

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

Richard J. Bloomer, PhD
Department of Health and Sport Sciences
The University of Memphis
Memphis, TN

Update on GlycoCarn™ Studies

- Bloomer RJ, Tschume LC, & Smith WA. **Glycine propionyl-L-carnitine modulates lipid peroxidation and nitric oxide in human subjects.** *The International Journal of Vitamin and Nutrition Research*, In Press.
- Smith WA, Fry AC, Tschume LC, & Bloomer RJ. **Effect of glycine propionyl-L-carnitine on aerobic and anaerobic exercise performance.** *International Journal of Sport Nutrition & Exercise Metabolism*, In Press.
- Bloomer RJ, & Smith WA. **Oxidative stress in response to aerobic and anaerobic power testing: Influence of exercise training and dietary carnitine supplementation.** *Applied Physiology, Nutrition, and Metabolism*, In Review.
- Bloomer RJ, Smith WA, Fisher-Wellman, KH, & Shastri, S. **Impact of glycine propionyl-L-carnitine on ischemia-reperfusion induced oxidative stress.** In Preparation.



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
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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
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Specific RONS

Reactive Oxygen Species	ROS
Superoxide ion	$O_2^{\bullet-}$
Ozone	O_3
Singlet oxygen	1O_2
Hydroxyl radical	OH^{\bullet}
Hydrogen peroxide	H_2O_2
Hypochlorous acid	$HOCl$
Alkoxyl radical	RO^{\bullet}
Peroxyl radical	ROO^{\bullet}
Hydroperoxyl radical	$ROOH^{\bullet}$
Reactive Nitrogen Species	RNS
Nitric oxide	NO^{\bullet}
Nitric dioxide	NO_2^{\bullet}
Peroxynitrite	$ONOO^{\bullet-}$

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
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Specific Protective Mechanisms

1. Antioxidant Enzymes

Superoxide dismutase
(Cu-ZnSOD; MnSOD)
Glutathione peroxidase
Catalase
Glutathione reductase
Glutathione S-transferase

2. Antioxidant Scavengers

Vitamins A, C, E
Thiols
Uric Acid
Bilirubin
Carotenoids
Flavonoids (quercetin, catechin, etc.)

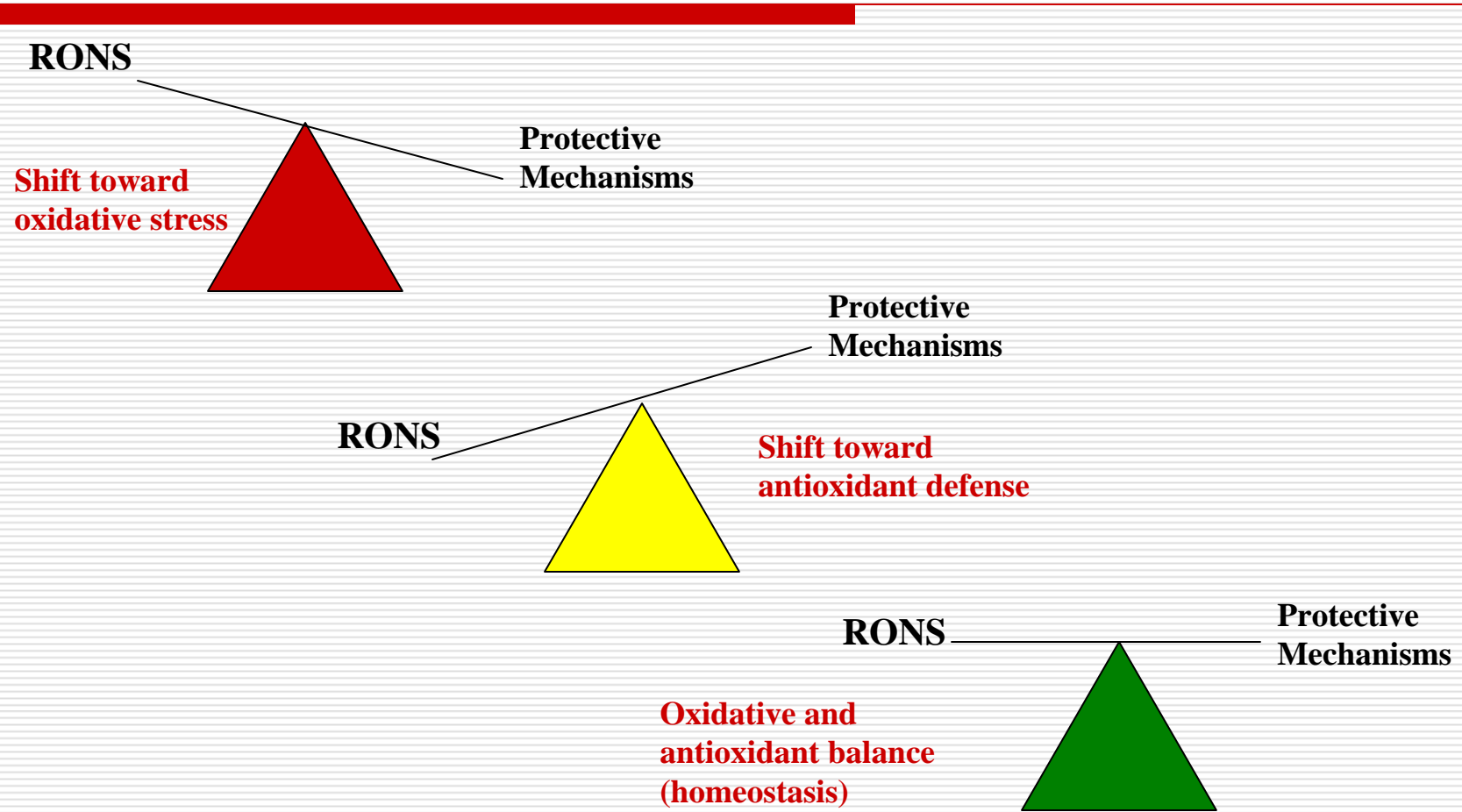
3. Metal Binding Proteins

Hemoglobin
Myoglobin
Ceruloplasmin
Ferritin
Lactoferrin
Metallothionein
Transferrin

4. Other Antioxidants

N-Acetyl-Cysteine
Copper
Zinc
Manganese
Selenium

Balance Needed for Optimal Physiological Functioning



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
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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, F₂-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)
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Metabolic Syndrome—involvement 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)
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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
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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
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Postprandial Oxidative Stress



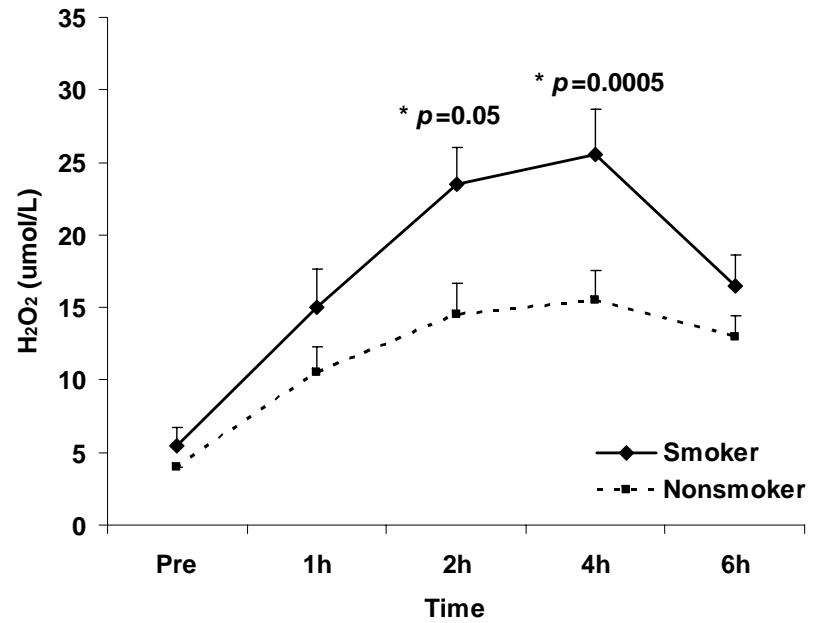
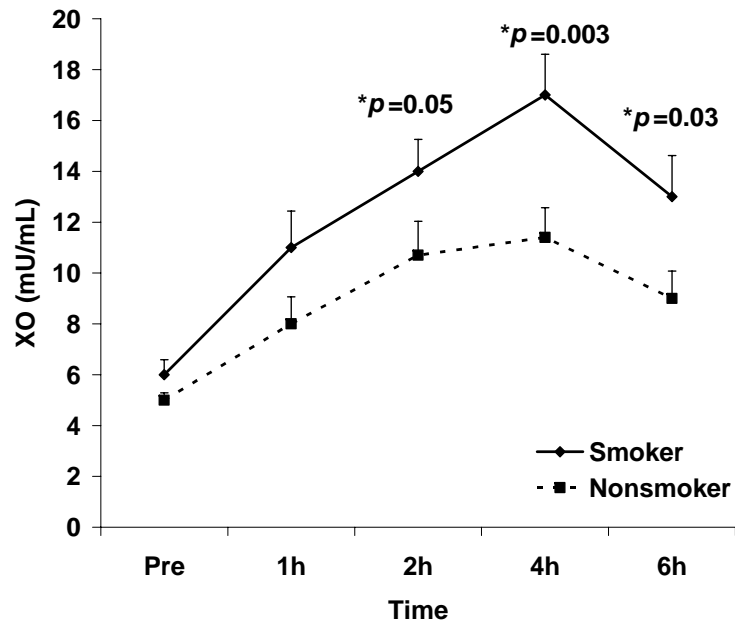
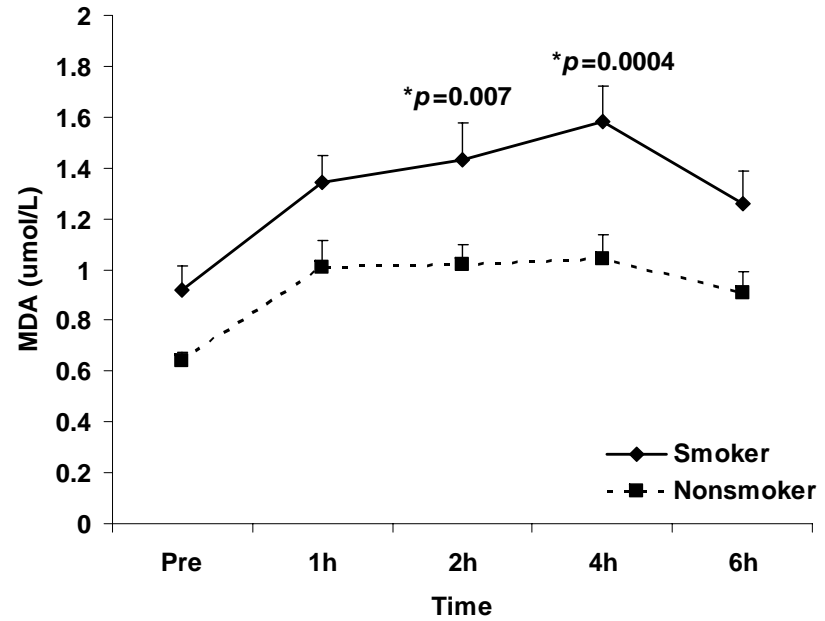
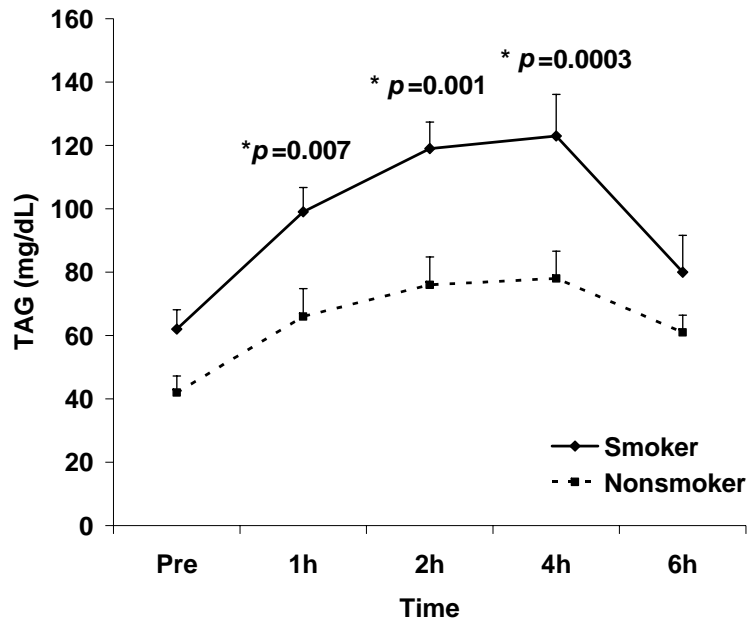
- Oxidative stress occurs following a meal high in kilocalories, saturated fat, and carbohydrate (For review please see Sies et al., 2005)
 - Peak response occurs between 3-4 hours post feeding
 - Mediated in large part due to blood triglyceride response to feeding
 - Also mediated by blood glucose response 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; Zilvermit, 1979)
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Postprandial Oxidative Stress

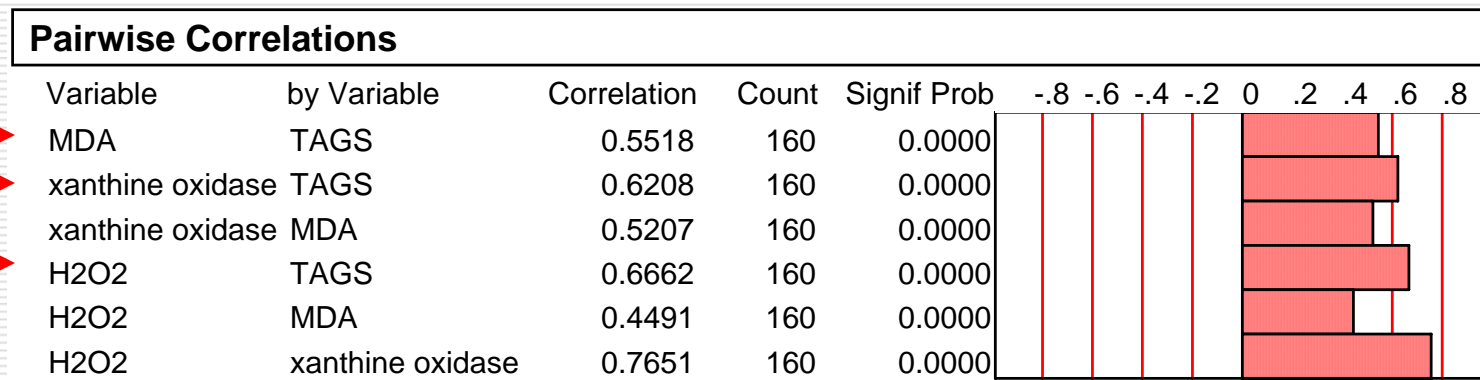


- Factors affecting the magnitude of postprandial oxidative stress:
 - Meal size and composition
 - Health disorders ([diabetes](#), CAD, smoking)
 - Gender??
 - Blood triglyceride: basal and **response to feeding**
 - Blood glucose: basal and response to feeding
 - Acute and chronic use of lipid/glucose lowering drugs/nutrients
 - Acute and chronic use of antioxidant supplements
 - Acute and chronic performance of exercise?
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Blood TAG and oxidative stress before and following intake of a high fat meal in smokers and nonsmokers. *Significant differences between smokers and nonsmokers using planned contrasts. Bloomer et al. *British Journal of Nutrition*. In Press.

Correlations between blood triglycerides and oxidative stress variables following intake of a high fat meal in men and women



Impact of lipid lowering strategies?

- Exercise
- Nutritional supplementation

Bloomer et al. *European Journal of Applied Physiology*. In Review.

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
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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
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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
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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)
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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



US patent:
6,703,042



- 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

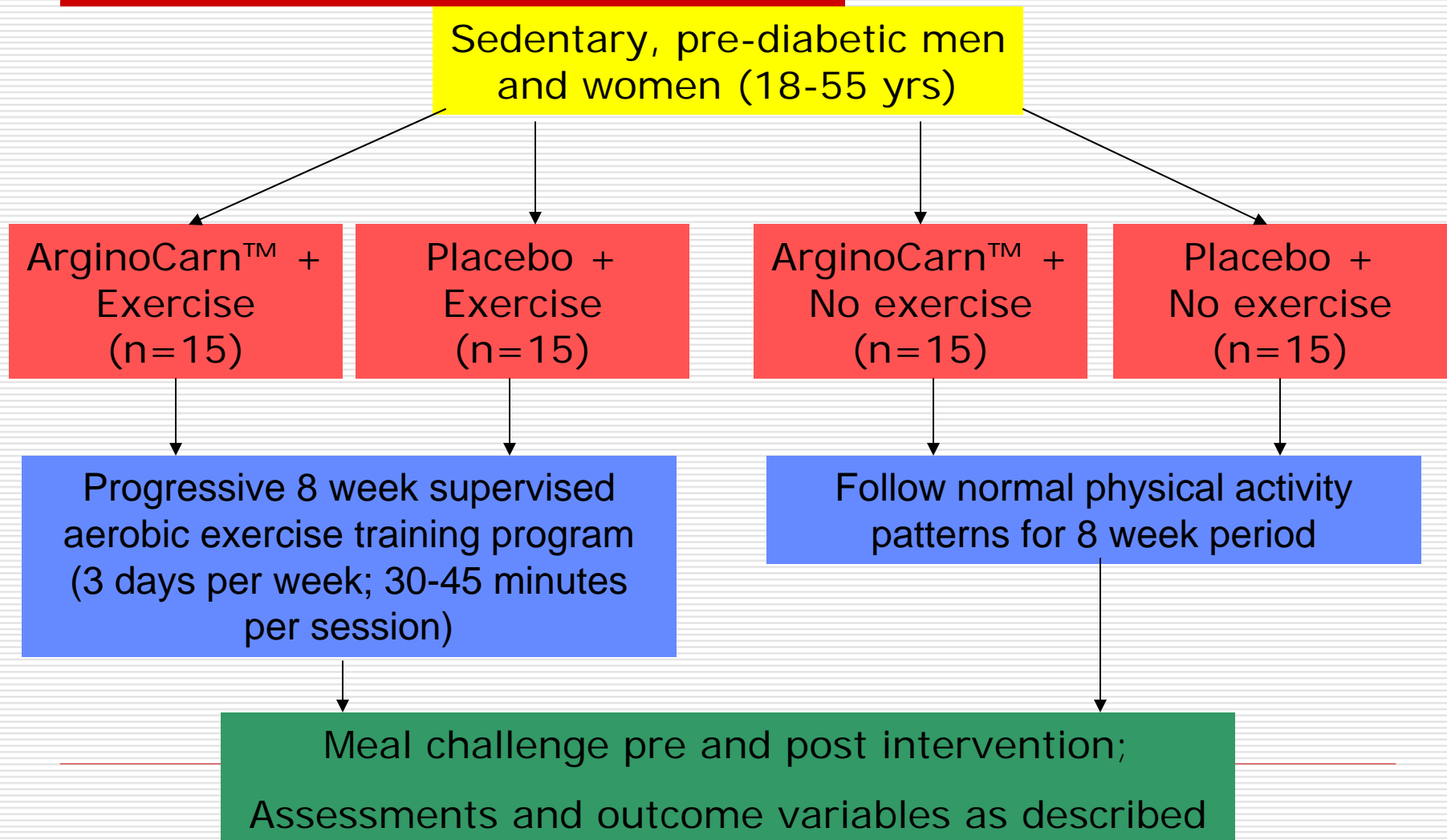
□ Purpose

To determine the independent and combined effects of exercise training and ArginoCarn™ on postprandial oxidative stress in a sample of pre-diabetic subjects

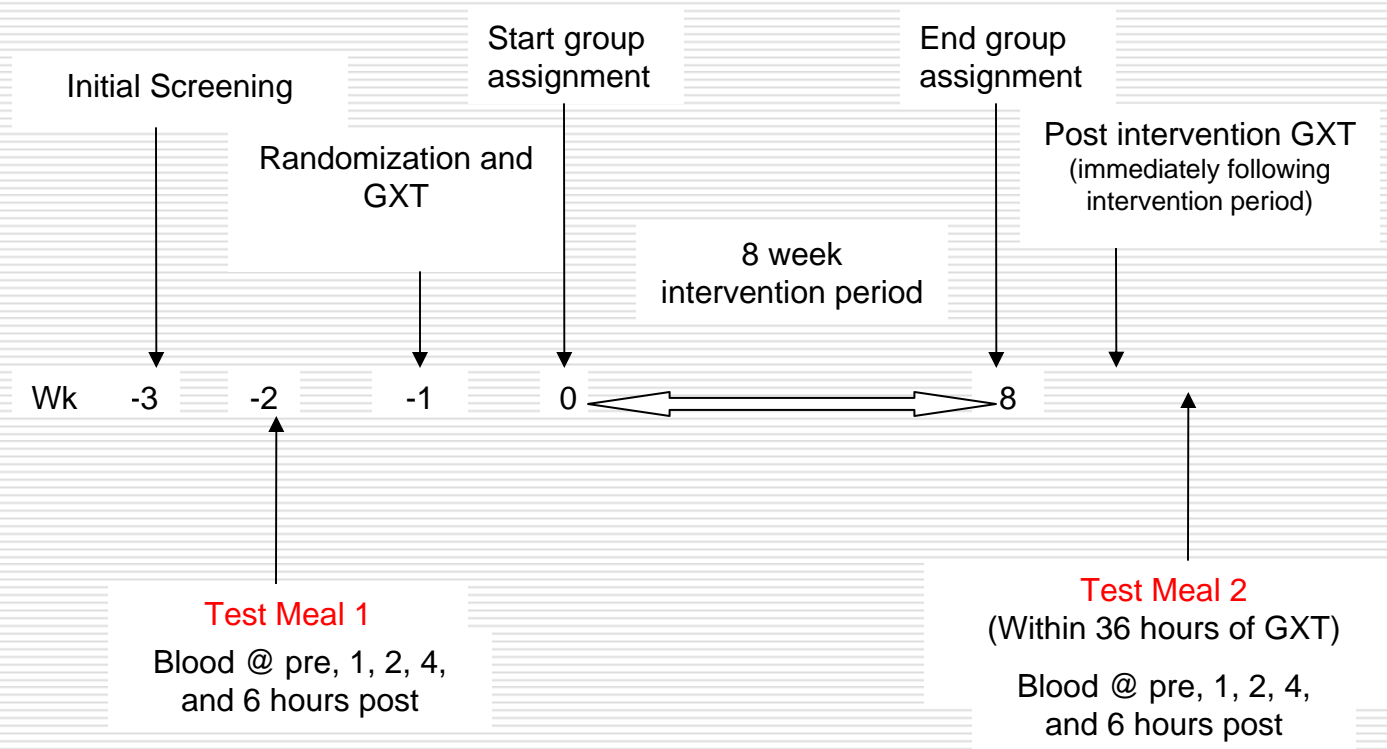
□ Hypothesis

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



Study Timeline



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
- VO_{2max} 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 neurotrophic factor
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Outcome Variables

Non-bloodborne variables

- Body weight
 - Body mass index
 - Body fat
 - Circumference measurements
 - Heart rate
 - Blood pressure
 - $VO_{2\max}$
 - Exercise time to exhaustion
-

Questions

Sigma-tau HealthScience, Inc. (Booth # 1205)

www.aminocarnitines.com
www.healthscienceusa.com
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Sigma-tau HealthScience

R. Bloomer

<http://coe.memphis.edu/HSS/CRML>

rbloomer@memphis.edu

[901-678-4341](tel:901-678-4341)

Laboratory-based product testing services available
