Heparin–decasaccharides impair the catabolism of chylomicrons

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On intravenous injection to rats, decasaccharides gave rise to a short-lived peak of lipoprotein lipase (LPL) activity, whereas octa- and hexasaccharides caused only marginal increases. In isolated hearts perfused by a single pass, decasaccharides released LPL more efficiently than conventional heparin on a mass basis. Octa- and hexasaccharides were much less efficient. Similar results were obtained for hepatic lipase, which was studied both in vivo and by liver perfusion. In the intact rat, the heparin fragments themselves disappeared rapidly from the circulating blood. The decay of hepatic lipase activity after the early peak roughly paralleled the decay of decasaccharide concentration, but for LPL the decay was faster, presumably because the liver extracted this lipase from plasma. To assess the lipase activities remaining in contact with blood a large dose of conventional heparin was injected at a series of times after the decasaccharides. LPL was decreased by 40% after 1 h. At that time, the LPL activity that could be released from isolated hearts by single-pass perfusion with heparin for 2 min (‘functional LPL’) was decreased by 75%. Chylomicrons labelled in vivo with [14C]oleic acid (primarily in triacylglycerols, providing a tracer for lipolysis) and [3H]retinol (primarily in ester form, providing a tracer for the particles) were injected intravenously to explore the effects of the LPL depletion on lipoprotein metabolism. Triacylglycerol lipolysis and particle clearance was markedly delayed from 30 min to 2 h after injection of decasaccharides. After 1 h the fractional catabolic rate was only one-third of the control value and the catabolism of chylomicron triacylglycerols by perfused hearts was delayed to a similar extent. Thus injection of decasaccharides leads to accelerated turnover of LPL with loss of functional LPL from extrahepatic tissues. This in turn leads to a period of delayed lipolysis and removal of chylomicron particles.

INTRODUCTION

Heparin releases lipoprotein lipase (LPL) and hepatic lipase into the circulating blood [1]. The presumed mechanism is that heparin forms soluble complexes with the two enzymes. Recently a molecular model for LPL was presented on the basis of coordinates from the X-ray structure for pancreatic lipase [2]. In this model a heparin–decasaccharide was fitted to the groove that makes up the presumed binding site. Model experiments have, however, shown that fragments as small as octasaccharides can compete with full-size heparin for binding to the lipases [3], and that decasaccharides, obtained from heparan sulphate [4] or from heparin [5], bind to LPL with high affinity. Merchant et al. [6] reported that decasaccharides release substantial lipase activity into plasma on injection. Braun and Severson [7] studied the release of LPL from cardiac myocytes and found that a low-molecular-mass (LMM) heparin [7] low-molecular-weight (LMW) heparin] preparation with mean molecular mass corresponding to decasaccharides caused an even higher release of LPL than conventional heparin. The sizes indicated by the above-mentioned studies, octa- to decasaccharides, are in the range of molecules present in commercial LMM heparin preparations [8].

In this paper we have studied the relation between the size of heparin fragments and their ability to release LPL into, and retain it within, the circulation. We also studied whether there might be a range of molecules that affect hepatic lipase more (or less) than they affect LPL. For this we prepared size-fractionated heparin fragments and injected them into rats. A recent study on the turnover of LMM heparin indicated that short heparin fragments rapidly leave the circulation [9]. To study this directly we labelled some of our fragments with 3H: 10 min after injection into rats less than 10% of the decasaccharides remained in blood. This, together with the fact that the liver extracts LPL [10], complicated the interpretation of whole-animal experiments. We therefore turned to organ perfusion to assess more directly the ability of the oligosaccharides to release the lipases from their binding sites. As a model organ with high LPL activity we chose the heart. For hepatic lipase we chose the liver. Taken together, the results show that decasaccharides, but not smaller heparin fragments, efficiently release both LPL and hepatic lipase.

A previous study showed that lipoprotein metabolism responds to heparin in a biphasic manner [11]. Early after heparin injection, blood concentrations of heparin and lipases are high, and lipolysis and clearance of triacylglycerol (TG)-rich lipoproteins are enhanced. Later, when heparin and lipase concentrations have decreased, both lipolysis and particle clearance are slower than in controls. A likely reason for this is that some of the LPL released into blood is taken up and degraded by the liver [10,12,13], so that the amount of LPL available in peripheral tissues is temporarily decreased. We speculated that injection of decasaccharides, even though they provide only a short pulse of heparin activity, might cause accelerated catabolism of LPL. This could give a model system in which to study the relation between the depletion of LPL activity and chylomicron metabolism with little or no heparin or LPL activity in the circulating blood. This turned out to be so, and we have studied the metabolism of doubly labelled chylomicrons at intervals after injection of decasaccharides.

MATERIALS AND METHODS

Handling of experimental animals, chylomicron preparation, experimental procedures, lipase assays and lipid extraction from the blood were as previously described [11,14]. Extraction of

Abbreviations used: FFA, free fatty acids; LMM heparin, low-molecular-mass heparin; LPL, lipoprotein lipase; TG, triacylglycerol.

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lipids from tissues was done by homogenization of the tissues in chloroform/methanol (15 vol; 2:1, v/v) [15]. The clearance of \(^3\)H-labelled oligosaccharides was followed by \(^\beta\)-scintillation counting of 150 \(\mu\)l of plasma added to 4 ml of scintillation liquid (Optiphase HiSafe III; LKB-Wallac, Uppsala, Sweden). Fasted male Sprague-Dawley rats weighing 190–210 g were used. All animal procedures were approved by the Animal Ethics Committee in Umeå.

**Heparin preparations**

Pig sodium heparin was from Novo Nordisk A/S (Gentofte, Denmark). Hexa-, octa- and decasaccharides were prepared from a side fraction from the manufacture of LMM heparin (tinzaparin sodium, Logiparin*) by ethanol precipitation and gel filtration as described by Larnkjær et al. [16]. Essentially size-homogeneous oligosaccharides were obtained through rechromatography by gel filtration. The average molecular masses for the conventional heparin, deca-, octa- and hexasaccharide preparations were 12700, 3000, 2400 and 1800 Da respectively. The anti-(factor Xa) activities of the deca-, octa- and hexasaccharides were 36.6, 10.7 and 3.0 units/mg (First International Standard for LMM Heparin, National Institute for Biological Standards and Control, London, U.K.), respectively. The preparations were administered on the basis of the same mass (3.25 mg/kg of body weight), which means that on a molar basis 4, 5 and 7.5 times as many molecules of deca-, octa- and hexasaccharides than conventional heparin were injected.

**Preparation of labelled oligosaccharides**

A sample of the starting material used for preparation of the oligosaccharides was labelled by Amersham International (Little Chalfont, Bucks., U.K.) by catalytic tritiation with tritium gas in columns and eluents described above. The specific radioactivities were 0.61, 0.69 and 0.96 mCi/mg respectively. The \(^{3}H\)oligosaccharides were diluted with cold oligosaccharides to obtain test solutions containing 7 \(\mu\)Ci and 3.25 mg per ml, corresponding to 2.2 \(\mu\)Ci/mg.

**Heart perfusion**

The heart was removed from anaesthetized rats and prepared for perfusion as described by Chajek-Shaul et al. [17]. An artificial ‘lung’ [18] was used so that the \(P_{\text{O}_2}\) entering the heart was 460–540 mmHg (61.3–72.0 kPa). The heart was washed free from blood by a single pass of medium A [136 mM NaCl/5.4 mM KCl/0.81 mM MgSO\(_4\)/0.98 mM MgCl\(_2\)/0.44 mM KH\(_2\)PO\(_4\)/1.33 mM Na\(_2\)HPO\(_4\)/1.3 mM CaCl\(_2\)/10 mM Hepes (pH 7.4)] supplemented with 11 mM glucose and 3 % (w/v) BSA and previously equilibrated with \(O_{\text{2}}/\text{CO}_2\) (19:1). The heart was then perfused retrogradely by recirculation of medium B (Eagle’s minimal essential medium modified with Earle’s salts) supplemented with 5 % (w/v) BSA/11 mM glucose/100 mM insulin for 10 min for stabilization. The pressure was maintained at 50–60 mmHg (6.7–8.0 kPa) by adjusting the pump speed, which resulted in a flow rate of 9–11 ml/min per g of heart.

**Liver perfusion**

For this, 200–250 g rats were used. The liver was perfused as described by Vilaró et al. [10] with minor modifications. The portal vein of an anaesthetized rat was cannulated and immediately perfused at a flow rate of 11–13 ml/min with medium A supplemented with 5.5 mM glucose and 1 % (w/v) BSA, and gassed with \(O_{\text{2}}/\text{CO}_2\) (19:1). The liver was then excised, placed in the perfusion chamber at 37°C and perfusion was continued with the same medium for 10 min to rinse out residual blood. The perfusion medium was then changed to medium B supplemented with 3 % (w/v) BSA and 5.5 mM glucose, which was recirculated for 10 min to allow the system to stabilize. Then the same medium containing deca-, octa- or hexasaccharides (59 \(\mu\)g/ml) was perfused in single-pass mode and 1 min fractions were collected into heparinized tubes (50 units/ml of medium) for determination of hepatic lipase activities.

**Calculations**

The SAAM II program (SAAM Institute, University of Washington, Seattle, WA, USA) was used for compartmental modelling. In the fitting process the means \(\pm\) S.D. for the rats were used. To calculate the fractional catabolic rates for the labelled glycosaminoglycans a mono-exponential decay was assumed. Statistical comparisons of data sets were done by Student’s two-tailed \(t\) test, with SPSS for Windows, version 6.1 (SPSS, Chicago, IL, U.S.A.)

**RESULTS**

**Properties of the oligosaccharides**

The oligosaccharides were prepared from a side fraction from the manufacture of tinzaparin sodium, which is produced by depolymerization of heparin with heparin lyase I from *Flavobacterium heparinum*. This enzyme cleaves the bond between sulphated iduronic acid and N-sulphated glucosamine giving rise to even-numbered split products (polymers of disaccharides). The identification as hexa-, octa- and decasaccharides was obtained by gel permeation chromatography–HPLC [19]. The oligosaccharides showed relatively narrow and symmetrical peaks (Figure 1). If the peak-broadening always seen in HPLC is taken into account, the overlaps between the peaks are small. Thus the oligosaccharides were essentially homogeneous with respect to size. They were, however, heterogeneous with respect to sulphation and content of \(N\)-acetylgalacosamine, and iduronic acid-to-glucuronic acid ratio [5,16]. The biological activities in the coagulation system were low, showing that the contents of anti-

![Figure 1 Oligosaccharide preparations](image-url)
Heparin–decasaccharides, lipases and chylomicrons

Release of lipase activities

Octa- and hexasaccharides released low, but significant, LPL and hepatic lipase activities (Figure 2). Decasaccharides gave rise to much larger peaks of both activities, corresponding to approx. 30% of the values observed with conventional heparin. With conventional heparin, LPL and hepatic lipase activities remained essentially unchanged until 1 h. With decasaccharides, the highest LPL activity was recorded at the first time point, 1 min. The activity then declined rapidly during the next 10 min and was close to the basal value after 1 h.

The lowest doses at which a substantial increase in LPL activity was recorded were 325, 3250, 13000 and 33 µg/kg of body weight for deca-, octa- and hexasaccharides and conventional heparin respectively (Figure 3). This means that 40-, 500- and 3000-fold as many deca-, octa- and hexasaccharide molecules as conventional heparin molecules were needed. In most cases the activities increased with the amount of the heparins until the largest dose used. For hepatic lipase this dose resulted in 35%, 12% and 5% of the activity obtained with conventional heparin for deca-, octa- and hexasaccharides respectively.

Clearance of oligosaccharides

After injection, decasaccharides disappeared rapidly from the circulating blood; less than 30% remained after 1 min (Figure 4). The disappearance tapered off to a much lower rate from about 10 min. Octa- and hexasaccharides followed similar curves, but the distribution phase was even faster. These results were fitted to a simple kinetic model in which a central compartment (blood and other spaces rapidly available to the tracer) is in reversible equilibrium with a peripheral compartment (extracellular and intracellular sites in tissues). Previous studies had suggested that loss from the system during the 2 h period that we studied would occur primarily through excretion in the urine; breakdown in tissues occurs relatively slowly [9]. Therefore catabolism was modelled as occurring from the central compartment. Kinetic parameters derived with the SAAM program are given in Figure 5.

A comparison of the results for hepatic lipase activity from Figure 2 with the results for decasaccharide concentrations in Figure 4 shows that hepatic lipase followed a similar time course.
The labelled oligosaccharides had been diluted with unlabelled preparations so that the same radioactivity (7.2 μCi/kg of body weight) for all three and the same chemical amount as in the experiment in Figure 2 (3.25 mg/kg of body weight) was injected. At the indicated times, blood samples were drawn for determination of remaining radioactivity. The first time point is 1 min. Results are means ± S.E.M. for five rats and are expressed as a percentage of the injected dose in blood, assuming a blood volume of 5.5% of body weight [27]. [3H]Hexasaccharides (○), [3H]octasaccharides (△), [3H]decasaccharides (▲). In the experiment with decasaccharides LPL activity was also determined in the blood samples. The inset compares these LPL activities (▲) with the decasaccharide concentration (○). For comparison the hepatic lipase activities from Figure 2 (●) are also plotted. Abbreviation: mU, m-units.

Figure 4 Clearance of oligosaccharides from the circulating blood

For each oligosaccharide, results from five rats (decasaccharides) or two rats (hexa- and octasaccharides) were fitted to an open two-compartmental model where the tracer is introduced into compartment 1, which is also the sampled compartment. Johansen et al. [9] used an open three-compartmental model to analyse the metabolism of tinzaparin sodium. The third compartment was slowly exchanging with compartment 1. They had results up to 504 h.

Release of hepatic lipase by heparin fragments in the perfused liver

Conventional heparin added to the perfusion medium rapidly released hepatic lipase from the liver. Perfusion with octa- or hexasaccharides resulted in a small release of hepatic lipase (5–10 m-units/min per g), corresponding to only a 2–3-fold increase over the spontaneous release (2–3 m-units/min per g) (Figure 7). Decasaccharides were more efficient. During the first 1 min of LPL activity had reached its peak at 1 min it decreased even faster than the decasaccharide concentration. This suggests that plasma LPL declined both because the heparin concentration driving the lipase into blood decreased and because substantial amounts of the lipase were cleared by the liver, as is known to occur from previous experiments [10,12,13].

Release of LPL by heparin fragments in heart perfusion

To probe the potency of the oligosaccharide preparations without the confounding effect of rapid removal of both the oligosaccharides themselves and of the released LPL activity, we used isolated hearts perfused by a single pass (Figure 6). Perfusion with conventional heparin resulted in a rapid burst of LPL activity within the first 2 min, followed by a slow continued release thereafter. Perfusion with octa- or hexasaccharides resulted in small but significant releases of LPL, less than 20% of the values obtained with conventional heparin (Figure 6). Decasaccharides, in contrast, were more efficient than conventional heparin. For instance, during the first 1 min decasaccharides released about 2.2 times as much activity as did conventional heparin (681 ± 39 compared with 313 ± 13 m-units/g; P < 0.001).

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perfusion about half as much hepatic lipase was released by decasaccharides as by conventional heparin, but after that there were no significant differences. The total release of hepatic lipase during the first 10 min was 8.8 units by decasaccharides compared with 10.1 units by conventional heparin (difference not significant).
Figure 9  Metabolism of chylomicrons at different times after injection of decasaccharides

Disappearance from blood of TG (a) and core label (b), and appearance of labelled oleic acid in unesterified form (c). (d) Lipolysis indexes (TG label divided by core label) of the chylomicrons remaining in the blood. First, decasaccharides (3.25 mg/kg of body weight) were injected into the tail vein without anaesthesia; then 5 min (\(\triangledown\)), 30 min (\(\blacksquare\)), 1 h (\(\bullet\)), 2 h (\(\bigcirc\)), 3 h (\(\bigtriangledown\)), or 4 h (\(\bigtriangleup\)) after the decasaccharides, chylomicrons (20 mg of TGs/kg of body weight) were injected into an exposed jugular vein. The rats had been anaesthetized 10 min before this. When the interval between the injections of decasaccharides and chylomicrons was less than 10 min, the rats were anaesthetized before the injection of decasaccharides. The rats were then kept anaesthetized throughout the period during which blood samples were taken (20 min). At 4 h the results were close to those for controls and are shown only for lipolysis indexes to avoid crowding the graphs. Controls (\(\square\)) were injected with saline 5 min before the chylomicrons. Results are means ± S.E.M. for five rats in each group.

5 min the rate of clearance decreased, despite the fact that there was substantial LPL activity in the circulating blood (compare with Figure 2). The decrease in clearance rate was maximal after 1 h (fractional catabolic rate 0.059 ± 0.018 pools per min compared with 0.157 ± 0.046 pools per min in controls; \(P < 0.01\)). The recirculation of label in FFA followed a similar pattern, i.e. lower levels from 5 min to 2 h (Figure 9c).

The removal of chylomicron particles, as determined from the disappearance of core label from blood, exhibited a monoe-xponential course as for TG label, but at a lower rate (Figure 9b). From 30 min to 2 h after injection of decasaccharides, particle removal was delayed significantly, with a maximal effect after 1 h (fractional catabolic rate 0.031 ± 0.012 pools per min compared with 0.081 ± 0.040 pools per min in controls; \(P < 0.005\)).

TGs are cleared both by hydrolysis and by uptake with the chylomicron particles. The extent of lipolysis (lipolysis index = remaining TG label divided by remaining core label) of the particles remaining in the circulating blood was calculated and plotted for each time (Figure 9d). In controls the lipolysis index was below 0.2 after 20 min, indicating that particles had lost 80% of their TGs. In rats that had received decasaccharides 30 min, 1 h or 2 h earlier the lipolysis indexes at 20 min after injection of the chylomicrons were much higher than in controls (0.78, 0.78 and 0.54 respectively).

**Uptake of chylomicrons in the liver**

The radioactivity remaining in liver 20 min after injection of chylomicrons is shown in Figure 10 (left panel). During the period of delayed particle removal (from 30 min to 2 h after decasaccharide treatment), the amount of core label found in the liver was markedly decreased but returned to the confidence interval for control values from 3 h on. The ratio of TG label to core label is illustrated in Figure 10 (right panel). From 5 min to 2 h after decasaccharide treatment, this index was higher than in the controls. The extrahepatic uptake of chylomicron particles or remnants, as estimated from the core label neither in the blood nor in the liver 20 min after injection of chylomicrons, increased from 14% in controls to 23%, 25% and 18% in rats that had received decasaccharides 30 min, 1 h or 2 h before, respectively.

**Metabolism of chylomicrons by the perfused heart**

In these experiments, decasaccharides (3.25 mg/kg of body weight) were first injected intravenously into fasted rats. After 5, 30, 50, 120 and 180 min, hearts were excised and prepared for perfusion, with three rats in each group. Chylomicrons labelled in vivo with \[^{14}C\]oleic acid (4 mg of TG; approx. 10⁶ d.p.m.) were added to the perfusate. The clearance of TG label was followed for 30 min.

The disappearance rate for TG label was similar for hearts from control rats and for 5, 120 and 180 min after decasaccharide treatment, corresponding to 87 ± 9, 104 ± 18, 89 ± 11 and 123 ± 8 μg of TG/min respectively, assuming that all TGs were metabolized at the same rate as those containing the labelled oleic acid. This means that approx. 70% of the chylomicron TGs were cleared during the 30 min of perfusion. If all clearance occurred
by hydrolysis it would require an LPL activity of at least 200 m-units, assuming that two fatty acids were split off from each TG with the formation of monoglycerides that were metabolized by other pathways [21]. This fits well with the ‘functional LPL’ assessed by 2 min of perfusion with heparin (345 m-units).

The rate of TG clearance was decreased for hearts of rats that had received decasaccharides 30 min and 1 h after the injection (40 ± 11 and 37 ± 8 μg of TG/min respectively). The difference was large: only approx. 20% of the chylomicron TGs were cleared from the medium during the perfusion, compared with the 70% cleared in controls.

**DISCUSSION**

This study confirms that heparin decasaccharides efficiently release LPL from its binding sites in vivo. In contrast with conventional or LMM heparin preparations, the decasaccharides rapidly left the circulation. Hence they provided a pulse of circulating heparin activity. Chylomicron clearance was slowed down and intravascular lipolysis was decreased, as illustrated by the smaller amount of radioactivity appearing in plasma FFA and by the higher lipolysis index of the particles. It took 3 h to restore TG clearance and to return to a similar pattern for radioactivity in plasma FFA to that in untreated rats.

Less than 10% of the decasaccharides remained in the circulation 10 min after injection. This is in agreement with a recent study on a related LMM heparin, tinzaparin sodium [9], if we take into account that that the preparation is more heterogeneous and contains approx. 20% molecules with the size of conventional heparin. That study showed that heparin fragments quickly leave the blood but remain intact in tissues for a relatively long time and that the elimination of short heparins is mainly through the kidney. In the present study the model parameters indicate an apparent distribution volume several times larger than the blood volume, and rapid exchange between the central and the peripheral compartment. The details of the distribution/exchange cannot be elucidated with the available results, but it is apparent that the oligosaccharides rapidly distribute throughout the body such that only a small proportion is present in blood after a few minutes. The fractional catabolic rates were similar for all three oligosaccharides, and were also similar to the catabolic rate estimated for tinzaparin sodium [9]. The derived rate constants predict that less than 40% of the oligosaccharides was lost from the organism during the first 2 h. Hence even though the plasma concentration of the injected oligosaccharides rapidly fell to low values, most of the injected material probably remained at tissue sites for the duration of our experiments.

The potency of the decasaccharides was unravelled by the studies with perfused hearts, where the oligosaccharide concentration could be kept constant and the catabolism of the released LPL was minimal. In this system the decasaccharides were actually more effective than conventional heparin on a mass basis. This is similar to the results of Braun and Severson [7], who found that an LMM heparin preparation with mean molecular mass of approx. 3000 Da (corresponding to decasaccharides) released more LPL from cultured cardiac myocytes than did conventional heparin. In our experiments there was about 4-fold more decasaccharide than conventional heparin molecules for the same mass. The release of LPL by octa- or hexasaccharides was only a few per cent of that with decasaccharides. This agrees with a recent study in vitro on the binding of heparin fragments to immobilized LPL [5], in which dissociation constants for deca-, octa- and hexasaccharides were found to be 53.1 ± 1.6, 187 ± 25 and 1880 ± 1300 nM respectively.

Decasaccharides were also the smallest heparin fragments that effectively released hepatic lipase. It is not evident that the same-sized fragments should be needed for hepatic lipase and for LPL. About half of the positively charged residues in the putative heparin-binding site in LPL are not present in hepatic lipase [22]. Hepatic lipase is eluted from heparin-agarose at lower salt concentrations than LPL, indicating that fewer charges are involved in the binding [23]. The tissue-binding sites must differ for the two enzymes. LPL is found in adipose tissue, muscles and many other extrahepatic tissues, but there is no hepatic lipase at these locations [24]. Hepatic lipase has its main functional site in the liver where the enzyme is turned over relatively slowly [25], in contrast with LPL, which soon becomes internalized and degraded after binding in the liver [10,13]. Further evidence for a difference in the binding came from a recent study on the effects of the polycation protamine [26]. Injection of protamine released hepatic lipase but only small amounts of LPL. Despite this, the two enzymes showed the same behaviour towards the heparin fragments.

The amount of LPL remaining in contact with blood, assessed by the injection of a large dose of conventional heparin, was decreased from 10 min to 4 h after decasaccharide injection. Of the times we studied, the largest decrease, approx. 40%, was noted at 1 h. This is similar to the depletions noted previously after the injection of conventional or LMM heparins [14]. The mechanism is presumably that LPL released into blood is taken up and degraded in the liver. This is based on earlier studies that have shown that when LPL is perfused through rat livers approx. 40% of the enzyme is cleared during a single pass [10,13]. After extraction by the liver, the enzyme soon loses its catalytic activity [13] and is later degraded [10].

We studied what would happen to the LPL that was not extracted by the liver. Would it bind back to the sites it came from when the decasaccharides disappeared from blood or would it bind to sites all over the vascular bed? To explore this we used...
the heart as a model tissue with high endogenous LPL activity. If binding back mimicked the original binding, depletion of heparin-releasable LPL in the heart should be proportional to overall depletion in the body. The depletion was, however, more pronounced in the heart. For instance, 1 h after decasaccharide treatment the amount of LPL released by a large dose of conventional heparin was decreased by 40 % in intact animals, but in the heart it was decreased by 65–80 %, depending on what time interval of the perfusion we consider. The implication of these results is that, when the decasaccharide concentration subsided, much of the LPL that had been released into blood was attached to sites where there is normally little or no LPL. This is in accord with results from experiments in which 131-I-labelled LPL was injected into rats [12]. Approx. 40 % was taken up by the liver. The remainder was distributed to all tissues of the body, but not in the same proportions as endogenous LPL activity [27].

These considerations on relocation of LPL give a clue to interpreting our finding that the effects of decasaccharides on chylomycin catabolism were more pronounced than expected from the decrease in total body heparin-releasable LPL activity. This was at its lowest at 1 h, but remained at 60 % of controls. In vitro, this LPL would hydrolyse the amount of chylomycin TG injected (4 mg in a 200 g rat) in less than 5 min. Nevertheless the fractional catabolic rate for TG clearance was decreased to one-third, and most of the clearance seemed to take place with particles that had undergone only little lipolysis. This statement is based on the much higher lipolysis index of the particles remaining in plasma, and on the higher ratio of TG to core label in the liver. For instance, at 10 min after injection, the particles circulating in blood had lost approx. 60 % of their TGs in control rats, but less than 10 % in rats given decasaccharides 1 h before. It is not evident why the moderate depletion of overall heparin-releasable LPL should have such a marked effect on chylomycin lipolysis. If, however, much of the LPL 1 h after decasaccharide treatment resided at sites that did not contribute much to chylomycin lipolysis, the retardation of lipolysis in vitro becomes more understandable. This hypothesis was supported by the results from heart perfusions. Clearance of chylomycin TGs was much slower in hearts from rats pretreated with decasaccharides. Hence our results support the view that there is a relation between chylomycin metabolism and endothelial LPL activity, but indicate that this relation holds only in tissues geared to metabolism of the released fatty acids. LPL relocated to other sites might be relatively ineffective.

The present results allow an estimate to be made of the turnover rate for functional LPL. Depletion of lipase activity and slowing down of chylomycin metabolism were most marked at 1 h. Most parameters had returned to near the control after 3 h, and after 4 h all were restored. If this reflects functional LPL, a maximal estimate for its turnover time is 2 h. This is of the same order of magnitude as some earlier estimates reached with different techniques [28,29], and supports the view that endothelial LPL is turned over rapidly.

Decasaccharides provided a model system for studying the effects of a pulse of heparin activity on lipoprotein metabolism. The effects demonstrated are, however, probably not confined to short heparins. Conventional heparin also leads to depletion of endothelial LPL, although the time scale differs between conventional and LMM heparins [11,14]. Heparin treatment in humans leads to delayed clearance of chylomycin TGs and particles [30]. The present study shows that this can be ascribed, at least in part, to a temporary depletion of endothelial LPL with impaired lipolysis of TG-rich lipoproteins. Whether there are additional effects caused by retention of heparin in tissues [9] requires further study.

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