Traumatic Brain Injury:

A Clinical Approach to Diagnosis and Treatment

by Mark L. Gordon, M.D.

Chapter 8: Supplements

Phase I - Neurotrauma

- Damage to the brain includes not only white and gray matters and their cellular constituents, but also an extensive vascular supply.
- Ischemia causing hypoxia/anoxia and hypoglycemia can initiate inflammatory pathways and further neuronal and glial death.
- Cavitation of the brain with the loss of brain tissue, is a progressive mechanism where areas of initial injury can continue to expand due to these underlying processes, led by all aspects of Oxidative Stress.

Cellular and Molecular Mechanisms of Glial Scarring and Progressive Cavitation: *In Vivo* and *In Vitro* Analysis of Inflammation-Induced Secondary Injury after CNS Trauma

. The Journal of Neuroscience, October 1, 1999, 19(19):8182–8198 Michael T. Fitch, Catherine Doller, Colin K. Combs, Gary E. Landreth, and Jerry Silver. Dept of Neurosciences and 2Alzheimer Research Lab, Case Western Reserve University School of Medicine, Cleveland, Ohio 44106

- Post-traumatic cystic cavitation, in which the size and severity of a CNS injury progress from a small area of direct trauma to a greatly enlarged secondary injury surrounded by glial scar tissue, is a poorly understood complication of damage to the brain.
- Persistent inflammation in the absence of significant physical damage within CNS white matter can result in an expanding astrocyte-free cavity surrounded by glial scarring and extracellular matrix proteoglycans and the secondary destruction of axons. A NPE condition.

Phase II - Neurotrauma

- mTBI represents 85% of the cases of head trauma.
- The injured is usually highly functioning with a slow progression of neuropsychiatric and physical complaints.
- Identification of hormonal deficiencies is simple as is the treatment, but the insidious and underlying "stealth" processes of **oxidative damage** can be missed or ignored.
- A number of impressive studies give us the support to venture on….

Mitochondrial Biogenesis

- Oxidative stress and cellular injury that damage mitochondria may impair the cell's capacity to generate sufficient ATP for homeostasis, ultimately leading to apoptosis or necrosis.
- The cell's energy supply is protected from conditions that damage mitochondria by an inducible transcriptional program of mitochondrial biogenesis that operates in large part through redox signals involving nitric oxide synthase, heme oxygenase-1 and CO systems.

Redox Regulation of Mitochondrial Biogenesis. Free Radic Biol Med. 2012 December 1; 53(11): 2043–2053. Claude A. Piantadosi, MD and Hagir B. Suliman, PhD, DVM. Depts of Medicine, Anesthesiology, and Pathology Duke University Medical Center and the Durham VA Medical Center Durham, NC 27710 USA

Mitochondrial Biogenesis

Initiators of Mitochondrial Biogenesis

- Embryonic development
- Cell division and repair
- Changes in physiological state
  - Exercise
  - Energy limitation
  - Cold stress
  - Calorie restriction (Hypoglycemia)
  - Hypoxia(?)
  - Sympathetic stimulation
  - Hormones
    - Erythropoietin
    - Leptin
    - Thyroid hormone

Inhibitors of Mitochondrial Biogenesis

- Oxidative/Nitrosative stress
- Hypoxia/ischemia
- Inflammation
- Apoptosis (AIF)
- Necrosis
- Parthanatos (PARP-1)
- BAX, Bcl2….. Cytochrome c

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Mitochondrial and the Theory of Aging

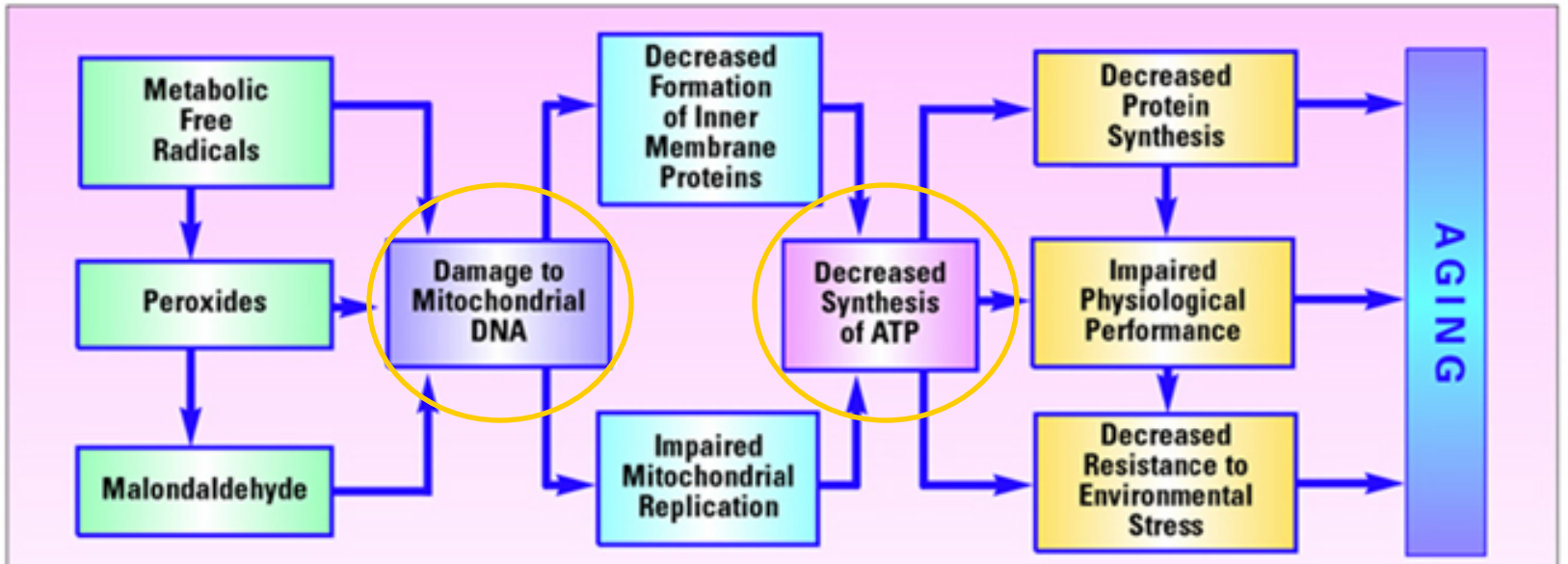


Fig. 1. Miquel's proposed model of aging, based on free radical-induced damage to mitochondrial DNA. This concept is consistent with the finding that free radicals, peroxides and malonaldehyde (which are injurious to DNA) are generated in the mitochondrial inner membrane. Accumulation of unrepaired damage to mitochondrial DNA leads to inadequate formation of inner membrane proteins, which interferes with mitochondrial replication. The resulting decline in ATP synthesis sets up a vicious cycle of mitochondrial loss, decline in energy production, decreased protein synthesis and impaired physiological performance. (Adapted from Fleming, J.E., Miquel, J., and Bensch, K.G. Age-dependent changes in mitochondria. *Molecular Biology of Aging*, 1985, Plenum, New York, 143-155.)

Reducing Agents

- Substances that have the ability to reduce other substances (gain electrons) are said to be reductive or reducing and are known as reducing agents, reductants, or reducers.
- The reducing agent transfers electrons to another substance, and is thus itself oxidized.
- And, because it "donates" electrons, the reducing agent is also called an electron donor.
- **Reduction is the *gain* of electrons**.

Oxidative Agents

- Substances that have the ability to oxidize other substances are said to be oxidative, oxidizers, or oxidizing agents.
- The oxidizing agent removes electrons from another substance, and is thus itself reduced.
- Because it "accepts" electrons, the oxidizing agent is also called an electron acceptor.
- Oxygen is the classical and quintessential oxidizer.
- Oxidation is the *loss* of electrons.

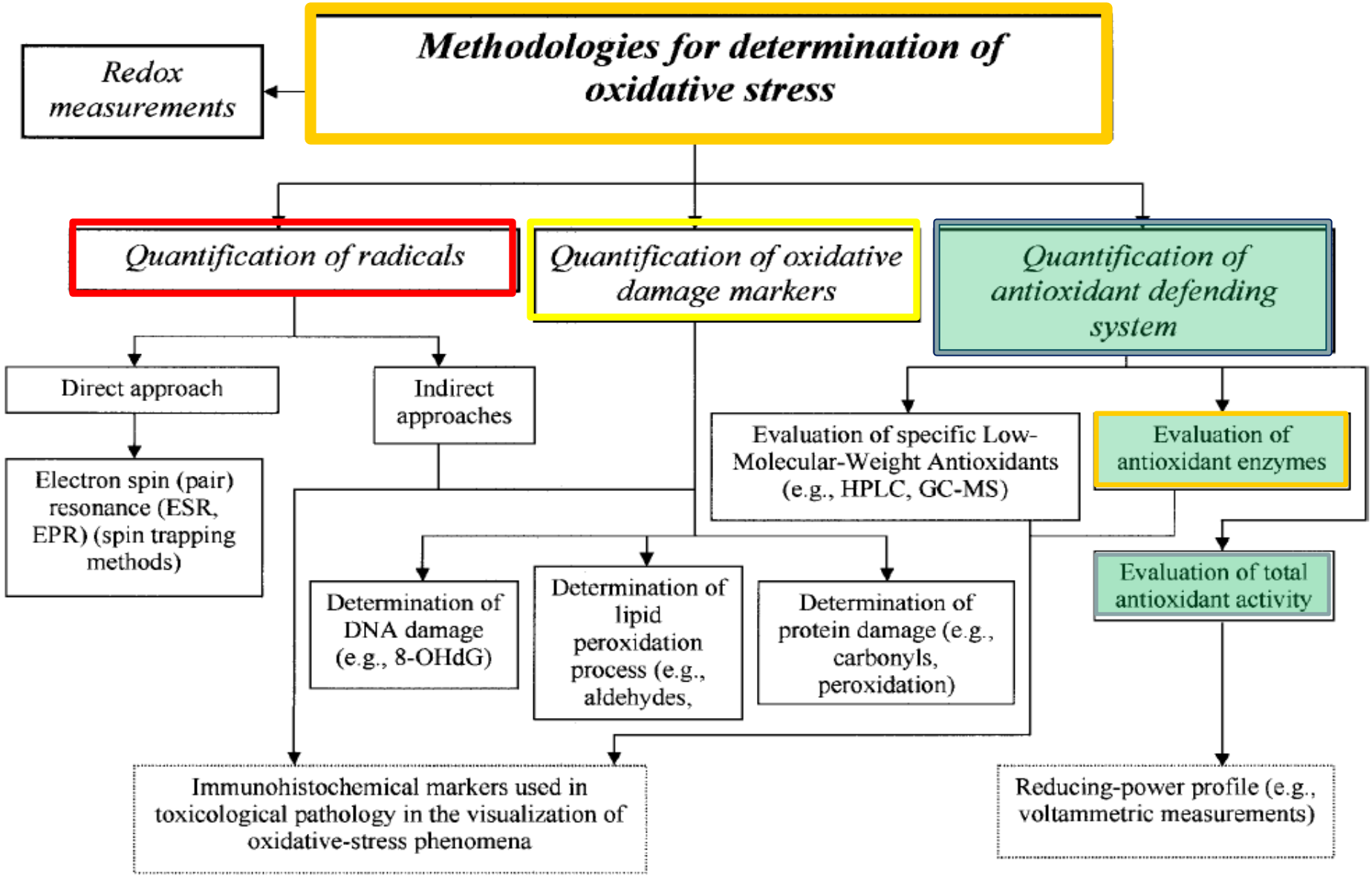
Free Radical & Oxidation

- Free radicals are highly reactive compounds which are mostly generated during cellular respiration and normal metabolism .
- Their possession of unpaired electrons in their outer shell causes them to be more reactive than their corresponding non-radicals.
- This is because they act as electron acceptors, and essentially "steal" electrons from other molecules and thereby modify their chemical structures – an action which is referred to as oxidation.

Disruption of the Balance

- Normally, a state of equilibrium exists between tissue oxidant and anti-oxidant activities.
- This balance can, however, be upset as a result of excessive free radical generation, depletion of endogenous antioxidants or failure to repair oxidative injury induced by reactive oxygen species.
- The resultant condition is referred to as **oxidative stress** and has been implicated in the disruption of neuronal homeostasis induced by traumatic brain injury.

Oxidative Stress Following Traumatic Brain Injury: Enhancement of Endogenous Antioxidant Defense Systems and the Promise of Improved Outcome. Eghwrudjakpor P O, *MBBS, DMS, FICS* Allison A B, *MBBS, FRCS, FICS* Department of Surgery University of Port Harcourt Teaching Hospital, Port Harcourt Nigeria

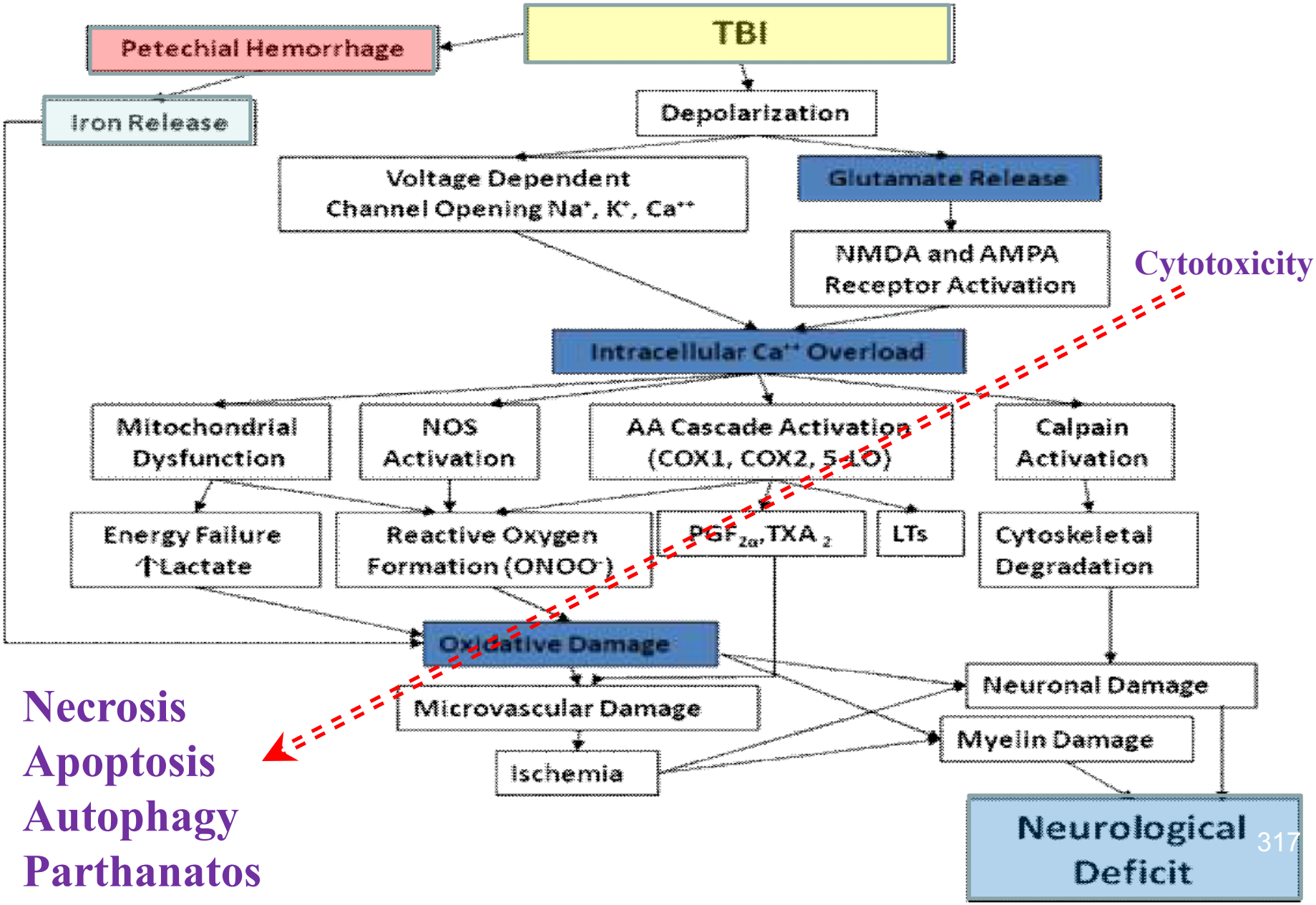

Methodologies for determination of oxidative stress
Redox measurements
Quantification of radicals
Quantification of oxidative damage markers
Quantification of antioxidant defending system
Direct approach
Indirect approaches
Electron spin (pair) resonance (ESR, EPR) (spin trapping methods)
Evaluation of specific Low-Molecular-Weight Antioxidants (e.g., HPLC, GC-MS)
Evaluation of antioxidant enzymes
Evaluation of total antioxidant activity
Determination of DNA damage (e.g., 8-OHdG)
Determination of lipid peroxidation process (e.g., aldehydes,
Determination of protein damage (e.g., carbonyls, peroxidation)
Immunohistochemical markers used in toxicological pathology in the visualization of oxidative-stress phenomena
Reducing-power profile (e.g., voltammetric measurements)

Antioxidant Therapies for Traumatic Brain Injury. Neurotherapeutics. 2010 January ; 7(1): 51. Edward D. Hall, Radhika A. Vaishnav, and Ayman G. Mustafa Spinal Cord & Brain Injury Research Center, University of Kentucky Medical Center

- Free radical-induced oxidative damage and membrane lipid peroxidation (LP) are one of the best validated secondary injury mechanisms in preclinical traumatic brain injury models.
- The disruption of the membrane phospholipid architecture, LP results in the formation of cytotoxic aldehyde-containing products that bind to cellular proteins on the cell membrane and impair their normal functions .

Gated-Ion Channels are impaired with LP of the cell membrane.

The full IMPACT of TBI



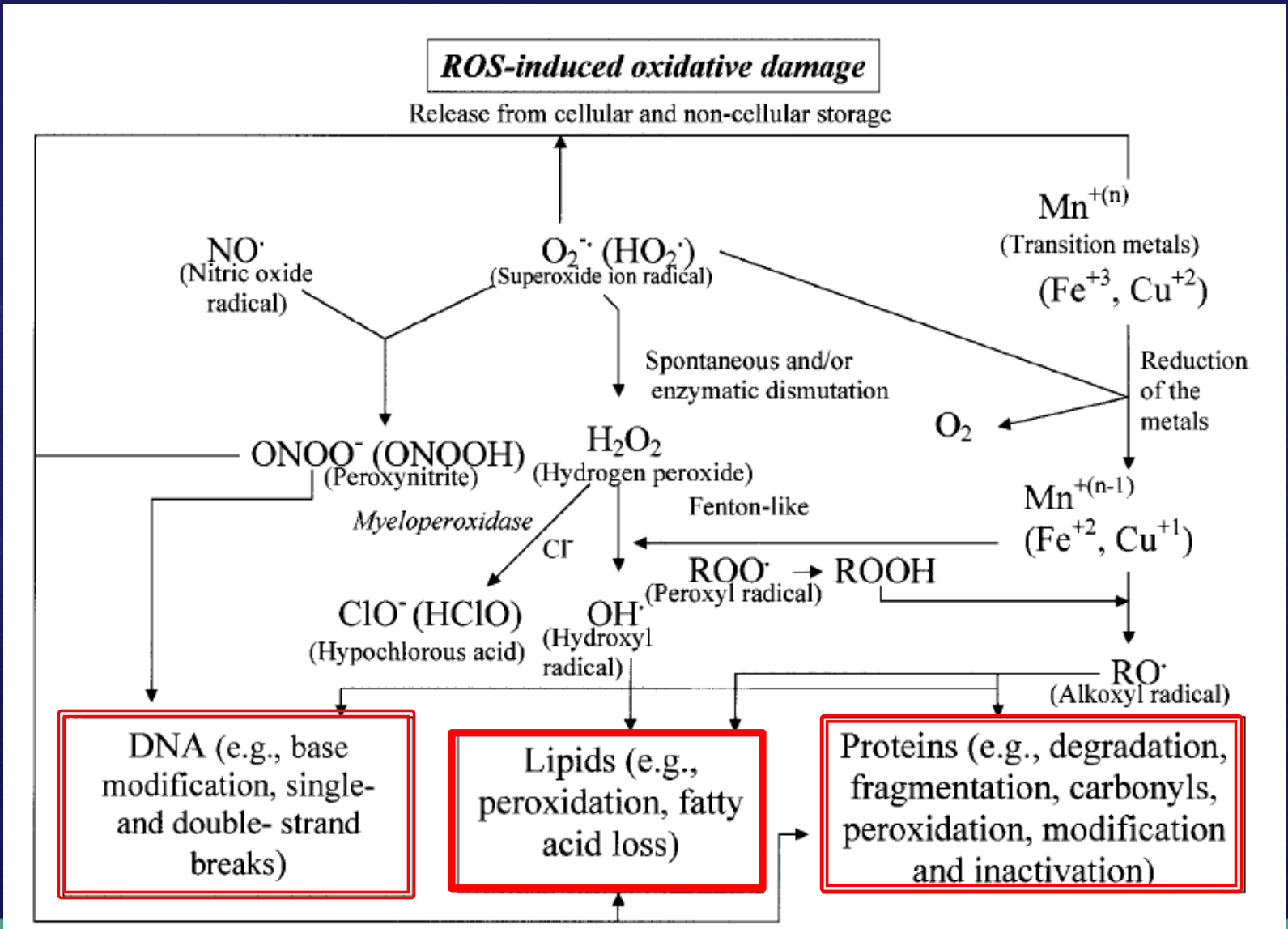
Oxidation of Biological Systems: Oxidative Stress Phenomena, Antioxidants, Redox Reactions, and Methods for Their Quantification.

Toxicol Pathol 2002 30: 620. Ron Kohen and Abraham Nyska

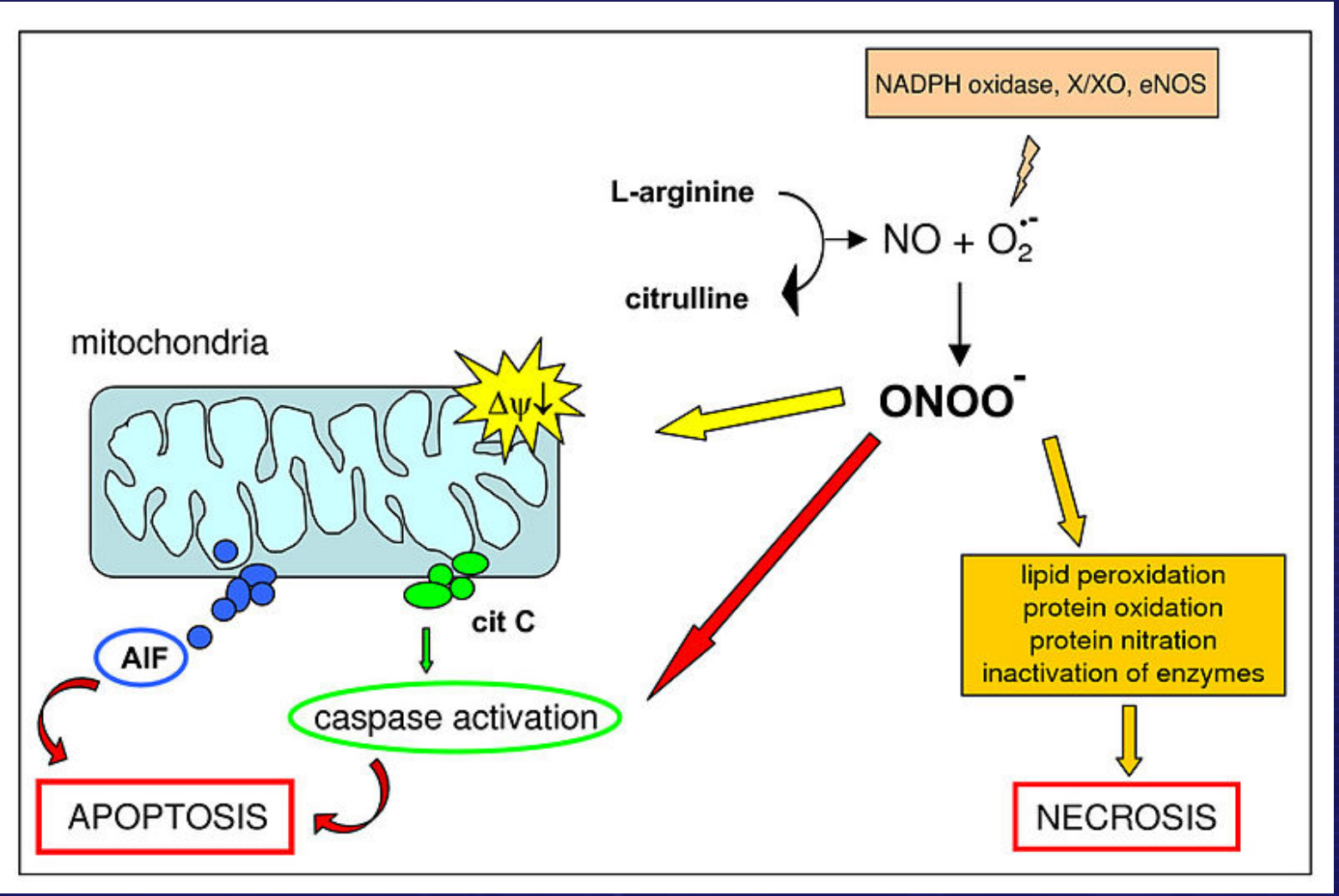
TABLE 1.—Radical and nonradical oxygen metabolites.

Name	Symbol
Oxygen radicals	
Oxygen (bi-radical)	$O_2^{\cdot\cdot}$
Superoxide ion	$O_2^{\cdot -}$
Hydroxyl	$OH^{\cdot}$
Peroxyl	$ROO^{\cdot}$
Alkoxyl	$RO^{\cdot}$
Nitric oxide	$NO^{\cdot}$
Nonradical oxygen derivatives	
Hydrogen peroxide	H_2O_2
(Organic peroxide)	ROOH
Hypochlorous acid	HOCL
Ozone	O_3
Aldehydes	HCOR
Singlet oxygen	$^1O_2$
Peroxynitrite	ONOOH

Reactive oxygen species (ROS)



Peroxynitrite



Melatonin

- *N-Acetyl-5-methoxytryptamine* (Melatonin) is produced in the pineal gland and readily crosses the blood brain barrier to enter neurons and glial cells.
- A potent scavenger of peroxyl and hydroxyl radicals, shown to prevent both initiation and propagation of lipid peroxidation and stimulate ***brain glutathione peroxidase***.
- Melatonin acts as an antioxidant in both ***lipophilic*** and ***hydrophilic*** environments because of its solubility properties.
- Recently it was shown that melatonin inhibits nitric oxide synthase (*NOS*), thus preventing the toxic effect obtained after its interaction with ***superoxide radicals***.

Melatonin: A Multitasking Molecule. Progress in Brain Research Volume 181, 2010, Pages 127–151. Russel J. Reiter, Dun-Xian Tan, Lorena Fuentes-Broto

- Melatonin has revealed itself as a ubiquitously distributed and functionally diverse molecule.
- Its ability to detoxify free radicals and related oxygen derivatives, melatonin influences the molecular physiology of cells via receptor-independent means.

Carnosine

- Carnosine (beta-alanyl-L-histidine) is a naturally-occurring, pluripotent, homeostatic agent – in both muscles and brain of humans.
- Carnosine is a dipeptide found at high concentrations in **glial and neuronal cells** throughout the brain.
- Because of its enrichment in histidine residues, carnosine has also been proposed as a chelator for divalent cations like Cu^{2+} and Zn^{2+}
- Carnosine can suppress amyloid-beta peptide toxicity, inhibit production of oxygen free-radicals, scavenge hydroxyl radicals and reactive aldehydes, and suppresses protein glycation.

Antioxidant activity of carnosine, homocarnosine, and anserine present in muscle and brain. Proc. Nat. Acad. Sci. USA Vol. 85, pp. 3175-3179, May 1988. Ron Kohen, Yorihiro Yamamoto, Ken C. Cundy, and Bruce N. Ames Department of Biochemistry, University of California, Berkeley, CA 94720

- Carnosine, anserine, and histidine protect phage against gamma-irradiation, which gives rise to oxidative DNA damage.
- Roles of carnosine, include chelation of metal ions, quenching of singlet oxygen, and binding of hydroperoxides.

Vitamin C

- Ascorbic acid is considered a powerful hydrophilic scavenger in biological fluids and tissues.
- Humans and primates have lost the ability to synthesize ascorbic acid and therefore rely on dietary sources.
- Vitamin C is distributed throughout the brain, and its concentration in the cerebrospinal fluid is about **tenfold higher than in the plasma.**
- The vitamin serves as a strong reducing agent by donating electron(s), thus directly neutralizing ROS.
- Vitamin C also acts to **recycle the Tocopherol radical** to its active reduced form.

Ascorbate and glutathione: the heart of the redox hub. Plant Physiology, January 2011, Vol. 155, pp. 2–18. Christine H. Foyer and Graham Noctor. Centre for Plant Sciences, Faculty of Biology, University of Leeds, Leeds LS2 9JT, United Kingdom (C.H.F.); and Institut de Biologie des Plantes, UMR CNRS 8618, Universite de Paris Sud 11, 91405 Orsay cedex, France (G.N.)

Ascorbate-Glutathione Cycle:

(1) H_2O_2 + Ascorbate $\xrightarrow{APX}$ H_2O + Monodehydroascrobate (MDA)

(2) MDA + NAD(P)H $\xrightarrow{MDAR}$ Ascorbate + $NAD(P)^+$

(3) Dehydroascorbate + GSH $\xrightarrow{DHAR}$ Ascorbate + GSSG

(4) GSSG + NAD(P)H $\xrightarrow{GR}$ GSH + $NAD(P)^+$

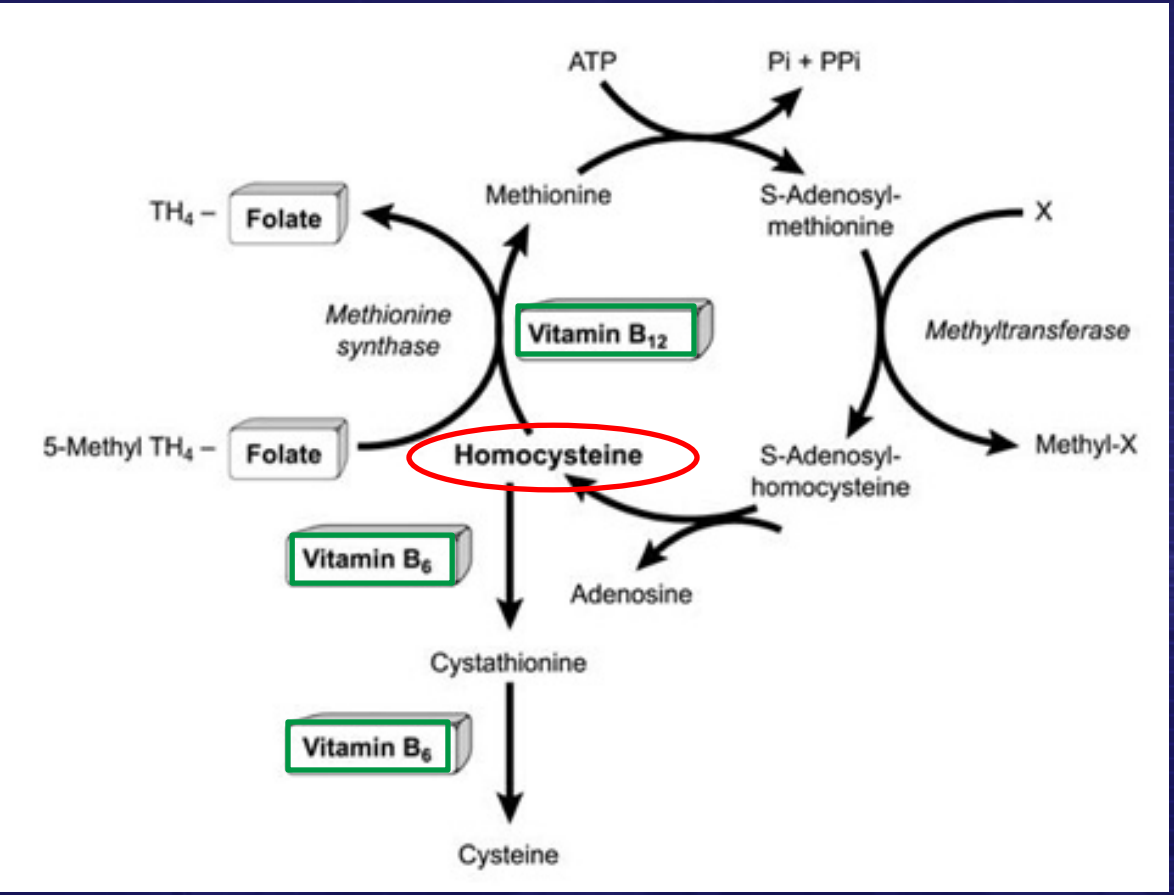
Vitamin B-12

- Vitamin B_{12}, also called cobalamin, is a water-soluble vitamin with a key role in the normal functioning of the brain and nervous system, and for the formation of blood cells.
- It is one of the eight B vitamins.
- It is normally involved in the metabolism of every cell of the human body, especially affecting DNA synthesis and regulation, but also fatty acid metabolism and amino acid metabolism.

Homocysteine Elicits a DNA Damage Response in Neurons That Promotes Apoptosis and Hypersensitivity to Excitotoxicity. The Journal of Neuroscience, Sept 15, 2000, 20(18):6920–6926. Inna I. Kruman, Carsten Culmsee, Sic L. Chan, Yuri Kruman, Zhihong Guo, LaRoy Penix, and Mark P. Mattson. Laboratory of Neurosciences, NIA, Baltimore, Maryland, and Sanders-Brown Research Center on Aging and Dept of Anatomy and Neurobiology, University of Kentucky, Lexington, Kentucky

- Homocysteine can induce neuronal apoptosis and can increase neuronal vulnerability to excitotoxicity by a mechanism involving DNA damage, PARP activation, and p53 induction.
- B-12 as well as B6 and Folate have been found to reduce the serum levels of HCY by increasing its conversion to cysteine.

Homocysteine Removal



Nicotinamide

- Nicotinamide (Vitamin B-3) is the amide form of nicotinic acid (niacin) and is currently used in the treatment of pellagra, a vitamin deficiency.
- It has been identified as a neuroprotective agent and has been shown to increase growth factors in Huntington's disease, reduce excitotoxicity in vitro, prevent cell death from oxidative damage both in vitro and in vivo, and improve behavioral outcomes following TBI and stroke.

The effects of nicotinamide on apoptosis and blood-brain barrier breakdown following traumatic brain injury. Brain Research. 2006 Dec 13;1125(1):185-93. Hoane MR, Kaplan SA, Ellis AL

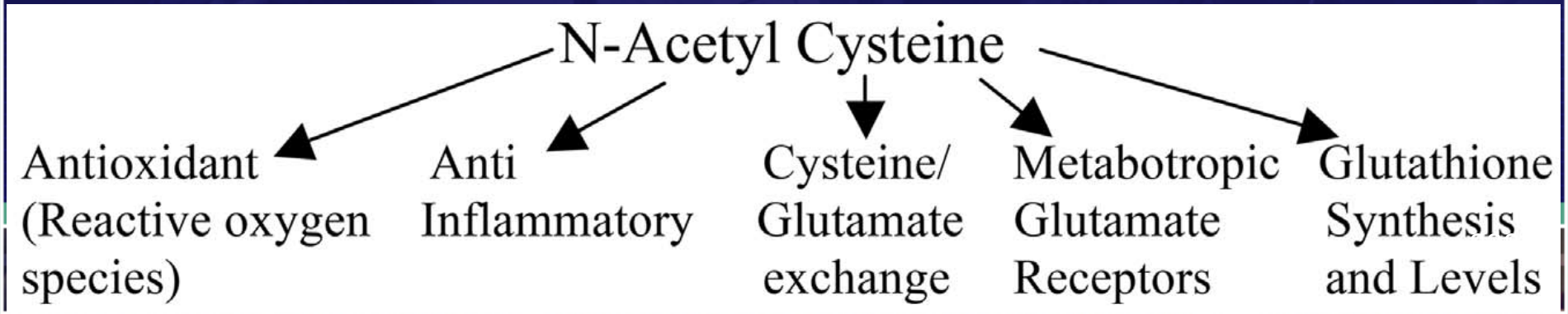
- Nicotinamide has been shown to protect against many of the pathophysiological factors associated with both ischemic and traumatic brain injuries.
- Nicotinamide reduced lesion cavity expansion 72 h following Cortical Contusion Injury.
- These findings suggest that nicotinamide reduces BBB breach and neuronal cell loss acutely following injury and that these reductions may account for the beneficial behavioral effects seen in previous studies.

N-Acetyl Cysteine

- A thiol acetylated cysteine that is a component of the tri-peptide Glutathione.
- NAC has anti-oxidant and free radical capabilities against Superoxides and Hydrogen peroxide, as well as hydroxyl radicals.

Efficacy of N-Acetyl Cysteine in Traumatic Brain Injury. PLOS ONE,April **2014**,Vol 9,4 , Katharine Eakin1, Renana Baratz-Goldstein, Chiam G. Pick, Ofra Zindel, Carey D. Balaban, Michael E. Hoffer, Megan Lockwood1, Jonathan Miller1, Barry J. Hoffer1, Dept of Neurosurgery, Case Western Reserve University School of Medicine, Cleveland, Ohio, USA, Dept of Anatomy and Anthropology, Sackler School of Medicine, Tel-Aviv University, Tel-Aviv, Israel, Dept of ENT, Neurobiology, Communication Sciences and Disorders, and Bioengineering, UofP, PA, USA, Dept of ENT, Spatial Orientation Center, Naval Medical Center San Diego, San Diego, Ca, USA, Graduate Program in Neuroregeneration, Taipei Medical University, Taipei City, Taiwan

- NAC has been shown to have antioxidant and neurovascular-protective effects after TBI.
- The researchers found that early post-injury treatment with NAC reversed the behavioral deficits associated with mTBI.



Amelioration of acute sequelae of blast induced mild traumatic brain injury by N-acetyl cysteine: a double-blind, placebo controlled study.

Michael E. Hoffer, Carey Balaban, Martin D. Slade, Jack W. Tsao, Barry Hoffer. Spatial Orientation Center, Dept of Otolaryngology, Naval Medical Center San Diego, California, USA, Depts of Otolaryngology, Neurobiology, Communication Sciences and Disorders, and Bioengineering, University of Pittsburgh, Pennsylvania, USA, Dept of Internal Medicine, Yale University, New Haven, Connecticut, USA, Wounded, Ill and Injured Directorate (M9), US Navy Bureau of Medicine and Surgery, Washington, D.C., USA, Dept of Neurosurgery, Case Western University, Cleveland, Ohio, USA.

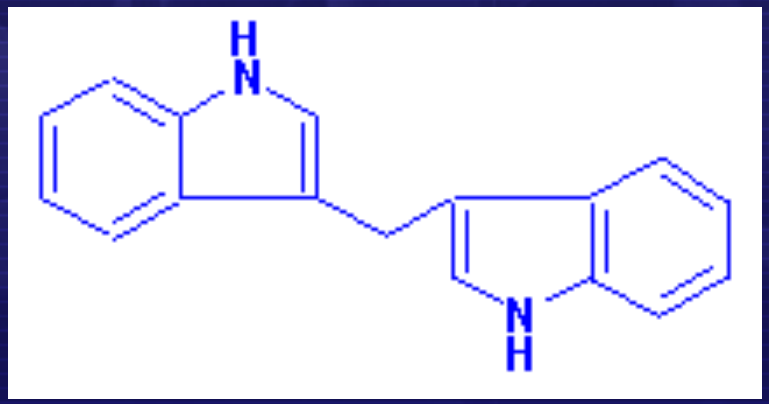
- A 4 gram loading dose was given followed by 2grams twice a day, then reduced to 1.5grams BID after 4 days.
- Early treatment with NAC resulted in a seven day symptom resolution rate of 86% as compared to 11% in those receiving placebo and began therapy between 24–72 hours after blast exposure.

Modulation of inflammation in brain: a matter of fat. Journal of Neurochemistry, 2007, 101, 577–599. Akhlaq A. Farooqui, Lloyd A. Horrocks, and Tahira Farooqui, *Depts of Molecular and Cellular Biochemistry, The Ohio State University, Columbus, Ohio,

- Cytokines are major effectors of the Neuroinflammatory cascade. They play an important role in neural cell response to infection and brain injury.
- Docosahexaenoic acid (DHA) is metabolized to resolvins and neuroprotectins. These lipid mediators inhibit the generation of prostaglandins, leukotrienes, and thromboxanes.
- A combination of NAC and Vitamin E (a,d,g) reduce the production of Nf Kappa B

Di-Indole-Methane

- A metabolite of indole–3–carbinol (I3C) found naturally in cruciferous vegetables like broccoli, kale and Brussels sprouts.
- Indole-3-carbinol (C9H9NO) is the subject of on-going biomedical research into its anti-carcinogenic, anti-oxidant, and anti atherogenic effects.



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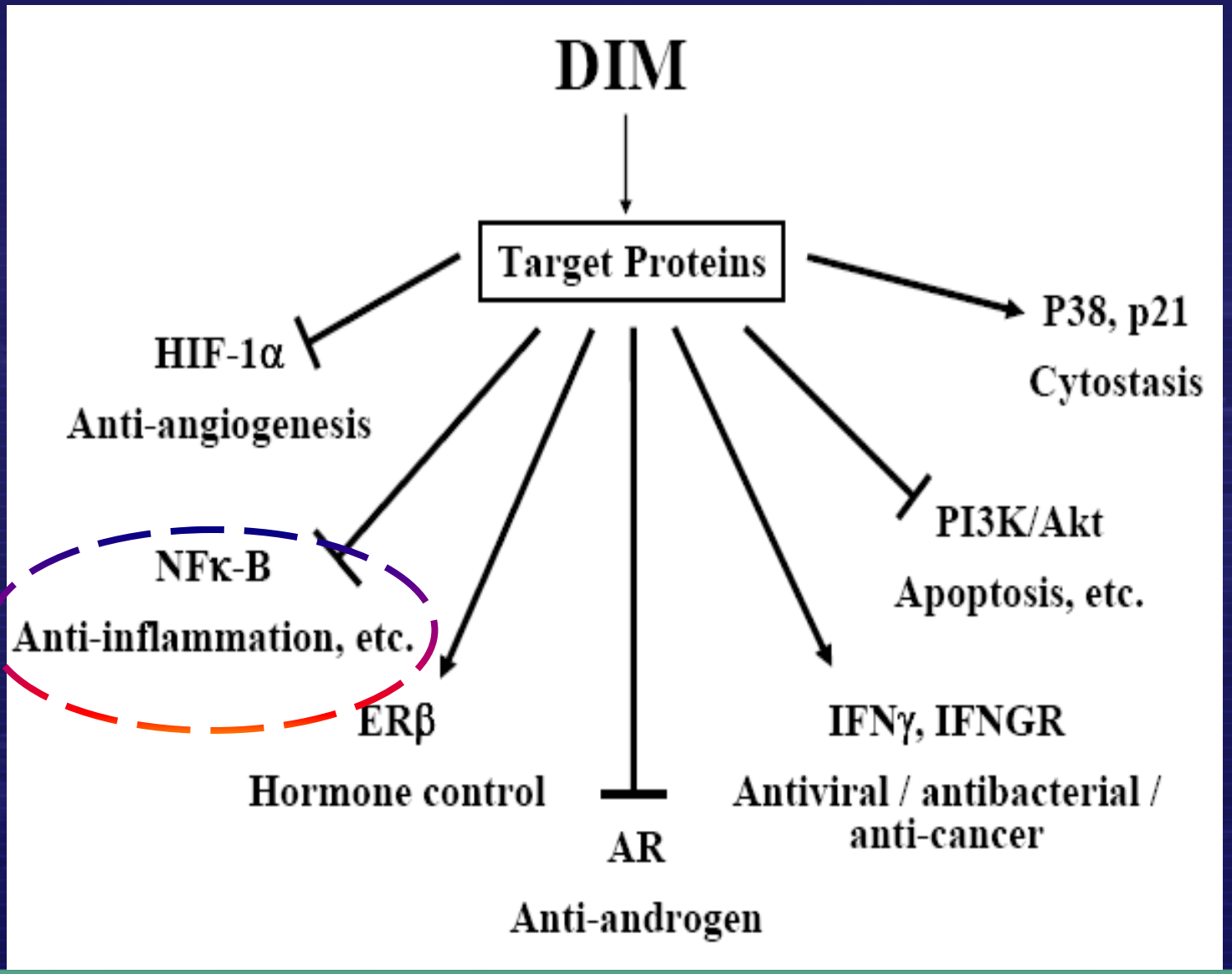
3,3'-Diindolylmethane Inhibits Lipopolysaccharide-Induced Microglial Hyperactivation and Attenuates Brain Inflammation.

Toxicological sciences 137(1), 158–167 2014. Hyo Won Kim, et al. WCU Biomodulation Major, Dept of Agricultural Biotechnology, Seoul National University, Korea; Center for Food and Bioconvergence, Seoul National University, Korea; Advanced Institutes of Convergence Technology, Seoul National University, Suwon Korea; Dept of Biological Sciences, Konkuk University, Seoul, Korea; and Research Institute of Bio Food Industry, Institute of Green Bio Science and Technology, Seoul National University, Pyeongchang ,Korea.

- Science indicates that microglial hyperactivation and neuro-inflammation are implicated in neurodegenerative diseases.
- DIM suppressed LPS-induced brain inflammation through the negative regulation (down-regulation) of the Nf-κappa-B signal pathway in microglia.

Article


DIM
Target Proteins
HIF-1α
Anti-angiogenesis
P38, p21
Cytostasis
NFκ-B
Anti-inflammation, etc.
PI3K/Akt
Apoptosis, etc.
ERβ
Hormone control
AR
Anti-androgen
IFNγ, IFNGR
Antiviral / antibacterial / anti-cancer

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Quercetin

- Quercetin, a natural polyphenolic flavonoid, is present in plants, including onions, apples, and berries.
- Similarity in the structure of quercetin to resveratrol and other flavonoid derivatives that have been shown to increase Mitochondrial Biogenesis (Mt-biogenesis).
- Quercetin increased mRNA expression of PGC-1α and SIRT1, mtDNA, and cytochrome c concentrations.
- An increase in Mt-biogenesis increases production of ATP.
- Quercetin contributes significantly to the protective effects of **neuronal cells from oxidative stress-induced neurotoxicity**.

Quercetin increases brain and muscle Mitochondrial biogenesis and exercise tolerance.

Am J Physiol Regul Integr Comp Physiol 296: R1071–R1077, 2009. J. Mark Davis, E. Angela Murphy, Martin D. Carmichael, and Ben Davis. Div. of Applied Physiology, Dept of Exercise Science and Dept of Com. Science and Disorders, Arnold School of Public Health, U. of South Carolina, Columbia, South Carolina. USA.

- Cerebral metabolism has important consequences on motivation, mood, fatigue, anxiety, depression, and central motor drive from the cortex; **ATP dependent.**
- Within 7 days of introduction of Quercetin, mitochondrial biogenesis with increased oxidative phosphorylation by facilitating transcription, translation, and replication are recorded. (Energy!!)

Protective Effect of Quercetin in Primary Neurons Against Aβ (1-42): Relevance to Alzheimer's Disease

Mubeen Ahmad Ansari, Hafiz Mohammad Abdul, Gururaj Joshi, Wycliffe O. Opii, and D. Allan Butterfield, Dept of Chemistry, Center of Membrane Sciences, Sanders-Brown Center on Aging, University of Kentucky, Lexington, KY 40536, USA

- Quercetin increases glutathione (GSH) levels and antioxidant enzyme function.
- Considerable attention has been focused on increasing the intracellular GSH levels in many diseases, including Alzheimer's disease (AD).
  - Amyloid beta-peptide, elevated in AD brain, is associated with oxidative stress and neurotoxicity.

Coenzyme Q10

- ***CoQ10*** is an essential co-factor of the electron transport chain as well as a potent free radical scavenger in lipid and mitochondrial membranes.
- Supplementation with CoQ10 increased cerebral cortex concentrations which resulted in a significant increase in cerebral cortex mitochondrial concentrations of CoQ10.
- Co-Q10 has been shown to exert neuroprotective effects useful in the treatment of neurodegenerative diseases and the sequelae associated with TBI.

Coenzyme Q10 administration increases brain mitochondrial concentrations and exerts neuroprotective effects. Proc. Natl. Acad. Sci. USA Vol. 95, pp.8892–8897, July 1998 Medical Sciences Coenzyme. Russell T Matthews, L. Yang, S. Browne, M. Baik, F. Beal., Neurochemistry Laboratory, Neurology Service, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114

- Lipid peroxidation leads to a decrease in coenzyme Q10 content and inactivation of respiratory chain enzymes, whereas administration of coenzyme Q10 preserves mitochondrial respiratory function.
- If defects in energy metabolism and oxidative damage play a role in the pathogenesis of neurodegenerative diseases then treatment with coenzyme Q10 could exert beneficial therapeutic effects. (A major component of TBI!)
- This study was able to show that oral administration of CQ10 increases both brain and brain mitochondrial concentrations.

Pyrroloquinoline Quinone (PQQ)

- Pyrroloquinoline Quinone (**PQQ**) is a micronutrient that is found in **soybeans, parsley and green pepper**.
- **PQQ as an antioxidant influences nerves and neurological health, as well as cognitive function.**
- PQQ supports several signal transduction pathways that are important in maintaining mitochondrial homeostasis and oxidative metabolism.
- When CoQ10 and PQQ used together there is a synergistic enhancement of functions for each

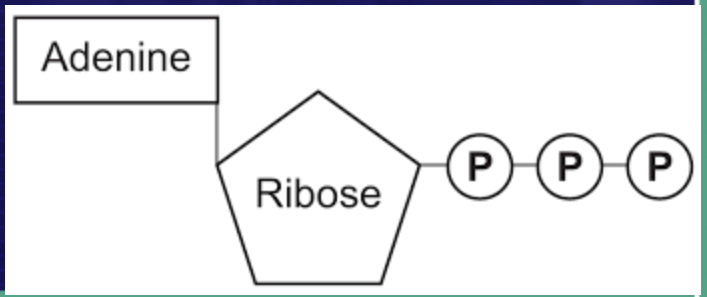
The Neuroprotective effect of Pyrroloquinoline Quinone (PQQ) on Traumatic Brain Injury. Journal of Neurotrauma 29:851–864. March 20, 2012. Lili Zhang, Jie Liu, Chun Cheng, Ying Yuan, Biyun Yu, Aiguo Shen, and Meijuan Yan

- PQQ has been classified as a new B vitamin.
- PQQ is an effective antioxidant in protecting mitochondrial lipid and protein, and it has been shown to protect mitochondrial functions against oxidative damage.
- PQQ has additional beneficial effects; anti-inflammatory, hepatoprotective, cardioprotective, and antioxidative properties.

Ribose

Ribose is a 5 carbon sugar that participates in a number of biological systems:

- Phosphorylated to become ATP, in fact the back bone of all energy molecules. (Energy)
- The core of RNA, mRNA, tRNA and DNA.
- Transport of inorganic phosphate into Oxidative Phosphorylation. (Energy - R-5-P)
- Poly (ADP-ribose) polymerase-1 (PARP-1), the DNA repair enzyme.



The use of D-ribose in Chronic Fatigue Syndrome and Fibromyalgia: a pilot study. Journal of alternative and complementary medicine. Volume 12 number 9. pages 857-862. 2006.

- Approximately 66% of patients experienced significant improvement while on D-ribose, with an average increase in energy on the VAS¥ of 45% and an average improvement in overall well-being of 30% ($p < 0.0001$).

❖ ¥VAS Testing for Pain evaluation in Adults.

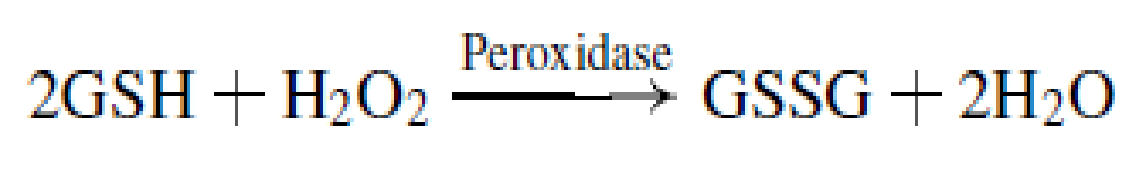
The NO/ONOO- Vicious Cycle Mechanism as the Cause of Chronic Fatigue Syndrome/Myalgic Encephalomyelitis.

Martin L. Pall, Professor Emeritus of Biochemistry and Basic Medical Sciences, Washington State University and Research Director, The Tenth Paradigm Research Group. 2009

- Cases of chronic fatigue syndrome(CFS) are reported to be initiated by nine different short-term stressors, each of which increase levels of nitric oxide(NO) in the body.
- Elevated nitric oxide, acting through its oxidant product, Peroxynitrite, initiates a local biochemical vicious cycle, the NO/ONOO-cycle, which is proposed to be the cause of CFS and related diseases.

Glutathione

- This tripeptide (glu-cys-gly) is the most abundant non-protein thiol found in the brain.
- Glutathione acts as an antioxidant via its capacity to serve as a substrate for the enzyme glutathione peroxidase and seems to mainly be found in **Astrocytes**.



Glutathione Efflux and Cell Death

. Antioxidants & Redox Signaling Volume 17, Number 12, 2012. Rodrigo Franco and John A. Cidlowski. Redox Biology Center and School of Veterinary Medicine and Biomedical Sciences, University of Nebraska-Lincoln, Lincoln, Nebraska. 3Laboratory of Signal Transduction, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, North Carolina.

- Glutathione (GSH) depletion is a central signaling event that regulates the activation of cell death pathways.
- Glutathione (GSH) depletion is a marker of **oxidative stress** and is a key event in the secondary phase of Traumatic Brain Injury.

Synthesis of the Antioxidant Glutathione in Neurons: Supply by Astrocytes of CysGly as Precursor for Neuronal Glutathione. The Journal of Neuroscience, January 15, 1999, *19*(1):562–569. Ralf Dringen, Brigitte Pfeiffer, and Bernd Hamprecht *Physiologisch-chemisches Institut der Universita, Germany*

- Deficiency of Brain Glutathione is associated with several diseases characterized by Neuronal Loss.
- Astrocytes release glutathione into the extracellular space to generate the dipeptide CysGly that is subsequently picked up and used by neurons as precursor for glutathione synthesis.
- Functional impairment of this process is directly associated with Inflammation.

Alpha Lipoic acid

- The lipid-soluble dithiol a-lipoate is absorbed from the diet, crosses the BBB, and is taken up by cells. It possesses a wide spectrum of activities as a metabolic antioxidant, and its reduced form, dihydrolipoate, exits the cells to act as an extracellular antioxidant.
- Alpha-lipoate scavenges a large variety of species including hydroxyl radicals, hypochlorous acid, NO, peroxynitrite, hydrogen peroxide, and singlet oxygen.
- Thus, α-lipoate and its reduced product act as potent antioxidants in both intra- and extracellular environments and shows **neuroprotective properties in traumatic brain injury**.

Antioxidant Therapies for Traumatic Brain Injury. Neurotherapeutics. **2010** January ; 7(1): 51. Edward D. Hall, Radhika A. Vaishnav, and Ayman G. Mustafa Spinal Cord & Brain Injury Research Center, University of Kentucky Medical Center

- α-Lipoic acid has lipid peroxyl radical (LOO•) scavenging effects, but functions indirectly via the regeneration of other endogenous electron-donating antioxidants including; **Vitamin E**, **Glutathione** and **Vitamin C**.

- Brain Cocktail: ALA, Qu, Vit E(a,d,g), NAC.

Pregnenolone

A. The MOTHER of ALL HORMONES
B. Precursor to DHEA/DHEA-s
C. Produced in adrenals **and brain.**
D. Can raise levels of steroids lower down or they can remain the same.
E. Neurosteroid for peak brain function!
    A. Found in highest concentrations in the brain!

Pregnenolone

A. Initiates the memory storage process by stimulating neuronal Adenylate Cyclase (**AC**).*
B. Regulates the timed-sequential flow of calcium ions through the cell membrane.
C. Calcium ion exchange may determine how memory is encoded by neurons.

*(T) → (DHT) → (3AAD) → (AC)

Neurosteroids in the Hippocampus: Neuronal Plasticity and Memory.

Stress 1997 Oct;2(1):65-78 Schumacher M, et al.

- There is accumulating evidence that Pregnenolone sulfate (Preg-s), has a strong influence on learning and memory processes, most likely by regulating neurotransmission in the hippocampus (CA1 cells).

Analysis of Neurosteroid levels in attention deficit hyperactivity disorder.

International Journal of Neuropsychopharmacol 2001 Sep;4(3):259-64. Strous RD et al.

- Inverse correlation between clinical symptoms and levels of DHEA-s and Pregnenolone in young male subjects aged 7-15 years with DSM-IV criteria of ADHD.

Pregnenolone Sulfate and Aging of Cognitive Functions: Behavioral, Neurochemical and Morphological Investigations. Hormones and Behavior 40, 215-217 (2001), Mayo M et al.

- Cerebral Pregnenolone correlated with cognitive performance and is improved with replacement in deficient older adults.
- Pregnenolone increases Acetylcholine in the amygdala, cerebral cortex and hippocampus.

Progesterone(P4) and Allopregnanolone(Allo) reduce inflammatory cytokines after traumatic brain injury. Exp Neurol **2004** Oct;189(2):404-12. He J; et al. Department of Psychology, Emory University, Atlanta, GA 30322, USA

- The excessive release of **IL-1β and TNF-α** is a major cause of cerebral edema, which in turn, can cause permanent neuronal loss and cognitive deficits.
- **Progesterone** attenuates the production of IL-1β and TNF-α, and this may be one mechanism by which P4/Allo reduces cerebral edema and promotes functional recovery from **Traumatic Brain Injury**.

The Neurosteroid Allopregnanolone is reduced in prefrontal cortex in Alzheimer's disease.

Biol Psychiatry. 2006; 60(12):1287-94. Marx CE; Trost WT; Shampine LJ; et. Al. Duke University Medical Center, Department of Psychiatry and Behavioral Sciences, Durham, North Carolina 27705, USA

- Postmortem AD subjects when compared to controls were found to have significantly diminished levels of Pre-frontal cortex (PFC) Allopregnanolone levels.
- Neurosteroids may have utility as candidate biomarkers in AD. (They now are!)

Low Pregnenolone sulphate plasma concentrations in patients with generalized social phobia. Psychology Medicine **2002** Jul;32(5):929-33. Heydari B; Le Melledo JM Department of Psychiatry, University of Alberta Hospital, Edmonton, **Canada**.

- Low Pregnenolone-s is associated with Social Phobia, and has been correlated in male patients who suffer from generalized anxiety disorder (GAD).

- MHC Note: The combination of a low to low-normal Pregnenolone and Prolactin appears to exacerbate all forms of anxiety secondary to an elevation in Dopamine.

Dehydroepiandrosterone

- DHEA is a steroid hormone secreted primarily by the adrenal glands and to a lesser extent by the **brain**, **skin**, **testes**, and **ovaries**.
- DHEA is classified as an adrenal androgen and is rapidly sulfated to DHEA-s, the predominant form found circulating in the plasma. (**Test for DHEA-s**).
- DHEA serves as precursor to androgens and estrogens and is low with anorexia, end-stage kidney disease, Diabetes, AIDS, adrenal insufficiency, auto-immune diseases, and in the critically ill.
- DHEA's secretion decreases from the age of 30 years and is already decreased by 60% at time of menopause

Dehydroepiandrosterone Sulphate: Action and Mechanism in the Brain,

Journal of Neuroendocrinology 24, 215–224. 2011. Y. Dong and P. Zheng. State Key Laboratory of Medical Neurobiology, Shanghai Medical College and Institutes of Brain Science, Fudan University, Shanghai, China.

- DHEA-s is one of the most important Neurosteroids in the brain having a concentration higher than peripheral system.
- DHEA-s has been shown to modulate a variety of synaptic transmission, including cholinergic, GABAergic, dopaminergic, and glutamatergic synaptic transmission.
- DHEA-s has memory enhancing, antidepressant and anxiolytic effects.
- Enhances myelin production from Oligodendrocytes.
- Reduction of Glia production of IL-6.

- MHC Note: The sole use of Testosterone will shut off DHEA production.

DHEA Stimulates Growth of Brain Cells. UCSF Researchers Conclude , Proceedings of the National Academy of Sciences, *Jeffrey Norris April 1998*

- In the April 14, 1998 issue of the Proceedings of the National Academy of Sciences, researchers at the University of California San Francisco report that in the brains of mice -- and very likely in the brains of humans as well:
- "DHEA and DHEA-s, **can stimulate the growth of nerve cell fibers needed to form electrical connections in the brain**. "

Oral DHEA supplementation modulates spontaneous and growth hormone-releasing hormone-induced growth hormone and insulin-like growth factor-1 secretion in early and late postmenopausal women. Fertil Steril 2001 Aug;76(2):241-8., Genazzani AD University of Pisa, Italy. 2001.

- DHEA significantly affects several endocrine parameters in early and late postmenopausal women.
- Our data support the hypothesis that DHEA treatment acts similarly to estrogen-progestin replacement therapy on the GHRH-GH-IGF-1 axis. *
- This suggests that DHEA is more than a simple "diet supplement" or "anti-aging product"; rather it should be considered an effective hormonal replacement treatment.

* High-Normal levels of DHEA-s increase GH release from the AP.

Diffuse Muscoskeletal Pain and Proximal Myopathy; Do Not Forget Hypovitaminosis D. Journal of Clinical Rheumatology • Volume 16, Number 1, January 2010. G.i Fabbriciani, MD, M. Pirro, MD, PhD, C. Leli, MD, A. Cecchetti, MD, L Callarelli, MD, Giuseppe Rinonapoli, MD, PhD Anna Maria Scarponi,MD, and E. Mannarino, MD

- Without VitD, only 10% to 15% of dietary calcium and about 60% of phosphorus is absorbed.
- Lack of VitD decreases muscular strength. Interaction of VitD with muscle VitD receptors (VID-r) exerts a positive influence on muscle mass and strength by promoting protein synthesis (Actin and Myosin).
- In addition VitD stimulates muscle cell uptake of inorganic phosphate, which is important for the production of energy-rich phosphate compounds such as ATP and creatine phosphate, vital for muscle contraction.

Effect of Vitamin D supplementation on testosterone levels in men.

Horm. Metab. Res. **43** (3): 223–5. **2011**. Pilz S, Frisch S, Koertke H, Kuhn J, Dreier J, Obermayer-Pietsch B, Wehr E, Zittermann A

- The male reproductive tract has been identified as a target tissue for Vitamin D, and previous data suggest an association of 25-hydroxyvitamin D [25(OH)D] with testosterone levels in men.
- The hormone vitamin D in levels of 400-1000 IU (10-25 mcg) can raise the testosterone level.

DL-Phenylalanine

- **As** an essential amino acid DLP is a precursor to the synthesis of neurotransmitters such as dopamine, norepinephrine, epinephrine, and serotonin.
- Researchers have concluded that L-phenylalanine can increase mental alertness, help control addictive substance abuse, promote sexual arousal, and release hormones that can curb appetite.
- D-phenylalanine is a non-nutrient amino acid that breaks down opiate-like substances known as enkephalins in the brain. Consequently, D-phenylalanine is able to modulate chronic pain.

dl-Phenylalanine versus imipramine: A double-blind controlled study. European achieves for psychiatry and clinical neuroscience **1979**, Vol. 227, #1, pp 49-58. Helmut Beckmann, Dieter Athen, Margit Olteanu, Reinhild Zimmer.

- In this double-blind, cross-over, wash-out study comparing the efficacy of dl-P against the tricyclic anti-depressant **Imipramine**,
- both patient groups using the Hamilton Depression Scale and the Bf-S self rating questionnaire, documented improvement on both portions of the study but without the side-effects on dl-P that were experienced with Imipramine.

Tocopherols and Tocotrienols

- **Vitamin E** is a collective term for eight naturally occurring compounds, four tocopherols (alpha-, beta-, gamma-, and delta-) and four tocotrienols (alpha-, beta-, gamma-, and delta-).
- Vitamin E is a potent, lipid-soluble, anti-oxidant with neuroprotective benefits.
- Pre-traumatic supplementation with alpha-tocopherol reduces TBI-induced lipid peroxidation, oxidative injury, and impairment in spatial memory.
- Gamma-tocopherol is the main anti-inflammatory component found to be more effective then alpha-tocopherol in scavenging free radicals and nitrogen oxygen species causing inflammation (RNS).

Modulation of inflammation in brain: a matter of fat. Journal of Neurochemistry, 2007, 101, 577–599. Akhlaq A. Farooqui, Lloyd A. Horrocks and Tahira Farooqui. Dept of Molecular and Cellular Biochemistry, and Entomology, The Ohio State University, Columbus, Ohio, USA

- Inhibiting transcription factor NFkB, preventing cytokine secretion, blocking the synthesis of prostaglandins, leukotrienes, and thromboxane, and modulating leukocyte trafficking.
- N-Acetyl Cysteine
- Quercetin
- PQQ
- Vitamin E (alpha, delta and gamma)

Vitamin E Protects Against Oxidative Damage and Learning Disability After Mild Traumatic Brain Injury in Rats.

Neurorehabil Neural Repair. 2010 March ; 24(3): 290–298 . Aiguo Wu, PhD1, Zhe Ying, BS, and Fernando Gomez-Pinilla, PhD, UCLA Brain Injury Research Center, Los Angeles, California, USA.

- Mixed Tocopherols (Vitamin E) can protect the brain against the effects of mild TBI on synaptic plasticity and cognition.
- The elevation of **superoxide dismutase** (SOD) and **Sir2** (silent information regulator 2) play an important role in resistance to oxidative stress and protection of neurosynaptic plasticity.

Eicosanoids (Omega 3 and 6)

- Omega-3 FAs are the major constituents of the cell membrane phospholipids, which suggest that supplementation of PUFAs, could help in reducing the irregular phospholipid metabolism that occurs during neuronal damage, as in TBI.
- There are three important physiological omega-3 FAs available, including alpha linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA).

Therapeutic use of omega-3 fatty acids in severe head trauma. Am J Emerg Med. 2013 January ; 31(1): 273.e5–273.e8. Michael Lewis, MD, et al, Brain Health Education and Research Foundation Arlington, VA.

- Arachidonic Acid, the primary N-6FA in the brain, is metabolized by cyclooxygenase (COX) and lipoxygenase (LOX) enzymes to pro-inflammatory eicosanoids that increase vascular permeability, local blood flow, infiltration of leukocytes, and production of proinflammatory cytokines.
- N-3FA attenuate release of these proinflammatory cytokines, decrease COX activity, inhibit formation of proinflammatory eicosanoids and cytokines, and increases levels of anti-inflammatory DHA.
- DHA (N-3FA), in particular, promotes neuronal survival, neurogenesis, neurite development, neuronal cell migration1, synaptogenesis, and modulation of inflammatory cascade.

Mucuna Pruriens

- Mucuna Pruriens (MP), also known as the Velvet Bean, contains L-DOPA, a precursor to the neurotransmitter *dopamine* in a 30% W/V.
- Dopamine seems to be an important neurotransmitter for pre-frontal functioning which is expressed by the term "*Executive Functions*" that is meant to represent a wide range of cognitive abilities.
- Patients with damage to the prefrontal cortex show impaired judgment, organization, planning and decision-making, as well as behavioral disinhibition and impaired intellectual abilities.

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Neuroprotective effects of the Anti-Parkinson drug Mucuna pruriens.

Phytotherapy Research. 2004 Sep;18(9):706-12. Manyam BV, Dhanasekaran M, Hare TA. Department of Neurology, Health Science Center College of Medicine, Temple, TX 76508, USA

- Mucuna pruriens significantly increased the brain's mitochondrial complex-I activity but did not affect the total monoamine oxidase (MAO) activity.
- Unlike synthetic levodopa treatment, **Mucuna Pruriens** treatment significantly restored the endogenous levodopa, dopamine, norepinephrine and serotonin content in the substantia nigra.
- This additional finding of a neurorestorative benefit by Mucuna pruriens on degenerating dopaminergic neurons in the substantia nigra may be due to increased complex-I activity and the presence of NADH and CoQ-10.

Mucuna pruriens in Parkinson's disease: a double blind clinical and pharmacological study. J Neurol Neurosurg Psychiatry 2004;75:1672–1677.
R Katzenschlager, A Evans, A Manson, P N Patsalos, N Ratnaraj, H Watt, L Timmermann, R Van der Giessen, A J Lees

- The rapid onset of action and longer effectiveness without concomitant increase in dyskinesia on Mucuna (seed powder) suggests that this natural source of L-dopa might possess advantages over conventional L-dopa preparations in the long term management of Parkinson's disease.

You Think?

Amantadine

- Amantadine affects the synthesis, accumulation, release, and reuptake of catecholamines in the central nervous system with a peak plasma level within 1-4 hours.
- Amantadine is rapidly absorbed, but it is not metabolized so that 90% is excreted unchanged in the urine.
- Amantadine causes release of dopamine from central neurons, facilitates dopamine release by nerve impulses, and delays the re-uptake of dopamine by neural cells.
- **Amantadine has profound NMDA receptor antagonistic effects, which may contribute to its neuroprotective effects early after injury.

Effects of dopaminergic combination therapy for frontal lobe dysfunction in traumatic brain injury rehabilitation

. BRAIN INJURY, 1999, VOL. 13, NO. 1, 63-68. David T. Burke, MD, et al. Department of Physical Medicine and Rehabilitation, Spaulding Rehabilitation Hospital, 125 Nashua Street, Boston, MA 02114, USA

- This trial demonstrated Amantadine's ability to positively affect frontal lobe dysfunction.
- Patients experienced decreases in agitation, fatigue, distractibility, rigidity and bradykinesia, and increases in general arousal, orientation, initiation, purposeful movement, attention & concentration, sequencing skills and processing/ response time.

Placebo-Controlled Trial of Amantadine for Severe Traumatic Brain Injury. N Engl J Med 2012;366:819-26. Joseph T. Giacino, Ph.D., John Whyte, M.D et al.

- Amantadine promotes dopaminergic activity by facilitating presynaptic release and blocking reuptake postsynaptically.
- Amantadine has the potential to restore an interrupted balance between glutamatergic and dopaminergic neurotransmitter systems, via its role as an NMDA antagonist-receptor inhibitor.

Statins: The bad and the very bad

- Statins are a class of drugs used to lower cholesterol levels by inhibiting the enzyme HMG-CoA reductase, which plays a central role in the production of cholesterol in the liver, which produces about 70 percent of total cholesterol in the body.
- Coenzyme Q10 (ubiquinone) levels are decreased in statin use. (WP)
- Statins may increase the risk of diabetes by 9%.
- Statins reduce the production of Cholesterol in the brain lowering Neurosteroid production.
- Peripheral production of all hormones from cholesterol; testosterones, progesterone, estrogens, vitamin D, cortisol, DHEA, and DHEA-s are all affected by statins.

Statins in Traumatic Brain Injury

. The Journal of the American Society for Experimental NeuroTherapeutics. Vol. 7, 62–73, Jan 2010. Elissa F. Wible* and Daniel T. Laskowitz. Depts of Medicine (Neurology), Anesthesiology, and Neurobiology, Duke University School of Medicine, Durham, North Carolina 27710

- The therapeutic effects of statins in brain injury may be divided according to mechanism from most acute to more chronic: acute lesional effects, anti-inflammatory and anti-excitotoxic effects, vascular and endothelial effects, anti-apoptotic effects, and effects on neurogenesis and angiogenesis.

Table 8.1: Statins Effects in Traumatic Brain Injury

ACUTE		SUBACUTE	CHRONIC
Cerebral Blood Flow	Neuroinflammation	Apoptosis	Angiogenesis
▼Microthrombosis	▼Inflammatory cytokines	▼Bax/Bcl-2	▲VEGF, VEGFR2
▼Platelet Activity	▲BBB Integrity	▼Caspase 3	▲Akt/eNOS
▼Von Willebrand Factor	▼Cerebral Edema	Neuronal Injury	Neurogenesis & Plasticity
▲Endothelial Integrity	▼Microglial Activation	▼Excitotoxicity Death	▲BDNF, VEGF
▲eNOS	▼Oxidative Stress		▲PI3K/Akt

Summary Statement

- Everyone has a degree of active cerebral inflammation due to free radicals.
- Selecting an initial starting point with supplements at the same time as starting a neurosteroid and neuroactive steroid replenishment protocol will enhance the process of recovery.
- As long as the Non-Permissive Environment exists there is little hope for improvement.