GILBERT S. OMENN, M.D., PH.D., GARY E. GOODMAN, M.D., M.S., MARK
JOHN BALMES, M.D., MARK R. CULLEN, M.D., ANDREW GLASS, M.D., JA
FRANK L. MEYSKENS, JR., M.D., BARBARA VALANIS, DR.P.H., JAMES H.
SCOTT BARNHART, M.D., M.P.H., AND SAMUEL HAMMAR,

Abstract *Background.* Lung cancer and cardiovascular disease are major causes of death in the United States. It has been proposed that carotenoids and retinoids are agents that may prevent these disorders.

Methods. We conducted a multicenter, randomized, double-blind, placebo-controlled primary prevention trial — the Beta-Carotene and Retinol Efficacy Trial — involving a total of 18,314 smokers, former smokers, and workers exposed to asbestos. The effects of a combination of 30 mg of beta carotene per day and 25,000 IU of retinol (vitamin A) in the form of retinyl palmitate per day on the primary end point, the incidence of lung cancer, were compared with those of placebo.

Results. A total of 388 new cases of lung cancer were diagnosed during the 73,135 person-years of follow-up (mean length of follow-up, 4.0 years). The active-treatment group had a relative risk of lung cancer of 1.28 (95 percent confidence interval, 1.04 to 1.57; P=0.02), as compared with the placebo group. The relative risk of death from cardiovascular disease was 1.26 (95 percent confidence interval, 1.03 to 1.54; P=0.03), and the relative risk of death from any cause was 1.17 (95 percent confidence interval, 1.03 to 1.33); of deaths from cardiovascular disease, 42 percent (95 percent confidence interval, 0.99 to 1.61). On the basis of these findings, the randomized trial was stopped 21 months earlier than planned; follow-up will continue through 1995.

Conclusions. After an average of four years of supplementation, the combination of beta carotene and vitamin A had no benefit and may have had a harmful effect on the incidence of lung cancer, cardiovascular disease, and death from lung cancer, cardiovascular disease, and any cause in smokers and workers exposed to asbestos. (N Engl J Med 1996;334:1150-5.)

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LUNG cancer is the leading cause of death from cancer in the United States, accounting for approximately 29 percent of deaths from cancer and 6 percent of all deaths.[1] New approaches are essential to prevent lung cancer in persons who have smoked cigarettes or who have had occupational exposure to asbestos. Twenty-nine percent of men and 25 percent of women who are 45 to 64 years of age currently smoke,[2] and at least 40 percent of men and 20 percent of women in this age group are former smokers.[3] An estimated 4000 to 6000 deaths from lung cancer per year are attributed to exposure to asbestos.[4,5]

On the basis of epidemiologic observations and laboratory studies, beta carotene and vitamin A have attracted wide interest as agents that may prevent lung cancer.[6-9] The Beta-Carotene and Retinol Efficacy Trial (CARET) is one of several recent trials to assess the chemopreventive efficac
and related agents.[10-13]

This report presents
CARET study, which coi
of the steering committe
to stop the trial's active
ditional end points is ex
five years.

Study Design

The study's strategy, desig
study findings, and recruitmen
where.[10,14] Briefly, CARET w
domization in Seattle in 1985
men with substantial occupati
randomly assigned in a 1:1 ra
15 mg of beta carotene per da
tive treatment) or placebo; the
with extensive histories of ciga
carotene per day, 25,000 IU of
ther vitamin (two-by-two desig
additional study centers in 19
domly assigned in a 1:1 ratio
The pilot groups receiving active agents were consolidated into a single group receiving a standard daily regimen of beta carotene plus 25,000 IU of retinol in the form of retinyl palmitate. Thus, in the pilot study with the cohort of smokers, th
jects were assigned to active treatment for every subject as
placebo; therefore, the rates rather than numbers of end po
be compared between active and placebo groups. The desig
for active intervention until late 1997 (110,000 person-years)
porting of results in 1998.

Eligibility, Recruitment, and Randomization

Workers exposed to asbestos were men 45 to 74 years
the pilot study and 45 to 69 years of age in the later peri

From the Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle (G.S.O., G.E.G., M.D.T., S.B.); the Departments of Environmental Health and Medicine, University of Washington, Seattle (G.S.O., G.E.G., S.B., S.H.); the Swedish Hospital Tumor Institute, Seattle (G.E.G.); the Department of Medicine, University of California at San Francisco, San Francisco (J.B.); the Department of Medicine, Yale University, New Haven, Conn. (M.R.C.); Kaiser Permanente Center for Health Research, Portland, Oreg. (A.G., B.V.); the Department of Medicine, University of Maryland, Baltimore (J.P.K.); and the Department of Medicine and Cancer Center, University of California at Irvine, Orange (F.L.M., III/K.). Address reprint requests to Dr. Omenn at the Fred Hutchinson Cancer Research Center, Division of Public Health Sciences, 1124 Columbia–MP859, Seattle, WA 98104.

Supported by grants (U01 CA63673, U01 CA63674, U01 CA47989, U01 CA46200, U01 CA46203, U01 CA46196, and U01 CA52596) from the National Cancer Institute.

*Other contributing authors were Carl Andrew Brodkin, M.D. (University of Washington, Seattle), Martin G. Chernick, M.D. (Yale University, New Haven, Conn.), James E. Grizzle, Ph.D. (Fred Hutchinson Cancer Research Center, Seattle), Marjorie Perloff, M.D. (National Cancer Institute, Bethesda, Md.), and Linda Rosenstock, M.D., M.P.H. (University of Washington, Seattle).

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α-Tocopherol and β-Carotene Supplements and Lung Cancer Incidence in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study: Effects of Base-line Characteristics and Study Compliance

*Demetrius Albanes, Olli P. Heinonen, Philip R. Taylor, Jarmo Virtamo, Brenda K. Edwards, Matti Rautalahti, Anne M. Hartman, Juni Palmgren, Laurence S. Freedman, Jaason Haapakoski, Michael J. Barrett, Pirjo Pietinen, Nea Malila, Eero Tala, Kari Liippo, Eija-Riitta Salomaa, Joseph A. Tangrea, Lyly Teppo, Frederic B. Askin, Eero Taskinen, Yener Erozan, Peter Greenwald, Jussi K. Huttunen**

Background
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The Journal of Nutrition
Nutritional Epidemiology

Antioxidant Supplementation Increases the Risk of Skin Cancers in Women but Not in Men[1]

Serge Hercberg,[2,3]* Khaled Ezzedine,[2,4] Christiane Guinot,[3,6] Paul Preziosi,[2] Pilar Galan,[2] Sandrine Bertrais,[2] Carla Estaquio,[2] Serge Briançon,[2] Alain Favier,[5] Julie Latreille,[1] and Denis Malvy[4]

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Abstract

This research aimed to test whether supplementation with a combination of antioxidant vitamins and minerals could reduce the risk of skin cancers (SC). It was performed within the framework of the Supplementation in Vitamins and Mineral Antioxidants study, a randomized, double-blinded, placebo-controlled, primary prevention trial testing the efficacy of nutritional doses of antioxidants in reducing incidence of cancer and ischemic heart disease in the general population. French adults (7876 women and 5141 men) were randomized to take an oral daily capsule of antioxidants (120 mg vitamin C, 30 mg vitamin E, 6 mg β-carotene, 100 μg selenium, and 20 mg zinc) or a matching placebo. The median time of follow-up was 7.5 y. A total of 157 cases of all types of SC were reported, from which 25 were melanomas. Because the effect of antioxidants on SC incidence varied according to gender, men and women were analyzed separately. In women, the incidence of SC was higher in the antioxidant group [adjusted hazard ratio (adjusted HR) = 1.68; $P = 0.03$]. Conversely, in men, incidence did not differ between the 2 treatment groups (adjusted HR = 0.69; $P = 0.11$). Despite the small number of events, the incidence of melanoma was also higher in the antioxidant group for women (adjusted HR = 4.31; $P = 0.02$). The incidence of nonmelanoma SC did not differ between the antioxidant and placebo groups (adjusted HR = 1.37; $P = 0.22$ for women and adjusted HR = 0.72; $P = 0.19$ for men). Our findings suggest that antioxidant supplementation affects the incidence of SC differentially in men and women. *J. Nutr.* 137: 2099–2105, 2007.

Introduction

Melanoma and nonmelanoma skin cancers (SC),[10] namely squamous cell carcinoma (SCC) and basal cell carcinoma (BCC), are the most common forms of malignancy in the Caucasian population (1) and sun exposure is thought to be the main established risk factor for all 3 types of tumor (2). An aging population, more intense exposure to UV rays due to depletion of the ozone layer, and sun exposure habits would appear to favor a higher incidence of skin malignancy (3).

Numerous studies have demonstrated the role of reactive oxygen species, also called free radicals, in skin carcinogenesis and the potential protective effect of antioxidants (4). Formation of free radicals in the skin can be enhanced by UV ra
cutaneous system has a very efficient interlinked
defense system for counteracting UV-induced exi
However, excessive exposure to sunlight or other s
light can overwhelm the skin's antioxidant capacity. A potentially interesting strategy for preventing UV exposure damage could be to boost the endogenous antioxidant system by oral intake of antioxidant vitamins and minerals. Although clinical trials have showed contradictory findings (5–7), oral antioxidant pills have been recommended for the prevention of sunburns and for their supposed photoprotective properties.

In particular, it has been suggested that nutrients such as β-carotene, ascorbic acid, vitamin E, selenium, and zinc may prevent such harmful effects of UV exposure because of their antioxidant ability (8). Clinical trials testing the impact of supplementation with high doses of antioxidants over long periods have, however, failed to reveal beneficial effects on the incidence of SC (9,10). For example, the Nutritional Prevention of Cancer trial, a double-blind, randomized clinical trial, was designed to test whether selenium (200 μg/d) could prevent nonmelanoma SC (NMSC) in 1312 individuals with an individual

[1] Author disclosures: S. Hercberg, K. Ezzedine, P. Preziosi, P. Galan, S. Bertrais, C. Estaquio, S. Briançon, A. Favier, and D. Malvy, no conflicts of interest; C. Guinot and J. Latreille, the C.E.R.I.E.S. is a research center on human skin founded by CHANEL.

[2] Abbreviations used: BCC, basal cell carcinoma; HR, hazard ratio; MSC, melanoma skin cancer; NMSC, nonmelanoma skin cancer; SC, skin cancer; SCC, squamous cell carcinoma; SU.VI.MAX, Supplementation en Vitamines et Minéraux Antioxydants study.

* To whom correspondence should be addressed. E-mail: hercberg@cnam.fr.

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The Efficacy and Safety of Multivitamin and Mineral Supplement Use To Prevent Cancer and Chronic Disease in Adults: A Systematic Review for a National Institutes of Health State-of-the-Science Conference

Han-Yao Huang, PhD, MPH; Benjamin Caballero, MD, PhD; Stephanie Chang, MD; Anthony J. Alberg, PhD, MPH; Richard D. Semba, MD, MPH; Christine R. Schneyer, MD; Renee F. Wilson, MSc; Ting-Yuan Cheng, MSc; Jason Vassy, MPH; Gregory Prokopowicz, MD, MPH; George J. Barnes II, BA; and Eric B. Bass, MD, MPH

Background: Multivitamin and mineral supplements are the most commonly used dietary supplements in the United States.

Purpose: To synthesize studies on the efficacy and safety of multivitamin/mineral supplement use in primary prevention of cancer and chronic

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gastric cancer and the overall mortality rate from cancer by 13% to 21%. In a French trial, combined supplementation with vitamin C, vitamin E, β-carotene, selenium, and zinc reduced the rate of cancer by 31% in men but not in women. Multivitamin and mineral supplements had no significant effect on cardiovascular disease

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ORIGINAL INVESTIGATION

LESS IS MORE

Dietary Supplements and Mortality Rate in Older Women

The Iowa Women's Health Study

Jaakko Mursu, PhD; Kim Robien, PhD; Lisa J. Harnack, DrPH, MPH; Kyong Park, PhD; David R. Jacobs Jr, PhD

Background: Although dietary supplements are commonly taken to prevent chronic disease, the long-term health consequences of many compounds are unknown.

Methods: We assessed the use of vitamin and mineral supplements in relation to total mortality in 38 772 older women in the Iowa Women's Health Study; mean age was 61.6 years at baseline in 1986. Supplement use was self-reported in 1986, 1997, and 2004. Through December 31, 2008, a total of 15 594 deaths (40.2%) were identified through the State Health Registry of Iowa and the National Death Index.

Results: In multivariable adjusted proportional hazards regression models, the use of multivitamins (hazard ratio, 1.06; 95% CI, 1.02-1.10; absolute risk increase, 2.4%), vitamin B_6 (1.10; 1.01-1.21; 4.1%), folic acid (1.15; 1.00-1.32; 5.9%), iron (1.10; 1.03-1.17; 3.9%), magnesium (1.08; 1.01-1.15; 3.6%), zinc (1.08; 1.01-1.15; 3.0%), and copper (1.45; 1.20-1.75; 18.0%) were associated with increased risk of total mortality when compared with corresponding nonuse. Use of calcium was inversely related (hazard ratio, 0.91; 95% confidence interval, 0.88-0.94; absolute risk reduction, 3.8%). Findings for iron and calcium were replicated in separate, shorter-term analyses (10-year, 6-year, and 4-year follow-up), each with approximately 15% of the original participants having died, starting in 1986, 1997, and 2004.

Conclusions: In older women, several commonly used dietary vitamin and mineral supplements may be associated with increased total mortality risk; this association is strongest with supplemental iron. In contrast to the findings of many studies, calcium is associated with decreased risk.

Arch Intern Med. 2011;171(18):1625-1633

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IN THE UNITED STATES, THE USE OF dietary supplements has increased substantially during the past several decades,[1-3] reaching approximately one-half of adults in 2000, with annual sales of more than $20 billion.[1,2] Sixty-six percent of women participating in the Iowa Women's Health Study[3] used at least 1 dietary supplement daily in 1986 at an average age of 62 years; in 2004, the proportion increased to 85%. Moreover, 27% of women reported using 4 or more supplemental products in 2004.[3] At the population level, dietary supplements contributed substantially to the total intake of several nutrients, particularly in elderly individuals.[1,2]

Supplemental nutrient intake clearly is beneficial in deficiency conditions.[4] However, in well-nourished populations, supplements often are intended to yield benefit by preventing chronic diseases. Results of epidemiologic studies[5-9] assessing supplement use and total mortality risk have been inconsistent. Several randomized controlled trials (RCTs),[10,11] concentrating mainly on calcium and vitamins B, C, D, and E, have not shown beneficial effects of dietary supplements on total mortality rate; in contrast, some[12,13] have suggested the possibility of harm. Meta-analyses[14,15] concur in finding no decreased risk and potential harm. Supplements are widely used, and further studies regarding their health effects are needed. Also, little is known about the long-term effects of multivitamin use and less commonly used supplements, such as iron and other minerals.

See Invited Commentary and Editor's Note at end of article

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Circulation
JOURNAL OF THE AMERICAN HEART ASSOCIATION
Heart Failure

Chronic Pulmonary Artery Pressure Elevation Is Insufficient to Explain Right Heart Failure

Harm J. Bogaard, MD, PhD; Ramesh Natarajan, PhD; Scott C. Henderson, PhD;

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Original Contribution

Protandim attenuates intimal ex vivo via a catalase-depend

Binata Joddar [a,b,c], Rashmeet K. Reen [b], Jay L. Zweier [b], Keith J. Gooch [a,b,*]

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[b] Davis Heart & Lung Research Institute, The Ohio State Univers
[c] RIKEN Nanomedical Engineering Laboratory, Wako-shi, Saitam
[d] Department of Surgery, The Ohio State University, Columbus,
[e] Department of Cardiothoracic Surgery, The Ohio State Univers
[f] Division of Pulmonary and Critical Care Medicine, Departmen

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Catalase
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Ex vivo culture
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US007241

(12) **United States Patent**
Myhill et al.

(10) **Patent No.:**
(45) **Date of Patent:**

(54) **COMPOSITIONS FOR ALLEVIATING INFLAMMATION AND OXIDATIVE STRESS IN A MAMMAL**

(75) Inventors: **Paul R. Myhill**, Castle Rock, CO (US); **William J. Driscoll**, Englewood, CO (US)

(73) Assignee: **Lifeline Nutraceuticals Corporation**, Englewood, CO (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35

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The Dietary Supplem Plasma Osteopontin a Oxidative Stress in Mus

Muhammad Mudda
Warren C. McClure, MS
Nicole L. Arevalo, MA
Rick E. Rabon, BA
Benjamin Mohr
Swapan K. Bose, BS, BPharm
Joe M. McCord, PhD
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PLoS one

Protandim, a Fundamentally New Antioxidant Approach in Chemoprevention Using Mouse Two-Stage Skin Carcinogenesis as a Model

Jianfeng Liu[1], Xin Gu[2], Delira Robbins[1], Guohong Li[3], Runhua Shi[4], Joe M. McCord[5], Yunfeng Zhao[1]*

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Abstract

Oxidative stress is an important contributor to cancer development. Consistent with that, antioxidant enzymes have been demonstrated to suppress tumorigenesis when being elevated both in vitro and in vivo, making induction of these enzymes a more potent approach for cancer prevention. Protandim, a well-defined combination of widely studied medicinal plants, has been shown to induce superoxide dismutase (SOD) and catalase activities and reduce superoxide generation and lipid peroxidation in healthy human subjects. To investigate whether Protandim can suppress tumor formation by a dietary approach, a two-stage mouse skin carcinogenesis study was performed. At the end of the study, the mice on a Protandim-containing basal diet had similar body weight compared with those on the basal diet, which indicated no overt toxicity by Protandim. After three weeks on the diets, there was a significant increase in the expression levels of SOD and catalase, in addition to the increases in SOD activities. Importantly, at the end of the carcinogenesis study, both skin tumor incidence and multiplicity were reduced in the mice on the Protandim diet by 33% and 57% respectively, compared with those on basal diet. Biochemical and histological studies revealed that the Protandim diet suppressed tumor promoter-induced oxidative stress (evidenced by reduction of protein carbonyl levels), cell proliferation (evidenced by reduction of skin hyperplasia and suppression of PKC/JNK/Jun pathway), and inflammation (evidenced by reduction of ICAM-1/VCAM-1 expression, NF-κB binding activity, and nuclear p65/p50 levels). Overall, induction of antioxidant enzymes by Protandim may serve as a practical and potent approach for cancer prevention.

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Serum Levels of Thiobarbituric Acid Reactive Substances Predict Cardiovascular Events in Patients With Stable Coronary Artery Disease

A Longitudinal Analysis of the PREVENT Study

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Gregory M. Preston, PhD,§ Jan Buch, MD,‡ R. Preston Mason, PhD*†
Beverly and Boston, Massachusetts; New York, New York; and Groton, Connecticut

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PLoS one

The Chemopreventive Effects of Protandim: Modulation of p53 Mitochondrial Translocation and Apoptosis during Skin Carcinogenesis

Delira Robbins[1], Xin Gu[2], Runhua Shi[3], Jianfeng Liu[1], Fei Wang[3], Jacqulyne Ponville[4], Joe M. McCord[5], Yunfeng Zhao[1]*

1 Department of Pharmacology, Toxicology and Neuroscience, Louisiana State University Health Sciences Center, Shreveport, Louisiana, United States of America, 2 Department of Pathology, Louisiana State University Health Sciences Center, Shreveport, Louisiana, United States of America, 3 College of Life Science, Jilin University, Changchun, Jilin Province, China, 4 Department of Chemistry, Nicholls State University, Thibodaux, Louisiana, United States of America, 5 Department of Medicine, University of Colorado at Denver and Health Sciences Center, Aurora, Colorado, United States of America, 6 Feist-Weiller Cancer Center, Louisiana State University Health Sciences Center, Shreveport, Louisiana, United States of America

Abstract

Protandim, a well defined dietary combination of 5 well-established medicinal plants, is known to induce endogenous antioxidant enzymes, such as manganese superoxide dismutase (MnSOD). Our previous studies have shown through the induction of various antioxidant enzymes, products of oxidative damage can be decreased. In addition, we have shown that tumor multiplicity and incidence can be decreased through the dietary administration of Protandim in the two-stage skin carcinogenesis mouse model. It has been demonstrated that cell proliferation is accommodated by cell death during DMBA/TPA treatment in the two-stage skin carcinogenesis model. Therefore, we investigated the effects of the Protandim diet on apoptosis; and proposed a novel mechanism of chemoprevention utilized by the Protandim dietary combination. Interestingly, Protandim suppressed DMBA/TPA induced cutaneous apoptosis. Recently, more attention has been focused on transcription-independent mechanisms of the tumor suppressor, p53, that mediate apoptosis. It is known that cytoplasmic p53 rapidly translocates to the mitochondria in response to pro-apoptotic stress. Our results showed that Protandim suppressed the mitochondrial translocation of p53 and mitochondrial outer membrane proteins such as Bax. We examined the levels of p53 and MnSOD expression/activity in murine skin JB6 promotion sensitive (P+) and promotion-resistant (P-) epidermal cells. Interestingly, p53 was induced only in P+ cells, not P- cells; whereas MnSOD is highly expressed in P- cells when compared to P+ cells. In addition, wild-type p53 was transfected into JB6 P- cells. We found that the introduction of wild-type p53 promoted transformation in JB6 P- cells. Our results suggest that suppression of p53 and induction of MnSOD may play an important role in the tumor suppressive activity of Protandim.

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Original Contribution

Synergistic induction of heme oxygenase-1 by the components of the antioxidant supplement Protandim

Kalpana Velmurugan [a,b], Jawed Alam [c], Joe M. McCord [d], Subbiah Pugazhenthi [a,b,*]

[a] Division of Endocrinology, Department of Medicine,
[b] Section of Endocrinology, Veterans Affairs Medical
[c] Department of Molecular Genetics, Ochsner Medica
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Original Contribution

The induction of human superoxide dismutase and catalase in vivo: A fundamentally new approach to antioxidant therapy

Sally K. Nelson [a,b], Swapan K. Bose [a], Gary K. Grunwald [c], Paul Myhill [d], Joe M. McCord [a,b,d,e]

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[b] Department of Medicine, University of Colorado Denver Health Sciences Center, Denver, CO 80262, USA
[c] Department of Preventive Medicine and Biometrics, University of Colorado Denver Health Sciences Center, Denver, CO 80262, USA
[d] Lifeline Therapeutics, Denver, CO, USA

Received 22 June 2005; revised 24 August 2005; accepted 28 August 2005

Abstract

A composition consisting of extracts of five widely studied medicinal plants (Protandim) was administered to healthy human subjects ranging in age from 20 to 78 years. Individual ingredients were selected on the basis of published findings of induction of superoxide dismutase (SOD) and/or catalase in rodents in vivo, combined with evidence of decreasing lipid peroxidation. Each ingredient was present at a dosage sufficiently low to avoid any accompanying unwanted pharmacological effects. Blood was analyzed before supplementation and after 30 and 120 days of supplementation (675 mg/day). Erythrocytes were assayed for SOD and catalase, and plasma was assayed for lipid peroxidation products as thiobarbituric acid-reacting substances (TBARS), as well as uric acid, C-reactive protein, and cholesterol (total, LDL, and HDL). Before

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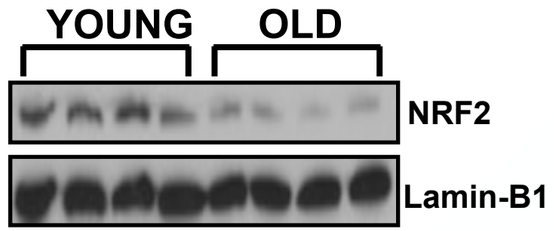
Oxidative Stress in Health and Disease: The Therapeutic Potential of Nrf2 Activation

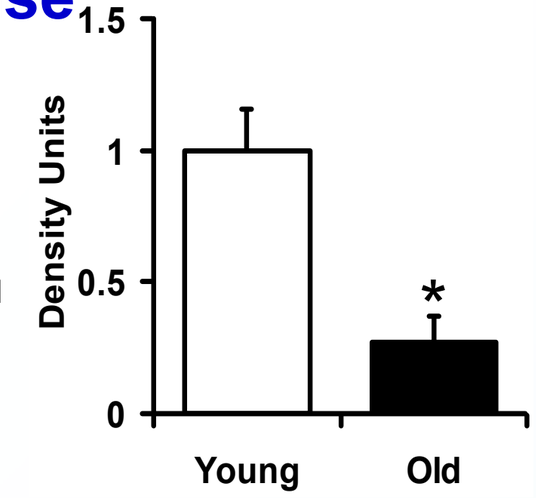
Brooks M. Hybertson,[a,b] Bifeng Gao,[a] Swapan K. Bose[a] and Joe M. McCord[a,b]

[a]Department of Medicine, Division of Pulmonary Science and Critical Care Medicine, University of Colorado at Denver, Aurora, CO 80045
[b]LifeVantage Corporation, 10813 S. Riverfront Parkway, South Jordan, UT 84095

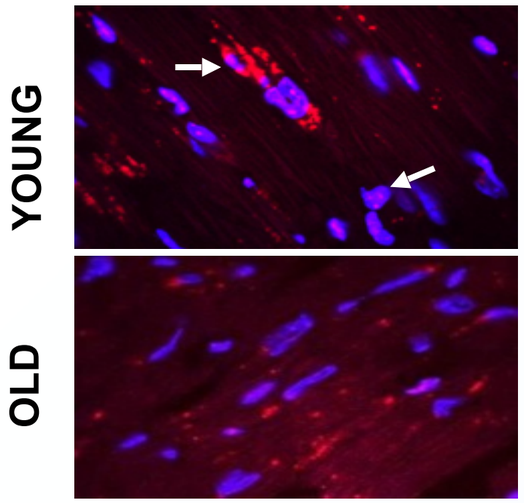
Nrf2 Declines with Age and Induces Oxidative Stress

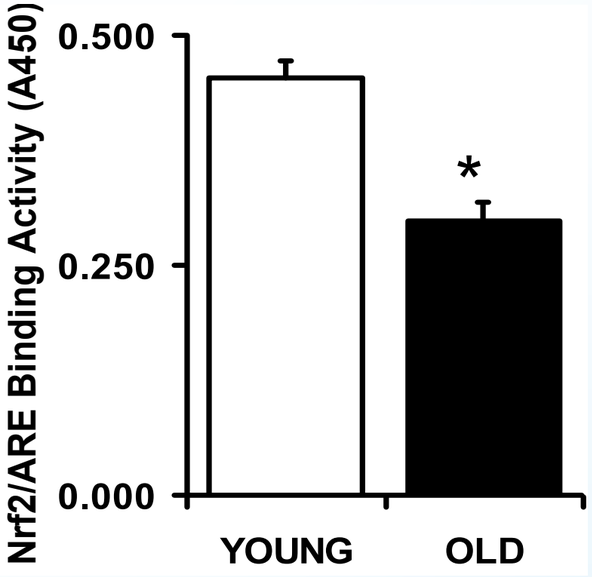
WT (C57/Bl6) Mouse



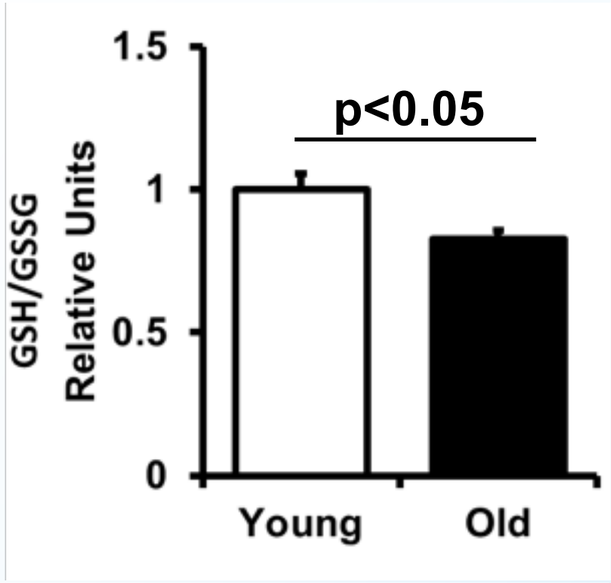


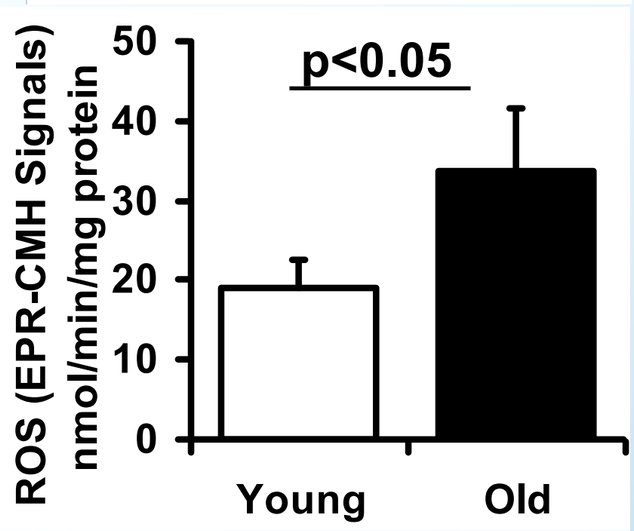
Nrf2





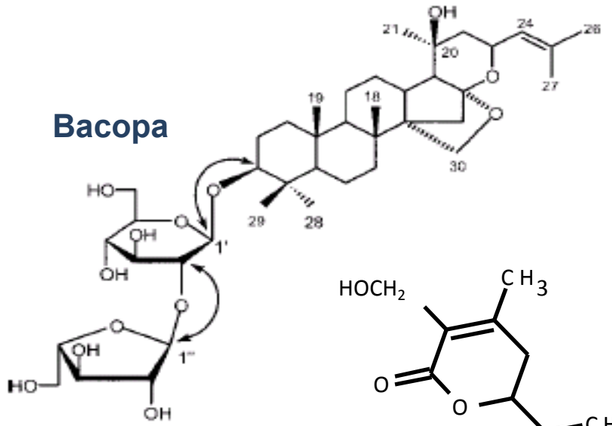
Increased oxidative stress



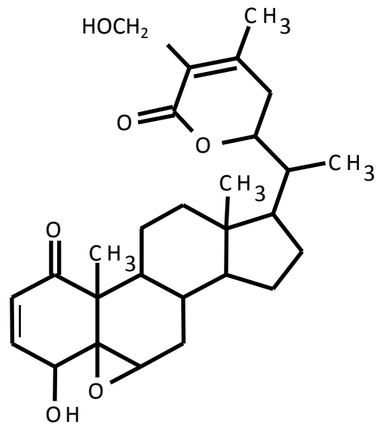


Nrf2 = a powerful "master regulator" of antioxidant enzymes and survival genes

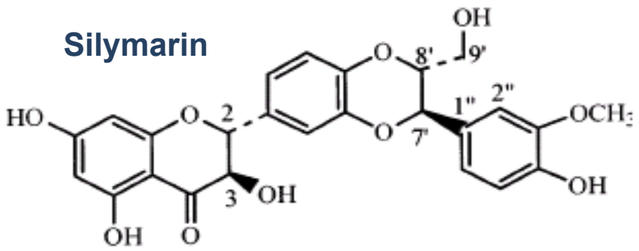
Bacopa

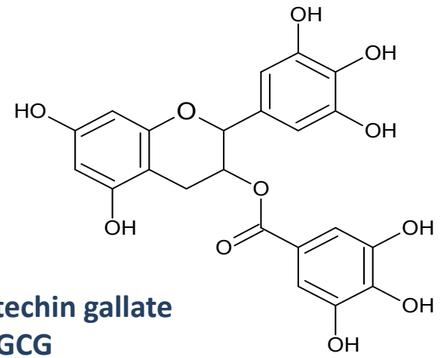


Withaferin A



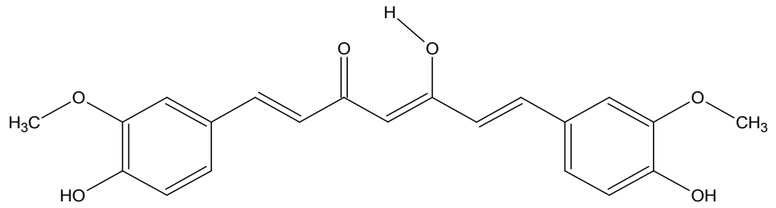
Silymarin

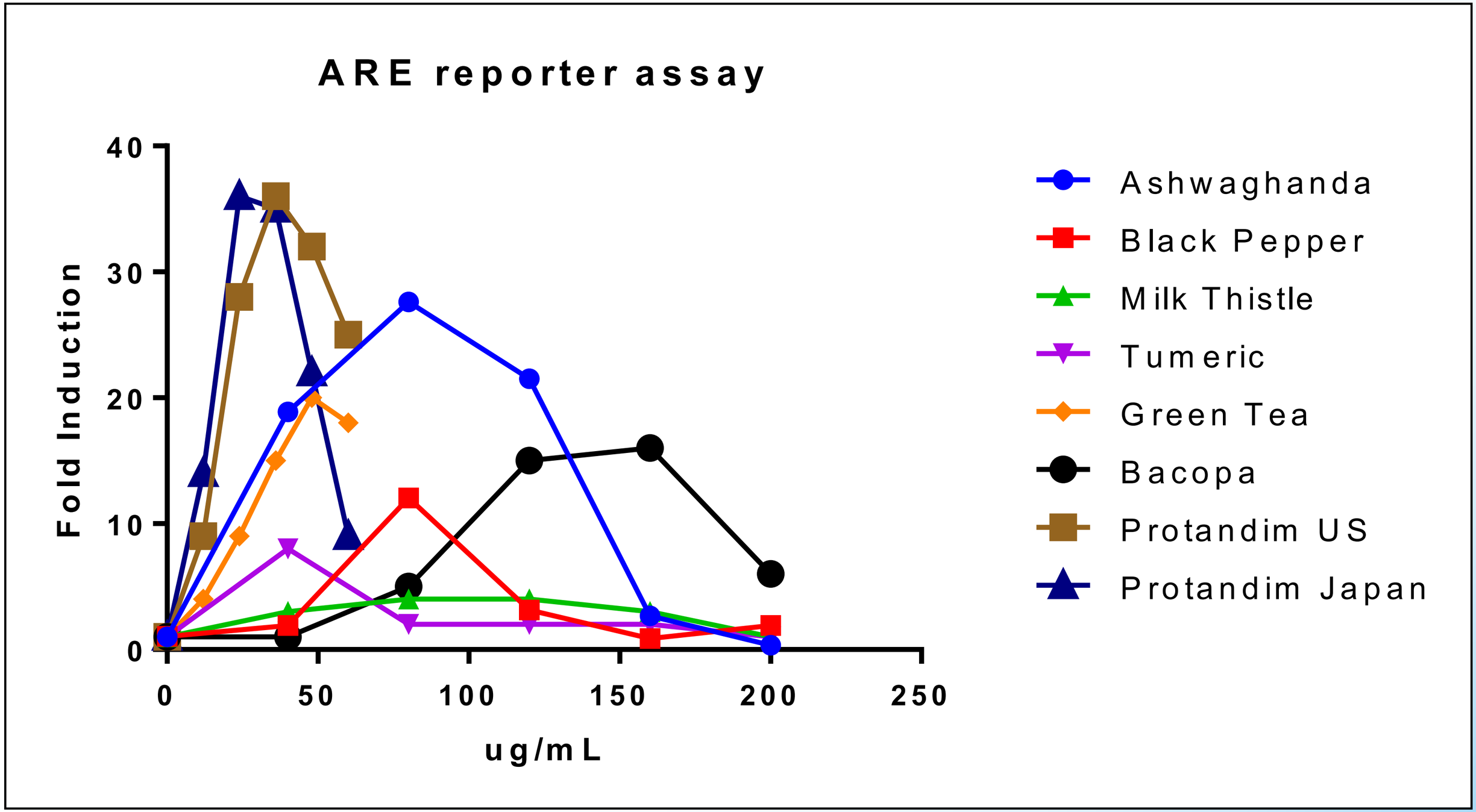




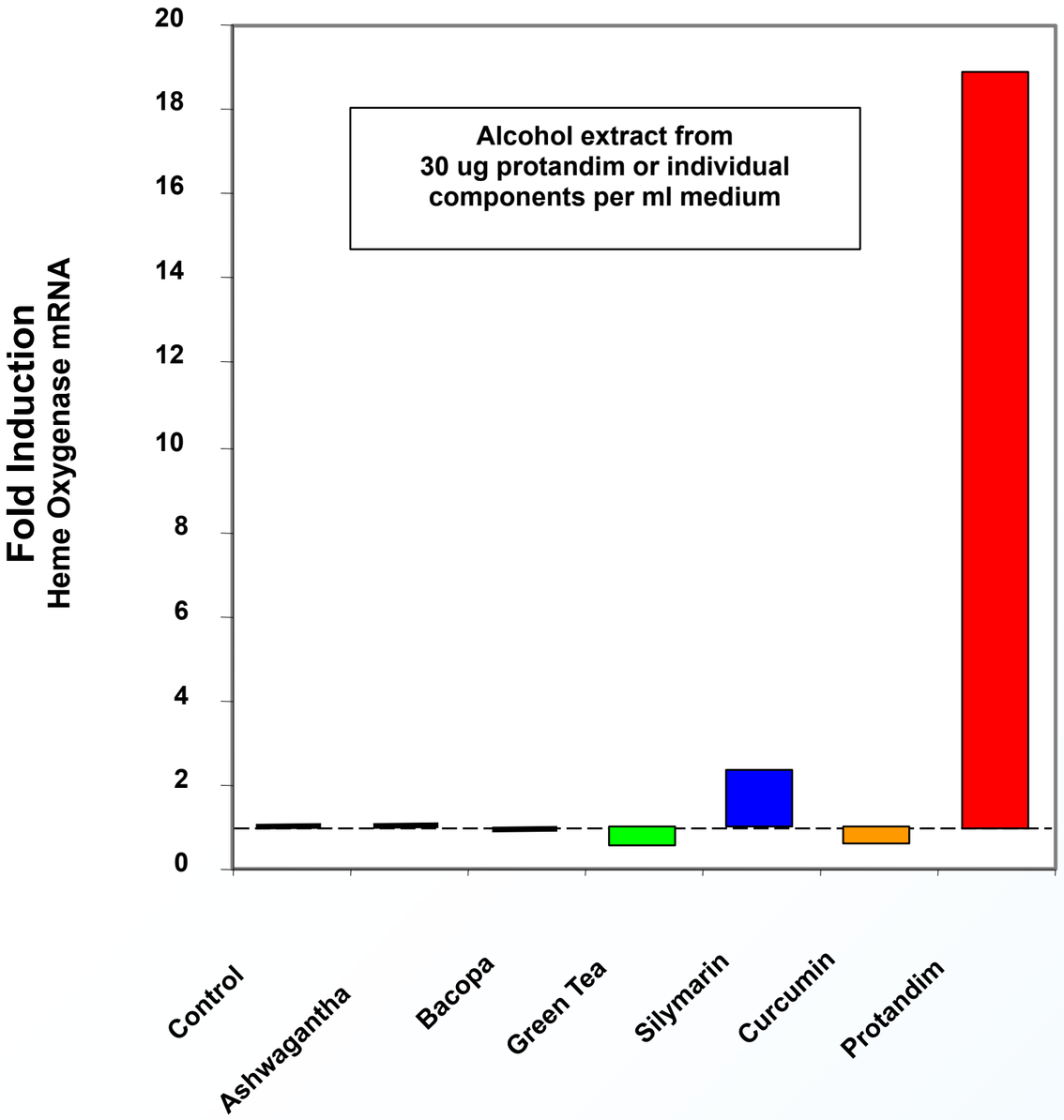
Epigallocatechin gallate
EGCG

Curcumin




ARE reporter assay
Fold Induction
40
30
20
10
0
0
50
100
150
200
250
ug/mL
Ashwaghanda
Black Pepper
Milk Thistle
Tumeric
Green Tea
Bacopa
Protandim US
Protandim Japan

SYNERGY = Action greater than the sum of the parts



All five ingredients together produced an **18-fold increase** in the expression of this antioxidant gene.

Protandim works ***18 times more effectively than the sum of its parts***.

LifeVantage FREEDOM

Patented Technology

First Patent Issued: July 10, 2007
Second Patent Issued: July 10, 2008
Third Patent Issued: August 25, 2009
Fourth Patent Issued: April 12, 2011

United States Patent
Myhill et al.

(10) **Patent No.: US 7,579,026 B2**
(45) **Date of Patent: *Aug. 25, 2009**

METHODS FOR ENHANCING ANTIOXIDANT ENZYME ACTIVITY AND REDUCING C-REACTIVE PROTEIN LEVELS

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Original Contribution

The induction of human superoxide dismutase and catalase in vivo: A fundamentally new approach to antioxidant therapy

Sally K. Nelson [a,b], Swapan K. Bose [a], Gary K. Grunwald [c], Paul Myhill [d], Joe M. McCord [a,b,d,*]

[a] *Webb-Waring Institute for Cancer, Aging and Antioxidant Research, University of Colorado Denver Health Sciences Center, Denver, CO 80262, USA*
[b] *Department of Medicine, University of Colorado Denver Health Sciences Center, Denver, CO 80262, USA*
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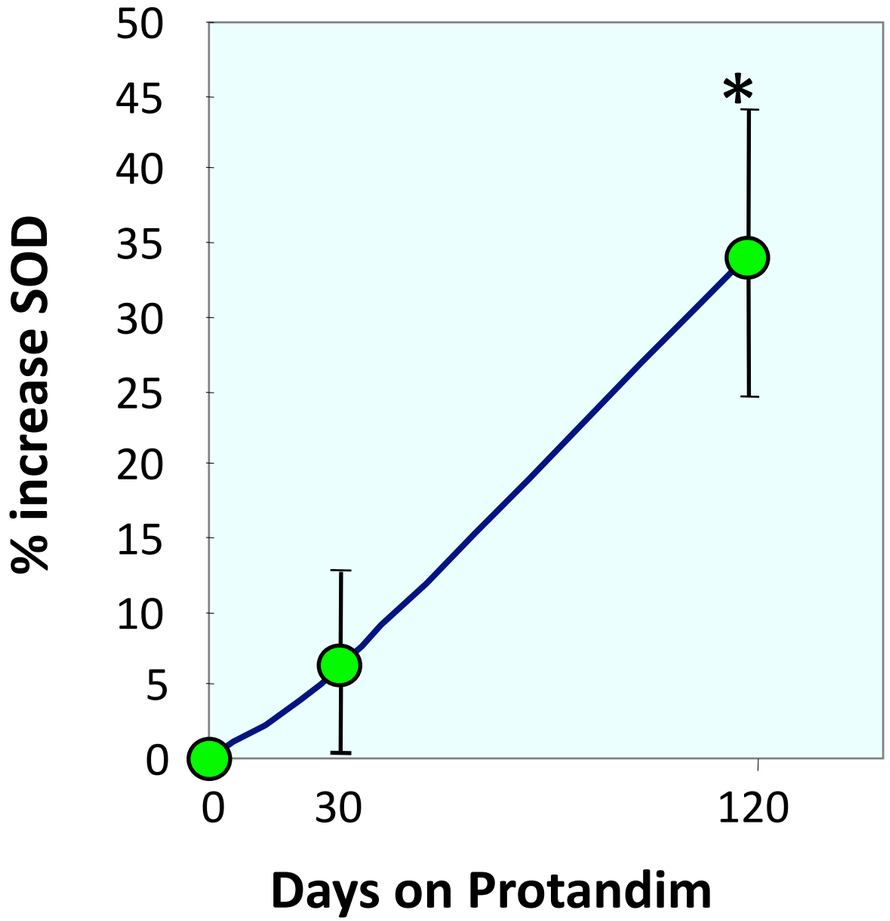
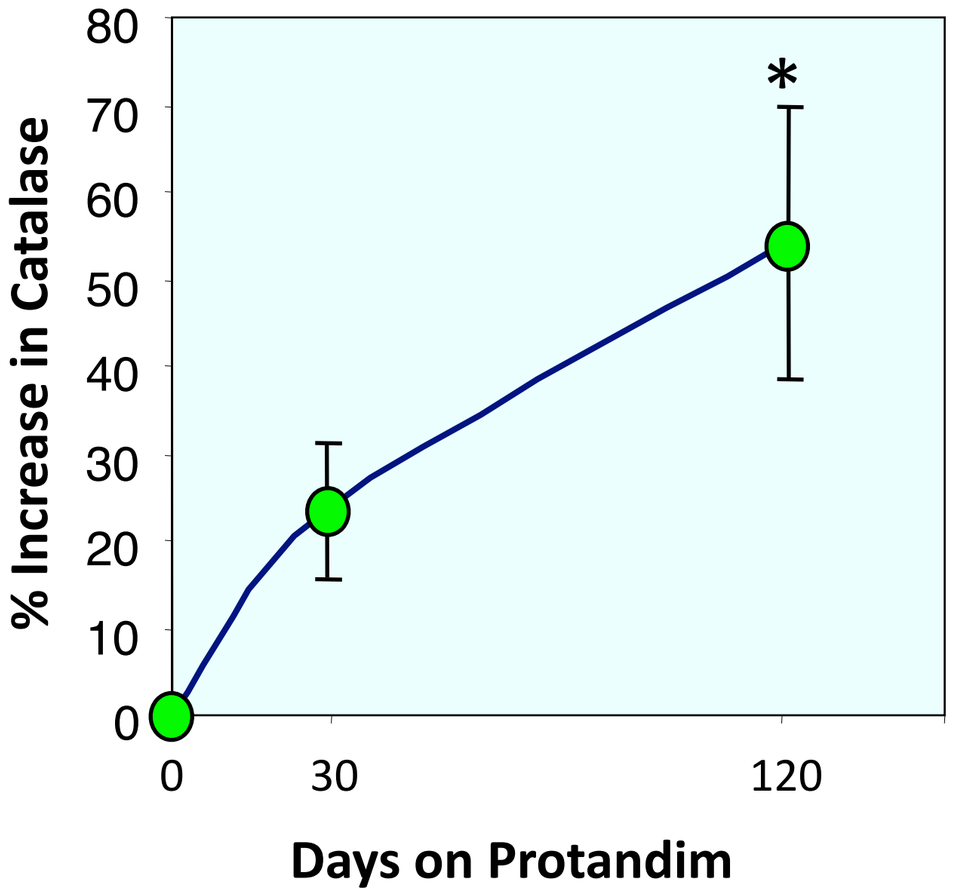
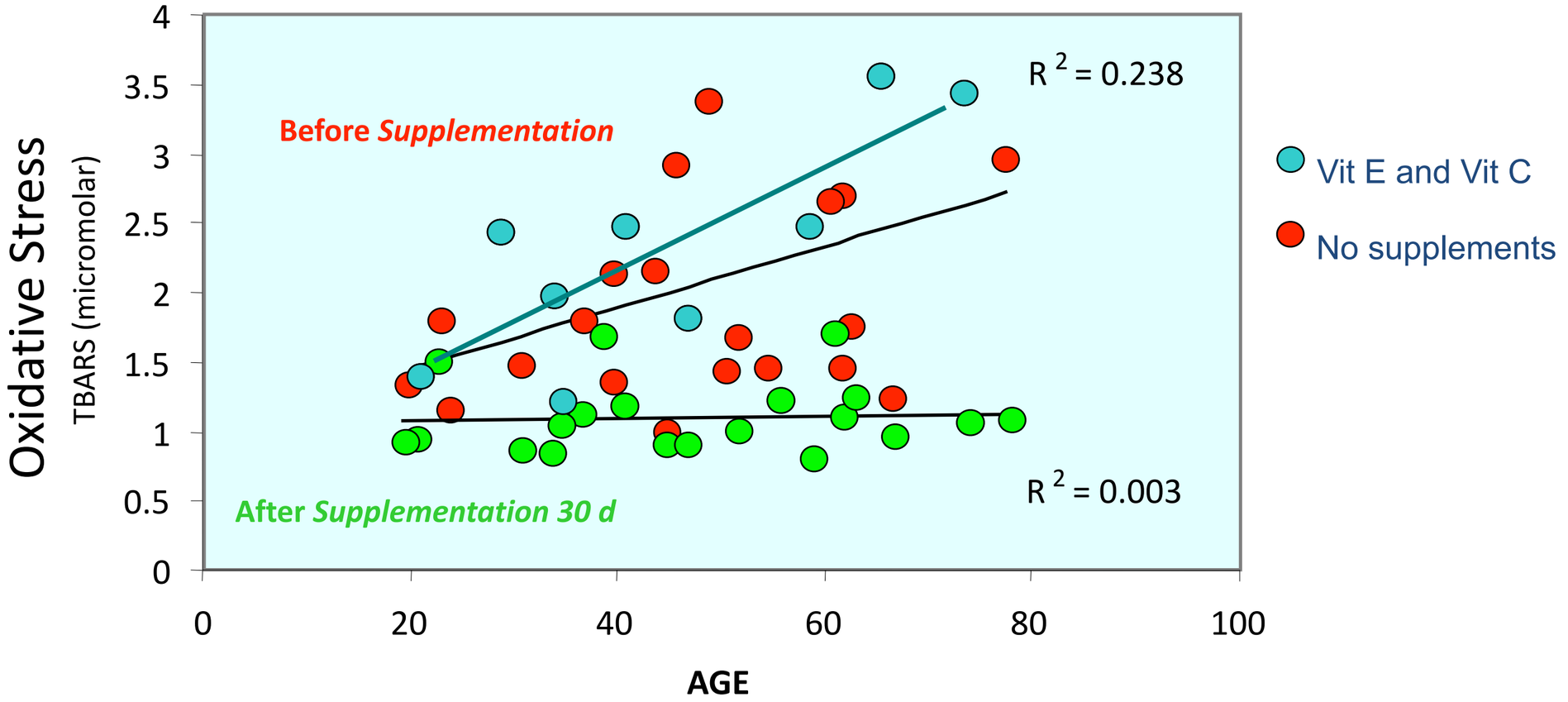




After 120 days…

SOD increased by **34%**

Catalase increased by **54%**



After 30 days…

“Remarkably, this age-dependent increase in TBARS was almost completely abolished by Protandim treatment (Fig. 1D), with an overall average reduction of the oxidative stress marker by 40%.”

Nutragenomics: Modifying gene expression with natural products

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Molecular Aspects of Medicine

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Oxidative Stress in Health and Disease: The Therapeutic Potential of Nrf2 Activation

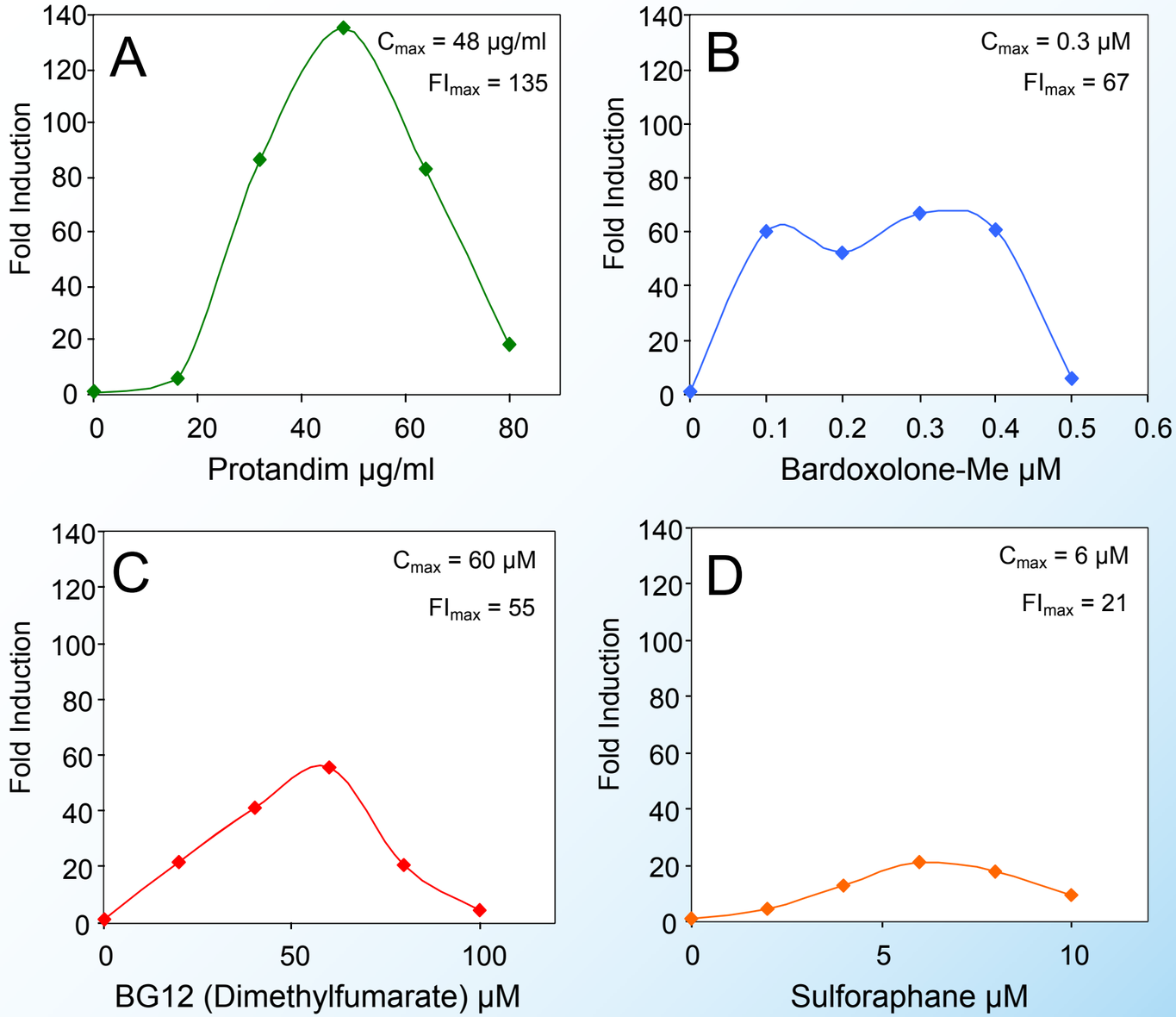
Brooks M. Hybertson,[a,b] Bifeng Gao,[a] Swapan K. Bose[a] and Joe M. McCord[a,b]

[a]Department of Medicine, Division of Pulmonary Science and Critical Care Medicine, University of Colorado at Denver, Aurora, CO 80045
[b]LifeVantage Corporation, 10813 S. Riverfront Parkway, South Jordan, UT 84095

LifeVantage FREEDOM

A side-by-side comparison of Nrf2 activating potencies

Agent	*Fold Induction*
Protandim	135
Bardoxolone Me	67
Dimethylfumarate	55
Sulforaphane	21



19 genes associated with atherosclerosis are regulated by Protandim

	Gene symbol	Gene title	Disease process
	Atherosclerosis (19 genes)		
	CTNNB1	catenin (cadherin-associated protein), beta 1, 88kDa	↑
*	**DHFR**	dihydrofolate reductase	↑
*	**EDN1**	endothelin 1	↑
*	**ITGB3**	integrin, beta 3 (platelet glycoprotein IIIa, antigen CD61)	↑
	MKI67	antigen identified by monoclonal antibody Ki-67	↑
	MMP11	matrix metallopeptidase 11 (stromelysin 3)	↑
	MMP14	matrix metallopeptidase 14 (membrane-inserted)	↑
	MMP2	matrix metallopeptidase 2	↑
*	**PDE7A**	phosphodiesterase 7A	↑
*	**PDE7B**	phosphodiesterase 7B	↑
	PLAU	plasminogen activator, urokinase	↑
*	**PTGS1**	prostaglandin-endoperoxide synthase 1	↑
	SCARB1	scavenger receptor class B, member 1	↑
*	**TUBB3**	tubulin, beta 3	↑
*	**NR3C1**	nuclear receptor subfamily 3, group C, member 1	↓
*	**PPARA**	peroxisome proliferator-activated receptor alpha	↓
	EGR1	early growth response 1	↑
	PTGS2	prostaglandin-endoperoxide synthase 2	↑
	SOAT1	sterol O-acyltransferase 1	↑

Of the 19 genes, 16 (84%) are modulated by Protandim in the opposing direction of the disease process

LifeVantage FREEDOM

28 genes associated with colon cancer are regulated by Protandim

	Gene symbol	Gene title	Disease process
	Colon carcinoma (28 genes)		
	ACLY	ATP citrate lyase	↑
	ANTXR1	anthrax toxin receptor 1	↑
	C20orf27	chromosome 20 open reading frame 27	↑
	CCNA2	cyclin A2	↑
	CHAF1A	chromatin assembly factor 1, subunit A (p150)	↑
	DHFR	dihydrofolate reductase	↑
	EFCAB11	chromosome 14 open reading frame 143	↑
	FEN1	flap structure-specific endonuclease 1	↑
	GINS2	GINS complex subunit 2 (Psf2 homolog)	↑
	MCM10	minichromosome maintenance complex component 10	↑
	MCM4	minichromosome maintenance complex component 4	↑
	RNASEH2A	ribonuclease H2, subunit A	↑
	SLIT2	slit homolog 2 (Drosophila)	↑
	SPC25	SPC25, NDC80 kinetochore complex component	↑
	TFRC	transferrin receptor (p90, CD71)	↑
	TK1	thymidine kinase 1, soluble	↑
	TMEM97	transmembrane protein 97	↑
	TRIP13	thyroid hormone receptor interactor 13	↑
	TUBB	tubulin, beta	↑
	TUBB3	tubulin, beta 3	↑
*	**TYMS**	thymidylate synthetase	↑
	UBA1	ubiquitin-like modifier activating enzyme 1	↑
	UNG	uracil-DNA glycosylase	↑
	VRK1	vaccinia related kinase 1	↑
	ABCD3	ATP-binding cassette, sub-family D (ALD), member 3	↓
	GLRX2	glutaredoxin 2	↑
	MICB	MHC class I polypeptide-related sequence B	↑
	NQO1	NAD(P)H dehydrogenase, quinone 1	↑

Of the 28 genes, 25 (89%) are modulated by Protandim in the opposing direction of the disease process

LifeVantage FREEDOM

66 genes associated with Alzheimer's are regulated by Protandim

	Gene symbol	Gene title	Disease process
	Alzheimer Disease (66 genes)		
	AGRN	agrin	↑
	ANP32A	acidic (leucine-rich) nuclear phosphoprotein 32 family, A	↑
	BAX	BCL2-associated X protein	↑
*	**BCHE**	butyrylcholinesterase	↑
	BGN	biglycan	↑
	BRCA1	breast cancer 1, early onset	↑
	CADPS2	Ca++-dependent secretion activator 2	↑
	CAPN1	calpain 1, (mu/I) large subunit	↑
	CCNB1	cyclin B1	↑
	CDC2	cell division cycle 2, G1 to S and G2 to M	↑
	CDK2	cyclin-dependent kinase 2	↑
	CDKN2A	cyclin-dependent kinase inhibitor 2A	↑
	CXCR4	chemokine (C-X-C motif) receptor 4	↑
	EIF4EBP1	eukaryotic translation initiation factor 4E binding protein 1	↑
	FOLH1	folate hydrolase (prostate-specific membrane antigen) 1	↑
	HOMER1	homer homolog 1 (Drosophila)	↑
*	**HRH1**	histamine receptor H1	↑
	IGF2	insulin-like growth factor 2 (somatomedin A)	↑
	IGFBP2	insulin-like growth factor binding protein 2, 36kDa	↑
	LDLR	low density lipoprotein receptor	↑
*	**MAOA**	monoamine oxidase A	↑
	NEFH	neurofilament, heavy polypeptide	↑
	NPDC1	neural proliferation, differentiation and control, 1	↑
	NRGN	neurogranin (protein kinase C substrate, RC3)	↑
*	**PREP**	prolyl endopeptidase	↑
	PROS1	protein S (alpha)	↑
*	**PTGS1**	prostaglandin-endoperoxide synthase 1	↑
	SELENBP1	selenium binding protein 1	↑
	TAGLN	transgelin	↑
	TGFB1	transforming growth factor, beta 1	↑
*	**TUBB3**	tubulin, beta 3	↑
*	**VKORC1**	vitamin K epoxide reductase complex, subunit 1	↑
	CANX	calnexin	↓

Genes 1-33

LifeVantage FREEDOM

66 genes associated with Alzheimer's are regulated by Protandim

	Gene symbol	Gene title	Disease process
	GCNT2	glucosaminyl (N-acetyl) transferase 2, I-branching enzyme	↓
	IDE	insulin-degrading enzyme	↓
	MMP1	matrix metallopeptidase 1 (interstitial collagenase)	↓
	NFE2L2	nuclear factor (erythroid-derived 2)-like 2	↓
	NR3C1	nuclear receptor subfamily 3, group C, member 1	↓
*	**PPARA**	peroxisome proliferator-activated receptor alpha	↓
	SLC6A6	solute carrier family 6, member 6	↓
	SYVN1	synovial apoptosis inhibitor 1, synoviolin	↓
	TSHZ1	teashirt zinc finger homeobox 1	↓
	TXN	thioredoxin	↓
	ACLY	ATP citrate lyase	↓
	ATAD2	ATPase family, AAA domain containing 2	↓
	BECN1	beclin 1, autophagy related	↓
	DHCR24	24-dehydrocholesterol reductase	↓
	FGF2	fibroblast growth factor 2 (basic)	↓
	HTRA1	HtrA serine peptidase 1	↓
	PRKCE	protein kinase C, epsilon	↓
	PRKDC	protein kinase, DNA-activated, catalytic polypeptide	↓
	SCD	stearoyl-CoA desaturase (delta-9-desaturase)	↓
	TUBB	tubulin, beta	↓
	UNG	uracil-DNA glycosylase	↓
	ATP1A1	ATPase, Na+/K+ transporting, alpha 1 polypeptide	↑
	CTSD	cathepsin D	↑
	GLRX	glutaredoxin (thioltransferase)	↑
	HMOX1	heme oxygenase (decycling) 1	↑
	IL6R	interleukin 6 receptor	↑
	NPTX1	neuronal pentraxin I	↑
	NQO1	NAD(P)H dehydrogenase, quinone 1	↑
	PHF1	PHD finger protein 1	↑
	PRKCD	protein kinase C, delta	↑
	PTGS2	prostaglandin-endoperoxide synthase 2	↑
	RANBP9	RAN binding protein 9	↑
	SOD1	superoxide dismutase 1, soluble	↑

Genes 34-66

Of the 66 genes, 43 (65%) are modulated by Protandim in the opposing direction of the disease process

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Original Contribution

Nrf2 activation: A potential strategy for the prevention of acute mountain sickness

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ABSTRACT

Reactive oxygen species (ROS) formed during acute high altitude exposure contribute to cerebral vascular leak and development of acute mountain sickness (AMS). Nuclear factor (erythroid-derived 2)-related factor 2 (Nrf2) is a transcription factor that regulates expression of greater than 90% of antioxidant genes, but prophylactic treatment with Nrf2 activators has not yet been tested as an AMS therapy. We hypothesized that prophylactic activation of the antioxidant genome with Nrf2 activators would attenuate high-altitude-induced ROS formation and cerebral vascular leak and that some drugs currently used to treat AMS symptoms have an additional trait of Nrf2 activation. Drugs commonly used to treat AMS were screened with a luciferase reporter cell system for their effectiveness to activate Nrf2, as well as being tested for their ability to decrease high altitude cerebral vascular leak in vivo. Compounds that showed favorable results for Nrf2 activation from our screen and attenuated high altitude cerebral vascular leak in vivo were further tested in brain microvascular endothelial cells (BMECs) to determine if they attenuated hypoxia-induced ROS production and monolayer permeability. Of nine drugs tested, with the exception of dexamethasone, only drugs that showed the ability to activate Nrf2 (Protandim, methazolamide, nifedipine, amlodipine, ambrisentan, and sitaxentan) decreased high-altitude-induced cerebral vascular leak in vivo. In vitro, Nrf2 activation in BMECs before 24 h hypoxia exposure attenuated hypoxic-induced hydrogen peroxide production and permeability. Prophylactic Nrf2 activation is effective at reducing brain vascular leak from acute high altitude exposures. Compared to acetazolamide, methazolamide may offer better protection against AMS. Nifedipine, in addition to its known vasodilatory activities in the lung and protection against high altitude pulmonary edema, may provide protection against brain vascular leak as well.

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- Funded by DARPA (Dept of Defense Advanced Research Projects Agency)
- Hypoxic high altitude environment can induce leaky blood vessels in lungs/brain
- "…Nrf2 activation either by Protandim or from 'off-target' effects of other compounds before high altitude or hypoxia exposure decreased cerebral vascular leak in vivo…"

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“**Protandim-mediated HO-1 induction** involved the presence of ARE sites in the HO-1 promoter and **nuclear translocalization of the transcription factor Nrf2**”

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Original Contribution

Synergistic induction of heme oxygenase-1 by the components of the antioxidant supplement Protandim

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ABSTRACT

Protandim is an antioxidant supplement that consists of five ingredients, namely, ashwagandha, bacopa extract, green tea extract, silymarin, and curcumin, each with known therapeutic properties. Protandim was formulated with the objective of combining multiple phytochemicals at low nontoxic doses to gain synergy among them. A recent clinical study demonstrated the in vivo antioxidant effects of Protandim (S.K. Nelson et al., 2006, *Free Radic. Biol. Med.* 40, 341–347). The objective of the present study was to determine if the components of Protandim induce heme oxygenase-1 (HO-1) in a synergistic manner in cultured MIN6 cells, a mouse β-cell line, and in SK-N-MC cells, a human neuroblastoma cell line. When the components of Protandim were tested alone at low doses, curcumin showed minimal induction, whereas the others were unable to induce the HO-1 promoter, assayed by transient transfection. All components together, however, produced a strongly synergistic induction of around three- to ninefold in a dose-dependent manner, greatly exceeding the sum of the parts. Similar findings were obtained for the expression of HO-1 at the mRNA and protein levels. Protandim-mediated HO-1 induction involved the presence of ARE sites in the HO-1 promoter and nuclear translocalization of the transcription factor Nrf2, which binds to ARE sites. The involvement of multiple signaling pathways, including PI3-kinase/Akt, p38MAPK, and PKCδ, in HO-1 induction seems to be the probable mechanism of synergy between the components of Protandim. There were significant increases in the levels of total glutathione in Protandim-treated cells. These findings suggest that the use of a combination of phytochemicals may be an efficient method for the induction of antioxidant enzymes.

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PLoS one

Protandim, a Fundamentally New Antioxidant Approach in Chemoprevention Using Mouse Two-Stage Skin Carcinogenesis as a Model

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Abstract

Oxidative stress is an important contributor to cancer development. Consistent with that, antioxidant enzymes have been demonstrated to suppress tumorigenesis when being elevated both in vitro and in vivo, making induction of these enzymes a more potent approach for cancer prevention. Protandim, a well-defined combination of widely studied medicinal plants, has been shown to induce superoxide dismutase (SOD) and catalase activities and reduce superoxide generation and lipid peroxidation in healthy human subjects. To investigate whether Protandim can suppress tumor formation by a dietary approach, a two-stage mouse skin carcinogenesis study was performed. At the end of the study, the mice on a Protandim-containing basal diet had similar body weight compared with those on the basal diet, which indicated no overt toxicity by Protandim. After three weeks on the diets, there was a significant increase in the expression levels of SOD and catalase, in addition to the increases in SOD activities. Importantly, at the end of the carcinogenesis study, both skin tumor incidence and multiplicity were reduced in the mice on the Protandim diet by 33% and 57% respectively, compared with those on basal diet. Biochemical and histological studies revealed that the Protandim diet suppressed tumor promoter-induced oxidative stress (evidenced by reduction of protein carbonyl levels), cell proliferation (evidenced by reduction of skin hyperplasia and suppression of PKC/JNK/Jun pathway), and inflammation (evidenced by reduction of ICAM-1/VCAM-1 expression, NF-κB binding activity, and nuclear p65/p50 levels). Overall, induction of antioxidant enzymes by Protandim may serve as a practical and potent approach for cancer prevention.

"Overall, induction of antioxidant enzymes by **Protandim** may serve as a practical and potent approach for **cancer prevention**"

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PLoS one

The Chemopreventive Effects of Protandim: Modulation of p53 Mitochondrial Translocation and Apoptosis during Skin Carcinogenesis

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Abstract

Protandim, a well defined dietary combination of 5 well-established medicinal plants, is known to induce endogenous antioxidant enzymes, such as manganese superoxide dismutase (MnSOD). Our previous studies have shown through the induction of various antioxidant enzymes, products of oxidative damage can be decreased. In addition, we have shown that tumor multiplicity and incidence can be decreased through the dietary administration of Protandim in the two-stage skin carcinogenesis mouse model. It has been demonstrated that cell proliferation is accommodated by cell death during DMBA/TPA treatment in the two-stage skin carcinogenesis model. Therefore, we investigated the effects of the Protandim diet on apoptosis; and proposed a novel mechanism of chemoprevention utilized by the Protandim dietary combination. Interestingly, Protandim suppressed DMBA/TPA induced cutaneous apoptosis. Recently, more attention has been focused on transcription-independent mechanisms of the tumor suppressor, p53, that mediate apoptosis. It is known that cytoplasmic p53 rapidly translocates to the mitochondria in response to pro-apoptotic stress. Our results showed that Protandim suppressed the mitochondrial translocation of p53 and mitochondrial outer membrane proteins such as Bax. We examined the levels of p53 and MnSOD expression/activity in murine skin JB6 promotion sensitive (P+) and promotion-resistant (P-) epidermal cells. Interestingly, p53 was induced only in P+ cells, not P- cells; whereas MnSOD is highly expressed in P- cells when compared to P+ cells. In addition, wild-type p53 was transfected into JB6 P- cells. We found that the introduction of wild-type p53 promoted transformation in JB6 P- cells. Our results suggest that suppression of p53 and induction of MnSOD may play an important role in the tumor suppressive activity of Protandim.

"Our results suggest that suppression of **p53** and induction of **MnSOD** may play an important role in the **tumor suppressive activity of Protandim**."

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informa
healthcare

The Dietary Supplement Protandim® Decreases Plasma Osteopontin and Improves Markers of Oxidative Stress in Muscular Dystrophy *Mdx* Mice

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ABSTRACT. Therapeutic options for Duchenne muscular dystrophy (DMD), the most common and lethal neuromuscular disorder in children, remain elusive. Oxidative damage is implicated as a pertinent factor involved in its pathogenesis. Protandim® is an over-the-counter supplement with the ability to induce antioxidant enzymes. In this

After 6 months on Protandim:

- TBARS reduced 48%
- Osteopontin reduced 57%
- PON1 increased 35%
- MRI signal reduced 38%

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Circulation
JOURNAL OF THE AMERICAN HEART ASSOCIATION

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Heart Failure

Chronic Pulmonary Artery Pressure Elevation Is Insufficient to Explain Right Heart Failure

Harm J. Bogaard, MD, PhD*; Ramesh Natarajan, PhD*; Scott C. Henderson, PhD; Carlin S. Long, MD; Donatas Kraskauskas, DVM; Lisa Smithson, BSc; Ramzi Ockaili, PhD; Joe M. McCord, PhD; Norbert F. Voelkel, MD

Background—The most important determinant of longevity in pulmonary arterial hypertension is right ventricular (RV) function, but in contrast to experimental work elucidating the pathobiology of left ventricular failure, there is a paucity of data on the cellular and molecular mechanisms of RV failure.

Methods and Results—A mechanical animal model of chronic progressive RV pressure overload (pulmonary artery banding, not associated with structural alterations of the lung circulation) was compared with an established model of angioproliferative pulmonary hypertension associated with fatal RV failure. Isolated RV pressure overload induced RV hypertrophy without failure, whereas in the context of angioproliferative pulmonary hypertension, RV failure developed that was associated with myocardial apoptosis, fibrosis, a decreased RV capillary density, and a decreased vascular endothelial growth factor mRNA and protein expression despite increased nuclear stabilization of hypoxia-induced factor-1α. Induction of myocardial nuclear factor E2-related factor 2 and heme-oxygenase 1 with a dietary supplement (Protandim) prevented fibrosis and capillary loss and preserved RV function despite continuing pressure overload.

Conclusion—These data brought into question the commonly held concept that RV failure associated with pulmonary hypertension is due strictly to the increased RV afterload. (***Circulation***. **2009;120:1951-1960.**)

Key Words: angiogenesis ■ heart failure ■ microcirculation ■ pressure ■ pulmonary heart disease

"[Protandim] prevented fibrosis and capillary loss and preserved [heart] function"

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Original Contribution

Protandim attenuates intimal hyperplasia in human saphenous veins cultured ex vivo via a catalase-dependent pathway

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ABSTRACT

Human saphenous veins (HSVs) are widely used for bypass grafts despite their relatively low long-term patency. To evaluate the role of reactive oxygen species (ROS) signaling in intima hyperplasia (IH), an early stage pathology of vein-graft disease, and to explore the potential therapeutic effects of up-regulating endogenous antioxidant enzymes, we studied segments of HSV cultured ex vivo in an established ex vivo model of HSV IH. Results showed that HSV cultured ex vivo exhibit an ~3-fold increase in proliferation and ~3.6-fold increase in intimal area relative to freshly isolated HSV. Treatment of HSV during culture with Protandim, a nutritional supplement known to activate Nrf2 and increase the expression of antioxidant enzymes in several in vitro and in vivo models, blocks IH and reduces cellular proliferation to that of freshly isolated HSV. Protandim treatment increased the activity of SOD, HO-1, and catalase 3-, 7-, and 12-fold, respectively, and decreased the levels of superoxide ($O_2^{\cdot-}$) and the lipid peroxidation product 4-HNE. Blocking catalase activity by cotreating with 3-amino-1,2,4-triazole abrogated the protective effect of Protandim on IH and proliferation. In conclusion, these results suggest that ROS-sensitive signaling mediates the observed IH in cultured HSV and that up-regulation of endogenous antioxidant enzymes can have a protective effect.

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"**Protandim blocks IH** and reduces cellular proliferation to that of freshly isolated HSV.

Protandim treatment **increased the activity of SOD, HO-1, and catalase** 3-, 7-, and 12-fold, respectively, and **decreased the levels of superoxide** and the lipid peroxidation product **4-HNE**."

Don’t Take Antioxidants Make Antioxidants

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