The Effect of Antioxidants on 8-Oxoguanine Levels in the Treatment of Feline Obesity and Human Breast Cancer

Justin A. Lemkul
Korinn E. Saker, DVM, Ph.D., DACVN
Amy E. Tanner, DVM
Virginia-Maryland Regional College of Veterinary Medicine
Virginia Polytechnic Institute and State University, Blacksburg, VA
SURP Symposium - August 4, 2006
Outline

• Background
• Purpose
• Feline obesity study
  – Experimental
  – Results
  – Conclusions
• Pomegranate study
  – Experimental
  – Results
  – Conclusions
Oxidative Stress and Disease

- Oxidative tissue damage can result from a persistent disease state
  - Obesity
  - Cancer
  - Atherosclerosis
  - Diabetes
- Damage to macromolecules from reactive oxidant species (ROS)
Obesity and Oxidative Stress

- Oxidative stress increases in obese state relative to non-obese state
- Weight loss = decrease in oxidative stress?
- Effects of *cis*-9, *trans*-11 conjugated linoleic acid (CLA)
  - Inhibition of lipoprotein lipase
  - Induction of carnitine palmitoyl transferase
  - Decrease in fat mass
  - Increase in lean muscle mass
Cancer and Oxidative Stress

• Cancer requires oxidative conditions for growth and proliferation
  – Stimulation of angiogenesis
  – Upregulation of VEGF, NF-κB, eicosanoid synthesis
  – Downregulation of TNF-α, MIF

• Polyphenols inhibit enzymes necessary for eicosanoid synthesis
  – Cyclooxygenase
  – Lipoxygenase

• Pomegranates are rich in antioxidants
Purposes

- To evaluate an 8-oxoguanine (8-oxoG) assay designed for human systems in feline white blood cells and breast cancer cells grown in culture
- Are 8-oxoG levels reduced when these systems are treated with antioxidant-enriched reagents?
The Cat - Beef Study

• Diet contents
  – Beef from pasture-finished and concentrate-finished cows
  – CLA, *trans*-11 vaccenic acid, saturated fats, omega-3 fatty acids, thiamin, riboflavin, \( \alpha \)-tocopherol

• 6 weeks at 50% MER

• Analysis
  – Body weight
  – DEXA
  – 8-oxoG Assay
Dual-Energy X-Ray Absorptiometry (DEXA)

- Principle: X-rays determine the difference between fat and lean
- Calculates % body fat and % lean muscle
Biotrin OxyDNA Assay

- Isolation of white blood cells from whole blood
- Methylene blue challenges the system
- FITC-conjugated binding protein
  - Selectively binds 8-oxoguanine
Results of DEXA Scans

- Cats in both groups lost weight at a similar rate
  - 0.751 kg for cats on Diet A
  - 0.705 kg for cats on Diet B
Results of the Biotrin OxyDNA Assay

% Change in 8-oxoG Levels After 6 Weeks

- Diet A: -10.9%
- Diet B: +15.3%
Cat - Beef Study Conclusions

• The CLA-rich diet appears to be influencing fat:lean content in these cats by reducing % body fat, increasing % lean muscle
• Loss of body fat is concomitant with reduction in 8-oxoG levels, and thus oxidative stress
• The 8-oxoG assay is reliable in this system (10.8% inter-assay variability)
The Pomegranate Study

- HPLC identification of compounds in juice
- Cell culture lines
  - T-47D: ER+
  - MDA-MB-231: ER-
- Cell proliferation
- 8-oxoG Assay
HPLC Characterization

• Total antioxidants = tannins + anthocyanins
  – Fresh Juice - 426 ppm
  – POM® Wonderful Juice - 713 ppm
Cell Proliferation Assay

- Doses of 0, 25, 35, 50, 75, 100 ppm total antioxidant
- Promega CellTiter 96® AQueous One Solution Cell Proliferation Assay
- Measured metabolic activity at 24 and 48 hr
Results of the Cell Proliferation Assays

T-47D cells treated with FJ after 48 hours

MDA-231 cells treated with FJ after 48 hours

T-47D cells treated with PWJ after 48 hours

MDA-231 cells treated with PWJ after 48 hours
Results of the Biotrin OxyDNA Assay

Reduction in 8-oxoG Levels in FJ-Treated Cells After 48 Hours

<table>
<thead>
<tr>
<th>FJ Dose (ppm)</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>-85.3%</td>
</tr>
<tr>
<td>100</td>
<td>-88.9%</td>
</tr>
</tbody>
</table>

Reduction in 8-oxoG Levels in PWJ-Treated Cells After 48 Hours

<table>
<thead>
<tr>
<th>PWJ Dose (ppm)</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>-84.0%</td>
</tr>
<tr>
<td>100</td>
<td>-61.5%</td>
</tr>
</tbody>
</table>

T-47D cells treated with FJ after 48 hrs.
(a) Control
(b) 50 ppm
(c) 100 ppm
Pomegranate Study
Conclusions

• The 8-oxoG assay is effective in this system (14.5% inter-assay variability)
• Both juices reduce cell proliferation/metabolic activity of both cell lines over 48 hours
• MDA-231 are more sensitive to juice treatment than T-47D
  – Greater reduction in proliferation
  – Greater reduction in 8-oxoG levels at 100 ppm than 50 ppm
Acknowledgements

- NSF MILES-IGERT
- POM® Wonderful, LLC
- Dr. William Swecker, Jr.
- Dr. Guillermo Scaglia
- Joan Kalnitsky
- Geraldine Magnin-Bissel
- The cats!
Thank You!