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EFFECTS OF AN ACUTE BOUT OF NEAR-MAXIMAL INTENSITY
EXERCISE ON THE CARDIAC ENZYMES
IN HUMAN SERA

THESIS

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By

Bernadette A. Goheen, B.A.

Denton, Texas

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The Cardiac Profile, a pattern of serum enzyme changes seen within seventy-two hours after an AMI, is diagnostic aid for detecting occurrence of infarcts. The effects of exercise stress on the Cardiac Profile aid clinicians in avoiding diagnostic errors in patients immediately after exercise.

Five male volunteers ran from six to ten miles. Serum enzyme levels were monitored serially three days before and five days after stress. Enzyme activity was determined spectrophotometrically and electrophoretically.

Significant increases in total CPK and LDH were seen. An LDH 'one-two flip' occurred eight hours after exercise. No MB-CPK was found following the run.

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CHAPTER I

INTRODUCTION

Alterations in serum enzyme levels have been characterized in a variety of pathological conditions. Clinicians have assessed the value of enzyme determinations on the basis of their specificity, sensitivity, and predictive value for the definitive diagnosis of disease states. Recently, research has revealed that exercise causes blood enzyme elevations similar to those seen after an acute myocardial infarction (AMI). The Cardiac Profile, a pattern of serum enzyme elevations seen within seventy-two hours after an AMI, has been a key diagnostic aid for detecting the occurrence and extent of damage of the infarct. Utilizing the highly specific and sensitive analysis of Creatine phosphokinase (CPK) and Lactate dehydrogenase (LDH) isoenzymes with the total enzyme determinations of CPK, LDH, and Glutamic oxaloacetate transaminase (GOT) the Cardiac Profile has provided clinicians with a prompt, but accurate, tool for the definitive diagnosis of AMI. The thrust of the study described here was to detail the effects of exercise on the activities of those enzymes used in the Cardiac Profile to better define the usefulness of this profile as a clinical indicator of disease.

Literature

Diagnostic Efficacy of Enzyme Determinations

Introduction.--Prompt, but accurate, identification of disease states has always been of primary importance to the physician. Serial estimation of elevated blood enzyme levels has been extensively utilized for the diagnosis of a variety of pathological conditions (92). Increased serum enzyme levels have been interpreted as indicators of cell necrosis and/or transudation of enzymes from intracellular to extracellular compartments. Knowledge of the possible organ and/or tissue source of specific enzyme release has aided the clinician in discerning the origin of disease.

Sensitivity and specificity.--According to Walter (92, p.2), "The value of serum enzyme determinations in the diagnosis of a specific disease should be assessed on the basis of both specificity and sensitivity of each test." The sensitivity of a specific enzyme analysis, expressed as the frequency of positive test results in patients with a particular disease (e.g.: myocardial infarction), has become a clinical indicator of the incidence of true positive diagnoses made from that test. Likewise; the specificity of a particular enzyme determination, expressed as the frequency of negative test results in patients (e.g.: myocardial infarction) without disease, has become a clinical indicator of the incidence of true negative diagnoses made from that test. Thus, any blood enzyme analysis which has

shown high specificity and sensitivity for the identification of a particular disease state, has become a valuable tool for the recognition of that disease, and has minimized the physician's chances of making a diagnostic error.

Predictive value.--In addition, Galen (23) has stressed the need for evaluation of the predictive value of each enzyme test. Expressed as the frequency of diseased patients among all patients with positive test results, the predictive value has become a clinical index of the prevalence of the disease in the population under study.

For example, if the test were a serum enzyme determination, we could know from its sensitivity and specificity that it was positive in ninety-five per cent of patients with AMI and negative in ninety-five per cent of non-AMI patients. We could then compare this enzyme test in the outpatient clinic and the coronary care unit. The prevalence of AMI, in the clinic, in all patients with chest pain, is five per cent, whereas, in the coronary care unit, it is fifty per cent in patients admitted to rule out AMI. When used in the clinic, the enzyme test would have a predictive value of fifty per cent: i.e., fifty per cent of the patients with positive results would have AMI and the remaining fifty per cent would not; and, therefore, their test results would be false positives. This same enzyme test, when used in the coronary care unit, would have a predictive value of ninety-five per cent: i.e., ninety-five per cent of the patients with positive results would have AMI with only five per cent of the results being false positives. (23, p. 142).

When used to solve diagnostic dilemmas, enzyme determinations have greatest predictive value in populations with a high incidence of the suspected disease.

General considerations.--Clinical laboratories must routinely establish normal distributions of enzyme activity

found among the non-diseased population. Detection of an enzyme activity which has fallen outside this range has been deemed a positive indicator of disease (23). However, variation in the range of enzyme levels considered "normal" in human populations has been attributed to: (1) the age, race, and sex of the individuals screened (35); (2) the type of blood specimens analyzed (e.g.: fasting, non-fasting, serum, plasma) (10,58,77); (3) the time of blood sampling in relation to previous physical activity (77), or onset of a pathological condition (92); and, (4) the quantitation and nature of the enzyme assay used (7,74). Thus, a good diagnostic enzyme determination frequently fails as a screening test for detection of disease due to a high incidence of false-positives resulting from intra-population variation, as well as to a low incidence of the disease in a selected population.

The major flaw of enzyme determinations has been the inability to achieve 100 per cent sensitivity and 100 per cent specificity at the same time. Many diagnostic tests have shown high sensitivity regarding the presence of a particular pathological condition (e.g.: LDH isoenzymes (95)). However, their specificity was low since the tests were not uniquely positive for that condition (92). The selection of an upper limit of normal for distinguishing between the disease versus non-disease populations has been the root of this problem. "It is impossible to select a cut-off point for enzyme activity that would afford complete discrimination

between the two groups." (23, p. 143). In the case of MI versus non-MI populations, Galen (23) has shown that when the enzyme results for each group were expressed as a frequency distribution, a classic over-lapping was apparent. (Figure 1) Hence, meaningful interpretation of an enzyme analysis has required an awareness of the limitations of this diagnostic procedure.

Isoenzymes.--Many enzymes have been shown to exist in several electrophoretically-distinct forms known as isoenzymes, or isozymes. Separate measurement of isoenzyme activity has become a valuable diagnostic tool due to the fact that the tissue specificity of the isoenzyme is usually greater than that of the total activity of the enzyme (92). Analysis of isoenzyme patterns from tissues, and normal and pathological sera has provided the necessary data base for clinical interpretation of various disease states (29). Recent development of commercial laboratory methods for electrophoretic separation and quantitation of CPK and LDH isoenzyme content has greatly enhanced the diagnostic efficacy of the corresponding total enzyme determinations for the definitive diagnosis of disease states.

The Cardiac Enzymes

In order to make a definitive diagnosis from enzyme determinations, clinicians must become acquainted with the specific time course of elevation and the maximum level of

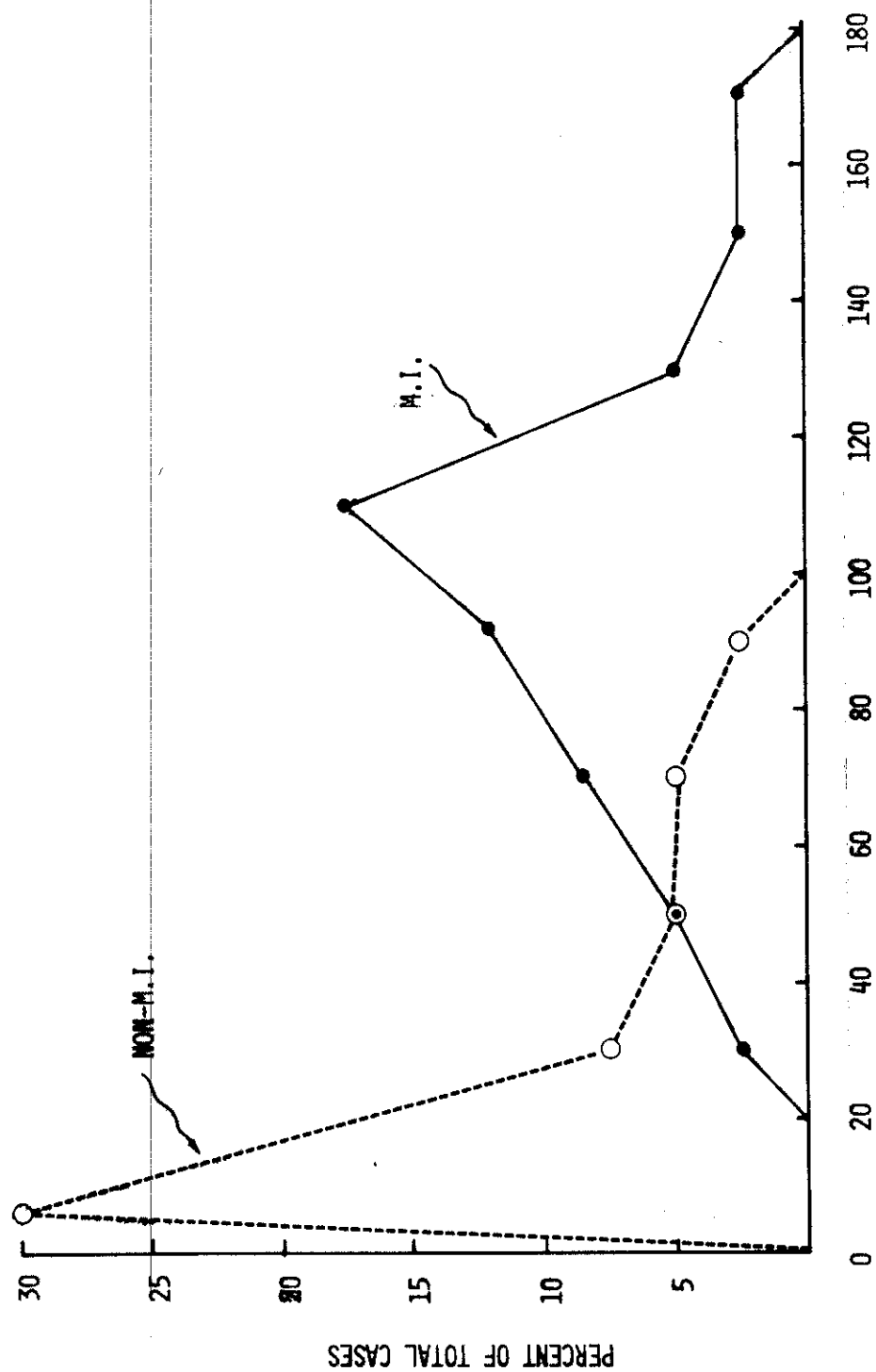


Fig. 1--Frequency distribution of enzyme results for MI versus non-MI populations. (23, p. 143)

SERUM AMYLASE UNITS

enzyme activity typically reported in particular disease conditions. Such patterns have been characterized for a variety of pathological states. A review of the disease states associated with increased serum levels of the cardiac enzymes follows.

Total CPK.--The occurrence of an increased level of Creatine phosphokinase (CPK), or Creatine kinase, or Creatine phosphotransferase (E.C.2.7.3.2.), has recently become a key diagnostic aid in the detection of acute myocardial infarct (AMI) (17,23,45,66,92), female carriers of Duchenne-type muscular dystrophy (MD), (30,45), and potential sufferers of malignant hyperthermia (21,99). In addition, increased serum CPK has been reported in cases of chronic alcoholism (21,23,45,92), cerebrovascular accidents associated with cerebral ischemia (23,45,92), muscle trauma due to surgery or frequent intramuscular injections (63,91,92), post-partum involuting uterus (21,31,45), and various neuromuscular disorders (23,42,45).

Total LDH.--Increased serum levels of total Lactate dehydrogenase (LDH) (E.C.1.1.1.27) have been demonstrated following AMI (23,38,45,66,92), myocarditis and other conditions resulting in damage to the myocardium (95), viral hepatitis (13,95), jaundice due to chlorpromazine and other drugs (88), extrahepatic biliary obstruction (13, 95), pernicious and megaloblastic anemias (19), and malignant neoplasms (95).

Total GOT.--Elevations of total Glutamate oxaloacetate transaminase (GOT), or Aspartate aminotransferase (E.C.2.6.1.1), have been associated with AMI (23,38,45,66,92), primary liver diseases (64), liver congestion secondary to congestive heart failure (23), skeletal muscle diseases (30), pulmonary embolus (64), biliary tract disease (30), pericarditis (64), and rapid tachyrrhythmias (23). Any general surgical procedure, shock of any origin, direct current cardioversion, and cardiac catheterization have caused elevations of CPK and LDH, as well as GOT (92).

CPK isoenzymes.--Creatine kinase, a dimeric protein, has been shown to exist in at least three isoenzyme combinations (each with a mass of approximately 82,000 daltons (15)) composed of subunits designated 'M' for muscle and 'B' for brain (the primary tissue sources of the isoenzymes). Until recently, little evidence has been presented to show that the two subunits of the enzyme might not behave identically. However, Price and Hunter (58) examined the effects of modifying reagents on the reactive thiol group of each subunit and found non-identical behavior of the subunits of rabbit CPK under "transition-state analogue" conditions.

Three isozymes have been isolated from human sera, namely: CPK I (BB), CPK II (MB), and CPK III (MM) (15,50,97). Upon electrophoresis (66), CPK I migrates most rapidly toward the anode, in the pre-albumin area, while CPK II exhibits

intermediate mobility with migration to the alpha-two-globulin area. CPK III migrates toward the cathode and is found in the gamma globulin zone. (A fourth isozyme, mitochondrial CPK, has been reported by Sobel et al. (79), Scholte (73), and Madsen (46).)

Analyses of human tissue extracts obtained from surgery have shown that CPK activity predominates in the brain, myocardium, and skeletal muscle (15,50,97). Isoenzyme profiling has revealed that brain tissue contains 100 per cent BB; skeletal muscle contains 96-100 per cent MM, 0-37 per cent MB, and 0-1 per cent BB; and, that adult myocardium contains 71-96 per cent MM, 4-27 per cent MB, and 0-2 per cent BB (36). The diagnostic value of CPK isoenzymes has been due to the high degree of tissue specificity obtained with this analysis. Thus, analysis of CPK isozymes in sera of patients with elevated total CPK activity has become a routine clinical procedure.

CPK III, the only form of the enzyme found in normal human sera, has been the isoenzyme most frequently responsible for elevated CPK levels. Clinical cases of increased serum CPK III activity have been reported following skeletal muscle damage (due to minor trauma) (62), surgery (23), intramuscular injections (63), and hypothyroidism (21,32,45). Detection of only the MM form of CPK in the sera has clinical relevance in that it has ruled out the presence of the two other isozymes.

Of the three isoenzymes, the MB form has proved to be the most pathognomic for disease. The detection of CPK II in the sera of patients suspected of sustaining an AMI has shown 94 per cent sensitivity and 100 per cent specificity for a positive diagnosis (55). The rapid return of MB levels to the normal range following onset of AMI has made this a particularly useful enzyme for the recognition of extensions of the infarct which occur subsequent to the initial episode (78). According to Wagner et al (91): "The ability to quantitate the MB-CPK fraction, its sensitivity and specificity, and its rapid appearance and disappearance from the serum has made this enzyme ideal for testing the feasibility of quantitating infarct size and for evaluating therapeutic interventions which are aimed at protection of the ischemic myocardium."

To date, the specificity of MB-CPK as an index of AMI has been impaired in only two cases: (1) cardiac surgery, which has caused elevated CPK II levels not attributable to ischemia; and, (2) muscular dystrophy, especially of the Duchenne-type, found in both children and adults (64). Clearly, the CPK-MB isoenzyme has become established as the most specific enzyme for disease now available.

An interesting clinical case, which may further enhance the specificity and sensitivity of CPK isozyme determinations in the diagnosis of AMI, was reported by Wevers et al (93). Three different MM and two different MB bands of CPK were detected in sera of patients with transmural infarctions

and elevated total CPK levels. Data gathered from hybridization experiments with this sera has led to speculation for the in vivo existence of two different 'M' subunits (M_1 and M_2) and two different 'B' subunits (B_1 and B_2). For ten hours after infarction only two MM bands were apparent in the sera. By twenty-four hours, a third MM band predominated while the two other MM bands had largely disappeared from the sera. Localization of the three MM and two MB isozymes in human tissues must be discerned before the significance of this study can be assessed.

The diagnostic significance of the BB form of CPK has yet to be established. Until recently, CPK I had been undetectable in human sera. Its apparent absence from the peripheral circulation, even following brain injury, had been attributed to the possible inability of the enzyme to cross the blood-brain barrier, or to the lability of the BB isozyme in the cerebrospinal fluid, or in the blood itself (65). In addition, improper methods for detection of CPK may have been employed. Bayer et al (3) state: "Of the methods for estimating CPK isoenzymes, only the ion-exchange procedure, and less well, electrophoresis are able to detect the presence of the BB isoenzyme. However, the advent of radioimmunoassay techniques for CPK isozyme separation (36) has increased the incidence of detection of BB-CPK in a variety of disorders.

Detection of CPK I in human sera has currently been reported: after surgery of the nervous system (37,44,54);

following kidney transplantation (12) during hemodialysis (24); in malignant hyperthermia (99); in patients undergoing aortocoronary bypass surgery (90); in Duchenne-type MD (81); and in patients with Oat-Cell Carcinoma (11).

Also, Bayer et al (3) reportedly found high activity of the BB isozyme in the spinal fluid of a patient suffering from severe brain damage. From these studies it has become apparent that detection of BB-CPK in the sera or spinal fluid will likely have future clinical relevance. Delineation of its relevance, in terms of specificity and sensitivity for the recognition of particular disease states, has yet to be elucidated.

Each of the isozymes of CPK have exhibited different kinetics of disappearance from the serum. Shell and Sobel (76) attributed differences in the rate and extent of release of each isozyme into the peripheral circulation after ischemia of a particular tissue to different intracellular loci, and different rates of inactivation during transit to the plasma space. In experiments with dogs, they established that the CPK disappearance from the circulation remained first order and constant with the same animal, despite marked hemodynamic derangements or intercedent myocardial infarction. This finding was of particular importance since individual variation of CPK disappearance during a study would impair enzymatic estimates of infarct size (64).

CPK

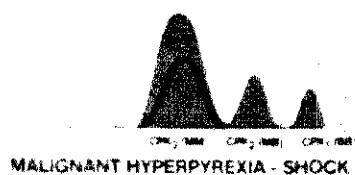
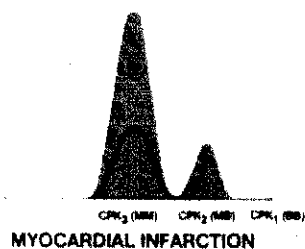
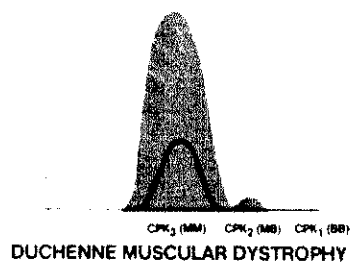
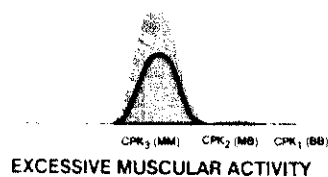
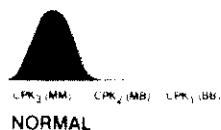


Fig.2--Characteristic electrophoretic patterns for CPK isoenzymes in normal and diseased states. (13, p. 2)

Functional studies concerning the role of CPK and its isoenzymes in metabolism have provided evidence that MM-CPK is the protein located in the M-line of the skeletal muscle myofibril (51,83,84). As suggested by Eppenberger et al (18), CPK isozymes may well turn out to be both structural proteins and enzymes.

In summary, CPK isoenzyme testing has become a valuable diagnostic tool for the detection of disease (Figure 2). CPK II has shown high specificity and sensitivity in the diagnosis of AMI. CPK III becomes valuable when used to rule out the presence of the two other isozymes in patients with increased total CPK activity. The clinical relevance of BB-CPK has yet to be established.

LDH isoenzymes.--Lactate dehydrogenase, a tetrameric protein, has been shown to exist in at least five isoenzyme combinations (each with a mass of 135,000 daltons (95)) composed of subunits designated 'H' for heart and 'M' for muscle. In general, tissues exhibiting aerobic metabolism have demonstrated faster-moving isoenzymes with more 'H' subunits, while the more anaerobic tissues demonstrated slower-moving isozymes with more 'M' type subunits (23). Distinctive physical properties, based on differences in amino acid content and catalytic properties (95), have been established for each LDH isoenzyme (95). Differences in substrate affinities as measured by Michaelis constants (K_m) for lactate and pyruvate, sensitivity

to heat denaturation, ability to utilize coenzyme analogues, affinity for the substrate analogue 2-oxobutyrate, and sensitivity to inhibitors such as urea and oxalate, have been incorporated in the methodology of diagnostic procedures (38,82,94,95). The five isoenzymes isolated from human serum have the following subunit compositions: LDH 1 (HHHH); LDH 2 (HHHM); LDH 3 (HHMM); LDH 4 (HMMM); and, LDH 5 (MMMM) (13). (A sixth isoenzyme, found in human testes and sperm also has the appearance of a tetramer, but has apparently been formed from a third, type "X", subunit (52).

Damage to only a small portion of tissue has generally been reflected in large elevations of serum LDH activity. This has been attributed to the fact that LDH activity is at least 100 times more concentrated in mammalian tissues than in the serum (88). When human tissue extract or sera is subjected to electrophoresis, the most anodic and fastest-moving fraction, LDH 1, migrated with a mobility similar to that of an alpha-one-globulin. LDH 5, the most cathodic and slowest-moving fraction, migrated similar to the gamma globulins. The three remaining hybrid fractions possessed intermediate mobilities (42). LDH 1 and 2 activity have shown predominance in the heart, kidney, brain, and erythrocytes. LDH 3 predominates in adrenal, thyroid, lymph node, pancreas, thymus, and spleen. LDH 4 and 5 predominate in liver and skeletal muscle (42,95). (The skeletal muscle patterns for LDH isozymes varied with the type of muscles.

The more aerobic muscles possessed a higher H/M ratio than the more anaerobic muscles (23,89,95).) Lung exhibits LDH activity evenly distributed among the five isozyme bands (95). The following distribution of LDH isozymes in human sera has been determined (26): LDH 1, 15-32 per cent; LDH 2, 25-44 per cent; LDH 3, 12-29 per cent; LDH 4 3-16 per cent; and, LDH 5, 3-16 per cent.

LDH isoenzyme testing has ubiquitous applications. It has become the most common isoenzyme analysis clinically utilized. Its significance has been shown in the diagnosis of: congestive heart failure, acute leukemia and lymphoma, malignant neoplasms, pernicious anemia, acute renal infarction, hepatic necrosis, acute myocardial infarction, skeletal muscle necrosis, inflammation or dystrophy (particularly of the Duchenne-type), shock with necrosis of various major organs, and brain necrosis (26,95) (Figure 3).

Unusual variants in LDH isozyme patterns have been reported in both normal and pathological sera of adults (3,48). In some instances, these variants were the result of complex formation between LDH and immunoglobulin (Ig)A or IgM (4). Others attributed this phenomena to a hereditary defect in the synthesis of one or other of the two subunits of the LDH molecule (8). Meany et al (48) reported a case in which a zone of LDH activity, having the same mobility as, but more diffuse, than LDH 4, was seen in an apparently healthy young woman. The presence of this variant zone was transient since

LDH ISOZYME ELECTROPHORETIC PATTERNS

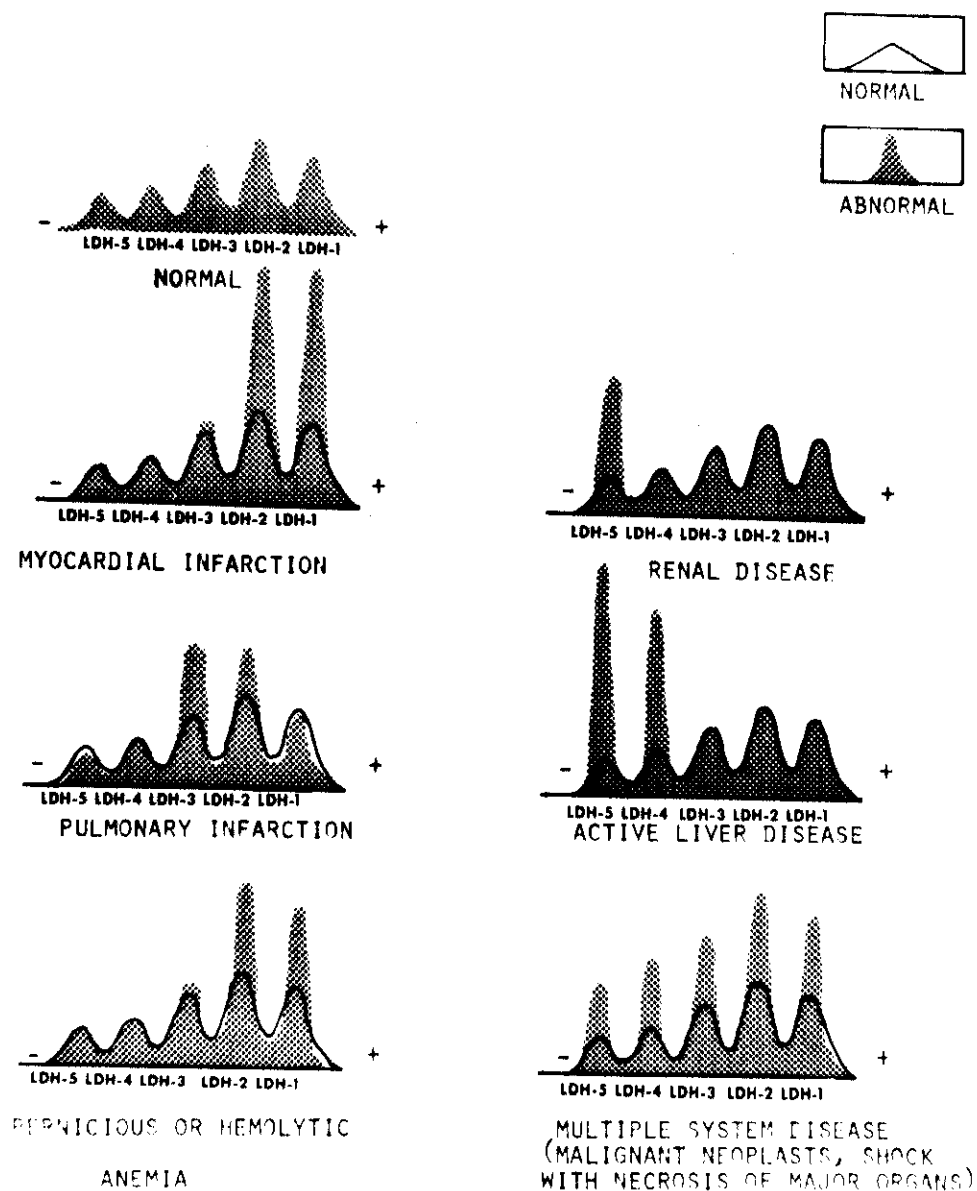


Fig.3--Characteristic electrophoretic patterns for LDH isoenzymes in normal and diseased states. (26, p. 10)

the isozyme pattern reverted to normal six weeks after it was noted. The significance of these observations has not been established.

One drawback of LDH isozyme determinations has been their lack of specificity for most disease conditions. Roberts and Sobel (64, p. 56) emphasized that: "Although profiles reflecting the predominance of one or more forms are relatively distinctive, all five LDH isoenzymes are present in most human tissues." For example, LDH 5 activity has been shown to rise early in certain liver diseases, such as hepatitis and carbon tetrachloride toxicity. However, this elevation was far from pathognomic of liver disease since acute skeletal muscle injuries or disease of skeletal muscle (e.g.: dermatomyositis) also have caused increased LDH 4 and 5 activity (82). Hence, additional testing has been required before the physician can make a definitive diagnosis following LDH isozyme testing.

According to Galen (23), the most important diagnostic parameter in interpretation of LDH isoenzyme data has been the relationship between isoenzyme fractions. Presence of the LDH 'one-two flip', indicated usually by a rise in total LDH activity, in which the ratio of LDH 1/LDH2 becomes greater than one, has been highly predictive for specific types of disease. For the diagnosis of AMI, LDH isoenzyme testing has demonstrated 90 per cent sensitivity and 95 per cent specificity (91). Detection of the 'one-two flip'

has only been reported following AMI, acute renal infarction, and hemolysis of blood caused by hemolytic anemia, prosthetic heart valves, or improper handling of blood samples following venipuncture (23).

Compared to CPK, the clearance rate of LDH from the serum following onset of an AMI has been much slower. LDH levels have typically risen twelve to twenty-four hours after chest pain, but remained elevated up to fourteen days later (64). CPK, on the other hand, has shown elevations within three hours after the infarct, and returned to normal serum CPK levels were resumed within thirty-six hours (64).

Serum and tissue LDH isoenzymes have also served as tools to investigate such normal processes as aging (27,89), sex differences (9), diurnal variations (35,80), genetic heterogeneity (87), ontogeny (47), and protein synthesis and degradation (22). Elucidation of these states in healthy subjects may eventually contribute to their application in medicine when these normal processes become disordered via disease (88).

In summary, LDH isoenzyme testing has ubiquitous applications in both the clinical setting and the research laboratory (Figure 4). Isozyme profiles have significantly narrowed the possible tissue sources of enzyme elevation, but have little diagnostic value by themselves. The most important parameter in interpretation of LDH isozyme data has been the relationship between the fractions. Presence

of the LDH 'one-two flip' has been highly predictive for disease.

The Cardiac Profile

History.--The Cardiac Profile, a serial pattern of serum enzyme alterations seen following acute myocardial infarction, has resulted from the combination of the highly specific and sensitive CPK and LDH isoenzymes analyses with the total enzyme determinations of CPK, LDH, and GOT. This profile has provided clinicians with a prompt, but highly accurate, means for detecting the occurrence of AMI (23). Utilization of serum total enzyme determinations for diagnosis of AMI was first reported by Karmen, Wroblewski, and LaDue (38), in 1955, when they characterized total LDH and total GOT alterations after infarction. Later, Dreyfus et al (17), in 1960, reported that elevations of total CPK were even more specific for AMI diagnosis. Wieme (94) and Wroblewski (82), in 1959 and 1960, introduced LDH isoenzyme testing as a means of improving the diagnostic specificity in AMI. In 1966, Van der Veen and Willebrands (85) presented CPK isoenzyme determinations as an even more specific and sensitive tool for AMI diagnosis. Serial analysis of the so-called 'cardiac enzymes' has become a routine procedure in current clinical medicine. The predictive value of the cardiac profile has been highest among patients suspected of sustaining an AMI whose chest pain was accompanied by equivocal electrocardiographic findings (92).

Characterization.--Following onset of acute myocardial infarction, total CPK activity elevates within three to six hours, reaches its peak within sixteen to twenty-four hours, and returns to normal levels within three to four days. Total LDH elevates within twelve to twenty-four hours, reaches maximal activity within three to six days, and resumes normal levels within ten to fourteen days. Total GOT elevates four to eight hours following infarction, peaks within eighteen to thirty-six hours, and returns to baseline within four days (64). Within a few hours after onset of infarction, LDH isoenzymes exhibit an increase in LDH 1 (92). Within twenty-four to forty-eight hours the characteristic LDH 'one-two flip' is apparent. (A substantial elevation of LDH 1 and 2, with LDH 1 almost as great as LDH 2, is also considered a positive pattern for AMI (13). CPK II is detectable in the sera within three hours after onset, exhibits peak activity within twelve hours, and usually disappears from the sera within thirty-six hours (92). Since CPK II activity never exceeds forty per cent of the total CPK activity, the sustained elevation of CPK activity thirty-six hours after the infarct is due to the continued presence of elevated CPK III activity (23) (Figure 4).

Diagnostic Implementation.--Clinicians routinely follow a three-sample drawing schedule for all admission Cardiac Profiles in order to: (1) (a) detect CPK II, as a result

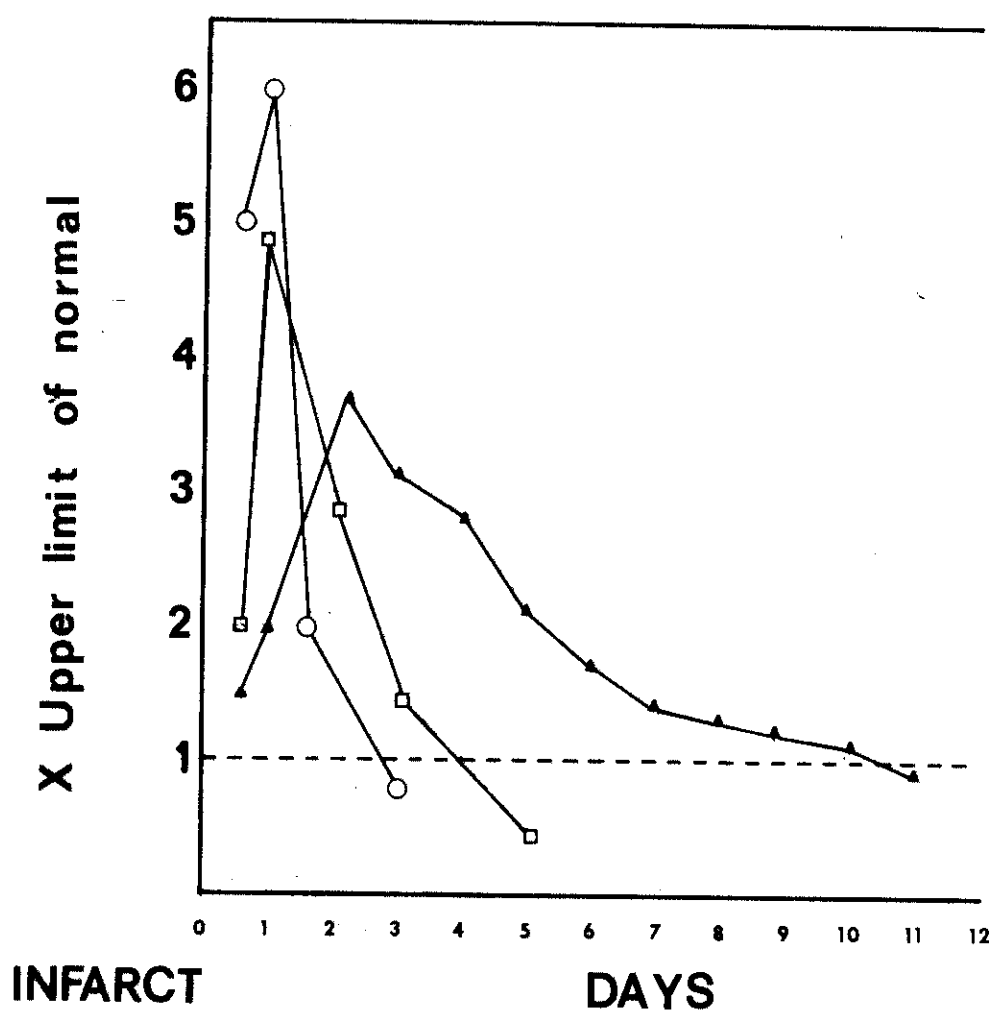


Fig. 4--Total enzyme activity of CPK (O), LDH (▲), and GOT (□) versus time following onset of acute myocardial infarction. (66, p. 23)

of AMI at or near its peak, (b) to gauge tissue damage, and (c) to note its subsequent reduction as an indication that the infarct was subsiding; (2) detect a positive LDH 'one-two flip' as confirmation of AMI; (3) detect a negative LDH 'one-two flip' in the presence of CPK II which indicates minimal cardiac damage; and/or (4) detect a positive LDH 'one-two flip' without positive CPK II, an indication that an infarct occurred more than twenty-four hours prior to admission (13). Accordingly; the first sample for Cardiac Profiling is drawn immediately upon admission; the second, six to thirteen hours after admission; and the third, twenty-four to thirty-seven hours post-admittance. The profile is discontinued after the third sample period unless (1) a new clinical episode is suspected, (2) reinfarction is indicated, or (3) a further aid for prognosis is desired. In these instances, a follow-up two to four-day profile is recommended (13).

According to Galen (23), total CPK determinations, when used to rule out AMI following onset of chest pain in patients with a high clinical index of suspicion, exhibit a sensitivity of ninety-six per cent, specificity of sixty-five per cent, and predictive value of seventy-five per cent. Total LDH determinations display a sensitivity of eighty-seven per cent, specificity of eighty-eight per cent, and predictive value of ninety per cent. Total GOT determinations demonstrate a sensitivity of eighty per cent, specificity of eighty per cent, and predictive value of eighty per cent. To be properly used,

the combined criteria of CPK and LDH isoenzyme analyses within the first forty-eight hours after MI must be assessed. Numerous studies (63,64,78) have shown CPK II to be a remarkably specific and sensitive index of myocardial damage with virtually 100 per cent diagnostic accuracy. Presence of the LDH 'one-two flip' has been positive for MI in sixty per cent of suspected cases at twenty-four hours, and eighty per cent of suspected cases at forty-eight hours (13).

In summary, alterations characteristic of the cardiac enzymes following an episode of acute myocardial infarction have been outlined. When used properly, cardiac profiling has been a key diagnostic aid for detection of the occurrence and extent of damage of the infarct. No other enzyme criteria have exhibited the high specificity, sensitivity, and predictive value obtained with the Cardiac Profile.

The Cardiac Enzymes and Exercise

Alteration of predictive value.--Exercise training, an adaptive process (33,70,98), has been shown to cause chronically elevated serum enzyme levels in both humans (57, 68) and animals (77). Consequently, the upper limit of normal enzyme activities for the members of the non-disease population who regularly exercise frequently exceeds that of the non-exercising members (69,77,86). The predictive value of enzyme determinations for distinguishing between the disease and non-disease populations becomes altered when

such upward shifts in enzyme distribution occur (23). In the clinical situation, elevated enzyme determinations following exercise may be falsely interpreted as positive indicators of disease (23). Analysis of the enzyme response to exercise becomes valuable to the clinician who wishes to avoid such diagnostic errors.

The training effect.--Physical conditioning has been shown to produce a "training effect" which results in a reduction of the degree of elevation of enzyme levels following exercise stress (1,56,59,96). Hollman et al (32), Schmidt and Schmidt (71), and Schwartz et al (74) have recently shown that the "training effect" may be related to the training of specific muscle groups rather than to general cardiovascular conditioning. According to Holloszy and Booth (33), and Yakovlev (98), skeletal muscle adaptation to exercise results in: (1) hypertrophy of the muscle cells; (2) enhancement of aerobic capacity; and, (3) increased anaerobic capacity. Assessment of the enzyme response to exercise in terms of different stages of adaptation would help researchers establish more definitive criteria for the "training effect".

Current research.--Recent studies have revealed elevations of the cardiac enzymes following physical exertion in humans and animals. Most of these studies reported alterations of total levels of the cardiac enzymes only. Concomitant

isoenzyme changes were rarely reported. The vast majority of these studies had one major pitfall--blood sampling was carried out for only a short time after exercise. (In most cases, sixty minutes post-exertion was the final sampling time). While there was some confusion with respect to patterns seen in studies limited to immediate post-exercise analyses, the few experiments that monitored serum enzyme levels for an extended period (greater than twenty-four hours post-exercise) consistently agreed that: (a) abnormally high elevations of the cardiac enzymes were evident (5,39,40, 57,61,72); (b) long-range monitoring before and after exercise was critical to observe the peak activities of total CPK, LDH, and GOT (1,20,25,39,53,57,61,68); (c) the time sequence of enzyme elevations was similar to that following AMI (39, 57,61,68); and (d) the degree and extent of increase in enzyme activity was dependent on the physical condition of the individual (1,25,96) and the type (14,43,70), duration (56,59,67), frequency (20,59) and intensity (75,59,67,70) of exercise performed. The clinical implications of this research are, presently, unknown. However, these studies suggest that the sensitivity, specificity, and predictive values of cardiac profiling may be impaired in the case of suspected cardiac patients who had recently engaged in exercise.

Rationale for this study.--To date, no attempt has been made to detail the complete seventy-two hour cardiac profile

following exercise stress in either humans or animals. Such a study would be of interest to diagnosticians who wish to better define the usefulness of the cardiac profile as a clinical indicator of disease. It would also provide further information regarding the enzyme response to exercise stress, a topic of concern to physicians currently involved in sports medicine. For the above reasons, the following investigation was undertaken.

Development of an experimental design for this study incorporated the following premises: (1) AMI is an acute, intensive form of pathological stress. A parallel form of physiological stress should entail an acute, intensive bout of physical exertion; and, (2) physical conditioning has been shown to reduce the enzyme response to exercise stress. Enzyme elevations would be of greater significance in trained, rather than untrained, individuals.

Similar studies.--King et al (39) have performed the only study, to date, which monitored the cardiac enzymes for a prolonged period following an acute, intense bout of exercise in humans. Following a one-hour game of handball, peak activities of total CPK, LDH, and GOT were noted at intervals similar to those seen after onset of an AMI. When comparing enzyme results between the experimental subjects and post-MI patients, it was observed that: (1) the rate of disappearance of CPK from the sera was slower

in the exercise subjects; and (2) the per cent increase of CPK activity after a short burst of exercise was generally not as great as that occurring after myocardial damage. Unfortunately, no isoenzyme were performed in this experiment.

Very few reports relating the changes of either CPK and/or LDH isoenzymes following exercise have appeared in the literature to date. None of the studies mentioned monitored enzyme levels for more than 120 minutes (7) post-exercise. LDH isoenzyme analyses in rats (6,16,25,56,59) have shown significant elevations of LDH 1, 2, and 5 following exhaustive swimming exercise in untrained animals, while the trained rats exhibited elevated LDH 5 activity only. Inconclusive results were obtained from the human LDH isozyme studies (5,67,74,96) due to wide variability between experimental conditions. However, these experiments consistently showed that physical conditioning causes alterations in the isoenzyme response to exercise. At this writing only two reports of CPK isoenzyme analyses following exercise have been found in the literature. Anderson (2) reported that elevated CPK activity in horses following exercise stress was due solely to increased CPK III activity. Klosak and Penney (40) reported decreased organ CPK activity in kidney, liver, lung, and brain following swimming exercise in rats. They suggested the forementioned organs to be possible sites

of enzyme release following strenuous exercise, rather than heart of skeletal muscle. Thus, although the tissue source of enzyme release following strenuous exercise had, logically, been assumed to be of muscular origin, this research indicates that other tissues (e.g., liver, kidney, lung, and brain) may also be possible sources of enzyme release. The discrepancies between these few studies suggest that cardiac isoenzyme profiling may extend into the research lab as a useful tool in determining the tissue origin of enzyme release after exercise.

Statement of the Problem

The purpose of this investigation was to detail the complete cardiac profile for seventy-two hours following an acute, intense bout of strenuous exercise in well-conditioned humans. Vigorous exercise, even in trained individuals, produces alterations in serum enzyme levels. Knowledge of the degree and extent of these changes on the cardiac profile, under closely designed experimental conditions, would better define the usefulness of the cardiac profile as a clinical indicator of disease and further the understanding of the enzyme response to exercise.

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CHAPTER II

METHODS AND MATERIALS

Five adult male volunteers, chronic joggers ranging in age from twenty-seven to thirty-six, served as subjects for this experiment. Anthropometric and stress test data (Table I) indicate the subjects were lean, middle-aged males in excellent cardiovascular condition. These data were obtained prior to the study following treadmill testing (Bruce Stress Test Protocol (13)) and per cent body fat determinations (combined skin-fold and underwater-weighing techniques (21)) performed by the staff at the Institute for Aerobics Research, 11811 Preston Road, Dallas, Texas 75230.

Each individual served as his own control for this eight-day experiment. All vigorous exercise was discontinued for the duration of the study (aside from that required on day 5). Twelve hour fasting blood samples were drawn via the antecubital vein using the vacutainer blood collection system on each of four consecutive mornings following cessation of exercise to establish baseline blood chemistry values. On the fifth day, blood specimens were collected one hour before and one hour after completion of a prolonged, near-maximal intensity run of six to ten miles, with an average time of eight minutes per mile. Subsequent blood sampling was performed at eight-

hour intervals for the remaining three days. All post-exercise blood specimens were drawn at least two hours post-prandial. Subjects were requested to maintain their normal diets, but abstain from smoking and intake of alcoholic beverages for the duration of the study. Lifestyles were routinely maintained except for the ingestion of stimulants/depressants and the withholding of vigorous exercise.

Following venipuncture, blood clotting and clot retraction were allowed to occur for 20-30 minutes at room temperature. After centrifugation for 15 minutes at 3500 rpm the serum was immediately isolated from the clot and stored in glass test tubes according to specifications for each blood analysis. (See below). All testing was performed within 24 hours after specimen collection.

Twenty-two blood chemistry parameters were obtained by subjecting sera to automated analysis on the Technicon SMAC (sequential Multiple Analysis--Computerized) operated by Ford, Lynn Medical Laboratories, Inc., of Denton, Texas 76201. Sera for this analysis was stored at room temperature for no longer than 24 hours (12). For this study only the cardiac enzyme determinations were reported. Enzyme activity was reported in International Units/liter (IU/l). The Technicon total CPK determination, Method No. SG4-0017PC6, was based on a modification of the automated procedure of Siegel and Cohen (17). According to this assay method, no significant deviation

in linear response occurs if the total CPK activity ranged from 0-1200 IU/l. The procedure for LDH determinations, Technicon Method No. SG4-0021PC6, was based on the method of Wacker et al (19), automated by Morgenstern et al (10), and modified by Morgenstern et al (11). There was no significant deviation in linear response when total LDH activity ranged from 60-600 IU/l. Technicon Method No. SG 4-0010PC6 for total GOT determinations was based on the manual method of Henry et al (5), automated by Kessler et al (8), and modified by Kessler et al (7). There was no significant deviation in linear response when total GOT activity ranged from 20-300 IU/l. These samples were automatically reanalyzed following sample dilution.

Simultaneous serum CPK and LDH isoenzyme separation was performed electrophoretically on cellulose acetate gels via methods and materials designed by Helena Laboratories, Inc., P.O. Box 752, Beaumont, Texas 77704. This was the most sensitive and specific technique commercially available to clinical laboratories at the time of this study (15). Sera for CPK isoenzymes was stored immediately after separation from the clot at -20°C (2,6). Sera for LDH isoenzyme analysis was stored at room temperature (2,4).

According to this procedure, sera was applied to cellulose acetate plates (60 x 45 mm), placed in a Helena Electrophoresis Chamber (catalog no. 1283) and run for 9 minutes at 350 volts

(5 watts/plate), at a room temperature, in Tris-Barbital buffer, (pH = 8.8, ionic strength = 0.009). These plates were subsequently sandwiched to the corresponding substrate-soaked plates and incubated at 40°C. Sandwiched LDH plates were incubated for 20 minutes, and then immediately scanned manually on the Helena Auto Scanner Flur-Vis on the visible mode using the 570 nm light filter. (The Helena LDH reagent, catalog no. 5909, contained a tetrazolium salt which was reduced during incubation and formed a colored formazan dye. This dye was detected spectrophotometrically with the densitometer.) Sandwiched CPK plates were incubated for 40 minutes, separated from the substrate plates, dried for 5-10 minutes at 55°C, allowed to cool for 20 minutes, and manually scanned on the fluorescent mode of the Flur-Vis. (The Helena CPK reagent, catalog no. 5130, contained the necessary reagents to cause a coupled reaction that, when in the presence of CPK, resulted in the reduction of NAD to NADH. The fluorescence of NADH, directly proportional to CPK activity, was measured fluorometrically.) Integration of isograms in terms of per cent distribution of isozymal bands was performed with the Helena Quick Quant II. Activities of each isoenzyme were quantitated by multiplying the per cent of isozyme distribution by the total enzyme activity.

Inaccurate quantitation of CPK isoenzyme activity has been reported in cases when: (1) the total CPK activity of

the serum is greater than 500 IU/l (3); (2) the serum is diluted prior to electrophoresis (1); or, (3) if excess substrate is not applied to the cellulose acetate plate prior to incubation (16). Thus, appropriate controls were applied to check the accuracy of the isoenzyme determinations. The per cent distribution of isoenzymes of LDH and CPK were not affected by 1:8 dilutions of samples containing total enzyme concentrations of 200, 600, 1700, and 1850 IU/l of CPK, and 200, 250, and 400 IU/l of LDH. The enzyme activities of all the specimens in this investigation were below the upper limit of control values. In addition, Helena's CPK Control, catalog no. 5131, containing predetermined quantities of luophylized CPK I, II, and III from rabbits, was used as a control sample each time a batch of CPK and/or LDH isoenzyme determinations were performed.

Since acute exercise has been shown to cause shifts in the plasma volume, which causes hemoconcentration (18), all total enzyme values post-exercise were corrected for plasma volume shifts by the protein method of King et al (9). (Total protein determinations were included in the SMAC analysis.)

Blood chemistry data served as dependent variables in the experimental design which required a one-way analysis of variance with repeated measures per enzyme test for each subject (14, 20). Statistically significant variations in post-exercise blood chemistry values with respect to baseline (pre-exercise) values were determined with the Duncan's

Multiple Range Test. Data was programmed into the IBM Model 360 Computer System at North Texas State University and all statistical comparisons were tested at the five per cent level of significance.

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CHAPTER III

RESULTS

Significant differences ($p < 0.05$) in all total enzyme activities were seen during the post-exercise enzyme assessments (Table II). Concomitant isoenzyme determinations exhibited statistically insignificant alterations ($p < 0.05$) although the qualitative changes were pertinent to this study (Table III). *Subsequent multiple comparisons of each time period with baseline values revealed that statistically significant differences ($p < 0.05$) were evidenced during the early time periods while no differences were seen during later time periods.

Figure 5 shows the effects of exercise on total CPK activity in each subject. The mean baseline level of total CPK was 119.8 ± 51.0 IU/l. Significant elevations ($p < 0.05$) were evidenced at eight hours and remained elevated for seventy-two hours post-exercise. The peak activity in total CPK, of 599.0 ± 574.81 IU/l, was seen at sixteen hours post-exercise. The large standard deviation seen in the post-exercise values was attributable to subject #5 who demonstrated a maximal CPK value of 1606 IU/l at the sixteen hour sampling time.

*(Due to circumstances beyond our control, no isoenzyme analyses were performed for subject #3.)

TABLE II

DESCRIPTIVE STATISTICAL VALUES FOR TOTAL ENZYME ACTIVITY
BEFORE AND AFTER NEAR-MAXIMAL INTENSITY EXERCISE

Time (Hours)	0	1	8	16	24
Total	119.80	202.20	437.20	599.00	581.60
CPK	± 50.91	± 98.28	± 303.65	± 574.81	± 524.02
Total	177.40	236.00	241.20	228.80	230.40
LDH	± 16.68	± 24.58	± 21.74	± 27.03	± 40.02
Total	29.00	35.60	35.80	38.40	43.20
GOT	± 4.36	± 13.28	± 8.50	± 12.97	± 16.42*

Enzyme activity reported in International Units/liter (IU/l)

*Statistical significance at 0.05 level when comparing indicated enzyme values with respective baseline values.

TABLE II--Continued

32	40	48	56	64	72
472.20	380.00	389.80	346.80	303.60	243.20
±	±	±	±	±	±
412.18	332.24*	360.56*	312.60	248.63	171.97
214.80	213.40	207.40	205.40	198.00	209.20
±	±	±	±	±	±
36.38*	38.87*	36.00*	38.54*	38.68	49.23*
39.40	33.80	37.20	32.80	29.40	30.40
±	±	±	±	±	±
14.87	18.89	12.83	13.44	11.41	10.74

TABLE III

DESCRIPTIVE STATISTICAL VALUES FOR CPK AND LDH ISOENZYMES
BEFORE AND AFTER NEAR-MAXIMAL INTENSITY EXERCISE

Time (Hr)	0	I	8	16	24	32	40	48	56	64	72
CPK ₂	10.25 ± 3.40	8.00 ± 4.40	4.50 ± 4.04	10.50 ± 4.65	11.00 ± 3.37	9.50 ± 3.42	7.50 ± 5.26	7.50 ± 5.07	7.25 ± 3.30	7.25 ± 3.20	7.75 ± 4.03
CPK ₃	89.75 ± 3.40	92.00 ± 4.40	95.50 ± 4.04	89.50 ± 4.65	89.00 ± 3.37	90.50 ± 3.42	92.50 ± 5.26	92.50 ± 5.07	92.75 ± 3.30	92.75 ± 3.20	92.25 ± 4.03
LDH ₁	28.75 ± 2.21	26.00 ± 4.24	27.75 ± 1.71	27.50 ± 3.11	27.50 ± 3.11	27.00 ± 2.71	26.50 ± 2.65	28.25 ± 0.50	28.50 ± 0.58	29.00 ± 2.16	29.25 ± 1.26
LDH ₂	33.75 ± 2.87	29.75 ± 2.75	26.00 ± 3.74	29.00 ± 2.94	31.25 ± 0.96	32.50 ± 1.29	34.25 ± 2.99	32.25 ± 1.26	32.00 ± 0.00	33.00 ± 1.41	32.75 ± 0.96
LDH ₃	27.75 ± 1.89	25.50 ± 0.58	28.50 ± 2.08	28.25 ± 1.71	25.25 ± 1.26	26.50 ± 0.58	28.75 ± 1.26	28.25 ± 0.50	28.25 ± 0.50	27.25 ± 0.50	26.75 ± 1.26
LDH ₄	5.50 ± 2.38	9.50 ± 2.38	11.00 ± 3.65	9.00 ± 1.83	8.00 ± 2.00	7.50 ± 1.73	7.00 ± 2.00	7.50 ± 1.73	7.50 ± 1.29	7.50 ± 0.58	7.50 ± 0.58
LDH ₅	4.25 ± 1.71	9.50 ± 4.80	6.50 ± 1.73	5.50 ± 2.08	8.00 ± 2.16	5.75 ± 1.50	3.50 ± 0.58	3.25 ± 0.50	3.25 ± 0.58	3.50 ± 1.29	4.25 ± 1.26

Isoenzyme activities expressed as percentage of total enzyme activity

No statistical significance found at 0.05 level when comparing indicated isozyme values with respective baseline value

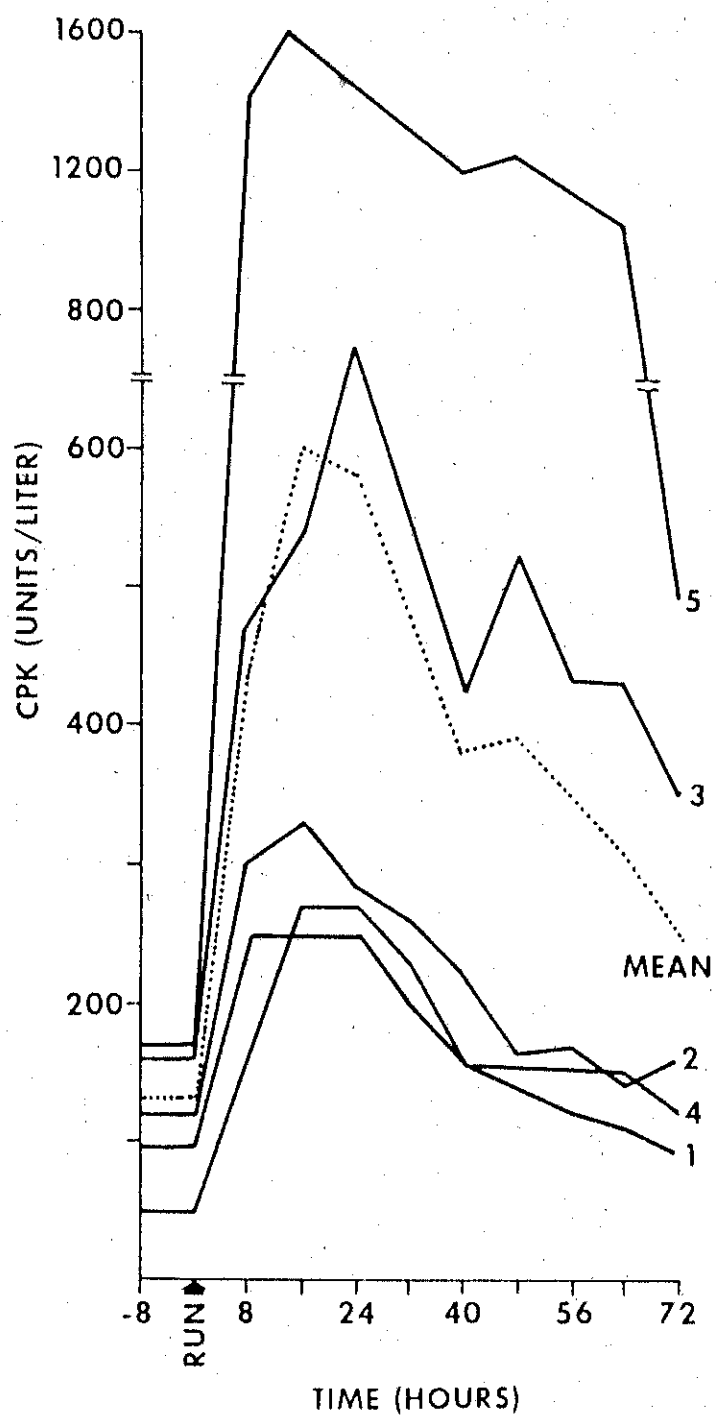


Fig.5--Total CPK activity versus time before and after near-maximal intensity exercise.

Figure 6 shows the effects of exercise on total LDH activity in each subject. The mean baseline level of total LDH was 177.4 ± 16.68 IU/l. Significant elevations ($p < 0.05$) were evidenced within one hour following exercise, and remained significantly elevated until twenty-four hours after the run. The peak activity in total LDH, 241.0 ± 21.74 IU/l, was seen eight hours following the maximal run. Peak LDH activity was not likely due to sample hemolysis. Hemolysis in several samples was apparent in all sera at the one hour sampling time only.

Figure 7 shows a summary of the effects of exercise on the mean total enzyme activity of CPK, LDH, and GOT. The mean baseline level of GOT was 29.0 ± 4.36 IU/l. The only significant ($p < 0.05$) elevation was found in the twenty-four hour sample (43.2 ± 16.42 IU/l). The rise in GOT during this time period, although statistically significant, is not viewed as physiologically significant. Clearly, exercise causes significantly greater increases in total serum CPK activity than in either LDH or GOT.

Figure 8 shows the effects of exercise on the isozyme pattern of CPK at a time of peak total CPK activity compared to the pre-exercise isozyme pattern. There was no clinically significant elevation seen in the MB, or cardiac type of CPK isozyme (CPK II). Hence the MM, or muscle type of CPK isozyme (CPK III) accounted for the qualitative increase in total CPK activity in each subject tested.

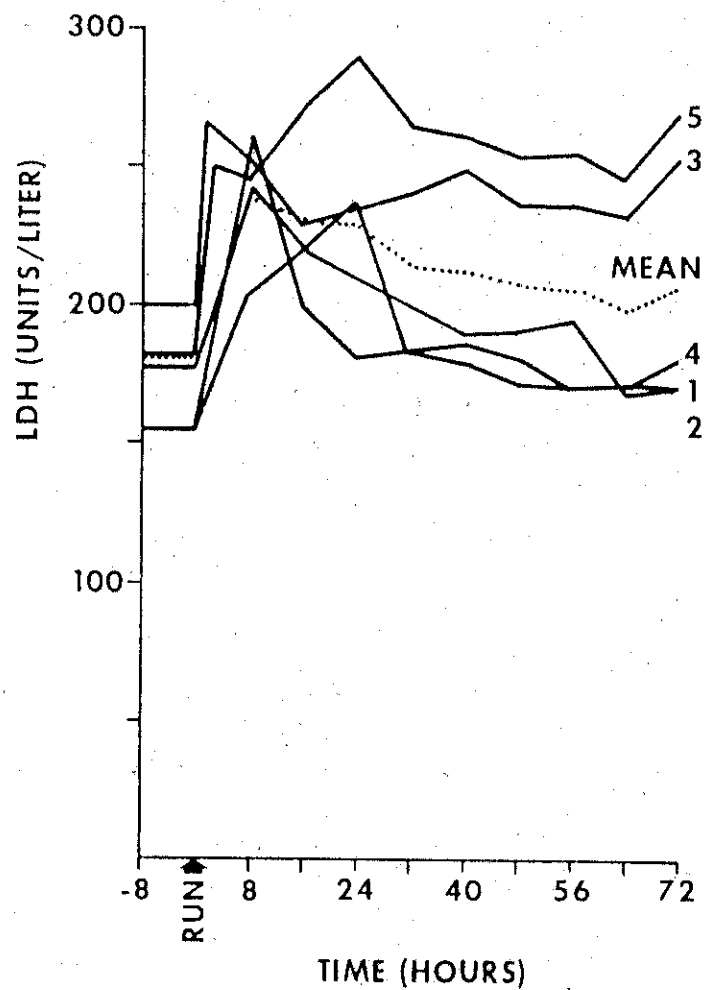


Fig. 6--Total LDH activity versus time before and after near-maximal intensity exercise.

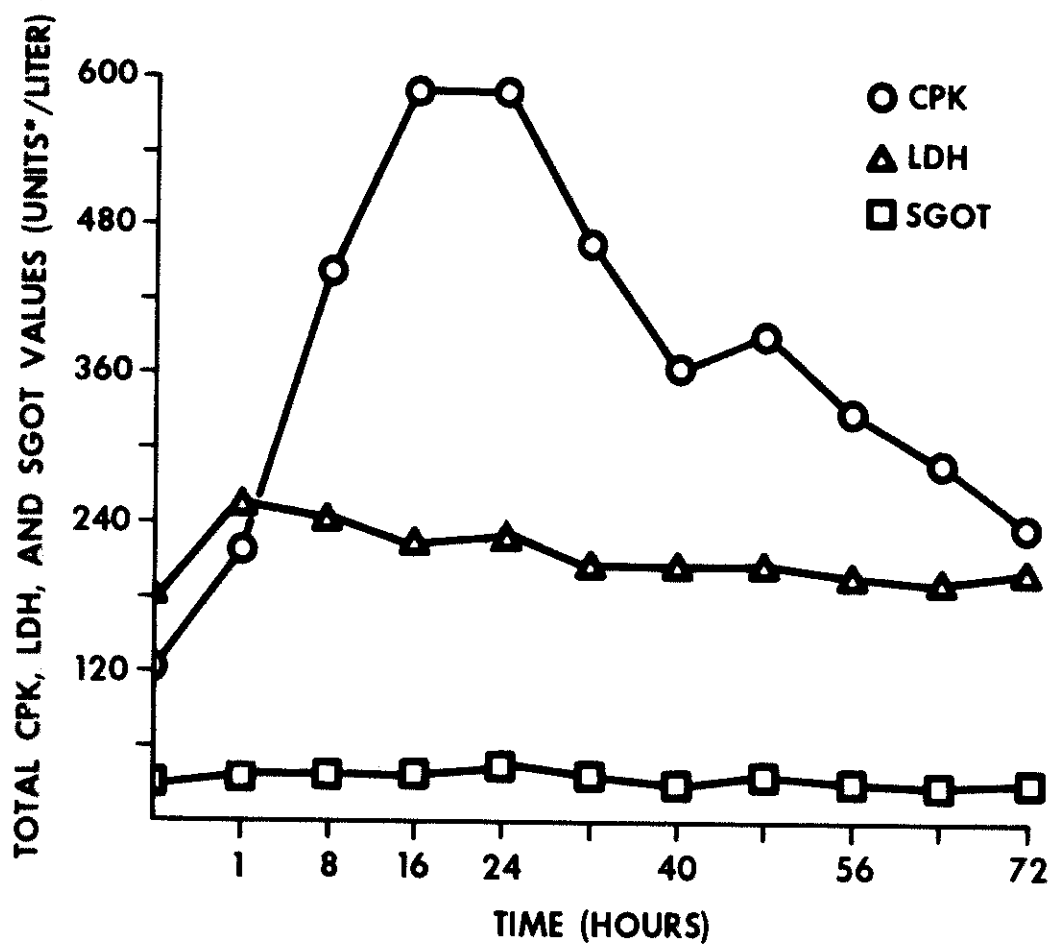


Fig. 7--Mean total CPK, LDH, and GOT activity versus time before and after near-maximal intensity exercise.

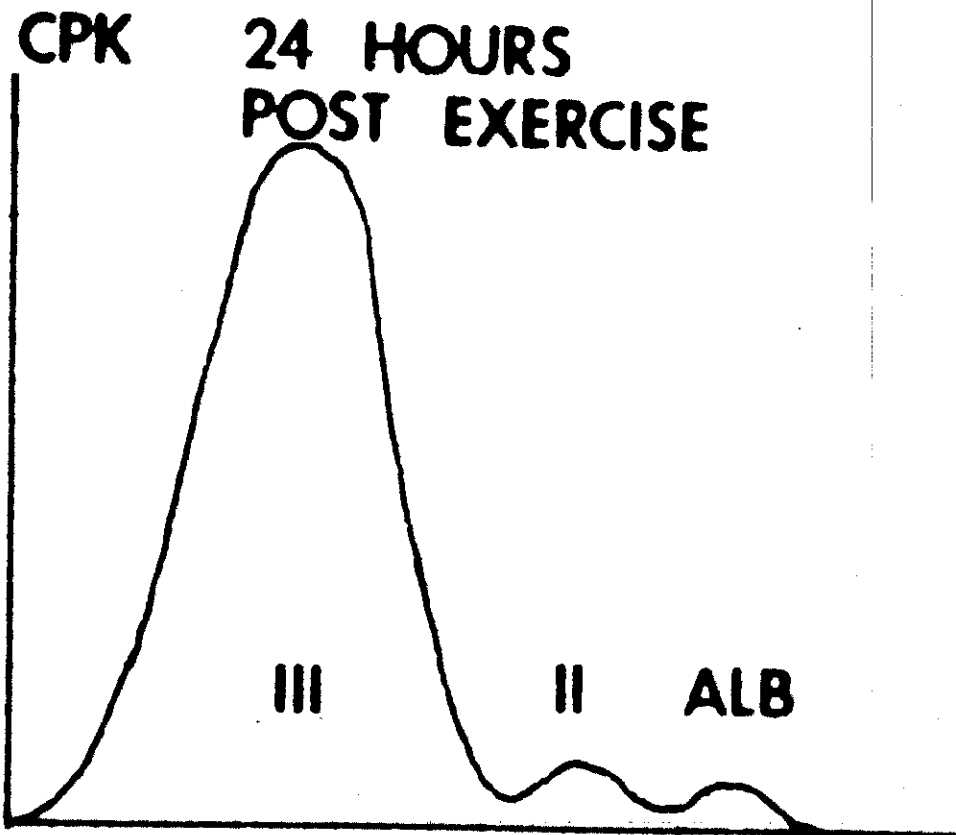
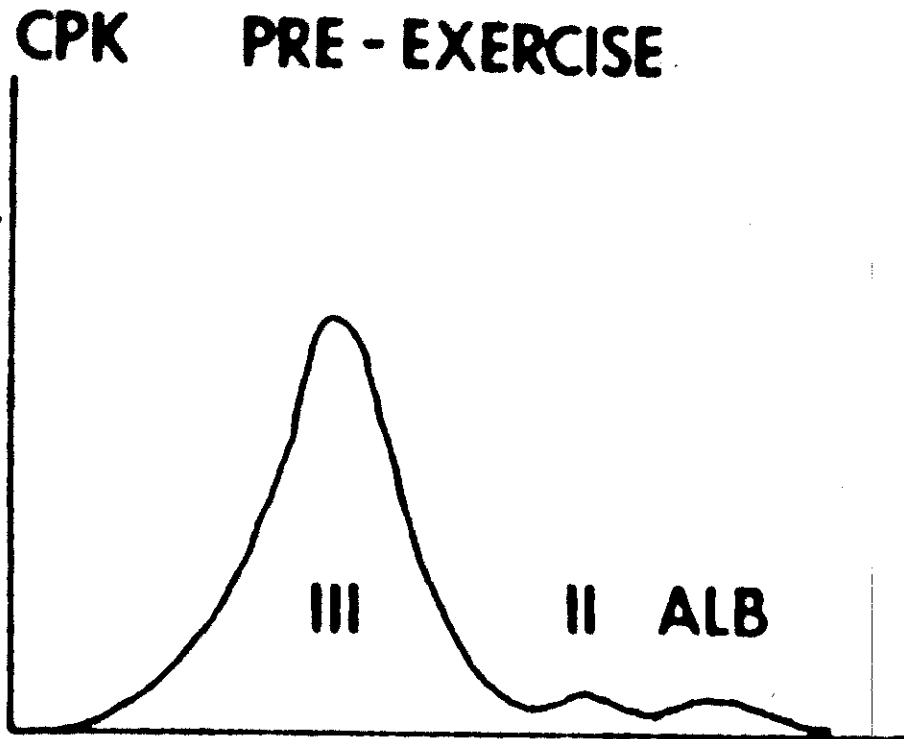


Fig. 8--Representative isoenzyme patterns for CPK activity before and twenty-four hours post-exercise.

Figure 9 shows the effects of exercise on the isozyme patterns of LDH, at the time of maximum total LDH activity (eight hours), compared to the pre-exercise isozyme pattern. Although total LDH activity showed a lower absolute increase than did total CPK, the elevations observed were reflected in the relative increase in per cent distribution of LDH 1. Hence, at eight hours, an LDH 'one-two flip' was seen.

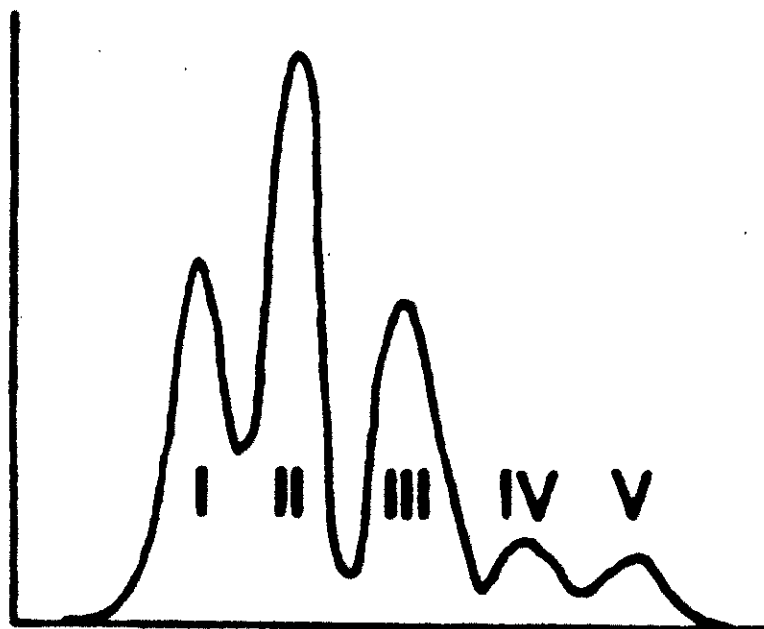
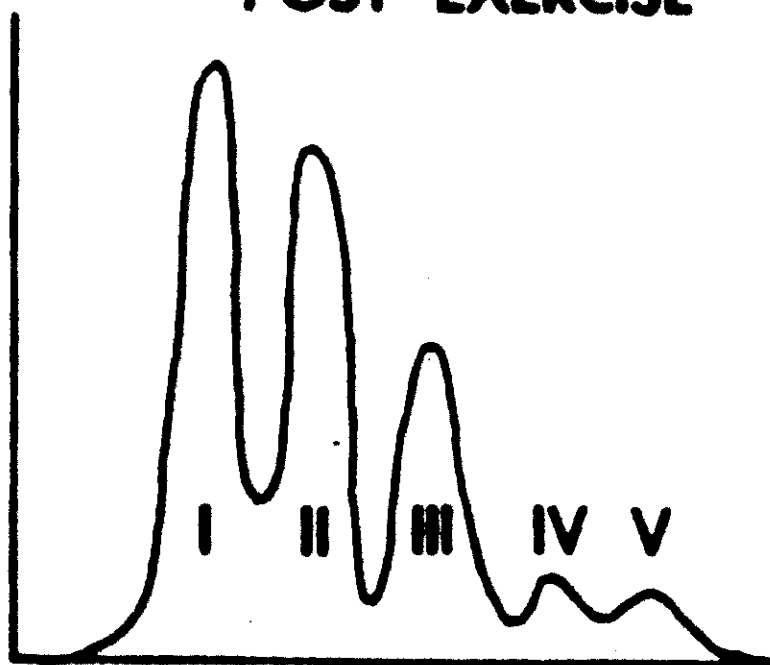
LDH PRE-EXERCISE**LDH 8 HOURS POST EXERCISE**

Fig. 9--Representative isoenzyme patterns for LDH activity before and eight hours post-exercise.

CHAPTER IV

CONCLUSION

Introduction

The study presented here details the effects of near-max exercise on selected serum enzymes in a group of fit, healthy volunteers. An acute bout of high intensity running exercise in trained individuals produces a change in the magnitude and sequence of serum enzyme release quite different from the enzyme pattern seen following myocardial infarction. Hence, the results generate an "Exercise Profile" which is clearly distinct from the "Cardiac Profile". (Table IV)

Both AMI and exercise cause an abnormally high amount of CPK release into the serum. Maximal total CPK activity occurs sixteen to twenty-four hours following either event. While AMI produces elevations in the activity of the CPK-MB isozyme, no evidence of such an elevation is seen after exercise.

Significant elevations in serum LDH also occur subsequent to AMI and exercise stress. Concurrent with maximum rise in total enzyme activity, an LDH 'one-two flip' is seen eight hours after exercise and forty-eight hours following infarction. Thus, maximum activity in total LDH following exercise is largely due to the increase in LDH 1, the cardiac form of

TABLE IV
SUMMARY OF RESULTS:
THE CARDIAC PROFILE VERSUS THE EXERCISE PROFILE

	CPK		LDH	
	Max Activity Post Event	Presence of MB-CPK	Max. Activity Post Event	1-2 Flip
Cardiac Profile	24 hours	Yes	48 hours	Yes
Exercise Profile	24 hours	No	8 hours	Yes

the isozyme. This pattern is similar to that seen post-AMI. However, unlike the Cardiac Profile, the total LDH activity in the Exercise Profile peaks forty hours sooner.

In contrast to CPK and LDH, the third cardiac enzyme, GOT, shows no abnormally high elevations anytime following exercise stress. A small, but clinically insignificant, peak in GOT activity is seen at the twenty-four hour post-exercise interval. Monitoring of GOT activity is of significance only to the Cardiac Profile.

Discussion of Results

The participants of the present study are considered to be well-trained individuals, yet near-maximum exercise still produces serum levels of the cardiac enzymes far above that considered normal. These results are in agreement with many workers who report a general increase in serum enzymes following running exercise of submaximal (5) and near-maximal (10,13) intensities. Physical conditioning results in a significant reduction in the degree of enzyme elevations post-exercise (17). Thus, total enzyme levels may be elevated to greater than normal levels even in trained individuals who participate in intensive running efforts.

King et al (11), recorded increases of serum enzymes following exercise stress in patterns similar to those following an AMI. Maximal CPK activity was reported at thirteen hours, LDH activity at ten hours, and GOT activity

at seven hours post-exercise. These results agree with the present study in that: (1) Maximal LDH activity was seen sooner than maximal CPK activity; (2) CPK activity exhibited the greatest increases above baseline levels than the other two enzymes, and: (3) GOT activity, although exhibiting slight elevations in both cases, showed no clinically or statistically significant changes.

In agreement with Anderson (1), the present study attributes the rise in total CPK activity solely to an increase in the muscle fraction (III) of CPK. The absence of cardiac CPK (II) is not surprising since there were no clinical signs of myocardial ischemia in any of these individuals (all subjects showed negative stress ECG's). Similarly, neither Anderson (1) nor Klosak and Penny (12) saw increases in the cardiac fraction (II) of CPK following exertion in horses or rats, respectively. Apparently, myocardial CPK is not affected by exercise stress. Thus, the absence of the cardiac fraction of CPK after exertion renders isozymal profiling a more significant diagnostic modality when distinguishing the Cardiac Profile from the Exercise Profile.

For the LDH isozyme patterns, no statistically significant increases ($p < 0.05$) in any one of the five isozymes is noted in this study. The only comparable studies to date, performed in rats, report significant elevations of LDH 5 following swimming exercise to exhaustion in trained animals

(2,4). This evidence further supports the suggestion made by several workers that the type duration, and intensity of exercise performed play a large part in determining the extent of LDH release from skeletal muscle.

Although not recognized as such, two other incidents of LDH 'one-two flips' after exertion have been recorded by Schwartz et al (15) and Doty et al (4). Schwartz recorded the LDH 'one-two flip' thirty minutes following bicycle ergometry at a high workload (720 kpm/min/30min) in sera of untrained human volunteers. Doty monitored plasma and tissue LDH changes following swimming exercise in untrained rats. The plasma 'one-two flip' was seen two hours after exertion. LDH activity in skeletal muscle and myocardial homogenates significantly decreased ($p < 0.05$) one hour post-exercise. Likewise, these 'flips' were both recorded at times when total LDH activity was greater than normal. It is questionable whether a valid comparison can be made between these cases and the present study since both groups of workers used untrained subjects in relatively short-term studies.

The presence of an LDH 'one-two flip' in the sera is a highly reliable criteria currently used for diagnosing the occurrence and extent of an AMI. To date, this study is the first to note the appearance of the 'flip' in sera of non-diseased individuals following exercise stress in which no hemolysis was apparent. Thus, further studies of similar, yet more specific, nature must be performed in order to better

define the usefulness of this isoenzyme phenomenon as a clinical indicator of disease.

Therefore, it may be stated that an acute, intense bout of running exercise in trained individuals manifests itself in an increase of specific serum enzyme activities. Three basic questions arise from the observations presented here: (1) Why was an LDH 'one-two flip' seen in the sera of a non-diseased exercise population? (2) What accounts for the differences between the Cardiac and the Exercise Profiles? (3) What are the clinical implications from this study?

In attempting to answer the first question, one must consider both the origin of LDH 1 and 2 release and the situations in which the 'one-two flip' commonly occurs. LDH 1 and 2 originate from the heart, brain, kidney, and erythrocytes (16). The appearance of an LDH 'one-two flip' in the sera may reflect: (a) a hemoconcentration effect; (b) one of the clinical disorders with which the flip is distinctly associated; or, (c) characteristic clearance rates of specific isozymes from the tissue or plasma.

Hemoconcentration is a common post-exercise occurrence in which the concentration of serum enzymes is elevated due to a decrease in plasma volume. The data for this study was corrected for plasma volume shifts using the protein method of King et al (11). Hence, the possibility of hemoconcentration as a cause for the observed 'flip' is ruled out.

In terms of diagnostic significance, the 'flip' is associated with cases of pernicious anemia, AMI, acute renal damage, and RBC hemolysis. AMI and pernicious anemia are ruled out since no further clinical manifestations of these disorders were seen.

Acute renal damage is possible, but unlikely, since the intensity of the run (a run of six to ten miles was performed; average time was 7.5 min/mi; $\text{VO}_{2\text{max}}$ averaged 50.5 ml/kg/min) could have produced an acute hypertension. Neither blood pressure monitoring nor urine analyses were performed at any time. None of the participants reported abnormalities in kidney functioning post-exercise. Thus, evidence that would support this theory (hematuria, oliguria, hypertension) is lacking.

RBC hemolysis, another frequent post-exercise phenomenon, was apparent only at the one-hour post-exercise sampling time. The LDH 'one-two flip' was seen at the eight hour post-exercise interval. Other blood chemistry parameters monitored via SMAC analyses (e.g., bilirubin, total protein, uric acid), do not indicate the presence of serum hemolysis at the time the 'flip' was seen in the sera. Thus, hemolysis does not account for the observed 'flip'.

At the present time, the most feasible explanation for the observed 'flip' is the existence of a characteristic clearance rate from the sera for each of the LDH isozymes.

As reviewed by Hess et al (8), the disappearance rates of enzymes generally resemble those of other proteins in following a bi-phasic exponential course. The first phase probably represents the distribution of the enzyme between the intravascular and extravascular compartments. The second phase is mainly due to the actual removal or inactivation of the enzyme and is a measure of its turnover rate. Although the disappearance of injected LDH isoenzymes has not been studied in man, Boyd (3) has performed this work in lambs. The half-lives of the two phases of disappearance of LDH activity from plasma in lambs is 2.0 hours and 8.0 hours for LDH 5 and 2.4 hours and 48.0 hours for LDH 1. These results indicate that, in order to maintain the same level in plasma, the LDH 5 isozyme must leak from tissues at a rate either 7.5 or 15 times greater than that of LDH 1.

If one assumes that exercise stress primarily involves skeletal muscle, then the supposition that LDH 5 clears the sera more quickly than LDH 1 in man seems a contradiction to the fact that an LDH 'one-two flip' occurred at the time of maximal LDH activity (eight hours) in the Exercise profile. However, the possibility exists that the heart may have been affected more by the stress of the exercise than the skeletal muscle in terms of increased LDH metabolism. Since the heart operates almost exclusively on aerobic metabolism, exercise stress may simply increase the production of pyruvate via the LDH reaction. Evidence to further substantiate this

theory is seen in the work of Gollnick et al (6) who recorded increased LDH activity in heart muscle of trained rats after swimming, whereas no change in skeletal muscle LDH was seen. In addition, Doty et al (4) reported significant declines in LDH activity in homogenates of skeletal muscle, myocardial and liver tissues within two to six hours following swimming exercise in untrained rats. As yet, the organ source(s) of enzyme release following exercise stress has not been established. However, the presence of the LDH 'one-two flip' in future studies may prove useful in the final determination.

Exercise and AMI, both forms of stress, induce changes in cellular metabolism which are reflected in characteristic serum enzyme elevation patterns referred to as the "Exercise Profile" and the "Cardiac Profile", respectively. Comparison of these profiles is useful when attempting to account for the differences between them. Notable differences between the two profiles include: (1) a forty-hour difference in maximal LDH activity; (2) no significant rise in GOT activity post-exercise; and, (3) absence of MB-CPK post-exercise. Notable similarities include: (1) maximal CPK activity seen twenty-four hours following both events; and (2) appearance of the LDH one-two flip at the time of optimum LDH activity after both AMI and exercise. Since the primary organs of involvement following these stresses are presently believed to be distinct (Exercise: skeletal

AMI; cardiac muscle), the differences in enzyme response may be generally attributed to the properties inherent to the specific muscle groups involved.

An apparent contradiction between the two profiles lies in the fact that maximal total CPK activity occurred at the same time interval, although maximal total LDH activity did not. However, one must consider that the circulatory supply to the myocardium is quite limited when compared to skeletal muscles. Very little collateral circulation is present in cardiac tissues. Upon onset of an AMI, the size of the tiny arterial and venous anastomoses increases to the maximum physical diameters within a few seconds (7). However, the blood flow through these collaterals is only one-half that needed to keep cardiac muscle alive. According to Guyton, the diameters of the collaterals do not enlarge further for the next eight to twenty-four hours (7). It seems that this occurrence should limit the size and amount of materials which can flow from the tissues to the peripheral circulation immediately after a heart attack. Assuming the biphasic course of enzyme release is present following both AMI and exercise, it seems likely that the difference in circulatory supply to myocardial and skeletal muscle may explain the forty hour difference between maximum LDH activity seen following the Cardiac versus the Exercise Profiles.

Consideration of the fact that GOT activity is significantly elevated only in the Cardiac Profile may indicate that

some type of membrane phenomenon is occurring. In the normal exercising heart, the membranes may be preferentially allowing the passage of specific types of molecules through the pores. This 'membrane specificity' may be due, at least in part, to the function and need for the particular enzyme in the metabolic processes occurring at the cellular level. In the present study, three different types of enzymes were involved--a kinase (CPK) (MW 82,000), a dehydrogenase (LDH), (MW 135,000), and an aminotransferase (GOT), (MW 90,000). Biochemical analysis of tissue and serum content of these enzymes in trained individuals following near-maximal intensity exercise has been performed but the results have not been consistent between different laboratories (9). Further work in this area of study will enhance the understanding of membrane changes in particular tissues due to exercise stress.

Similar observations have been made by Strawn (14) as a result of an in vivo study in dogs. Via perfusion hypothermia, induction of cardiac enzyme release was performed without adversely affecting the myocardium. A general increase in LDH and CPK, but not GOT was seen whenever the body environment was lowered below a transition temperature (25°C). It was speculated that a change in the lipid portion of the membrane occurs somewhere in the heart, going from liquid to gel to crystalline state. It was further suggested that this is not an irreversible condition

and that the heart may function even better, in some cases, at lower temperatures. Further studies must be performed in order to confirm this hypothesis. The answer to this question lies in determining the cause of enzyme release from its tissue source as well as the differences in membrane integrity in the normal versus diseased states.

The absence of MB-CPK in the Exercise Profile suggests that anaerobic activity is not significantly elevated in cardiac tissue due to exercise stress. Holloszy and Booth (9) have shown that the training effect of exercise differs between cardiac and skeletal muscles.

"In contrast to skeletal muscle, the heart does not undergo an adaptive increase in respiratory capacity in response to endurance exercise. Instead, the heart hypertrophies, myocardial contractility is enhanced, and resistance to hypoxia is increased. Because heart muscle contracts continuously and has the highest capacity for aerobic metabolism of any mammalian muscle, it seems reasonable that the levels of activity of the enzymes for the generation of ATP and for the hydrolysis of ATP during muscle contraction are the optimal ones for continuous, vigorous contractile activity. The heart appears to obtain its energy essentially completely from aerobic metabolism, taking up lactate, rather than forming it." (9, pp. 285-286).

Since MB-CPK does appear in the Cardiac Profile, the assumption can be made that pathological stress (AMI) may produce different tissue specific enzymes responses than physiological stress (exercise).

Possible reasons for the appearance of the LDH 'one-two flip' have been previously discussed. The fact that the 'flips' occurred at the time of maximal LDH activity in both instances provides further support to the suggestion

that the clearance rates of the individual LDH isozymes is responsible for this phenomenon.

Several clinical implications have arisen as a result of this study: (1) the specificity, sensitivity, and predictive value of CPK isozymal profiling in the diagnosis of disease is further enhanced due to the absence of MB-CPK in the Exercise Profile; (2) despite its relative utility, the Cardiac Profile does not account for the effects of exercise on levels of serum CPK and LDH. Hence, uncertainty is created in the interpretation of enzyme patterns obtained from suspected cardiac patients who have recently engaged in exercise; (3) enzyme levels may be significantly elevated in non-diseased individuals; (4) the ubiquity of LDH isozyme profiling is further demonstrated due to the appearance of the LDH 'one-two flip' in the Exercise Profile; and, (5) monitoring of serum CPK levels may serve as a useful index of stress.

SUMMARY

In conclusion, it is of interest to compare the Cardiac Profile with the Exercise Profile on an hour-by-hour basis. As seen in Figure 10, differences in isozyme patterns are evident at each hour of blood analysis following either MI or near-maximal intensity exercise. Although the LDH 'one-two flip' occurs in both patterns, this change, which is at present unexplained (there was no apparent hemolysis), occurs

forty hours sooner following exercise than AMI. Additionally, in both instances, CPK accounts for the greatest proportional increase in total serum enzyme activity and both appear maximal within twenty-four hours after either AMI or exercise. While the relatively high increase in MB-CPK following AMI serves as a distinctive characteristic of the Cardiac Profile, it is evident that uncertainty may arise in the definitive diagnosis of AMI by Cardiac Profiling unless the exercise history of the subject is known.

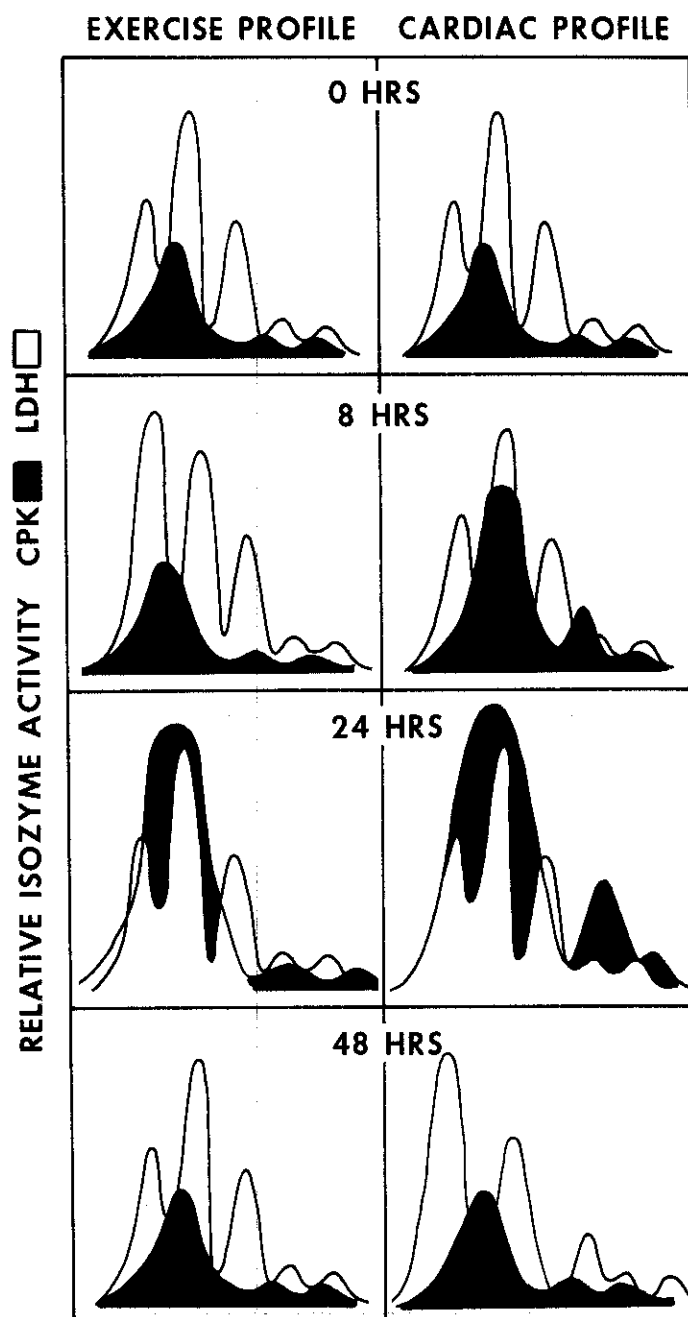


Fig. 10--CPK and LDH isozymal variations over time. The Cardiac Profile versus the Exercise Profile.

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