Attenuating Postprandial Oxidative Stress in Pre-Diabetics: Potential Influence of Exercise and Acetyl L-Carnitine Arginate Dihydrochloride (ArginoCarn™: US patent: 6,703,042)

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Memphis, TN
Update on GlycoCarn™ Studies


Outline

- Oxidative Stress Defined
  - Importance in biological systems
  - Common targets of RONS
  - Association with health and disease
    - Metabolic Syndrome

- Postprandial Oxidative Stress
  - Overview
  - Methods to attenuate
    - Physical exercise
    - Nutritional supplements

- Acetyl L-Carnitine Arginate Dihydrochloride
  - Study design and methods

- Questions
Oxidative Stress Defined

- **Oxidative Stress**
  - Condition in which the quantity of reactive oxygen and nitrogen species (RONS) exceeds the physiologic capacity of the system to render these RONS inactive

- **Reactive Oxygen and Nitrogen Species (RONS)**
  - Products of normal cellular metabolism
  - Increased with acute physical, psychological, and environmental stress

- **RONS countered by protective mechanisms**
  - Endogenous antioxidant defenses
  - Exogenous (dietary) antioxidants
# Specific RONS

<table>
<thead>
<tr>
<th>Reactive Oxygen Species</th>
<th>ROS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superoxide ion</td>
<td>O$_2^-$</td>
</tr>
<tr>
<td>Ozone</td>
<td>O$_3$</td>
</tr>
<tr>
<td>Singlet oxygen</td>
<td>$^1$O$_2$</td>
</tr>
<tr>
<td>Hydroxyl radical</td>
<td>OH$^-$</td>
</tr>
<tr>
<td>Hydrogen peroxide</td>
<td>H$_2$O$_2$</td>
</tr>
<tr>
<td>Hypochlorous acid</td>
<td>HOCL</td>
</tr>
<tr>
<td>Alkoxy radical</td>
<td>RO$^-$</td>
</tr>
<tr>
<td>Peroxy radical</td>
<td>ROO$^-$</td>
</tr>
<tr>
<td>Hydroperoxy radical</td>
<td>ROOH$^-$</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reactive Nitrogen Species</th>
<th>RNS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitric oxide</td>
<td>NO$^-$</td>
</tr>
<tr>
<td>Nitric dioxide</td>
<td>NO$_2^-$</td>
</tr>
<tr>
<td>Peroxynitrite</td>
<td>ONOO$^--$</td>
</tr>
</tbody>
</table>
Protective Mechanisms

- Despite constant production and exposure, RONS do not always lead to cell damage
- Protective mechanisms serve to either minimize RONS formation, or neutralize their damaging effects once formed
## Specific Protective Mechanisms

<table>
<thead>
<tr>
<th>1. Antioxidant Enzymes</th>
<th>3. Metal Binding Proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superoxide dismutase</td>
<td>Hemoglobin</td>
</tr>
<tr>
<td>(Cu-ZnSOD; MnSOD)</td>
<td>Myoglobin</td>
</tr>
<tr>
<td>Glutathione peroxidase</td>
<td>Ceruloplasmin</td>
</tr>
<tr>
<td>Catalase</td>
<td>Ferritin</td>
</tr>
<tr>
<td>Glutathione reductase</td>
<td>Lactoferrin</td>
</tr>
<tr>
<td>Glutathione S-transferase</td>
<td>Metallotheinein</td>
</tr>
<tr>
<td></td>
<td>Transferrin</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>2. Antioxidant Scavengers</th>
<th>4. Other Antioxidants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamins A, C, E</td>
<td>N-Acetyl-Cysteine</td>
</tr>
<tr>
<td>Thiols</td>
<td>Copper</td>
</tr>
<tr>
<td>Uric Acid</td>
<td>Zinc</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>Manganese</td>
</tr>
<tr>
<td>Carotenoids</td>
<td>Selenium</td>
</tr>
<tr>
<td>Flavonoids (quercetin, catechin, etc.)</td>
<td></td>
</tr>
</tbody>
</table>
Balance Needed for Optimal Physiological Functioning

RONS

Shift toward oxidative stress

Protective Mechanisms

Protective Mechanisms

Shift toward antioxidant defense

RONS

Oxidative and antioxidant balance (homeostasis)

Protective Mechanisms

Protective Mechanisms
Importance of RONS in Biological Systems

Regulation of a variety of key molecular and cellular mechanisms

1. Signal transduction
2. Immune response (inflammation)
3. Apoptosis
Common Targets of RONS: Specific Cellular Damage

- **Proteins**
  Specific modifications (e.g., conversion of phenylalanine residues to o-tyrosine and of tyrosine to dityrosine) or more global modifications (carbonyl derivatives)

- **Lipids** (*lipid peroxidation—autocatalytic process involving degradation of PUFAs through a chain reaction*)
  Measurements include conjugated dienes, lipid hydroperoxides TBARS, MDA, F$_2$-isoprostanes, etc.

- **DNA**
  Damage may occur to both mitochondrial and nuclear DNA, and may involve DNA strand breaks & oxidative base modifications (8-hydroxy-2'-deoxyguanosine formation)

- **Others:** Antioxidants (thiols, vitamins, etc.)
Association with Health & Disease

Clinical conditions linked to increased oxidative stress

- Aging
- Atherosclerosis (coronary artery disease, ischemic stroke)
- Diabetes (diabetic retinopathy, diabetic neuropathy)
- Chronic Inflammation (autoimmune, rheumatoid arthritis)
- Cancer (colon, breast, prostate, lung, skin)
- Neurodegenerative (Parkinson’s, muscular dystrophy, multiple sclerosis, Alzheimer’s, Down’s syndrome, Amyotrophic lateral sclerosis)
- Red Blood Cell (sickle cell anemia, hemolytic anemia)
- Pulmonary (asthma, emphysema, pneumonia, COPD)
- Gastrointestinal (pancreatitis, inflammatory bowel disease)
- Kidney
- Liver
- Eye (cataractogenesis, retinopathy, macular degeneration)
- Skin (thermal injury, contact dermatitis)
- Nutritional Deficiency (kwashiorkor)
Metabolic Syndrome—involvedment of RONS?
(Defined by the American Heart Association)

- Characterized by a single person having multiple metabolic risk factors such as:
  - Insulin resistance or glucose intolerance (the body can’t properly use insulin or blood sugar)
  - Abdominal obesity (excessive fat tissue in and around the abdomen)
  - Atherogenic dyslipidemia (blood fat disorders — high triglycerides, low HDL cholesterol and high LDL cholesterol — that foster plaque buildups in artery walls)
  - Elevated blood pressure
  - Proinflammatory state (e.g., elevated C-reactive protein in the blood)
  - Prothrombotic state (e.g., high fibrinogen or plasminogen activator inhibitor–1 in the blood)
Metabolic Syndrome

- **Usually diagnosed by:**
  - Elevated fasting glucose:
    - Equal to or greater than 100 mg/dL
  - Elevated waist circumference:
    - Men — Equal to or greater than 40 inches (102 cm)
    - Women — Equal to or greater than 35 inches (88 cm)
  - Elevated triglycerides:
    - Equal to or greater than 150 mg/dL
  - Reduced HDL (“good”) cholesterol:
    - Men — Less than 40 mg/dL
    - Women — Less than 50 mg/dL
  - Elevated blood pressure:
    - Equal to or greater than 120/80 mm Hg
Metabolic Syndrome

Recommendations:

- Weight loss to achieve a desirable weight (BMI less than 25 kg/m²)

- Increased physical activity, with a goal of at least 30 minutes of moderate-intensity activity on most days of the week

- Healthy eating habits that include reduced intake of saturated fat, trans fat and cholesterol
Postprandial Oxidative Stress

- Oxidative stress occurs following a meal high in kilocalories, saturated fat, and carbohydrate. (For review please see Sies et al., 2005)
  - Peak response occurs between 3-4 hours post feeding
  - Mediated in large part due to blood triglyceride response to feeding
  - Also mediated by blood glucose response to feeding

- Chronic postprandial status (lipemia)
  - Positively correlated with superoxide production
  - Increased lipemia and oxidative stress, coupled with impaired nitric oxide production, is associated with endothelial dysfunction
  - All above are considered significant risk factors to atherogenesis (Ceriello et al., 2002; Zilversmit, 1979)
Postprandial Oxidative Stress

Factors affecting the magnitude of postprandial oxidative stress:
- Meal size and composition
- Health disorders (diabetes, CAD, smoking)
- Gender??
- Blood triglyceride: basal and response to feeding
- Blood glucose: basal and response to feeding
- Acute and chronic use of lipid/glucose lowering drugs/nutrients
- Acute and chronic use of antioxidant supplements
- Acute and chronic performance of exercise?
Correlations between blood triglycerides and oxidative stress variables following intake of a high fat meal in men and women

**Pairwise Correlations**

<table>
<thead>
<tr>
<th>Variable by Variable</th>
<th>Correlation</th>
<th>Count</th>
<th>Signif Prob</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA TAGS</td>
<td>0.5518</td>
<td>160</td>
<td>0.0000</td>
</tr>
<tr>
<td>xanthine oxidase TAGS</td>
<td>0.6208</td>
<td>160</td>
<td>0.0000</td>
</tr>
<tr>
<td>xanthine oxidase MDA</td>
<td>0.5207</td>
<td>160</td>
<td>0.0000</td>
</tr>
<tr>
<td>H2O2 TAGS</td>
<td>0.6662</td>
<td>160</td>
<td>0.0000</td>
</tr>
<tr>
<td>H2O2 MDA</td>
<td>0.4491</td>
<td>160</td>
<td>0.0000</td>
</tr>
<tr>
<td>H2O2 xanthine oxidase</td>
<td>0.7651</td>
<td>160</td>
<td>0.0000</td>
</tr>
</tbody>
</table>

Impact of lipid lowering strategies?
- Exercise
- Nutritional supplementation

Attenuating postprandial oxidative stress: Role of physical exercise

- **TAG Processing**
  - Lower fasting TAG in trained vs. untrained individuals
  - More efficient processing of TAG following high fat meals in trained vs. untrained individuals (Cohen et al., 1989)
    - Reduced chylomicron-TAG half-life
    - Increased activity of lipoprotein lipase, the rate limiting enzyme for serum TAG removal
Attenuating postprandial oxidative stress: Role of physical exercise

- **Glucose Processing**
  - Lower fasting glucose in trained vs. untrained individuals
  - More efficient processing of glucose following high carbohydrate meals in trained vs. untrained individuals
    - Increased insulin sensitivity and responsiveness
      - Insulin receptor number may increase
    - Increased GLUT4 protein content and translocation
Attenuating postprandial oxidative stress: Role of physical exercise

- **Antioxidant Protection**
  - Sufficient exercise stimulus (intensity and duration) allows for an up-regulation in endogenous antioxidant defenses (Ji, 2002; Ji et al., 2006; Powers et al., 1999)
  - The generation of RONS appears to be the “signal” needed to allow for such adaptations
    - Acute exercise » ROS production » up-regulation of endogenous antioxidant protection
    - Improved conditioning » more tightly coupled ETC » less oxygen leakage » lower XO activity » less superoxide/H₂O₂
  - To date, all studies involving exercise have focused on postprandial lipemia (TAG) or glycemia and not oxidative stress, with one exception:
    - McClean et al., 2007 (impact of *acute* exercise)
      - 1 hr aerobic exercise @ 2 hrs post-high fat meal
        - Increased SOD, attenuated TAG and LOOH
Attenuating postprandial oxidative stress: Role of nutritional supplementation

- **Antioxidants**
  - A few studies have shown favorable effects with either acute or long-term antioxidant supplementation
  - Many studies have focused on endothelial function exclusively
  - Data are somewhat limited in relation to blood oxidative stress biomarkers
  - Few studies have used clinical populations
    - Need for additional studies using various antioxidants (alone or in combination) to attenuate postprandial oxidative stress
      - Outcome variables to include both blood biomarkers and clinical measures (e.g., endothelial function)
Attenuating postprandial oxidative stress: Role of nutritional supplementation

- Lipid and Glucose Lowering Agents
  - A few studies have shown favorable effects with lipid and glucose lowering drugs
  - Data are limited in relation to blood oxidative stress biomarkers
    - Need for studies using lipid and glucose lowering nutritional supplements to attenuate postprandial oxidative stress
      - Outcome variables to include both blood biomarkers and clinical measures (e.g., endothelial function)
New Product Development

- ArginoCarn™ (acetyl L-carnitine arginate dihydrochloride)
  - 45% acetyl L-carnitine
  - 39% arginine

- AminoCarnitines™: molecules including a combination of L-carnitine + specific amino acid

- Metabolic performance of L-carnitine is related to precursors such as arginine, glycine, taurine and lysine
  - Arginine: Substrate for NO biosynthesis
    - NO involved in insulin signaling and glucose transport

- ArginoCarn™ may function as an antioxidant, a lipid lowering agent, and a glucose disposing agent; ultimately providing potential related to cardiovascular protection
Effect of aerobic exercise and acetyl L-carnitine arginate dihydrochloride on postprandial oxidative stress in pre-diabetics

A Randomized, double-blind, placebo controlled, intervention trial
Study Purpose and Hypothesis

□ Purpose
To determine the independent and combined effects of exercise training and ArginoCarn™ on postprandial oxidative stress in a sample of prediabetic subjects

□ Hypothesis
Postprandial oxidative stress will be attenuated in subjects who are assigned to exercise or ArginoCarn™ alone, as compared to no exercise or placebo; and further attenuated in those assigned to exercise + ArginoCarn™
Study Design

Sedentary, pre-diabetic men and women (18-55 yrs)

- ArginoCarn™ + Exercise (n=15)
  - Progressive 8 week supervised aerobic exercise training program (3 days per week; 30-45 minutes per session)

- Placebo + Exercise (n=15)

- ArginoCarn™ + No exercise (n=15)

- Placebo + No exercise (n=15)
  - Follow normal physical activity patterns for 8 week period

Meal challenge pre and post intervention;
Assessments and outcome variables as described
Study Timeline

Initial Screening

Randomization and GXT

Start group assignment

End group assignment

Blood @ pre, 1, 2, 4, and 6 hours post

Wk -3

Wk -2

Wk -1

Wk 0

Wk 8

8 week intervention period

Test Meal 1
Blood @ pre, 1, 2, 4, and 6 hours post

Test Meal 2
(Within 36 hours of GXT)
Blood @ pre, 1, 2, 4, and 6 hours post

Post intervention GXT
(immediately following intervention period)
Methods

- **Pre and Post Intervention Assessments**
  - Health history, drug/dietary supplement usage, physical activity and diet questionnaires
  - Anthropometric variables and blood glucose
  - Resting heart rate and blood pressure
  - VO2max and exercise time to exhaustion

- **Exercise Training**
  - Supervised program of aerobic exercise
    - 3 days per week; 30-45 minutes; intensity and progression based on ACSM guidelines

- **Nutritional Supplementation**
  - ArginoCarn™—3 grams per day
  - Placebo
Test Meal: Consumed in fasted state

- Whole milk, ice cream, and heavy whipping cream
- Size: relative to body mass
- 1.2 g of fat and CHO per kg BM
- 0.25 g of protein per kg BM
- Approx 17 kcal per kg BM
- Example: 80kg (176 lb) person would consume 1360 kcal
- Consumed within 15 min
- 0 hr @ beginning of meal

7 day diet and activity records maintained immediately prior to each test meal
Outcome Variables:
pre meal, 1, 2, 4, & 6 hours post meal*

- **Blood oxidative stress***
  - Xanthine oxidase activity
  - Hydrogen peroxide
  - Malondialdehyde
  - Trolox equivalent antioxidant capacity

- **Other bloodborne variables**
  - Nitric oxide*
  - Insulin*
  - Glucose*
  - Triglyceride*
  - Total, HDL, and LDL cholesterol
  - HbA1c
  - C-reactive protein
  - Brain-derived neurotrophin factor
Outcome Variables

- Non-bloodborne variables
  - Body weight
  - Body mass index
  - Body fat
  - Circumference measurements
  - Heart rate
  - Blood pressure
  - VO\textsubscript{2}\text{max}
  - Exercise time to exhaustion
Questions

Sigma-tau HealthScience, Inc. (Booth # 1205)
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www.healthscienceusa.com
www.livingtonics.com
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