Effect of exercise and dietary polyunsaturated fat upon prevention of L-isoproterenol-induced myocardial infarction

David L. Crandall

Iowa State University

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INFARCTION.

IOWA STATE UNIVERSITY, PH.D., 1979
Effect of exercise and dietary polyunsaturated fat upon prevention of L-isoproterenol-induced myocardial infarction

by

David L. Crandall

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1979
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ABSTRACT

Degree of infarction in trained and sedentary rats fed iso-
calorically a Teklad rat chow diet or a chow + safflower oil diet
(50% calories from oil) was determined by measurement of the LDH-1
isozyme. After a 12-week treadmill training period (30 min/day; 20
m/min), all rats received two subcutaneous injections of L-isopro-
terenol hydrochloride (70 mg/kg) 24 hours apart, the first dose being
administered 24 hours after the last exercise period. Twenty-four
hours after the final injection, rats were anesthetized, bled by
venipuncture, and sacrificed. At time of sacrifice, there was no
significant difference in body weight between all groups (P > .05).
Exercised, fat-fed rats exhibited significantly lower concentrations
of serum LDH-1 when compared to animals in the other experimental
groups (P < .05). Serum triglyceride concentration was depressed
significantly in exercised, fat-fed rats when compared to rats in other
experimental groups (P < .05). In addition, exercised, fat-fed rats
exhibited significantly less cardiac lipid while only nonexercised,
fat-fed rats exhibited significantly more hepatic lipid when compared
to all other groups (P < .05). Our results indicate that the high
intake of polyunsaturated fatty acids as safflower oil and exercise
decreased the severity of the myocardial infarcts induced by L-
isoproterenol.
INTRODUCTION

Of all the daily crises that mankind encounters, it appears that attainment of harmony with the environment is increasingly becoming the foremost priority. Unfortunately, as society becomes more advanced through technological discoveries, it seems that science can barely keep abreast of replenishing natural resources and combating new diseases that also arise. This combination of technological advancement and environmental disunion is widely recognized by contemporary man. Through various media sources, the public is becoming increasingly aware of the fact that at least part of these new pathological conditions are caused by both naturally occurring and synthetic dietary substances.

Although cancer-producing agents are widely publicized, a United States citizen is nearly twice as likely to die of coronary heart disease than of cancer (U. S. Bureau of the Census, 1977). Actually, various disease conditions of the cardiovascular system account for three of the four most frequent causes of death in the United States: acute myocardial infarction, renal disease, and stroke (Wessler, 1974). Complete understanding of the cause of these particular pathological conditions of the circulatory system has yet to be elucidated. However, data exist in sufficient quantity to establish exercise as a beneficial deterrent to development of many detrimental cardiovascular anomalies.

As debate continues at various levels of government concerning
regulation of synthetically produced dietary substances, scientific research is also beginning to be directed toward the determination of the beneficial effects of naturally occurring dietary substances upon the state of human health. For example, the detrimental effects of large quantities of saturated fat as found in beef tallow have been presumed for well over a decade. In contrast, the apparent beneficial effects of naturally occurring polyunsaturated fatty acids are more recently being investigated.

The combination of the fact that many food additives, commonly used prescription drugs, and other widely distributed synthetic substances are carcinogenic, contrasted with the beneficial effects of naturally occurring substances, serves as a basis for the research contained herein. Although at this time it is not imaginable that the entire gamut of synthetic substances can be completely replaced by naturally occurring substances with similar results, evidence does exist to support the substitution of some naturally occurring substances into the diet with an actual increase in the state of health of the individual. Explicitly, therefore, the purpose of this research is to:

1. determine the beneficial effect of naturally occurring polyunsaturated fatty acids upon the cardiovascular system; specifically, if polyunsaturated fatty acids can decrease the development and subsequent severity of drug-induced myocardial infarction;
2. determine whether the high concentration of dietary polyunsaturated fatty acids needed to produce beneficial effects in the cardiovascular system can at the same time produce detrimental effects elsewhere in the body;

3. determine if an exercise-training program and dietary polyunsaturated fatty acids can produce an additive beneficial effect upon the prevention of the development of myocardial ischemia and infarction;

4. and to determine whether an exercise-training program is necessary to counteract the possible detrimental effects of high concentrations of dietary lipid.
LITERATURE REVIEW

Relationship of Body Weight to Cardiovascular Fitness and Exercise

In the simplest terms, body weight is regulated by a combination of caloric intake and expenditure. Although the specific mechanisms of body weight regulation are beyond the scope of this manuscript, it is important to note that the weight of an individual is closely related to incidence of myocardial infarction and at the same time changes during exercise-training.

Much of the knowledge concerning the relationship between body weight and cardiac fitness is contained in a now classic investigation begun in 1950 known as the Framingham study. In this study the relationship between body weight and cardiovascular fitness was investigated in 5209 men and women aged 30 to 59 years (Gordon and Kannel, 1976). Ultimately, the results of this study indicate that if everyone were at optimal weight by height, there would be 25 percent less coronary heart disease and 35 percent less congestive failure and brain infarctions.

The affect of exercise upon body weight generally produces an initial weight loss followed by weight stability (Bjorntorp, 1976). The initial loss in body weight may be due to a decrease in triglyceride deposits in peripheral tissues and a decrease in fat cell size (Froberg et al., 1972). Also, it has been reported that a voluntary decrease in food intake may directly effect the body weight loss during exercise (Crews et al., 1969).
The interrelationship between cardiac fitness, degree of exercise-training, and body weight is extremely important. A change in any of these parameters will directly affect the others and must therefore be precisely measured and regularly monitored during experimentation in exercise and cardiovascular physiology.

Effect of Exercise upon the Cardiovascular System

Exercise-training induces several significant morphological changes in cardiac muscle. Regular physical activity causes an initial loss of cardiac fat accompanied by an increase in muscle mass. Other morphological adaptations include a functional adaptive mechanism of cardiac muscle to an increase in the functional load known as myocardial hypertrophy. The exercise-induced mechanism of cardiac hypertrophy has yet to be determined, but hearts subjected to aortic constriction exhibit both an increase in total ribonucleic acid content and increased heart weight (Dowell et al., 1976; Fanburg et al., 1972). Endurance exercise-training also increases the cardiac output, and exercise-trained hearts perform more cardiac work and attain a higher systolic pressure than those of sedentary controls (Scheuer and Stezoski, 1972). In addition, exercise-training will increase the cardiac stroke volume (Saltin, 1971). Because of this increase in stroke volume, the conditioned rat heart is more capable of maintaining its performance during ischemic or hypoxic conditions when compared with the untrained heart (Beroshn and Scheuer, 1978).

The mechanism primarily responsible for the increased stroke
volume in the trained heart is the increase in myocardial contractility (Mitchell and Wildenthal, 1971). This increased contractility produces a subsequent increase in myocardial tension development and may be attributed to an increase in the availability of extracellular Ca\(^{+2}\) to the heart cell (Tibbits et al., 1978). Additionally, the suggestion has been made that the greater contractility of the trained heart may be caused by an increase in the activity of myosin ATPase (Bahn and Scheuer, 1975).

The decrease in heart rate, known as resting bradycardia that is exhibited by physically trained individuals, is well documented and has recently been reviewed by Scheuer and Tipton (1977). This decrease in heart rate may be caused by extrinsic factors including increased vagal tone and a reduction in sympathetic activity (Badeer, 1975). Intrinsic factors, too, may be involved in the production of resting bradycardia. For example, the heart of an exercise-trained rat exhibits decreased sensitivity to norepinephrine because of a possible decrease in the quantity or sensitivity of myocardial beta-receptors (Hughson et al., 1977; Scheuer and Tipton, 1977).

Another beneficial morphological adaptation produced by exercise is stimulation of the increased development of the coronary vasculature. Daily swimming as well as treadmill running will increase circulation to the heart through increased collateral circulation (Denenberg, 1972). Although the mechanism of the exercise-induced
increase in collateral circulation has yet to be elucidated, recent advances in experimental technique such as the use of radioactive microspheres hopefully will aid in the furthering of knowledge concerning myocardial blood flow.

Morphological, Enzymatic, and Hormonal Adaptations to Exercise

Because of the energy costs of exercise, certain adaptations in substrate utilization are required during training. Because depletion of glycogen stores is one factor leading to early exhaustion when an untrained individual is subjected to exercise, a primary adaptation to training is glycogen-sparing (Bergstrom et al., 1967). One method for glycogen-sparing by trained individuals involves the derivation of a greater proportion of energy from the oxidation of fat and less from carbohydrate (Issekutz et al., 1966). For example, men subjected to prolonged running exhibit a decrease in the respiratory quotient and an increase in the plasma free fatty acid concentration indicating a change in the primary source of energy from carbohydrate to lipid (Costill et al., 1971a).

Changes occurring in the concentration of lipids in both blood and tissue are indicative of exercise-training. Plasma triglyceride concentration decreases with exercise because of an increased uptake of plasma triglyceride by peripheral tissues and a decrease in the formation of triglyceride in the liver (Carlson and Froberg, 1969). This observed decrease in triglycerides and increase in glycerol in the plasma reflects the utilization of peripheral
tissue lipid stores for energy during exercise (Havel et al., 1964). Circulating concentrations of triglycerides during exercise also are regulated by decreased absorption from the gut.

The concentration of plasma free fatty acids may reflect the extent of physical conditioning. Short bouts of exercise in the untrained individual will produce an increase in the concentration of plasma fatty acids. In healthy, sedentary individuals, high postabsorptive values of plasma free fatty acids are indicative of poor physical fitness (Costill et al., 1971b). The trained muscle exhibits increased capacity for aerobic metabolism however, and extracts more fatty acids from the circulation for oxidation. During exercise, triglyceride fatty acids are rapidly removed from the circulation and converted to a form that can be used for oxidative metabolism. Recycling of triglyceride fatty acids of very low density lipoproteins is reduced during exercise (Jones and Havel, 1967). The net effect of exercise-training upon plasma lipid concentration is a decrease in free fatty acids in the resting condition.

Morphological adaptations during exercise are partially responsible for the increased capacity for maximum oxygen utilization and subsequent increase in use of lipid as an energy source. The development of secondary collaterals is promoted by hypoxia, pressure gradients, increased flow volume, and increased flow velocity. Under the influence of these stimuli, exercise could increase the blood flow to the heart and skeletal muscle through increased
collateral development (Barmeyer, 1976). Additionally, exercise induces an increase in total mitochondrial protein by increasing both the number and size of mitochondria in skeletal muscle (Gollnick and King, 1969). The occurrence of a similar type of increase in heart muscle has yet to be substantiated.

The capability of increasing oxygen utilization also is reflected by the increase in the activity of several oxidative enzymes. The exercise-induced increase in the respiratory capacity of rat gastrocnemius muscle is illustrated by a subsequent increase in the activity of cytochrome oxidase, succinate dehydrogenase, and citrate synthase and in the capacity to oxidize pyruvate and malate (Baldwin et al., 1975; Fitts et al., 1975). A training program also produces significant changes in the activity of enzymes of fatty acid and ketone oxidation. Molé et al. (1971) reported a doubling of the activity of palmityl CoA synthase, carnitine palmityl transferase, and palmityl CoA dehydrogenase in gastrocnemius muscle of exercise-trained rats. Increases in the concentration of these enzymes imply that the muscle of the exercise-trained individual has an increased capacity to activate, transport, and catabolize long-chain fatty acids. The enzymes of ketone oxidation that increase significantly in activity with exercise include 3-hydroxybutyrate dehydrogenase, 3-ketoacid CoA transferase, and acetyl CoA thiolase (Winder et al., 1974).

Exercise-training also induces several hormonal changes that
influence the glycogen-sparing effect. Trained individuals exhibit a decreased catecholamine release together with decreased concentrations of circulating growth hormone and insulin. The insulin decrease releases inhibition upon lipolysis with a concomitant decrease in utilization of glycogen as an energy source (Rennie and Johnson, 1974). Conversely, the concentration of plasma insulin increases markedly in rats subjected to both a high-fat diet and exercise. This free fatty acid stimulated increase in insulin has an antagonistic effect toward glucagon secretion. In this manner elevated plasma concentrations of free fatty acids serve to spare glycogen stores (Costill et al., 1977).

Adaptations of the heart to an exercise program are not as marked as those of other tissues such as skeletal muscle and adipose tissue. It often has been stated that the adaptive changes produced by exercise concerning the ability to oxidize lipids efficiently for energy tend to make skeletal muscle more like heart muscle. Although some data suggest an exercise-induced adaptive increase in the quantity of cardiac mitochondria, it is generally agreed that the capacity for myocardial oxidative metabolism is sufficient to meet the demands of exercise without a further increase in mitochondrial number (Oscai et al., 1971).

Although exercise produces drastic changes in the activity of certain respiratory enzyme in skeletal muscle, the changes found in cardiac muscle are much less spectacular. For example, experimental
evidence suggests that carbohydrate utilization by the heart does not change with exercise-training and that important enzymes of glycolysis also remain unaltered (Scheuer and Stezoski, 1972; York et al., 1975). Interestingly, the only cardiac enzyme that has been reported to increase with exercise is lactate dehydrogenase (Gollnick and Heart, 1961). The precise effect, however, of exercise upon cardiac glycogen and carbohydrate utilization in general is presently unresolved. Segel and Mason (1978) found no increase in cardiac glycogen concentration of exercised rats while Scheuer et al. (1970) found a significantly increased glycogen concentration under similar conditions.

Glucose, ketone bodies and fatty acids are removed readily from the blood by the heart and then rapidly catabolized. However, hearts from normal animals utilize fatty acids as the major substrate for energy at all work loads. The source of these fatty acids is either the plasma free fatty acids or lipoprotein triacylglycerols. The extent of incorporation of fatty acids into neutral glycerides is increased in the mildly ischemic heart, but severe ischemia can result in the accumulation of free fatty acids or acyl CoAs ultimately producing deleterious effects (Gilbertson, 1977).

Antithrombotic Effects of Polyunsaturated Fatty Acids

Arterial thrombosis is the main lethal complication of atherosclerosis, is involved in atherogenesis, and is causal toward the production of ischemia leading to myocardial infarction. Investigations
into the role of thrombosis have been limited until recently because of the lack of suitable technique. In the last decade, however, new techniques together with the elucidation of the pathway for arterial thrombus formation have allowed an acceleration of research into this important clinical problem.

The formation of a thrombus involves two processes: formation of loose platelet thrombi and fibrin formation. Briefly, the former process involves the adhering of circulating platelets to subendothelial tissue which becomes exposed to the blood after vessel damage. The adhered platelets release adenosine diphosphate (ADP) which serves to cause platelet aggregation. Platelet aggregation itself can cause the release reaction, thus the formation of thrombi is a self-propagating process (Mustard and Packham, 1970). The latter process or the intrinsic coagulation system is induced by collagen and platelets. Coagulation is accelerated by platelet factor 3, a phospholipid which becomes available concomitant with the platelet release reaction. The end product of the clotting process, fibrin, serves to produce a stabilized thrombus (Sixma and Nijessen, 1970).

The influence of dietary fat upon thrombus formation has not been completely resolved. Generally speaking, experimentation suggests that saturated fat enhances arterial thrombus formation while unsaturated fat is either antithrombotic or has no effect whatsoever.

The antithrombotic effect of polyunsaturated fat is dependent
upon concentration in the diet, length of feeding, and degree of unsaturation. However, of all types of polyunsaturated fat tested, a diet rich in linoleic acid (50% of calories as linoleic acid) exhibited the strongest antithrombotic effect, with the maximum effect occurring after five weeks of feeding (Hornstra, 1975). This antithrombotic effect of linoleic acid is substantiated further by a long-term clinical study conducted in Helsinki, Finland. Over a twelve-year period, the use of a diet containing 12 percent of calories as linoleic acid significantly decreased mortality from coronary heart disease in male mental patients when compared to a diet containing 4 percent of calories as linoleic acid (Miettinen et al., 1972). The anti-aggregating effect of a high linoleic acid diet also has been observed in children with type II hyperlipoproteinemia and in geriatric patients with a high incidence of diabetes mellitus and coronary heart disease (Fleischman et al., 1973). The beneficial effect in the geriatric patients was evident after two weeks of feeding, but, with subsequent return to the normal diet, the initial level of aggregation was reached within two weeks.

A possible mechanism for the antithrombotic effect of linoleic acid is through the synthesis of prostaglandins (PG). Prostaglandins are a unique group of cyclic fatty acids with widespread and potent biological effects involving practically every organ system. Discovered independently in the 1930's by several different research groups, the prostaglandin synthesizing system is found both in the higher animals and in lower animals such as corals and mussels.
Prostaglandins were all but ignored for almost forty years, but in the 1960's the degree of research associated with prostaglandin structure and function increased greatly. For example, in 1961 a single article related to PG appeared in the literature while in 1972 over 600 articles were published dealing with these compounds (Lee, 1974). To date, literally thousands of journal articles have been published concerning the structure and function of prostaglandins.

The immediate precursors of prostaglandins are essential unsaturated fatty acids. For example, the precursor of PGE$_1$ and PGF$_{1a}$ is dihomo-γ-linolenic acid, and for PGE$_2$ and PGF$_{2a}$, is arachidonic acid. The conversion of these unsaturated fatty acids to prostaglandins is catalyzed by prostaglandin synthase (Lee, 1974). Because linoleic acid is a precursor to both of the aforementioned fatty acids, it can serve as a precursor to most prostaglandins.

The biological actions of prostaglandins are undoubtedly the most varied of any naturally occurring compound. These actions include reported effects upon the cardiovascular system and the development of myocardial ischemia. Gudbjarnason and Hallgrimsson (1976) reported a dietary modification of the fatty acid composition of cardiac lipids because increased dietary polyunsaturated fatty acids were shown to replace less unsaturated fatty acids in cardiac phospholipids. Additionally, mortality following isoproterenol-induced infarction increased with decreasing amounts of arachidonic acid in cardiac phospholipids. The E-prostaglandins have a dramatic
effect upon the dilatation and decreased resistance of the coronary arteries (Boroyan, 1976). Forster (1976) has stated that prostaglandins possess two regulative functions in the heart: coronary artery dilatation and inhibition of the development of arrhythmia. Tanz et al. (1977) also reported a prostaglandin reversal of tachycardia in the rabbit but noted that this effect was species specific. Certain prostaglandins are formed and released from the heart within minutes of the onset of hypoxia or ischemia (Block et al., 1975). Also, bradykinin and prostaglandins act synergistically as the natural stimulus for excitation of the sensory receptors that initiate signalling of pain associated with myocardial ischemia (Barzak et al., 1976). However, Logan et al. (1977) could find no protective effect in rats subjected to myocardial ischemia after a one-month diet containing 20 percent safflower oil.

Although conflicting data are commonplace in the literature, the positive attributes of prostaglandins far outweigh the negative aspects with respect to the development of cardiovascular disease. Unfortunately, prostaglandin administration hardly represents a therapeutic alternative in patients with myocardial infarction. The vasoactive properties of many prostaglandins are strong enough to produce hypotension and shock. Prostaglandins may act as feedback inhibitors of catecholamines and might therefore inhibit the catecholamine-stimulated lipolysis in adipose tissue and the heart (Mjos et al., 1976). This decreased lipolysis would reduce myocardial
free fatty acid uptake and utilization and therefore limit myocardial ischemia.

The effects of different polyunsaturated fatty acids upon the heart are well-described, but the mechanisms underlying these effects are ill-defined. The common factor in the literature that seems to interrelate the beneficial effects of polyunsaturated fatty acids with respect to cardiovascular disease is the platelet membrane. Most of the special properties of the blood platelets, including their ability to display shape change, procoagulant activity, the release of stored substances from organelles, and aggregation, involve membrane reactivity or are directly or indirectly dependent upon it. The fact that platelet activity is stimulated by a variety of agents, most of which are unable to penetrate the cell, makes it obvious that the platelet membrane has unique properties (Luscher, 1977).

Recent investigations suggest that an intermediate product formed during prostaglandin synthesis in stimulated platelets serves as an intercellular messenger to promote aggregation and the platelet release reaction (Willis, 1974; Hamberg et al., 1974). The labile aggregation stimulating substance (LASS) probably consists of two cyclic endoperoxides, PGG$_2$ and PGH$_2$. As one of the major constituents of platelet phospholipids, arachidonic acid is the principal precursor for LASS. Therefore, an important step in the synthesis of LASS is the cleavage of fatty acids from phospholipids in the membrane by phospholipase A and exposure of the platelets to aggregating agents.
(Smith and Silver, 1973). Gerrard et al. (1976) have shown that linoleic acid inhibits platelet aggregation through inhibition of the platelet enzyme that converts arachidonic acid to LASS. In similar experiments, the tendency for thrombosis also was related to the concentration of linoleic acid in the diet. This finding definitely suggests that the anti-thrombotic effect of dietary linoleic acid is mediated by its lowering effect on the arachidonic/linoleic acid ratio in platelet phospholipids (Hornstra and Haddeman, 1977).

Isoproterenol-induced Infarction as a Model for Cardiovascular Research

Although the toxic effects of large doses of catecholamines have been well known for a number of years, Rona et al. (1959) were the first investigators to administer catecholamines for the sole purpose of producing experimental myocardial necrosis. Because of the potency and specificity in the production of myocardial infarcts, the synthetic catecholamine isoproterenol hydrochloride was chosen of which the L-isomer is the most potent (Rosenblume et al., 1966).

The infarcts produced by isoproterenol closely resemble spontaneously occurring myocardial infarcts (Rona et al., 1959). Similar results were found by Wexler and Kittinger (1963) where they described the infarcted areas of rat heart as appearing blanched and yellow-brown caused by cell death and leukocytic infiltration.
Capillary dilation and myocardial edema caused by increased capillary permeability along with mitochondrial swelling and myocardial fiber disintegration also were observed. These investigators also noted elevated plasma concentrations of lactate dehydrogenase (LDH) and creatine phosphokinase (CPK) which are common clinical methods for substantiating the occurrence of myocardial infarction.

It is well-documented that isoproterenol acts selectively on the beta receptors of the myocardium to produce a positive ionotropic and chronotropic effect (Brink et al., 1971; Petery and Mierop, 1977). This increase in heart activity is induced in part by the increased intracellular concentration of cAMP and also by an elevation of cyclic AMP-dependent protein kinase activity (Haber and Wrenn, 1976; Byus et al., 1976). The precise mechanism has yet to be elucidated, however.

Injections of isoproterenol increase both the energy demand and oxygen requirements of the rat heart by increasing the work of the heart (Rona et al., 1959). Isoproterenol simultaneously acts on the peripheral vascular system producing massive vasodilation in the muscular beds. This vasodilation drastically reduces peripheral resistance, resulting in a greatly reduced blood pressure (Innes and Nickerson, 1975). The decrease in blood pressure produced by isoproterenol is in turn responsible for a decrease in the perfusion of the myocardium where flow to the endocardium is most seriously affected (Beznak and Hacker, 1963).
Therefore, isoproterenol produces infarcts due to increased heart rate and myocardial contractility which concomitantly produces an increased oxygen demand by the myocardium. The hypofunctioning coronary circulation, however, will not allow the oxygen demand to be met causing hypoxic conditions that result in myocardial ischemia and irreversible infarction.

A relationship exists between the severity of isoproterenol-induced infarction and body weight. Although the precise mechanism for the increased sensitivity with increased body weight is unknown, investigators have suggested that the amount of body fat and not the catabolism of isoproterenol or activity of the myocardial beta-receptors is involved (Balazs et al., 1972).

Interrelationship between Dietary Polyunsaturated Fatty Acids, Physical Condition, and Development of Myocardial Ischemia

The development of myocardial ischemia and infarction can be due, at least in part, to the formation of thromboemboli in the coronary circulation. Arterial thrombosis is a major contributor to three of the four most frequent causes of death (acute myocardial infarction, renal disease, and stroke) in the United States (Wessler, 1974). Prevention of ischemia can be partly resolved by administration of drugs that inhibit platelet aggregation. Recent experimental evidence has shown, however, that dietary factors such as polyunsaturated fatty acids also inhibit the normal clotting
In addition to chemical prevention of ischemia and infarction, exercise too protects the myocardium (Scheuer and Tipton, 1977). The effect of exercise on the rate of oxidation of saturated fat is well documented (Zierler, 1976). Exercise-trained rats have a greater capacity to oxidize stored cardiac lipid (Jones and Havel, 1967); in addition, they exhibit lower concentrations of serum triglycerides and free fatty acids (Carlson and Froberg, 1969). Because the amount of total serum lipids is correlated directly with incidence of myocardial infarction, the decrease produced by exercise has become instrumental toward the widespread use of physical training as a mechanism for prevention of myocardial reinfarction (Paffenbarger et al., 1970; Brunner and Meshulam, 1969).

The replacement of dietary saturated fat by PUFA could produce several manifestations. The ability of animals to oxidize PUFA is greater than the ability to oxidize saturated fatty acids (Cenedella and Allen, 1969). Also, PUFA's, especially linoleic acid, are precursors to prostaglandins. Theoretically, the increase of precursors of PG in the diet can increase the biosynthesis of prostaglandins (Galli et al., 1977). Additionally, release of certain classes of PG's into blood plasma increases during myocardial ischemia (Galli et al., 1977). Also, PG's reverse ventricular tachycardia caused by myocardial ischemia (Forster, 1976) and regulate coronary circulation (Boroyan, 1976).
MATERIALS AND METHODS

Male Sprague-Dawley rats were individually housed at 25 ± 3°C in a room with a controlled light-dark cycle (14 h light, 10 h dark). Water was provided ad libitum. Rats, 100 days old, were randomly divided into the following four groups: exercise-trained rats that were fed a high-fat diet (Fat-Ex), nonexercised rats fed a high-fat diet (Fat-C), exercise-trained rats fed a low-fat diet (Chow-Ex), and nonexercised rats fed a low-fat diet (Chow-C). Fifty percent of the calories in the high-fat diet were supplied by safflower oil with the remaining calories being supplied by Teklad rat chow. Rats in all four groups received an equal amount of calories per gram of body weight. Rats fed the low-fat diet were given only the Teklad rat chow. The experimental period was of 10 weeks duration, and rats were weighed each week.

Exercised rats were run on a motor-driven treadmill at an 8° incline at 20 m/min for 30 min/day and 6 days/wk for a 10-wk experimental period. A similar but slightly less intense exercise program has been shown previously to be sufficiently strenuous to result in physical training of rats (Severson et al., 1978). At the end of the 10-wk experimental period, all rats received two subcutaneous injections of L-isoproterenol hydrochloride (70 mg/kg of body weight) 24 hours apart, the first dose being administered 24 hours after the last exercise period in the exercised rats.

Twenty-four hours after the second injection of isoproterenol,
rats were anesthetized lightly with sodium pentobarbital (25 mg/kg of body weight). The abdominal cavity of each rat was opened and livers were clamped with Wollenberger tongs that had been maintained in liquid nitrogen. Liver samples were stored at -20°C before analysis. Blood was withdrawn from the vena cava using plastic syringes, placed in heparinized centrifuge tubes, and centrifuged for 15 min at 1400 X G. The prepared plasma samples then were placed in sealed plastic tubes and stored at 4°C before analysis. All enzymatic assays were completed within 12 h of sample collection.

Total lactate dehydrogenase (LDH) activity in the plasma was determined by the method of Wacker et al. (1956) with minor modifications (Calbiochem, La Jolla, California). Electrophoretic separation of plasma LDH isozymes was performed on cellulose poly-acetate strips according to the method of Preston et al. (1965). The relative percentage of each isozyme was determined by densitometric methods. The activities of specific isozymes then were calculated by multiplying the total plasma LDH activity by the relative percentage of the specific isozyme. Concentration of solvent-extractable lipid in liver and heart was determined in an aliquot of each tissue by using two successive extractions with chloroform-methanol (2 to 1, v/v) and one with diethyl ether (Schneider, 1945). Pericardial adipose tissue had been dissected from the hearts before homogenization. Assays of glycogen concentration in liver samples were performed according to the method of
Seifter et al. (1950). Plasma triglyceride concentrations were determined according to the method of Mendez et al. (1975).

Data were analyzed using the Statistical Analysis System (Barr and Goodnight, 1972). Comparisons of treatment means were made using the Duncan's multiple range test (Snedecor and Cochran, 1967). Means were considered significantly different if P < 0.05.
RESULTS

To minimize the effect of dietary energy intake on the parameters measured in this experiment, all rats were fed the same number of calories per unit of body weight. Although body weights of individual rats fluctuated somewhat, there was no difference in the mean body weights of rats of each group at the initiation or at the termination of the 10-wk experimental period (Table 1).

Table 1. Mean body weight of rats before and after 10-week experimental period

<table>
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<th>Final Body Wt.</th>
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<td>334.9 ± 10.1</td>
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<td>Fat-C</td>
<td>11</td>
<td>324.7 ± 11.9</td>
<td>363.5 ± 12.6</td>
</tr>
<tr>
<td>Chow-Ex</td>
<td>15</td>
<td>350.6 ± 10.2</td>
<td>328.9 ± 10.8</td>
</tr>
<tr>
<td>Chow-C</td>
<td>14</td>
<td>342.1 ± 10.5</td>
<td>342.6 ± 11.2</td>
</tr>
</tbody>
</table>

\textsuperscript{a}N = No. of rats per group.

\textsuperscript{b}Values are means ± SE. No significant difference (p < 0.05) between initial and final body weights for each group.

Severity of Infarction

The severity of the isoproterenol-induced infarction was assayed by determining the activity of lactate dehydrogenase (LDH) and of the LDH-1 isozyme in plasma collected 24 hours after the second isoproterenol administration.
After 10 weeks on the experimental diets and exercise regimen, total LDH activity was greater in the plasma of Chow-C rats than in plasma of rats of other groups (Table 2). Total LDH activities in rats of the Fat-Ex, Fat-C, and Chow-Ex groups were similar. LDH-1, the major isozyme of LDH in heart, was lower in plasma of Fat-Ex animals than in the rats of the other three groups, which had similar activities of LDH-1 in plasma.

Table 2. Total LDH and LDH-1 activity in plasma of rats after 10-week experimental period

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Total LDH Activity</th>
<th>LDH-1 Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat-Ex</td>
<td>16</td>
<td>39.9 ± 9.5^bμU/ml</td>
<td>1.7 ± 2.3^d</td>
</tr>
<tr>
<td>Fat-C</td>
<td>11</td>
<td>72.9 ± 11.4</td>
<td>15.9 ± 2.9</td>
</tr>
<tr>
<td>Chow-Ex</td>
<td>15</td>
<td>53.4 ± 9.8</td>
<td>11.7 ± 2.5</td>
</tr>
<tr>
<td>Chow-C</td>
<td>14</td>
<td>110.0 ± 10.1^c</td>
<td>13.6 ± 2.6</td>
</tr>
</tbody>
</table>

^aN = No. of rats per group.

^bValues are means ± SE.

^cSignificantly different from other values in column (p < 0.05), which are similar (p > 0.05).

^dSignificantly different from other values in column (p < 0.05), which are similar (p > 0.05).

Concentration of Hepatic and Cardiac Lipid

Effect of concentration of fat in the diet and exercise on lipid content of liver and heart of rats was determined. Fat-C rats
exhibited a greater concentration of hepatic lipid at the end of the 10-week experimental period, but there was no difference in hepatic lipid concentration of rats in the Fat-Ex, Chow-Ex and Chow-C groups (Table 3). Cardiac lipid concentration was lower in Fat-Ex rats than in the Chow-Ex rats; but rats in both exercise-trained groups exhibited less cardiac lipid than the nonexercised control rats.

Table 3. Concentration of total lipid in livers and hearts of rats after the 10-week experimental period

<table>
<thead>
<tr>
<th>Group</th>
<th>N\textsuperscript{a}</th>
<th>Hepatic Lipid (%) of wet wt.</th>
<th>Cardiac Lipid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat-Ex</td>
<td>17</td>
<td>10.6 (\pm 0.4)\textsuperscript{b}</td>
<td>8.3 (\pm 0.3)\textsuperscript{d}</td>
</tr>
<tr>
<td>Fat-C</td>
<td>11</td>
<td>12.7 (\pm 0.5)\textsuperscript{c}</td>
<td>12.4 (\pm 0.4)</td>
</tr>
<tr>
<td>Chow-Ex</td>
<td>15</td>
<td>10.5 (\pm 0.4)</td>
<td>11.2 (\pm 0.3)\textsuperscript{e}</td>
</tr>
<tr>
<td>Chow-C</td>
<td>14</td>
<td>10.7 (\pm 0.4)</td>
<td>12.9 (\pm 0.3)</td>
</tr>
</tbody>
</table>

\textsuperscript{a}N = No. of rats per group.
\textsuperscript{b}Values are means \(\pm SE\).
\textsuperscript{c}Significantly different from other values in column \((p < 0.05)\), which are similar \((p > 0.05)\).
\textsuperscript{d}Significantly different from other values in column \((p < 0.05)\).
\textsuperscript{e}Significantly different from values for Fat-C and Chow-C groups in column \((p < 0.05)\).
Plasma Triglyceride and Hepatic Glycogen Concentration

Plasma triglyceride concentration was lower in Fat-Ex rats than in rats of other three groups (Table 4). Chow-Ex rats had lower concentration of plasma triglycerides than did the sedentary control (Chow-C) animals but greater than did the Fat-Ex rats.

Table 4. Plasma triglyceride and hepatic glycogen concentrations in rats after 10-week experimental period

<table>
<thead>
<tr>
<th>Group</th>
<th>N a</th>
<th>Plasma triglyceride</th>
<th>Hepatic glycogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat-Ex</td>
<td>17</td>
<td>20.6 ± 3.7 b,c mg/dl</td>
<td>27.5 ± 5.3 mg/g wet wt.</td>
</tr>
<tr>
<td>Fat-C</td>
<td>11</td>
<td>40.7 ± 4.6</td>
<td>11.3 ± 6.6 e</td>
</tr>
<tr>
<td>Chow-Ex</td>
<td>15</td>
<td>36.1 ± 3.9 d</td>
<td>33.3 ± 5.6</td>
</tr>
<tr>
<td>Chow-C</td>
<td>14</td>
<td>52.5 ± 4.1</td>
<td>49.1 ± 5.8 f</td>
</tr>
</tbody>
</table>

N = No. of rats per group.

Values are means ± SE.

Significantly different from other values in column (p < 0.05).

Significantly different from values for Fat-Ex and Chow-C groups in column (p < 0.05).

Significantly different from values for Chow-Ex and Chow-C groups in column (p < 0.05).

Significantly different from other values in column (p < 0.05).

Liver glycogen concentration was lower in Fat-C rats than in rats of other three groups (Table 4). Conversely, glycogen concentration in rats of Chow-C group was greater than those in
other three groups. There was, however, no difference in hepatic glycogen concentration of Fat-Ex and Chow-Ex rats.
DISCUSSION

In general, initiation of an exercise program causes an initial decrease of body weight in the untrained animal; this initial change is followed by an attainment of body weight stability (Bjorntorp, 1976). Aging, sedentary rats fed ad libitum, however, will continue to exhibit an increase in body weight. Obesity has been designated as an important risk factor associated with susceptibility to coronary heart disease. Therefore, to adequately determine the effect of dietary polyunsaturated fatty acids (PUFA's) and exercise on reduction of incidence or severity of myocardial infarction, body weight should not be an influencing factor. Furthermore, there exists a direct relationship between the body weight of rats and their cardiotoxicity to isoproterenol (Balazs et al., 1972). Therefore, all rats in our experiments were pair-fed equal amounts of calories per kilogram of body weight, resulting in insignificant differences between average body weights for each group of rats at the termination of the experiment (Table 1).

Isoproterenol-produced infarct-like lesions in the rat heart closely resemble spontaneously occurring myocardial infarcts (Wexler and Kittinger, 1963). Also, LDH-1 isozyme activity in blood plasma is a commonly accepted method for quantitation of the severity of induced infarcts. As shown in Table 2, the activity of plasma LDH-1 was lowest in the exercised rats that were fed a diet high in safflower oil. The rats fed the high safflower oil diet, but not
exercised, had similar activity to the chow-fed rats. On the basis of LDH-1 activity in plasma, the isoproterenol-induced infarcts in rats fed the chow diet or the high-fat diet, but not exercised, were 6 to 9 times more severe than in the rats fed high-fat diet and exercised. Exercise had no effect on severity of isoproterenol-induced infarcts in the chow-fed rats.

Our results suggest that the protective effect against myocardial infarcts by exercise is dependent on the concentration of polyunsaturated fatty acids in the diet. Probably, the high concentration of PUFA's, especially linoleic acid, in the high-fat diet increased the concentration of PUFA in lipids of body tissues. Therefore, more PUFA were available to, directly or indirectly, inhibit the blood clotting process and, thus, possibly contribute to the decrease in the severity of the isoproterenol-induced infarct.

The precise mechanism of the PUFA effect upon platelet aggregation is yet to be elucidated, but several possible mechanisms exist. First, Pickart and Thaler (1976) reported a lesser stimulation of fibrinogen synthesis in liver slices incubated with linoleic acid than in those incubated with palmitate. A second possible antithrombotic effect of dietary linoleic acid involves an increase in the concentration of linoleic acid in platelet membranes. Stimulation of platelets by aggregating agents activate platelet phospholipase A, which, in turn, releases arachidonic acid from phospholipids in platelet membranes (Schoene and Iacono, 1974). Arachidonic acid
then is converted enzymatically to prostaglandin endoperoxides (Hamberg and Samuelsson, 1973). Most of these endoperoxides, in turn, are converted to thromboxane A₂, which causes platelet aggregation (Hamberg et al., 1975). The activated phospholipase is not specific to the release of arachidonic acid from the platelet phospholipids, but can cause the release of other ω-fatty acids as well, especially linoleic acid. Because linoleic acid can compete for the enzyme system that catalyzes the conversion of arachidonic acid to thrombogenic endoperoxides, it therefore can inhibit the arachidonic acid-induced platelet aggregation and the endoperoxide-induced aggregation (Gerrard et al., 1976). In addition, Hornstra and Haddeman (1977) recently have suggested that the anti-thrombotic effect of dietary linoleic acid is mediated by its reduction of the arachidonic acid to linoleic acid ratio in phosphatidyl choline in platelet membranes.

An important aspect of high concentrations of dietary fat is the effect upon concentration of lipids in several tissues, especially liver and heart. Pawar and Tidwell (1967) reported an increase in hepatic lipid in male rats fed a diet of 20% safflower oil for a twelve-week period. Isoproterenol as well can produce an increase in hepatic lipid concentration because Judd and Wexler (1969) observed a striking fatty infiltration of the liver and hyperlipidemia in male rats injected with isoproterenol by the same method used in this study. Our data agree with these findings for
Fat-C animals. However, Fat-Ex rats exhibited no increase in hepatic lipid concentration when compared to Chow-Ex and Chow-C rats. Increased catabolism of lipid for ATP generation during exercise as a mechanism for lowering concentrations of lipid in several tissues is well documented (Jones and Havel, 1967). Increased lipid catabolism could also lower the concentration of hepatic lipid in Fat-Ex rats.

Cardiac lipid concentration was lower in both Fat-Ex and Chow-Ex rats than in rats of the nonexercised groups. Heart muscle is well adapted for lipid catabolism because of its high concentration of mitochondria and its rich supply of oxygen. Additionally, Parizkova (1969) has reported that cardiac muscle from trained rats exhibits an increased lipoprotein lipase activity, which, in turn, would aid in cardiac lipid catabolism.

Safflower oil and dietary PUFA, usually, cause a decrease in plasma lipid concentration in mammals (Pawar and Tidwell, 1967). Due to increased energy requirement and increased uptake of lipids by muscle, exercise, too, causes a decrease in plasma lipid concentration (Jones and Havel, 1967). These findings agree well with our data as plasma triglyceride concentration was lower in exercised rats than that in sedentary controls. Liver glycogen concentration was 2.5-fold lower in the nonexercised than in the exercised rats fed a high fat diet. Glycogen concentration in livers of exercised rats was lower than in livers of the nonexercised rats when the low fat diet was
fed. There was, however, no difference in hepatic glycogen concentration due to dietary regimen in those animals that were exercised. This lack of difference between Fat-Ex and Chow-Ex rats could be produced by several mechanisms. An increase in plasma free fatty acids directly before an exercise bout serves to spare liver glycogen (Rennie et al., 1976). Fat-Ex rats were provided lipid in the diet, but free fatty acid concentration would be increased in both exercising groups as lipolysis is increased to provide energy needed for muscular contraction. Additionally, glycogen repletion is greater in exercised animals (Baldwin et al., 1975).

Even though exercise decreased plasma and cardiac lipid concentrations, the decrease was significantly greater in those animals fed a high-fat diet. Together with the antithrombotic effect of PUFA discussed earlier, it appears that exercise, through adequate catabolism of lipid, can produce tissue lipid concentrations at least comparable to those of an exercise program undertaken on a more normal dietary regimen.
SUMMARY

Determination of plasma lactate dehydrogenase activity, plasma triglyceride concentration, liver glycogen concentration, and the lipid content of heart and liver were used to characterize metabolic changes occurring in male rats subjected to a high-fat diet and exercise. Additionally, the beta catecholamine isoproterenol was used to determine the effect on the aforementioned metabolic parameters in response of myocardial ischemia and infarction.

Rats were divided into two groups and treatments: animals that were exercised and fed a standard Teklad rat chow diet (Chow-Ex), animals that were not exercised and fed Teklad rat chow (Chow-C), animals that were exercised and also received 50 percent of their caloric intake from safflower oil (Fat-Ex), and animals that were fed the high-fat diet, but were not exercised (Fat-C). Chow-Ex and Fat-Ex animals were physically trained 30 minutes per day, 6 days per week for a period of 10 weeks on a motor driven treadmill. The treadmill was operated at an 8° incline and a speed of 20 meters per minute. This intensity of exercise approximates to 70 percent of \( V_{\text{max}} \) of oxygen consumption. All animals were housed individually and body weight was maintained throughout the experimental period by pair-feeding. At the end of the 10 week period, all rats received two subcutaneous injections of L-isoproterenol hydrochloride (70 mg/kg of body weight) 24 hours apart, the first dose being administered 24 hours after the last exercise period in Fat-Ex and
Chow-Ex rats.

Results of this study were as follows:

1. Only those animals that were exercised and fed a high-fat diet (Fat-Ex) exhibited depressed plasma concentration of LDH-1, the heart isozyme.

2. Plasma triglyceride concentration was significantly lower in Fat-Ex rats when compared to all other groups.

3. There was no difference in hepatic glycogen concentration between Fat-Ex and Chow-Ex rats.

4. Concentration of hepatic or cardiac lipid was not significantly increased by the high-fat diet in those animals that were exercised.

Data accumulated in this study imply that dietary polyunsaturated fatty acids in the form of safflower oil will decrease the severity of myocardial infarcts in exercised but not sedentary rats and that exercise did not reduce the severity of myocardial infarcts in chow-fed rats. Additionally, the exercise training program was necessary to maintain the concentration of hepatic and cardiac lipid at normal levels in those animals that were fed the high-fat diet.
LITERATURE CITED


ACKNOWLEDGEMENTS

I would sincerely like to thank my co-major professors, Dr. Yola Forbes and Dr. Donald Beitz, for their encouragement and advice throughout the completion of my doctoral requirements. I also thank the other members of my committee, Dr. Allen Trenkle, Dr. Jerry Young and Dr. James Redmond.

I could never adequately express the thoughts and feelings I have toward Dr. David Griffith, who passed away during the completion of this work. Of all the knowledge that I obtained through our relationship, I feel the most important is the way in which he treated all students and faculty with kindness and fairness. He was truly an individual that can never be replaced.

Finally, I wish to thank my parents for their love and support that was always very present throughout my graduate educational process.
Table 5. Plasma cholesterol concentration and clotting time of blood of rats after 10-week experimental period.

<table>
<thead>
<tr>
<th>Group</th>
<th>N&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Plasma Cholesterol (mg/dl)</th>
<th>Clotting Time (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat-Ex</td>
<td>17</td>
<td>146.3 ± 4.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.2 ± 0.1</td>
</tr>
<tr>
<td>Fat-C</td>
<td>11</td>
<td>151.8 ± 9.9</td>
<td>3.6 ± 0.5</td>
</tr>
<tr>
<td>Chow-Ex</td>
<td>14</td>
<td>144.1 ± 3.5</td>
<td>4.2 ± 0.4</td>
</tr>
<tr>
<td>Chow-C</td>
<td>15</td>
<td>168.6 ± 5.2</td>
<td>4.0 ± 0.4</td>
</tr>
</tbody>
</table>

<sup>a</sup>N = No. of rats per group.

<sup>b</sup>Values are means ± SE. No significant difference (p < 0.05) among or between treatment groups for plasma cholesterol concentration or blood clotting time.

The high-fat diet consisted of 448 g of Hy-Vee brand safflower oil mixed with 1 kg of Teklad rat diet that had previously been ground. According to label information, a diet mixed in this manner would contain 3844 Kcal from oil and 3844 Kcal from the Teklad rat diet, i.e., 50 percent of the calories consumed from an evenly mixed diet would be provided by safflower oil.