THE EFFECT OF DIETARY CAYENNE PEPPER SUPPLEMENTATION ON THE METABOLIC DYSREGULATION OF RATS IN VIVO

Running Head: Effects of cayenne pepper on metabolic dysregulation

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Abstract

Background: Metabolic dysregulation is a hallmark of diseases such as Type 2 diabetes, Cancer, and Cardiovascular disease (1).

Purpose: In this study, we assessed whether dietary capsaicin in the form of cayenne pepper improves the risk factors of metabolic dysregulation in atherogenic diet-fed rats with inflammation.

Procedure: Forty rats were divided into four groups with 50% consuming a control atherogenic diet, and the other 50% consuming a cayenne pepper (2.9%;0.015% capsaicin) atherogenic rat diet for 4 weeks. One group from each diet type were administered an inflammation inducing agent (DSS) after the four week process. All four groups were then tested for inflammatory markers (C-reactive Protein), lipid profile, glucose tolerance, serum albumin, epididymal fat and various organ weights.

Results: Treatment effect caused significance in food (p=0.004) and water (p=0.016) intake during DSS. Diet effect accounted for significance of final body weight (p=0.045), final weight gain (p=0.022), epididymal fat measurement (p=0.037), triglycerides (p=0.031), total cholesterol (p=0.038), high-density lipoprotein (p=0.045), low-density lipoprotein (p=0.031), and C-reactive protein (p=0.043). No significance found (p<0.05) in initial body weight, food or water intake before or after DSS treatment, liver, spleen, or kidney weight, albumin, or glucose.

Conclusion: Cayenne pepper reduces the risk factors of metabolic dysregulation by favorable changes of body weight gain, body fat, lipid profile and inflammation.
1. Introduction:

Capsaicin, a pungent principle of hot peppers, is habitually consumed in Central and South America as a food, spice and for topical treatment. Capsaicin has a thermogenic effect in animals, involving activation of TRPV1 receptors (2) that produce increased sympathetic nervous efferent activity (3) thus suppressing body fat accumulation. Studies have shown capsaicin additionally elicits anti-inflammatory properties by inhibiting the release of proinflammatory mediators such as C-reactive protein (CRP)(1). When ingested, dietary capsaicin is absorbed into the body and distributed to high-density lipoprotein (HDL) in larger amounts than to low-density lipoprotein (LDL) (4). Moreover, it acts as an antioxidant, participating in the primary defense mechanisms against oxidative stress and affecting lipid metabolism and favorable blood lipid profiles (5). Dietary capsaicin has also been found to lower fasting glucose, insulin, and reduce the impairment of glucose tolerance in obese mice (6). The combined affect of these mechanisms offers great promise in the areas of obesity prevention, Type 2 diabetes, Cardiovascular health, and inflammatory-inducing pathologies such as cancer. This led us to test the involvement of capsaicin in these mechanisms that cause metabolic dysregulation, in order to assess its pharmacological interaction through supplementation.

The primary aim of this investigation was to attempt to test the efficacy of these mechanisms of capsaicin from the source of Cayenne pepper. Cayenne pepper—also referred to as red
hot chili pepper—is a common dietary source of capsaicin in the western world. In this study, we tested the hypothesis that cayenne pepper reduces the risk factors of metabolic dysregulation by favorable changes of body weight gain, lipid profile, glucose control and inflammation. We examined whether the capsaicin in cayenne pepper modulates serum CRP, albumin, glucose, total cholesterol (TC), triglyceride (TG), HDL, and LDL markers in laboratory rats provided with equal nutrient supply.
2. Materials and methods:

2.1 Animals

Forty, pathogen-free, Sprague Dawley rats (aged 21 days) were individually housed in wire-bottomed cages under controlled conditions (12 hour light-dark cycle, humidity 40-45%, temperature 20-24°C). Rats were kept in a San Diego State University research facility. The animals had access to both food and water at all times. All training and procedure for animal use were approved and conducted by the San Diego State University animal subjects committee.

2.2 Diets

Rats maintained a normal rat pellet diet for 2-3 days during acclimation. Rats were divided into four groups of ten, consuming diets (Table 1) consisting of 33% sugar, 21% fat by weight, and 3% cholesterol by weight from either a no cayenne (control), or cayenne for 4 weeks. Ground cayenne pepper (82,879 HU)(TradeWinds, Amerifoods trading Co. Los Angeles, CA) was used for the cayenne diet. 100 gram cayenne pepper composition consisted of: 318.00 kcal, 12.01g protein, 17.27 g lipid, 56.63 g Carbohydrates (27.20 g fiber, 10.34g sugar), other vitamins and minerals (USDA National Nutrient Database for Standard Reference, Release 23 [2010]). Heat Unit was established by the Nutritional Analysis Center (Eurofins Scientific Inc. Des Moines, IA).
2.3 Animal treatment
At the completion of the experiments, the food was removed from the cages for overnight fasting, and half of the rats (10 control, and 10 cayenne) were given the 3% DSS (Dextran sodium sulfate) with their water for 24 hours. DSS is an inflammation-inducing agent. The remaining half was given plain water for 24 hours. For the following 48 hours, all rats were fasting with regular water. The animals were then euthanized and blood was collected from the rat carcass into labeled test tubes.

2.4 Collection
Blood was allowed to clot at room temperature and then was centrifuged for 15 minutes at 1200 x g at 2-8°C. Until analyzed, serum was stored at -70°. Liver, kidney, spleen, epididymal fat pads, and the large intestine were dissected and weighed by one sole researcher to avoid error. Serum albumin, glucose, TC, TG, and HDL-cholesterol were assessed using kits from Stanbio (Boerne, TX). To calculate LDL-cholesterol we subtracting HDL-cholesterol from the total cholesterol; then the total TG was divided, and five was subtracted from the previous number obtained.

Serum CRP was assessed using a solid phase sandwich ELISA (enzyme linked immunosorbent assay)(BD Biosciences, San Jose, CA). Samples were diluted and added to the coated 96-well plate, to bind to a CRP antibody (Ab), after the appropriate incubation. Wells were then washed and “sandwiched” with the addition of anti-rat CRP as antibody-antigen-antibody lineup. After another
washing of the wells and an additional incubation period, a color-producing solution was added determining the presence of CRP. The reaction was then interrupted with a stop solution, which changes the color at which point, absorbance was measured at a wavelength of 450 nm on a spectrophotometer/ELISA reader.

2.5 Statistical analysis
All data was analyzed to evaluate the effects of diets and treatments on weight, food intake, water intake, lipid profiles, albumin, glucose, and CRP using the two way ANOVA procedure (diet and treatment) using SPSS software (IBM, Armonk, New York). Statistical significance was considered with the criterion of an alpha level of p<0.05.
3. Results:

Both the food intake during DSS treatment and the water intake during DSS treatment was found to be significantly different between the non-DSS treated and the DSS treated groups (p<0.02) (Table 2). Final body weight, final weight gain, C-reactive protein, and epididymal fat were significantly different between the control diet and the cayenne diet groups (p<0.05) (Table 2). TG (P=0.031), TC (p=0.038), HDL (p=0.045), and LDL (p=0.031) were significantly different (p<0.05) between the control diet and the cayenne diet groups.

There were no evident differences between the serum albumin and serum glucose markers from any group. Initial body weights before DSS treatment, food weights before DSS treatment, food weight after DSS treatment, and water before DSS treatment were all found to not be of any significance. Liver weight, spleen weight, and kidney weight were all assessed and found not significantly different among groups (Table 2).

Figure 1 demonstrates a comparison of the effects of the control diet with no DSS treatment with the control diet with DSS treatment, with the cayenne pepper diet with no DSS treatment, and the cayenne pepper diet with DSS treatment on the serum lipid profile of the animal subjects. Cayenne pepper diet groups, regardless of whether they were DSS treated or non DSS treated,
showed a reduction in TG, TC, and LDL in comparison to the control diet \((p<0.05)\). Moreover, the cayenne diet showed an increase in HDL when compared to the control diet \((p<0.05)\).
Discussion:

Cardiovascular disease, obesity, and type 2 diabetes share metabolic dysregulatory mechanisms characterized by glucose tolerance, and chronic subacute inflammation. Several lines of research suggest that it is possible to target these mechanisms with pharmacological interventions, such as with capsaicin supplementation (1).

In a recent study, during 2 weeks (daily) administration of capsaicin (10mg/kg-body weight/day), fat accumulation was suppressed, suggesting that capsaicin promotes energy metabolism (7). In a similar study, treatment with capsinoids (active molecule in capsaicin) (6mg/d) was associated with fat loss, and increase in fat oxidation that was nearly significant (8). It was further noted that capsaicin was found to be a useful phytochemical for attenuating obesity-related complications and obesity-induced inflammation (9). In a study by Spiller et al., capsicum pepper juice (0.25-2.0g kg(-1)) was able to discourage the neutrophil migration towards the inflammatory process (10). Additionally, it has been found to have significant anti-inflammatory activity, inhibiting leukocyte migration (11).

These findings were all consistent with the results of this study, revealing that dietary capsaicin significantly decreased rat epididymal fat and CRP-receptor marked inflammation.

In a study by Ahuja et al., regular consumption of chilli for 4 weeks increased the resistance of serum lipoproteins to
their oxidation state (12). Medvedeva et al. published that paprika carotenoids (containing capsaicin) suppressed the oxidation of LDL, lowered LDL, and inhibited the transformation of cholesterol to oxidized products (13). A Capsaicin treatment was found to decrease LDL and TG, and increase HDL with no noticeable affect on serum total cholesterol. Liver glycogen was also found to decrease after the capsaicin treatment (14,15).

The results from this study showed that dietary capsaicin lowered TG levels, TC levels, LDL cholesterol levels, and raised HDL cholesterol levels. This data correlated with the literature cited thus far with the exception of Lee et al. finding no noticeable serum TC change. This could be due to the short duration of the mentioned study as it was only 3 days. Our results were analyzed after 4 weeks, giving the pharmacological properties of capsaicin time to effectively report the change in TC.

Further results showed no significance in the areas of organ (liver, spleen, kidney) weight, serum albumin and blood glucose levels.

C-reactive protein (CRP) did show significance due to a diet affect. This was most likely due to the fact that CRP is an inflammatory marker that was inhibited by the capsaicin in the cayenne pepper group (1).

In a study by Kang et al. dietary capsaicin decreased glucose levels in the plasma (16). It was conducted by solely administering an atherogenic diet for 2 weeks before any capsaicin supplementation was added. Perhaps the duration of time
was enough to ensure an obese state in the animals that was not yet reached in our test subjects. Our supplementation of cayenne pepper was also conducted concurrently with the atherogenic diet. Future research examining the effects on non-obese in comparison to obese subjects should be conducted in order to determine the specific effects on each weight category.

The results from this study also found a significant difference in the food and water intake amounts during DSS treatment. Perhaps this is due to the possible inflammatory affect on thirst and hunger. There were no other results of significance reflecting the DSS treatment in our study.

In summary, this research demonstrated that capsaicin suppressed fat accumulation and oxidation (7,8), inhibited the inflammatory process (9-11), lower TC (12,13), and lower LDL and TG (12-15) suggesting that capsaicin may reduce metabolic dysregulatory processes. More importantly, we observed in this study that dietary capsaicin in the source of cayenne pepper elicited similar significance in all findings.

In conclusion, cayenne pepper proved to be an efficient source of dietary capsaicin by reducing the risk factors of metabolic dysregulation by favorable changes of body weight gain, body fat, lipid profile and inflammation, further suggesting that it may contribute to decreasing one’s risk for obesity, cardiovascular disease, and inflammatory-inducing pathologies such as cancer.
Acknowledgements:

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Literature Cited:

7. Ohnuki K, Haramizu S, Oki K, Watanabe T, Yazawa S, Fushiki T. Administration of capsiate, a non-pungent capsaicin analog, promotes energy metabolism and suppresses body fat


13. Medvedeva NV, Andreenkov VA, Morozkin AD, Sergeeva EA, Prokof’ev IU, Misharin AIu. [Inhibition of oxidation of


Table 1. Composition of experimental diets*

<table>
<thead>
<tr>
<th>Ingredient (g)</th>
<th>Control %</th>
<th>Cayenne pepper %</th>
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<tbody>
<tr>
<td>Cornstarch</td>
<td>12.30</td>
<td>11.60</td>
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<tr>
<td>Sucrose</td>
<td>33.00</td>
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<tr>
<td>Cellulose</td>
<td>5.00</td>
<td>4.00</td>
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<tr>
<td>Casein</td>
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<td>19.60</td>
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<tr>
<td>Corn oil</td>
<td>5.00</td>
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<tr>
<td>Anhydrous milkfat</td>
<td>16.00</td>
<td>15.9</td>
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<tr>
<td>Cholesterol</td>
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<td>3.00</td>
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<tr>
<td>Salt mix, AIN-76</td>
<td>3.50</td>
<td>3.50</td>
</tr>
<tr>
<td>Vitamin mix, AIN-76</td>
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<tr>
<td>Methionine</td>
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<tr>
<td>Sodium cholate</td>
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</tr>
<tr>
<td>Choline chloride</td>
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<td>0.40</td>
</tr>
<tr>
<td>Cayenne pepper**</td>
<td>0.00</td>
<td>2.90</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 weight, 3% cholesterol by weight
**Cayenne pepper (0.015% capsaicin)
Table 2. Initial body weight, final body weight, final weight gain, food intake during DSS* treatment, water intake during DSS* treatment, liver weight, spleen weight, kidney weight, epididymal fat weight and C-reactive protein (CRP) measurements. **

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control without DSS</th>
<th>Control with DSS</th>
<th>Cayenne without DSS</th>
<th>Cayenne with DSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body wt (g)</td>
<td>47.87 ± 1.64</td>
<td>48.03 ± 1.41</td>
<td>48.00 ± 1.29</td>
<td>48.04 ± 1.16</td>
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<tr>
<td>Final body wt (g)</td>
<td>230.53 ± 6.01(^a)</td>
<td>229.18 ± 4.8(^a)</td>
<td>217.60 ± 6.83(^b)</td>
<td>218.33 ± 5.01(^b)</td>
</tr>
<tr>
<td>Final wt gain (g)</td>
<td>182.66 ± 4.67(^a)</td>
<td>181.15 ± 3.83(^a)</td>
<td>170.13 ± 5.81(^b)</td>
<td>170.28 ± 5.14(^b)</td>
</tr>
<tr>
<td>Food intake (g/d)</td>
<td>16.32 ± .63(^a)</td>
<td>14.22 ± .42(^b)</td>
<td>15.68 ± .40(^a)</td>
<td>14.20 ± .79(^b)</td>
</tr>
<tr>
<td>Water intake (g/d)</td>
<td>25.29 ± .83(^a)</td>
<td>19.66 ± 1.04(^b)</td>
<td>27.50 ± 4.79(^a)</td>
<td>20.96 ± 1.22(^b)</td>
</tr>
<tr>
<td>Liver (g)</td>
<td>15.1322 ± 65854</td>
<td>15.0630 ± .42818</td>
<td>14.1822 ± .43054</td>
<td>14.1444 ± .56762</td>
</tr>
<tr>
<td>Spleen (g)</td>
<td>1.04 ± .07</td>
<td>1.06 ± .06</td>
<td>.97 ± .06</td>
<td>.92 ± .04</td>
</tr>
<tr>
<td>Kidney (g)</td>
<td>2.00 ± .07</td>
<td>1.99 ± .04</td>
<td>1.93 ± .07</td>
<td>2.01 ± .09</td>
</tr>
<tr>
<td>Epididymal fat (g)</td>
<td>1.7100 ± .15226(^a)</td>
<td>1.6622 ± .11025(^a)</td>
<td>1.4700 ± .07881(^b)</td>
<td>1.4760 ± .10081(^b)</td>
</tr>
<tr>
<td>CRP (ug/ml)</td>
<td>67.23 ± 11.86(^a)</td>
<td>92.86 ± 15.61(^a)</td>
<td>58.66 ± 6.48(^b)</td>
<td>59.69 ± 7.71(^b)</td>
</tr>
</tbody>
</table>

* DSS (Dextran sodium sulfate) an inflammation-inducing agent.

**Data are presented as mean ± SE (standard error). Data in rows with varying superscript letters are statistically different (p<0.05).
Figure legend

Figure 1: Serum lipid concentrations of rats fed the control diet with no DSS treatment, versus the control diet with DSS treatment, versus the cayenne pepper diet with no DSS treatment, and versus the cayenne pepper diet with DSS treatment. Data are presented as mean ± SE (standard error). TG: triglyceride; TC: total cholesterol, HDL: high-density lipoprotein cholesterol; LDL: low density lipoprotein cholesterol. Statistical differences determined by a two way ANOVA.
Figure 1