Lipoprotein Lipase. Mechanism of Formation of Triglyceride-Rich Remnant Particles from Very Low Density Lipoproteins and Chylomicrons†

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ABSTRACT: The catalytic rate of membrane-supported lipoprotein lipase has been determined for chylomicron and very low density lipoprotein substrates during the formation of triglyceride-depleted ("remnant") particles. Both lipoprotein species and their generated remnant products were competitive substrates for lipase activity. Remnant formation from each species was associated with decreasing k_c but an unchanged apparent K_m. This finding was confirmed from the rate plot of total triglyceride catabolism by lipase at low substrate concentrations. When compared with the major very low density lipoprotein fraction (S_f 100–400), a fraction isolated from plasma with a lower flotation rate (S_f 40–100) had a lipid composition and decreased k_c compatible with this representing a physiological remnant particle.

Both chylomicrons and very low density lipoproteins are substrates for lipoprotein lipase at the rat heart endothelial membrane (Delcher et al., 1965; Borensztajn and Robinson, 1970). Chylomicron triglyceride is hydrolyzed at a significantly greater rate by both the membrane-supported and solubilized lipase species (Fielding and Higgins, 1974a). The rate of activity of this lipase is increased by a specific polypeptide (C-terminal glutamic acid) which is a component of the protein moiety of both lipoprotein substrates (Havel et al., 1973a) and whose kinetic effect at physiological pH is limited to increasing the catalytic constant k_c[1] of lipolysis (Fielding, 1973). During the catabolism of these lipoproteins, transfer of at least part of the lipase cofactor protein component to nonsubstrate lipoproteins of the plasma has been reported (Eisenberg and Rachmilewitz, 1973). Earlier studies have suggested that lipoprotein lipase is active in only the initial stages of triglyceride depletion and that generated "remnant" particles, still containing much of the original triglyceride content of the particle, are the end product of enzyme activity (Redgrave, 1970). On the other hand, lipoprotein lipase, at least at low substrate concentrations, could degrade almost completely the triglyceride moiety of very low density lipoproteins (Fielding and Higgins, 1974b). The purpose of the present study has been to investigate the basis of substrate selection by rat heart membrane lipase in the presence of both intact substrates and remnant particles.

Experimental Section

Lipoprotein Preparation. Animal donors were male Sprague-Dawley rats, 300–350 g. Chylomicrons (S_f 400–5000)[1] were isolated by ultracentrifugation from animals carrying a cannula in the mesenteric lymph duct and fed triglyceride–lecithin emulsion (Intralipid, Vitrum) mixed with 1-[^{14}C]- or 9,10-[^{3}H]palmitic acid (Amersham-Searle) as palmitate–albumin complex (Felts and Masoro, 1959). After an initial centrifugation at 9.5×10^4 g-av. min to remove the largest triglyceride particles (S_f >5000), the fraction required was obtained by further centrifugation at 0.61×10^6 g-av. min. This fraction was washed twice more by flotation under the same conditions. Very low density lipoproteins (S_f 100–400 or 40–100) were isolated from the plasma of animals injected 30–40 min previously with radioactive palmitate–albumin complex. The first of these fractions was obtained by centrifugation for 4.86×10^6 g-av. min (after discarding the 0.61×10^6 g-av. min fraction) and the second by a further centrifugation at 1.27×10^7 g-av. min. Both were washed by a second flotation under the same conditions. The purified lipoproteins were analyzed for their content of protein and lipid classes as described previously (Fielding and Higgins, 1974a). The composition of the chylomicron and very low density lipoprotein (S_f 100–400) fractions was not significantly different from that reported earlier. Composition of the smaller plasma lipoprotein fraction (percent by weight) was as follows (five preparations ± SD): triglyceride, 69.2 ± 0.4; phospholipid, 10.2 ± 2.0; protein, 12.7 ± 1.3; unesterified cholesterol, 4.4 ± 0.3; cholesteryl ester, 3.4 ± 1.0. Analysis of triglyceride radioactivity was by liquid scintillation counting with [^{14}C]- or [^{3}H]toluene as internal standard of lipid extracts separated by thin-layer chromatography on silica gel layers on glass plates developed in hexane–diethyl ether–acetic acid (83:16:1, v/v).

Perfusion Procedure. Isolated rat hearts from animals fasted overnight were perfused by recirculation by a modification of the procedure of Morgan et al. (1961) as previously described (Fielding and Higgins, 1974a). Specifically, hearts were perfused in a recirculating system with a medium containing either single lipoprotein species (chylomicrons, very low density lipoproteins, or generated triglyceride-depleted particles) or mixtures of these, as described below, in the presence of albumin (3% w/v, bovine fraction V) (Reheis), rat plasma [from which endogenous very low density lipoprotein or very low density + low density lipo-

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[1] Abbreviations used are: S_f, flotation index at solvent density 1.063 g/ml; k_c' and K_m', catalytic constant and apparent Michaelis constant for membrane-bound lipoprotein lipase (Fielding and Higgins, 1974a).

proteins had been removed by previous centrifugation at *d* 1.006 or 1.063 g/ml (10% v/v)], and glucose (5 mg/ml) in a medium of Krebs–Ringer bicarbonate buffer (pH 7.4) which was gassed continuously with 95% O_2–5% CO_2. Under these conditions the rate of lipoprotein lipase mediated hydrolysis of lipoprotein triglyceride, determined as described below, was maintained for at least 60 min. Neither the lipolytic rate nor the composition of remnant particles obtained by perfusion showed changes when higher concentrations of rat plasma (up to 30% v/v) were incorporated into the perfusion medium.

The rate constants determined for the membrane-supported lipase species were not significantly different from the same constants determined for the solubilized lipase species (Fielding and Higgins, 1974a). In most experiments hearts were perfused with a sequence of media containing different lipoprotein species. Triglyceride hydrolysis was determined by assay of sequential duplicate or triplicate 0.1-ml samples of perfusion medium at five or six time points over a 15-min period. The rate of lipase activity was expressed as micromoles of substrate depletion per milliliter of recirculating medium (corrected to the original perfusion volume). Triglyceride depletion in these experiments was limited to 10% and usually to 5% of initial substrate concentration. Under the experimental conditions described there was no release of lipase into the medium in the absence of added heparin.

Preparation of Remnant Particles. Triglyceride-depleted "remnant" particles were generated by pre-perfusion in the recirculating system until 10–90% of original triglyceride content had been removed. Prepared particles for chemical analysis of protein and lipid components were isolated from contaminating lipoproteins and other plasma protein by gel filtration at 3–5° (Sata et al., 1972). Columns of Sepharose 2B (Pharmacia) (50 × 2 cm) were equilibrated with 0.154 *M* NaCl–0.01% sodium azide. Samples of perfusion medium (2 ml) containing intact or remnant lipoproteins were chromatographed with a flow rate of 15–20 ml/hr. Column fractions were assayed for protein and triglyceride and for triglyceride radioactivity. Triglyceride radioactivity of both intact and remnant chylomicrons was completely separated from low and high density lipoprotein and was recovered in the column void volume. Remnants from very low density lipoprotein, prepared in the presence of plasma ($d > 1.063$), were completely separated from other plasma proteins by gel column chromatography. Recovery of added lipoprotein triglyceride radioactivity was complete (98–101%). The composition of the purified lipoprotein particle was unchanged by a second chromatographic separation under the same conditions. Formation of remnant particles was also followed by analysis of the flotation pattern of samples of perfusion medium in a linear sucrose gradient (Pinter and Zilversmit, 1962). Samples (0.5 ml) mixed with 0.1 ml of 82% sucrose (w/v) were layered between a cushion of 0.5 ml of 20% sucrose and a 9.5-ml linear gradient (5–10% sucrose) in 0.154 *M* NaCl in the centrifuge tube of the Spinco SW-41 rotor. The gradients were centrifuged for between 15 min and 2 hr at 17,500 or 35,000 rpm depending on the flotation index of the particles under investigation. The triglyceride content of fractions collected dropwise after centrifugation was then determined as described above.

Determination of Kinetic Constants. Apparent k_c' and K_m' values for membrane-associated lipoprotein lipase were determined at high ($[S] \gg K_m'$) and low ($[S] \ll K_m'$) substrate concentrations for single substrates (Fielding and Higgins, 1974a). Since lipase was reactive with both chylomicrons and very low density lipoproteins the removal rates of these substrates from mixtures of lipoprotein species, under competitive conditions, were given by:

$$\frac{v_1}{v_2} = \frac{k_c'(1)K_m'(2)[S_1]}{k_c'(2)K_m'(1)[S_2]} \quad (1)$$

where v_1 and v_2 are the component reaction velocities with substrates 1 and 2 at concentrations $[S_1]$ and $[S_2]$ and where $k_c'(1)$ and $k_c'(2)$ and $K_m'(1)$ and $K_m'(2)$ are the apparent catalytic and Michaelis constants for the membrane-supported lipase with each lipoprotein species. The experimental removal ratio determined for substrate pairs carrying alternate radioactive labels was compared with the calculated ratio determined from eq 1. A second investigation of the kinetic dependence of lipoprotein triglyceride content was made from the plot of [S] vs. time at low substrate concentrations ($[S] \ll K_m'$) when:

$$v = k_c'[S][E]/K_m' \quad (2)$$

such that at constant enzyme concentration, [E], modification of k_c' or K_m' will be expressed as a deviation from the linear plot of first-order kinetics. In the present research these procedures have been compared to determine the dependence of the kinetic constants of lipoprotein lipase on the triglyceride content of the lipoprotein substrate.

Results

Formation of Remnant Particles by Lipoprotein Lipase. Chylomicrons or very low density lipoproteins were hydrolyzed by lipase in the presence of plasma and albumin. As shown in Table I the composition of the substrate particles in the course of hydrolysis was consistent with the formation of particles of decreasing diameter, containing a core of triglyceride and cholesteryl ester and a surface layer of phospholipid, unesterified cholesterol, and protein (Sata et al., 1972). In particular, analysis of component lipid classes indicated that triglyceride depletion was associated with removal of phospholipid but little or no transfer away of unesterified cholesterol. There was only a minor reduction in the content of total protein. Since lipase in the present experiments was determined as the rate of triglyceride depletion it was important to determine to what extent triglyceride–cholesteryl ester exchange, as described by Nichols and Smith (1965), might contribute to the loss of substrate triglyceride during remnant formation. As shown in Table I, there was a small net entry of cholesteryl ester into the triglyceride-rich fraction during remnant formation. However, it can be readily calculated that even if this entry is completely reciprocal for triglyceride on a molar basis (rather than representing minor activity of lecithin:cholesterol acyltransferase with triglyceride-rich substrate) (Akanuma and Glomset, 1968), no more than 5% of triglyceride loss from very low density lipoprotein would be by this mechanism, and much less from chylomicrons (Table I). It is possible, however, that this process may be of greater significance in the formation of very small remnant particles (triglyceride content < 10%) not studied in this research, although other evidence (see below) suggests that particles of this composition may be quantitatively unimportant in triglyceride hydrolysis by lipoprotein lipase. Both lipolytic rate and the composition of the generated remnant particles were unchanged by the substitution of lipoprotein-depleted plasma ($d > 1.063$) for plasma ($d > 1.006$) in the perfusion medium.

Table I: Lipid and Protein Composition of Intact and Remnant Lipoproteins.[a]

Relative Triglyceride Content	Very Low Density Lipoproteins				Chylomicrons			
	1.0	0.76	0.54	0.25	1.0	0.60	0.25	0.19
Triglyceride	205 (80.7)	156 (78.2)	110 (75.5)	52 (55.1)	761 (93.2)	460 (90.2)	190 (85.3)	146 (82.1)
Phospholipid	21 (8.5)	18 (8.8)	15 (10.6)	11 (11.8)	32 (3.9)	29 (5.7)	16 (7.0)	13 (7.5)
Protein	18 (7.3)	16 (7.8)	14 (9.5)	17 (18.5)	20 (2.4)	16 (3.2)	13 (5.9)	14 (8.2)
Free cholesterol	4 (1.7)	4 (2.1)	4 (2.9)	4 (3.8)	2 (0.3)	2 (0.4)	2 (0.7)	1 (0.8)
Cholesteryl ester	4 (1.7)	6 (3.0)	7 (4.8)	10 (10.8)	2 (0.2)	2 (0.5)	3 (1.1)	3 (1.5)

[a] Lipids were determined as previously described (Fielding et al., 1974), and analyses were reproducible ± 3% in each case. In the experiment shown, samples were taken for analysis at zero time and after 35, 70, and 90 min of perfusion as described in the Experimental Section, in the presence of $d > 1.063$ plasma. Values presented are means of duplicate analyses from experiments representative of five experiments with chylomicrons and four with very low density lipoproteins in each case analyzing the composition of the original lipoprotein and three generated remnant particles. Experimental values are expressed as micrograms of lipid/milliliter of perfusate. Values in parentheses represent compositions by weight. Triglyceride is expressed as triolein, phospholipid in terms of a mol wt of 750, protein as albumin, and cholesteryl ester as cholesteryl oleate.

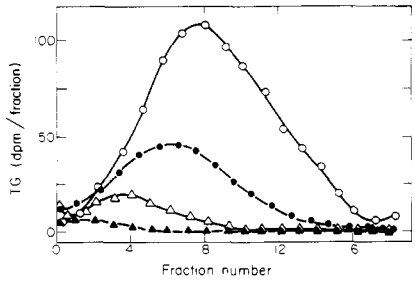

FIGURE 1: Flotation of intact and remnant very low density lipoproteins in a sucrose density gradient. Centrifugation was for 1 hr at 35,000 rpm. Samples of perfusion medium layered under the gradient contained the following proportions of original triglyceride content: (○) 1.0; (●) 0.49; (△) 0.13; (▲) 0.04.

Table II: Hydrolysis of Chylomicron and Plasma Lipoprotein Triglyceride.[a]

Lipoprotein Concn (mM)		Rel Hydrolysis Rate (a/b)	
Very Low Density Lipoproteins (a)	Chylomicrons (b)	Calcd	Exptl
0.89	0.05	17.5	16.6; 16.8
1.11	0.12	8.3	7.1; 8.4
1.13	0.22	4.6	4.8; 4.1
0.85	0.31	2.5	3.0; 3.6
1.19	1.06	1.0	1.1; 1.5

[a] Values are from two experiments. Relative rate is the ratio between the hydrolytic rates of very low density lipoproteins and chylomicrons. Calculated relative reaction rates were determined using k_c' values of 1.80×10^3 min^{-1} (very low density lipoprotein) and 3.16×10^3 min^{-1} (chylomicrons) and apparent K_m' values of 0.053 mM (very low density lipoprotein) and 0.083 mM (chylomicrons) (Fielding and Higgins, 1974a).

Further analysis of remnant formation was carried out by sucrose density gradient centrifugation (Figure 1). The results obtained indicated formation of a continuous distribution of particle sizes as a result of catabolism from the original particle population. That is, lipolysis was taking place both with intact particles and also with particles whose triglyceride content had already been hydrolyzed in part by prior action of the enzyme. The probable mechanism of this reaction is discussed below. Subsequent experiments analyzed the factors regulating the rate of reaction of substrates at the membrane site as a function of their residual triglyceride content.

Competitive Hydrolysis of Lipoprotein Triglyceride. Triglyceride hydrolysis was determined in media containing both very low density lipoproteins (S_f 100–400) and chylomicrons. K_m' and k_c' for these substrates have been previously reported (Fielding and Higgins, 1974a). K_m' for very low density lipoproteins (S_f 40–100) was not significantly different from that of the larger plasma lipoprotein fraction. k_c' was significantly lower [$1.1 \pm 0.1 \times 10^3$ min^{-1} vs. $1.8 \pm 0.2 \times 10^3$ min^{-1}; three experiments].

The initial rates of hydrolysis of plasma lipoproteins and chylomicrons were determined using media containing mixtures of these species in different proportions. The ratio between the removal rates obtained experimentally was compared with the ratio calculated for competitive substrate hydrolysis using the kinetic constants for each substrate and substituting into eq 1. As shown in Table II a satisfactory correlation was obtained between experimental and calculated values.

K_m' and k_c' Dependence on Lipoprotein Triglyceride Content. Particles containing from 10 to 90% of their original triglyceride content were prepared from chylomicrons or very low density lipoproteins as described above. The apparent kinetic constants of each species were determined as previously. As shown in Figure 2 removal of triglyceride from lipoprotein substrates was associated with a significant reduction in k_c' after hydrolysis of about one-third of triglyceride content had been catalyzed. There was no significant change in apparent Michaelis constant during triglyceride depletion for the particles used in these experiments. K_m' for very low density lipoprotein triglyceride in these experiments was 0.06 ± 0.02 mM (three experiments). The value for their generated remnant particles was 0.07 ± 0.01 mM. K_m' for intact chylomicrons (four experiments) was 0.09 ± 0.01 mM and K_m' for their remnant particles was 0.10 ± 0.02 mM. These values are similar to those previously reported in other experiments for the intact lipoprotein particles (Fielding and Higgins, 1974a).

Competitive Hydrolysis of Remnant Lipoprotein Triglyceride. The initial removal rates of triglyceride from in-

Table III: Hydrolysis of Chylomicron and Remnant Lipoprotein Triglyceride.[a]

Lipoprotein Concn (mM)		Rel Hydrolysis Rate (a/b)	
Chylomicron (a)	Remnant (b)	Calcd	Exptl
0.423	0.009	64.8	61.5 ± 3.5
0.430	0.020	29.0	26.8 ± 1.2
0.381	0.030	17.1	14.1 ± 2.0
0.427	0.053	10.9	11.5 ± 0.3
0.667	0.128	7.1	7.6 ± 0.4

[a] Values are means ± standard deviations for triplicate determinations. Calculated kinetic constants for chylomicrons were as in Table II. The remnant lipoprotein contained 20.0% of original triglyceride content and k_c(remnant)/k_c(chylomicron) was 0,74. Apparent K_m was unchanged by remnant formation. Relative hydrolysis rate is the ratio between the hydrolysis rates with intact and remnant lipoprotein substrates.

tact and remnant lipoprotein particles were determined using media containing mixtures of these substrates in different proportions. The experimental ratio was compared with the calculated value using the k_c' for the remnant particle as shown in Figure 2. As shown in Table III a satisfactory agreement was obtained between these values. Similar results were obtained for intact and remnant very low density lipoproteins. These results indicated competitive removal of intact and remnant lipoproteins as well as lipoproteins of dietary (chylomicron) and endogenous (very low density lipoprotein) origin.

Course of Remnant Formation by Lipase. As previously described, the initial course of triglyceride removal at low substrate concentrations accurately followed predicted first-order kinetics. In the present research the course of formation of particles containing only a small proportion of initial triglyceride content was determined. As shown in Figure 3 the formation of small remnant particles was associated with nonlinear kinetics, involving a decreased first-order removal rate.

Discussion

The membrane lipoprotein lipase system appears to represent in the heart the only pathway for the hydrolysis of circulating lipoprotein triglyceride (Borensztajn and Robinson, 1970). Our earlier research determined some properties of this system for the initial hydrolysis of triglyceride from very low density lipoprotein and chylomicron substrates (Fielding and Higgins, 1974a). In the present research, investigation has been made of the remnant particles which are the product of more extensive hydrolysis. Since both lipoprotein particles contain many tens of thousands of triglyceride molecules (Dole and Hamlyn, 1962) lipase activity must evidently proceed with the intermediate formation of partially degraded lipoprotein particles. Continued hydrolysis could take place with the particle retained at the enzyme site, or it could be released into the medium for competitive further reaction at a rate which was a function of its concentration and kinetic properties (eq 1). Analysis of the chemical composition and flotation characteristics of the recirculating medium during remnant formation indicated a decreasing mean particle size and suggested that the latter mechanism was the case. The alternative explanation, that there was a preferential initial removal of larger particles, was not borne out by two other pieces of information. Firstly, experiments carried out in the presence of both intact and remnant lipoproteins (Table III) indicat-

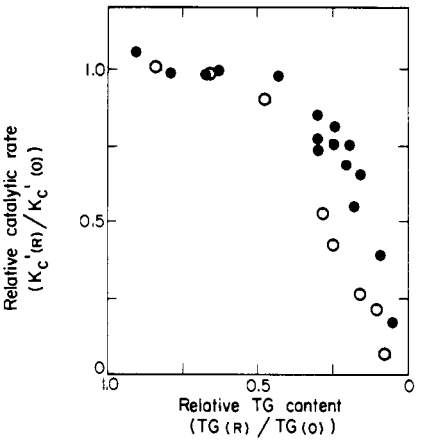

FIGURE 2: Dependence of the catalytic constant (k_c') of lipase on triglyceride content of the substrate particle. The rate constant of the remnant particle relative to that of the intact lipoprotein of the same species was determined from the zero-order removal rates at high substrate concentration ([S] > K_m): (●) chylomicrons; (○) very low density lipoprotein (S_f 100–400). Each point represents the mean value from duplicate determinations for a single experiment: (R) remnant particle; (O) original lipoprotein.

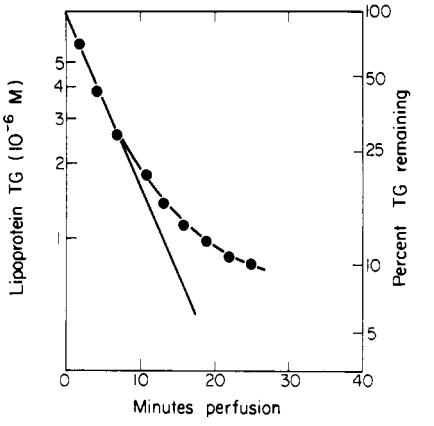

FIGURE 3: Triglyceride depletion from very low density lipoprotein (S_+ 100–400) at low ([S] < K_m) substrate concentration (initial concentration 0.0078 mM triglyceride). Each point represents the mean of duplicate determinations. The indicated slope is the linear regression for hydrolysis of the initial 50% of triglyceride content.

ed concomitant hydrolysis of triglyceride from both intact and smaller substrate particles. Secondly, the earlier study (Fielding and Higgins, 1974a) indicated that the reaction velocity with large and small intact chylomicrons was the same. It therefore seems likely that remnant formation takes place by the formation and release of smaller intermediate particles. Heart lipase was reactive with both major lipoprotein species (Fielding and Higgins, 1974a) and, as shown in Table II, reaction in mixtures of these lipoproteins took place according to competitive kinetics. Similarly, competitive interaction was found for lipoproteins and their generated remnant particles (Table III). Hence, the rate of triglyceride clearance by heart lipase is a function of the proportions of very low density lipoproteins and chylomicrons present in the medium and on the proportions of intact and triglyceride-depleted substrates (or on the rate of entry of intact particles into the system). These proportions are determined by the k_c' dependence of particle triglyceride content (Figure 3). As described in the earlier study, the catalytic constants used may be underestimated in each case by about 10% because of uncertainties in the estimate

of the maximal reaction velocity with this preparation. A further uncertainty lies in the fact that triglyceride depletion itself, during the assay of kinetic constants, causes a further change in the values of these parameters, particularly with small remnant particles. However, when substrate hydrolysis was limited to 5–10% of original triglyceride content in each medium, this error was minimized and the general agreement between experimental and calculated values for remnants and intact lipoprotein substrates suggests that the assumptions made are reasonable.

The catabolism of lipoprotein substrates by lipase was accompanied by a decrease in k_c'. It has been previously suggested that lipoprotein catabolism may be accompanied by the transfer of lipase cofactor protein to the high density lipoprotein class (Rubinstein and Rubinstein, 1972; Eisenberg and Rachmilewitz, 1973). This process is reversed by the reaction of plasma with triglyceride-rich particles (Havel et al., 1973b). Furthermore, the mechanism of activation of lipase by cofactor protein in the presence of synthetic lipids is associated with an increased k_c but no significant change at physiological pH in apparent K_m (Fielding, 1973). It is therefore likely that the changed kinetic properties of remnant particles during triglyceride depletion may be associated, at least in part, with the loss of cofactor protein although other factors, such as the content of arginine-rich polypeptide (Shore et al., 1974) or the increased cholesterol:phospholipid ratio (Fielding, 1970), may also play a role. The activity of lipase with synthetic lipids is also dependent upon the proportions of cofactor proteins and an inhibitor protein component of the natural lipoprotein substrates in human plasma (Brown and Baginsky, 1972). However, the analogous component of rat very low density lipoprotein is rapidly removed from the particle during triglyceride hydrolysis (Eisenberg and Rachmilewitz, 1973). Such inhibition probably does not contribute in a major way to the decreased rate of reaction of lipase with the remnant particles.

The dependence of k_c' on remnant triglyceride content was also indicated by the plot of [S] vs. time at low substrate concentrations. Since K_m' was unchanged, decreased k_c' during remnant formation should be associated with a decreased removal rate (eq 2). This was found to be the case in the present experiments (Figure 3). In this connection, it is of interest that whole animal studies have reported complex kinetics of tracer triglyceride removal from very low density lipoprotein. The initially steep first-order plot was followed by a second shallower phase which has been correlated with clearance of remnant particles formed by lipase into the liver (Redgrave, 1970). However, comparison of the plot shown in Figure 3 for the isolated heart with that obtained for the same lipoprotein species in the intact animal (Figure 1; Fielding and Higgins, 1974a) shows evident similarities. This result raises the possibility that the nonlinear removal of plasma lipoprotein triglyceride in the whole animal, as in the isolated heart, may be the result of the changing kinetic properties of the substrate particles during remnant formation in the course of reaction with a single enzyme system, the lipoprotein lipase of the endothelium. Any selectivity by the liver in lipoprotein clearance would be superimposed on this pattern. In this connection it is relevant that unless the level of enzyme at the capillary surface is constant (eq 2), the opportunity for remnant generation by an alternative pathway exists. In the perfused heart it has been demonstrated that the level of reactive lipoprotein lipase remains constant during the perfusion period.

Figure 2 indicates a similar plot of the k_c' dependence of triglyceride content for very low density lipoproteins and chylomicrons. While both large and small chylomicrons from lymph have the same kinetic properties (Fielding and Higgins, 1974a) these have not been exposed to lipase activity. On the other hand small very low density lipoproteins (S_f 40–100) had a significantly lower k_c' than particles of S_f 100–400. Comparison of the composition of the former with remnant particles produced in the heart (Table I) indicates that these have about 50% of the triglyceride content of the larger intact very low density lipoproteins. The observed k_c' value of the S_f 40–100 particles is compatible with an origin in the larger class (Figure 2) and these may represent remnant particles formed in vivo. However, it is also possible that smaller very low density lipoprotein particles secreted from the liver, unlike small chylomicrons, have different kinetic properties from larger particles of the same origin. The fraction of smaller very low density lipoproteins (S_f 40–100) might then contain both intact small particles and remnants from larger endogenous substrate particles.

This study indicates that remnant particles formed from triglyceride-rich lipoproteins by lipoprotein lipase have no fixed composition and their catabolism depends on the relative proportions of intact and remnant lipoproteins present in the medium and their kinetic properties. The plot of k_c' dependence of triglyceride content can be used to determine the relative removal rates of the different lipoprotein species.

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Interaction of Carbohydrate Binding Sites on Concanavalin A-Agarose with Receptors on Adipocytes Studied by Buoyant Density Method†

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ABSTRACT: The interaction of concanavalin A (Con A) with isolated adipocytes was studied using Con A-Sepharose beads in the affinity binding buoyant density method previously used to study insulin receptors. Free Con A-Sepharose beads could be separated from the bound beads (cell–bead complexes) by sedimentation of the high density beads and floatation of the low density complexes. Sedimented and total beads could be determined by counting the radioactivity associated with [^{125}I]Con A coupled in tracer amounts to the beads. Various lines of evidence demonstrated the high specificity of binding. Soluble Con A, but neither insulin nor any of the other proteins tested, inhibited and reversed the binding of Con A-Sepharose to the cells. Whereas treatment of Con A- (and insulin-) derivatized beads with anti-insulin antiserum, and cells with trypsin, readily inhibited binding of insulin-Sepharose to cells, neither treatment inhibited Con A-Sepharose binding. According to the relative extents of inhibition and reversal of binding exhibited by 15 different carbohydrates, the saccharide binding sites on Con A-Sepharose appeared virtually identical with the known sites on free Con A. Protein-containing components of cell ghosts that were solubilized with Triton X-100 appeared to correspond to the Con A-Sepharose receptor sites on the basis of their ability to bind to Con A-Sepharose columns, be eluted with methyl α-D-mannopyranoside (MeMan) and be precipitated by the free lectin and redissolved by MeMan. According to (a) Normarski interference contrast microscopic examination of the topographical distribution of Con A-Sepharose beads and cells surrounding and bound to each other, and (b) absence of any apparent morphological changes in the cells due to binding, it is suggested that extensive clustering ("cap" or "macropatch" formation) of Con A receptors did not occur on the adipocyte as a consequence of the interaction of the cells with the Con A-Sepharose beads.

Although plant lectins have been widely used to probe the structure and function of cell surfaces of a variety of mammalian cells (reviewed by Lis and Sharon, 1973), there have been relatively few reports on the adipocyte as a target cell for these studies. Interest in the effects of, and binding sites for, lectins on the adipocyte has recently been stimulated by the finding of insulin-like activity exhibited by the interaction of concanavalin A[1] with these cells (Czech and Lynn, 1973; Cuatrecasas and Tell, 1973).

Persistent doubts expressed (Lambert et al., 1972; Hamlin and Arquilla, 1974) about the validity of ^{125}I-radioiodinated insulin as a biologically active and relevant ligand to study the insulin receptor prompted us to search for alternative ligands and to develop new methodology to study membrane receptor sites. Our previous findings of specific and reversible binding of insulin-agarose (insulin-Sepharose) beads to intact isolated adipocytes that led to the development of an "affinity-binding buoyant density" assay procedure to study insulin receptor sites (Katzen and Soderman, 1973; Soderman et al., 1973), suggested that an analogous procedure, utilizing Con A-Sepharose, could be applied to study the interaction of Con A with these cells. The particular advantage of adipocytes as a target for Con A-Sepharose beads is the ability of these low density cells in physiological media to float the derivatized beads bound to the cells. The unbound beads would otherwise sediment.

In earlier studies with immobilized Con A, it was found that intact cells specifically bound to Con A-derivatized nylon fibers or agarose beads could not be released (eluted) without distortion of the cells by physical or mechanical methods (Edelman et al., 1971). Because of these and other known limitations of affinity chromatographic-like binding (Cuatrecasas and Anfinsen, 1971; Shaltiel and Er-el, 1973; Katzen and Vlahakes, 1973), studies on any specific type of cells require that a determination be made of the specificity and reversibility of the binding of the derivatized support to those cells. The present study is intended to demonstrate the specific and reversible binding of Con A-Sepharose beads to intact adipocytes for the purpose of applying the affinity-binding buoyant density procedure to the study of the interaction between the saccharide binding sites on Con A and

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[1] Abbreviations used are: Con A, concanavalin A; Con A (or insulin)-Sepharose, Con A (or insulin) covalently coupled to Sepharose 4B (agarose); MeMan, methyl α-D-mannopyranoside; AIS, anti-insulin antiserum.