Attenuating Postprandial Oxidative Stress in Pre-Diabetics: Potential Nutritional Aspects of Acetyl-L-Carnitine Arginate DiHcl (ArginoCarn®) for Metabolic Disorders

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Outline

- Oxidative Stress Defined
  - Importance in biological systems
  - Reactive Oxygen and Nitrogen Species (RONS) and Common targets
  - 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 summary of results

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.)

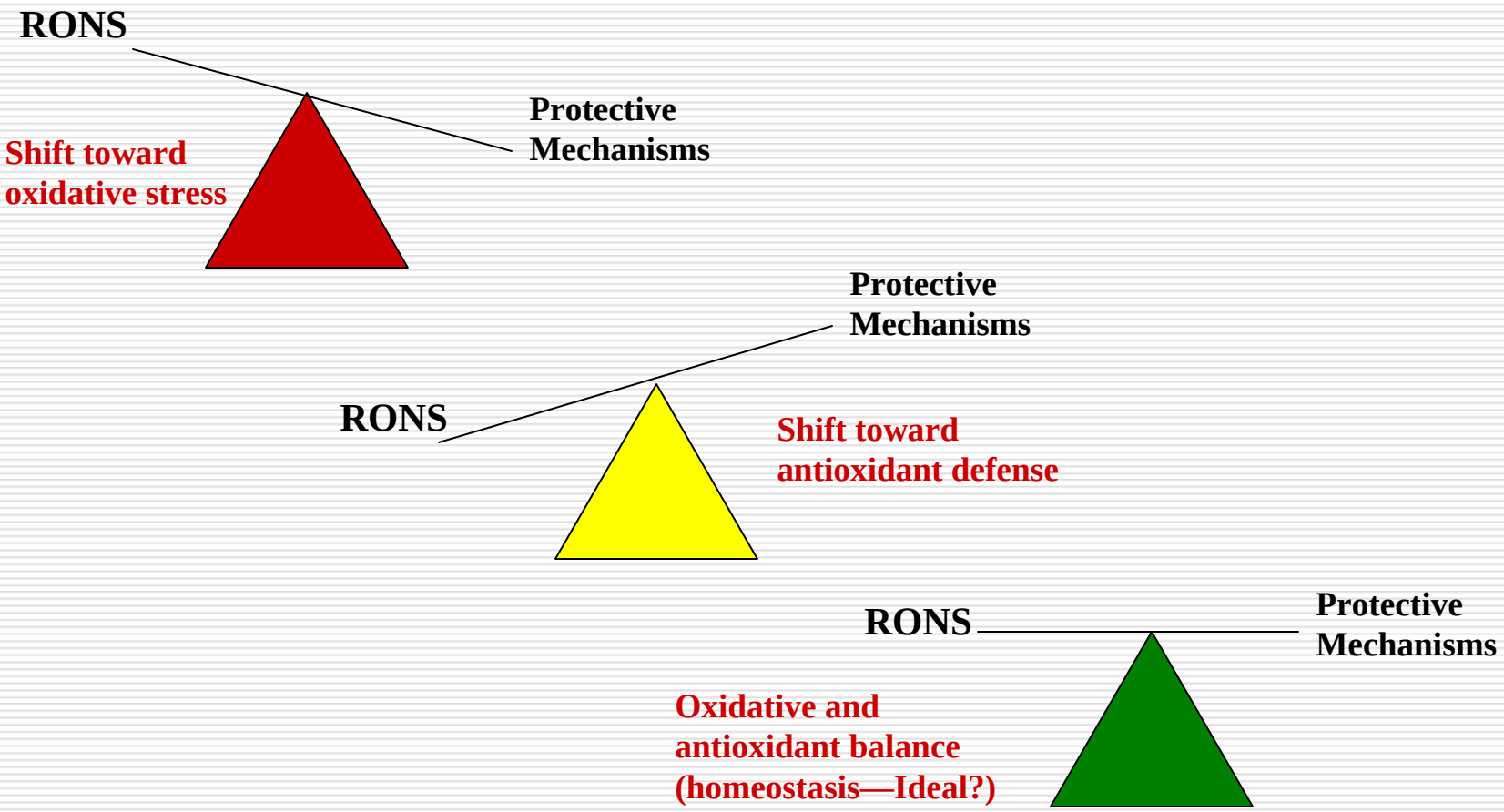
3. Metal Binding Proteins

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
    1. Insulin signaling
    2. Muscle force production
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 *associated with* 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 $mg \cdot dL^{-1}$
  - 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 $mg \cdot dL^{-1}$
  - Reduced HDL (“good”) cholesterol:
    - Men — Less than 40 $mg \cdot dL^{-1}$
    - Women — Less than 50 $mg \cdot dL^{-1}$
  - Elevated blood pressure:
    - Equal to or greater than 120/80 mmHg

Metabolic Syndrome

- Recommendations:
  - Weight loss to achieve a desirable weight (BMI less than 25 $kg \cdot m^{-2}$)
  - 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, as well as highly processed, high carbohydrate foods
    - Ingestion of above strongly predicts RONS production

Postprandial Oxidative Stress

- Oxidative stress occurs following a meal high in kilocalories, saturated fat, and carbohydrate (Sies et al., 2005)
  - Peak response occurs between 2-4 hours post feeding (meal dependent)
  - Mediated in large part due to blood triglyceride and glucose response to feeding
    - Excess substrate leads to overproduction of RONS (electron transport)
- Chronic postprandial status (hyperlipidemia and hyperglycemia)
  - Positively correlated with superoxide production
  - Increased lipemia and glycemia associated with increased oxidative stress
    - Coupled with impaired nitric oxide production—associated with endothelial dysfunction
  - All above are considered significant risk factors to atherogenesis (Ceriello et al., 2002; Zilversmit, 1979)

Postprandial Oxidative Stress

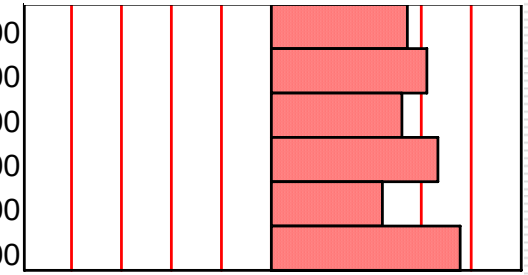
- Factors affecting the magnitude of oxidative stress:
  - Meal size
  - Macronutrient composition and type (Protein<Saturated fat; maltodextrin and dextrose similar)*
  - Sex (Women<Men)*
  - Race (African Americans<Caucasians)*
  - Body mass (Normal weight<Obese)*
  - Cigarette smoking (Nonsmokers<Smokers)*
  - Health disorders (Healthy<Diseased: diabetes, CAD)
  - 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 performance of exercise?
  - Acute and chronic use of antioxidant supplements

*Recent findings from Bloomer and colleagues

Correlations between blood triglycerides and oxidative stress variables following intake of a high fat meal (whipping cream, milk, ice cream) 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?

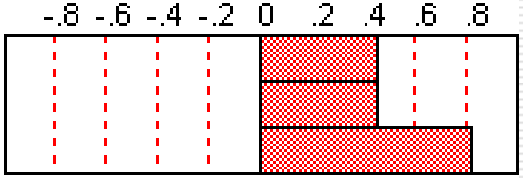
- Exercise
- Nutrient intake/supplementation

Bloomer et al. Medicine and Science in Sports & Exercise. In Review.

Correlations between blood glucose and oxidative stress variables following intake of a high carbohydrate meal (dextrose) in men

Pairwise Correlations

Variable	by Variable	Correlation	Count	Signif Prob
MDA	glucose	0.4536	45	0.0018
H2O2	glucose	0.4606	45	0.0015
H2O2	MDA	0.8317	45	0.0000



Impact of glucose lowering strategies?

- Exercise
- Nutrient intake/supplementation

Fisher-Wellman et al. In Progress.

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 is 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 radical leak » 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

- Agents with scientific support (blood glucose regulation primarily—see O'Keefe et al., JACC 2008)
  - *Cinnamon*
  - *Vinegar*
  - *Nuts and oils*
  - *Alcohol*
- Data are limited in relation to blood oxidative stress biomarkers
  - Need for studies using lipid and glucose lowering nutrients/nutritional supplements 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

- Antioxidants
  - A few studies have shown favorable effects with either acute (at time of meal ingestion) or long-term (weeks prior to meal ingestion) antioxidant supplementation
    - Many studies have focused on endothelial function exclusively
    - Data are 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)

ArginoCarn® (acetyl-L-carnitine arginate dihydrochloride, USP)

Sigma-tau HealthScience

AminoCarnitine®

ArginoCarn® USP

US patent: 6,703,042

- 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 Nitric Oxide (NO) biosynthesis
    - NO involved in insulin signaling and glucose transport
- ArginoCarn® may function as an antioxidant, a blood flow stimulating agent, and a glucose disposing agent; ultimately providing support related to cardiovascular protection & metabolic syndrome

Effect of oral acetyl-L-carnitine arginate on resting and postprandial blood biomarkers in pre-diabetics

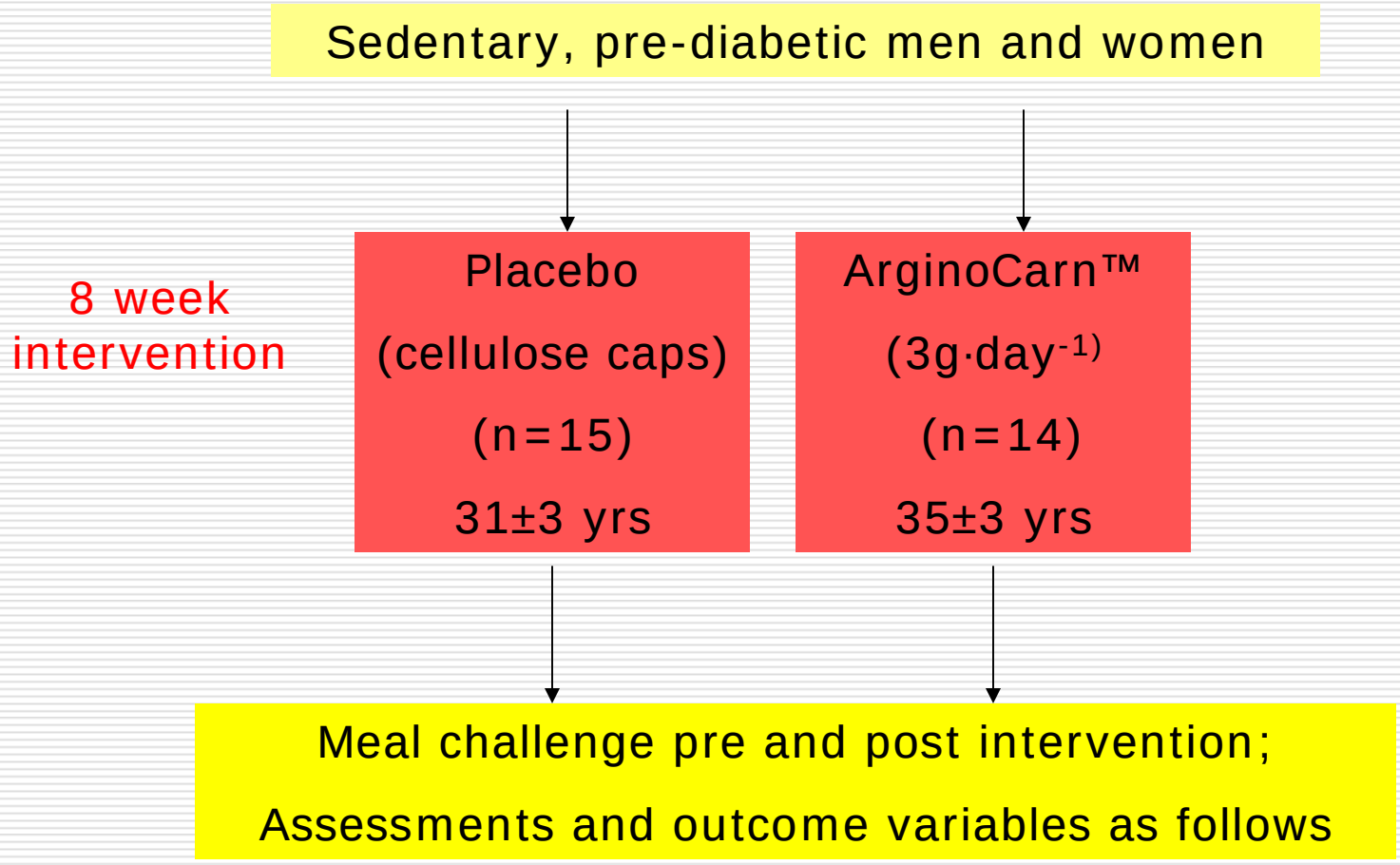
A Randomized, double-blind, placebo controlled, intervention trial

THE UNIVERSITY OF MEMPHIS®

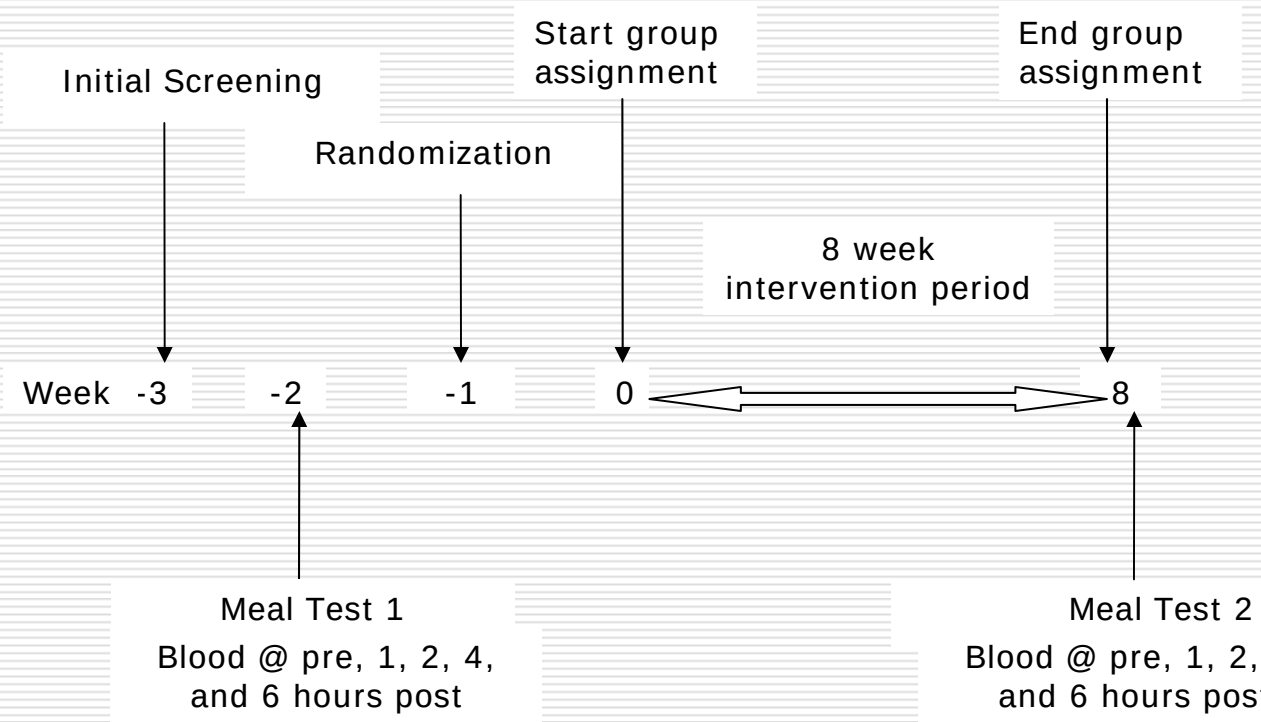
Study Purpose

To determine the effects of acetyl-L-carnitine arginate DiHcl, USP (ArginoCarn®) on resting and postprandial biomarkers of glucose and lipid metabolism, as well as oxidative stress.

Study Design



Study Timeline



Methods

- Pre and Post Intervention Assessments
  - Health history, drug/dietary supplement usage, physical activity and diet questionnaires
  - Resting heart rate and blood pressure
  - Anthropometric variables
  - Bloodborne variables
    - Lipids
    - Glucose
    - HbA1c
    - Insulin
    - Nitrate/Nitrite
    - C-Reactive Protein
    - Oxidative Stress variables

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
  - Nitrate/Nitrite
- Other bloodborne variables
  - Glucose*
  - Triglyceride*
  - Insulin
  - HbA1c
  - C-reactive protein
  - Total, HDL, and LDL cholesterol

Results Overview*

- ArginoCarn®, but not placebo, resulted in an increase in nitrate/nitrite from pre to post intervention
  - 25.4±1.9 to 30.1±2.8 $\mu mol \cdot L^{-1}$
  - Post intervention values greater compared to placebo ($p=0.01$)
- ArginoCarn® resulted in slight improvements (non-statistical significance noted;$p>0.05$) in:
  - Glucose (109±5 to 103±5 $mg \cdot dL^{-1}$)
  - HbA1c (6.6±1.1 to 6.2±1.2%)
  - HOMA-IR (3.3±1.3 to 2.9±1.2)

*Bloomer et al. Nutrition and Metabolism. In Review.

Results Overview*

- Area under the curve (AUC) postprandial data were not statistically different between ArginoCarn® and placebo for any variable ($p>0.05$)
  - Nitrate/nitrite demonstrated a moderate effect size (r = 0.35) for increase from pre (139.50±18.35 $\mu mol \cdot L^{-1} \cdot 6hr^{-1}$) to post (172.40±21.75 $\mu mol \cdot L^{-1} \cdot 6hr^{-1}$) intervention with ArginoCarn®
    - The magnitude of decrease in nitrate/nitrite following feeding was not as pronounced as with placebo

*Bloomer et al. Nutrition and Metabolism. In Review.

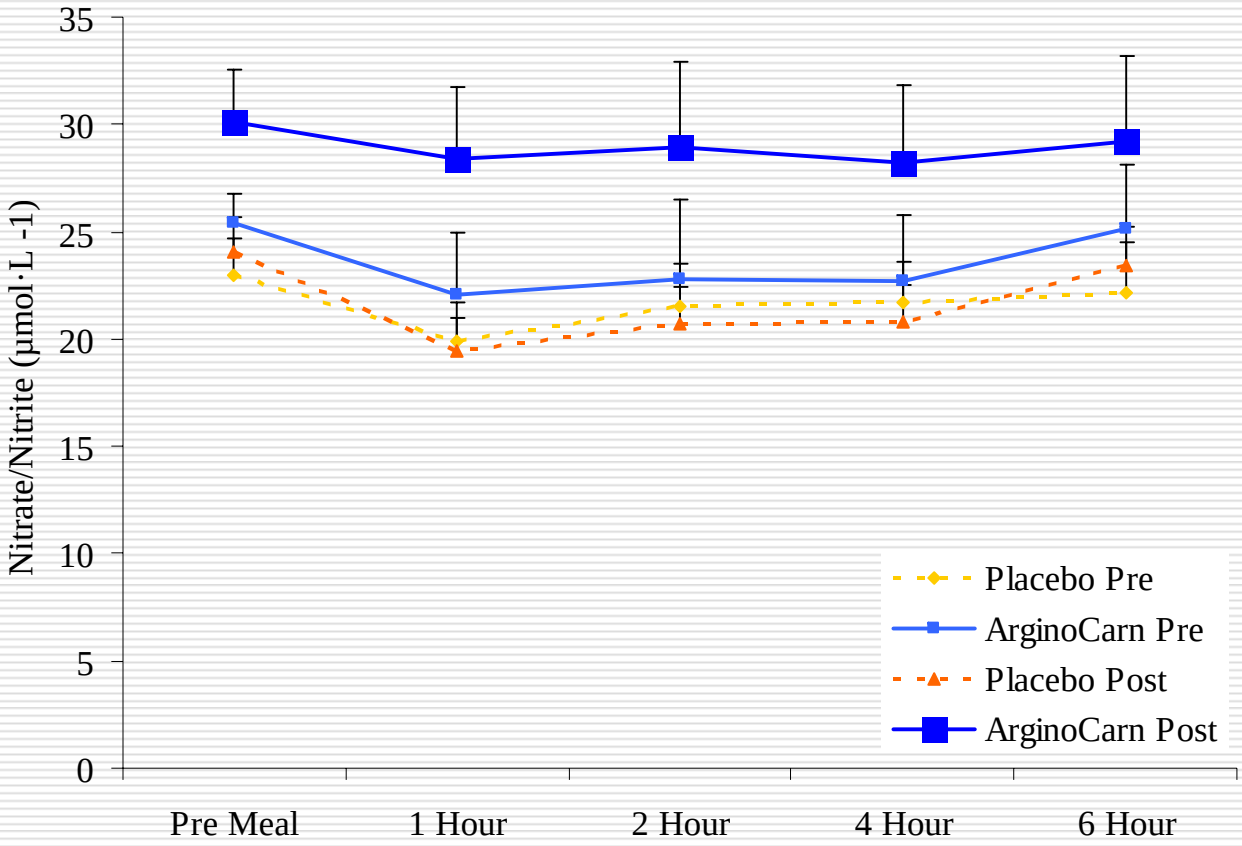
Descriptive characteristics and bloodborne data of pre-diabetic subjects

Variable	Pre Intervention ArginoCarn	Post Intervention ArginoCarn	Pre Intervention Placebo	Post Intervention Placebo
Age (yrs)	31±3	31±3	35±3	35±3
Height (cm)	172±3	172±3	169±3	169±3
Weight (kg)	85±4	83±5	91±5	90±4
BMI ($kg \cdot m^{-2}$)	28.5±1.9	27.8±1.7	31.7±2.1	32.6±2.4
Body Fat (%)	28±2	26±3	28±2	27±2
Waist (cm)	94±4	92±4	101±5	100±5
Hip (cm)	109±4	106±4	113±5	111±6
Waist:Hip	0.87±0.05	0.87±0.03	0.89±0.04	0.90±0.05
Resting HR (bpm)	73±2	71±2	73±2	72±3
Resting SBP (mmHg)	123±1	121±1	119±1	118±2
Resting DBP (mmHg)	79±1	78±1	80±1	80±1
Glucose ($mg \cdot dL^{-1}$)	109±5	103±5	112±6	111±5
HbA1c (%)	6.6±1.9	6.2±2.2	6.7±1.8	6.6±2.2
Insulin ($\mu U \cdot mL^{-1}$)	12.1±1.8	11.4±2.1	12.4±1.9	12.7±2.0
HOMA-IR	3.3±1.3	2.9±1.2	3.4±1.4	3.5±1.6
Total Cholesterol ($mg \cdot dL^{-1}$)	195±13	192±14	184±14	179±15
HDL-C ($mg \cdot dL^{-1}$)	51±7	48±6	47±4	50±5
LDL-C ($mg \cdot dL^{-1}$)	127±13	132±11	123±8	117±10
CRP ($ng \cdot mL^{-1}$)	1432±342	1389±328	1652±408	1738±425

Data are mean±SEM.
No statistically significant differences noted ($p>0.05$).

*Findings may be significantly different in frank diabetics rather than in pre-diabetic subjects (e.g., glucose, insulin, HbA1c)

Nitrate/Nitrite of pre-diabetic subjects before and after an eight week intervention of ArginoCarn® or placebo



Data are mean ±SEM.

Discussion and Explanations for Findings

- Data related to nitrate/nitrite provide support for previous findings of improved flow mediated dilation (FMD) with carnitine intake, as nitric oxide is known to facilitate vasodilatation by acting on vascular smooth muscle cells.
  - eNOS gene expression has been demonstrated to be increased within cultured human endothelial cells following carnitine incubation
  - The antioxidant effects of carnitine can provide protection against nitric oxide destruction via superoxide (forming peroxynitrite)

Discussion and Explanations for Findings

- Failure to note significant antioxidant effects may be due to:
  - Dosage of ArginoCarn®, in conjunction with the relatively large nutrient load, was not adequate to provide protection against the massive oxidative insult incurred by the test meal
  - Deficiencies in postprandial glucose and lipid metabolism of our subjects likely contributed to lack of effects
    - Pre-diabetic and overweight/obese

Conclusions

- Eight weeks of supplementation with ArginoCarn® results in:
  - An increase in resting nitrate/nitrite in pre-diabetic subjects, which may allow for higher overall circulating nitrate/nitrite following intake of a high kilocalorie, high fat and carbohydrate meal
  - A slight (but non-significant) improvement in glucose metabolism, as evidenced by minor decreases in blood glucose, insulin, and HbA1c

Future Directions

- Future work may consider the use of
  - A higher dosage of ArginoCarn®
  - Smaller test meals
  - The use of metabolically normal test subjects

Questions

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