Effect of fish oil on mitochondrial respiration in isoproterenol induced myocardial infarction in rats

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Received 9 August 2000; revised 3 September 2001

Following isoproterenol treatment mitochondrial lipid peroxidation, phospholipase activity, lactate and calcium increased significantly, while activities of tricarboxylic acid cycle enzymes, enzymes of respiratory chain and ATP production showed decline. Oxidative phosphorylation was also affected on isoproterenol treatment with significant reduction in all the variables. Fish oil pretreatment in isoproterenol treated rats showed improved mitochondrial energy metabolism. The results suggest cardioprotective effect of fish oil.

Lipid peroxides are believed to inhibit production of prostacyclin\(^5\); this explains why fish oil at increasingly higher doses offers lesser cardioprotection. The n-3 fatty acids, viz. eicosapentaenoic acid and docosahexaenoic acid of fish oil being highly unsaturated fatty acids, get incorporated in the membrane phospholipids and offer protection only at low doses. Helgi et al\(^6\) reported myocardial protection in dogs by daily feeding them with fish oil containing 0.06 mg of eicosapentaenoic acid per kg body weight for 6 weeks. Zhu et al\(^7\) have reported that long term dietary fish oil supplementation significantly reduces myocardial infarct size whereas short term supplementation has no effect. Keeping these in view studies have been undertaken with 0.05 ml dose of fish oil for 45 days pretreatment period and administration of 60 mg/kg body weight isoproterenol. To confirm the induction of myocardial infarction histopathological studies have been done and the levels of marker enzymes like creatinine phosphokinase (CPK) and lactate dehydrogenase (LDH) have been estimated\(^4\).

Mitochondrial respiration refers to all those processes concerned with the uptake of oxygen and associated production of ATP, including the activity of the citrate cycle and the respiratory chain.

During ischaemia the subsequent generation of lipid peroxides and hydroperoxides results in initiation of chain reactions that could damage the mitochondrial membranes. The pathophysiological consequences of lipid peroxidation are development of alterations in membrane integrity and permeability of mitochondrial and sarcolemmal membranes. These alterations result in altered electrolyte levels including calcium entry which results in phospholipase activation, further ATP depletion and irreversible injury\(^3\).

Materials and Methods

Male Wistar rats weighing 100-150 g were housed 6 per cage at 27±2°C with constant humidity (55%), on a 12 hr light/dark cycle. The animals were provided with Hindustan Lever Pellet diet and water ad libitum. They were divided into 4 groups. Group I animals did not receive any treatment and group II animals were given subcutaneous injection of isoproterenol hydrochloride (IPH; 60 mg/kg body wt) dissolved in 0.1 ml normal saline at an interval of 24 hr for 2 days\(^6\). Group III animals were given 0.05 ml fish oil orally by gastric intubation for 45 days. Group IV animals received both fish oil and isoproterenol (at the end of fish oil treatment i.e., on 45 day) in the above mentioned dosages.

After 12 hr of the second injection of IPH, the animals were sacrificed by cervical decapitation. Blood was collected in ice cold saline containers without any anticoagulant and the serum was separated. Immediately after the sacrifice, the rats were dissected, heart was removed and washed in ice cold saline. About 500 mg of the tissue was weighed and homogenized in 5 ml of 0.25M ice cold sucrose solution at 4°C in a Potter-Elvehjem homogenizer. Mitochondria were isolated from the heart as per Johnson and Lardy\(^7\) the mitochondrial pellets were suspended in Tris-HCl buffer (0.1 M, pH 7.4) and used for estimation of enzyme activities and protein.

The activities of TCA cycle enzymes, viz. succinate dehydrogenase\(^8\), isocitrate dehydrogenase\(^8\), malate
dehydrogenase\textsuperscript{10} and α-ketoglutarate dehydrogenase\textsuperscript{11} and of respiratory marker enzymes cytochrome C oxidase\textsuperscript{12} and NADH dehydrogenase\textsuperscript{13} were assayed. The protein content of heart mitochondria\textsuperscript{14}, phospholipase\textsuperscript{15}, lipid peroxidation\textsuperscript{16}, plasma lactic acid\textsuperscript{17} were estimated.

The oxidation of sodium succinate was followed by an oxygen electrode according to the method of Katyare \textit{et al.}\textsuperscript{18}. Mitochondrial calcium was estimated in Perkin-Elmer 2380 atomic absorption spectrophotometer. Mitochondrial ATP concentration was measured by the method of Williamson and Corkey\textsuperscript{19}.

Isoproterenol hydrochloride, menhaden fish oil, bovine serum albumen, α-ketoglutarate, NAD, NADP, cytochrome \textit{c}, ATP, rotenone, N-phosphatidyl choline, hexokinase and glucose-6-phosphate dehydrogenase were obtained from Sigma Chemical Co. St. Louis, MO, USA. All other chemicals used were of analytical grade and purchased locally.

**Results and Discussion**

The results are presented in Tables 1 - 3 and Fig. I.

During normal oxygenation, cytoplasmic NADH\textsubscript{2} is removed by malate-aspartate cycle which depends on continued mitochondrial respiration. In hypoxia or ischaemia, intramitochondrial NADH\textsubscript{2} increases as a result of impaired β-oxidation due to decreased electron transport. NADH\textsubscript{2} accumulation in the cytosol means more protons so that intracellular acidosis is promoted. Pyruvate dehydrogenase, located on the mitochondrial membrane, is inhibited by NADH\textsubscript{2} so that entry to citrate cycle is inhibited, more lactate forms, and the ratio of NADH\textsubscript{2} to NAD increases further. NADH\textsubscript{2} also accumulate in the mitochondria with adverse effects (i) dehydrogenase enzymes are inhibited at several sites so that any residual activity of the citrate cycle is decreased and (ii) intra mitochondrial calcium increases\textsuperscript{20}.

Lactate is taken up by the aerobic heart and is produced during anaerobiosis; its release into coronary sinus blood is sometimes used as a sign of myocardial ischemia\textsuperscript{21}. Increased neutral lactate in severe ischemia causes decreased contractile activity in the ischaemic zone\textsuperscript{22}, promotion of mitochondrial damage\textsuperscript{23}, decrease of the action potential duration\textsuperscript{24} and inhibition of glycolysis at the level of glyceraldehyde 3 phosphate dehydrogenase\textsuperscript{25}.

Increased external lactate in ischaemic myocardium decreases internal myocyte \(pH\) by inhibiting lactate/proton cotransporter\textsuperscript{26}. The decrease in internal \(pH\) increases cytosolic calcium by exchange of Na\textsuperscript{+}/H\textsuperscript{+} and Na\textsuperscript{+}/Ca\textsuperscript{2+}. The adverse effects of high external levels of neutral lactate could contribute to the overall mechanism of ischaemic damage\textsuperscript{27}.

The increase in lactate content and mitochondrial calcium in isoproterenol treated rats in the present study may have inhibited the dehydrogenases of the TCA cycle enzymes in these animals. During ischemia pronounced enhancement of lipid peroxidation was seen in mitochondria. The dehydrogenases of TCA cycle enzymes could have been affected by the free radicals produced on isoproterenol treatment.

Cytochrome \textit{c} oxidase and NADH dehydrogenase have an absolute requirement of cardiolipin\textsuperscript{28}. Decrease in the activity of cytochrome \textit{c} oxidase and NADH dehydrogenase in isoproterenol administered rats could be due to enhanced phospholipid degradation resulting in the nonavailability of cardiolipin for their functional activity.

**Table I—Effect of fish oil on mitochondrial TCA cycle enzyme activities in isoproterenol treated rats**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Isocitrate dehydrogenase\textsuperscript{1}</th>
<th>α-Ketoglutarate dehydrogenase\textsuperscript{2}</th>
<th>Succinate dehydrogenase\textsuperscript{3}</th>
<th>Malate dehydrogenase\textsuperscript{4}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>754.6 ± 40.2</td>
<td>74.6 ± 5.9</td>
<td>251.3 ± 16.1</td>
<td>339.12 ± 27.6</td>
</tr>
<tr>
<td>Group II</td>
<td>540.4 ± 43.7***</td>
<td>49.8 ± 3.7***</td>
<td>155.7 ± 11.2***</td>
<td>256.7 ± 22.5***</td>
</tr>
<tr>
<td>Group III</td>
<td>761.4 ± 33.8\textsuperscript{m}</td>
<td>76.8 ± 6.7\textsuperscript{m}</td>
<td>247.4 ± 18.2\textsuperscript{n}</td>
<td>346.72 ± 214\textsuperscript{n}</td>
</tr>
<tr>
<td>Group IV</td>
<td>643.8 ± 52.3**</td>
<td>63.7 ± 4.1**</td>
<td>216.8 ± 12.4**</td>
<td>314.7 ± 22.5**</td>
</tr>
</tbody>
</table>

Student's t-test : Group II and III vs Group I. Group IV vs Group III.

\(p\) values ** < 0.01; *** < 0.001; \textsuperscript{29} - non significant

Units:

\textsuperscript{1}nmole of α-ketoglutarate formed/hr/mg protein

\textsuperscript{2}nmole of potassium ferrocyanide formed/hr/mg protein

\textsuperscript{3}nmole of succinate oxidised/min/mg protein

\textsuperscript{4}nmole of NADH oxidised/min/mg protein
Isoproterenol treated rats showed increase in phospholipase activity in heart mitochondria, this could also be the reason for enhanced phospholipid degradation apart from lipid peroxidation.

Decreased lactate content in fish oil treated rats was reported by Meng et al. Lower phospholipase activity in n-3 rich cardiomyocytes compared to n-6 rich cardiomyocytes was reported by Nalbone et al. The enrichment in membrane n-3 PUFA of the fish oil heart could partially affect phospholipase activation during ischaemia and reperfusion and reduces membrane damage and enzyme leakage. In this connection the ischaemia induced increase in free calcium could be an important factor.

Feeding rats with fish oil results in the incorporation of n-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid into the membrane phospholipids which are poor substrates for oxidation. Decreased membrane lipid peroxidation and improved antioxidant defence on EPA feeding was reported by Abraham Demoz et al.

Thus increase in TCA cycle enzymes found in fish oil supplemented rats could be due to (i) decreased plasma lactic acid content, and (ii) decreased mitochondrial Ca²⁺ resulting in decreased phospholipase activity leading to decreased phospholipid degradation. The antioxidant nature of EPA also plays an important role here.

In ischaemic mitochondria, the measurement of oxidative phosphorylation revealed marked depression in all variables i.e., ADP/O ratio, respiratory control index and rate of succinate oxidation. The decreased oxygen uptake may be due to impairment in myocardial oxygen production.

The increased free fatty acids and other lipid metabolites resulting from impaired oxidation of fatty

<table>
<thead>
<tr>
<th>Groups</th>
<th>Lipid peroxides a</th>
<th>NADH dehydrogenase b</th>
<th>Cytochrome c oxidase c</th>
<th>Phospholipase d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>179.52 ± 7.4</td>
<td>140.7 ± 8.7</td>
<td>3.12 ± 0.2</td>
<td>0.31 ± 0.032</td>
</tr>
<tr>
<td>Group II</td>
<td>287.65 ± 16.8***</td>
<td>95.76 ± 10.1***</td>
<td>1.71 ± 0.1***</td>
<td>0.52 ± 0.036***</td>
</tr>
<tr>
<td>Group III</td>
<td>176.39 ± 8.2 NS</td>
<td>147.6 ± 12.3 NS</td>
<td>2.97 ± 0.2 NS</td>
<td>0.33 ± 0.029 NS</td>
</tr>
<tr>
<td>Group IV</td>
<td>211.37 ± 6.4***</td>
<td>124.3 ± 11.3***</td>
<td>2.62 ± 0.3***</td>
<td>0.40 ± 0.037***</td>
</tr>
</tbody>
</table>

Student’s t-test : Groups II and III vs Group I. Group IV vs Group III.

***P < 0.001, NS - non significant

Units :  
 a n mole of MDA/mg protein,  
b n mole of NADH oxidised/min/mg protein,  
c O.D x 10-2/min/mg protein,  
d n mole of free fatty acid liberated/min/mg protein.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mitochondrial calcium a</th>
<th>Lactic acid b</th>
<th>ATP c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>99.56 ± 11.2</td>
<td>21.5 ± 2.5</td>
<td>2.54 ± 0.19</td>
</tr>
<tr>
<td>Group II</td>
<td>187.8 ± 12.8***</td>
<td>40.7 ± 2.8***</td>
<td>1.37 ± 0.14***</td>
</tr>
<tr>
<td>Group III</td>
<td>96.7</td>
<td>19.7 ± 1.5 NS</td>
<td>2.41</td>
</tr>
<tr>
<td>Group IV</td>
<td>115.6 ± 10.8***</td>
<td>28.3 ± 3.11</td>
<td>1.97 ± 0.17***</td>
</tr>
</tbody>
</table>

Student’s t-test : Groups II and III vs Group I. Group IV vs Group III.

* P < 0.05; ***P < 0.001.

Units :  
a mmol 10⁴/ 100 mg protein.  
b mg/dl  
c nmole/mg protein.
Fish oil treatment results in increased β-oxidation of fatty acids. Decreased phospholipid degradation due to decreased phospholipase activity and low cytosolic and mitochondrial Ca²⁺ concentration in fish oil fed rats may all contribute to improved mitochondrial energy metabolism.

Acknowledgement
The authors are thankful to CSIR, New Delhi for financial assistance.

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