Adaptations of “Free Radical Biology” to Acute and Chronic Exercise

Outline
1. Mitochondrial production of oxidants during acute exercise
2. Production of oxidants by xanthine oxidase during exhaustive exercise
3. Exercise, Muscle Injury (Delayed Onset Soreness) Inflammation and Oxidative stress. Inflammation and production of oxidants by oxidases
4. Exercise Training and Longevity in Animals and Humans
5. Adaptations and Mechanisms with Exercise Training; Potential benefits as countermeasures to aging
6. Exercise and Physical Activity; Effects on Longevity in Humans
7. Reactive Oxygen Species and effects on muscle contractility (brief)
8. Exercise as a model to study cell signaling (brief)
Generation of Radicals: Main Source

- **Mitochondrial respiratory chain**
  ('Electron Transport Chain')
  
e.g. superoxide radical; $\text{H}_2\text{O}_2$, hydroxyl radical;

**More Oxygen More Radicals??**

**“Radical” Reactions**

- $\text{O}_2 + e^{-} \rightleftharpoons \text{O}_2^-$
- $\text{O}_2 + 2e^- + 2\text{H}^+ \rightarrow \text{H}_2\text{O}_2$
- $\text{O}_2^- + \text{O}_2^- + 2\text{H}^+ \rightarrow \text{SOD} \rightarrow \text{H}_2\text{O}_2 + \text{O}_2$
- $\text{H}_2\text{O}_2 + 2\text{GSH} \rightarrow \text{GPX} \rightarrow \text{H}_2\text{O} + \text{GSSG} + \text{ROH}$
- $2\text{H}_2\text{O}_2 \rightarrow \text{Catalase} \rightarrow 2\text{H}_2\text{O} + \text{O}_2$
- $\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{HO}^- + \text{HO}^- + \text{Fe}^{3+}$
- $\text{O}_2^- + \text{NO}^- \rightarrow \text{ONOO}$
- $\text{O}_2^- + \text{M}^{n+} \rightarrow \text{O}_2 + \text{M}^{(n-1)+}$

Background:

- What are mechanisms to reduce radical production during acute exercise?
- Comparing data from isolated mitochondria with the hypothetical *in vivo* ("physiological exercise") situation.
- Keep in mind!!!
  - Oxygen tension
  - State 3 vs. State 4
  - Tissue specificity!!! i.e. Heart vs. Muscle

Quantification of radical generation via:

1. **Substrate Type**
   ('fuel')

2. **“Stress” on mitochondria**
   (rest vs active state)

**Generation of Radicals**

- **Mitochondrial Stress:**
  I. Resting (substrate only) $\Rightarrow$ **STATE 4**
  II. Active (substrate + ADP) $\Rightarrow$ **STATE 3**
RESULTS: STATE 4 (resting)

- COMPLEX I +/or III
  - contain O₂ radical generator(s)
- Pyr/Mal as substrate produce $\uparrow \text{H}_2\text{O}_2$ than Succinate

INHIBITOR EFFECT

RESULTS: STATE 3 (active)

- COMPLEX I produces $O_2$ radicals
  * appears to be ONLY COMPLEX containing $O_2$ radical generator for this state*

NB. Mitochondria operate in ‘flux’ of states between 3 and 4; rarely at either end of the range for a prolonged period.

*SUMMARY: RADICAL GENERATION*

- COMPLEX I - always involved (regardless of substrate + state)
- COMPLEX II - partial involvement
- COMPLEX III - involved in STATE 4

*Data from heart mito; differences exist between tissue type.
Conclusions

- O$_2$ radicals mainly generated at COMPLEX I in STATE 4 + 3
- ↑ radical production NOT necessarily proportional to O$_2$ consumption
- ↑ radical formation with exercise and age inconclusive
  (Comparative Studies ⇒ O$_2$ radical generation ↓ in long lived vs short lived animals)

Background

- Studies with isolated mitochondria show that during normal respiration there is production of partially reduced oxygen species in the electron transport chain
- It is estimated that the release of reactive oxygen species accounts for about 1-5% of the oxygen consumed during respiration

Hypothesis

- An increase in exercise-induced mitochondrial oxidative metabolism could result in an increase in oxidative stress
- Oxidative stress could therefore increase mitochondrial protein oxidation

Oxidative Stress in the Mitochondria

- Oxidized Proteins
- Oxidized Amino Acids

Plasma
- Oxidized Amino Acids

Kidneys
- filtration / reabsorption

Urine
- Oxidized Amino Acids
- Thiol Metabolites

Proposed formation and removal of oxidized amino acids in the mitochondria after acute exercise
Animals and Exercise Protocol

- **Animals**
  - Male Wistar Rats
  - Fasted Overnight

- **Exercise**
  1 bout of 30 min. weight of 1.5% of body weight used
  3 bouts of 30 min (STRESS FULL and EXHAUSTIVE EXERCISE)

**Design**

1) Controls (Placed in Metabolic Cages for Urine Collection)

2) Acute Exercise (Immediately Sacrificed)

3) Acute Exercise (Placed in Metabolic Cages for Urine collection)

Conclusions

- Exercise is a physiological relevant oxidative stress
- This study provides the first direct evidence of hydroxyl radical formation in the mitochondria of exercising animals
- Oxidized amino acids may be recognized by proteolytic enzymes degraded, released, and excreted into the urine
- Markers for oxidative stress in urine may be useful for non-invasive assessment of several disease states

Free-radical production in exercise

- At rest, about 2% of the Oxygen consumed by mitochondria is not converted into water but forms ROS.
- Thus it was assumed that during exercise there would be an increase in mitochondrial ROS production.
- This is not the case. During exercise, ROS formation by the mitochondria is negligible. **Mitochondria are very efficient in reducing the radical leak in State 3 (active state + ADP)**
- Possible explanation?
  - Alternate source of ROS production outside the mitochondria.
  - Xanthine Oxidase.
    - Past research has shown that Xanthine Oxidase levels correlate well with ROS levels and cellular damage.

Production of oxidants during exhaustive exercise; Role of xanthine oxidase

- Sastre and Vina; Free Radicals in Exhaustive Physical Exercise: Mechanism of Production, and Protection by Antioxidants

Radical production in exercise: Past research

![Figure 1. Linear relationship between oxidized (GSSG)-to-reduced glutathione (GSH) and lactate-to-pyruvate ratios in blood from humans subjected to physical exercise. Human blood samples were collected at rest and then 45 min after exercise (n = 10). (Reproduced with permission from reference (25).)
The role of Xanthine Oxidase in the production of ROS in exhaustive exercise: Protection by allopurinol

Mechanism of free-radical production in exercise

Protection of ROS generation by allopurinol

- Allopurinol inhibits xanthine oxidase, a likely source of ROS production during exhaustive exercise.

- The substrates for xanthine oxidase:
  - Xanthine
  - Hypoxanthine

- Hypoxanthine derives from the degradation of ATP via AMP.

- Therefore, the substrates needed for xanthine oxidase are available only after exhaustive exercise.

Radical production in exercise

- Exhaustive exercise increases blood xanthine oxidase levels, which leads to ROS production.

- Exhaustive exercise is also associated with an increase in glutathione oxidation (Quintanilha et al.).
  - There is a linear increase between exercise intensity (blood lactate, CK levels) and the oxidation of glutathione (Sastre et al.).
  - Glutathione oxidation (GSSG) is a ‘marker’ for oxidative damage.

- Inhibiting xanthine oxidase activity with allopurinol reduces glutathione oxidation, and therefore oxidative damage.

- Does Xanthine Oxidase play a role in the oxidation of glutathione?

- When Xanthine oxidase is inhibited by allopurinol, what happens to oxidized glutathione (GSSH) levels?
Exercise, Muscle Injury (Delayed Onset Soreness) Inflammation and Oxidative stress


**Initial mechanical injury**

1. **Muscle damage**
2. **Inflammatory response**
3. Oxygen free radicals
4. Phagocyte infiltration
5. Lysosomal proteases

Human Oxidative Stress- Inflammatory Model

- Human model to rapidly and safely test the efficacy of a variety of anti-inflammatory and antioxidant compounds (pharmaceutical or non-pharmaceutical).
- Used the eccentric portion of a bicep curl to elicit muscle damage
- Subjects performed 3 sets of ten repetitions with 80% of their eccentric maximum
- Untrained Male Subjects

Clinical Symptoms

- Severe pain, decrease in range of motion of the injured arm, and edema characterize this type of exercise-induced injury for several days post-injury.

Blood Parameters

- The increases in LDH and CK are comparable with plasma levels of patients who have suffered from a heart infarct.

**Antioxidant intervention and muscle damage**

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### Interleukin-6 and Myeloperoxidase

![Graph showing Interleukin-6 and Myeloperoxidase levels over time](image)

### Superoxide Dismutase

\[ 2O_2^* + 2H^+ \rightarrow \text{SOD} \rightarrow H_2O_2 + O_2 \]

![Graph showing Superoxide Dismutase activity over time](image)

### Oxidative Stress

![Graph showing Oxidative Stress levels over time](image)

### Publications

**Exercise and Inflammation**


**Exercise and Longevity in Rodents and Humans**

Can we retard aging and increase lifespan?
**Common ways to exercise rodents**

- Swimming
- Treadmill running
- Voluntary wheel running

**Forced Treadmill Running**

- Can control duration, intensity, and frequency of running
- ↑ in oxidative capacity of skeletal and cardiac muscle
- BUT…can elicit adaptations indicative of chronic stress (Moraska et al., 2000)
  - adrenal gland hypertrophy and thymic involution
  - ↓ serum CBG levels
  - ↓ lymphocyte proliferation and antigen-specific IgM production

**Voluntary Wheel Running**

- Increases mean lifespan (Holloszy et al., 1993, 1997, & 1998)
- Prevents stress-induced behavioral depression & immunosuppression (Moraska et al., 2001)
- ↓ body weights and enhanced survival (Narath et al., 2001)

**Survival Data (Narath et al., 2001)**

**Variations in running activity**

Antioxidants have no effect on longevity
- Exercise increase mean life-span


- Voluntary wheel running has been shown to increase mean lifespan, but unlike caloric restriction, does not increase maximal lifespan.
- Food restriction combined with voluntary wheel running on survival when compared to sedentary pair-weight caloric restricted controls.

Group A Runners
Group B Sedentary
Group C Runners + R
Group D Restricted

Findings regarding Longevity

- Voluntary wheel running individuals lived slightly but significantly longer than sedentary ad lib controls and sedentary pair-fed controls (mean lifespan 1012, 924, 926 days respectively), but not compared to caloric restricted pair-weight sedentary controls (mean lifespan 1113 days).
- Although, voluntary running improved survival it did not cause an extension in lifespan, unlike CR, and did not lead to a reduction in observed malignancies, unlike CR.

Conclusion

- The survival curves of the two groups were virtually identical, with no evidence of an increased early mortality in the exercise group (see Holloszy and Schechtman, 1991).
- The main finding of this study was that while exercise did not interfere with the extension of maximal lifespan due to caloric restriction, there was no additive or synergistic beneficial effect of voluntary exercise in tandem with caloric restriction on maximal lifespan in these rats.

- The results of this study provide no support for the concept that increased energy expenditure decreases longevity.

Can we exercise too much? What will this do to your maximum life span potential?

Lessons from flies

- Liang-Jun Yan and Rajinder S. Sohal
  Prevention of Flight Activity Prolongs the Life Span of the Housefly,
  Musca Domestica, and Attenuates the Age-Associated Oxidative Damage to Specific Mitochondrial Proteins

Other Interesting Facts about Drosophila Melanogaster or fruit Flies

- Crawlers vs. flyers
- Promiscuous Males vs. Females (males with multiple females)
- Promiscuous Females vs. Males???? – off course not tested, but could give interesting results!! Theses or Dissertation?

Free radical Biology; Adaptations to Exercise and Training


What happens to mitochondrial efficiency and deletions as well as oxidant and antioxidant balance?
Key Points

- Oxygen consumption is the same in Trained and Untrained animals
- \( \text{H}_2\text{O}_2 \) production was lower in State 3 and State 4 (Succinate as substrate). Per Mitochondria!!!
- Overall production \( \text{H}_2\text{O}_2 \) production was not different in State 3 or State 4 (Succinate)
- Overall production \( \text{H}_2\text{O}_2 \) production was slightly increased in State 3 or State 4 (Pyr-Mal)
- In this study no changes in antioxidant defenses, however this is swim exercise
Long-term wheel running

- ↓ lipid peroxidation & ↑ CAT activity in heart (Kim et al., 1996a)
- ↑ CAT, GPX, GSH & mito. membrane fluidity in liver (Kim et al., 1996b)
- ↓ urinary o,o'-dityrosine & ↑ skeletal muscle GPX & MnSOD (Leeuwenburgh et al., 1999)

Aging and exercise training in skeletal muscle: responses of glutathione and antioxidant enzyme systems.


Adaptations of glutathione antioxidant system to endurance training are tissue and muscle fiber specific.


GPX and SOD Adaptations following treadmill exercise training

- Treadmill training increases GPX and SOD activity in the Deep Vastus Lateralis, but not in the soleus muscle

Exercise as a Countermeasure to Aging; Mechanisms

- Delays onset of morbidity & mortality
- How???
  - ↓ oxidant production (Venditti et al., 1999)
  - ↑ antioxidant enzyme activity (Leeuwenburgh et al., 1994 & 1997; Powers et al., 1993 & 1994; Venditti et al., 1996)
  - ↓ oxidative damage, ↑ DNA repair & 20S proteasome activity (Radak et al., 1999, 2000, & 2002)

Evidence of training effects on the antioxidant network in skeletal muscle

- Glutathione peroxidase activity ↑ (Ji, 2002)
- SOD protein and activity ↑ (Suzuki et al. 2000, Ji, 2002)
- Catalase → (Powers & Sen, 2000)
- Muscle glutathione (GSH) content ↑ (Powers & Sen, 2000)
Long-term wheel running

- Gene expression (heart) - 137 genes sig. affected by age (Bronikowski et al., 1999)
- Most were assoc. with inflammatory and stress response
- 32 mos. of wheel running attenuated age-related changes in 70 of the 137 genes

Potential benefits from life long exercise under investigation

- Increase in mitochondrion function
- Decrease oxidant production, specifically a decrease in free radical leak in Complex I and or Complex III
- Decrease mitochondrial damage
- Decrease in mitochondrial deletions
- Removal of protein aggregates by proteosome activities
- Removal of damaged mitochondria by lysosomal autophagy (-imperfect autophagy)

Exercise & Longevity

Humans; The Human Story

Disease prevention

Historical Perspective

- Occupational = Leisure-time physical activity
- 1940’s: Morris et al.
  - Bus drivers vs. conductors
  - Postal service workers vs. civil servants
- Initial findings
  - ↑ physical activity yields ↓ CHD

This is occlusive coronary atherosclerosis. The coronary at the left is narrowed by 60 to 70%. The coronary at the right is even worse with evidence of previous thrombosis with organization of the thrombus and recanalization such that there are three small lumens remaining.
Historical details through 1978

- Physically inactive had
  - 50% higher incidence of CHD
  - 50% higher all-cause mortality
- Physical activity ‘protected’ independently of other risk factors:
  - Smoking, ↑ BP, ↑ BMI, Parental history of CHD, ↓ stature, hypercholesterolemia

Epidemiological studies

- College Alumni Health Study
- Harvard Alumni Health Study
- San Francisco Longshoremen
- Is exercise important to live longer?
- And How Much Exercise?

Caloric Expenditure

- I. Caloric Expenditure
  - 1-2 kcal/min (light work)
  - < 5 kcal/min (housework, driving, etc)
  - > 10 kcal/min (hard running)
  - ACSM Advise to expend:
    - 150-300 (kcal) per exercise session
    - 800-900 (kcal) per week

Harvard Alumni Health Study

- Harvard Alumni Health Study (Paffenbarger ’86 ’93 ’95)
  - Mortality lowest (↓ 54%) for those (men) expending 3000-3500 kcal/wk in physical activity
  - Initial ↓ mortality risk (↓ 22%) at >500 kcal/wk (n=17,000)
  - At >2000 kcal/wk (300 kcal/day):
    - 16% decline in mortality
    - Compare to avoiding: Smoking: 23% decline

- >2000 vs <500 kcal/wk physical activity increases mean lifespan about 2 yrs (3%)
‘Fins’ in the 7 Countries Study (’87)

- Heavy physical activity (> 5km/d walking, farming profession etc.) improved mortality by 35% during 1st 10 years of study only.
- “premature mortality but not max life span can be improved by physical activity” (n=600)

Smoking negated physical activity benefit

Getting fit seems to be the key.. To reducing your risk

- previous physical activity does not reduce relative mortality risk (Harvard alumni)
- Contrasted by Aerobics center (’95):
  1st vs 2nd Exam relative risk
  Unfit* fit ↓ 45%
  Fit unfit ↓ 48%
  Fit fit ↓ 67%

*unfit = lowest 20% for TET (very unfit)
- Each 1 minute added to GXT = ↓ 10%

Reactive Oxygen Species and Potential effects on Muscle contractility (Brief)

ROS and contractile force in unfatigued skeletal muscle

Antioxidants and contractile force in unfatigued muscle

Antioxidants and contractile force in muscle fatigue
Cellular redox state and contractile force

A: Basal conditions  
B: + ROS (small amounts)  
C: + ROS (large amounts)  
D: + antioxidants


Cell Signaling and Exercise Training

- Endogenous antioxidants  
- Alimentary antioxidants  
- Antioxidant enzymes  
  - Glutathione and thioredoxin system  
  - Superoxide dismutase (SOD), catalase  
- Antioxidant stress proteins  
  - Heat shock proteins: HSP25, HSP27, HSP70  
  - Heme oxygenase-1 (HO-1)

Exercise and HO-1 expression in rat muscle

60 min exhaustive treadmill running


Exercise and HO-1 expression in human skeletal muscle

One-legged knee extensor exercise (60-90 min)  
4 h cycling exercise (60% VO2max)


Exercise and HSP27 expression in human skeletal muscle

Repeated eccentric contractions M. biceps brachii


Exercise and phosphorylation of MAPKs in rat muscle

Antioxidants and exercise-induced MAPKerk1/2 phosphorylation

Exercise and binding activity of NF-κB and AP-1 in rat skeletal muscle

Exemplary signaling pathways sensitive to ROS

Conclusions

Summary

Reactive oxygen species (ROS) - good or bad guys?

Long-lived species "CR" diet and life-long exercise habits will remain powerful interventions to reach maximum lifespan potential

Humans (~77M ~80-F years) Maximum ~122y