Food for Thought:
Pomegranate Juice Science for Promoting Health & Wellness
Today’s Speaker

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Specialty Areas: Behavioral Neuroscience, Experimental Psychology
Disclosure

• POM Wonderful

• NIH P01 Program Project Grant (*Center for Brain Hemorrhage Studies*; 2014-2019)
Agenda

Pomegranates 101
- Fruit Anatomy
- Whole-Pressed Process
- AOX Potency
- Bioavailability
- Mechanism of Action

Memory & Cognition Science
- Key Plant Compounds
- Normal Aging in the Brain and Cognitive Decline
- Dementia
- Role of Diet
- Preclinical Models
- Animal Data
- Human Trials
- Synergistic Mechanisms for Neuroprotection

Appendix
POM 101

Brain

Athlete

Nutrition
Anatomy of a Pomegranate
Whole-Pressed POM Juice

Arils (seeds + juice sacs)
- Anthocyanins
- Hydrolyzable tannins

Rind & Pith
- Hydrolyzable tannins
- Ellagic acid

Juice
(16° Brix)

OTHER
- Flower (persistent calyx, aka crown): Mainly hydrolyzable tannins, with a smaller amounts of ellagic acid and trace anthocyanins
- Seeds (oil): Fatty acids (e.g. punicic, linoleic, linolenic), no phenolics

Antioxidant Potency

Total phenolics as gallic acid equivalents (GAEs)

Antioxidant potency as average of four assays: DPPH, ORAC, TEAC, FRAP
Areas of Interest

- Neuroprotection
- Vascular Health
- Microbiome & Gut Health
- Skin Health
- Athletic Performance
Polyphenols are Metabolized by Gut Microbiota

Espin et al., eCAM, 2013.
Ellagitannins are metabolized by the microbiota

**Bioavailability**

Pharmacokinetic profile of ellagic acid after consumption of 180ml pomegranate juice (318mg punicalagins & 12 mg of free EA). Means ± SD (n=18)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± SD</th>
<th>2.5% quantile</th>
<th>97.5% quantile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rate constant of elimination, ( \mu\text{mol}/\text{h} )</td>
<td>0.98 ± 0.1</td>
<td>0.7</td>
<td>1.15</td>
</tr>
<tr>
<td>Rate constant of absorption, ( \mu\text{mol}/\text{h} )</td>
<td>1.08 ± 0.14</td>
<td>0.92</td>
<td>1.51</td>
</tr>
<tr>
<td>( T_{\text{max}} ), \text{h}</td>
<td>0.98 ± 0.06</td>
<td>0.86</td>
<td>1.09</td>
</tr>
<tr>
<td>( C_{\text{max}} ), ( \mu\text{mol}/\text{L} )</td>
<td>0.06 ± 0.01</td>
<td>0.05</td>
<td>0.07</td>
</tr>
<tr>
<td>AUC, ((\text{mmolxh})\text{L}^{-1})</td>
<td>1.17 ± 0.02</td>
<td>0.13</td>
<td>0.21</td>
</tr>
<tr>
<td>( T_{1/2E} ), \text{h}</td>
<td>0.71 ± 0.09</td>
<td>0.6</td>
<td>0.98</td>
</tr>
</tbody>
</table>

Numbers of Subjects with EA and EA Metabolites detected in urine samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ellagic Acid</th>
<th>Urolithin A-glucuronide</th>
<th>Urolithin B-glucuronide</th>
<th>Dimethylellagic Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>0/18 (0%)</td>
<td>0/18 (0%)</td>
<td>0/18 (0%)</td>
<td>0/18 (0%)</td>
</tr>
<tr>
<td>By 24 hr</td>
<td>5/18 (28%)</td>
<td>11/18 (61%)</td>
<td>3/18 (17%)</td>
<td>15/18 (83%)</td>
</tr>
<tr>
<td>By 48 hr</td>
<td>0/18 (0%)</td>
<td>16/18 (89%)</td>
<td>5/18 (28%)</td>
<td>0/18 (0%)</td>
</tr>
</tbody>
</table>

Propered Mechanism of Action

Pomegranate Juice

Polyphenol metabolism in the Gut

Oxidative Stress

Prebiotic Activity
Healthy Microbiome

Reactive Oxygen Species
Subclinical Inflammation

Protect Nitric Oxide Function
Support Cellular & Tissue Health

Neuroprotection
Circulatory Function
Muscle Health

Memory & Cognition
Neonatal Brain
Health & Wellness
Strength Recovery

Health & Wellness
Insights into MOA

Pomegranate Juice Extended Lifespan and Decreased Intestinal Fat Deposition in C. elegans Model Organism

Jolene Zheng¹, Mingming Wang¹, Chenfei Gao², Steven B. Heymsfield³, Roy J. Martin¹, Michael J. Keenan¹, John W. Finley¹, Frank L. Greenway³, Fredric M. Enright³ and Zhaoping Li⁴.¹School of Nutrition and Food Sciences,²Pennington Biomedical Research Center,³School of Animal Sciences, Louisiana State University, Baton Rouge, LA, ⁴Department of Nutrition, University of California, Davis, CA.

Introduction

Pomegranate juice (PJ) is rich in polyphenols, ellagic acid metabolites, and hydroxy-dihydrobenzopyran-4-one derivatives. Consumption of pure juice shows antioxidant properties, enhances the growth of gut bacteria, increases short chain fatty acids, prevents Clostridium difficile infections, augments memory function and increases brain activity in humans.

Hypothesis: PJ extends lifespan and reduces intestinal fat deposition in C. elegans model organism.

Methods

Dose response of PJ on lifespan and IFD

Dose response of POMxm on lifespan and IFD

Discussions

PI dose-dependently extended lifespan at lower doses, which was reduced at higher doses in wild-type (N2) and in FOXO knockout (daf-16) mutant to a lesser extent. IFD was decreased in both strains (P<0.05). PJ, with less sugar content, extended lifespan and reduced IFD, and excessive sugar reversed the beneficial effect of low dose PJ. These effects were partially mediated by daf-16 in the C. elegans model organism. Pomegranate extract (POMxm) treatment dose-dependently extended lifespan without the “biphasic shape”. Intestinal fat deposition (IFD) was decreased at lower doses and increased at higher doses.

References

Acknowledgements

The nematode strains used in this work were provided by the Caenorhabditis Genetics Center, which is funded by the NIH National Center for Research Resources (NCRR).
In response to environmental neuronal, humoral, or mechanical stimuli (e.g., ACh, bradykinin, or shear stress), NO is synthesized in endothelial cells (EC) from L-arginine (L-Arg) by activated form of endothelial NO synthase.

NO diffuses to neighboring vascular smooth muscle cells which regulates numerous target proteins (e.g., \( I_{KCa} \) channel current, \( I_{CaL} \) L-type Ca\(^{2+} \) channel current, \( I_{CaP} \) sarcolemma Ca\(^{2+}\)-ATPase pump, and myosin light chain phosphatase (MLCP)), and leads to vascular smooth muscle cell relaxation, “vasodilation”.

The mechanism of action appears to include:

- Correction of perturbed eNOS expression & NO production
- Protection of NO from oxidative destruction
- Protection of NO mediated biological action
Phytochemicals

Vitamins / Minerals / Carbohydrates

Some have strong odors and/or bitter tastes to protect from metabolic/environmental insults

- Terpenes (e.g., carotenoids)
- Phenols (e.g., flavonoids, tannins, anthocyanins)
- Organosulfurs

Consuming these seems to confer similar beneficial properties

Many currently available pharmaceuticals have roots in traditional herbal medicines
Aging Brain and Memory

• A steady decline in brain functions are seen starting about age 30 especially in many types of memory with aging.

• There are changes to the brain though loss of brain cells is minor until age 20. The brain is made of nerves which send out branches called axons. These axons are like wires that conduct electricity and they are coated with a fat called myelin. The length of these wires in the brain shorten 10% per decade after age 20.

• Inflammation may have a role in loss of memory function.
Age Increases Risk for Cognitive Decline

• Life expectancy at birth: 47 years in 1900; 78 years today.
• Approximately 40% of people age 65 and older have age-associated memory impairment.
• Risk of dementia is 10% by age 65 and older and nearly 50% by age 85 and older.
The Aging Brain
The Aging Brain
The Aging Brain

[Diagram of a brain with labeled regions: Frontal lobe, Parietal lobe, Occipital lobe, Hippocampus, Medial view of the brain showing severe AD]
Aging in the Brain

- Clumps together in plaques and causes inflammation, oxidative stress, etc.

- Cleavage by α-secretase:
  - prevents Aβ production
  - produces sAPPα
  - neuroprotective

- β + γ-secretase (enzymes) clip APP to produce Aβ

- α-secretase site lies within the “Aβ” domain
Epidemiological studies suggest that:

• “Western” diet (high carbohydrate / high fat) increases risk.

• “Mediterranean” diet (a lot of fruits and vegetables) may decrease risk or slow progression.

• People whose diets included curry performed significantly better on neuropsychological tests of cognitive performance.

• Daily consumption of phenol-rich fruits and vegetables (or juices) appear to support cognitive function.

Isolated dietary phytochemicals do not seem to be as effective:

• A lower incidence of AD is associated with high intake of dietary vitamins E and C (but not supplements).
Rationale for Study

Epidemiological studies link antioxidant consumption and cognitive protection.

Pomegranate juice shown to have highest antioxidant capacity among fruit juices (using multiple antioxidant assays).

Nitric oxide impacts blood flow.

Previous clinical trials of antioxidant use in normal aging and Alzheimer’s disease yield mixed results.

Studies examining mechanisms of polyphenol treatment in humans are limited.

Preclinical Models

Experimental preclinical studies have provided evidence that various aspects of AD can be manipulated by plants and their phytochemicals. However, few experimental clinical trials have been published assessing the effects of plants or phytochemical compounds in humans.

Recent study showed that a grape / blueberry polyphenol-rich diet prevented age-related cognitive decline in mice.

My 2006 study was first to show a cognitive and physical benefit of pomegranate juice in a mouse model of Alzheimer’s disease.
Pomegranate juice decreases amyloid load and improves behavior in a mouse model of Alzheimer’s disease

Richard E. Hartman, a,* Aartie Shah, b,c Anne M. Fagan, b,c Katherine E. Schwetye, b,c Maia Parsadanian, b,c Risa N. Schulman, d Mary Beth Finn, b,c and David M. Holtzman b,c,d,e,*
How?

Enhanced α-secretase processing of Aβ

Control

Pomegranate

β-CTF

α-CTF

→ less neurotoxic Aβ

→ more neuroprotective sAPP-α
How?

Enhanced Neurogenesis
Further Experimental Evidence in Animal Models

- 2 other papers using pomegranate in mouse models of Alzheimer’s disease
  - other plants have effects on plaque / cognitive decline in similar models (tea, caffeine, garlic, blueberries)
- Pomegranate also effective for:
  - rat models of TBI
  - mouse models of stroke
- we have data from mice showing that a grape-enhanced diet reduces the size of a TBI

\[ p < 0.05 \]
Experimental Evidence in People

Research Article

Pomegranate Supplementation Protects against Memory Dysfunction after Heart Surgery: A Pilot Study

Susan A. Ropacki,⁠¹ Sapna M. Patel,⁠² and Richard E. Hartman⁠²

Average performance by domain

- Working
- Immediate
- Delayed
- Retention

Z-score ± SEM

- Improved compared to baseline
- Impaired compared to baseline

Placebo
Pomegranate

Ropacki, eCAM, 2013
Effects of Pomegranate After Ischemic Stroke

**Pilot Data**

- **Improved Cognitive Recovery**
- **Improved Physical Recovery**
- **Cognitive / Physical Recovery**
- **Decreased Length of Hospital Stay**

Data to be presented at Society for Neuroscience Annual Meeting, 2017.
Consumption of anthocyanin-rich cherry juice for 12 weeks improves memory and cognition in older adults with mild-to-moderate dementia

Katherine Kent¹ · Karen Charlton¹ · Steven Roodenrys² · Marijka Batterham³ · Jan Potter⁴ · Victoria Traynor⁵ · Hayley Gilbert² · Olivia Morgan² · Rachelle Richards²
Pomegranate Juice Augments Memory and fMRI Activity in Middle-Aged and Older Adults with Mild Memory Complaints

Susan Y. Bookheimer,1,2 Brian A. Renner,1,2 Arne Eksstrom,1,2 Zhaoping Li,1,2 Susanne M. Henning,1,2 Jesse A. Brown,1,2 Mike Jones,1,2 Teena Moody,1,2 and Gary W. Small1,2

Early Clinical Data

Bookheimer et al., eCAM, 2013
Abstract. Despite increasing emphasis on the potential of dietary antioxidants in preventing memory loss and on diet as a precursor of neurological health, rigorous studies investigating the cognitive effects of foods and their components are rare. Recent animal studies have reported memory and other cognitive benefits of polyphenols, found abundantly in pomegranate juice. We performed a preliminary, placebo-controlled randomized trial of pomegranate juice in older subjects with age-associated memory complaints using memory testing and functional brain activation (fMRI) as outcome measures. Thirty-two subjects (28 completers) were randomly assigned to drink 8 ounces of either pomegranate juice or a flavor-matched placebo drink for 4 weeks. Subjects received memory testing, fMRI scans during cognitive tasks, and blood draws for peripheral biomarkers before and after the intervention. Investigators and subjects were all blind to group membership. After 4 weeks, only the pomegranate group showed a significant improvement in the Buschke selective reminding test of verbal memory and a significant increase in plasma trolox-equivalent antioxidant capacity (TEAC) and urolithin A-glucuronide. Furthermore, compared to the placebo group, the pomegranate group had increased fMRI activity during verbal and visual memory tasks. While preliminary, these results suggest a role for pomegranate juice in augmenting memory function through task-related increases in functional brain activity. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3736548/
# Subject Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean (SD)</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Pomegranate</td>
<td>Placebo</td>
</tr>
<tr>
<td></td>
<td>( n = 15 )</td>
<td>( n = 13 )</td>
</tr>
<tr>
<td>Mini-mental state examination</td>
<td>28.0 (1.5)</td>
<td>27.8 (1.5)</td>
</tr>
<tr>
<td>Age, y</td>
<td>63.1 (8.0)</td>
<td>62.0 (7.8)</td>
</tr>
<tr>
<td>Female, no. (%)</td>
<td>11 (73.3)</td>
<td>10 (76.9)</td>
</tr>
<tr>
<td>TEAC baseline</td>
<td>1712 (299)</td>
<td>1927 (461)</td>
</tr>
<tr>
<td>Buschke selective reminding test</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recall</td>
<td>85.0 (11.7)</td>
<td>86.5 (12.5)</td>
</tr>
<tr>
<td>Consistent long-term retrieval</td>
<td>52.8 (19.9)</td>
<td>55.8 (25.5)</td>
</tr>
</tbody>
</table>

Bookheimer et al., eCAM, 2013
Methods

Subjects required to remain on low polyphenol diet for 1 week before baseline and throughout trial

- Restricted fruits and vegetables, onions, tea, chocolate, dried beans

Placebo drink

- Flavor-matched, containing sugar, citric acid and food color to match the calories, taste, and color of the pomegranate juice

Subjects given 1 week of juice and visited clinic each week to return empty bottles

- Ensured compliance
- Brief weekly meetings with dietitian reinforced low polyphenol diet

Bookheimer et al., eCAM, 2013
Measurement Tools: Functional MRI
Measurement Tools: Item Memory and Which Store was Seen

Original store façade (from encode phase)  Altered store façade

Bookheimer et al., eCAM, 2013
Clinical Results. Plasma Antioxidant Activity and Pomegranate Metabolites

1. The PJ group had a significantly higher chemical measurement of antioxidant activity after day 28 compared with baseline than the placebo group (unpaired t = 2.8, df = 25, p < 0.05).

2. Similarly, plasma urolithin A-glucuronide increased significantly in the PJ group, but not in the placebo group (unpaired t = 3.75; df = 24, p < 0.001).

Early Clinical Data

• n=28
• 4 weeks
• 8oz PJ, daily

Bookheimer et al., eCAM, 2013
Clinical Results. Memory Tests

1. Within-group analysis of changes following treatment: The **PJ group performed significantly better in memory testing compared to baseline (alternating test forms; p=0.017)**. No significant difference found for the placebo performance scores (p>0.3).

2. Between groups: The $t_2$ vs. $t_1$ improvement in memory scores was significantly greater in the PJ group compared to placebo on the total recall measure ($2$-tailed $t=2.3; p=0.029$). PJ group recalled more items on consistent long-term retrieval compared to controls (p=0.022).

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**Clinical Study**
- n=28
- 4 weeks
- 8oz PJ, daily

Bookheimer et al., eCAM, 2013
Clinical Results. Brain Imaging Studies (Visual Memory fMRI task between groups T2 vs T1)

Significant activation in visual pathways (within-group means for both groups and at both time points) including bilateral occipital cortex extending into temporal fusiform and parahippocampal gyrus, as well as activation in subcortical region across the 3 task conditions, consistent with visual memory processing and spatial navigation.

The PJ group showed greater fMRI activation than the placebo group in the t2 versus t1 contrast (p=0.05), located bilaterally in regions of the basal ganglia and thalamus.

At a lower threshold of Z=1.7 (p<0.05), additional regions in the left inferior frontal gyrus and left middle frontal gyrus were recruited.

In contrast and as predicted, no brain regions were more active in t2 versus t1 for the placebo group compared to pomegranate.

Figure 3: Visual memory fMRI task: between-groups T2 versus T1 ANOVA. Regions showing greater activation for t2 > t1, for the pomegranate juice group > placebo group (group by time interaction), were found bilaterally in the basal ganglia and thalamus, including caudate, putamen, and pallidum (ANOVA, Z > 2.0, P = .05, corrected for multiple comparisons). Additional regions significant at an uncorrected threshold (Z > 1.7) are listed in Table 2.
Clinical Results. Brain Imaging Studies (Visual Memory fMRI task between groups T2)

Secondary analysis comparing PJ versus placebo groups for the visual memory versus control.

This analysis directly compares visual memory processing between groups after treatment. The PJ group showed significantly greater activation than the placebo group in right occipital and right fusiform regions, extending into parahippocampal cortex (p=0.05). These regions are typically engaged during visual navigation memory tasks, indicating greater recruitment of visual memory regions in the PJ group.
**Clinical Results.** Brain Imaging Studies (Verbal Memory fMRI task)

Verbal Memory - Only the PJ group showed significant activation \((p=0.05)\) in the post-treatment session compared to pretreatment for any brain regions for the learn versus rest condition (using paired \(t\)-tests). Activation was not observed in the placebo group.

![Brain imaging study images](image)

*Figure 7: Verbal memory fMRI task: activations for the pomegranate group for \(t_2 > t_1\). For the pomegranate group in the verbal memory task, there were significant activations between sessions, in the left inferior temporal gyrus and right occipital lobe (paired \(t\)-test between time points, \(Z > 2.0, P = .05\), corrected for multiple comparisons). No regions were more active for \(t_2\) versus \(t_1\) in the placebo group.*

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**Clinical Study**
- \(n=28\)
- 4 weeks
- 8oz PJ, daily

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Bookheimer et al., eCAM, 2013
Summary of Findings

Pomegranate juice intervention activated only memory-related brain regions and did not result in decreases in activation.

No activation increases observed in the control group.

Immediate and long-term verbal memory improved.

Taken together, results support the hypothesis that drinking 8 ounces of pomegranate juice each day for one month may increase task-related brain activation and improve memory ability in older adults with pre-existing mild memory complaints.
Proposed Model for Neuroprotection: Synergistic Mechanisms

- Punicalagins
- Reduce Oxidative Stress
- Anti-inflammatory by suppressing genes that produce stress-related proteins (e.g., NfκB)
- Improve Synaptic Function (ginkgo) / Prevent damage (curcumin)
- Inhibit Neuronal Death
- Increase Neurogenesis
Proposed Model for Neuroprotection: Synergistic Mechanisms

Protect from Aβ exposure
- Ginkgo
- Omega-3s
- Cannabinoids
- Nicotine

Reduce Aβ levels in brain
- Prevent Aβ production
- Increase α-sec processing (punicalagins, ginkgo, tea, huperzine-a, omega-3s)
- Decrease β-sec processing (punicalagins, luteolin, folic acid)
Proposed Model for Neuroprotection: Synergistic Mechanisms

- Prevent Aβ aggregation
  - Curcumin
  - Garlic
  - Tea
  - Nornicotine

- Bind to Aβ and prevents binding to other Aβ
  - Cannabinoids
  - Galantamine
  - Huperzine-a

- Bind to and inhibits AChE activity, reducing aggregation

- Clear Aβ from the brain into periphery
  - Resveratrol
Summary

Aging associated with slow, gradual (or sometimes acute) insults to the brain, leading to oxidative stress / inflammation, synaptic impairment & loss, neurotransmitter deficits, hypersensitivity to damage, neuronal death.

Regular consumption of fruits / vegetables / dietary phospholipids / wine may support cognitive health.

- phytochemicals achieve these results by a wide variety of mechanisms
- a varied diet may provide more protection than supplemental vitamins

Healthy lifestyles can lower the risk of certain chronic diseases, and scientists are very interested in the possibility that a healthy lifestyle might have a beneficial effect on Alzheimer’s as well.*

- A varied healthy diet and exercise supports this goal

* NIH. Preventing Alzheimer’s Disease: What Do We Know?
Thank You & Stay in Touch

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APPENDIX
Scientific Literature Update
42 articles relevant to pomegranate science published over the last year, 29 of which are articles on urolithins


- **Urolithin A attenuates ox-LDL-induced endothelial dysfunction partly by modulating microRNA-27 and ERK/PPAR-γ pathway.** Han et al., Mol Nutr Food Res. 2016 Sep;60(9):1933-43.

- **Effects on Nitric Oxide Production of Urolithins, Gut-Derived Ellagitannin Metabolites, in Human Aortic Endothelial Cells.** Spigoni et al., Molecules. 2016 Aug 2;21(8).

- **Urolithins inhibit LPS-induced inflammation in RAW 264.7 murine macrophages.** Piwowarski et al., Mol Nutr Food Res. 2015 Nov;59(11):2168-77.


Note: The full body of scientific research on pomegranates is not included in this presentation, only selected studies are depicted for illustration purposes. Note, that research is preliminary and further research is needed on larger populations to establish causation.
Pomegranate’s Exercise Literature

Four Pre-Clinical Studies

1. Anti-fatigue effects of polyphenols extracted from pomegranate peel.

2. Pomegranate and green tea extracts protect against ER stress induced by a high-fat diet in skeletal muscle of mice.

3. Urolithin A induces mitophagy and prolongs lifespan in C. elegans and increases muscle function in rodents.

4. Pomegranate extract prevents skeletal muscle of mice against wasting induced by acute TNF-α injection.

Eight Clinical Trials

1. Ellagitannin consumption improves strength recovery 2-3 d after eccentric exercise.

2. The effect of pomegranate juice supplementation on strength and soreness after eccentric exercise.

3. Effects of pomegranate extract on blood flow and running time to exhaustion.
   Trexler ET, et al., Appl Physiol Nutr Metab. 2014 Sep;39(9):1038-42. [UNC-Chappel Hill]

4. Effects of Differing Dosages of Pomegranate Juice Supplementation after Eccentric Exercise
   Machin DR et al., Physiology Journal. Volume 2014, Article ID 271959, 7 pages. [POM with Coyle]

5. Impact of Polyphenol Antioxidants on Cycling Performance and Cardiovascular Function
   Trinity JD, et al., Nutrients 2014, 6, 1273-1292 [POM with Coyle]

6. Effects of Pomegranate Juice in Circulating Parameters, Cytokines and Oxidative Stress Markers in Endurance-Based Athletes.
   Fuster-Munoz et al., Nutrition. Dec 2015

7. Effects of pomegranate extract on blood flow and vessel diameter after high-intensity exercise in young, healthy adults.

8. Pomegranate Supplementation Accelerates Recovery of Muscle Damage and Soreness and Inflammatory Markers after a Weightlifting Training Session.