Vitamin C Supplementation May Lower Body Burdens of Persistent Organic Pollutants

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ECL-DTSC
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Outline

• Background
• Method
• Results
• Future Directions
Original Vitamin Study:

Investigators:

Dr. Gladys Block, professor of Epidemiology and Public Health Nutrition, UCB.

Dr. Nina Holland, adjunct professor in School of Public Health, UCB.

Objectives

• To investigate whether vitamin C or E could reduce C-reactive protein (CRP) and oxidative stress levels.
Study Population:

• Participants were recruited between Jan. 2005 and March 2006 from San Francisco, Berkeley and Oakland, CA.

• Exclusion criteria:
  – Age (<18 years);
  – First/second hand smokers;
  – Pregnancy or breastfeeding;
  – Disease conditions;
  – Use of certain prescription medications;
  – Consumption of supplements (Iron, vitamins)
  – Body weight $\geq 300$ pounds or height $> 75$ inches.

• 396 were enrolled (age = 44 years, male = 34.6%).
Study Design Chart

396 Randomized
137 male
259 female

138 assigned to placebo
50 male
88 female

128 assigned to vitamin C
42 male
86 female

130 assigned to vitamin E
45 male
85 female

1000 mg/day vitamin C
800 IU/day vitamin E

2 months
Population Samples/Measurement

• Fasting venous blood:
  – Protected from light, maintained at <15°C, and processed within 6 hours.
• Waist and hip circumference.
• Body fat and BMI.
• Plasma ascorbic acid (vitamin C levels).
• Total or HDL, LDL chol. and trig.
• Malondialdehyde (MDA) conc. at baseline.
• Plasma F₂-isoprostane (F2-isop.) conc.
• C-reactive protein (CRP).
• ........
Publications

• The effect of Vitamin C and E on biomarkers of oxidative stress depends on baseline level (2008, Block, G).
  – Reduced plasma F2-isop. at the higher base levels.
  – Vitamin C having higher impact
  – Oxidative stress is elevated in obesity and may be a major mechanism for obesity related diseases.

• Vitamin C treatment reduces elevated C-reactive protein (2009, Block, G).
  – Vitamin E effect was not significant.
Pilot Study—Background

• Obesity:
  – Risk factor for many chronic diseases.
  – Sharp increase in the past 30 years worldwide (especially women and lower SES population).
  – Diet imbalance and genetic predisposition don’t explain.

• Emerging evidence:
  – POPs wide spread and population high exposure.
  – POP are persistent, lipophilic and accumulated in adipose tissue.
  – POPs are EDC and have toxic effects. POPs may be “Obesogens”, especially low-dose/lower molecular weight POPs.

• Antioxidant intervention:
  – It’s very difficult to remove the POPs from body.
  – Vitamin C may be a simple, safe and efficient remediation approach.
Why Vitamin C

• Vitamin C (ascorbic acid, AA):
  – One of the most natural antioxidants.
  – Can be formed in living tissue or supplemented.

• Significant biochemical function
  – Affects xenobiotic biotransformation by speeding up the microsomal hydroxylation process.
  – Reacts with free radicals, particularly those derived from oxidative stress, and enhances the solubility of xenobiotics to eliminate them in urine.

• Animal studies:
  – Vitamin C supplementation may directly reduce the levels of chlorinated hydrocarbons and PCBs as well as the adverse effects.
Study Design

- 15 healthy women (Vitamin C group).
  - 8 obese/overweight subjects.
  - 7 non-obese.

- 2 subjects have both serum and plasma.
  - test differences between two different matrices.

- POP levels need to be measured in the pilot study.
Study Objectives and Hypotheses

• If POP levels are associated with BMI levels.

• If there are POP level differences between plasma and serum.

• If vitamin C supplementation can reduce the POP levels.
Agilent GC-MSMS using electron ionization and multiple reaction monitoring.

- DB-5ms column (30 m × 0.25 mm, 0.25μm i.d.)
- ALL analytes were successfully and simultaneously measured.
Analytical Method Cont.

• Batch size = 20
  — 14 Samples
  — 2 Lab Blank Controls (LBC)
  — 2 Matrix Spiked Controls
  — 2 Standard Reference Material Controls

• Analytes = 29
  — 7 OCPs (HCB, b-BHC, oxychloridane, t-nonachlor, p,p’-DDE, o,p’-DDT, p,p’-DDT)
  — 5 PBDEs (BDE-47, 99, 100, 153, and 154)

• MDL (method detection limit)
  — Defined as 3 times the standard deviation (SD) of the lab blank concentration from current study.
Analytical Method Cont.

Twenty Nine Measured Compounds Plus 5 Internal Standards Separated Within 30 Minutes

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Counts x10^5

Acquisition Time (min)
Analytical Method Cont.

- **Recoveries**: surrogate spikes in SRM, matrix spiked controls and all samples were within ±25% error ranges.

- **Recovery correction**:
  - Surrogate---TCMX, PCB14, PCB65, 165, and BDE139
  - PCB and OCP values were corrected based on the PCB65.
  - PBDE values were corrected based on the BDE139.

- **Surrogate standard variation**:
  - PCB65 and BDE139 had percent coefficient variation (C.V.%) of 11% and 12%, respectively. C.V.% = standard deviation/average*100
  - PCB65 and BDE139 showed no differences in samples of before and after vitamin C supplementation.

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<td>%change geomean</td>
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Data Analysis

• Log transformation of the POP levels given that the levels have log normal distributions.

• Pearson’s correlation coefficient.

• Paired t-test on 15 pair of samples.
Results

• Lipid
• POP and BMI levels
• POP levels in serum and plasma
• POP levels before and after vitamin C supplementation
Lipid Measurement

Lipid measurements of before and after vitamin C intervention

Lipid average value before = 6.16 mg/mL
Lipid average value after = 5.84 mg/mL

---5.2% decrease
Lipid normalization or not?

Reasons not to normalize and use only wet weight based data:

• Fasting blood were obtained in both before and after vitamin C supplementation.

• Total lipid values dropped slightly after vitamin C supplementation.
  – May cover up vitamin C effects due to over adjustment.

• Lipid measurement were done in two different time points, therefore may introduce measurement variations.
PCB, OCPs and PBDE Levels

• All PCBs and OCPs were correlated except PCB101.

• PCBs/OCPs and PBDEs were not correlated.

• PBDEs were correlated with each other.
POP Levels and BMI

- Log transformed POP levels were tested correlations against BMI baseline levels.

- Most of the PCBs and OCPs except PCB101, oxyclordane, o,p’-DDT had strong positive correlations with BMI levels.

- No correlations found in PBDEs and BMIs.

- No correlations found in changes of POPs and BMI levels after vitamin C supplementation. A negative correlation was found in changes of BDE153 (corr = -0.5292, p=0.0425) and BMI levels.
Strong correlation of log transformed POP levels (PCBs and OCPs but not PBDEs) and BMI levels.

Correlations between PCB153, p,p'-DDE and BMIs

- Corr = 0.9099
- Corr = 0.9806
Two subjects have both plasma and serum samples.

PCBs in duplicate samples (plasma and serum) for before and after vitamin C intervention

PBDEs in duplicate samples (plasma and serum) for before and after vitamin C intervention

OCPs in duplicate samples (plasma and serum) for before and after vitamin C intervention
## POPs and Vitamin C

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• Statistical Analysis: Paired t-test
Possible Mechanism

Vitamin C

From: Recent Advances in Environmental Toxicology and Health Effects, Edts Larry W. Robertson and Larry G. Hansen, 2001
Conclusion

• Strong correlations were found between PCBs/OCPs and BMI.

• POP levels were identical in both serum and plasma.

• Small pilot study showed significant drop in 9 out of 29 measured POPs after vitamin C supplementation.
Future Plans and Remarks

• Include 30-35 more pairs of participants and confirm the findings.

• If confirmed, vitamin C supplementation may have important public health implications by improving the health of obese and overweight people.
Acknowledgement

We thank all the participants who provided samples.

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Disclaimer

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Thank You!