Discussion
HISTOPATHOLOGICAL STUDIES

The histopathological examination revealed a marked damage to cardiac muscle with increased degenerative changes in isoproterenol treated rats (Plate II). Heart sections from rats treated with fishoil alone showed apparently normal architecture (Plate III).

The degenerative changes were very much reduced in the heart sections of IPH treated rats which received fishoil pretreatment (Plate IV). This study confirms the protective efficacy of fishoil pretreatment on isoproterenol induced toxic changes in heart.

ELECTROCARDIOGRAPHY

In healthy adult rats myocardial infarction was induced by the subcutaneous injection of 60 mg/kg body wt of isoproternol hydrochloride (IPH). ECG studies in IPH treated rats showed abnormalities related to myocardial ischaemia.

The protective effect of fish oil in myocardial infarction induced rats was also studied.

Normal ECG of a rat resembles in essential detail that of a man.

In control rats a normal P wave and no Q waves was observed and every P wave was followed by a narrow QRS of normal contour. The heart rate was regular.
Test principle: ELISA/1-step sandwich assay using streptavidin technology

Sample → Incubation Solution → Washing Solution → Substrate-chromogen solution → Absorbance

Immunoreaction → Separation step → Indicator reaction → Measurement/evaluation

- Troponin T in sample
- Streptavidin
- biotinylated anti-troponin-T antibodies
- anti-troponin-T antibody-POD conjugate

Fig. 5.1
In isoproterenol treated rats, a higher rate was observed with decreased PR interval. Similar findings in IPH induced myocardial infarction was reported by Rona et al. (1959). Increased heart rate means increased cardiac metabolism with resultant increase in O₂ consumption leading to hypoxia. Decreased PR interval denotes decreased calcium clearance.

In rats treated with fish oil alone the heart rate was reduced with increased PR interval.

In rats pretreated with fish oil and then administered isoproterenol, there was a reduction in the heart rate with prolonged PR interval.

ω₃ fatty acids reduced heart rate and could therefore indirectly protect against ischaemic arrhythmias by lowering cardiac metabolism and allowing more time for the high cytosolic calcium levels to be cleared (Billman GE 1994).

ω₃ fatty acid infusion significantly prolonged P wave-R wave intervals. The PR interval, which reflects atrioventricular nodal conduction, is critically dependent on calcium influx and as a result is significantly increased by the infusion of calcium channel antagonists, such as, verapamil or dilitiazazen (Billman, 1992). Similar findings have been observed in the preset study.

HAEMORHEOLOGY

The cardiovascular effects of n-3 fatty acids seem to comprise the potency of delaying atherosclerosis. The mechanisms involved are possibly
manifold varying from actions on platelets, plasma lipid pattern, endothelial function etc.

Based on the close relationship between cardiovascular risk factors and haemorheology (Chien et al., 1987) it has been postulated that both atherogenesis and blood rheology might have some common denominator (Ernst et al., 1986). Adsorption phenomena taking place on surfaces which are in contact with blood plasma might constitute such a common denominator. These phenomena are a basic mechanism of early atherogenic changes and are the cause for abnormalities in blood rheology. Thus rheological variables might be an easily accessible marker for early atherosclerotic processes.

The flow properties of blood are mainly determined by plasma viscosity as well as the number and mechanical properties of blood cells. An increase in blood viscosity occurs with acute myocardial infarction, which is attributable to haemoconcentration. A positive correlation is observed between blood viscosity and parameters like hematocrit, fibrinogen, globulin as well as total lipid concentration (Schabitz et al., 1983).

During acute myocardial infarction there is an increase in the number of erythrocytes, haemoglobin content, reticulocyte quantity, haematocrit indices, mass of circulating erythrocytes and intensity of erythropoiesis (Tarasov, 1976).

The significant increase in red blood cell count, haemoglobin content and haematocrit values following isoproterenol treatment when compared to
control is in accordance with the observation of Tarasov (1976) during myocardial infarction.

This could be due to the circulatory impairment of blood to the heart resulting in hypoxia, which stimulates erythrocytosis. Increase in erythrocytosis during acute myocardial infarction can lead to haemolysis. In response to haemolysis there is an increase in erythropoiesis, which is a compensatory mechanism of $O_2$ insufficiency normally accompanying acute myocardial infarction (Tarasov, 1976).

The enhanced haematocrit value during acute myocardial infarction is a consequence of haemoconcentration and increased blood viscosity (Hershberg et al., 1972).

The pretreatment with fish oil in isoproterenol treated rats resulted in a significant decrease in red blood cell count, haemoglobin content and haematocrit values compared to rats treated with isoproterenol alone.

Decreased blood viscosity and haematocrit values in fish oil treated rats were reported earlier by Ernst (1989).

The cutaneous bleeding time provides an overall estimate of the platelet-vessel wall interaction (Harker et al., 1972). The bleeding time has been shown to be shortened in the acute phase of myocardial infarction (Milner et al., 1985). Several factors control bleeding time and these include platelet count, packed cell volume blood pressure and vascular reactivity.
Studies have shown that the mean platelet volume is increased in acute myocardial infarction (Martin et al., 1983; Cameron et al., 1983; Trowbridge et al., 1987). In vitro studies indicate that large platelets are more reactive haemostatically than small ones (Thompson et al., 1982) and that large platelets produce more thromboxane B₂ in response to stimulation with collagen or thrombin (Jakubowski et al., 1983). Mean platelet volume has been shown to be inversely correlated to the bleeding time in patients with ischaemic heart disease (Kristensen et al., 1988).

The significant decrease observed in bleeding and clotting time following isoproterenol treatment compared to control is in accordance with the observation made by Kristensen et al. (1988). The activation of β-adrenergic receptor on isoproterenol treatment could be the reason for the observed shortened clotting time. There was a significant increase in the platelet count on isoproterenol treatment which is in accordance with the report of Manjula et al. (1992). This could be due to the synthesis of higher percentage of small platelets and rapid consumption of medium and large sized platelets following acute myocardial infarction resulting in the net increase in platelet volume (Sewel et al., 1984).

In rats pretreated with fish oil and then administered isoproterenol there was a significant decrease in platelet content with concomitant increase in bleeding and clotting time. A decrease in platelet count after supplementation with n-3 fatty acids has been reported (Goodnight et al., 1981; Hay et al., 1982; Lorenz et al., 1983; Von Schacky et al., 1985). In several
studies on healthy volunteers (Sanders et al., 1981; Thorngren et al., 1981; Von Lossonczy et al., 1978; Ahmed et al., 1984; Atkinson et al., 1987) and in patients with coronary atherosclerosis (Saynor et al., 1984) or peripheral atherosclerosis (Knapp et al., 1986) supplementation with n-3 PUFAs has been shown to prolong the bleeding time.

Platelet adhesiveness to the vascular endothelium may be the major determinant of bleeding time. EPA is a potent inhibitor of human platelet adhesion (Li et al., 1991). EPA augments the release of EDRF (endothelium derived relaxing factor) activity and this could be the reason for the observed prolongation of bleeding time (Gwenda Mark et al., 1994).

ESR is not dependent upon anyone factor but is the result of a complex interplay among various influences. The most important being plasma proteins, chiefly fibrinogen and globulins. Epidemiological studies suggest that high clotting factor levels, especially factor VII and fibrinogen may be of causal significance in coronary heart disease (Meade et al., 1980; Kannel et al., 1987).

In isoproterenol treated rats a significant increase in fibrinogen content and ESR was observed. The fish oil pretreatment in isoproterenol treated rats resulted in a significant reduction in the fibrinogen content and ESR compared to rats treated with isoproterenol alone. Hostmark et al. (1988) have reported a significant (P < 0.05) decrease in plasma fibrinogen concentrations.

Leucocytes are functionally important cells in inflammation and monocytes have now been shown to be involved in the initial stages of
atherosclerosis (Ross, 1986; Schwartz et al., 1986; Mitchinson et al., 1987). High levels of circulating neutrophil leucocytes may be a risk factor for the development of atherosclerotic complications (Friedman et al., 1974; Grimm et al., 1985; Ernst et al., 1987) and neutrophil activity may be an important determinant of infarct size in AMI. Neutrophils may contribute to the tissue injury following AMI, possibly by release of pro-inflammatory mediators such as LT, free oxygen radicals and hydrolytic enzymes (Lucchesi et al., 1986; Moncada et al., 1986; Werns et al., 1987).

A significant increase in the leucocyte count observed in isoproterenol treated rats is in accordance with the report of Gryglewski et al. Among the leucocytes-the neutrophil count showed a significant increase. The increase in leucocyte count could be due to leucocytosis which is related to the necrotic process and its magnitude. β adrenergic activation following isoproterenol treatment results in leucocytosis (Gryglewski et al., 1971).

Ernst et al. (1987) suggested the correlation between high leucocyte count and the risk of myocardial infarction.

There was a significant reduction in the neutrophil and increase in lymphocyte fractions of the leucocytes in fish oil pretreated and isoproterenol administered rats when compared to rats treated with isoproterenol alone. Decreased levels of circulating neutrophils have been reported by Knapp et al. (1986). This leads to reduced production of \( \text{LTB}_4 \). The n-3 PUFAs seem to enhance lymphocyte proliferation. This could be due to reduced level of \( \text{LTB}_4 \),
as LTB₄ suppresses T lymphocyte function (Rola-Pleszczynski et al., 1982; Payan et al., 1983; Atluru et al., 1984).

MARKER ENZYMES AND GENERAL BIOCHEMICAL PARAMETERS

Troponin-T is considered as a cardiac marker. Under physiological conditions, no myocardial troponin-T is present in serum. Only after degradation of the contractile actin-troponin complex—for example, following severe ischaemia or cell necrosis—are measurable concentrations of troponin-T found in the blood. Troponin-T estimation is done clinically (1) for diagnosis of acute myocardial infarction and monitoring its course, (2) prognosis assessment in patients with unstable angina pectoris (3) monitoring progress in thrombolytic therapy, (4) Tropnin-T is considered as a cardiac marker due to its complete cardiac specificity.

Advantages of its usage as a cardiac marker include:

1. Low upper reference range limit (0.1 ng/ml)
2. Large signal to noise ratio
3. Independent of muscle mass
4. Independent of noncardiac diseases
5. Independent of coincident skeletal muscle injury/disease

It remains elevated for 5-7 days.
Elevated serum troponin value in unstable angina (Hamm et al., 1992) and acute myocardial infarction (Katus et al., 1991) have been reported earlier. Isoproterenol administered rats showed a significant (p<0.001) increase in serum troponin level.

The significantly (P < 0.001) reduced troponin-T value in fish oil pretreated group compared to isoproterenol alone treated group confirms cardiac protection from fish oil.

The myocardial activity of LDH is high enough to make it unlikely that it could be a controlling step for lactate metabolism by the heart. There are five LDH isoenzymes, named in order of rapidity of their electrophoretic migrations. Each isoenzyme is a tetrameric unit composed of four subunits of the H or M type. The H type, predominating in the heart muscle, is also known as LDH₁ or as α-hydroxybutyrate dehydrogenase because α-hydroxybutyrate can replace lactate as substrate. In acute myocardial infarction, an estimate of infarct size may be formed by measuring the rate of appearance and disappearance in the blood of the LDH cardiac isoenzyme (LDH₁), with peak values 35 to 43 hours after the onset of symptoms (Hermens and Witteveen, 1977).

The LDH isoenzyme pattern of control rats show very faint bands which means the myocardium is intact and there is no demage. The LDH isoenzyme pattern of isoproterenol treated rats show prominent bands of the various fractions of LDH. The increased LDH fraction in serum of isoproterenol treated rats could be due to the leakage of the enzyme from damaged
myocardium. The less prominent bands of the LDH isoenzyme pattern in fish oil pretreated and isoproterenol administered rats could be due to relatively less damage to the myocardium which confirm the cardioprotective effect of fish oil.

Decreased oxygen availability in ischaemic myocardium leads to increased tissue LDH₁ content (Ballo et al., 1968) this isoenzyme shift could be due to the molecular ‘adaptation’ of myocardium to the mechanical stress or ischaemia. It may serve as a compensatory mechanism in the failing heart as LDH₅ favours anaerobic metabolism compared with LDH₁ (Dawson et al., 1964).

Relative myocardial ischaemia due to increased myocardial oxygen demand coupled with hypotension and microvascular spasm could be the possible explanation for the pathogenesis of cardiomyopathy in rats treated with isoproterenol (Blasig et al., 1985). Thus, increased LDH₁ in isoproterenol treated animals might indicate relative hypoperfusion and compensatory mechanism adapted by the failing heart.

Serum enzymes - creatine kinase, lactate dehydrogenase and transaminases are the diagnostic indicators of myocardial infarction (Hearse et al., 1979). Isoproterenol treated rats showed extensive necrosis due to lipid peroxidation. An increase in the activity of these enzymes in serum is due to the leakage of enzymes from the heart as a result of necrosis induced by isoproterenol (Manjula et al., 1992). Reduced necrotic changes in fish oil treated animals could be the reason for the decreased activities of the marker
enzymes namely creatine kinase, lactate dehydrogenase and transaminases in Group IV animals.

Increase in serum uric acid in our experimental condition could be due to excessive degradation of purine nucleotides and proteolysis as evidenced by Iriama (1987).

The reduction of infarct size in a rat model seemed to correlate with altered platelet function and EPA and DHA levels in platelets (Zhu et al., 1994). Fish oil possess antiplatelet activity (Leaf et al., 1988). Antithrombotic effect of fish oil could be due to reduced release of vasoconstrictive thromboxane A$_2$ and increased levels of vasodilatory and antiaggregatory prostacyclin (Schacky et al., 1985). It has been shown that prostacyclin is a valuable agent for protecting myocardial tissue during and after myocardial ischaemia (Lefer et al., 1978). Increased levels of prostacyclin could be the major contributor to fish oil mediated protective effect in rats (group IV).

Fish oil modifies the composition of membrane phospholipids and increase n-3/n-6 ratio (Al Makdess et al., 1995). Antioxidant potential of EPA was reported by Abraham Demoz et al. (Abraham Demoz et al., 1992). The protective efficacy of fish oil in the present study could be due to the antioxidant nature of the EPA which is incorporated in the membrane phospholipids of fish oil pretreated rats.

Na$^+$ K$^+$ATPase and Ca$^{2+}$ATPase play significant roles in the contraction and relaxation cycles of the cardiac muscle by maintaining normal
ion levels within the myocytes. Changes in the properties of these ion pumps affect the cardiac function. In fact, the failure of the cell membrane to maintain normal transmembrane ionic distribution through ion pumps is considered to be a major event in the pathogenesis of ischaemia and arrhythmias (Vajreswari and Narayana Reddy, 1992).

During β adrenergic stimulation cyclic AMP phosphorylates at several sites on C terminal chains of the calcium channel and increases the probability of opening of the calcium channel (Varadi et al., 1995). This leads to increase in both Ca\(^{2+}\)ATPase activity and cytosolic calcium. This might be the reason for enhanced activity of Ca\(^{2+}\)ATPase and increased concentration of Ca\(^{2+}\) in myocardial tissue with concomitant decrease in serum Ca\(^{2+}\) level in isoproterenol treated rats.

The affinity of ATP for magnesium is higher than that of ADP, so that cytosolic magnesium increases during ATP hydrolysis (Leyssens, 1996). During prolonged ischaemia as there is depletion of ATP there will be corresponding decrease in magnesium as well.

ATP sensitive K\(^+\) channel is closed during physiological conditions when ATP is high. As ATP breaks down during ischaemia opening of the K\(^+\) channel is promoted (Ferraro, 1996) leading to decreased concentration of the same in the myocardial tissue. Our findings are in line with the above report, i.e., myocardial tissue K\(^+\) concentration decreased and that of serum level was high on isoproterenol treatment. During ischaemia Na\(^+\) K\(^+\)ATPase activity and phospholipid content has been reported to decrease in heart (Kayawake, 1982).
The decrease is attributed to the accelerated phospholipid catabolism as a result of activation of phospholipases. The increased peroxidation of membrane phospholipid releases the free fatty acid by the action of phospholipase A₂. Elevated myocardial FFA result in inhibition of several enzyme systems non competitively such as Na⁺ K⁺ATPase etc (Ahmed and Thomas, 1971). Inhibition of the sodium pump may precipitate an excess of internal sodium (Jennings et al., 1986).

A significant reduction in the Na⁺ K⁺ATPase activity in tissue and serum Na⁺ level were observed in isoproterenol treated rats whereas the tissue Na⁺ levels were found to be increased significantly.

Fish oil pretreatment in isoproterenol administered rats seems to protect the heart from calcium overload by reducing the activities of Ca²⁺ and Mg²⁺ATPases and increasing Na⁺ K⁺ATPase activity. The myocardial tissue and serum levels of various elements like Na⁺, K⁺, Ca²⁺ and Mg²⁺ were maintained at near normal levels.

Feeding rats with n-3 fatty acid rich fish oil results in an increase of the n3 to n6 ratio in the membrane phospholipids (Al Makdessi, 1994). The eicosa pentaenoic acid (EPA) which is incorporated in the membrane phospholipids affects various membrane bound ATPases by decreasing the cyclic AMP concentration (Ming Fong Chen et al., 1994) and phospholipase activity (Grynberg et al., 1992) in myocardial tissue. A 30% decrease in oxalate facilitated ATP dependent Ca⁺ uptake and concomitantly impaired Ca²⁺ATPase activity was found in fish oil fed rats (Taffet et al., 1993).
EPA and even more effectively, docosa hexaenoic acid (DHA) specifically modulate the calcium currents through dihydropyridine sensitive L-type calcium channels (Hallaq et al., 1992). DHA directly inhibits the major cardiac voltage sensitive, rectifier potassium channel of heart cells (Honore et al., 1994).

Therefore changes in fatty acid composition of the membrane can influence Ca\(^{2+}\) signalling either by influencing receptor mediated transmembrane signalling process or by influencing Ca\(^{2+}\) pumps and carriers that are initially involved in the excitation contraction coupling.

Hypoglycemia was observed in rats administered with isoproterenol. Isoproterenol administration followed by β receptor binding activates phosphorylase kinase leading to glycogenolysis and lipolysis (Gross & Mayer, 1975). Mueller and Ayres (1978) showed a link between the magnitude of the sympathetic response in humans and myocardial infarction, accompanied by a significant increase in hormones like cortisol and corticosterone which control and conserve the amount of glucose in blood during stress thereby leading to hypoglycemia (Mason, 1968).

Fish oil pretreated rats with isoproterenol showed near normal levels of glucose. Improved glucose tolerance and decreased activities and (G6PDH) glucose-6 phosphate dehydrogenase and G-6-phosphatase activities were reported earlier by Meng-Tsen et al., (1995).
Wexler and Kitlinger (1965) observed significant increase in aldosterone production during stress and β-adrenergic action, which could account for the intense sodium and water retention by the kidney and the hydrothorax condition. The ongoing catabolism and renal pathophysiology could account for the intense sodium and water retention by the kidney and the hydrothorax condition. The ongoing catabolism and renal pathophysiology could account for the substantial and sharp increase in urea and creatine levels in the experimental rats after isoproterenol administration.

Isoproterenol administered rats which received fish oil pretreatment showed improved renal physiology which reflected in low values of creatine and urea in these animals. Fish oil has been shown to protect against cyclosporine induced nephrotoxicity in rats and in renal transplant recipients (Homan et al., 1993, Busnach et al., 1998). Protection against gentamycin induced nephrotoxicity is reported by Grauer et al. (1996).

Cardiac hypertrophy i.e., enlargement of heart has been observed in isoproterenol administered rats. An increase in RNA and DNA content followed by an increase in tissue protein content with concomitant decrease in serum protein levels after isoproterenol administration suggests that the reparative processes are in progress.

The increased DNA content in isoproterenol treated rats is probably attributable to fibroblast cells as cardiac muscle cells donot undergo mitotic division (Smits et al. 1992). Increased proliferation of interstitial cells, presumably cardiac fibroblasts and increased collagen content have been
reported in experimental left ventricular dysfunction (Smits et al., 1992). The decreased DNA content in fish oil pretreated rats with isoproterenol shows that fish oil might have inhibitory effect on cell proliferation. This correlates well with the earlier reports demonstrating the antiproliferative effect of fish oil (Finstad et al., 1994)

Increased protein synthesis following experimental myocardial infarction as a part of repair process which may be stimulated after cellular necrosis has been reported by Lochner et al. (1971). Protein synthesis is preceded and accompanied by enhanced RNA synthesis (Koide and Rabinowitz, 1969).

Wood et al. (1971) suggested that this early rise in RNA synthesis could be a primary event which leads to hypertrophy at a later phase.

Decreased heart weight and RNA content in fish oil pretreated rats with isoproterenol suggests that fish oil treatment could remove the stimulus for hypertrophy.

ANTIOXIDANT STATUS

Increased level of lipid peroxide can be a causative factor for the irreversible damage to myocardial membrane during necrosis (Kako, 1987). The increased content of lipid peroxides in serum will enhance platelet aggregation (Hamberg et al., 1974) which is usually observed in myocardial infarction and suppress biosynthesis of prostacyclin (Salmon et al., 1978).
Prostacyclin is a protective factor against the development of vascular disease due to its vasodilation effect.

The increased levels of "TBA reactants" observed in the heart of animals subjected to isoproterenol stress indicates the excessive formation of free radicals and activation of lipid peroxidation system. Increased free radical formation can result in the origin of irreversible damage to the heart (Biemond et al., 1986). Glutathione is an antioxidant and is a substrate of Glutathione peroxidase which metabolises hydrogen peroxide (Xia et al., 1985). Isoproterenol treated rats show a decrease in the level of glutathione in blood and heart. This may be due to the exposure of myocardium to a large flux of hydrogen peroxide and hydroxy radicals. Fish oil pretreatment maintains the glutathione level to normal.

Glutathione depletion in isoproterenol treated rats may be due to its increased utilisation in protecting SH containing proteins from lipid peroxides. The unavailability of glutathione reduces the activity of GPx and GST in isoproterenol treated rats (Litov et al., 1981).

A decrease in the level of SOD in isoproterenol treated rats may be due to the involvement of superoxide free radicals in myocardial cell damage. These reactive free radicals especially H$_2$O$_2$ apart from damaging the myocardial cell also modify the aminoacids and metal ions of the enzyme SOD resulting in the loss of its activity and accumulation of superoxide anion. Superoxide anion inturn creates modification of various cellular constituents and inactivate catalase and reduce its activity (Sinet et al., 1981).
Excess activation of biomembrane lipid peroxidation and suppression of the function of the system of antioxidant defence occurring in necrosis of the myocardium in experimental setting appeared to be generally preventable by preliminary administration of PUFAs. It is suggested that the ability of the n-3 PUFAs to modulate the properties of the biological membranes might account for the above effects (Kresiun et al., 1996).

Fish oil modifies the composition of membrane phospholipids (Hartog et al., 1987; Gudbjarnason et al., 1977; Al Makdessi et al., 1994) and increases both n-3/n-6 ratio and the double bond index (Lamers et al., 1987; Abraham Demoz et al., 1992).

Abraham Demoz et al (1992) reported significant increase in the activities of catalase, glutathione-S-transferase and glutathione peroxidase after EPA feeding. They also reported lower levels of lipid peroxides and an increase in reduced glutathione content.

Ceruloplasmin, a copper donor and a ferroxidase catalyses the oxidation of Fe(II) to Fe(III), the Fe(III) form of iron is used up for the production of various haem proteins. Ceruloplasmin also donates copper ions to superoxide dismutase, an enzyme that scavenges superoxide anion. Thus ceruloplasmin also acts as an inhibitor of lipid peroxidation (Halliwell et al., 1986). A significant decrease in ceruloplasmin activity was evident in the isoproterenol treated rats (Biemond et al., 1984) and the activity was maintained near normal in fish oil pretreated animals.
Increased free iron is usually associated with free radical production. (Sreekriya et al., 1998) Accelerated free iron concentration in isoproterenol treated rats can be attributed to decreased iron binding capacity. Free iron released from proteins play an important role in lipid peroxidation. Fish oil pretreated rats showed a decreased level of free iron and this could be due to antioxidant nature of EPA which is incorporated in the membrane phospholipids of fish oil treated rats.

LIPIDS AND LIPOPROTEINS

Excess of lipids in the blood is considered to accelerate the development of arteriosclerosis and is a risk factor in myocardial infarction. Accordingly reduction of high blood lipid levels by diet or drugs is used as a preventive measure in people at risk.

Isoproterenol induced myocardial infarction leads to hyperlipidemia (Saleema Mathew et al., 1981). The increase in total lipids observed in isoproterenol treated rats is an evidence for an hyperlipidemic effect (Wexler et al., 1963). High levels of circulating cholesterol and its accumulation in heart tissue are well associated with cardiovascular disturbances. A rise in serum cholesterol reflects the concentration of LDL levels. There is a positive correlation between the risk of developing ischaemic heart disease and serum LDL level and a negative one with those of HDL cholesterol. Isoproterenol treatment has been shown to increase the levels of serum LDL and decrease that of HDL significantly. Consequently the affecting the ratio of LDLC/HDLC.
Hyper triglyceridemia is a prominent feature of isoproterenol induced cardiovascular disturbances (Saleema Mathew et al., 1981).

Diets supplemented with EPA rich fish oil have been described to lower effectively serum triglycerides, total, VLDL and LDL cholesterol in normolipidemic (Von Lossonezy et al., 1978; Saynor et al., 1984) and hyperlipidemic subjects (Philipson et al., 1985; Nestel et al., 1984). HDL cholesterol was increased. Our results are in line with the above reports. It has been suggested that the hypotriglyceremic effect of fish oil results from an increase in beta oxidation which reduces the hepatic pool of fatty acids available for esterification. These conclusions were based on the observation that in rats fish oil feeding inhibits the hepatic synthesis and secretion of triacylglycerols while promoting ketogenesis (Wong et al., 1984; Topping et al., 1987). Peroxisomes are capable of degrading very long chain fatty acids such as those present in fish oil (Osmundsen, 1982). Fish oil has previously been shown to increase peroxisome beta oxidation in rats possibly by enzyme induction (Flatmark et al., 1988) or by inhibiting the degradation of fatty acid oxidase (Horie et al., 1989). Although some EPA is oxidized by peroxisomes most is probably oxidised by mitochondria (Christensen et al., 1986).

On feeding the rats with fish oil EPA and DHA are avidly incorporated into phospholipids of the membranes and are poor substrates for oxidation (Sanders et al., 1989). Increased formation of phospholipid by EPA has been reported by Wong et al. (1985) and it is due to reduced triacylglycerol synthesis and/or lysophospholipid acylation in the presence of this fatty acid.
EPA decreases synthesis of triacylglycerol and cholesterol ester primarily by affecting esterification of diacylglycerol and cholesterol. EPA decreases hepatic formation of triacylglycerol and cholesterol ester primarily by inhibiting the activities of microsomal ADGAT and ACAT. EPA itself is a poor substrate for these enzymes and it decreases incorporation of other fatty acids into triacylglycerol and cholesterol ester (Rustan et al., 1989).

Kiyoshi Mizuguchi et al. (1993) reported reduced intestinal cholesterol absorption on EPA-E treatment. EPA could inhibit acyl CoA cholesterol acyltransferase (ACAT) enzyme which catalyzes the important step in the regulation of intestinal cholesterol absorption (Suckling et al., 1985).

An EPA rich diet decreases HMG-CoA reductase activity in rat (Choi et al., 1989) and increases biliary secretion in bile duct cannulated rats (Balasubramaniam et al., 1985; Chautan et al., 1990; Smit et al., 1991). EPA causes modification of serum lipoproteins such that their clearance from serum is increased.

A significant increase in free fatty acid and a decrease in phospholipid in isoproterenol treated rats might have been due to the breakdown of membrane phospholipids. The increased peroxidation of polyunsaturated fatty acids is recognized as the possible biochemical mechanisms for the genesis of membrane injury in the myocardium.

The increased peroxidation of membrane phospholipids release free fatty acids by the action of phospholipase A₂ (Singal et al., 1983). Ca^{2+} ion have
been reported to be one of the inducers of phospholipase A$_2$. So the observed increase in free fatty acid concentration could have been due to increased PLA$_2$ activity and calcium ion concentration in isoproterenol treated rats.

Accelerated membrane phospholipid degradation resulting in cell injury and ultimately cell death in isoproterenol treated rats have been reported by Biemond et al. (1986) and Julicher et al. (1984). This is probably due to the defects in membrane systems which regulates Ca$^{2+}$ availability (Shen et al., 1972).

Pretreatment with fish oil was observed to increase the level of phospholipid and decrease the level of free fatty acids in the heart tissue. Reduced PLA$_2$ activity was observed in fish oil treated rats. EPA and DHA gets incorporated into membrane phospholipids on fish oil feeding and are poor substrates for oxidation. Increased phospholipid formation on EPA treatment has been reported by Wong et al. (1985).

Fish oil feeding modifies the composition of the membrane phospholipids and increases the n$_3$/n$_6$ ratio (Almakdesh et al., 1994), n-3 PUFA have been shown to reduce the PLA$_2$ activity (Grynberg et al., 1992) and consequently cystosolic Ca$^{2+}$ concentration. Thus fish oil treatment modulates the membrane bound PLA$_2$ activity by modifying the membrane phospholipid fatty acid composition.

The reduced level of free fatty acid in fish oil pretreated animals could be the result of membrane protective action of the fish oil.
Dietary n-3 PUFA alter the membrane lipid composition in organelles of several organs and play important roles in the regulation of lipid metabolism even in circulating lipoproteins.

**Mitochondrial metabolism**

"Mitochondrial respiration" refers to all those processes concerned. With the uptake of oxygen and the associated production of ATP, including the activity of the citrate cycle and the respiratory chain. Besides the evident need for oxygen, there are three important regulators of the respiratory rate: (1) the supply of ADP that regulates respiration by its rate of transfer into the mitochondria, (2) the ratio of NAD/NADH$_2$ in the mitochondria, which regulates the activity of citrate cycle dehydrogenases and (3) the mitochondrial calcium concentration, which also regulates these dehydrogenases (Brown et al., 1992).

During normal oxygenation, cytoplasmic NADH$_2$ is removed by the malate-aspartate cycle which depends on continued mitochondrial respiration. In hypoxia or ischaemia, intramitochondrial NADH$_2$ increases as a result of impaired β-oxidation due to decreased electron transport. NADH$_2$ accumulation in the cytosol means more protons so that intracellular acidosis is promoted. Pyruvate dehydrogenase, located on the mitochondrial membrane, is inhibited by NADH$_2$ so that entry to citrate cycle is inhibited, more lactate forms, and the ratio of NADH$_2$ to NAD increases further. NADH$_2$ also accumulates in the mitochondria with adverse effects (1) there is inhibition of dehydrogenase enzymes at several sites so that any residual activity of the
citrate cycle is decreased and (2) intra mitochondrial calcium increases Lehninger et al. (1978).

Lactate is taken up by the aerobic heart and produced during anaerobiosis, so that in its release into coronary sinus blood it is sometimes used as a sign of myocardial ischemia (Gertz et al., 1980). Increased neutral lactate in severe ischaemia causes decreased contractile activity in the ischaemic zone (Tennant, 1935), promotion of mitochondrial damage (Armiger et al., 1974), decrease of the action potential duration (Wissner, 1974) and inhibition of glycolysis at the level of glyceraldehyde 3 phosphate dehydrogenase (Revetto et al., 1975).

Cairns et al. (1993) showed that 20 mmol/L external neutral lactate decreased internal myocyte pH by only 0.24 pH units, probably because of inhibition of the outward transport of protons as the high external lactate inhibited lactate/protons cotransporter. The decrease in internal pH could by Na⁺/H⁺ and N⁺/Ca²⁺ exchange substantially increase cytosolic calcium with adverse consequence. The adverse effects of high external levels of neutral lactate could contribute to the overall mechanism of ischaemic damage (Cross et al., 1995).

Calcium is an important controller of mitochondrial respiration. Calcium uptake by the mitochondria occurs by a uniporter system that is not linked to the transport of any other ion. Inward transport of calcium is increased when cytosolic calcium increases. This uptake of calcium ions by mitochondrial matrix effectively requires energy to pump the protons out to
balance the charge brought in with calcium ions. The fact that mitochondria contain much less calcium than previously though suggests that the main function of calcium transfer in and out of the mitochondria is to regulate internal matrix calcium and thereby kreb's cycle activity (Carafoli, 1988).

Calcium release from mitochondria occurs by an antiporter system, whereby two sodium ions are taken up for each calcium ion released. This carrier system is electrically neutral. There are separate pathways and separate control mechanism for calcium uptake and release. The flux of calcium ions can be varied in either direction so that mitochondrial pool of calcium can act as a calcium buffer for the cytosol. The uptake of excess calcium in conditions of cytosolic calcium overload is serious. It impairs the proton gradient across the mitochondrial membranes thereby reducing the ability of mitochondria to synthesis ATP.

Increased myocardial lactic acid concentration and increased mitochondrial calcium were observed in isoproterenol treated rats. Decreased activities of TCA cycle enzymes were observed in isoproterenol treated rats. Decreased activities of TCA cycle enzymes were observed in isoproterenol treated rats.

The increase in lactic acid formation and increased mitochondrial calcium might have inhibited the dehydrogenases of TCA cycle in isoproterenol treated rats resulting in their decreased activity. Fish oil supplementation decreased myocardial lactic acid and mitochondrial calcium levels. Increased
activity of the TCA cycle enzymes in heart mitochondria have been observed in fish oil pretreated animals compared to isoproterenol treated animals.

Decreased lactic acid formation in fish oil treated rats was reported by Meng Tsan et al. (1995).

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 would be the development of alterations in membrane integrity and permeability of mitochondrial and sarcolemmal membranes. These alterations would result in altered electrolyte levels including calcium entry which would result in phospholipase activation, ATP depletion and irreversible injury (Burton et al., 1984).

Succinate dehydrogenase is located in the inner mitochondrial membrane and other dehydrogenases of the TCA cycle enzymes namely α keto glutarate, isocitrate and malate dehydrogenases are present in the matrix compartment of mitochondria. Dehydrogenases are 'SH' group containing enzymes and are readily inactivated on exposure to free radicals (Manjula et al., 1993).

In the present study the mitochondrial lipid peroxide content was found to be increased in isoproterenol treated rats. The dehydrogenases of TCA cycle could have been affected by the free radicals produced on isoproterenol treatment (Nicolay et al., 1985).
Cytochrome c oxidase and NADH dehydrogenase are the enzymes involved in the electron transport chain and are located in the inner mitochondrial membrane. Cytochrome C oxidase and NADH dehydrogenase have an absolute requirement of cardiolipin (Dudnik et al., 1980).

During ischaemia, pronounced enhancement of lipid peroxidation was seen in mitochondria. Activation of lipid peroxidation correspond with changes in the lipid composition which included a decrease in the level of total and readily oxidisable lipid i.e. cardiolipid. Decrease in the activity of cytochrome C oxidase and NADH dehydrogenase in isoproterenol administered rats could be due to enhanced phospholipid degradation resulting in the non availability of cardiolipin for their functional activity. Isoproterenol treated rats have been reported to show increase in phospholipase activity in heart mitochondria (Kondo et al., 1987).

We also observed an increased phospholipase activity in isoproterenol treated rats. Feeding rats with n3 PUFA rich fish oil results in an increase of n-3 to n-6 PUFA ratio in the membrane phospholipids of the cardiac myocytes (Almakdessi et al., 1994).

Changes in incorporation of PUFAs in the plasma membrane phospholipids of cardiomyocytes have been shown to affect receptor mediated PLA$_2$ and PLC$\beta$ signalling on many steps of the way.

By changing the fattyacyl composition of the phospholipids, the endogenous substrates for the membrane associated PLC-\(\beta\) and PLA$_2$ are
changed and subsequently also their products which results in altered events down stream of these signalling enzymes: activation of distinct PKC isoenzymes, regulation by non esterified PUFAs of Ca\textsuperscript{2+} channels, and changed rate of the formation of distinct eicosonoids. Additionally it has become clear that the membrane dynamic characteristics in terms of membrane fluidity and cholesterol concentration can be modified upon altered n-3 and n-6 PUFA incorporation in the phospholipids. This could likely have conservances for the receptor function of G proteins and the activity of the signal transducing phospholipases as well (Henriette et al., 1996).

Lower phospholipase A\textsubscript{2} activity in n-3 rich cardio myocytes in culture compared to n-6 rich cardiomyocytes was reported (Nalbone et al., 1988). The enrichment in membrane n-3 PUFA of the fish oil fed heart could partially affect phospholipase A\textsubscript{2} activation during ischaemia and reperfusion and reduce membrane damages and enzyme leakage. In this connection, the ischaemia induced increase in free calcium could be an important factor (Miyata et al., 1992).

Feeding rats with fish oil results in the incorporation of n-3 fatty acids, EPA and DHA into the membrane phsospholipids which are poor substrates for oxidation. Decreased membrane lipid peroxidation and improved antioxidant defence on EPA feeding was reported by Abraham Demoz et al. (1993).

Thus increase in the activity of TCA cycle enzymes as well as cytochrome oxidase and NADH dehydrogenase of respiratory chain found in
fish oil supplemented rats could be due to decrease in lactic acid content of cardiac myocytes, decreased mitochondrial Ca$^{2+}$ level resulting in decreased phospholipid degradation. The antioxidant nature of EPA also plays an important role here.

Oxidative phosphorylation is the process of energy coupling in the respiratory chain which results in the formation of ATP from ADP and phosphate at the expense of free energy yielded by electron transport to oxygen. The phenomenon in which the rate of electron transport is controlled by the concentration of ADP is called respiratory control. Respiratory control ratio is the ratio of the rate of respiration of mitochondria in the presence of ample ADP (state 3) to the ratio of respiration in the absence of ADP (state 4). This respiratory control ratio is a useful measure of the integrity of isolated mitochondria (Lehninger et al., 1993).

In ischaemic mitochondria, the measurement of oxidative phosphorylation revealed marked depression in all variables (the ratio of O$_2$ consumed to ADP phosphorylated to ATP) i.e. ADP/O ratio, respiratory control index and rate of succinate oxidation. The decrease in respiratory activity during state 3 respiration indicated severe impairment of electron transport capability (Kaul et al., 1990).

A significant decrease was observed in the oxidation of succinate in state 3 (+ADP) and state 4 (-ADP) in the heart mitochondria of isoproterenol treated rats. The ATP content, respiratory control index and ADP/O ratio also
showed a significant decrease. The decreased oxygen uptake may be due to an impairment in myocardial oxygen production (Jarmakani et al., 1978).

For oxidative phosphorylation to proceed requires a continuous supply of (1) oxygen delivered by the coronary circulation (2) protons and electrons delivered by citric acid cycle and (3) ADP.

Isoproterenol treatment has been reported to cause tissue hypoxia where there is oxygen demand. The production of protons and electrons is also affected as there is reduced activity of TCA cycle enzymes in isoproterenol treated rats (Manjula et al., 1993).

Isoproterenol induced myocardial infarction results in hyperlipidemia (Saleena Mathew et al., 1981) resulting from impaired $\beta$ oxidation of fatty acids. The increased free fatty acids (Singal et al., 1983) and other lipid metabolites may inhibit the activity of adenine nucleotide translocase resulting in the deprivation of ADP for oxidative phosphorylation.

Finally increased mitochondrial Ca$^{2+}$ in isoproterenol treated animals (Saetersdal et al., 1982) may disturb the proton gradient across the mitochondrial membrane thereby affecting ATP production.

Fish oil pretreatment resulted in an increase in the oxidation of succinate, ADP/O ratio, ATP content and respiratory control index when compared to isoproterenol treated rats.
Improved aortic flow and better recovery of the cardiac pump function of fish oil fed rats in ischaemia reperfusion model was observed by Demaison et al. (1994). This was associated with a better preservation of the cellular integrity (Demaison et al., 1994).

Fish oil treatment results in increased β oxidation of fatty acids. Decreased phospholipid degradation due to decreased phospholipase activity and low cytosolic mitochondrial Ca^{2+} concentration in fish oil fed rats might contribute to improved mitochondrial energy metabolism. Thus dietary fish oil by increasing the n3/n6 polyunsaturated fatty acid ratio in membrane phospholipids appears to increase the membrane resistance to ischaemia, which insure a better recovery of mitochondrial energy metabolism and aortic flow in isoproterenol treated rats.

**Lysosomal alterations**

Lysosomes are a distinct group of cytoplasmic organelles, known to occur in numerous animal tissues and characterized by their content of a variety of acid hydrolases (De Duve, 1959). Lysosomal enzymes are important mediators of acute myocardial infarction and its release into cytoplasm stimulate the inflammatory mediators like oxygen radicals, prostaglandin etc. (Ravichandran et al., 1991).

A significant decrease was observed in the activities of β-D-glucuronidase, β-D-N-acetyl glucosaminidase, acid phosphatase and cathepsin D in the lysosomes of heart in isoproterenol treated rats. A concomitant
increase in the activities of lysosomal hydrolases was observed in serum and heart of these animals. These observations are in accordance with that of Ravichandran et al. (1991).

Elevated levels of lysosomal hydrolases have been reported in the serum of patients with MI (Welman et al., 1978) and in myocardial infarction induced in experimental animals (Mathew et al., 1982). Elevated lysosomal enzymes in the extracellular fluid occurs as a result of decreased lysosomal stability (Niebes et al., 1975). This eventually affects the metabolism of different connective tissue constituents viz glycosaminoglycan, glycoprotein & collagen.

Oxygen free radicals generated during ischaemia, damage the myocardium through the release of lysosomal enzymes apart from their effect on cardiac damage (Karla et al., 1994). As isoproterenol administered rats show increased lipid peroxidation, this could be one of the reasons for the decreased lysosomal membrane stability.

Decreased stability of the lysosomal membrane resulting in irreversible damage has been reported by Decker (1978). Isoproterenol induced myocardial infarction results in increased lysosomal hydrolase activities which in turn causes tissue damage and infarcted heart (Ravichandran et al., 1991).

The increased activities of glycohydrolases and cathepsin D in heart indicate the possible infiltration of inflammatory cells at the site of infarctions. During ischaemia when heart muscle cell undergoes irreversible damage
degereation occurs and proteolysis of necrotic myocardium occurs with a coincident influx of inflammatory cells at the infarcted site (Bolli et al., 1981).

During ischaemia increased neutral lactate in the cytosol decreases the myocyte pH resultant acidosis. The decrease in internal pH could, by Na⁺/H⁺ and Na⁺/Ca²⁺ exchange, substantially increase cytosolic calcium with adverse consequences. Increased acidity in the cell activates the lysosomes. Increased lactate leading to increased acidity in the cell and increased tissue calcium were observed in isoproterenol treated rats (Fig.4.14 and Fig.4.15).

Increased lipid peroxidation on isoproterenol treatment results in phospholipase activation in lysosomal membrane leading to decrease in membrane fluidity (Mathew et al., 1982). Altered membrane integrity has been suggested as a major factor in the development of cellular injury during myocardial ischaemia (Burton et al., 1977; Burton et al., 1980; Jennings, 1976; Willerson et al., 1977).

Two components associated with membrane injury, increased Ca influx and phospholipid degradation were intensely investigated (Chien et al., 1981; Chien et al., 1978; Farber et al., 1978; Flekenstein et al., 1974; Reimer et al., 1977; Shen et al., 1972). There is increasing evidence that intracellular accumulation of calcium may be the ultimate mediator of ischaemic cell deaths (Flekenstein et al., 1974; Shen et al., 1972) and that alternations in membrane phospholipids could be responsible for changes in membrane permeability allowing increased calcium entry (Chien et al., 1981; Chien et al., 1978).
Thus increased intracellular calcium increases phospholipase activity which in turn results in phospholipid degradation, membrane permeability alterations and irreversible cellular injury in isoproterenol induced myocardial infarction in rats.

In fish oil pretreated rats the lysosomal enzyme activities were increased in the lysosomes of the heart and decreased significantly in the serum and heart. This reflects the membrane integrity of the lysosomes in these animals. Prostacyclin has been reported to stabilise the lysosomal membrane (Schorr et al., 1982). n-3 fatty acid rich fish oil is reported to be non inflammogenic (Billar et al., 1988; Endres et al., 1989). It increased the production of PGI$_2$ (Decaterina et al., 1990).

Feeding rats with n-3 fattyacid rich fish oil results in an increase of n$_3$/n$_6$ ratio in the membrane phospholipids. The EPA which is incorporated in the membrane phospholipids affects various membrane bound ATPases and phospholipase activity in myocardial tissue.

Lower phospholipase activity of n$_3$ rich cardio myocytes compared to n$_6$ rich cardiomyocytes was reported by Nalbone et al. (1990). Antioxidant effect of EPA was reported by Abraham Demoz et al. (1992). Fish oil decreased myocardial calcium accumulation (Hallaq et al., 1992) and lactic acid formation (Meng Tsan et al., 1995).

In fish oil pretreated animals (1) the decreased inflammation (2) increased PGI$_2$ production (3) antioxidant nature (4) decreased acidity (5)
decreased intracellular Ca\textsuperscript{2+} (6) decreased phospholipase activity and (7) decreased phospholipid degradation might contribute to the increased lysosomal membrane integrity. Thus by stabilizing the lysosomal membrane fish oil offers multifaceted protection to the heart.

From the present study, it is concluded that fish oil modifies membrane phospholipid composition and increases n3/n6 ratio. These n3 fatty acids in turn offer cardioprotection by their ability to modulate biological membranes.
Summary
Coronary artery disease, the leading cause of death in the world, poses a major socio economic problem which has prompted extensive research into both preventive and therapeutic measures. Among the former are dietary modifications, including fish oil supplements which have been strongly advocated to prevent the development of atherosclerosis.

Administration of fish oils rich in n-3 polyunsaturated fatty acids to humans has been recognised as a preventive or therapeutic approach for the treatment of cardiovascular disease based on their ability to modulate a diverse range of factors contributing to cardiovascular disease. The objective of the present study is to evaluate the protective effect of fish oil pretreatment on biochemical alterations in isoproterenol induced myocardial infarction.

Histopathological studies revealed marked degenerative changes in heart sections from rats treated with isoproterenol alone. The degenerative changes were less pronounced in isoproterenol administered rats which received fish oil pretreatment.

The ECG pattern of isoproterenol administered rats showed abnormalities related to myocardial ischaemia. The electro cardiographic changes were restored towards normalcy on fish oil pretreatment with isoproterenol.
In isoproterenol treated rats there was a rise in RBC count, WBC count, haemoglobin, haematocrit, platelet count, ESR and Fibrinogen levels with a significant decrease in bleeding and clotting times. The fish oil pretreatment altered the isoproterenol induced rheological changes favourably.

Serum Troponin-T values were increased significantly in IPH treated rats, IPH administered rats which received fish oil pretreatment showed significant reduction in serum troponin-T value compared to IPH alone treated rats.

Serum LDH isoenzyme pattern showed increased LDH$_1$ fraction (typical of myocardial infarction) in isoproterenol treated rats. In fish oil pretreated isoproterenol administered rats the LDH isoenzyme pattern was close to that of control rats.

The activities of serum enzymes LDH, CPK and transaminases were significantly increased in Isoproterenol treated rats. Concomitant decrease in the activities of the above enzymes was noted in myocardial tissue of rats after isoproterenol treatment. The altered activities of various enzymes mentioned above were maintained at near normal in fish oil pretreated group.

Isoproterenol administration leads to decrease in blood sugar. An increase in blood urea and serum creatine and uric acid were observed after isoproterenol administration. Fish oil pretreatment prevented the IPH induced hypoglycemic condition of the rats and also decreased values of urea, creatine and uric acid were observed in that group.
There was a significant increase in the heart weight, tissue protein, RNA and DNA contents of the heart tissue in IPH treated rats. Whereas fish oil pretreatment in isoproterenol administered rats brought about significant reduction in heart weight, protein, DNA and RNA contents of heart tissue.

IPH administration is found to decrease Na⁺K⁺ATPase level and increase Ca²⁺ATPase and Mg²⁺ATPase levels in myocardial tissues. Fish oil treatment has been found to maintain the activities of Ca²⁺ATPase, Mg²⁺ATPase and Na⁺K⁺ATPase which were altered during isoproterenol treatment.

Calcium, magnesium, sodium and potassium were found to be altered in the serum when subjected to β-adrenergic stimulation. Potassium levels were found to be increased markedly whereas sodium, calcium and magnesium were significantly lowered. Significant elevation of sodium and calcium levels with concomitant decrease in the levels of potassium and magnesium were observed on IPH administration in myocardial tissue. Fish oil pretreatment prevented the alteration in tissue and serum levels of various elements namely sodium, potassium, calcium and magnesium upon isoproterenol administration.

The biochemical lesion due to the activation of lipid peroxidation and decrease in antioxidant status are significantly implicated in experimental myocardial infarction induced by isoproterenol. The protective effect of fish oil is achieved by decreasing the peroxide concentration and the normalization of the antioxidant defence enzymes.
In IPH administered rats the lipid levels in serum registered a significant rise in total cholesterol, ester, free cholesterol, triglyceride, phospholipid and free fatty acid levels. Whereas in myocardial tissue there was a significant increase in total cholesterol, ester cholesterol, free cholesterol, triglyceride and free fatty acid levels. The phospholipid content of myocardial tissue was found to be significantly decreased. Isoproterenol induced changes in serum and myocardial lipids were altered favourably on fish oil pretreatment.

In Isoproterenol treated rats there was significant increase in lipid peroxidation and significant decrease in the activities of mitochondrial TCA cycle enzymes and respiratory chain enzymes. Pretreatment with fish oil has been observed to maintain the levels of lipid peroxides, TCA cycle enzymes and respiratory chain enzymes to near normal.

The succinate oxidation decreased in state 3 and state 4 in isoproterenol treated rats. The ATP content, respiratory control ratio and the ADP/O ratio were also decreased. In rats which were pretreated with fish oil these changes were maintained at near normal.

The plasma lactic acid content was increased in Isoproterenol treated rats. Fish oil pretreated rats showed significant decrease in plasma lactic acid content compared to rats treated with Isoproterenol alone.

A significant decrease in the activities of D-glucoronidase, D-N-acetylglucosaminidase acid phosphatase and cathepsin D in the lysosomes of
heart was observed in isoproterenol treated rats. A concomitant increase in the activities of lysosomal hydrolases was observed in serum and heart of these animals. In fish oil pretreated isoproterenol administered rats the lysosomal enzyme activities were increased in the lysosomes of the heart and decreased significantly in the serum and heart.

CONCLUSION

The above results confirm the multifaceted protection offered by fish oil to the heart.

To optimize clinical benefits, appropriate dietary modifications should always be encouraged concurrently with the use of fish oil or fish oil derived n-3 PUFAs. Such a plan should include restriction of total dietary fat and cholesterol intake and an increase in the ratio of polyunsaturated to saturated fat, adequate physical exercise, lifestyle modifications and, if necessary, other medications, into a comprehensive cardiovascular revitalization strategy. This strategy is likely to be cost-effective and can extend beyond restoring cardiac functionality to extended survival and improved quality of life.