Release of Clearing Factor Lipase (Lipoprotein Lipase) in Vivo and from Isolated Perfused Hearts of Alloxan Diabetic Rats

E. Aktin, M.D., and H. C. Meng, M.D., Ph.D., Nashville

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/21/3/149/346575/21-3-149.pdf by guest on 09 December 2024

SUMMARY

The release of clearing factor lipase (CFL) into the blood stream after heparin injection and from hearts during perfusion with a heparin-containing fluid was studied using normal and alloxan diabetic rats. In addition, the effect of alloxan diabetes on heart tissue lipase activity and the "repletion" of this enzyme in previous "depleted" animals by pre-injection of heparin were also investigated. The results show that the plasma CFL activity after an injection of a standard dose of heparin and that of the medium incubated with heart slices or homogenates in the presence of heparin was significantly decreased in alloxan diabetic rats. CFL release from isolated perfused hearts of alloxan diabetic rats was comparable to that from hearts of normal animals during the short perfusion period of five to ten minutes. However, the enzyme release from hearts of alloxan diabetic rats was considerably decreased during prolonged perfusion. Addition of insulin and glucose to the fluid perfusing hearts of diabetic animals did not correct the defect. Insulin treatment restored the CFL activity in plasma, heart slices, homogenates, and perfusates to normal levels. The CFL release into the blood stream or from perfused hearts of rats previously treated with heparin was decreased when rats were given a second injection of this agent or when rats were killed and hearts were perfused less than two hours after the first heparin administration. This decrease was more marked in diabetic than in normal rats. The "repletion" of CFL in hearts of normal rats was completed in three hours with an overshoot four hours after heparin injection. The CFL release from perfused hearts of diabetic rats remained at a low, depleted level even eight hours after heparin administration. The heart tissue lipase activity in rats with heparin pretreatment was also decreased; this decrease was more marked in diabetic than in normal animals. The "repletion" of tissue lipase was also much slower in diabetic rats. Omission of heparin in the incubation medium of heart slices and the perfusion fluid markedly decreased the lipase release especially that from the perfused heart; the decrease was very slight in the incubation medium of heart homogenates. Tissue and perfusate lipase were inhibited by sodium chloride and protamine.
DIABETES *21*:149-56, March, 1972.

It is known that a variety of myocardial tissue preparations contain a heparin "activated" lipase which hydrolyzes the triglyceride in chylomicrons and in very low-density lipoproteins.[1-10] Kessler[8] has reported an increase in lipase activity of heart acetone powder and in lipase release into the incubation medium from hearts of alloxan diabetic rats. Based on an elevation of glycerol release, an increase in lipolysis in hearts of alloxan diabetic rats has also been implicated.[11] Kreisberg[12] has reported, however, that the hydrolysis, uptake, and oxidation of chylomicron tripalmitin ^{14}C by isolated perfused hearts of alloxan diabetic rats are decreased as compared with normal hearts: these results suggested a decrease in lipase activity. Preliminary observations obtained in our laboratory have shown the lipase release from isolated perfused hearts of alloxan diabetic rats was decreased.[13,14] A decrease in clearing factor lipase activity of postheparin plasma has also been reported.[15,16] Some investigators have found no changes, however, in postheparin plasma lipolytic activity in patients with diabetes mellitus.[17-19] The present work was designed to extend studies of the effect of alloxan diabetes on the release and "repletion" of clearing factor lipase in the rat heart.

From the Department of Physiology, Vanderbilt University, School of Medicine, Nashville, Tennessee, 37203. Dr. Aktin's present address: Capa Noroloji Klinigi, Topkapi, Istanbul, Turkey.

METHODS AND MATERIALS

Animals: Normal and alloxan diabetic male rats of Sprague-Dawley strain, weighing 250 to 280 gm., were used. All rats were fed Purina rat chow ad libitum before use. For the preparation of diabetic rats, alloxan (50 mg./kg. of body weight) dissolved in normal saline was injected through a tail vein. Blood was obtained through a cut of the tail for the determination of glucose before and forty-eight hours after alloxan. Glucose levels of 250 mg. per 100 ml. or higher were judged evidence for diabetes.

In some experiments, diabetic rats were treated with insulin beginning three days after the injection of alloxan. Diabetes was considered under control with insulin dosages of 5 to 15 units and blood glucose concentrations of 120 mg. per 100 ml. or less in three successive measurements.

Perfusion of rat heart. Rats were anesthetized by an intraperitoneal injection of Nembutal (50 mg./kg. of body weight). The procedure used for heart perfusion was as described previously.[21] It was necessary to isolate and cannulate the heart as quickly as possible to avoid clotting of blood in the coronary bed.

Krebs-Ringer bicarbonate buffer, pH 7.4, containing normal rat serum (5 per cent v:v) and heparin (10 μg./ml.), was used as a perfusion fluid. It was gassed with a mixture of 95 per cent O_2-5 per cent CO_2 before and during perfusion. In some experiments glucose (200 mg./100 ml.) and crystalline insulin (10 mU./ml.) were added to the perfusion fluid.

Hearts, after excision and cannulation, were perfused in an open system with Krebs-Ringer bicarbonate buffer for three minutes to wash out any residual blood from coronary vessels. After washing, a perfusion fluid containing rat serum and heparin at the concentrations indicated above was used. Perfusion was carried out either in an open (nonrecirculating) or closed (recirculating) system.

Plasma lipase activity. For the determination of plasma lipase activity, blood was withdrawn fifteen minutes after the injection of heparin (1 mg./kg. of body weight, intravenously). The optimal heparin dosage and time of blood withdrawal for maximal plasma lipase activity were based on results obtained in our laboratory (Aktin and Meng, unpublished data). Then, 4.8 ml. of blood were withdrawn by cardiac puncture into a syringe containing 0.2 ml. of a 10 per cent sodium citrate solution. Blood was centrifuged at 800 *g* at 4° C. for fifteen minutes. Plasma obtained was used for the measurement of lipase activity.

Heart slices for lipase activity. After blotting and weighing, half of the heart was sliced with a sharp razor blade. Special attention was given to obtain slices of equal thickness which was between 1.0-2.0 mm. and reproducible. Slices were incubated in 5 ml. of Krebs-Ringer phosphate buffer[22] (0.1M, pH 7.4) containing rat serum (5 per cent v:v) without or with heparin (25 μg./ml.) in a Dubnoff metabolic shaker at 37° C. under air for one hour. At the end of this period 2 ml. of the incubation medium were taken for lipase assay.

Heart homogenates for lipase activity. Half of the heart was blotted dry, weighed and homogenized with an Omnimixer in 10 ml. of Krebs-Ringer phosphate buffer (pH 7.4) containing rat serum (5 per cent v:v) in the absence or presence of heparin (25 μg./ml.) for thirty seconds. The mixture was incubated in a Dubnoff metabolic shaker at 37° C. under air for one hour. Two milliliters of homogenate were used for the measurement of lipase activity.

Measurement of lipase activity. Two milliliters of perfusate or equal volume of incubation medium of tissue slices were added to 3 ml. of an assay system which consisted of 1.7 ml. of phosphate buffer (pH 7.4), 0.7 ml. of a 1 per cent coconut oil emulsion (diluted Ediol), 0.4 ml. of 25 per cent bovine serum albumin adjusted to pH 7.4 with sodium hydroxide and 0.2 ml. of normal rat serum. The mixture was incubated in a water bath at 37° C. for one hour. Two milliliters of this mixture were used for free fatty acid (FFA) determination before and at the end of incubation.

The assay system used for the determination of homogenate lipase activity contained 5.4 ml. of phosphate buffer (pH 7.4), 1.4 ml. of 1 per cent coconut oil emulsion, 0.8 ml. of bovine serum albumin (25 per cent) 0.4 ml. of normal rat serum and 2 ml. of tissue homogenate.

For the determination of plasma lipase activity 1 ml. of plasma was incubated for one hour in an assay system containing 2.9 ml. of phosphate buffer (pH 7.4), 0.7 ml. of coconut oil emulsion and 0.4 ml. of bovine serum albumin with a total volume of 5 ml.

The incubation system, incubation time and FFA determination for the lipase activity of tissue homogenate and of plasma were the same as described for tissue slices. The FFA were measured according to the method of Dole[23] using Nile blue as an indicator. The lipase activity was calculated as the difference of FFA produced in μmoles between, before and after incubation. The lipase activity is expressed as μmoles of FFA produced per unit weight of tissue or volume of plasma per unit time.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/21/3/149/346575/21-3-149.pdf by guest on 09 December 2024

TABLE 1
Lipase activity of plasma, heart slices and homogenates of normal, diabetic and insulin-treated diabetic rats*

Enzyme source	Lipase activity†		
	Normal	Diabetic	Insulin-treated
Plasma	11.63±0.34	8.95±0.14	11.76±0.10
Heart slices	20.94±1.71	11.85±0.72	21.57±0.63
Heart homogenates	173.97±6.52	132.94±4.81	164.18±3.21

*Five to seven rats were used in each group. Values are means ± S.E.
† Plasma: μmoles FFA/ml. plasma/hr. Heart slices and homogenates: μmoles FFA/gm. wet tissue/hr.

Statistical analyses of the results were made according to Li.[24]

Bovine serum albumin Fraction V used in this study was obtained from the Nutritional Biochemicals Corporation (Cleveland, Ohio) and heparin, 10 mg./ml. and 100 U./mg. was purchased from The Upjohn Co. (Kalamazoo, Michigan). Insulin (NPH and crystalline) from Eli Lilly Company (Indianapolis, Indiana), was used and 50 per cent Ediol, kindly supplied by Riker Laboratories, was used as a triglyceride substrate after appropriate dilution.

RESULTS

Lipase activity of plasma, heart slices and homogenates of normal, diabetic and insulin-treated diabetic rats. As shown in table 1, there was a marked decrease in plasma and heart tissue lipase activity in alloxan diabetic rats. The differences between the lipase activity of plasma, heart slices and homogenates of normal and diabetic animals were statistically significant ($p < 0.001$). Insulin treatment of diabetic rats reversed these changes to normal levels.

Lipase release from normal and diabetic rat hearts perfused with heparin. Figure 1 shows there was no difference in lipase release from hearts of normal and diabetic animals during the first five minutes of perfusion. However, the enzyme release from diabetic rat hearts was considerably decreased during subsequent prolonged periods of perfusion. The lipase activity of perfusate collected from diabetic hearts during the five minutes of perfusion was 53.75 ± 3.46 μmoles FFA as compared to 26.61 ± 2.82 μmoles FFA/gm. of wet tissue for that of perfusate obtained during thirty-five minutes of perfusion. This difference is highly significant ($p < 0.001$). The perfusate lipase activity of normal rat hearts was only slightly decreased after thirty-five minutes of perfusion although further decrease was observed after prolonged perfusion. The lipase release during 35, 65, and 95 min. of perfusion from diabetic rat hearts was significantly lower than that from hearts of normal animals; the p values are less than 0.005, 0.01 and 0.005, respectively.

Figure 1 also shows the remaining lipase activity in the hearts after ninety-five minutes of perfusion with a heparin-containing fluid. In spite of prolonged perfusion, myocardial tissue contained a substantial amount of lipase activity. The difference in homogenate lipase activity between normal and alloxan diabetic rat hearts is statistically significant ($p < 0.001$).

The addition of glucose and insulin to the perfusion fluid did not restore the lipase activity of diabetic rat hearts to that of normal animals (figure 1).

Pattern of lipase release from normal, alloxan diabetic and insulin-treated alloxan diabetic rat hearts perfused

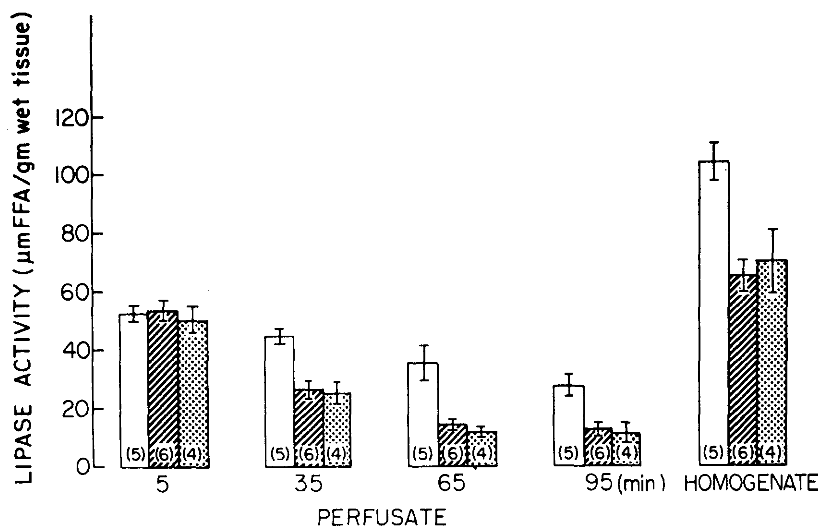

FIG. 1.
Perfusate and homogenate lipase activity of normal and alloxan diabetic rat heart. Hearts were cannulated and perfused as described in "Methods and Materials." The perfusion fluid was a Krebs-Ringer bicarbonate buffer, pH 7.4, containing normal rat serum (5 per cent v:v) and heparin (10μg/mg.). In some experiments glucose (200 mg./100 ml.) and insulin (10 mU./ml.) were added to the perfusion fluid perfusing hearts from alloxan diabetic rats. All hearts were perfused in a closed perfusion system with recirculation of the buffered fluid. Perfusate was taken for the determination of lipase activity at the time-intervals indicated. Heart was homogenized after ninety-five minutes of perfusion and homogenate lipase activity was measured. □, normal rats, ▨, alloxan diabetic rats, ▩ alloxan diabetic rats perfused with the basal fluid containing glucose and insulin. Figures in parentheses are the number of hearts perfused.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/21/3/149/346575/21-3-149.pdf by guest on 09 December 2024

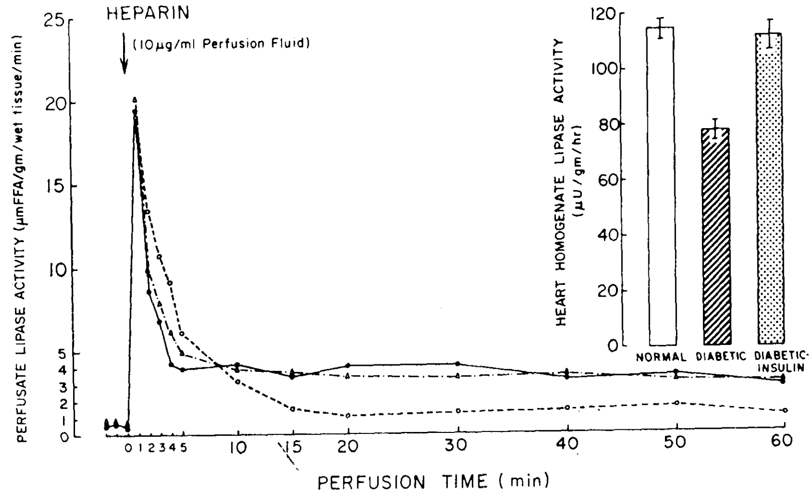

FIG. 2

Clearing factor lipase release (perfusate lipase activity) from hearts of normal and alloxan diabetic rats. Hearts of normal, alloxan diabetic and insulin-treated alloxan diabetic rats were perfused with a Krebs-Ringer bicarbonate buffer, pH 7.4, containing normal rat serum (5 per cent v:v) and heparin (10 µg./ml.) in an open perfusion system without recirculation of the fluid. Perfusate was taken for lipase activity at the time-intervals indicated. ●—●, normal rats; O - - - O, alloxan diabetic rats; △ — • — • △, insulin-treated alloxan diabetic rats. Hearts were homogenized after sixty minutes of perfusion, and homogenate lipase activity was determined. The results are the averages of four to six hearts.

with heparin. Figure 2 shows that the patterns of lipase release into the perfusion fluid from normal and diabetic rat hearts were similar during the first ten minutes of perfusion. Both groups showed a sharp rise immediately after perfusion with a fluid containing heparin. The peak of lipase activity was reached in one minute, and the decline was very rapid. After ten minutes of perfusion, the lipase activity of perfusates from diabetic rat hearts was further decreased reaching to the baseline levels. On the other hand, the perfusate lipase activity of normal hearts was maintained at a constant level of approximately 3 to 4 µmoles FFA/gm. of wet tissue/minute throughout the perfusion period.

The homogenate lipase activities of perfused normal and diabetic hearts were 114.69 ± 3.70 and 77.70 ± 3.63 µmoles FFA/ gm. of wet tissue/hour, respectively, the difference being statistically significant ($p < 0.001$).

Figure 2 also shows that insulin treatment of alloxan diabetic rats corrected the decrease in lipase release observed during prolonged perfusion of hearts and homogenate lipase activity of diabetic rats.

Plasma lipase activity of normal and diabetic rats in response to repeated heparin administration. This experiment was designed to investigate the rate of restoration of plasma lipase activity in normal and diabetic rats. For this purpose, rats were pretreated with heparin injection to "deplete the lipase store." A second heparin injection was given at various time intervals after the first.

In nonheparin pretreated normal rats, heparin admin-

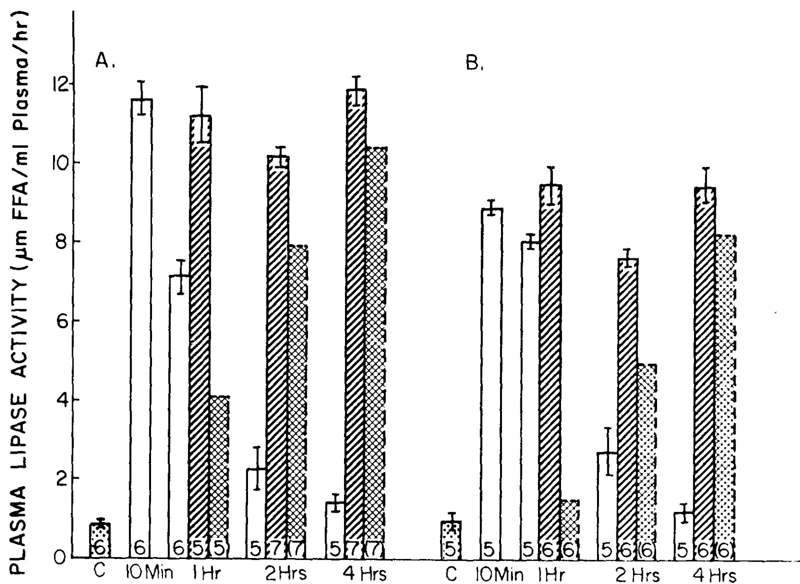

FIG. 3

Plasma clearing factor lipase activity after intravenous injection of heparin (1 mg./kg. of body weight). Blood samples after the first heparin were drawn at various time-invals as indicated. The second heparin injection was given at 60, 120 or 240 min. after the first. All blood samples were drawn ten minutes after the second heparin administration. Therefore, the blood samples taken after the second heparin injection corresponded to 70, 130 and 250 min. after the first. A. Normal rats. B. Alloxan diabetic rats. ▒ control, plasma lipase activity before heparin administration. □, lipase activity of plasma obtained at various time-intervals after the first heparin injection. ▨ Lipase activity measured at various time-intervals after the first heparin injection and ten minutes after the second. ▓, plasma lipase activity calculated by subtracting the lipase activity measured after the first heparin injection from that obtained after the second at respective time-intervals; this is considered as the true lipase activity in response to the second heparin administration. Figures shown at the bottom of the bars represent the number of experiments.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/21/3/149/346575/21-3-149.pdf by guest on 09 December 2024

TABLE 2

Lipase activity of heart slices and homogenates of heparin-pretreated normal and diabetic rats*

Experimental group	Lipase activity† Heart slices Normal	Heart slices Diabetic	Homogenates Normal	Homogenates Diabetic
Control (no heparin)	20.94±1.71	11.87±0.72	173.96±6.51	132.96± 4.83
Heparin injected 10 min. before sacrifice	18.28±1.45	10.35±0.92	146.29±5.24	102.64± 1.80
Heparin injected 4 hrs. before sacrifice	25.63±0.97	11.00±1.45	176.67±6.52	119.88±11.72

* Five to nine rats were used in each group. Values are means ± S.E.
† μmoles FFA/gm. wet tissue/hr.

istration was followed by a marked increase in plasma lipase activity measured ten minutes after injection; the lipase level decreased as time progressed and returned to the control level at the end of four hours (figure 3A, open bars).

Normal rats with pre-injection of heparin responded to the second administration of this agent with a rise in plasma lipase activity at the time intervals indicated (figure 3A, hatched bars). The increase in lipase activity in response to the second heparin administration determined two hours after the first injection was slightly lower than that in response to the first. The difference in plasma lipase activity obtained ten minutes after the first heparin injection and that measured two hours later in response to the second heparin administration was statistically significant ($p < 0.005$).

The lipase release in response to the second injection of heparin alone, (figure 3A, crossed bars), which is calculated by subtracting the lipase activity obtained after the first heparin injection from that measured after the second injection at respective time intervals, was markedly reduced at one hour, but progressively increased to the normal level four hours after the first heparin administration.

The plasma lipase activity of alloxan diabetic rats in response to a single heparin injection and that after the second injection showed a similar course as that of normal animals but at reduced levels (figure 3B, open and hatched bars). The lipase release which is truly due to the second injection of heparin (figure 3B, crossed bars) was insignificant one hour after the first heparin administration in diabetic rats.

Lipase activity of heart slices and homogenates of heparin pretreated normal and diabetic rats. Table 2 shows that the tissue lipase activity of hearts from diabetic rats was considerably decreased as compared to that from normal animals. Heparin injection into rats ten minutes before sacrifice caused a decrease in lipase activity of heart slices and homogenates from both normal and diabetic rats. In the rats killed four hours after heparin injection, the lipase activity of both slices and homogenates of hearts from normal rats had returned to the control values. However, the lipase activity of heart homogenates from diabetic rats remained at a lower level as compared to that of diabetic animals given no heparin injection.

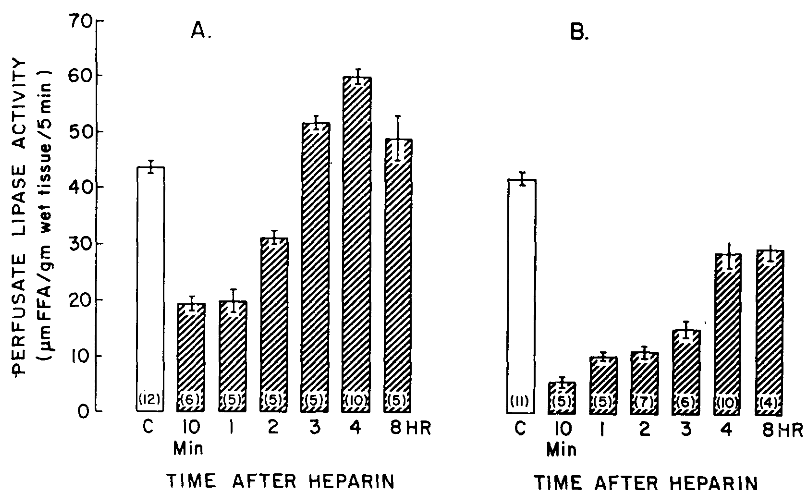

FIG. 4.

Perfusate clearing factor lipase activity. All hearts were perfused for five minutes with a Krebs-Ringer bicarbonate buffer containing normal rat serum (5 per cent v:v) and heparin (10 μg./ml.) in a closed system with recirculation of the perfusion fluid. A. Perfusate lipase activity from hearts of normal rats. B. Perfusate lipase activity from hearts of alloxan diabetic rats. ☐, rats received no heparin pretreatment. ▨, rats received heparin injection (1 mg./kg., i.v.) and hearts were perfused at various timed intervals after heparin. Figures in parentheses are the number of hearts perfused.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/21/3/149/346575/21-3-149.pdf by guest on 09 December 2024

TABLE 3

Comparison of lipase activity of rat heart with and without added heparin*

Heparin‡	Perfusate	Lipase activity† Homogenate	Slice
0	0.33±0.06	156.47±19.11	10.89±0.68
+	5.51±0.30	175.82±14.20	23.11±3.81

* Averages of four experiments.
† Slice and homogenate were pre-incubated for one hour with heparin (25 mg./ml.). Perfusion fluid contains 10 μg heparin/ml.
‡ Lipase activity is expressed as μmoles FFA/gm. wet tissue/hr., or μmoles FFA/ml. perfusate/hr.

Perfusate lipase activity of heparin pretreated normal and diabetic rat hearts. In an attempt to investigate the effect of heparin pretreatment on perfusate lipase activity, rats were first given an injection of heparin (1 mg./kg.). The animals were killed at various time intervals after heparin and hearts were perfused with a heparin-containing fluid immediately after sacrifice. All hearts were perfused for five minutes, because of the fact that the greatest lipase release was observed during this period (figures 1 and 2). As seen in figure 4A and 4B (open bars) nonheparin pretreated normal and diabetic animals showed no significant difference in perfusate lipase activity. However, the lipase activity of perfusate from hearts of normal rats was reduced to 19.48 ± 1.45 μmoles FFA/gm. of wet tissue ten minutes after heparin injection while that from hearts of diabetic animals was only 5.60 ± 1.20 μmoles FFA/gm. of wet tissue which is significantly lower than that of normal rats ($p < 0.001$). A significant difference was also observed between the lipase activity of hearts from normal and diabetic rats 1, 2, 3, 4, and 8 hr. after injection of heparin, ($p < 0.001$). The lipase activity of perfusate from normal rat hearts had returned to their control (nonheparin injected) levels three hours after heparin injection. The maximal level of 60.00 ± 1.41 μmoles FFA/gm. wet tissue was observed four hours after heparin administration; the enzyme activity was significantly higher than that of the control value ($p < 0.001$). This "overshoot" of perfusate lipase activity was invariably observed in hearts of normal rats. In diabetic rats, however, the perfusate lipase activity was only 28.84 ± 2.72 μmoles FFA/gm. of wet tissue four hours after heparin administration. It never reached the control level even eight hours after the heparin injection ($p < 0.001$), and the "overshoot" of lipase activity was never observed.

Properties of lipase of heart slice, homogenate and perfusate.

1. Omission of heparin. Omission of heparin from the perfusion fluid or from the pre-incubation medium of heart slices caused a substantial decrease in lipase activity. However, the lipase activity of heart homogenates was only slightly reduced in the absence of heparin in the incubation medium (table 3).

2. Effect of inhibitors. Table 4 shows that sodium chloride (0.6 and 1.0 M) and protamine sulphate (1 mg./ml.) exerted an inhibitory effect on lipase activity of heart slices, heart homogenates and perfusate.

DISCUSSION

The results of the present study confirm our previous report that the clearing factor lipase activity of plasma, perfusate, slice and homogenate of heart from alloxan diabetic rats is decreased.[13-16] However, the findings are at variance with those of Kessler[8] who found that diabetes produced an increase in lipase release from incubated myocardial tissue in the presence of heparin. As reported by Borensztajn et al.,[25] the difference in experimental procedure such as tissue preparation (fresh homogenate or acetone-ether dry powder), the presence or absence of serum in the assay system, etc., may have contributed, in part, to the conflict in the results. However, Kreisberg[12] has shown that the hydrolysis,

TABLE 4

Effect of NaCl and protamine on the lipase activity of heart slices, homogenates and perfusate*

Addition	Slices† Lipase activity	Slices† Inhibition (%)	Homogenates† Lipase activity	Homogenates† Inhibition (%)	Perfusate† Lipase activity	Perfusate† Inhibition (%)
None	23.11±3.81	0	175.82±14.20	0	5.51±0.30	0
NaCl (0.6 M)	7.69±0.92	66.7	101.45± 8.13	42.3	1.47±0.41	73.4
NaCl (1.0 M)	6.56±0.54	71.9	45.68± 4.93	74.1	0.88±0.21	84.0
Protamine sulfate (1 mg./ml.)	6.21±0.81	73.1	32.40± 3.71	81.0	0.83±0.10	84.9

* Average of four experiments for each preparation.
† Slices and homogenates were pre-incubated for one hour with heparin (25 μg./1 ml.) prior to lipase assay. Perfusion fluid contained 10 μg. heparin/ml.
‡ Lipase activity is expressed as μmoles FFA/gm. wet tissue/hr. or μmoles FFA/ml. perfusate/hr.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/21/3/149/346575/21-3-149.pdf by guest on 09 December 2024

uptake and oxidation of chylomicron tripalmitin-C-14 by isolated perfused hearts of alloxan diabetic rats were decreased as compared to those of normal hearts; these results suggest a decrease in clearing factor lipase activity. Meng and Goldfarb[16] have also shown that the clearing activity of postheparin plasma of alloxan diabetic and depancreatized rats was markedly reduced. A similar difference in lipase activity of hearts of fasted rats has also been reported: Several investigators,[2,3,7,10] have found that the lipase release from and activity of the myocardial tissues in the fasted state are increased. However, the observations of Robinson and Jennings[26] and Meng et al.[13] do not agree with these results. Again, the difference in clearing factor lipase activity of hearts from fasted rats observed by various investigators may be due to differences in the assay system and in the tissue preparation used.

It has been proposed that clearing factor lipase is in or very close to the vascular bed.[27,28] In a recent publication, Ho et al.[29] observed that lipase activity of perfusate from rat hearts perfused with a heparin-containing fluid reached the maximum level within ten seconds. The present work shows that after the rapid release, the lipase which may be localized in or near the vascular bed seems to be "depleted." However, a low rate of enzyme release continues for as long as ninety-five minutes, the longest perfusion period carried out. The total amount of lipase release during the period of prolonged perfusion is considerable when the duration of perfusion is taken into consideration. It is speculated that the clearing factor lipase may be distributed in two pools: One is in or near the vascular bed. The enzyme in this pool can be released very quickly by appropriate stimuli. The pool size is small and can be "depleted" readily. It serves as a temporary storage site. The other pool is localized or associated with cells where lipase synthesis takes place. The size of this pool is large and serves as a site for synthesis and storage. The enzyme may be transferred from the latter to the former site at a slow rate. It seems reasonable to postulate that the amount of the "quick releasing" lipase is very similar in normal and diabetic rats. However, the amount of this enzyme in the large pool differs markedly in these two conditions; the rates of transfer and/or synthesis of lipase are significantly slower in diabetic than in normal animals.

In the present study, perfusion of diabetic rat hearts in vitro with a fluid containing insulin and glucose failed to restore the perfusate lipase activity to normal levels. Salaman and Robinson[30] and Wing et al.[31] found that the low clearing factor lipase activity of fasted rat adipose tissue increased progressively when the tissue was incubated in a medium containing glucose, insulin, amino acid mixture and some minerals. The failure to correct the defect in lipase release from hearts of diabetic rats perfused with a fluid containing added insulin and glucose may be due to the short period of perfusion and/or to the absence of amino acids and minerals. However, in insulin controlled diabetic rats, lipase activity of plasma, heart slices, homogenates and perfusate was similar to that observed in normal rats.

It is interesting to note that the lipase release from isolated perfused hearts of rats that were given heparin injection two hours or less prior to perfusion was markedly decreased in both normal and diabetic animals; the decrease in the latter group was more marked than that in the former. However, the "repletion" of the "quick releasing" lipase from the perfused hearts was completed in three hours, and an overshoot was observed in four hours after heparin injection in normal rats. However, the lipase release remained at "depleted" levels even eight hours after heparin administration in diabetic animals. On the other hand, "repletion" of plasma, heart slice and homogenate lipase activities had reached to or toward the preheparin level in four hours in both normal and diabetic rats. Thus, the "repletion" of lipase activity after "depletion" following heparin injection occurs also in diabetic animals but at a slower rate. In addition, there may be a defect in the transfer of this enzyme from the pool of synthesis to that for release.

It is known that lipoprotein lipase extracted from acetone powders of rat hearts by Korn is inhibited by NaCl and protamine.[32] The observations made in this study show that lipase activity of heart slices, homogenates and perfusate was also significantly inhibited by these agents. The results suggest that the clearing factor lipase is similar to the enzyme reported by Korn.

ACKNOWLEDGMENT

This study was supported in part by National Institutes of Health grants HE-04372 and AM-07462.

REFERENCES

[1] Korn, E. D.: Clearing factor, a heparin-activated lipoprotein lipase. I. Isolation and characterization of the enzyme from normal rat heart. J. Biol. Chem. *215*:14, 1955.

[2] Zemplenyi, T., and Grofnetter, D.: Fatty acid release on incubation of lipemic serum with rat tissues. The influence of heparin and fasting. Arch. Int. Pharmacodyn. Ther. *122*:57-66, 1959.

[3] Hollenberg, C. H.: The effect of fasting on the lipoprotein lipase activity of rat heart and diaphragm. J. Clin. Invest. *39*: 1282-87, 1960.

[4] Bjorntorp, P., and Furman, R. H.: Lipolytic activity in rat heart. Amer. J. Physiol. *203*:323-26, 1962.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/21/3/149/346575/21-3-149.pdf by guest on 09 December 2024

[5] George, J. C., and Iype, P. T.: Lipase activity in pigeon heart muscle particulate fractions and its metabolic significance. Amer. J. Physiol. *204*:165-67, 1963.

[6] Schnatz, D. J., Ormsby, J. W., and Williams, R. H.: Lipoprotein lipase activity in human heart. Amer. J. Physiol. *205*: 401-04, 1963.

[7] Alousi, A. A., and Mallow, S.: Effects of hyperthyroidism, epinephrine, and diets on heart lipoprotein lipase activity. Amer. J. Physiol. *206*:603-09, 1963.

[8] Kessler, J. I.: Effect of diabetes and insulin on the activity of myocardial and adipose tissue lipoprotein lipase of rats. J. Clin. Invest. *42*:362-67, 1963.

[9] Zemplenyi, T.: The lipolytic and esterolytic activity of blood and tissue and problems of atherosclerosis. *In* Advances in Lipid Research, edited by R. Paoletti and D. Kritchevsky. New York: London, Academic Press, 1964, p. 235-95.

[10] Nikkila, E. A., Torsti, P., and Penttila, O.: Effects of fasting, exercise and reserpine on catecholamine content and lipoprotein lipase activity of rat heart and adipose tissue. Life Science *4*:27-35, 1965.

[11] Randle, P. J., Garland, P. B., Hales, C. N., and Newsholme, E. A.: The glucose fatty-acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. The Lancet *1*:785-89, 1963.

[12] Kreisberg, R. A.: Effect of diabetes and starvation on myocardial triglyceride and free fatty acid utilization. Amer. J. Physiol. *210*:379-84, 1966.

[13] Meng, H. C., Aktin, E., Crass, M. F. III, and Ghosal, M.: Some factors affecting release of clearing factor lipase from isolated perfused rat heart. *In* Drugs Affecting Lipid Metabolism, edited by W. H. Holmes, L. A. Carlson and R. Paoletti. New York, Plenum Press, 1969, p. 261-78.

[14] Aktin, E., and Meng, H. C.: Depletion and repletion of heparin-released lipase in normal and diabetic rats. Circulation (Supplement 2) *32*:1, 1965.

[15] Ho, R. J., and Meng, H.C.: Preliminary study of lipemia clearing defect in alloxan-diabetic rat. Fed. Proc. *19*:13, 1960.

[16] Meng, H. C., and Goldfarb, J. L.: Heparin-induced lipemia clearing in rats—Role of the pancreas in its production. Diabetes *8*:211-17, 1959.

[17] Denborough, M. A., and Patterson, B.: Clearing factor, fibrinolysis and blood lipids in diabetes mellitus. Clin. Sci. *23*:485-88, 1962.

[18] Jones, D. P., Plotkin, G. R., and Arky, R. A.: Lipoprotein lipase activity in patients with diabetes mellitus, with and without hyperlipemia. Diabetes *15*:565-70, 1966.

[19] Wilson, D. E., Schreibman, P. H., and Arky, R. A. Postheparin lipolytic activity in diabetic patients with a history of mixed hyperlipemia—Relative rates against artificial substrates and human chylomicrons. Diabetes *18*:562-66, 1969

[20] Huggett, A. St. G., and Nixon, D. A.: Enzymatic determination of blood glucose. Biochem. J., *66*:12p, 1957.

[21] Crass, M. F., III, and Meng, H. C.: Serum requirement for release of heparin-induced lipase from perfused rat heart Amer. J. Physiol. *206*:610, 1964.

[22] Umbreit, W. W., Burris, R. H., and Stauffer, J. F.: Manometric Techniques, Burgess Publishing Company, 4th ed., 1964, 1. 132.

[23] Dole, V. P.: A relationship between non-esterified fatty acids in plasma and the metabolism of glucose. J. Clin. Invest. *35*:150-54, 1956.

[24] Li, J. C. R.: Introduction of Statistical Inference. Edwards Brothers, Inc., Ann Arbor, Mich., 1959.

[25] Borensztajn, J., Otway, S., and Robinson, D. S.: Effects of fasting on the clearing factor lipase (lipoprotein lipase) activity of fresh and defatted preparations of rat heart muscle. J. Lipid Res. *11*:102-10, 1970.

[26] Robinson, D. S., and Jennings, M. A.: Release of clearing factor lipase by the perfused rat heart. J. Lipid Res. 6:222-27, 1965.

[27] Robinson, D. S.: The clearing factor lipase and its action in the transport of fatty acids between the blood and the tissues. *In* Advances in Lipid Research, edited by R. Paoletti and D. Kritchevsky. New York: London, Academic Press, 1963, p. 133-82.

[28] Ho, S. J., Ho, R. J. and Meng, H. C.: Comparison of heparin-released and epinephrine-sensitive lipases in rat adipose tissue. Amer. J. Physiol. *212*:284-90, 1967.

[29] Ho, R. J., Aktin, E., and Meng, H. C.: Lipase release induced by Compound 48/80 in rat and perfused rat heart. Amer. J. Physiol. *210*:299-304, 1966.

[30] Salaman, M. R., and Robinson, D. S.: Clearing-factor lipase in adipose tissue. A medium in which the enzyme activity of tissue from starved rats increases in vitro. Biochem. J. 99:640-47, 1966.

[31] Wing, D. R., Salaman, M. R., and Robinson, D. S.: Clearing-factor lipase in adipose tissue. Factors influencing the increase in enzyme activity produced on incubation of tissue from starved rats in vitro. Biochem. J. 99:648-56, 1966.

[32] Korn, E. D.: Clearing factor, a heparin-activated lipoprotein lipase. II. Substrate specificity and activation of coconut oil. J. Biol. Chem. *215*:15-26, 1955.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/21/3/149/346575/21-3-149.pdf by guest on 09 December 2024