The effect of carotenoids and flavones on oxidative stress in endothelial cells

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Cardiovascular Mortality Risk Doubles with Each 20/10 mmHg Increment in Systolic/Diastolic BP*

*Individuals aged 40–69 years

BP Reduction of 2 mmHg Decreases the Risk of Cardiovascular Events by 7–10%

- Meta-analysis of 61 prospective, observational studies
- 1 million adults
- 12.7 million person-years

2 mmHg decrease in mean SBP

7% reduction in risk of ischemic heart disease mortality
10% reduction in risk of stroke mortality

Vascular wall

In the medial layer, collagen deposits increase, smooth-muscle cells diminish, and fibrous tissue increases.

The intimal layer thickens.

Lumen decreases.

Endothelial dysfunction.
Blood vessels:

- Vessel lumen
- Endothelium
- Tunica intima
- Smooth muscle cells
- Tunica adventitia
Endothelial function

- Dilation vs Constriction
- Thromboresistance vs Thrombosis
- SMC inhibition vs SMC proliferation
- Anti-inflammation vs Proinflammation

1 ½ kg.
6 tennis courts
Semi-permeable
Imbalance in Factors Affecting Vascular Tone and Structure

Constrictors/
Growth Promoters
Angiotensin II
Catecholamines
Endothelin-1
ROS
Cytokines
EDCF

Dilators/
Growth Inhibitors
Nitric Oxide
Prostacyclin
Bradykinin
EDHF

Nephron destruction nd renal failure

Vascular tone and structure

EDHF = endothelium-derived hyperpolarizing factors
ROS = reactive oxygen species
EDCF = endothelium-derived constricting factors
ROS Reduces the Biological Effects of NO

\[ \text{L-Arginine} \rightarrow \text{eNOS} \rightarrow \text{NO} + O_2^- = \text{OONO}^- \]

L-Citrulline

Fibroblast
VSMC

MΦ
PMN

Afferent Arteriole
Oxidative Stress: Endothelial Dysfunction and CAD/Renal Risk Factors

- Hypertension
- Diabetes
- Smoking
- LDL
- Homocysteine
- Estrogen deficiency

$\uparrow O_2^*$
$\uparrow H_2O_2$

Endothelial Cells and Vascular Smooth Muscle

Endothelial Dysfunction

- Apoptosis
- Leukocyte adhesion
- Lipid deposition
- Vasoconstriction
- VSMC growth
- Thrombosis

www.hypertensiononline.org
Endothelial dysfunction
Fibrinoid necrosis
Figure 1: Range of hypertensive cardiovascular disease from prehypertension to target-organ damage and end-stage disease.
Reactive oxygen species and endothelial dysfunction

Ang II $\rightarrow$ Reduced NO bioactivity

- ROS
- NO

Macrophages

Endothelium

- Chemotaxis factors (MCP-1)
- Selectins
- ICAMs
- Vasodilation

VSMC

Regulatory Functions of the Endothelium

**Normal**
- Vasodilation
  - NO, PGI₂, EDHF, BK, Č-NP
- Thrombolysis
  - tPA, Protein C, TF-I, vonWF
- Platelet Disaggregation
  - NO, PGI₂
- Antiproliferation
  - NO, PGI₂, TGF-β, Hep
- Lipolysis
  - LPL

**Dysfunction**
- Vasoconstriction
  - ROS, ET-1, TxA₂, A-II, PGH₂
- Thrombosis
  - PAI-1, TF, TxA₂
- Adhesion Molecules
  - CAMs, Selectins
- Growth Factors
  - ET-1, A-II, PDGF, bFGF, ILGF, Interleukins
- Inflammation
  - ROS, NF-κB
How to Assess ED

- Endothelium-dependent vasodilation
  - Acetyl choline or post-ischaemic FMD*
  - Coronary or forearm arteries

- Intima-media thickness (IMT)

- Microalbuminuria

- Plasma markers
  - ADMA, CRP, adhesion molecules

- Clinical diagnosis
  - flow-mediated dilatation (FMD)
  - asymmetric dimethylarginine (ADMA)
Can we prevent endothelial dysfunction?

Are there treatments for endothelial dysfunction?

Is it a reversible process?

Prehypertension = endothelial dysfunction?
Correcting Endothelial Dysfunction

- Risk factor modification (BP, DM, Smoking)
- Exercise and weight loss
- Blockade of the RAS- ACE Θ / ARB
- LDL reduction, HDL augmentation.
- PPAR-γ agonists
- Antioxidants
- Reducing homocysteine levels
- Improving insulin sensitivity
- Lowering CRP
- L-arginine.
Life style modifications

• Lose weight, if overweight
• Limit alcohol intake
• Increase physical activity
• Reduce salt intake
• Stop smoking
• Limit intake of foods rich in fats and cholesterol: DASH Diet

JNC VII and ESH recommendation
Blood Pressure Reductions Resulting from Various Lifestyle Modifications
Trials of Hypertension Prevention – Phase I

- Systolic Blood Pressure
- Diastolic Blood Pressure

<table>
<thead>
<tr>
<th>Measure</th>
<th>Net Mean Change (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight Loss</td>
<td>-5.67 kg</td>
</tr>
<tr>
<td>Reduced Sodium</td>
<td>-58.45 mmol/24 h</td>
</tr>
<tr>
<td>Added Calcium</td>
<td>1.22 mmol/24 h</td>
</tr>
<tr>
<td>Added Potassium</td>
<td>44.4 mmol/24 h</td>
</tr>
</tbody>
</table>

*All values are averages and are statistically significant at $P < 0.01$.*

Trials of Hypertension Prevention Collaborative Research Group.
Effect of Antihypertensive Monotherapy Is Augmented by Lifestyle Modifications

Diet-Exercise-Weight Loss Intervention Trial

Systolic Blood Pressure

Diastolic Blood Pressure

*Low-calorie, low-sodium diet and exercise.
†A single antihypertensive drug.

Health benefit of carotenoids

• Epidemiological studies
• Clinical data
• Clinical studies
• Animal experiments
• In vitro models
Epidemiologic evidence

High serum values of carotenoids such as α-carotene, β-carotene, and lycopene were found to be significantly associated with low hazard ratios for cardiovascular disease mortality.

Japan 3061 people; 12 years follow-up

Ito et al  J Epidemiol, 2006. 16, 154-60
The Kuopio Ischaemic Heart Disease Risk Factor Study
725 middle-aged men free of CVD at the study baseline

Low plasma lycopene concentration is associated with increased intima-media thickness of the carotid artery wall and excess incidence of acute coronary events and stroke:

Men in the lowest quartile of serum levels of lycopene had a 3.3-fold (P < 0.001) risk of the acute coronary event or stroke as compared with others

Rissanen et al
2. Br J Nutr, 2001 85, 749-54
Clinical Data
Decreased Oxidized LDL concentrations after consumption of the tomato meals

![Graph showing decreased Oxidized LDL concentrations after consumption of tomato meals compared to control meals. The graph includes time points at 0, 180, 240, and 360 minutes, with lower OxLDL levels observed after consuming the tomato meal.]
Decreased LDL oxidation after tomato ext consumption

Fatty meal containing 30 mg lycopene in the form of tomato oleoresin (LycoMato)

Aviram et al. Antioxidants & Redox Signaling 2, 492, 2000
IL-6 concentrations at baseline & after consumption of tomato and control meals.
Consumption of tomato meal increased Flow-Mediated Dilation (FMD)
Phytoene

Phytofluene

ζ-carotene

Lycopene
Lycopene 7%

Beta-carotene 0.2%

Phytoene 0.7%

Phytofluene 0.7%

Other carotenoids 0.3%

Tocopherols (Vit E) 2%

Phytosterols 0.7%

Other carotenoids 0.3%

LYC-O-MATO®

tכְׁלוּת חומרי המזון השומניים במאיצי עגבניות

LYC-O-MATO®

 Hebrew text: "תכולת חומרי המזון השומניים במיצוי עגבניות LYC-O-MATO®"
Clinical studies
Aims

Examining the effects of tomato lycopene on:

- Systolic and diastolic blood pressure
- Biochemical parameters:
  Serum lipids and lipoproteins
- Oxidative stress markers
Results

Systolic blood pressure

Significant reduction of SBP has been achieved as early as the sixth week of Cardi-O-Mato administration, -4.7 mm Hg and 10 mmHg on the eight week.
Significant reduction in DBP was demonstrated as early as the fourth week (-1.27 mm Hg, p=0.029) and 5 mmHg on the 8th week.
Results

Thiobarbituric acid reactive substances (TBARS)

Engelhard Y. Paran E. Am. Heart J 2006, 151: 100
Conclusions

- Tomato lycopene can reduce systolic and diastolic blood pressure significantly in newly diagnosed never treated mild hypertensives.

- The same effect of tomato lycopene was recorded in mild to moderate treated patients.

- Patients were compliant with the treatment at least for the relatively short term of these studies (12-16 weeks).

- No side effect was observed in any of the patients in all studies.
Study design
Prehypertensives

The study consists of three phases:

- **Phase I** Single-blind *placebo* run–in period for 4 weeks.
- **Phase II** Double-blind 5 arm parallel group for 8 weeks of:
  1. *Cardi-O-Mato 5mg*
  2. *Cardi-O-Mato 15mg*
  3. *Cardi-O-Mato 30mg*
  4. *Lycopene 15 mg*
  5. *Placebo*
- **Phase III** Long term treatment for 3 months with *Cardi-O-Mato 15mg*
Dose-response study in prehypertensive subjects

Study design

Placebo run in

Cardi-O-Mato 5mg

Cardi-O-Mato 15mg

Cardi-O-Mato 30 mg

Lycopene 15 mg

Placebo

phase I

4 w

phase II

8 weeks

phase III

16 weeks
Systolic blood pressure changes during phase I-II of the study

Significant reduction of SBP after 4 weeks already with Cardi-O-Mato 15 and 30 mg
Conclusions

• Tomato lycopene (Cardi-O-Mato) in doses 15mg and 30 mg reduced
  - SBP by 9 and 7mmHg compared to 1.97 mmHg by placebo or synthetic lycopene
  - DBP was reduced by 4.15 and 3.8 mmHg compared
    to 0.7 and -0.9 mmHg with synthetic lycopene and placebo
• The dose of 5mg caused very mild non-significant reduction in SBP and DBP
What is the mechanism for the antihypertensive effect of the tomato lycopene?

- At what point the anti-oxidant property is interferes with the vasoconstrictive forces?
- Are these actions restricted to the endothelium?
- Is there one active component that does all the work or is there a unique combination of micronutrients that responsible for the effect?
Effect of Lyc-O-Mato® Supplementation on Endothelial Function and Oxidative Stress

Clinical trial setting

• Healthy frequent smoking men (n=126) were randomized to receive placebo, Lyc-O-Mato® pills (6 or 15 mg lycopene) daily for 8-week

Endpoints:

• Endothelial function as measured by reactive hyperemia peripheral arterial tonometry (RH-PAT)
• Oxidative stress measured by plasma superoxide dismutase (SOD) activity; Alkaline comet assay for DNA damage in circulating lymphocytes; Plasma sVCAM-1, sICAM-1 and LDL particle size

Lyc-O-Mato® Increased lycopene blood level

Dose dependent increase in lycopene level

Changes in adhesion molecules ICAM and VCAM

Endothelial Function improved after Lyc-O-Mato® consumption

Endothelial Function measured by Reactive Hyperemia Peripheral Arterial Tonometry (RH-PAT)

Systolic blood pressure was reduced after Lyc-O-Mato® consumption

Regulatory Functions of the Endothelium

**Normal**

- **Vasodilation**
  - NO, PGI$_2$, EDHF, BK, C-NP

- **Thrombolyis**
  - tPA, Protein C, TF-I, vonWF

- **Platelet Disaggregation**
  - NO, PGI$_2$

- **Antiproliferation**
  - NO, PGI$_2$, TGF-β, Hep

- **Lipolysis**
  - LPL

**Dysfunction**

- **Vasoconstriction**
  - ROS, ET-1, TxA$_2$, A-II, PGH$_2$

- **Thrombosis**
  - PAI-1, TF, TxA$_2$

- **Adhesion Molecules**
  - CAMs, Selectins

- **Growth Factors**
  - ET-1, A-II, PDGF, bFGF, ILGF, Interleukins

- **Inflammation**
  - ROS, NF-κB
Anti-inflammatory effect in animal models

studies carried out in:

• in vitro – mouse macrophages
• in vivo – animal models:
  Paw edema in rats
  Peritonitis in mice

By Prof Rachel Levy
Ben-Gurion University
Endothelial dysfunction

Endothelial cells

Endothelin

NO

Carotenoids?

Vascular tone
Effect of carotenoids on NO induction and ET-1 secretion

![Graphs showing NO induction and ET-1 secretion](image)

**p-eNOS ser1177**

**Fold induction**  
1.0  1.5  1.8  1.6

**eNOS**

**ET-1 secretion** (% of basal)

Armoza & Paran  J Hypertension Dec 2012
Adhesion of leukocytes

Diagram showing the process of leukocyte adhesion involving capture, rolling, firm adhesion, and transmigration. Key components include leukocytes, endothelium, selectins, Immunoglobulin, and chemotactic factors.
Endothelial dysfunction

Endothelial cells

Endothelin

NO

Carotenoids?

Vascular tone

Anti-adhesive
Adhesion assay

Endothelium

Neutrophils

Calcein-AM stained Neutrophils

Total

Adherence
1. Lycopene, Lutein and tomato oleoresin inhibit the adhesion of white blood cells to stimulated EC

![Graph showing inhibition of adhesion](image)

**EA.hy 926 (cell-line)**
hUVEC (primary cell culture)

Adhesion (fold induction)

% of inhibition

No cytokine
control
oleoresin
lycopene
lutein
lutein

TNFα 10 ng/ml

Armoza, & Paran J Hypertension Dec 2012
Adhesion of leukocytes

selectins
P-selectin (C,I)
E-selectin (I)

Immunoglobulin
ICAM-1 (C,I)
ICAM-2 (C)
VCAM-1 (C,I)

NFkB
2a. Carotenoids and tomato-oleoresin reduce expression of adhesion molecules (ICAM-1 & VCAM-1) in stimulated EC

**A** EA.hy 926 cells

<table>
<thead>
<tr>
<th></th>
<th>ICAM-1 expression (%) of control</th>
<th>VCAM-1 expression (%) of control</th>
</tr>
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<tbody>
<tr>
<td>cal</td>
<td>[Image]</td>
<td>[Image]</td>
</tr>
<tr>
<td>basal veh oleo lyc lut</td>
<td>[Image]</td>
<td>[Image]</td>
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TNFα (10 ng/ml)

**B** hUVEC

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TNFα (10 ng/ml)

** p<0.001, * p<0.05 vehicle vs. basal
## p<0.001, # p<0.05 treatments vs. vehicle

Armoza, & Paran J Hypertension Dec 2012
Effects of carotenoids on TNF-α-induced NF-κB activation in endothelial cells

Armoza, & Paran. J Hypertension Dec 2012
To further investigate whether carotenoids act through regulation of NF-κB pathway, the effects of the carotenoids on dislocation of NF-κB-complex components (IκB, p65 and p50) were tested. Thirty minutes following exposure to TNF-α, dramatic reduction in cytoplasm IκB was detected in both cell types.
Reduction in cytoplasm IkB

Armoza, & Paran J Hypertension Dec 2012
Reduction in the production of the subunits P65 and P50

E

p-65 (nuc)  Lamin

F

p-65 expression (% of basal)

basal  veh  oleo  lyc  lut

TNF-α

#  *  **

G

p-50 (nuc)  Lamin

H

p-50 expression (% of basal)

basal  veh  oleo  lyc  lut

TNF-α

#  *  **  **

Armoza, & Paran  J Hypertension Dec 2012
Conclusions

(in-vitro models)

- Carotenoids attenuate the adhesion of neutrophils to stimulated endothelial cells.
- Carotenoids reduce expression of adhesion molecule ICAM-1
- Carotenoids inhibit activation of transcription factor NFkB

Carotenoids inhibit vasoconstrictive and pro-inflammatory effects and improve endothelial function
Thank you