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# Effect of saturated, ω-3 and ω-6 polyunsaturated fatty acids on myocardial infarction

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*Dietary fatty acids have cholesterol lowering, antiatherogenic, and antiarrhythmic properties that decrease the risk of myocardial infarction (MI). This study was designed to study the effects of various oils rich in either polyunsaturated (ω-3 or ω-6) fatty acids (PUFA) or saturated fatty acids (SFA) on the severity of experimentally induced MI. Male albino Sprague-Dawley rats (100–150 g;* n = *20) were fed diets enriched with fish oil (ω-3 PUFA), peanut oil (ω-6 PUFA), or coconut oil (SFA) for 60 days. Experimental MI was induced with isoproterenol. Mortality rates; serum enzymes aspartate amino transferase; alanine amino transferase; creatine phosphokinase (CPK); lipid profiles in serum, myocardium, and aorta; peroxide levels in heart and aorta; activities of catalase and superoxide dismutase; and levels of glutathione were measured. The results demonstrated that mortality rate, CPK levels, myocardial lipid peroxides, and glutathione levels were decreased in the ω-3 PUFA treated group. Maximum increase in parameters indicative of myocardial damage was seen in the coconut oil group. These findings suggest that dietary ω-3 PUFA offers maximum protection in experimentally induced MI in comparison to ω-6 PUFA and SFA enriched diets. SFA was found to have the least protective effect.* (J. Nutr. Biochem. 10:338–344, 1999) 

**Keywords:** myocardial infarction; atherosclerosis; fatty acids; malondialdehyde; hydroperoxides; catalase; superoxide dismutase

## Introduction

Myocardial infarction (MI) is the acute condition of necrosis of the myocardium that occurs as a result of imbalance between coronary blood supply and myocardial demand.[1] Various theories have been proposed to elucidate the pathogenesis of this critical condition. One theory suggests that there is increased production of malondialdehyde (MDA), which is a measure of lipid peroxidation, and a transient inhibition of protective enzymes such as superoxide dismutase (SOD) in both MI and unstable angina.[2] Elevated nonesterified fatty acids in plasma have been shown to stimulate platelet aggregation and have been hypothesized to contribute to the progression of tissue injury in MI by increasing tissue oxygen requirements and promoting vascular occlusions.[3] Free radical induced lipid peroxidation has been proposed as an etiologic factor in cell membrane damage, atherosclerosis, cancer, MI, and aging.[4–6] Increased levels of free fatty acids (FFA) also have been shown to activate microsomal lipid peroxidation, which may be an important event in cellular necrosis and damage.[6]

Pharmacologic agents have been tested for their effects on the severity of MI induced by isoproterenol [1(3,4 dihydroxyphenyl 2-isopropyl amino ethanol], a synthetic catecholamine and β-adrenergic agonist that has been reported to cause severe oxidative stress in the myocardium, resulting in infarct-like necrosis of the heart muscle.[7–9] Dietary fatty acids may offer a protective role in MI development. The type and nature of fat are known to influence serum lipid levels in humans and experimental animals.[10–12] The antiatherogenic effect of various fatty acids has been reported in numerous studies. The majority of studies indicate that saturated fatty acids (SFA) accelerate atherosclerosis whereas monounsaturated and polyunsaturated fatty acids (PUFA) have cholesterol lowering and antiatherogenic actions.[13–17] Fats containing ω-3 fatty acids

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have been shown to be more protective than ω-6 fatty acids from an atherogenic point of view.[18] A recent review suggested that ω-3 fatty acids prevent coronary heart disease (CHD).[19] This is evidenced by the low rate of CHD in Greenland Eskimos who consume high amounts of fish seal and whale fats, which contain ω-3 fatty acids, docosahexaenoic acid, and eicosapentaenoic acid.[19] Parks et al.[20] compared diets containing high levels of fish oil with diets containing the same amount of SFA in African green monkeys. The results showed that coronary artery atherosclerosis and aortic atherosclerosis were reduced by fish oil. In the aorta, the fish oil effect on atherosclerosis was characterized by a decrease in both free and esterified cholesterol content. Fish oil intake may reduce plasma fibrinogen concentration and positively modify the fatty acid blood profile of healthy young men.[21] A recent study showed that dietary supplementation with fish oil significantly increases endothelial function in peripheral small arteries of hypercholesterolemic patients.[22] Unesterified ω-3 PUFAs were able to protect cardiac myocytes from induced arrhythmias.[23] Reports are also available on the protective effects of free PUFA on arrhythmias.[24–26]

The purpose of this study was to determine the effect of specific types of fatty acids on the severity and progression of MI. Biochemical parameters including cholesterol, phospholipids, and FFA, which are indicators of lipid peroxidation in MI, and the enzymes aspartate amino transferase (AST), alanine amino transferase (ALT), and creatine phosphokinase (CPK) were monitored to assess MI damage.

## Materials and methods

### *Animals and diet*

The experimental protocol was approved by the Ethical Committee for Conduction of Animal Studies at the University of Kerala. Male albino Sprague-Dawley rats weighing from 100 to 150 g were randomly divided into three dietary groups. The rats were fed defatted pellets (Gold Mohur, Mumbai, India) enriched with 10% by weight either peanut oil (ω-6 PUFA), coconut oil (SFA), or fish oil (ω-3 PUFA). The oils were purchased from the local market at Trivandrum. Analysis of fatty acid composition of different oils was done by gas chromatography and is shown in *Table 1*.[27] For this study the lipid extracts of the oils were converted into fatty acid methyl esters using $BF_3$ methanol. Fatty acid methyl ester peaks were identified by injecting authentic standard mixtures of fatty acid methyl esters.

The diet and water were provided ad libitum. After 60 days on the respective diets, the animals were randomly allocated into control and experimental groups of 20 animals in each group.

### *Myocardial infarction procedures*

MI was induced in the experimental group ($n = 10$) by subcutaneous injection of isoproterenol (30 mg/100 g) twice with an interval of 24 hours between injections. Rats administered isoproterenol had the expected symptoms of shock, tachycardia, dyspnea, anuria, and prostration. Mortality was monitored and rats that survived after the second injection were sacrificed for histologic examination. The animals in each group were fasted overnight and sacrificed by decapitation. The tissues (heart and aorta) were removed and cleaned in saline and stored in ice for various measurements. Blood was collected and serum was separated from the blood after centrifugation (3,000 rpm × 15 minutes).

**Table 1** Fatty acid composition in different oils

| | Peanut oil | Coconut oil | Fish oil |
|---|---|---|---|
| Saturated | | | |
| C 12:0 | — | — | — |
| 14:0 | — | 48.90 | 11.0 |
| 16:0 | 14.90 | 21.96 | 26.7 |
| 18:0 | 2.95 | 6.00 | 4.1 |
| Total | 17.85 | 76.86 | 41.8 |
| Monoenes | | | |
| 16:1 | — | — | 13.6 |
| 18:1 | 41.96 | 18.20 | 12.6 |
| Total | 41.96 | 18.20 | 27.25 |
| PUFA | | | |
| 18:2 (ω-6) | 40.20 | 5.33 | 1.70 |
| 18:3 | — | — | — |
| 20:3 (ω-6) | — | — | 2.5 |
| 20:4 (ω-6) | — | — | 5.0 |
| 20:5 (ω-3) | — | — | 13.8 |
| 22:5 (ω-6) | — | — | 1.2 |
| 22:6 (ω-3) | — | — | 6.8 |
| Total | 40.20 | 5.33 | 31.0 |

CPK, AST, and ALT were determined as previously described.[6] Cholesterol, FFA, and phospholipid levels were measured in the serum, heart, and aorta as previously described.[28] The levels of lipid peroxides (i.e., MDA, hydroperoxides, and conjugate dienes) were measured in the heart and aorta according to previously published methods.[6] Activities of catalase, SOD, and the level of glutathione also were determined in the heart tissue.[29]

### *Statistical tests*

Statistical significance was calculated by Student's *t*-test and a *P*-value of less than 0.05 was considered significant.[30]

## Results

### *Mortality*

Survival rate was higher in rats fed with ω-3 PUFA enriched diet (9 rats surviving) than in the ω-6 PUFA group (7 rats surviving), but the SFA group had the lowest survival rate

**Table 2.** Serum AST, ALT, and CPK enzyme activities

| Groups | AST* | ALT† | CPK‡ |
|---|---|---|---|
| ω-6 PUFA | 142.9 ± 4.9 | 47.0 ± 1.7 | 316.8 ± 12.4 |
| ω-6 PUFA+iso | 245.7 ± 12.6[a] | 150.9 ± 6.7[a] | 463.8 ± 26.0[a] |
| SFA | 145.9 ± 5.2 | 52.7 ± 1.9 | 304.0 ± 11.6 |
| SFA+iso | 267.2 ± 15.6[a] | 175.1 ± 10.9[a] | 508.2 ± 28.5[a] |
| ω-3 PUFA | 139.9 ± 4.5 | 48.9 ± 1.7 | 291.6 ± 10.9 |
| ω-3 PUFA+iso | 193.3 ± 10.8[a,b,c] | 157.9 ± 10.0[a] | 410.4 ± 23.4[a,b] |

Note: Values are means ± SEM from six animals.
*Micromoles of oxaloacetic acid liberated/min/L serum.
†Micromoles of pyruvate liberated/min/L serum.
‡Micromoles of creatine liberated/min/L serum.
[a]$P < 0.05$, compared with respective controls.
[b]$P < 0.05$, compared with ω-6 polyunsaturated fatty acid (PUFA) + isoproterenol (iso).
[c]$P < 0.05$, compared between saturated fatty acid (SFA)+iso and ω-3 PUFA+iso.
AST–aspartate amino transferse; ALT–alanine amino transferase; CPK–creatine phosphokinase.

**Table 3** Serum lipid levels

| Groups | Cholesterol (mg/100 mL) | Free fatty acids (mg/100/mL) | Phospholipid (g/100/mL) |
|---|---|---|---|
| ω-6 PUFA | 72.3 ± 2.4 | 76.6 ± 2.6 | 0.173 ± 0.007 |
| ω-6 PUFA+iso | 98.4 ± 3.7[a] | 103.3 ± 4.3[a] | 0.208 ± 0.009[a] |
| SFA | 88.5 ± 3.4 | 85.4 ± 2.9 | 0.191 ± 0.008 |
| SFA+iso | 124.4 ± 4.9[a,b] | 145.2 ± 6.6[a,b] | 0.293 ± 0.16[a,b] |
| ω-3 PUFA | 74.4 ± 2.8 | 87.9 ± 3.4 | 0.169 ± 0.006 |
| ω-3 PUFA+iso | 109.4 ± 4.1[a] | 115.3 ± 5.4[a,c] | 0.254 ± 0.012[a] |

Note: Values are means ± SEM from six animals.
[a]$P < 0.05$, compared with respective controls.
[b]$P < 0.05$, compared with ω-6 polyunsaturated fatty acid (PUFA)+isoprenterenol (iso).
[c]$P < 0.05$, compared between saturated fatty acid (SFA)+iso and ω-3 PUFA+iso.

(6 animals surviving). Mortality was 40% in the SFA oil group, 30% in the ω-6 PUFA group, and 10% in the ω-3 PUFA fed group.

### *Serum AST, ALT, and CPK activities*

Serum ALT and CPK activities (*Table 2*) were similar in the SFA and the ω-3 PUFA groups compared with the ω-6 PUFA group. With administration of isoproterenol (iso), the activities of these enzymes were elevated compared with the appropriate controls ($P < 0.001$). When compared with the ω-6 PUFA+iso group, no significant changes in enzyme levels of ALT and CPK were observed in the SFA+iso group. The activity of enzyme AST in the ω-3 PUFA+iso group was decreased significantly compared with the SFA+iso and ω-6 PUFA groups ($P < 0.05$). When compared with the SFA+iso group, the activity of enzyme CPK in ω-3 PUFA+iso showed a significant decrease in activity ($P < 0.05$).

### *Serum lipids levels*

The effects of diet and isoproterenol on the serum lipid profile are shown in *Table 3*. The concentration of serum cholesterol was increased in the SFA+iso group compared with the ω-6 PUFA+iso group ($P < 0.01$). The levels of FFA and phospholipid were increased significantly in the SFA+iso group compared with the ω-6 PUFA+iso group ($P < 0.01$). FFA levels were significantly decreased in the ω-3 PUFA+iso group compared with the SFA+iso group ($P < 0.05$). Phospholipid levels were increased in ω-3 PUFA+iso group compared with ω-6 PUFA+iso group ($P < 0.05$).

### *Myocardial and aortic lipid levels*

The concentration of lipids in the heart are presented in *Table 4*; concentrations of lipids in the aorta are presented in *Table 5*. The level of cholesterol in heart tissue was increased in all of the isoproterenol groups compared with the appropriate controls; however, significance was demonstrated only in the SFA+iso and ω-3 PUFA groups ($P < 0.05$). The myocardial cholesterol level showed a significant increase in the SFA+iso group compared with the ω-6 PUFA group ($P < 0.05$). Phospholipid levels in heart and aorta were the highest in the SFA+iso group. The level of FFA in heart was increased significantly in the SFA+iso group compared with the ω-6 PUFA+iso group. In the aorta, FFA were significantly decreased in the ω-3 PUFA group compared with the SFA+iso group ($P < 0.01$).

### *Lipid peroxides levels*

Lipid peroxide levels in the heart are presented in *Table 6*; those in the aorta are presented in *Table 7*. Administration of isoproterenol increased the level of MDA in heart and aorta when compared with the respective controls. The levels of MDA were decreased significantly in ω-3 PUFA+iso group in heart tissue compared with both the ω-6 PUFA+iso and SFA+iso groups ($P < 0.01$). Levels of MDA in the aorta were decreased in the SFA+iso group and increased in the ω-3 PUFA+iso group compared with the ω-6 PUFA+iso group ($P < 0.05$). The level of MDA in the aortas of the ω-3 PUFA+iso animals was increased significantly compared with the SFA+iso group ($P < 0.001$).

The concentration of hydroperoxides in isoproterenol-administered groups was increased in both the heart and aorta compared with the appropriate controls. When compared with the ω-6 PUFA+iso group, the level of hydroperoxides in the heart showed no significant change, whereas in the aorta, both the SFA+iso and ω-3 PUFA+iso groups showed a significant decrease ($P < 0.001$). With respect to SFA+iso group, ω-3PUFA+iso group showed a significant decrease ($P < 0.05$) only in aortic hydroperoxides.

**Table 4** Myocardial lipid levels

| Groups | Cholesterol (mg/100 g tissue) | Free fatty acids (mg/100 g tissue) | Phospholipid (g/100 g tissue) |
|---|---|---|---|
| ω-6 PUFA | 189.7 ± 7.6 | 480.0 ± 17.3 | 2.84 ± 0.10 |
| ω-6 PUFA+iso | 207.2 ± 12.9 | 632.3 ± 28.9[a] | 3.06 ± 0.18 |
| SFA | 226.5 ± 10.2 | 500.0 ± 28.8 | 2.09 ± 0.07 |
| SFA+iso | 290.0 ± 18.8[a,b] | 947.0 ± 44.8[a,b] | 3.91 ± 0.25[a,b] |
| ω-3 PUFA | 180.6 ± 6.1 | 779.2 ± 34.0 | 2.53 ± 0.09 |
| ω-3 PUFA+iso | 241.7 ± 15.7[a] | 973.0 ± 44.8[a,b] | 3.63 ± 0.24[a] |

Note: Values are means ± SEM from six animals.
[a]$P < 0.05$, compared with respective controls.
[b]$P < 0.05$, compared with ω-6 polyunsaturated fatty acid (PUFA)+isoprenterenol (iso).
[c]$P < 0.05$, compared between saturated fatty acid (SFA)+iso and ω-3 PUFA+iso.

**Table 5** Myocardial lipid levels

| Groups | Cholesterol (mg/100 g tissue) | Free fatty acids (mg/100 g tissue) | Phospholipid (g/100 g tissue) |
|---|---|---|---|
| ω-6 PUFA | 154.7 ± 5.9 | 897.7 ± 35.0 | 1.021 ± 0.035 |
| ω-6 PUFA+iso | 164.5 ± 10.2 | 980.5 ± 44.1 | 1.186 ± 0.075 |
| SFA | 160.0 ± 5.8 | 778.0 ± 28.0 | 1.110 ± 0.05 |
| SFA+iso | 197.7 ± 12.5 | 1,103.6 ± 50.8[a] | 2.013 ± 0.130[a,b] |
| ω-3 PUFA | 129.5 ± 4.8 | 798.9 ± 27.2 | 1.190 ± 0.065 |
| ω-3 PUFA+iso | 147.6 ± 9.4 | 822.9 ± 35.4[c] | 1.480 ± 0.095[c] |

Note: Values are means ± SEM from six animals.
[a]$P < 0.05$, compared with respective controls.
[bb]$P < 0.05$, compared with ω-6 polyunsaturated fatty acid (PUFA)+isoprenterenol (iso).
$P < 0.05$, compared between saturated fatty acid (SFA)+iso and ω-3 PUFA+iso.

Although there was an increase in the level of conjugated dienes in the heart and aorta of isoproterenol administered groups compared with their controls, there was no significant change in both groups; that is, SFA+iso and ω-3 PUFA+iso showed no significant change compared with ω-6 PUFA+iso group.

### *SOD and catalase activities and glutathione levels in heart tissue*

A significant decrease in the activity of the enzymes SOD and catalase was found in all the isoproterenol groups compared with the respective controls (*Table 8*). Similar activities of catalase were shown in ω-6 PUFA+iso and ω-3 PUFA+iso groups, whereas decreased activity was shown in the SFA+iso group. SOD activity was significantly decreased in the SFA+iso group ($P < 0.05$). When compared with the ω-6 PUFA+iso and SFA+iso groups, SOD activity was significantly increased in the ω-3 PUFA+iso group ($P < 0.05$).

Although there was an increase in the level of glutathione content in heart tissue of all the isoproterenol groups compared with the appropriate controls, there was no significant change in both the ω-3 PUFA+iso and SFA+iso groups compared with the ω-6 PUFA+iso group. With respect to the SFA+iso group, the glutathione level was significantly decreased in the ω-3 PUFA+iso group ($P < 0.05$).

## Discussion

The increased survival rate following isoproterenol administration and decreased serum AST and CPK levels clearly indicate that a diet enriched with fish oil offers better protection against experimental MI than peanut oil or coconut oil. The increased survival rate of the ω-3 PUFA+iso group may due to the lowering of cholesterol and FFA levels in serum and heart. Myocardial and aortic cholesterol, FFA, and phospholipids were elevated in the SFA+iso group compared with the ω-6 PUFA+iso and ω-3 PUFA+iso groups. The ω-3 PUFA+iso group showed minimum elevation in these parameters in the aorta over the SFA+iso and ω-6 PUFA+iso groups. The increase in serum, myocardial, and aortic lipid levels may be a contributing factor in the development of atherosclerosis and subsequent MI. Our findings are in accordance with previous studies by Spady and Wollett,[31] which indicated that replacement of a saturated fat rich diet with a fish oil rich diet in rats reduces low density lipoprotein (LDL) cholesterol concentration as effectively as a reduction in dietary saturated fat. Fish oil was found to increase LDL receptor activity and LDL clearance significantly more than SFA.

The levels of FFA, the substrate for microsomal lipid peroxidation, showed maximum concentration in the serum and aorta of the SFA+iso group. The increased level of serum FFA in the SFA+iso group may be due to increased lipolysis as suggested by a previous study by Saleena et al.[32]

**Table 6** Myocardial lipid peroxide levels

| Groups | Malondialdehyde (mM/100 g tissue) | Hydroperoxide (mM/100 g tissue) | Conjugated dienes (mM/100 g tissue) |
|---|---|---|---|
| ω-6 PUFA | 0.695 ± 0.023 | 19.29 ± 0.66 | 10.90 ± 0.380 |
| ω-6 PUFA+iso | 1.5998 ± 0.085[a] | 50.66 ± 2.99[a] | 16.90 ± 1.000[a] |
| SFA | 0.715 ± 0.030 | 27.05 ± 0.89 | 10.40 ± 0.364 |
| SFA+iso | 1.906 ± 0.114[a] | 59.04 ± 3.50[a] | 15.16 ± 0.894[a] |
| ω-3 PUFA | 0.674 ± 0.020 | 37.60 ± 1.32 | 11.62 ± 0.420 |
| ω-3 PUFA+iso | 1.096 ± 0.060[a,b,c] | 54.96 ± 3.22[a] | 15.64 ± 0.910[a] |

Note: Values are means ± SEM from six animals.
[a]$P < 0.05$, compared with respective controls.
[b]$P < 0.05$, compared with ω-6 polyunsaturated fatty acid (PUFA)+isoprenterenol (iso).
[c]$P < 0.05$, compared between saturated fatty acid (SFA)+iso and ω-3 PUFA+iso.

**Table 7** Aortic lipid peroxide levels

| Groups | Malondialdehyde (mM/100 g tissue) | Hydroperoxide (mM/100 g tissue) | Conjugated dienes (mM/100 g tissue) |
|---|---|---|---|
| ω-6 PUFA | 1.344 ± 0.050 | 189.45 ± 6.30 | 52.41 ± 1.83 |
| ω-6 PUFA+iso | 1.868 ± 0.065[a] | 236.97 ± 13.3 | 57.47 ± 2.70 |
| SFA | 1.136 ± 0.020 | 55.43 ± 0.89 | 35.24 ± 1.27 |
| SFA+iso | 1.238 ± 0.040[b] | 140.4 ± 7.70[a,b] | 61.21 ± 3.43[a] |
| ω-3 PUFA | 1.282 ± 0.040 | 57.75 ± 1.96 | 30.97 ± 1.05 |
| ω-3 PUFA+iso | 2.200 ± 0.080[a,b,c] | 113.36 ± 6.6[a,b,c] | 60.10 ± 3.25[a] |

Note: Values are means ± SEM from six animals.
[a]$P < 0.05$, compared with respective controls.
[b]$P < 0.05$, compared with ω-6 polyunsaturated fatty acid (PUFA)+isoprenterenol (iso).
[c]$P < 0.05$, compared between saturated fatty acid (SFA)+iso and ω-3 PUFA+iso.

Although the myocardial FFA levels were increased significantly in the ω-3 PUFA+iso group compared with the SFA+iso group, a significant decrease in the level was observed in aortic FFA. The aortic FFA level was maximum in SFA+iso group. This increased FFA level in the aorta would be a consequence of increased cholesterol levels. The increased delivery of cholesterol to this tissue can lead to changes in the permeability of the cell membrane including mitochondrial membrane.[33] This can cause impairment in anion transport in the mitochondrial membrane, resulting in interference with malate aspartate shuttle, which is a mechanism for mitochondrial oxidation of nicotinamide adenine dinucleotide (NADH). Under these circumstances, NADH could be oxidized by means of an alternate route that channels acetate into fatty acid biosynthesis. Elevated nonesterified fatty acids have been shown to stimulate platelet aggregation and may contribute to the progression of tissue injury in MI by both increasing tissue oxygen requirements and promoting vascular occlusion.

The production of lipid peroxide is closely associated with myocardial damage due to isoproterenol. It is known that the desaturation of the cell membrane obtained by enrichment with PUFA exposes the cell to the toxic effects of free radicals, which leads to lipid peroxidation.[34] MDA as determined in this study represents a reliable index of lipid peroxidation in MI induced by isoproterenol. The ω-3 PUFA+iso group showed a significant decrease in the MDA levels in the heart but increased levels were observed in the aorta. The decreased levels of MDA in the heart suggest decreased necrosis. MDA is a breakdown product of unsaturated fatty acids. Maintenance of normal function in the presence of oxygen largely depends on the efficiency of tissue protection against free radical mediated oxidative stress. Oxygen derived free radicals are produced continuously in the cell during cellular metabolism and a variety of mechanisms such as increased oxygen concentration, redox cycle of biochemical substances, or excessive phagocyte activation may modulate the oxidative stress. Increased levels of lipid peroxides in the aorta may be related to increased formation of eicosanoids. A previous report suggested that fish oil enhances the formation of prostacyclin (and to a lesser degree, thromboxane) in tissues, which may have a possible protective effect.[35] The increased concentration of aortic lipid peroxides in the ω-3 PUFA group may be a compensatory mechanism to protect the tissue during the recovery phase. Lipid peroxidation causes a disturbance of the structural organization of lipoprotein, and as a consequence, there is an increase of LDL cholesterol-donating ability and a decrease of high density lipoprotein (HDL) cholesterol-accepting ability. The greater the amount of LDL oxidized, the more cholesterol is transported to erythrocytes. The greater the level of HDL peroxidation, the

**Table 8** Myocardial catalase, superoxide dismutase enzyme activities, and glutathione levels

| Groups | Catalase (unit × 10/mg protein*) | Superoxide dismutase (unit/mg protein†) | Glutathione (mg/100 g tissue) |
|---|---|---|---|
| ω-6 PUFA | 8.80 ± 0.51 | 11.97 ± 0.67 | 29.64 ± 1.0 |
| ω-6 PUFA+iso | 6.97 ± 0.24[a] | 8.52 ± 0.31[a] | 39.84 ± 1.6[a] |
| SFA | 7.90 ± 0.45 | 10.72 ± 0.61 | 22.20 ± 0.6 |
| SFA+iso | 5.17 ± 0.19[a] | 6.40 ± 0.23[a,b] | 43.96 ± 1.9[a] |
| ω-3 PUFA | 8.30 ± 0.47 | 10.03 ± 0.58 | 27.13 ± 0.8 |
| ω-3 PUFA+iso | 6.97 ± 0.25[a] | 9.90 ± 0.36[b,c] | 34.53 ± 1.1[a,c] |

Note: Values are means ± SEM from six animals.
*unit = velocity constant/sec.
†unit = the enzyme concentration required to inhibit the optical density at 560 nm chromogen production by 50% in 1 minute.
[a]$P < 0.05$, compared with respective controls.
[b]$P < 0.05$, compared with ω-6 polyunsaturated fatty acid (PUFA)+isoprenterenol (iso).
[c]$P < 0.05$, compared between saturated fatty acid (SFA)+iso and ω-3 PUFA+iso.

stronger HDL's cholesterol-accepting function is suppressed. Hence lipid peroxidation can play an important role in lipoprotein modifications, which makes them susceptible to atherogenesis.[36]

Oxidative stress can damage many biological molecules; indeed, protein and DNA are often more significant targets of injury than are lipids, and lipid peroxidation often occurs late in the injury process.[37] Experimental evidence indicates that free radical-mediated lipid peroxidation can induce endothelial cell injury or dysfunction.[34] Reactive oxygen species, including peroxidized lipids capable of initiating cell injury, may be generated within endothelial cells, present in plasma compounds, or derived from neutrophils. Lipid peroxidation could initiate or promote the process of atherosclerosis lesion formation by directly damaging endothelial cells and by enhancing the adhesion and activation of neutrophils and the susceptibility of platelets to aggregate. Endothelial cell injury by lipid hydroperoxides also could increase the uptake of LDL into the vessel wall. These events and other cellular dysfunctions may individually or collectively initiate and/or help to sustain the development of atherosclerosis.

SOD and catalase, two key scavenging enzymes for the superoxide radical and hydrogen peroxide, were decreased in the heart of the SFA+iso group compared with ω-6 PUFA+iso group. A decrease in the activity of these enzymes can lead to the formation of oxygen and hydrogen peroxide, which in turn can form the toxic hydroxy radical [$OH^{\cdot}$]. The decrease in activities of SOD and catalase may be due to myocardial cell damage. The increased activity of the myocardial enzymes catalase and SOD is associated with decreased levels of peroxidation in the ω-3 PUFA+iso and ω-6 PUFA+iso group. This can result in decreased formation of toxic intermediates. Other investigators have reported that changes in aortic antioxidant defense mechanism affect lipid peroxidation.[37] Cholesterol fed rabbits with massive vascular lipid infiltration reportedly have antioxidant mechanisms that are stressed as evidenced by increased activities of SOD and glutathione peroxidase, and increased levels of total thiol compounds, whereas others are depressed as evidenced by decreased enzyme activities of catalase, glutathione reductase, and glutathione transferase. This potentially reduces or increases vascular susceptibility to oxidative injury.[38]

Glutathione is an important substrate for the enzyme glutathione peroxidase. Increased activity of this enzyme suggests increased utilization of glutathione and therefore its level decreases in tissues. Thus, the decreased level of glutathione in the ω-3 PUFA group suggests its increased utilization. An increase in the concentration of a reductant (such as glutathione) can result in increased lipid peroxidation, which can lead to increased damage to the tissue. Therefore, the increased level of the reductant in the SFA group may result in increased lipid peroxidation, leading to damage of the myocardium. Conversely, the decreased level of this reductant in ω-3 PUFA groups may therefore result in decreased lipid peroxidation and decreased damage. Hence, fish oil has a better effect on the lipid profile in the aorta and maximum antiatherogenic effect, and coconut oil has the poorest protective action.

## Conclusion

This study showed that dietary intake of ω-3 fatty acids as fish oil can protect the myocardium from experimentally induced MI. This is documented by a lower mortality rate, a significant reduction of the levels of enzymes known to be markers of myocardial damage, decreased atherogenic effect as evidenced by decreased level of tissue lipids in aorta, decreased myocardial lipid peroxides, and decreased glutathione levels in the fish oil treated group. Adverse changes in lipid profile, enzymatic levels, and peroxide concentrations were seen in the coconut oil treated group, which offered least protection from experimentally induced MI.

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