binding is approximately twofold (Figures 1 and 2), this would indicate that monomer binds reduced coenzyme with twice the affinity of dimer. A fluorimetric study of NADH binding to the enzyme, performed at enzyme concentrations under which the enzyme was primarily in the dimeric form even after coenzyme was bound, could not discern a twofold difference in dissociation constants. On the other hand, oxidized coenzyme binding at low enzyme concentrations should show marked effects, since the results of Figures 1 and 2 indicate that the dimer binds NAD^+ at least an order of magnitude more tightly than the monomer. If the dissociation constant of a monomer–dimer equilibrium is affected by coenzyme binding, the two processes must be thermodynamically linked (Ackers and Halvorson, 1974). Regardless of the pathway, the affinity of NAD^+ for dimer will be much greater than for monomer. This aspect of the enzyme reaction mechanism is currently being investigated.

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Lipoprotein Lipase: Evidence for High- and Low-Affinity Enzyme Sites

C. J. Fielding

ABSTRACT: The kinetic constants for membrane-supported lipoprotein lipase have been determined for the enzyme active in lipoprotein triglyceride catabolism in perfused heart and adipose tissues, using a nonrecirculating system. Heart endothelial lipoprotein lipase reacted as a single population of high-affinity substrate binding sites (K_m' 0.07 mM triglyceride). K_m' (apparent Michaelis constant for the supported enzyme species) was independent of flow rate and the enzyme was rapidly released by heparin, suggestive of a superficial membrane binding site. Lipoprotein lipase active in perfused adipose tissue had significantly different kinetic properties, including a low substrate affinity (K_m' 0.70 mM triglyceride), diffusion dependence of K_m' at low flow rates, and slow release of enzyme by heparin. Adipose tissue may contain a small proportion of high affinity sites. While only a small proportion of total heart tissue lipoprotein lipase was directly active in triglyceride hydrolysis, this study suggests that the major part of lipoprotein lipase in adipose tissue may be involved in the hydrolysis of circulating lipoprotein triglyceride.

In a previous study (Fielding and Higgins, 1974) it was shown that the kinetic activity of membrane-supported lipoprotein lipase, based on a theoretical treatment for flow through an enzyme column (Lilly et al., 1966) followed predicted Michaelis–Menten kinetics for several lipoprotein substrates in a recirculating perfusion system. Such techniques provide a means of investigating the behavior of membrane–enzyme complexes whose lability has precluded their isolation, as is the case for the lipoprotein lipase system. They can also be used to determine the effect of the support environment, for example its electrical charge, on the kinetic characteristics of enzyme systems (Goldstein, 1972). Although the lipoprotein lipase system was apparently the first in which a natural membrane system was studied in this way, the techniques had been previously validated for several systems of soluble enzymes which had been immobilized on cellulose or polyacrylamide supports (Lilly et al., 1966; Goldman et al., 1971; Bunting and Laidler, 1972). It can be shown that, at low flow rates, while maximum reaction velocity is unchanged, the apparent Michaelis constant for the membrane-associated enzyme species becomes flow dependent below a limiting rate (Hornby et al., 1968; Sundaram et al., 1970). The apparent K_m'[1] value is dependent upon the rate constant of the en-

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[1] Abbreviations used in this paper are: S_f, flotation index at solvent density 1.063 g/ml; V_{max}', V_{max}, maximal reaction velocities for the supported and soluble enzyme species; K_m', K_m, apparent Michaelis constants for the supported and soluble enzyme species.

zyme at the membrane support and the diffusion coefficient of the substrate. In view of the high molecular weight, and low diffusion coefficient, of the natural lipoprotein substrates of this lipase system (Dole and Hamlyn, 1962), it was of interest to determine whether lipoprotein lipase activity is flow dependent. In the recirculating system previously reported, the reservoir solute must be in equilibrium with the reaction space for accurate rate determination (Fielding and Higgins, 1974). This limits the investigation of flow dependence to systems with high flow rates or very low levels of enzyme activity. This is not the case for nonrecirculation perfusion (Lilly et al., 1966), and equations can be generated which are valid for any flow rate (Hornby et al., 1968). The purpose of the present study was to validate the technique of noncirculation perfusion to determine the kinetic constants of lipoprotein lipase activity in perfused adipose tissue. The effects of flow rate on K_m' have also been determined for perfused heart and adipose tissues.

Experimental Section

Materials. Animal donors were male Sprague-Dawley rats. Donors for heart tissue weighed 300–350 g and were fasted overnight. Donors for adipose tissue (with free access to food) normally weighed 260–300 g but, to obtain kinetic values for a wide range of tissue lipase levels, a few experiments for comparative purposes were carried out with the former group, as specified. [*N*-Sulfonate-^{35}S]heparin, [1-^{14}C]- and [9,10-^{3}H]palmitic acid were purchased from Amersham-Searle (Chicago, Ill.). Insulin (crystalline) (24.3 IU/mg) was from Sigma (St. Louis, Mo.) and albumin (bovine, fraction V) was purchased from Reheis, Chicago, Ill.

Lipoprotein Preparation. Chylomicrons (flotation index $(S_f)^1$ 500–4000) (Dole and Hamlyn, 1962) were prepared from mesenteric lymph of donors prepared by cannulation of the mesenteric lymph duct and duodenum. The duodenum was infused with triglyceride–lecithin emulsion (Intralipid) (2.5% w/v triglyceride) mixed with 10–20 μCi/ml of radioactive palmitic acid (as albumin–palmitate complex) at a flow rate of 2 ml/h. Lymph recovered via the mesenteric duct was fractionated by preparative ultracentrifugation as previously described (Fielding and Higgins, 1974). Specifically, the lymph was centrifuged at 4 °C in the 40-rotor of a Spinco L3-50 ultracentrifuge for $9.5 \times 10^4 g$-min to remove the largest particles and then the major remaining chylomicron fraction recovered by flotation after a further centrifugation for $0.61 \times 10^6 g$-min. Particles recovered were repurified by low- and high-speed centrifugation under the same conditions, and their composition was determined in terms of protein content and content of the major lipid classes. This composition was not significantly different from that previously reported (Fielding and Higgins, 1974). More than 93% of chylomicron radioactivity was recovered in the triglyceride moiety as determined after extraction and chromatography. Lipoprotein solution (0.2 ml) was extracted with 0.75 ml of 1:2 v/v chloroform–methanol, and then the phases were separated by addition of 0.25 ml of 0.154 M NaCl and the same volume of chloroform. Portions of the organic phase after mixing and centrifugation were fractionated by thin-layer chromatography on glass plates coated with silica gel (Merck, Elmsford, N.J.) developed in hexane–diethyl ether–acetic acid 83:16:1 v/v. Chemical determination of triglyceride was according to Carlson (1963). Determination of triglyceride radioactivity was by liquid scintillation counting with [^{3}H]- or [^{14}C]toluene as internal standard. Specific radioactivity in these experiments was $1.5–3.5 \times 10^5$ dpm/μmol triglyceride.

Perfusion Procedures. Perfusion of epididymal adipose tissue was as described by Ho and Meng (1964). Perfusion was carried out in a medium of Krebs–Ringer bicarbonate buffer containing 10% v/v of rat plasma (which had been previously depleted of triglyceride-rich lipoproteins by centrifugation for $1.65 \times 10^8 g$-min), 3% w/v albumin, pH 7.4 (previously dialyzed against 1000 volumes of 0.154 M NaCl), glucose (0.25 mg/ml), insulin (100 μU/ml), and chylomicrons (0.05–2.0 μmol of triglyceride/ml). The salt concentration of the medium was adjusted by addition of 0.154 M solutions of $CaCl_2$, KH_2PO_4, KCl, $MgSO_4$, and $NaHCO_3$ to maintain the proportions present in Krebs–Ringer bicarbonate buffer, and it was continuously gassed with 95% O_2–5% CO_2. Perfusion medium was passed via the spermatic artery into the tissue vascular space, which was maintained in a perfusion chamber at 37 ± 0.5 °C. The outflow was collected from the spermatic vein. Blood was washed from the tissue with perfusion medium at a flow rate of 0.45 ml/min for 5 min. Subsequent perfusion was at a flow rate of 0.05–0.6 ml/min. Tissue wet weight was 1.1–1.5 g (mean 1.24 ± 0.15 g) for 260–300 g animals. Perfusion period was up to 75 min. Perfusion pressure was 20–30 mmHg. No lipoprotein lipase activity was released from the perfused tissue in the absence of added heparin, as described below. O_2 consumption and CO_2 production were maintained essentially constant throughout the perfusion period. For a flow rate of 0.45 ml/min, inflow O_2 pressure was 530–560 mmHg and outflow O_2 was 120–140 mmHg. Corresponding figures for CO_2 were 17 and 36–41 mmHg. In preliminary experiments, it was shown that there was no significant difference between the wet weights of contralateral epididymal fat pads. The wet weight of the perfused pad was therefore compared after perfusion with that of the nonperfused contralateral tissue. In some experiments, the dry wt/wet wt ratio of each was compared. There was no significant decrease in this ratio during perfusion. In this study perfused pads had a final wet weight which was 102 ± 4% the weight of the nonperfused tissue from the same animal. In some experiments adipose tissue was perfused with the same medium containing heparin (10 μg/ml; 10^4 dpm/μg). Fractions (0.2–0.5 ml) were collected and assayed for lipoprotein lipase activity and heparin radioactivity. Lipase released was assayed in medium containing 2.0 μmol/ml chylomicron triglyceride for 30 min at 37 °C. For each fraction control assays were immediately extracted with chloroform–methanol as described above. Residual lipase activity after perfusion with heparin (normally for 20 min) was determined after homogenization of the tissue in 50% plasma–0.154 M NaCl. The homogenate was extracted with 200 volumes of acetone at 0–2 °C and then the precipitate was filtered and washed with the same volume of acetone at 25 °C and 200 ml of diethyl ether under the same conditions. Remaining organic solvent was removed under vacuum and the dried tissue extracted with 50% plasma–0.154 M NaCl (Fielding, 1968). The tissue homogenate, from which other lipase activities had been removed by the delipidation procedure (Korn, 1955), was assayed with the chylomicron-containing medium described above. Enzyme activity is expressed as micromoles of triglyceride hydrolyzed per minute at 37 °C and pH 7.4. Values from the perfused and nonperfused contralateral tissues were compared. In experiments where the tissue had been perfused with heparin, perfusate and residual lipase activities

of the perfused tissue were compared with the total activity of the nonperfused tissue from the same animal. The apparent Michaelis constant for lipoprotein lipase activity solubilized by heparin was determined from the double reciprocal plots of $1/v$ vs. $1/S$ for at least five experimental points for reaction with chylomicron triglyceride at pH 7.4. The stimulation by heparin of adipose tissue lipase (2.0 ± 0.1 fold) under these experimental conditions was not significantly different from that previously determined for the endothelial heart lipase (Fielding and Higgins, 1974), and this correction factor has been applied in determining reaction velocities in the presence of heparin.

Isolated rat hearts were perfused without recirculation by the modified Langendorff procedure described by Morgan et al. (1961). The perfusion medium was the same as that used for adipose tissue except that insulin was not present and the glucose concentration was 0.5 mg/ml. These conditions were those previously shown to maintain endothelial lipoprotein lipase activity, and its triglyceride hydrolysis rate, unchanged during perfusion for at least 90 min. Hearts were perfused with chylomicrons (0.02–1.0 μmol of triglyceride per ml) with the medium described. Effluent triglyceride concentration was determined after extraction by thin-layer chromatography as described above for adipose tissue. Endothelial lipoprotein lipase, released by perfusion with 10 μg per ml of heparin, was assayed in the perfusate for hydrolytic activity with chylomicron triglyceride as described above.

In both perfused adipose and heart tissues, the effects of medium triglyceride concentration upon hydrolysis rate were determined by passing sequentially through the tissue media containing 4–7 different chylomicron triglyceride concentrations. In the case of adipose tissue, where the flow rate was low, samples were assayed sequentially throughout the perfusion period. In heart tissue, with more rapid flow rates, each reservoir was washed for 1–4 min through the tissue before sampling of the triglyceride concentration of the inflow and outflow media.

Determination of Kinetic Constants. From the Michaelis–Menten equation:

$$(s_0 - s_t) = k_{cat}(E/v)t + K_m \ln (s_t/s_0) \quad (1)$$

where s_0 is the initial concentration of substrate, s_t the final substrate concentration, E the enzyme content, v the reaction volume, and k_{cat} and K_m the catalytic and Michaelis constants for the reaction.

For substrate flow through an enzyme column at rate Q

$$v = Qt \quad (2)$$

where t is the time spent within the reaction space.

For maximal reaction velocity

$$V_{max} = k_{cat}E \quad (3)$$

$$(s_0 - s_t) = V_{max}'/Q + K_m' \ln (s_t/s_0) \quad (4)$$

where V_{max}' and K_m' are the corresponding kinetic values for the membrane supported enzyme. For a constant flow rate Q, the plot of $(s_0 - s_t)$ has a slope K_m' and an intercept of V_{max}'/Q. If V_{max}' is independent of flow rate (Hornby et al., 1968; Sundaram et al. 1970), then, at a constant s_0, K_m' can be determined for any Q (eq 4). To determine K_m', V_{max}' at constant flow rate s_0 was varied to give $s_t = f(s_0)$. Using the same enzyme system and constant s_0, K_m' was determined as a function of Q.

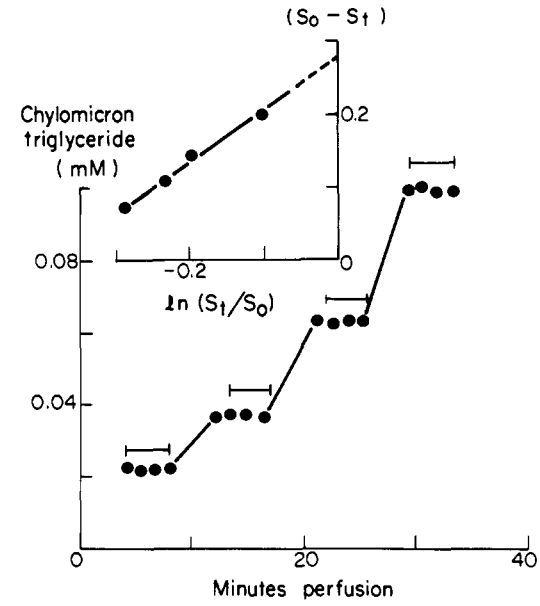

FIGURE 1: Removal of triglyceride by the perfused heart as a function of medium triglyceride concentration. Reservoirs contained 0.03, 0.05, 0.078, and 0.12 mM of chylomicron triglyceride. After passage of medium for 2 min, samples were taken at 45 s intervals for analysis of triglyceride content (s_t) together with the same number of samples of reservoir medium (s_0). Insert: the plot of ln (s_t/s_0) vs. $(s_0 - s_t)$. K_m' for this experiment was 0.07 mM triglyceride and calculated V_{max}', −0.059 μmol of triglyceride/min.

Results

Triglyceride Hydrolysis at Constant Flow Rate. Triglyceride removal from perfused adipose tissue remained essentially constant during a perfusion period of at least 60 min under the conditions specified. Over longer periods removal rates declined. Triglyceride removal from the nonrecirculating heart system with the same medium was also unchanged over a 60-min period. These conditions were confirmed for the range of flow rates used in these experiments. K_m' and V_{max}' were determined for a range of initial substrate (s_0) concentrations at a fixed flow rate under conditions giving a proportional triglyceride removal $(s_0 - s_t)/s_0$ of from 0.08 to 0.40. Previous studies with the perfused heart had shown that the kinetic properties of chylomicrons remain almost unchanged in the course of triglyceride hydrolysis until more than 50% of initial triglyceride content has been hydrolyzed (Higgins and Fielding, 1975). In both heart and adipose tissues, predicted Michaelis–Menten kinetics were accurately followed in experiments using 4–6 successive medium triglyceride concentrations (Figures 1 and 2). In heart tissue K_m' was similar to the value determined by recirculation perfusion (Table I). However, the value for adipose tissue lipoprotein lipase was greater by an order of magnitude (0.70 mM triglyceride vs. 0.07 mM triglyceride). Maximum reaction velocities were calculated from the intercept of the plots of ln (s_t/s_0) vs. $(s_0 - s_t)$ for a known flow rate. These were compared with the heparin-released and residual enzyme activities assayed at the end of the perfusion period.

Release of Lipoprotein Lipase by Heparin. Heparin rapidly released lipoprotein lipase activity from the perfused heart. As shown in Table I, maximal reaction velocity determined from perfusion data was satisfactorily correlated with the level of total activity releasable by heparin from the heart. Release was complete within 2 min. This repre-

Table I: Kinetic Constants of High- and Low-Affinity Lipoprotein Lipases.[a]

Enzyme Source	V_{max}'	V_{max}	V_{max}'/V_{max}	K_m'	K_m[d]	K_m'/K_m
	(μmol of triglyceride/min)			(mM triglyceride)		
Heart (HA)	0.06 ± 0.01	0.06 ± 0.01[b]	1.03	0.07 ± 0.01	0.08 ± 0.01	0.88
Adipose tissue (LA)	0.12 ± 0.02	0.11 ± 0.01[c]	1.09	0.70 ± 0.10	0.23 ± 0.02	3.10

[a] Values are means ± for four experiments. Heart high-affinity (HA) activity was determined at 4.0 ml/min flow rate and adipose tissue low-affinity (LA) activity at 0.45 ml/min. [b] For the fraction released by heparin, assayed at pH 7.4, 37 °C. [c] Total adipose tissue lipase activity, assayed at pH 7.4, 37 °C. The contribution for high-affinity activity has not been subtracted. [d] For soluble lipoprotein lipase, released by heparin. In the case of perfused heart, activity released within 1 min was assayed with chylomicron substrate; in the case of perfused adipose tissue, activity collected at periods between 15 and 45 min post-heparin was assayed under the same conditions. HA: high-affinity lipase of heart; LA: low-affinity lipase of adipose tissue.

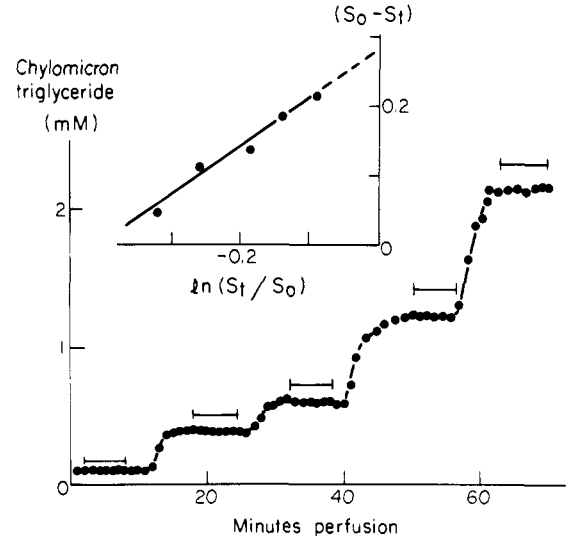

FIGURE 2: Removal of triglyceride by perfused adipose tissue as a function of medium triglyceride concentration. Reservoirs contained 0.15, 0.53, 0.74, 1.47, and 2.32 mM triglyceride. Samples were collected each minute for analysis of medium triglyceride (s_t) together with samples of each reservoir (s_0). Insert: the plot of ln (s_t/s_0) vs. ($s_0 - s_t$). K_m' for this experiment was 0.68 mM triglyceride and calculated V_{max}', −0.12 μmol of triglyceride/min.

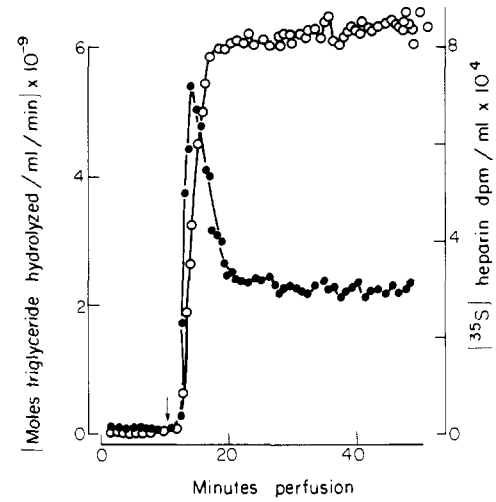

FIGURE 3: Release of lipoprotein lipase from perfused adipose tissue by heparin: (○) heparin radioactivity; (●) lipase activity (mol × 10^{-9} of triglyceride hydrolyzed ml^{-1} min^{-1}) at pH 7.4, 37 °C. Perfusion rate was 0.45 ml/min and heparin was added to the reservoir medium at the point indicated (↓).

sented about one-third of the total of heparin-released plus residual activity assayed under the same conditions (0.32 ± 0.05; mean ± SD, four experiments). Heparin also released lipase from adipose tissue but, as shown in Figure 3, in this case the pattern of release was quite different. Heparin initially rapidly released a small peak of activity. The apparent K_m of this enzyme fraction, as measured with activity released within 3 min post-heparin, was 0.08 ± 0.01 mM triglyceride. This value was not significantly different from the value found for the activity released by heparin from the heart. Activity was then released from adipose tissue at a constant rate for the remainder of the perfusion period (up to 40 min). K_m for solubilized lipase released between 15 and 40 min post-heparin was significantly greater than for the fraction rapidly released (0.23 ± 0.02 mM triglyceride; Table I).

Preliminary experiments indicated no significant difference in the levels of lipoprotein lipase activities in the nonperfused tissue and the perfused tissue before heparin (0.12 ± 0.02 vs. 0.11 ± 0.02 μmol triglyceride hydrolyzed per min; four experiments). As shown in Table II, the sum of solubilized and residual lipase activities in the perfused tissue correlated well with the enzyme content of the contralateral, nonperfused tissue. Overlap of the initial peak and residual solubilized activity prevented exact measurement of the former but it obviously represented only a minor fraction of the activity releasable from adipose tissue. On the other hand, comparison of the V_{max}' value obtained from the perfusion studies indicated that this was well correlated, in the case of adipose tissue, with the whole lipoprotein lipase content of the tissue determined after acetone-ether extraction (Table I).

Flow Rate Dependence of K_m'. The flow dependence of K_m' was determined by nonrecirculation perfusion of heart and adipose tissues as described under Methods. Adipose tissue was perfused at flow rates of from 0.045 to 0.5 ml/g of wet weight and, in the case of heart tissue, the rate was 0.6–2.5 ml/g of wet weight. For comparative purposes, results were expressed in terms of flow rate per ml of perfused volume/min, using total perfusion volume values of 0.029 ml/g of wet weight for adipose tissue (Mayerle and Havel, 1969) (converting from total blood volume for a hematocrit of 0.48) and of 0.42 ml/g of wet weight for heart tissue (Morgan et al., 1961). As shown in Figure 4, K_m' for heart endothelial lipase was independent of flow rate over the entire experimental range including the physiological rate of about 4 ml/ml of perfused volume/min. On the contrary, K_m' for adipose tissue lipoprotein lipase in the perfused tissue was flow dependent at rates below about 0.3 ml/g of

Table II: Soluble and Residual Fractions of Adipose Tissue Lipoprotein Lipase.

Experiment	Perfused Tissue			Nonperfused Tissue
	Soluble Enzyme[a]	Residual Enzyme	Total	Total
1	0.071	0.312	0.398	0.383
2	0.019	0.069	0.088	0.091

[a] Values are for the highest and lowest enzyme activities of five experiments. Mean ratio of soluble to total enzyme in all experiments was 0.21 ± 0.04 when soluble enzyme was the total activity released during 20 min perfusion with medium containing heparin (10 μg/ml). Experiment 1 was for tissue for a 350-g animal; experiment 2 was representative of four experiments with animals weighing 200–250 g. Residual tissue enzyme and total enzyme from the nonperfused tissue were determined after delipidation with acetone–ether (Fielding, 1968). Enzyme activity is expressed as μmol of triglyceride hydrolyzed/min at 37 °C.

wet weight, and K_m' at the physiological flow rate of about 0.07 ml/g of wet weight (Mayerle and Havel, 1969) was about 1.5 times the minimum value of 0.70 mM triglyceride found at the limiting flow rate, i.e., about 15 times the value for the active fraction of heart lipoprotein lipase under the same conditions.

Discussion

In this study the kinetic constants of reactive lipoprotein lipase in perfused heart and adipose tissues have been obtained using a novel treatment for nonrecirculation perfusion. In the former case values have been compared with those obtained previously by recirculation perfusion (Fielding and Higgins, 1974). The major assumptions made in the present research appear to be as follows. The enzyme space was assumed to be represented by the tissue plasma volume; i.e., the volume occupied by the enzyme itself was negligible, and the reactive lipase was located at or near the membrane boundary. However, this assumption affects V_{max}' but not K_m' (Lilly et al., 1966). Secondly, it was assumed that maximal reaction velocity was independent of flow rate. This is predicted by theory (Sundaram et al., 1970) and has been borne out in practice in other nonrecirculation column systems (Hornby et al., 1968). Thirdly, it has been assumed that the kinetic properties of lipase reacting with triglyceride remain unchanged in the course of removal of at least 40% of initial triglyceride content. This has been shown directly for heart endothelial lipase (Higgins and Fielding, 1975). In the case of adipose tissue lipase this has not been measured directly but, because particles of almost identical composition are produced during chylomicron remnant formation in the perfused heart and by the total extrahepatic tissues (Mjos et al., 1975), the assumption seems a reasonable one. If the dependence of k_{cat}' on residual triglyceride content was greater for adipose tissue, then the difference in K_m' values between heart and adipose tissues would be increased.

In both tissues ln (s_t/s_0) vs. $(s_0 - s_t)$ gave linear plots as predicted by eq 4 (Figures 1 and 2), suggesting the presence of only one major reactive enzyme species in each case. For heart tissue, K_m' for endothelial (active) lipase was 0.07 ± 0.01 mM triglyceride by nonrecirculation perfusion. This value can be compared with values of 0.09 ± 0.01 mM by recirculation perfusion and 0.08 ± 0.01 mM for the same enzyme after solubilization (Fielding and Higgins, 1974).

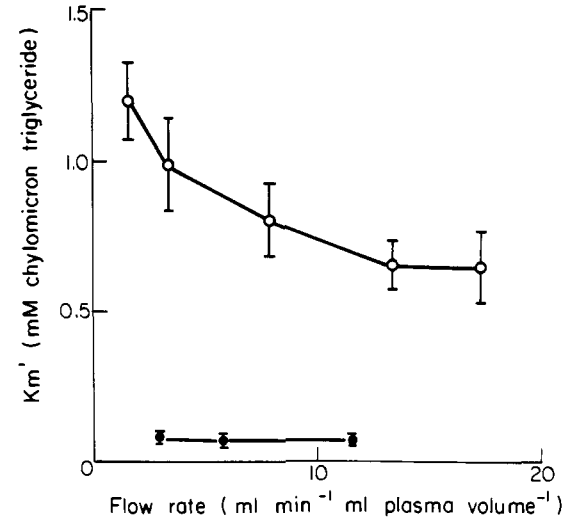

FIGURE 4: Flow-dependence of apparent Michaelis constant (K_m') for perfused heart and adipose tissues. Flow rates/min were converted to flow rates per ml plasma space/min (Mayerle and Havel, 1969; Morgan et al., 1961). Values are calculated from s_t values at constant s_0 after determination of V_{max}'/Q from the plot of ln (s_t/s_0) vs. $(s_0 - s_t)$ as described under Methods. Values for each point are means ±SD: (●—●) perfused heart; (○—○) perfused adipose tissue.

In this tissue the presence of a single population of high-affinity enzyme sites is suggested by (1) the absence of a slowly released enzyme population, (2) the absence of residual catabolic activity after release of the high-affinity fraction by heparin (Fielding and Higgins, 1974), and (3) the competitive kinetics shown for removal of triglyceride from lipoprotein substrates with different kinetic characteristics when mixtures of these were catabolized by this lipase fraction (Higgins and Fielding, 1975).

For enzyme in perfused adipose tissue, K_m' was significantly greater (0.7 ± 0.1 mM triglyceride). Additionally the relationship between the active enzyme fraction, determined kinetically, and activity released by heparin was quite distinct. Since the activity of enzyme in contralateral pads was not significantly different before and after perfusion, total lipase activity of the tissue was maintained under the conditions described; this was also shown by the proportional removal of triglyceride maintained at fixed s_0. Since the sum of perfusate activity after heparin and residual lipase activity was equivalent to the total activity of the nonperfused tissue (Table II), lipase assayed after delipidation quantitatively represented the activity present and unchanging in the course of perfusion. However, as shown in Table I the maximal rate of triglyceride hydrolysis, determined kinetically, was similar to the total lipoprotein lipase activity of this tissue. It seems, therefore, possible that the major part of this enzyme activity in the tissue is directly involved in the catabolism of circulating triglyceride. As shown in Figure 3 a small amount of activity was rapidly released by heparin with a substrate affinity (0.08 ± 0.01 mM triglyceride) not significantly different from that of heart endothelial lipoprotein lipase. However, this is unlikely to provide any major part of effective adipose tissue triglyceride hydrolytic capacity because this would require its catalytic constant to be much greater at the tissue site than in solution, which would be quite contrary to the earlier result with high affinity lipase activity (Fielding and Higgins, 1974). The effect of any major contribution of this lipase in perfused adipose tissue would also be to decrease the apparent Michaelis constant determined from low substrate con-

centrations. This was found not to be the case (Figure 2), confirming the minor contribution of high affinity enzyme sites to activity in this tissue.

In perfused adipose tissue, K_m' at the lowest flow rates (about 0.04 ml/g of wet weight tissue) was about 1.5 times the minimum value. Formally, decreased apparent substrate affinity, in terms of increased K_m', could result either from partition of substrate between the bulk and supporting phases or because the reaction at the supported site was diffusion limited. Only the latter would be flow dependent although partition, or steric changes induced by solubilization of the enzyme, could result in differences between the K_m' of the soluble and supported lipase species. In the case of low-affinity lipase, K_m' was significantly lower after solubilization (Table I). Both these effects are compatible with the concept that lipase at low-affinity sites is located in an environment away from the bulk plasma phase. This is also suggested by the slow release of lipase by heparin from these sites into the plasma space. It has been shown that in adipose tissue the major part of tissue lipoprotein lipase was associated with the fat cell fraction when adipocytes were dissociated with collagenase. The total recovery of enzyme in these experiments was about 75% of that present in the original tissue (Rodbell, 1964). Since evidence discussed above suggests that a major part of enzyme activity in adipose tissue may be directly reactive in the hydrolysis of perfused triglyceride, it appears that the low-affinity sites may be associated with the adipocytes themselves, perhaps at the fat cell membrane. However, this study does not rule out the possibility that the activity observed is a function of a smaller fraction of highly active enzyme, at a less superficial endothelial site.

In the perfused heart, lipoprotein lipase activity was independent of flow rate, kinetic constants were not significantly modified on solubilization, and the active enzyme fraction was rapidly released by heparin. These results confirmed and extended the previous data which suggested that high-affinity lipase sites were located at the endothelial vascular surface (Fielding and Higgins, 1974). This comparison of results from heart and adipose tissues suggests that lipoprotein lipases from high- and low-affinity sites have quite distinct kinetic properties but that both can be recovered in post-heparin perfusate and presumably, in the post-heparin plasma of the intact animal.

Some properties of such a mixed enzyme system in the whole animal can be predicted. At low substrate concentrations, available lipoprotein triglyceride would be hydrolyzed preferentially by the high affinity sites, located in heart, and perhaps other muscle tissues, and provide unesterified fatty acids for oxidation. At high substrate concentrations total hydrolysis rate would be mainly determined by the level of enzyme present in the low-affinity sites of adipose tissue, providing fatty acid for re-esterification and triglyceride storage. In this way the kinetic properties of the enzyme system itself would contribute to regulation of hydrolytic rate in different tissues, as a function of circulating triglyceride substrate concentration.

Nonrecirculation perfusion experiments, such as those described here, can be used for obtaining values of the kinetic constants for the different enzyme populations, and in that way provide a means of predicting overall reaction rates for different conditions of substrate concentration.

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