EFFECTS OF WATERMELON POWDER ON RISK FACTORS OF
CARDIOVASCULAR DISEASE AND INFLAMMATION IN ATEROGENIC-
DIET FED RATS

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GRADE: 91/100

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Abstract

Objective

To determine if watermelon powder improves the risk factors of CVD and inflammation in atherogenic diet-fed rats.

Hypothesis

Watermelon powder will reduce the risk factors of CVD and inflammation by increasing antioxidant.

Methods

Forty male Sprague-Dawley rats were equally divided into four groups that included two atherogenic diets (control diet and watermelon powder diet) and two treatments (DSS and no DSS). Body weight, food intake, and water intake were measured. After four weeks, the rats were euthanized and blood was collected for serum total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol, CRP, LDH, and total antioxidant assessment.

Results

The watermelon powder group had favorable effects on triglycerides, total cholesterol, LDL-cholesterol, LDH, CRP, and total antioxidant capacity compared to the control group (p<0.05). The weights of the liver, spleen, and epididymal fat did not show any significant difference between groups.

Conclusion

Watermelon powder demonstrated to have favorable effects on triglycerides, total cholesterol, LDL-cholesterol, LDH, CRP, and total antioxidant capacity compared to the
control group, suggesting that it may reduce inflammation and the risk for developing cardiovascular disease.

Key Words: watermelon; inflammation; atherogenic diet; cardiovascular disease; citrulline

Introduction

Cardiovascular disease (CVD) is the number one killer of men and women in the United States [1]. Genetics, age, sex, and race are uncontrollable risk factors for CVD [2]. Controllable risk factors for CVD include physical inactivity, smoking, obesity, high LDL-cholesterol, low-HDL cholesterol, and diet [2]. The typical Western high-fat, high-sugar, high-cholesterol atherogenic diet is a significant factor for growing number of CVD cases in the United States. Diet, as previously mentioned, can be changed to decrease the risk, or severity, of CVD. Research has shown that a diet rich in fruit and vegetables is beneficial for the prevention of cardiovascular disease, the number one killer of men and women in the United States [3] and [4]. Inflammation is also associated with CVD. There have been continuous studies on the benefits of consuming certain fruits and vegetables to lower risk of cardiovascular disease and lower inflammation [5].

There is researching indicating that the amino acid citrulline has been therapeutically effective in improving cardiovascular function [6]. Citrulline is made in the body and is found naturally in fruits such as watermelon. Citrulline is a precursor to nitric oxide, which dilates blood vessels, thus increasing blood flow. Watermelon
(Citrullus lanatus or C. lanatus) is a good source of citrulline and may be useful in treating and preventing cardiovascular disease. In a recent study, researchers concluded that watermelon decreased body weight, decreased cholesterol, and decreased inflammation in LDL-deficient mice [7]. Additionally, watermelon contains the antioxidant lycopene. There has been research on the anti-inflammatory effects of lycopene [5]. However, the effects of watermelon on inflammation have not been determined.

The purpose of this study was to determine if watermelon powder improves the risk factors of CVD and inflammation in atherogenic diet-fed rats. The hypothesis was that watermelon powder would reduce the risk factors of CVD and inflammation by increasing antioxidant.

Materials and methods

Animals and diets

Forty male Sprague-Dawley rats (Harlan, Placentia, CA) were housed individually in wire-bottomed cages on a 12-hour light-dark cycle in a research room at San Diego State University. Both temperature and humidity were controlled at approximately 20-24°C and 40-45% humidity. Water and food were available at all times for the animals. The procedures and training for use of the animals were conducted and approved by the San Diego State University animal subjects committee.

Rats were divided into four groups, comprising of two diets (Table 1), and followed by two different inflammation-inducing treatments. One group of twenty rats received an atherogenic diet consisting of 33% sucrose, 21% fat, 3% cholesterol, and 2%
watermelon powder by weight; while the other group of twenty rats received the same atherogenic diet (33% sucrose, 21% fat, 3% cholesterol), but instead of watermelon powder it contained 2% maltodextrin placebo (the placebo consisted of sucrose, glucose, and fructose at a ratio of 2:2:1 that matched the nutrient level of the watermelon powder). The watermelon powder was generously donated by Dr. Figueroa from Florida State University (Tallahassee, FL). The watermelon powder consisted of sieved and freeze-dried watermelon solids and its origin was Milne Fruit Products (Prosser, WA). Each group was on its diet for a span of 4 weeks. After the 4 week duration, ten rats from the watermelon powder group and ten rats from the placebo group were given the inflammation inducing agent 3% dextran sodium sulfate (DSS) in their water supply for a 48 hour period, followed by regular water for another 48 hour period. The other ten rats from each the watermelon powder group and the placebo group did not receive the DSS treatment and continued receiving regular water for each of the two 48 hour periods.

At the end of the study, the food was removed from the cages, and the animals were euthanized. Blood was collected from the rat carcass into labeled test tubes, allowed to clot at room temperature, and centrifuged for 15 minutes at 1200 x g at 2-8°C. Serum was stored at -70°C until time of analysis. Epididymal fat pads, kidneys, liver, and spleen were dissected and weight by one sole researcher to avoid error. Serum total cholesterol, triglycerides, and HDL-cholesterol were measured using a kit from StanBio (Boerne, TX). LDL-cholesterol was calculated by subtracting HDL cholesterol from the total cholesterol; then the total triglycerides was divided by five and was subtracted from the previous number attained. Serum glucose was measured using a kit from StanBio (Boerne, TX). C-reactive protein (CRP) was measured using an ELISA (Enzyme-Linked
Immunosorbent Assay) kit from BD Biosciences (San Jose, CA). Rat serum samples were added to the plates in the ELISA kit that were pre-coated with Stellar KLH and incubated for 30 minutes. The plates were then washed and an HRP (horseradish peroxidase) conjugate was added and then incubated for an additional 30 minutes. After the incubation, the plates were washed again and the substrate TMB is added to cause the sample to turn blue. The plates were then incubated for an additional 20 minutes, and then a stop solution is added to cause the blue to turn a yellow color. The results were then read at 450nm with a spectrometer. Lactate dehydrogenase (LDH) and Creatine Kinase (CK) were both measured using kits from StanBio (Boerne, TX). Total antioxidant levels were measured by a kit by Sigma (St. Louis, MO). First, Trolox working solution is made to make a Trolox standard curve. Then, 3% hydrogen peroxide solution is added to ABTS substrate solution to make an ABTS substrate working solution. Next, to produce the Trolox standard curve, Trolox standard was added to myoglobin working solution in its wells in a 96 well plate. The test samples and myoglobin working solution were added to its wells in the 96 well plate. ABTS substrate working solution was then added to each well and incubated for five minutes. Stop solution was then added to each well and mixed. The end absorbance was then read at 405nm with a spectrometer.

Statistical analysis

All data was analyzed by ANOVA procedure using SPSS (IBM, Armonk, NY) to evaluate the effects of the diets and treatment on weight, food intake, water intake, lipid
profiles, serum glucose, CRP, LDH, CK, and total antioxidant levels. Data was presented as Mean± SE. An alpha level of p<0.05 was considered significant.

Results

Initial body weight, final body weight (before DSS treatment), weight gain (before DSS treatment), food intake (before DSS treatment), food intake (after DSS treatment), and water intake (before DSS treatment) were not found to be statistically different among the groups (Table 2). However, final body weight (after DSS treatment) showed a significant reduction in weight in the DSS group compared to the non-DSS group (p=0.003). Final body weight (last day) also had a significant result as the DSS group weighed less compared to the non-DSS group (p=0.007). There was a significant difference in weight gain (during DSS treatment) where the non-DSS group gained more weight than the DSS group (p=0.001). Weight gain (grand) showed a significant difference in that the non-DSS group gained more weight than the DSS group (p=0.003). There was a significant difference in food intake (during DSS treatment) where the non-DSS group ate more than the DSS group (p<0.001). Water intake (during the treatment) showed a significant difference where the non-DSS group drank more water than the DSS group (p<0.001). Water intake (after DSS treatment) had a significant different as the DSS group drank more compared to the non-DSS group (p=0.020).

Liver weight and epididymal fat weight were not found to be statistically different among the groups (Table 2). However, spleen weight was found to be statistically significant in that the spleen weight in non-DSS group was higher than the DSS group (p=0.049). Kidney weight showed a significant difference in both the different diet
groups and the DSS treatment groups (Table 2). The DSS group showed a lower kidney weight compared to the non-DSS group (p=0.019). In addition, the control diet group had a lower kidney weight than the watermelon diet group (p=0.024).

Serum glucose was not found to be statistically different among the groups. However, many statistically significant data was found. Figure 1 shows the effects of the diets on lipid profiles. Triglycerides were found to be significantly higher in the control diet group compared to the watermelon diet group (p=0.031). Total cholesterol was found to be significantly higher in the control diet group compared to the watermelon group (p<0.001). Additionally, total cholesterol was found to be significantly higher in the DSS group compared to the non-DSS group (p=0.011). There was a significant difference in HDL-cholesterol where the non-DSS group had a higher test result than the DSS group (p=0.041). LDL-cholesterol was found to be lower in the watermelon diet group compared to the control diet group (p<0.001), and was found to be lower in the non-DSS group compared to the DSS group (p=0.008). Figure 2 demonstrates that there was a significant difference of LDH in diet groups where the watermelon diet group had lower LDH compared to the control diet group (p<0.001). CRP was found to be significantly different in both diet groups and treatment groups (Figure 3). CRP was significantly lower in the watermelon diet group compared to the control diet group (p=0.001), whereas CRP was significantly lower in the non-DSS group compared to the DSS group (p<0.001). Total antioxidant capacity was found to be significantly different in both diet groups and both treatment groups (Figure 4). Total antioxidant capacity was found to be significantly greater in the watermelon diet group compared to the control diet group.
(p=0.049). In addition, the non-DSS group was found to have an increase in total antioxidant capacity compared to the DSS group (p=0.015).

**Discussion**

Risk factors for cardiovascular disease include unfavorable lipid profiles, inflammation, physical inactivity, and an atherogenic diet. More specifically, cardiovascular disease risk rises when there is increased oxidative stress on epithelial cells causing epithelial dysfunction [8] & [9]. Both oxidative stress and epithelial dysfunction may lead to atherogenesis, the typical cause of cardiovascular disease [8]. Atherogenesis occurs when the walls of the arteries begin to harden and narrow due to the oxidation of LDL-cholesterol, which forms plaque in the artery walls and induces inflammation [10]. Nitric oxide has been found to be a vasodilator as well as a regenerator of endothelial tissue, thus relieves atherogenesis and improves cardiovascular function [11]. In the body, nitric oxide is made by the enzyme nitric oxide synthase from arginine, an amino acid [12]. Arginine also produces the amino acid citrulline as a by-product, and citrulline can be deiminated to arginine to make more nitric oxide, which may decrease the development of atherogenesis. However, citrulline is not only found in the body, but also in food, such as watermelon. This suggests that watermelon may be used as a functional food to prevent atherogenesis and cardiovascular disease.

Arginine is considered a non-essential amino acid; however, there are populations that may benefit from supplementation such as those at risk for cardiovascular disease. Unfortunately, high dose supplementation of arginine may be potentially dangerous because it is primarily metabolized by the liver [13]. Citrulline, on the other hand, is not
metabolized by the liver but rather by the kidneys, which makes it a potentially safe supplement for those at risk of cardiovascular disease. In a study that was conducted on rats that received a citrulline supplement, an arginine supplement, or no supplement, the group that received the citrulline supplement group showed the highest arginine levels as well as the most improved nitrogen balance after the conclusion of the study [13]. This study demonstrates to have increased nitric oxide via arginine in the body, taking supplementary citrulline, such as watermelon powder, would be most beneficial. In addition, in a study conducted by Poduri et al, they had two groups of mice that each received a high saturated fat diet, where one group received a watermelon extract supplement and the other group received a carbohydrate placebo, and found the group that received the watermelon extract supplement had increased plasma citrulline levels [7].

As mentioned, one of the causes of atherogenesis is the oxidation of LDL-cholesterol. In addition to nitric oxide producing citrulline, watermelon contains the potent antioxidant carotenoid lycopene. Lycopene is an anti-inflammatory agent, which may inhibit development of atherogenesis. Additionally, one cup (154g) of watermelon provides 12.5mg (21%DV) of the antioxidant vitamin C [14]. Lycopene by itself has been shown to lower inflammation; however, there was a study showing that when both lycopene and vitamin C were found naturally in a food item, that they may have synergistic effects that combine to be more effective in lowering inflammation [5]. Because watermelon provides both lycopene and vitamin C, it makes watermelon a promising functional food in terms of decreasing risk of cardiovascular disease.
The results of this study have shown that supplementation of watermelon powder appears to decrease some of the risk factors for cardiovascular disease. Triglycerides, total cholesterol, LDL-cholesterol, LDH, CRP, and total antioxidant capacity all had favorable changes with the supplementation of watermelon powder. Like this study, Poduri et al also found in this study that watermelon supplementation decreased total cholesterol and LDL-cholesterol levels [7]. However, unlike this study, Poduri et al concluded that watermelon extract reduced weight gain and decreased fat mass [7]. These differences in the two similar studies demonstrate that more similar studies should be preformed. However, the similar findings show assuring results that watermelon supplementation may reduce the risk of cardiovascular disease.

In summary, this study showed that supplemental watermelon powder has favorable effects on triglycerides, total cholesterol, LDL-cholesterol, LDH, CRP, and total antioxidant capacity compared to the control placebo group. These advantageous effects may be due to the nitrogen balancing citrulline content in the watermelon, as well as its antioxidants lycopene and vitamin C. Citrulline is deiminated into arginine that produces nitric oxide, which is a vasodilator. This may decrease the development of atherogenesis. Additionally lycopene and vitamin C may work synergistically and reduce inflammation more efficiently, thus reducing the development of cardiovascular disease. Further research over a longer duration is needed to determine the long-term effects of watermelon supplementation on cardiovascular disease.

In conclusion, the watermelon powder group appeared to have more favorable effects on triglycerides, total cholesterol, LDL-cholesterol, LDH, CRP, and total antioxidant capacity compared to the control placebo group, which suggests that
watermelon supplementation may decrease inflammation and the risk of cardiovascular disease.
Acknowledgements:

The authors wish to acknowledge the contributions of the Spring 2013 Advanced Nutrition Laboratory students at San Diego State University who assisted in conducting and evaluating this research.
References


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‘sentinel’ (watermelon) extract reduces atherosclerosis in LDL receptor-deficient 


April 27, 2013.


Table 1. Composition of experimental diets*

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Control-placebo (%)</th>
<th>Watermelon powder (%)</th>
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</thead>
<tbody>
<tr>
<td>Cornstarch</td>
<td>23.30</td>
<td>12.30</td>
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<tr>
<td>Sucrose</td>
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<td>Cellulose</td>
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</tr>
<tr>
<td>Casein</td>
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<td>20.00</td>
</tr>
<tr>
<td>Corn oil</td>
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<td>5.00</td>
</tr>
<tr>
<td>Dairy butter</td>
<td>16.00</td>
<td>16.00</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>3.00</td>
<td>3.00</td>
</tr>
<tr>
<td>Salt mix, AIN-93G</td>
<td>3.50</td>
<td>3.50</td>
</tr>
<tr>
<td>Vitamin mix, AIN-93G</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.30</td>
<td>0.30</td>
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<td>Sodium cholate</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>Choline chloride</td>
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<td>0.40</td>
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<tr>
<td>Placebo</td>
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<td>0.00</td>
</tr>
<tr>
<td>Watermelon powder</td>
<td>0.00</td>
<td>0.20</td>
</tr>
<tr>
<td>Total</td>
<td>100.00</td>
<td>100.00</td>
</tr>
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</table>

*33% sugar, 21% fat by wt, 3% cholesterol by wt
Table 2. Initial and final body weights, weight gain, food intakes, water intakes, and organ weights (liver, spleen, kidney, epididymal fat) for control-noDSS, control-DSS, watermelon-noDSS and watermelon-DSS fed rats*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control-noDSS</th>
<th>Control-DSS</th>
<th>Watermelon-noDSS</th>
<th>Watermelon-DSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight (g)</td>
<td>59.83±1.05</td>
<td>59.80±0.93</td>
<td>59.79±0.88</td>
<td>59.80±0.84</td>
</tr>
<tr>
<td>Final body weight (g, before DSS treatment)</td>
<td>238.70±4.30</td>
<td>239.15±3.48</td>
<td>238.65±5.64</td>
<td>239.93±3.69</td>
</tr>
<tr>
<td>Final body weight (g, right after treatment)</td>
<td>254.80±5.08a</td>
<td>240.80±4.09b</td>
<td>254.80±5.83a</td>
<td>238.64±3.60b</td>
</tr>
<tr>
<td>Final body weight (g, last day)</td>
<td>258.56±5.34a</td>
<td>242.41±4.19b</td>
<td>258.43±5.97a</td>
<td>245.41±4.49b</td>
</tr>
<tr>
<td>Weight gain (g, before DSS treatment)</td>
<td>179.12±3.69</td>
<td>179.35±3.03</td>
<td>178.86±5.19</td>
<td>180.13±3.41</td>
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<tr>
<td>Weight gain (g, during DSS treatment)</td>
<td>195.22±4.17a</td>
<td>181.00±3.48b</td>
<td>195.01±5.40a</td>
<td>178.84±3.31b</td>
</tr>
<tr>
<td>Weight gain (g, grand)</td>
<td>198.98±4.48a</td>
<td>182.61±3.61b</td>
<td>198.64±5.56a</td>
<td>185.61±4.22b</td>
</tr>
<tr>
<td>Food intake – before treatment (g/d)</td>
<td>16.10±0.26</td>
<td>16.04±0.28</td>
<td>16.48±0.42</td>
<td>16.44±0.24</td>
</tr>
<tr>
<td>Food intake – during DSS treatment (g/d)</td>
<td>17.61±0.39a</td>
<td>14.21±0.36b</td>
<td>17.67±0.42a</td>
<td>13.30±0.42b</td>
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<tr>
<td>Food intake – after DSS treatment (g/d)</td>
<td>18.22±0.46</td>
<td>15.44±0.70</td>
<td>17.49±0.45</td>
<td>17.57±1.38</td>
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<tr>
<td>Water intake – before DSS treatment (g/d)</td>
<td>17.18±0.43</td>
<td>23.75±0.86</td>
<td>24.12±1.43</td>
<td>26.28±1.70</td>
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<tr>
<td>Water intake – during DSS treatment (g/d)</td>
<td>26.66±0.56a</td>
<td>21.81±1.08b</td>
<td>28.39±1.79a</td>
<td>19.55±2.08b</td>
</tr>
<tr>
<td>Water intake – after DSS treatment (g/d)</td>
<td>29.71±1.04a</td>
<td>36.97±3.58b</td>
<td>31.55±1.71a</td>
<td>46.65±8.24b</td>
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<tr>
<td>Liver weight (g)</td>
<td>19.3140±0.77649</td>
<td>17.8240±0.46407</td>
<td>18.511±1.29088</td>
<td>17.3010±0.51609</td>
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<tr>
<td>Spleen weight (g)</td>
<td>1.1470±0.06708a</td>
<td>1.1333±0.04113b</td>
<td>1.2400±0.05664a</td>
<td>1.0256±0.05140b</td>
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<tr>
<td>Kidney weight (g)</td>
<td>2.0210±0.5800b</td>
<td>1.8990±0.01980d</td>
<td>2.1760±0.07712b</td>
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<tr>
<td>Epididymal fat weight (g)</td>
<td>1.8889±0.12690</td>
<td>1.7510±0.13910</td>
<td>1.8325±0.10862</td>
<td>1.7320±0.15674</td>
</tr>
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</table>

*Data are presented as mean ±SE (standard error). Data in rows with varying superscript letters are statistically different (p<0.05).
Figure 1. Serum lipid concentrations of rats fed watermelon powder with no DSS treatment, watermelon powder with DSS treatment, placebo control with no DSS treatment, and placebo control with DSS treatment. Bars represent means ± SE. Bars with different superscripts differ significantly at P < 0.05.

Figure 2. Effects of watermelon on lactate dehydrogenase. Bars represent means ± SE. Bars with different superscripts differ significantly at P < 0.05.
Figure 3. Effects of watermelon on total antioxidant capacity. Bars represent means ± SE. Bars with different superscripts differ significantly at P <0.05.

Figure 4. Effects of watermelon on C-reactive protein. Bars represent means ± SE. Bars with different superscripts differ significantly at P <0.05.