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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NATURAL PRODUCTS EXPO EAST

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.

GlycoCarn™

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

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

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.)

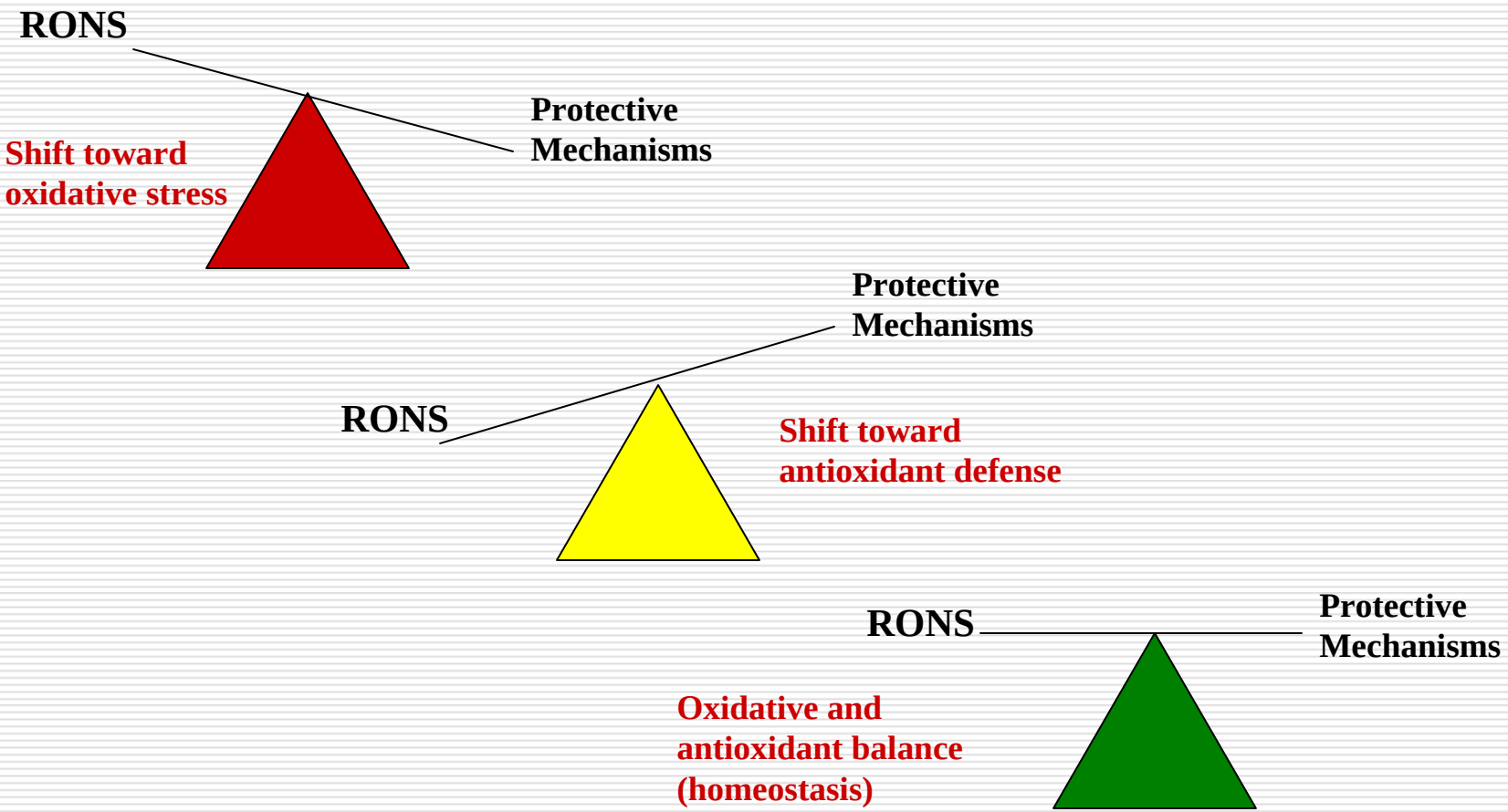
3. Metal Binding Proteins

Hemoglobin
Myoglobin
Ceruloplasmin
Ferritin
Lactoferrin
Metallotheinein
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

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—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)

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/m^2)
  - 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

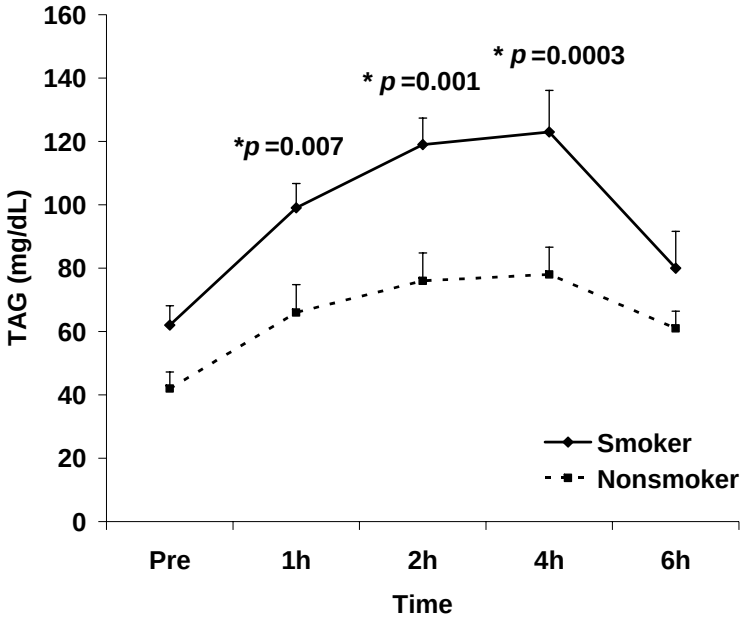
Postprandial Oxidative Stress

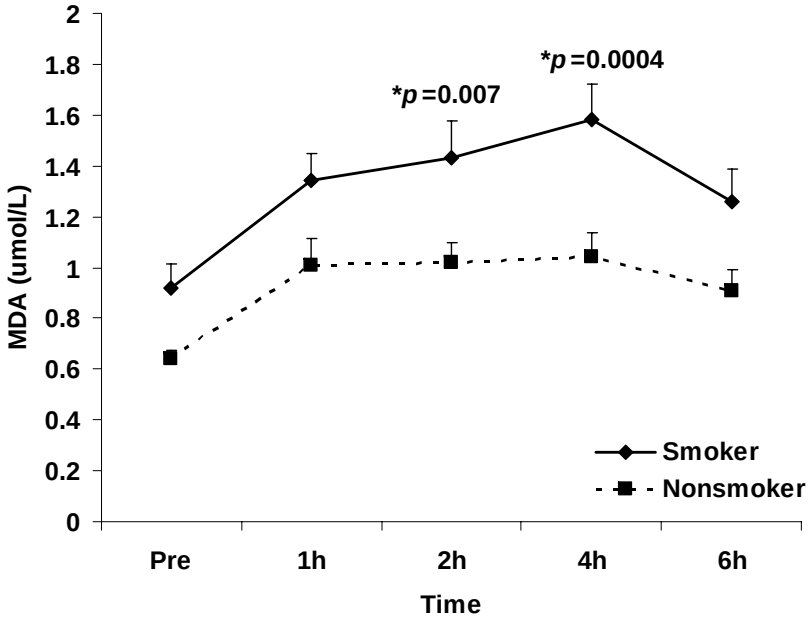
- Oxidative stress occurs following a meal high in kilocalories, saturated fat, and carbohydrate
  (For review please see Sies et al., 2005)
  - Peak response occurs between 3-4 hours post feeding
  - Mediated in large part due to blood triglyceride response to feeding
  - Also mediated by blood glucose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

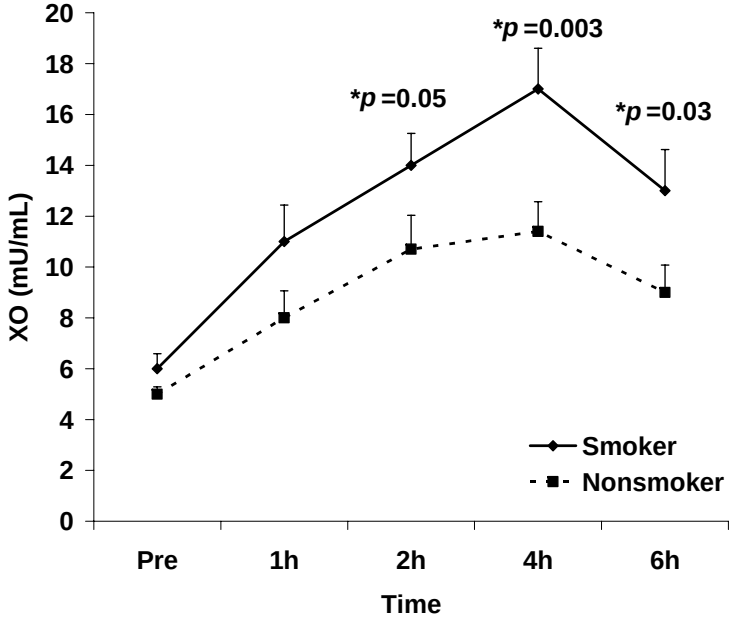
- Chronic postprandial status (lipemia)
  - Positively correlated with superoxide production
  - Increased lipemia and oxidative stress, coupled with impaired nitric oxide production, is associated with endothelial dysfunction
  - All above are considered significant risk factors to atherogenesis (Ceriello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

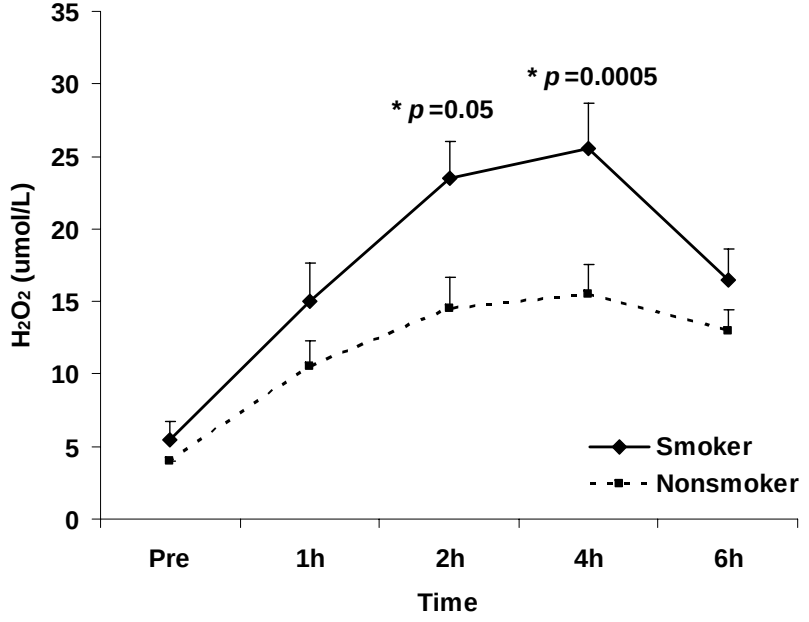
Postprandial Oxidative Stress

- 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?







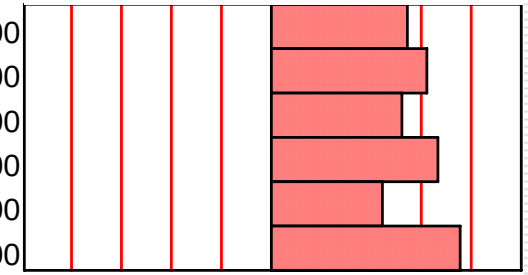


Blood TAG and oxidative stress before and following intake of a high fat meal in smokers and nonsmokers. *Significant differences between smokers and nonsmokers using planned contrasts. Bloomer et al. *British Journal of Nutrition*. In Press.

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

Pairwise Correlations

Variable	by Variable	Correlation	Count	Signif Prob
MDA	TAGS	0.5518	160	0.0000
xanthine oxidase	TAGS	0.6208	160	0.0000
xanthine oxidase	MDA	0.5207	160	0.0000
H2O2	TAGS	0.6662	160	0.0000
H2O2	MDA	0.4491	160	0.0000
H2O2	xanthine oxidase	0.7651	160	0.0000



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

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/H_2O_2
- 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

Sigma-tau HealthScience

AminoCarnitine™

ArginoCarn™ USP

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

THE UNIVERSITY OF MEMPHIS®

Study Purpose and Hypothesis

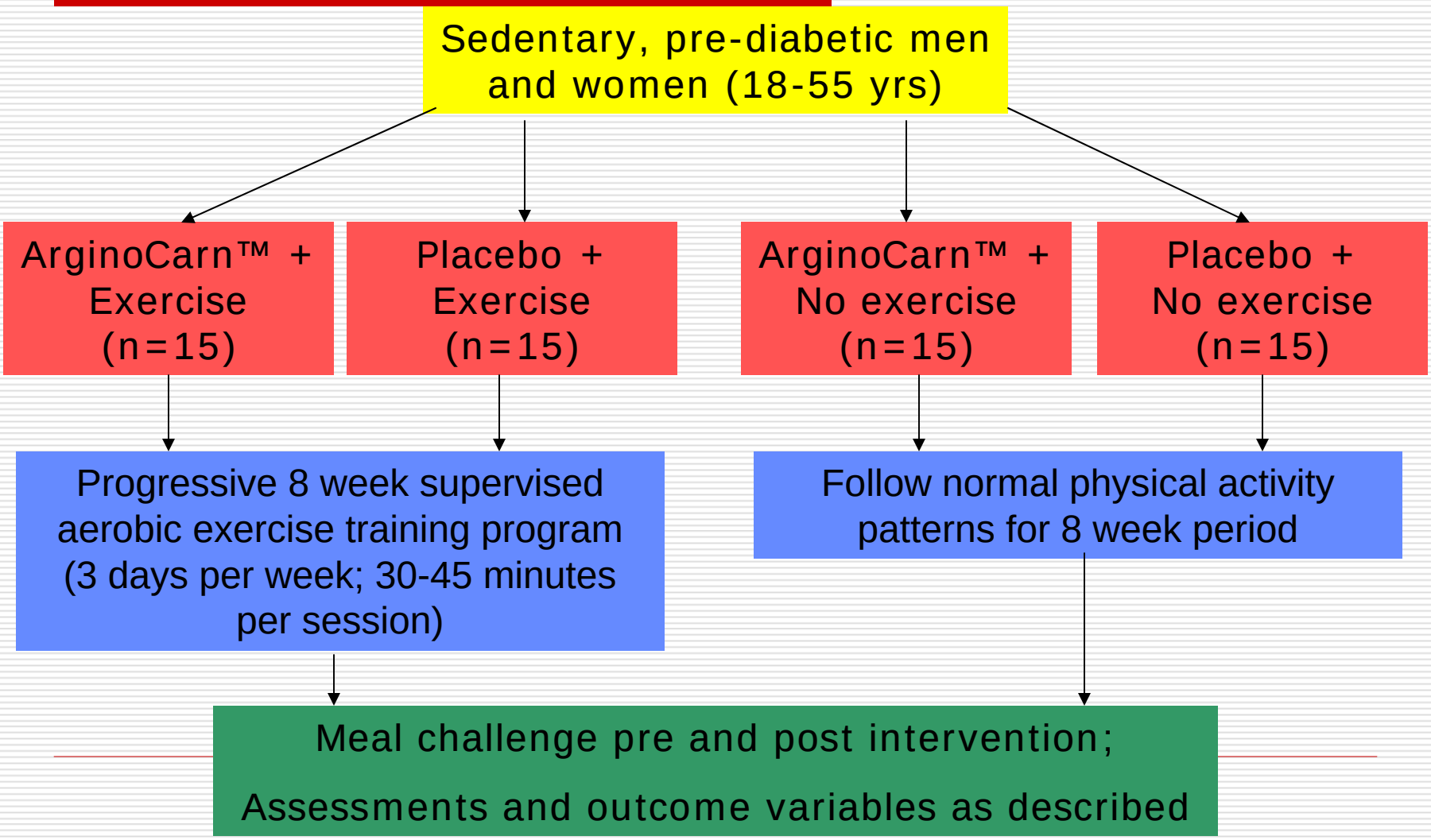
- Purpose

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

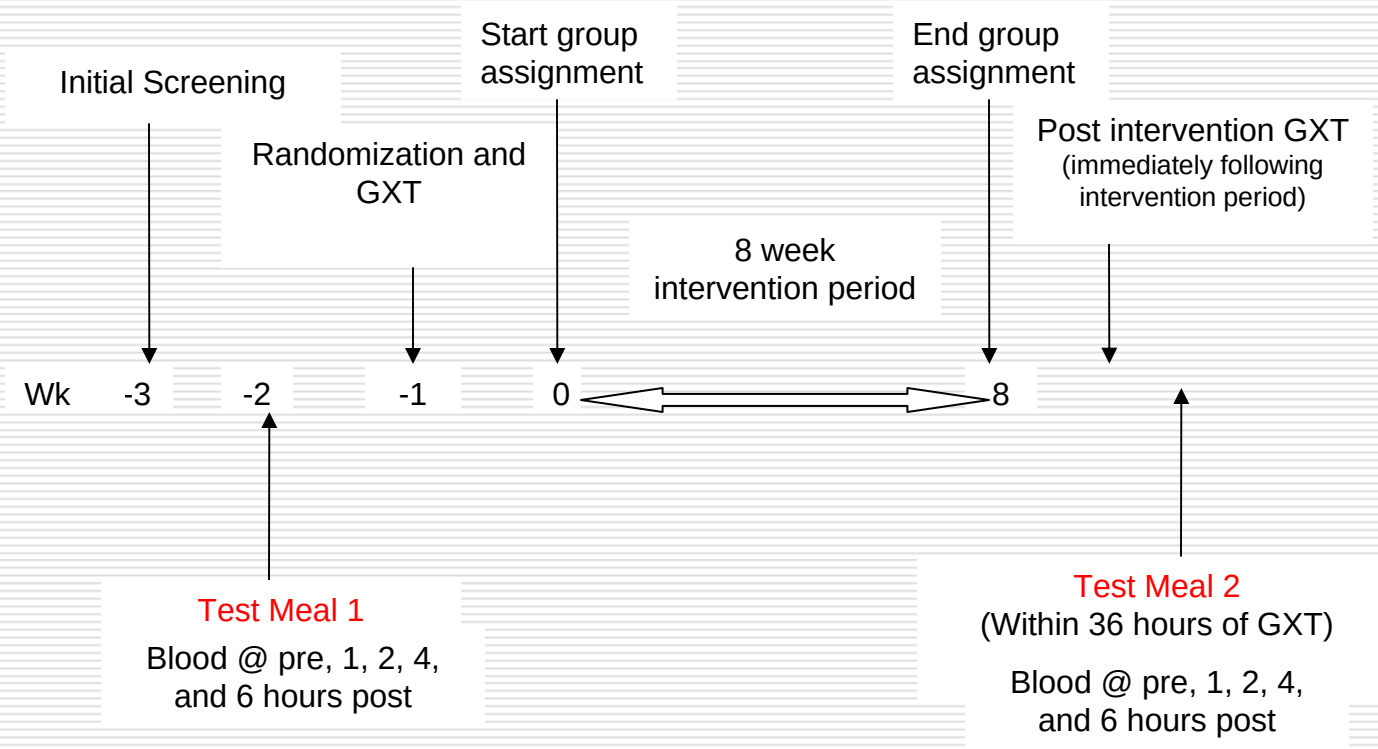
- Hypothesis

  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

Outcome Variables

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

Questions

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