Effects of oral supplementation with L-citrulline on recovery from myocardial infarction in mice

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Since 1900, cardiovascular disease has been the leading cause of death in every year except 1918.

CVD kills more people each year than the next five leading causes of death combined.
What happens during a heart attack?

1. The left coronary artery (LCA) becomes blocked

2. The tissue of the left ventricle is deprived of blood

Picture by J. Heuser. Wikimedia Commons
What happens in the *left ventricle* during a heart attack?

- Changes in mRNA transcription (Harpster et al.)
  - Arginase production goes up
  - Nitric oxide synthase production goes down

- This means less arginine is converted to nitric oxide and is instead converted to ornithine
What happens in the left ventricle during a heart attack?

- Up regulated enzymes
- Down regulated enzymes
Question

• Nitric oxide is a vasodilator and improves contractility

• Nitric oxide production goes down during MI

• Would increased levels of nitric oxide make it easier to survive a heart attack?
Would increased levels of nitric oxide make it easier to survive a heart attack?

• Questions to answer

1. Can the concentration of arginine be artificially increased in the blood? My project was to answer Question 1.

2. Does a higher concentration of arginine lead to more nitric oxide in heart tissue?

3. Does the heart function better physiologically as a result of having more arginine?

This data was evaluated and I will present some of the results.
Q1. Can the concentration of arginine be artificially increased in the blood?

- Problem: getting dietary arginine past the liver
  - This problem was observed by Shulman et al.

- Possibility: give dietary citrulline
Q1. Can the concentration of arginine be artificially increased in the blood?
## Experimental Samples

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 28</th>
<th>Day 56</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart attack</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Citrulline fed</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Water fed</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td>No heart attack</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Citrulline fed</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>Water fed</td>
<td>10</td>
<td>4</td>
</tr>
</tbody>
</table>
Procedure

1. Mice were fed citrulline (once/day for 56 days)

2. Blood was collected at Day 28 and 56. Plasma was separated.

3. Plasma was diluted with 0.25x PBS
4. Diluent was centrifuged through a filter

5. Eluent was aliquotted and derivatized

6. Derivative was analyzed by amino acid analysis, HPLC

7. Data was analyzed for significant results by 3-way ANOVA
Results

• The observed plasma amino acid levels were comparable to published values

• The amino acid levels between males and females showed no significant difference by T-test

• There were no significant changes in the plasma concentration of arginine, citrulline, or ornithine between mice that were gavaged with water versus mice gavaged with citrulline.
Discussion

• The observed plasma amino acid levels were comparable to published values
  – This suggested our amino acid analysis procedure was accurate and precise

• Amino acid levels between males and females showed no significant difference by T-test
  – So values from males and females were combined for the 3-way ANOVA (analysis of variance)
• Our analysis would have detected a change in plasma amino acid concentration of 30% or greater.
  – Thus, within that limit, there were no significant changes for arginine, citrulline, or ornithine plasma concentrations upon citrulline gavage.
Would increased levels of nitric oxide make it easier to survive a heart attack?

• Questions to answer

1. Can plasma arginine and citrulline concentrations be increased by dietary supplementation with citrulline?  
   No

2. Do higher blood levels of arginine lead to more nitric oxide in cardiomyocytes?  
   ???

3. Does the heart show improved recovery from a myocardial infarction if the animal is provided with supplemental dietary citrulline?  
   Yes
Q3. Does the heart show improved recovery from a myocardial infarction if the animal is provided with supplemental dietary citrulline?

Of the mice who received a heart attack...

mice who received citrulline showed better fractional shortening than mice who received water

• There did seem to be an effect of citrulline feeding
  – But this effect was not apparent in the blood arginine, ornithine, or citrulline concentrations

• If the experiment were to be repeated
  – Look for nitric oxide directly
  – Look for changes in amino acids in tissues rather than blood
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References


• Schulman, SP. et al. L-Arginine Therapy in Acute Myocardial Infarction, the Vascular Interaction With Age in Myocardial Infarction (VINTGE MI) Randomized Clinical Trial. *JAMA*. 2006; 295(1):58-64.