Effect of dietary hempseed intake on cardiac ischemia-reperfusion injury


1Canadian Centre for Agri-food Research in Health and Medicine, St. Boniface Hospital Research Centre, Department of Physiology, Faculty of Medicine, 2Faculty of Pharmacy, University of Manitoba, Winnipeg, Canada; and 3College of Medicine and Medical Sciences, Arabian Gulf University, Kingdom of Bahrain

Submitted 18 September 2006; accepted in final form 9 November 2006

Hempseed contains a high proportion of the PUFAs linoleic acid (LA) and α-linolenic acid (ALA), which may have opposing effects on postischemic heart performance. There are no reported data concerning the cardiovascular effects of dietary hempseed intake. A group of 40 male Sprague-Dawley rats were distributed evenly into four groups that were fed for 12 wk a normal rat chow supplemented with hempseed (5% and 10%), palm oil (1%), or a 10% partially delipidated hempseed that served as a control. Plasma ALA and LA levels were significantly elevated in the rats fed these diets compared with control. After the dietary interventions were completed, postischemic heart performance was evaluated by measuring developed tension, resting tension, the rates of tension development and relaxation, and the number of extrasystoles. Hearts from rats fed a hempseed-supplemented diet exhibited significantly better postischemic recovery of maximal contractile function and enhanced rates of tension development and relaxation during reperfusion than hearts challenged by an ischemia-reperfusion insult.

MATERIALS AND METHODS

Guidelines for the ethical care and treatment of animals used in this study were approved by the University of Manitoba Animal Care Committee in accordance with the Canadian Council on Animal Care. Male Sprague-Dawley rats were fed one of four different diets for 12 wk. All rats weighed ~250 g when they were started on their designated diets. A regular rat chow was supplemented with 5% (5H) or 10% (10H) hempseed or 1% palm oil [saturated fat (SF) diet]. The SF diet was chosen to simulate the fat content of a 10H-supplemented diet. In addition, palm oil contains primarily saturated fatty acids, not omega-3 fatty acids. A regular rat chow supplemented with hempseed to alter the response of hearts to ischemia-reperfusion challenge.
with 10% delipidated hempseed (DLH) was used as the control diet in this study. Rats fed with regular rat chow alone were not considered to be a fair control group because the chow was not supplemented, as were all of the other diets. In addition, it contained a significant amount of both omega-3 and omega-6 fatty acids.

After completion of the dietary intervention, rats were anesthetized with a ketamine-xylazine mixture before the heart was removed for in vitro perfusion. The heart was perfused with a standard Krebs buffer at a rate of 10 ml/min at 37°C, pH 7.4, with the Langendorff retrograde perfusion technique (15). Apicobasal displacement (contractile tension) was recorded with a force transducer that was connected to a chart recorder, as described previously (18). The atria were resected, and electrodes were used to maintain the heart rate at 200 beats/min. Global ischemia was induced by turning off the peristaltic pump. Ischemia was induced for 10 min, followed by 45 min of reperfusion. The contractile performance of the heart was assessed through the following parameters: developed tension, resting tension, the rates of tension development and its dissipation (relaxation) (±dT/dt), and the incidence of extrasystoles.

Plasma LA, ALA, and γ-linolenic acid (GLA) levels were measured by gas chromatography. Briefly, blood samples were collected in vacutainer tubes containing EDTA. Plasma was obtained by centrifuging the blood sample at 1,800 g for 5 min at 4°C. The plasma was aliquoted into Eppendorf tubes and stored at −80°C. The fatty acids were then extracted from the plasma sample and derivatized with the method of Lepage and Roy (14). A Varian CP-3800 gas chromatograph equipped with a flame ionization detector and a Varian CP-Sil 88 capillary column (60 m × 0.25 mm × 0.20 μm) was used. One microliter of the benzene layer was injected with a CP-8400 autosampler at a split ratio of 1:50. The flow rate of the helium carrier gas was 1.5 ml/min. The initial oven temperature was programmed at 80°C and was held there for 1 min, then raised by 30°C/min to 140°C, further increased by 5°C/min to 225°C, and held there for 10 min. The total run time for each sample was 30 min. Heart and diet fatty acid levels were detected with the same derivatization technique and chromatographic parameters described above. However, prior lipid extraction with the Folch technique (1) was required before this could be accomplished. The fatty acid contents of the sample were identified by comparison with an authentic standard, GLC-462 (Nu-Chek Prep, Elysian, MN).

Data were analyzed by a one-way analysis of variance test followed by a Duncan’s multiple range post hoc test. Statistical significance was determined at P < 0.05.

RESULTS

Animal weights. The rats gained a significant amount of weight over the course of the study. Rats weighed ~250 g at the start of the study and grew to 613 ± 17, 628 ± 23, 644 ± 19, and 603 ± 14 g in the DLH, SF, 5H, and 10H groups, respectively, by the end of the 12-wk dietary intervention. There were no significant differences in the weights of the animals due to the four different dietary interventions after 12 wk.

Diet composition. The diet nutritional composition differed only in the total fat content as a result of the inclusion of hempseed or palm oil (Table 1) compared with the control DLH diet.

Diet fatty acid levels. Saturated fatty acids were significantly increased in the SF diet (2.71 ± 0.03%) relative to the DLH (0.64 ± 0.02%) and hempseed (5H: 0.93 ± 0.03%; 10H: 0.72 ± 0.02%) diets. Levels of LA and ALA were significantly higher for the hempseed-supplemented groups relative to the other two diets, with the 10H group significantly higher than the 5H group (Table 2). Even though GLA levels were present in trace amounts compared with LA and ALA levels, significant differences in GLA levels were still observed among the different dietary groups. Only the 10H and SF diets were significantly different from the DLH diet, whereas both hempseed groups differed significantly from the SF group. In addition, the 10H diet displayed significantly greater levels of GLA relative to the 5H group. The SF and the DLH diet groups were also significantly different. ALA and GLA levels, but not LA levels, were significantly decreased in the SF diet compared with the DLH diet (Table 2).

Plasma fatty acid levels. Blood was collected from the rats after 12 wk of dietary intervention, and plasma was separated and analyzed for its fatty acid composition. No significant differences were observed in the plasma LA levels of rats fed a 5H- or 10H-supplemented diet compared with the other two groups (Fig. 1A). The plasma levels of ALA and GLA were significantly elevated in both the 5H- and 10H-supplemented rats relative to SF- and DLH-fed rats (Fig. 1B and C). In addition, the levels of plasma ALA in the 10H-supplemented rats were significantly higher than those observed in the 5H-supplemented rats (P < 0.05). There were no statistically significant differences between the SF and DLH groups in either fatty acid. The other physiologically important fatty acid species, eicosapentaenoic acid (EPA, 20:5ω-3) and docosahexaenoic acid (DHA, 22:6ω-3), were not changed by the dietary interventions (data not shown).

Table 1. Nutritional composition of experimental diets

<table>
<thead>
<tr>
<th>Ash (g)</th>
<th>Prot (g)</th>
<th>Fiber (g)</th>
<th>Fat (g)</th>
<th>CHO (g)</th>
<th>TDN (g)</th>
<th>DE (g)</th>
<th>GE (g)</th>
<th>NEF (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLH</td>
<td>6.82</td>
<td>26.4</td>
<td>6.6</td>
<td>6.4</td>
<td>53.8</td>
<td>81.58</td>
<td>3.7</td>
<td>4.69</td>
</tr>
<tr>
<td>SF</td>
<td>6.96</td>
<td>24.4</td>
<td>4.1</td>
<td>10.7</td>
<td>53.9</td>
<td>89.56</td>
<td>4.01</td>
<td>4.85</td>
</tr>
<tr>
<td>5H</td>
<td>7.14</td>
<td>25.5</td>
<td>5.3</td>
<td>7.7</td>
<td>54.4</td>
<td>84.37</td>
<td>3.8</td>
<td>4.72</td>
</tr>
<tr>
<td>10H</td>
<td>6.9</td>
<td>26.8</td>
<td>6.2</td>
<td>8.8</td>
<td>51.3</td>
<td>84.49</td>
<td>3.82</td>
<td>4.82</td>
</tr>
</tbody>
</table>

Table 2. Linoleic, α-linolenic, and γ-linolenic fatty acid content in diets

<table>
<thead>
<tr>
<th></th>
<th>LA (mg/kg)</th>
<th>ALA (mg/kg)</th>
<th>GLA (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLH</td>
<td>16.91 ± 0.76</td>
<td>2.31 ± 0.11</td>
<td>0.28 ± 0.01</td>
</tr>
<tr>
<td>SF</td>
<td>18.02 ± 0.20</td>
<td>1.42 ± 0.01</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>5H</td>
<td>20.58 ± 0.90</td>
<td>3.29 ± 0.16</td>
<td>0.32 ± 0.02</td>
</tr>
<tr>
<td>10H</td>
<td>24.35 ± 1.49</td>
<td>4.89 ± 0.36</td>
<td>0.60 ± 0.05</td>
</tr>
</tbody>
</table>

Values (in mg fatty acid methyl esters/g diet) are means ± SE; n = 3. LA, linoleic acid; ALA, α-linolenic acid; GLA, γ-linolenic acid. *P < 0.05 relative to DLH; †P < 0.05 relative to SF; ‡P < 0.05 relative to 5H.
DHA were not changed by the dietary interventions (data not shown).

Cardiac performance during ischemia-reperfusion. After 12 wk of dietary administration, there were significant changes in the response of the hearts to the ischemia-reperfusion insult. As shown in Fig. 3, ischemia resulted in a significant loss of developed tension in hearts from all groups. Reperfusion induced an immediate recovery in developed tension. This recovery had leveled off within 20–30 min of reperfusion in all groups. Developed tension did not recover for the first 35 min of reperfusion in the control rats fed a delipidated hempseed diet. At 40 min of reperfusion, there was a small ~5% recovery of tension detected. This was in sharp contrast to the other groups. There was a significantly better recovery of developed tension in the hempseed-supplemented groups compared with the lipid-depleted hempseed group during the reperfusion period. The 10H-supplemented group tended to exhibit the best recovery, but this was not statistically different from the 5H-supplemented group. The animals fed the SF diet had a significantly slower recovery of cardiac developed tension during reperfusion and did not achieve as great a maximal recovery of developed tension.

Ischemia resulted in a significant loss in the rates of tension development (Fig. 4) and its dissipation (relaxation) (Fig. 5) in
hearts from all groups. Reperfusion induced a recovery in cardiac tension generation and relaxation. Qualitatively, the response of these contractile parameters was similar to that observed for maximal developed tension. The rats fed a DLH diet did not exhibit any recovery in $\frac{dT}{dt}$ until 40 min of reperfusion, and even then it was minimal. In contrast, there was a statistically significant increase in the rates of tension development and relaxation in the hempseed-supplemented groups after 15 min of reperfusion compared with the control, delipidated hempseed-supplemented group. The animals fed the 10H-supplemented diet exhibited the best recovery, but this was not statistically different from the values obtained for the group of rats supplemented with 5H. Again, the SF-supplemented diet led to a slower recovery of both $+\frac{dT}{dt}$ and $-\frac{dT}{dt}$ compared with the hempseed diets.

Ischemia induced a small increase in cardiac resting tension in all of the hearts examined (Fig. 6). Reperfusion resulted in a much larger, immediate rise in resting tension. Resting tension increased to $\approx 180\%$ of preischemic values, and this gradually dissipated over time during reperfusion. There was no statistically significant difference in the increase in resting tension between groups.
tension among any of the dietary interventions during ischemia or during reperfusion at any of the time points examined ($P < 0.05$).

Evidence of arrhythmias was observed in the form of extrasystoles, which were rarely observed in hearts perfused with normal perfusion medium before ischemia. On induction of ischemia, extrasystoles were also infrequent (Fig. 7). There were no significant differences in the incidence of extrasystoles among the groups during ischemia. The incidence of extrasystoles increased in frequency during the reperfusion period compared with the ischemic period, but there were no differences in incidence among the groups.

**DISCUSSION**

LA, an omega-6 fatty acid, is enriched in hempseed. 5H and 10H supplementation were not sufficient to successfully increase the plasma levels of LA in these rats. This is surprising in view of its content in the hempseed. However, it is not totally unexpected, as this result is similar to effects observed in a clinical study (11) in which subjects ingested hempseed oil capsules over a 3-mo period. Subjects did not achieve significant changes in plasma LA content despite the dietary dosage of LA. It was suggested (11) that LA is absorbed less efficiently than other fatty acid species, and the data from the present study would extend this interpretation to rats as well. Hempseed is also enriched (although to a lesser degree) in the omega-3 fatty acids ALA and GLA. ALA and GLA levels in the plasma of rats fed a hempseed-enriched diet were elevated. Our dietary intervention, therefore, induced a selective absorption of omega-3 over omega-6 fatty acids in the rats despite a higher content of omega-6 fatty acids in the hempseed. The increases in plasma ALA and GLA were specific to the hempseed-supplemented groups. Dietary intervention with lipid-depleted hempseed- and palm oil-supplemented diet did not induce increases in the plasma levels of ALA or GLA. These increases in plasma fatty acid content were transferred to cardiac tissue only in the case of ALA. ALA levels were elevated in cardiac tissue after dietary supplementation with hempseed. To our knowledge, this is the first report that these omega-3 fatty acids are effectively absorbed and then selectively deposited within the heart with hempseed supplementation of the diet.

Because our model used isolated hearts to assess cardiac performance during ischemia-reperfusion, the effects of the high circulating ALA levels observed in vivo were removed. The protective effects of the diet, therefore, were likely largely dependent on changes to the cardiac tissue itself. Of course, we cannot rule out the possibility that changes in the vasculature of the heart may be involved in the effects observed. It is also possible that these changes in ALA may not be directly responsible for the effects observed but may have induced the generation of another signaling molecule that itself is responsible for the beneficial action. However, we believe it is still possible to make two general conclusions from our work. First, it is clear that any cardioprotective effects are due to endogenous cardiac stores of ALA, and other fatty acid species that were unchanged by dietary hempseed (including LA, GLA, EPA, and DHA) do not participate in this action. Second, the cardioprotective potential of dietary hempseed may be more pronounced in vivo because of the availability of both circulating and endogenous tissue stores of ALA. Thus the cardioprotective potential of dietary hempseed supplementation during and ischemia-reperfusion challenge to the heart may actually be underestimated in the present study.

We have demonstrated for the first time in this study that dietary hempseed represents an effective, unique method to significantly alter the levels of ALA in the heart. We have also demonstrated for the first time that dietary hempseed will confer beneficial cardioprotective effects in hearts subjected to ischemia-reperfusion challenge. Because ALA was the only fatty acid species altered in the heart by the hempseed supplementation, it is clear that the beneficial effects were induced by ALA. This extends the data of Pepe and McLennan (20), who showed that fish oil improves posts ischemic recovery of cardiac contractile function. In their study, EPA and DHA were attributed to be the primary mechanism whereby fish oil was effective. Our study represents the first study that demonstrates that dietary hempseed through ALA may also confer a cardioprotective effect to the posts ischemic heart. Other sources of ALA include flaxseed, canola, soybeans, walnuts, and some fish like salmon (20). Because LA levels did not increase in the rats in response to hempseed ingestion, the data cannot address one of the prevailing contentions in the literature today that LA and omega-6 fatty acids have a negative cardiovascular effect. One of the potential limitations of the study is that the hearts were not blood perfused, and any negative side effects induced by LA through, for example, a stimulation of platelet aggregation, would be avoided in our system. However, preliminary data (not shown) suggest that dietary hempseed induced an inhibition of platelet aggregation, so it is unlikely that blood perfusion would have revealed any negative side effects.

Despite an extensive history of its use in the diet, few data currently exist in the peer-reviewed literature regarding the biological effects of hempseed. The highest concentration of hempseed that was used in this study was a 10% supplementation. Without any clinical studies on hempseed, it is difficult to relate this to levels of hempseed ingestion in humans. However, we believe that the 5–10% hempseed supplementation levels chosen in this study have relevance to human diets.
DIETARY HEMPSEED INTAKE AND CARDIAC ISCHEMIA

R1203

Similar levels of dietary supplementation with flaxseed (10%) are thought to adequately mimic dietary interventions in humans (1). This may represent an upper limit for the diet where compliance is an issue. Higher hempseed concentrations in the diet may not be practical. However, with hempseed ingestion a common practice over many centuries, serious complications may not be a realistic concern. Without detailed investigations in humans, this is merely conjecture at this time. Further investigations in both animal and human trials are required.

ACKNOWLEDGMENTS

We are grateful to Shawn Crew and Hemp Oil Canada for providing the hempseed for this study.

GRANTS

This study was supported by a grant from the Canadian Institutes for Health Research. M. Richard was a recipient of a Canadian Graduate Scholarship for this study.

REFERENCES