Myocardial Lipoprotein Lipase Levels in Hamsters with Congestive Heart Failure* (32686)

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Lipoprotein lipase (LPL) also known as clearing factor or postheparin lipase has been found in many tissues (1). Heart muscle is a rich source of this enzyme and several recent studies have shown the effects of exercise, diet, and drugs on myocardial LPL levels (2-5).

All BIO 14.6 inbred hamsters are afflicted with a hereditary dystrophy-like myopathy affecting all animals (6). The heart muscle is also involved and histological examination reveals in all animals over 30 days of age a marked and uniform degree of myocardial degeneration (7). The majority of these animals eventually exhibit an apparent compensatory cardiac hypertrophy and subsequent congestive heart failure. This experimental animal model was used in the present studies to determine whether elevation of the myocardial LPL level might be an adaptive change of the failing myocardium.

In addition, LPL from healthy hamster hearts was also characterized by some basic enzymatic studies.

Materials and Methods. Animals. Inbred hamsters of both sexes of the myopathic BIO 14.6 line, housed in air-conditioned rooms, and given free access to food (Old Guilford Mouse and Rat Breeder Pellets) and water, were used. Two groups of animals were selected from this strain—the first, animals showing signs of severe congestive heart failure and the second, symptom-free animals of comparable age. In addition, inbred hamsters of the LSH strain (known to be free of myocardial disease) and random-bred animals (also free of myocardial disease) were also studied.

Preparation of tissue powder. Hearts were removed from animals killed by pentabarbital. All animals were sacrificed between 10:00 AM and noon. The hearts were blotted free of blood and their wet weights were recorded. Tissue powder was prepared individually from each heart by extraction with acetone in a Virtis-45 homogenizer at the temperature of dry ice. The resulting samples of dried tissue powder were stored in a desiccator at −10°C. To prepare enzyme solutions, tissue powder was extracted with 0.025 *N* NH_4OH (1 ml/50 mg) at 0°C for 30 min, centrifuged at 17,000 rpm in a refrigerated centrifuge, and the supernatant fluid used. The protein content of a portion of the supernatant fluid was determined by the method of Lowry *et al.* (8). Although the lipolytic activity of powder extracted and assayed 2 weeks after dehydration of heart tissue remains the same as that of freshly extracted powder, all samples were assayed within 10 days of dehydration.

Enzyme assay. LPL was measured in digest mixtures of the following composition: 1.4 ml of 0.25 *M* ammonia buffer, pH 8.6 containing 70 mg bovine serum albumin; 0.4 ml substrate, consisting of equal portions of normal human serum, and a 1:10 aqueous dilution of Ediol, after preincubation of the mixture at 37°C for 30 min; and 0.1 ml of water or an aqueous solution containing 2 μg heparin; 0.1 ml heart powder extract.

The assay mixtures were incubated for 60 min at 37°C. Duplicate 0.4-ml samples of the digest were taken at 0 and 60 min and the amount of liberated fatty acid was measured by a single-phase microtitration system described elsewhere (9). The data are expressed as microequivalents of fatty acid released in 60 min per milligram protein in 0.1 ml of heart powder extract.

The substrate requirement of hamster heart LPL was examined in the following way. Ediol was diluted to contain 1, 2, 4, 10, 20, or 50 mg triglyceride/0.2 ml solution. Portions of these dilutions were preincubated

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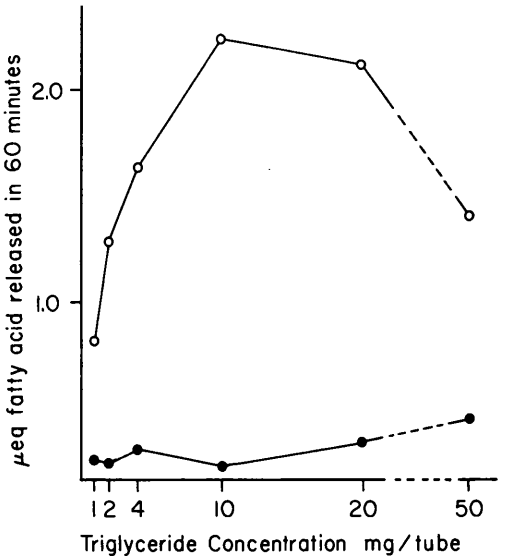

FIG. 1. Substrate requirement of lipoprotein lipase from hamster heart. O———O, triglyceride emulsion preincubated with human serum; ●———●, triglyceride emulsion preincubated with saline.

with equal parts of either human serum or saline and then used as substrate in the assay system described above.

The effects of heparin, NaCl, NaF, and protamine sulfate on the lipolytic activity of hamster heart extract were measured. These compounds were dissolved in the buffer and incorporated in the assay.

Results. Lipoprotein lipase from hamster heart requires a protein-bound triglyceride substrate as seen in Fig. 1. Like LPL from mouse or rat heart, it is activated *in vitro* by low concentrations of heparin with a maixmum effect occurring at a heparin concentration of 2 μg/2 ml digest. The hamster enzyme is inhibited by NaCl, NaF, and protamine sulfate, as shown in Table I. Lipoprotein lipase extracted from hamster heart, thus, behaves very much like this enzyme from other sources.

The influence on heart LPL of strain and of presence or absence of congestive heart failure in myopathic animals is shown in Table II.

The level of LPL activity in myopathic hamsters without symptoms of congestive heart failure was somewhat lower than that in the two nonmyopathic groups. The degree

TABLE I. Activation and Inhibition of Hamster Heart Lipoprotein Lipase.

Test material	Activity (% of control value)
Heparin, 1 μg	121
Heparin, 2 μg	132
Heparin, 10 μg	115
NaCl, 1 *M*	5
NaF, 1 *M*	21
NaF, 0.2 *M*	78
Protamine sulfate, 400 μg	8
pH optimum	8.3

of activation by heparin was also less than in the other groups. The most striking finding is the marked (almost twofold) elevation in LPL level in the myocardia of animals with severe congestive heart failure, as compared with symptom-free animals of the same inbred strain and age. This increase was evident in assays both with ($p<.001$) and without ($p=.01$) added heparin. Increased body and heart weights of this group reflect the severity of the congestive heart failure.

Discussion. The inbred LSH strain was chosen as a control because it was genetically unrelated to the BIO 14.6 strain and free of myocardial disease. In addition, random-bred hamsters, representing the type most commonly used in other laboratories, were studied. Although there are significant differences in myocardial LPL activity between these three groups (myopathic, LSH, and random-bred), the biological meaning of these differences is unknown.

The marked elevation of LPL level observed in myopathic hamsters in congestive heart failure could not be related to any factor other than the presence of the disease. Histological study shows that all the animals of this strain, at the ages studied, exhibit approximately the same amount of myocardial degeneration. There was a poor correlation between the degree of elevation of LPL and the severity of the disease as manifested by increased body weight (due to edema) and increased heart weight.

The level of activity of heart lipoprotein lipase has been shown to be elevated by exercise (5), fasting (2) diabetes (3) chronic epinephrine treatment (4) and hyperthyroid-

TABLE II. Myocardial LPL Activity in Four Groups of Hamsters; Two Healthy Groups, Symptom-free Myopathic Hamsters, and Myopathic Hamsters in Congestive Heart Failure.

Group	Number of animals	Age in weeks (range)	Body weight (gm)	Heart weight (mg)	μeq Fatty acid released in 60 min[a]	
					Without heparin	With heparin
Inbred LSH	12	31 (26–39)	113 ± 5.4[b]	369 ± 13.4	1.58 ± 0.12	2.02 ± 0.13
Random-bred	6	43 (36–49)	119 ±10.4	397 ± 32.4	1.37 ± 0.25	1.79 ± 0.14
Inbred BIO 14.6 myopathic, symptom-free	10	25 (10–36)	111 ± 3.7	359 ± 19.2	1.24 ± 0.09	1.35 ± 0.09
Inbred BIO 14.6 myopathic, in congestive heart failure	15	25 (16–34)	165 ± 9.1	518 ± 25.1	2.10 ± 0.22	2.66 ± 0.16

[a] Per milligram of protein in 0.1 ml of heart powder extract.
[b] All averages are ± SE.

ism (10). Hypothyroidism produced by propylthiouracil administration and high-fat or high-carbohydrate diets (10) decreased its level.

Although those metabolic manipulations were capable of modifying cardiac LPL levels, experimental production of cardiac hypertrophy alone was without effect. Mallov and Alonsi (10) suggested that increased LPL activity might constitute an adaptive change of the heart to increased work load, perhaps enabling the heart more readily to utilize fatty acid as an energy source. However, cardiac hypertrophy induced in rats by either aortic constriction or corticosteroid-salt-produced hypertension was without effect on LPL activity (10). The data presented here demonstrate that the hypertrophy of the myocardium in myopathic hamsters is accompanied by a marked elevation in LPL level. This appears to be a primary adaptive response of the failing myocardium since no other biochemical defect to which LPL might respond has been noted in these animals. Failure to induce alteration in LPL in hypertrophied rat heart may be due to the lack of adequate stimuli. Or it may be that the hamster response is not caused by the hypertrophy alone.

Slack *et al.* (11) reported significantly lower levels of postheparin serum LPL in men with ischaemic heart disease and proposed a possible relationship between their findings and the etiology of the disease. The elevation of clearing factor reported in patients in congestive failure (12) did not occur in the hamster with congestive heart failure. Postheparin serum from hamsters in failure and from symptom-free animals showed a wide range of lipolytic activity without significant difference between the two. This is not surprising if the LPL elevation, seen in animals in failure, is restricted to the heart, since the heart contributes only a minor portion of the circulating LPL engendered by heparin administration (13).

It is concluded that the elevated LPL seen in the hypertrophied myocardium of the myopathic hamster is probably one of several adaptive changes induced in the failing heart.

Summary. The levels of lipoprotein lipase (LPL) in the myocardium of healthy hamsters and of hamsters afflicted with an inherited dystrophy-like disease were examined. There was a marked elevation in myocardial LPL activity in myopathic hamsters in severe congestive heart failure as compared to symptom-free animals of the same strain and age. LPL from hamster myocardium was enzymatically similar in many respects to myocardial LPL from other species.

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Consequences of Natural Exposure to Rubella during Pregnancy* (32687)

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Although rubella is usually a mild disease in children or adults, the serious consequences to the fetus in the form of congenital anomalies following maternal infection early in pregnancy have led to extensive studies designed to prevent the disease during that period. Since control through artificial, active immunization, a promising likelihood in the near future, is not currently available, reliance has been placed on passive immunization with pooled lots of human gamma globulin. In spite of the known rubella antibody content of such preparations, the data on effectiveness of this procedure have been conflicting. Reduction in the incidence of clinical disease resulting from administration of gamma globulin to exposed individuals has been reported (1–6), but the lowest attack rates have clearly been related to the injection of larger quantities shortly after, or even prior to exposure. Similarly, a decreased incidence of defects in children exposed during fetal life has been correlated with earlier injection of their mothers (7). Variation in effectiveness of different lots of gamma globulin has been observed (8) and others have reported no significant effect on the incidence of infection (9, 10). It is notable, however, that subclinical infection has been demonstrated serologically in recipients of gamma globulin (6, 10) and that the incidence of viremia in a small group of inoculated subjects was found to be the same as in uninoculated controls (9, 10). These findings suggest that attempted passive immunization, while frequently suppressing clinically recognizable disease, may allow the virus to cross the placenta and damage the fetus.

This report describes the incidence of clinical and subclinical rubella with associated effects on infants in relation to the administration of gamma globulin to women exposed during pregnancy.

Materials and Methods. Paired specimens of human sera which had been collected during the second or third month of pregnancy and at the time of delivery were available as part of a larger and continuing prospective study of the etiology of congenital anomalies (11, 12). During the years 1964 and 1965, which were characterized by a marked increase in clinical rubella in the area, 347 women in the study had reported an exposure to the disease. The degree of these exposures was difficult to evaluate, especially since many had occurred prior to the first visit to an obstetrician and shortly before the collection of the first blood specimen. One hundred and twenty-nine women gave a history of such ex-

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