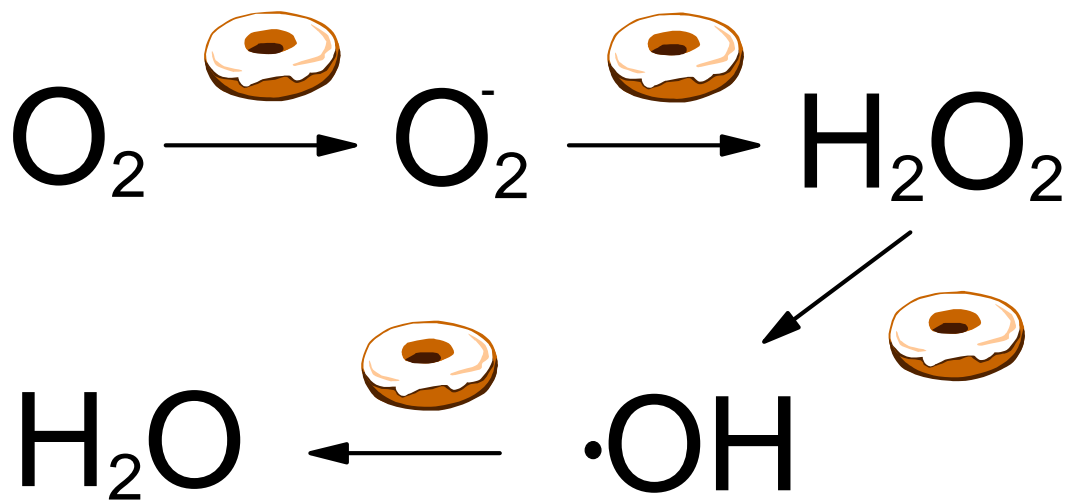


The Sunrise School: Protein Oxidation as Marker



NOAA

Feeding Donuts to a Bear



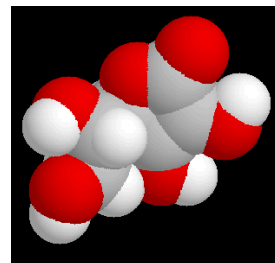
You Suspect a Bear is Around



What Now ????

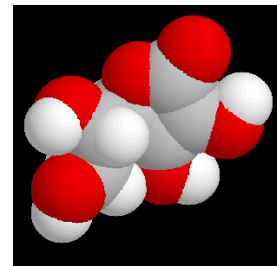
How to Assess Oxidative Stress or Damage ?

- Increased antioxidant proteins
 - ▶ Gene chip *or* Northern *or* Western *or* Activity
- Changes in low MW antioxidants
 - ▶ vitamins C *or* E *or* Glutathione
- Macromolecular damage
 - ▶ DNA *or* Lipid *or* Protein

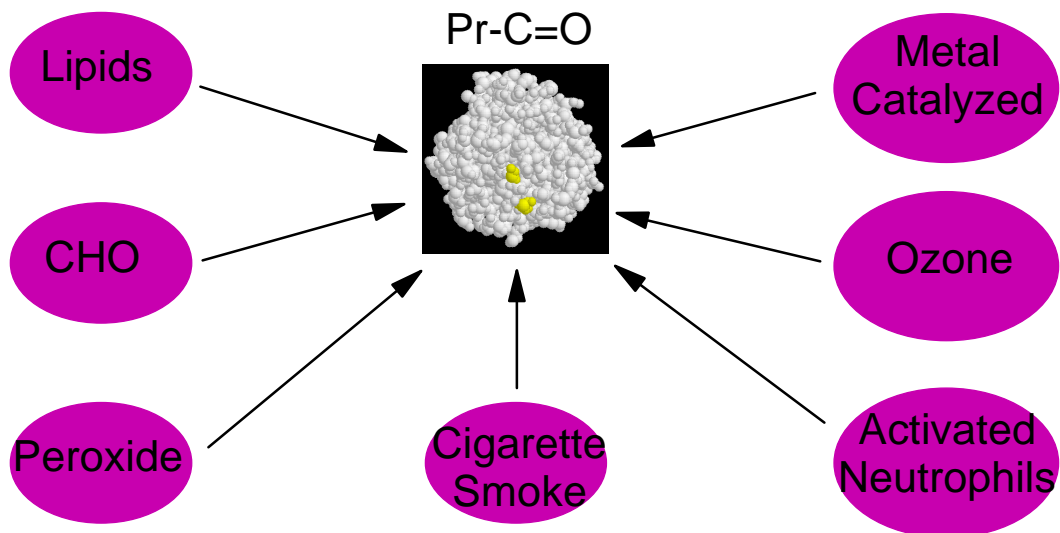


Which protein oxidation assay is best for your needs ?

- Direct versus Indirect
- Global versus Specific



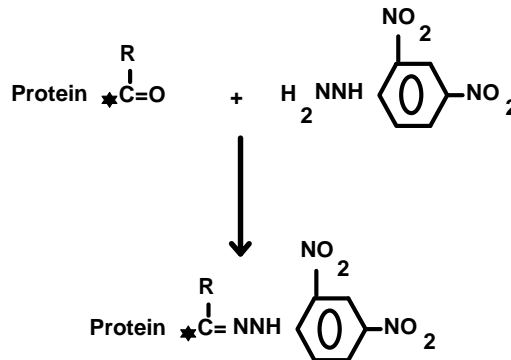
Protein Carbonyl is a Generalized Marker of Oxidation Reactions



Assessing Oxidative Stress: Protein Carbonyl

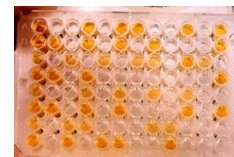
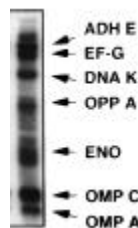
- A Generalized Marker for Oxidative Changes

- Carbonyl by 2,4 DNPH assay

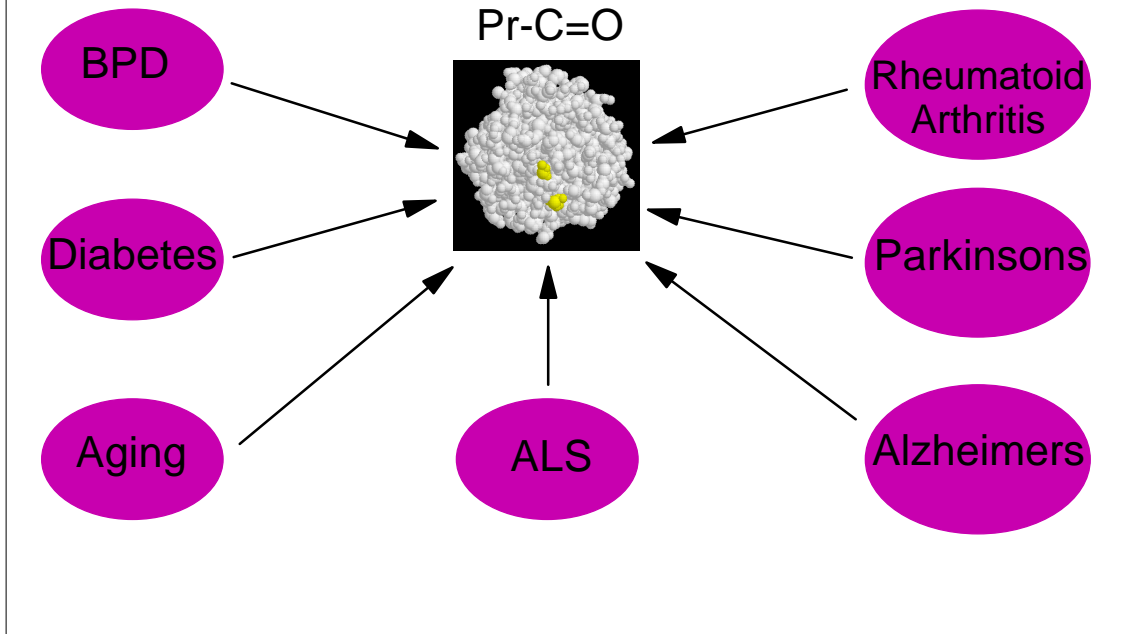


Protein Carbonyl: Choose Your Assay Style

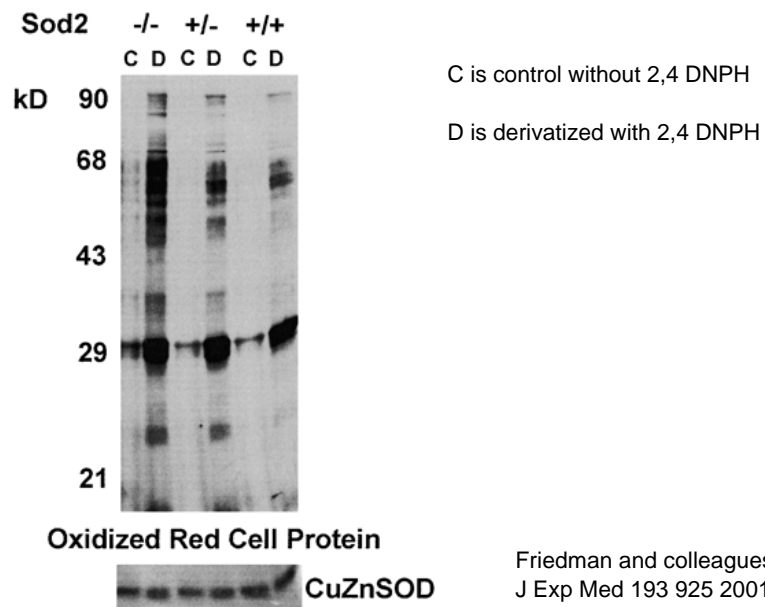
- Test Tube / Spectrophotometric
- Quantitative
- Batch - especially with 96-well
- HPLC
- Quantitative; smaller sample; can automate
- Serial
- Western (1D or 2D)
- Sensitive
- Commercial kit
- ELISA
- Convenient for batching
- Commercial kit



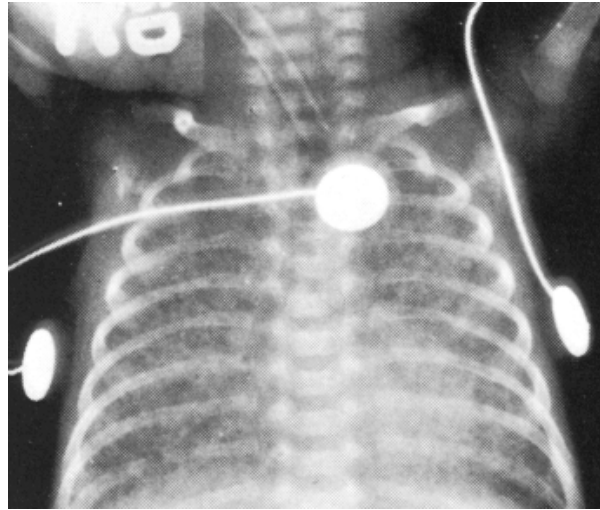
Protein Carbonyl is Elevated in Many Diseases



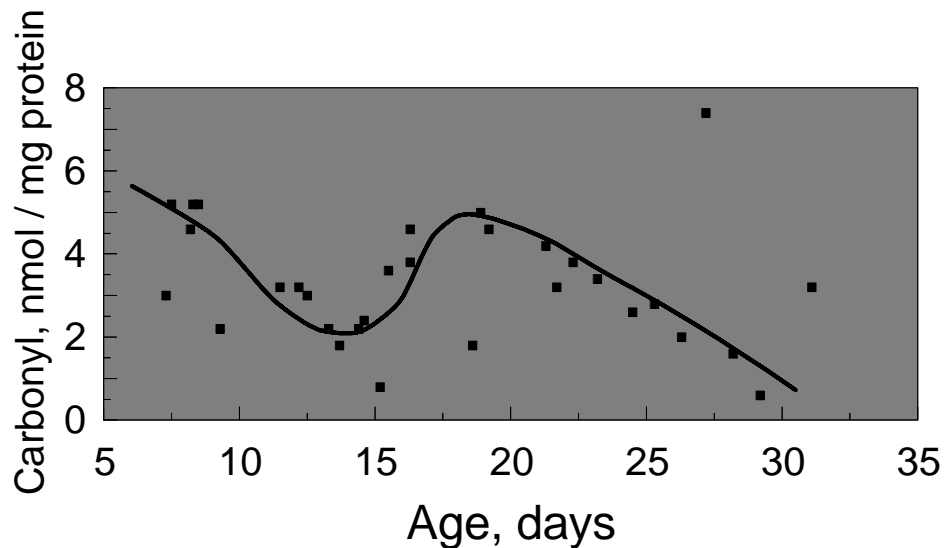
Red Cell Protein Carbonyl Increases in MnSOD Knockouts



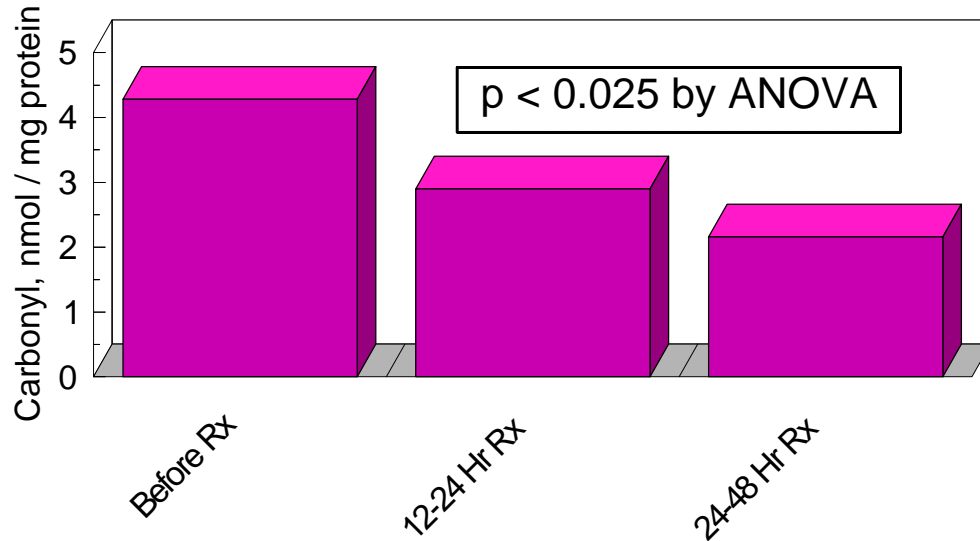
Monitor Oxygen Toxicity Clinically: Neonatal



BAL Carbonyl in a Premature 730 g, 24 week gestation

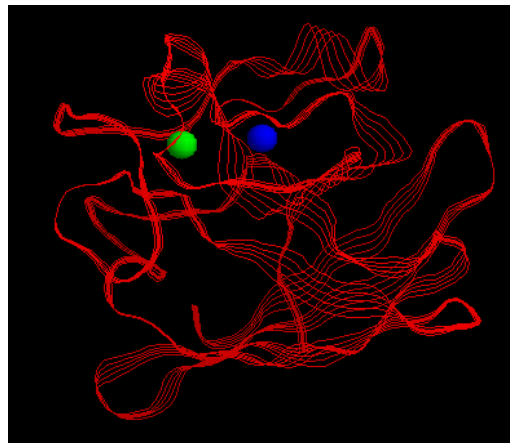


Monitor Therapy: Carbonyl Drops with Steroid Rx



Assessing Role in Disease: The ALS Example

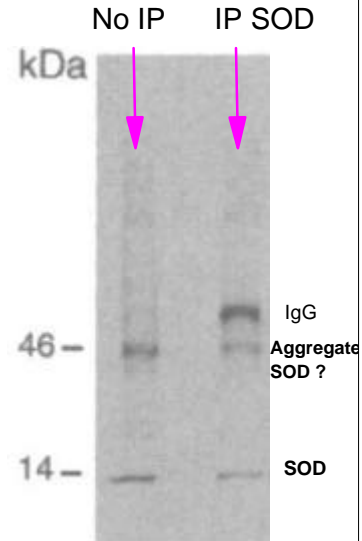
- Lou Gehrig disease (ALS) - sporadic or inherited
- Mutations in Cu-Zn SOD cause toxic gain of function
- Expressing mutant SOD in mice causes disease



Assessing Role in Disease: The ALS Example

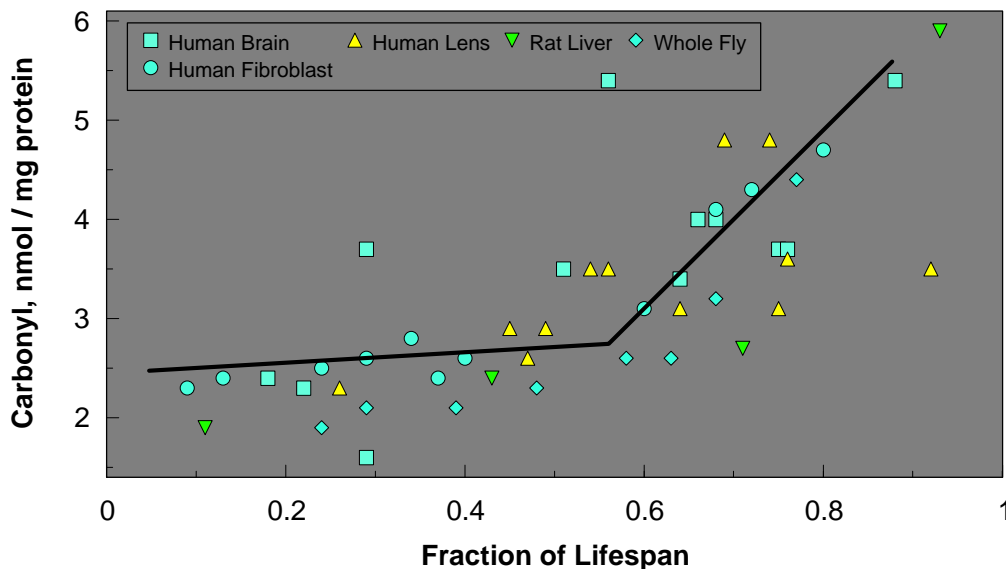
- Intracellular aggregates of SOD occur in ALS
- Protein carbonyl increased in ALS mice
- Carbonyl increase precedes clinical disease

Carbonyl Blot



G93A spinal cord: SOD IP, Carbonyl Westerr
Andrus et al J Neurochem 71:2041 1998

Oxidative Damage during Aging: Burden Increases in Last 1/3



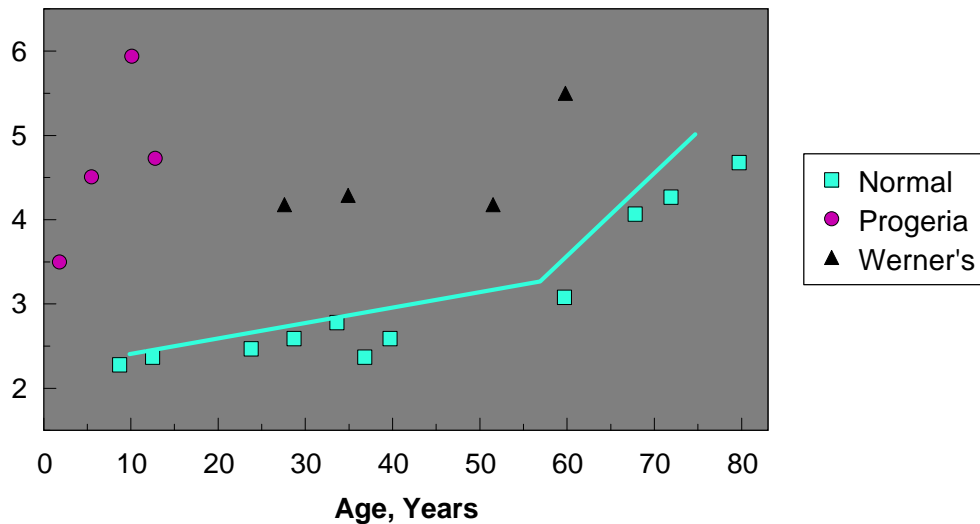
Premature Aging Syndromes: Hutchinson-Guilford; Werner's



Daniel P.F. Adoue
Centre de Medecine Geriatrique
Toulouse, France

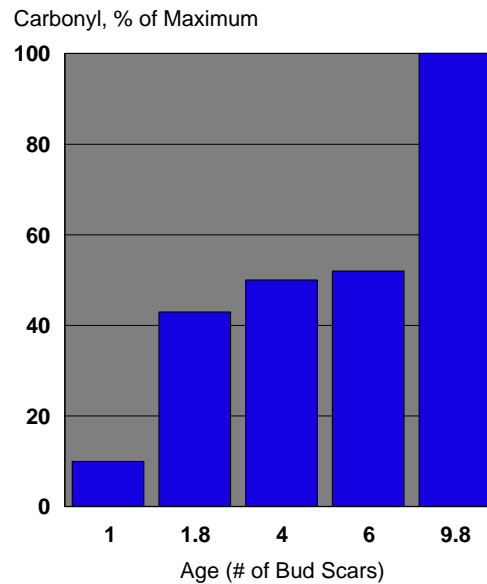
Protein Carbonyl Is Increased in Disorders of Aging

C=O, nmol / mg protein



Yeast "Age" Too

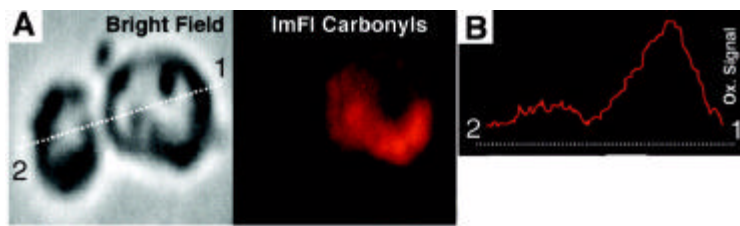
- "Mother" yeast bud off smaller "daughter" cells
- Mothers stop reproducing after ~30 deliveries -- in 5 days!
- Carbonyl increases with age



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Like All Mothers, Yeast Sacrifice for their Daughters

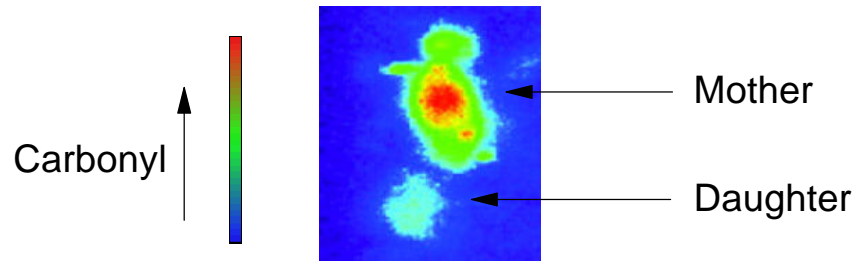
- Mothers retain oxidized proteins which accumulate during aging



Aguilaniu et al Science 299 1751 2003

Like All Mothers, Yeast Sacrifice for their Daughters

- Proteins oxidatively damaged by paraquat are also retained by Mother



Aguilaniu et al Science 299 1751 2003

Oxidized Protein: Correlation or Causation ?

- Close to a thousand papers reporting protein carbonyl levels
- Protein carbonyl is a validated marker for damaged cells
- Might oxidized protein be the **cause** of cellular and organ dysfunction ?
- Feedforward amplification is possible (vicious cycle)

