Biochem. J. (1982) **206**, 647–654
Printed in Great Britain

Hydrolysis of plasma triacylglycerol-rich lipoproteins from immature and laying hens (*Gallus domesticus*) by lipoprotein lipase *in vitro*

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(*Received 17 May 1982/Accepted 20 May 1982*)

Very-low-density (VLD) lipoproteins and portomicrons were isolated from the plasma of immature and laying hens and their size, lipid composition and susceptibility to hydrolysis by lipoprotein lipase were compared. In agreement with other studies, VLD lipoproteins from laying hens were found to be smaller and have a different lipid composition than VLD lipoproteins from immature hens. Portomicrons from immature and laying hens had mean diameters of about 150 nm and similar lipid compositions. Hydrolysis of VLD lipoproteins from immature hens, and portomicrons from immature and laying hens, proceeded rapidly until at least 40% of the substrate had been used. In contrast only 1–15% of laying-hen VLD-lipoprotein triacylglycerol was readily hydrolysed and further hydrolysis occurred slowly. The limited susceptibility of laying-hen VLD lipoproteins appeared to be due to their low content of lipoprotein lipase activator apoprotein, which occurred despite an abundance of activator in the high-density lipoproteins of laying-hen plasma. The results provide further evidence that the liver of the laying hen synthesizes specialized lipoproteins. Their limited susceptibility to hydrolysis by lipoprotein lipase is probably a major factor in ensuring transport of lipid to yolk rather than to other tissues. The form of transport of dietary lipid, however, is similar in immature and laying hens.

Triacylglycerol-rich lipoproteins form about 30% of the weight of the hen's egg yolk. They are synthesized in the liver in response to oestrogens and transported in the plasma to the ovary, where they are incorporated into the oocyte by receptor-mediated endocytosis (Perry & Gilbert, 1979; Krumins & Roth, 1981). A hen laying regularly deposits up to 5 g of lipid in yolk each day, but little is known of how the yolk lipoprotein precursors are directed to the ovary or how such transfer is integrated with other aspects of lipid metabolism.

Lipid transport in the plasma of immature hens and male chickens is similar to that in mammals, except that dietary fat is transported via the portal vein as portomicrons (Bensadoun & Rothfeld, 1972) rather than through the lymphatic system. VLD, LD and HD lipoproteins of immature-hen or rooster plasma have similar sizes, lipid composition and densities to their mammalian counterparts (Chapman, 1980) and the metabolic relationships between the lipoprotein classes appear to be the same (Behr *et al.*, 1981).

Abbreviations used: VLD lipoproteins, very-low-density lipoproteins; LD lipoproteins, low-density lipoproteins; HD lipoproteins, high-density lipoproteins.

The triacylglycerol-rich lipoproteins that accumulate in the plasma of laying hens or oestrogen-treated chicks are different from VLD lipoproteins of immature-hen or -rooster plasma. They are smaller (Chapman *et al.*, 1977; Kudzma *et al.*, 1979) and have different lipid composition (Gornall & Kucsis, 1973), a lower electrophoretic mobility (Evans, 1974) and a broader density distribution (Kudzma *et al.*, 1979). Moreover, whereas VLD lipoproteins from immature-hen plasma contain at least six major apoproteins (Kudzma *et al.*, 1979; Griffin, 1981) those from the plasma of laying hens or oestrogen-treated chicks contain only two (Chan *et al.*, 1976; Williams, 1979). One of these, the high-molecular-weight apo-B, occurs in both immature- and laying-hen VLD lipoproteins. The other, apo-VLDL-II, is synthesized only in response to oestrogens (Wiskocil *et al.*, 1980) and is probably absent from both immature-hen and rooster VLD lipoproteins.

In the present paper we examine whether the differences between immature- and laying-hen plasma VLD lipoproteins affect their hydrolysis by lipoprotein lipase *in vitro*. The properties and susceptibility to hydrolysis of portomicrons from immature and laying hens are also compared.

0306-3283/82/090647-08$01.50/1

Experimental

Materials

Intralipid (10%) was obtained from KabiVitrum, Ealing, London, U.K. Bio-Gel A-150m (100–200 mesh) was obtained from Bio-Rad Laboratories, Watford, Herts., U.K. Bovine serum albumin [Cohn Fraction V; Sigma (London) Chemical Co., Poole, Dorset, U.K.] was depleted of fatty acids by using activated charcoal (Chen, 1967).

Chickens

Plasma lipoproteins were isolated from immature (10–12 weeks old) or laying hens (25–40 weeks old) from the Poultry Research Centre's S- and T-lines. Both strains are derived from commercial hybrids bred for egg-laying performance, the S-line from White Leghorn stock and the T-line from a Rhode Island Red/Light Sussex cross. Hens were fed normal commercial-type diets appropriate for their ages which contained about 2% (w/w) fat. Portomicrons were prepared from the plasma of hens fed diets containing 15% (w/w) added corn oil for at least 4 days before being killed.

Isolation of lipoproteins

Hens were anaesthetized by intravenous injection of sodium pentobarbitone (Expiral; Abbott Laboratories, Queenborough, Kent, U.K.) and blood collected from the jugular vein. EDTA was added to a final concentration of about 2mg/ml of blood and all subsequent operations were performed at 0–4°C. Blood from two or three hens was usually combined, though in some experiments VLD lipoproteins were isolated from individual laying hens. Plasma was prepared by centrifugation at 1000 *g* for 10 min. VLD lipoproteins were isolated by centrifuging plasma at 150000 *g* for 18h in an MSE 6 × 14 ml swing-out rotor after overlaying with at least 3ml of 0.154M-NaCl/10mM-Tris/HCl/1mM-EDTA, pH7.4. HD lipoproteins were isolated by sequential centrifugation of plasma adjusted to solvent densities of 1.063 and 1.225 by addition of solid KBr. VLD lipoproteins and LD lipoproteins were removed by centrifugation at 150000 *g* for 20h. HD lipoproteins were recovered by centrifugation at 150000 *g* for 40h and dialysed against 0.154M-NaCl/10mM-Tris/HCl/1mM-EDTA, pH7.4. Portomicrons were isolated by centrifugation at 150000 *g* for 2h after overlaying with 0.18M-Tris/HCl/1mM-EDTA, pH8.9, and further purified by gel filtration on a Bio-Gel A-150m column (40cm × 2.6cm) equilibrated with the same buffer containing 0.02% NaN_3. Lipoproteins eluting in the void volume were concentrated by centrifugation at 150000 *g* for 2h and dialysed against 0.154M-NaCl/10mM-Tris/HCl/1mM-EDTA, pH7.4. When necessary lipoproteins were concentrated further using Centriflow CF25 ultrafiltration cones (Amicon Corp., Lexington, MA, U.S.A.).

Gel filtration of lipoproteins

This was performed at 4°C on a column (60cm × 2.6cm) of Bio-Gel A-150m that had been equilibrated with 0·18M-Tris/HCl/1mM-EDTA, pH8.9, containing 0.02% NaN_3. Lipoprotein preparation (5–10ml) was applied to the column, which was then eluted with buffer at a rate of 12ml/h.

Delipidation of lipoproteins

Lipids were removed from lipoproteins as described by Scanu & Edelstein (1971). Lipoproteins concentrated to about 1mg/ml were extracted with 50vol. of ethanol/diethyl ether (1:3, v/v) at 0°C for 4h. Precipitated protein was recovered by centrifugation and washed once with 20 vol. of ethanol/diethyl ether (1:3, v/v) and once with 20 vol. of diethyl ether at 0°C. Residual diethyl ether was removed by evaporation under N_2 and apoproteins were dissolved in 0.154M-NaCl/10mM-Tris/HCl/1mM-EDTA, pH7.4.

Purification of lipoprotein lipase

Lipoprotein lipase was partly purified from acetone/diethyl ether powders of adipose tissue from 7-week-old male broiler chickens by heparin-affinity chromatography. Purification was performed at 0–4°C and all buffers contained 20% (w/v) glycerol. Powders were extracted with 1.2M-NaCl/10mM-sodium phosphate, pH6.5 (Bensadoun *et al.*, 1974; Kompiang *et al.*, 1976) for 1h and insoluble material removed by centrifugation at 18000 *g* for 20 min. The clear supernatant was dialysed for 18h against 3 vol. of 10mM-sodium phosphate, pH6.5, and applied to a column (12cm × 4cm) of heparin–Sepharose 4B (Iverius, 1971) that had been equilibrated with 0.3M-NaCl/10mM-sodium phosphate, pH6.5. The column was washed with 3 vol. of the same buffer and the enzyme was eluted with 1.2M-NaCl/10mM-sodium phosphate, pH6.5. Fractions containing lipoprotein lipase activity were combined and dialysed against 10mM-sodium phosphate, pH6.5. Recovery of applied activity was normally greater than 50% with an approx. 50-fold increase in specific activity. Enzyme activity was stable for several months at −70°C.

Assay of lipoprotein lipase

Lipoprotein lipase activity towards activated Intralipid or isolated lipoproteins was determined by measuring release of fatty acids, usually by indirect or direct titration. Intralipid was activated by incubation with an equal volume of immature hen serum for 1h at 37°C. Reaction mixtures for indirect assays contained 10μmol of substrate triacylglycerol and 30mg of fatty acid-free bovine

serum albumin in 1ml of 0.154M-NaCl/50mM-Tris/HCl/5mM-$CaCl_2$, pH8.0. Reactions were started by addition of enzyme and incubated at 37°C for 1 h. Fatty acids were extracted as described by Dole & Meinertz (1960) and titrated aginst 0.01M-NaOH with palmitate as standard. In some experiments the assay volume was reduced to 250μl and fatty acids were determined colorimetrically as described by Laurell & Tibling (1966) except that diethyldithiocarbamate was used as colour-developing reagent and zeolite was used to remove phospholipids.

The conditions for assay of activity by direct titration of liberated fatty acids were similar to those used by Chung & Scanu (1974). Reaction mixtures contained 2, 5 or 10μmol of substrate triacylglycerol and 60mg of fatty acid-free bovine serum albumin in 2ml of 0.154M-NaCl/5mM-$CaCl_2$/2mM-Tris/HCl, pH8.0. Incubations were performed at 37°C under N_2 and pH was monitored using a digital pH meter (model 501; Orion Research, Cambridge, MA, U.S.A.) equipped with a semi-micro electrode. pH was adjusted to 8.00 after a pre-incubation of 5 min and reactions were started by addition of enzyme. Fatty acids released were titrated continuously by addition of 0.01M-NaOH from an autoburette (model ABU 12; Radiometer, Copenhagen, Denmark). Extraction and estimation of fatty acids in samples taken during trial incubations confirmed that H^+ release was due to liberation of fatty acids.

Electron microscopy

Plasma lipoproteins were diluted with buffer containing 10g of ammonium acetate, 0.35g of ammonium carbonate and 5 mg of EDTA/litre, pH7.2, and either negatively stained with 2% (w/v) sodium phosphotungstate, pH7.0, as described by Forte *et al.* (1968), or fixed with OsO_4 vapour and shadowed with platinum as described by Jones & Price (1968). The size of lipoproteins with diameters less than 40 nm was determined from electron micrographs (final magnification, ×71000) of negatively stained preparations. The sizes of larger lipoproteins were determined from electron micrographs (×12500 or 27000) of shadowed preparations; measurements of flattened particles were not included. Values for lipoprotein diameter are means ± S.D. of at least 400 measurements.

Other methods

Protein was determined by the method of Lowry *et al.* (1951) with bovine serum albumin as standard. Samples were extracted with chloroform to eliminate light scattering due to suspended lipid. Lipoprotein triacylglycerol was measured by using a semi-automated fluorimetric method (Kessler & Lederer, 1965). Lipoprotein phospholipid was determined by measuring phosphorus content (Bartlett, 1959) of chloroform/methanol extracts (Bligh & Dyer, 1959). Total and free cholesterol was measured enzymically (Allain *et al.*, 1974) in the presence and absence of cholesterol esterase.

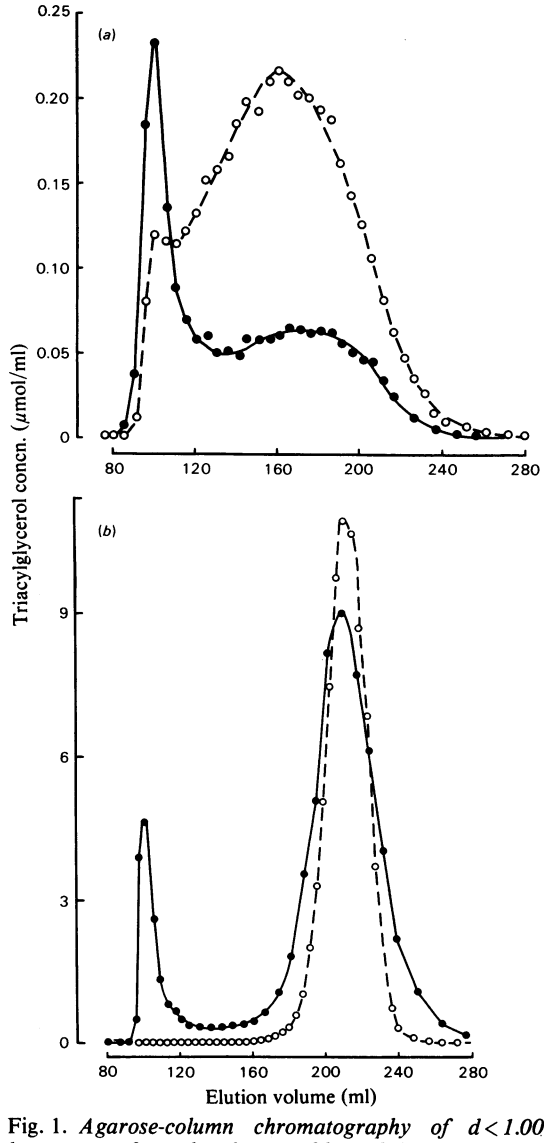

Fig. 1. *Agarose-column chromatography of d<1.006 lipoproteins from the plasma of hens fed on normal and high-fat diets*

Lipoproteins of relative density less than 1.006 were isolated by centrifugation of 20ml of plasma from S-line hens fed on standard diets (○) or on diets supplemented with 15% (w/w) corn oil (●) and fractionated by chromatography on a Bio-Gel A-150m column (60cm × 2.6cm) as described in the Experimental section. (*a*) Immature hens; (*b*) laying hens. Similar results were obtained with lipoproteins from T-line hens.

Results

Characteristics of triacylglycerol-rich lipoproteins of hen plasma

The sizes of lipoproteins isolated from the plasma of immature and laying hens were examined by electron microscopy and gel filtration and the elution patterns of typical separations on Bio-Gel A-150m are shown in Fig. 1. Almost all the triacylglycerol in the plasma of laying hens fed the standard diet (which contained a relatively low level of fat) was present in small VLD lipoproteins with a mean diameter of 30 ± 5 nm. VLD lipoproteins from the plasma of immature hens fed the standard diet were larger and had a greater range of size. Much larger lipoproteins, presumed to be intact and partly degraded portomicrons, accumulated in the plasma of hens fed diets supplemented with 15% (w/w) corn oil. They could be recovered by centrifuging plasma at 150000 *g* for 2h but further purification by gel filtration was necessary to remove contamination by VLD lipoproteins. Although VLD lipoproteins from immature and laying hens were different in size and lipid composition, portomicrons from immature and laying hens were very similar (Table 1). Their mean diameters of about 155nm are similar to that reported for portomicrons isolated from functionally hepatectomized roosters injected with Triton WR-1339 (Bensadoun & Rothfeld, 1972) but almost twice that of 'newly synthesized' portomicrons from roosters injected with anti-lipoprotein lipase antiserum (Bensadoun & Kampiang, 1979). However, in both these studies portomicron size was estimated from flotation rate rather than by electron microscopy.

Susceptibility of lipoproteins to hydrolysis

In preliminary investigations into the susceptibility of VLD lipoproteins and portomicrons to lipoprotein lipase we compared initial rates of hydrolysis. Assays contained 10μmol of substrate triacylglycerol/ml; the rate of fatty acid release was usually constant until at least 5% of the substrate had been hydrolysed. The initial rate of hydrolysis of VLD lipoproteins from the plasma of laying hens was similar to that of activated Intralipid (Table 1), as previously reported by Husbands (1972). Activity towards both these substrates had an optimum at pH8.0 and was similarly stimulated by Ca^{2+} and inhibited by EDTA and high NaCl concentration. The initial rate of hydrolysis of VLD lipoproteins from immature hen plasma was also similar to that of activated Intralipid, but portomicrons from both immature and laying hens were hydrolysed up to twice as rapidly (Table 1).

Major differences in the susceptibility of VLD lipoproteins from immature and laying hens became apparent when hydrolysis was allowed to continue. Hydrolysis of VLD lipoproteins from the plasma of immature hens, and portomicrons from immature and laying hens, proceeded rapidly until at least 40% of lipoprotein triacylglycerol had been hydrolysed, with only a gradual decrease in rate of fatty acid release as hydrolysis progressed. In contrast only a part of the triacylglycerol of laying-hen plasma VLD lipoproteins was readily hydrolysed and further hydrolysis occurred slowly (Fig. 2). VLD lipoproteins from some laying hens were more susceptible than those from others, but there was no obvious difference between S- and T-line hens. The proportion of VLD-lipoprotein triacylglycerol readily hydrolysed varied from 1 to 15% with a mean of about 10%. The subsequent low rate of hydrolysis was not due to denaturing or irreversible absorption of enzyme because addition of a susceptible substrate (i.e. activated Intralipid) produced an immediate and substantial increase in activity. The proportion of laying-hen VLD lipoproteins readily

Table 1. *Characteristics of d < 1.006 lipoproteins from immature and laying hens*

Lipoproteins were isolated from S-line hens as described in the Experimental section. Initial rates of hydrolysis were determined by direct titration of liberated fatty acids and expressed relative to the initial rate of hydrolysis of activated Intralipid by the same amount of enzyme. Values for lipid composition and initial rates of hydrolysis are means ± S.D. of three or four experiments. Values for laying hens that are significantly different (Student's *t* test) from those for immature hens are indicated by * ($P < 0.01$). Values for lipoprotein size are means from single preparations ± S.D.

	VLD lipoproteins		Portomicrons	
	Immature	Laying	Immature	Laying
Lipid composition (%)				
Triacylglycerol	60.0 ± 2.9	67.5 ± 3.3*	89.8 ± 1.4	89.2 ± 1.0
Phospholipid	16.7 ± 0.6	25.1 ± 2.4*	6.4 ± 1.2	7.1 ± 0.9
Cholesterol	17.4 ± 2.6	6.6 ± 1.0*	3.8 ± 1.0	3.7 ± 0.4
Cholesteryl ester	6.0 ± 0.5	0.8 ± 0.1*		
Cholesterol/phospholipid	1.04 ± 0.20	0.26 ± 0.03*		
Diameter (nm)	58 ± 37	30 ± 5	156 ± 45	155 ± 50
Initial rates of hydrolysis by lipoprotein lipase	1.14 ± 0.39	1.05 ± 0.14	1.74 ± 0.43	1.65 ± 0.79

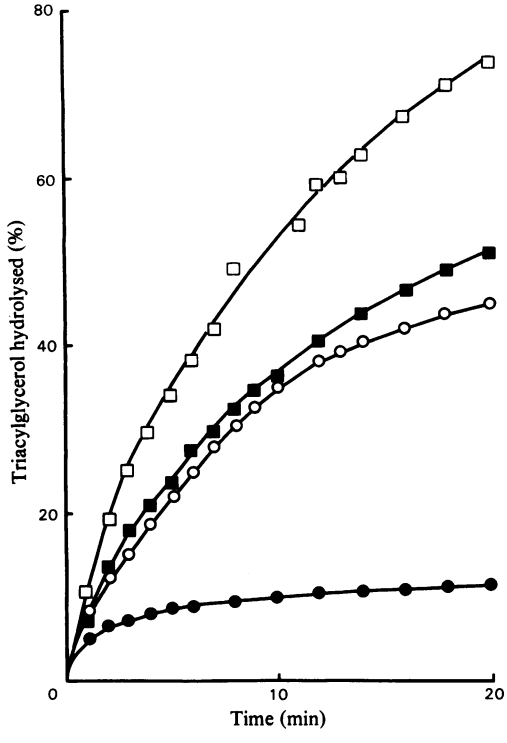

Fig. 2. *Hydrolysis of VLD lipoproteins and portomicrons from immature- and laying-hen plasma by lipoprotein lipase*

The hydrolysis of lipoproteins isolated from the plasma of T-line hens was followed by direct titration of liberated fatty acids as described in the Experimental section. Incubations contained 2 μmol of substrate triacylglycerol and reactions were started by addition of 2 μg of lipoprotein lipase protein. ○ and ●, VLD lipoproteins; □ and ■, portomicrons. ○ and □, immature hens; ● and ■, laying hens. Similar results were obtained in experiments with lipoproteins from S-line hens.

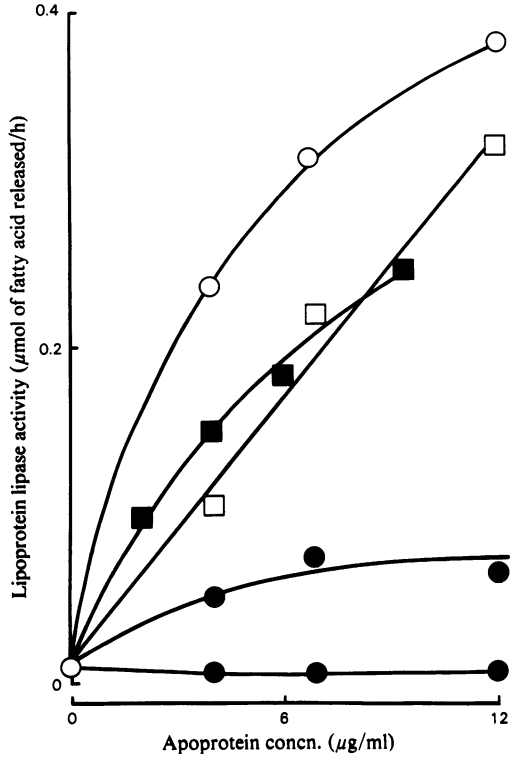

Fig. 3. *Lipoprotein lipase activator content of triacylglycerol-rich lipoproteins from immature and laying hens*

Lipoprotein apoproteins were pre-incubated for 1 h at 37°C with 2.5 μmol of Intralipid triacylglycerol in 250 μl of 0.154 M-NaCl/50 mM-Tris/HCl/5 mM-$CaCl_2$, pH 8.0, containing 3% (w/v) bovine serum albumin. Reactions were started by addition of 0.5 μg of lipoprotein lipase protein and terminated after 1 h. Fatty acids were extracted and estimated colorimetrically as described in the Experimental section. ○ and ●, VLD apolipoproteins; □ and ■, portomicron apoproteins. ○ and □, Immature hen; ● and ■, laying hen. Data for laying-hen VLD lipoproteins are the results of incubations with two apoprotein preparations from individual hens. Other apolipoproteins were prepared from blood pooled from two or three hens.

hydrolysed was increased slightly in the presence of 5 mM-Ca^{2+} and reduced by about 50% in 1 mM-EDTA, but was unaffected by pre-incubation with HD lipoproteins.

Lipoprotein lipase activator content of lipoproteins

Normal metabolism of mammalian plasma VLD lipoproteins *in vivo* is dependent on their content of a specific lipoprotein lipase-activator apoprotein, apo-C-II (Breckenridge *et al.*, 1978). The avian equivalent of apo-C-II has not yet been positively identified, but the ability of isolated lipoproteins and their apoproteins to activate artificial substrates such as Intralipid is probably a reliable estimate of their activator content. By this criterion, VLD lipoproteins from immature-hen plasma and portomicrons from immature and laying hens contain substantial amounts of activator apoprotein, whereas VLD lipoproteins from laying-hen plasma contain little or none (Fig. 3). Both immature- and laying-hen plasma are, however, potent activators of lipoprotein lipase, with most of their activating ability residing in their HD lipoproteins (Fig. 4). The lower activator content of laying-hen serum is probably largely due to its lower HD lipoprotein concentration because HD lipoproteins purified from

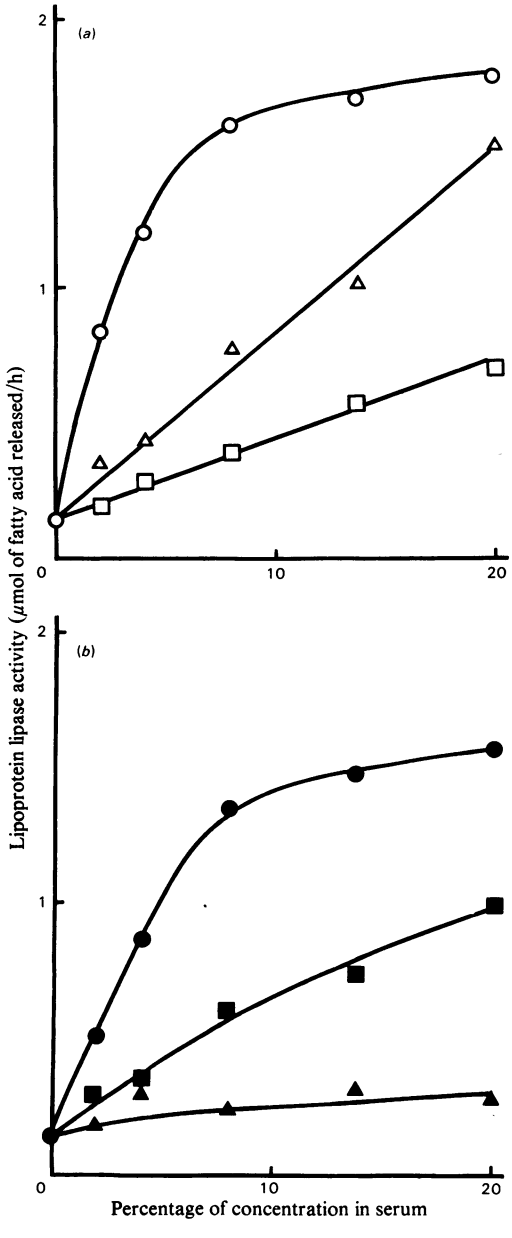

Fig. 4. *Distribution of lipoprotein lipase activator in immature- and laying-hen serum*

Blood was allowed to clot for 1 h at about 20°C and serum was recovered by centrifugation at 1000 *g* for 10 min. Lipoproteins of relative density less than 1.063 (□ and ■), high-density lipoproteins (○ and ●) and a residual fraction with density greater than 1.225 (△ and ▲) were isolated by sequential centrifugation. Lipoproteins were extracted with diethyl ether/ethanol (3:1, v/v) as described in the Experimental section and their apoproteins were pre-incubated with 10 μmol of Intralipid triacylglycerol in 1 ml of incubation medium as described in the legend to Fig. 3. The residual fraction was pre-incubated with Intralipid without extraction. Reactions were started by addition of 2 μg of lipoprotein lipase protein and terminated after 1 h. Non-esterified fatty acids were determined by titration after extraction. (*a*) Immature hen; (*b*) laying hen. Immature-hen serum contained 0.1 mg of VLD-plus-LD lipoprotein-apoprotein and 1.8 mg of HD lipoprotein-apoprotein per ml. Laying-hen serum contained 3.7 mg of VLD-plus-LD lipoprotein-apoprotein and 1.1 mg of HD lipoprotein-apoprotein per ml.

immature- and laying-hen plasma have the same activating ability when expressed per mg of protein (results not shown).

Discussion

The metabolism of plasma VLD lipoproteins from laying and immature hens has been studied *in vivo* by Bacon *et al.* (1978). They found that the rate of clearance from the circulation of immature hens was lower for laying-hen VLD lipoproteins than it was for immature-hen VLD lipoproteins. They concluded that the low rate of clearance of triacylglycerol from the circulation of laying hens was at least partly due to some property of their VLD lipoproteins. The results described in the present paper strongly suggest that this property is a low content of lipoprotein lipase activator apoprotein, which limits the susceptibility of VLD lipoprotein to hydrolysis by lipoprotein lipase.

Plasma VLD lipoproteins deficient in activator occur in some patients with hyperlipoproteinaemia, type 1 (Breckenridge *et al.*, 1978), and in guinea-pigs (Fitzharris *et al.*, 1981). However, in these instances activator is also absent from the plasma and normal rates of hydrolysis of VLD lipoprotein triacylglycerol can be restored by incubation with apo-C-II. Plasma VLD lipoproteins from laying hens have a low content of activator despite an abundance of activator in their plasma HD lipoproteins. Transfer of apo-C-II from HD lipoproteins to VLD lipoproteins in mammals occurs after secretion of nascent VLD lipoproteins from the liver (Nestruck & Rubenstein, 1976) and this is probably also the case in the immature hen or rooster. The affinity of artificial substrates and lipoproteins for activator apoproteins is probably determined by physicochemical conditions at their surface. The properties that distinguish laying-hen plasma VLD lipoproteins and that could account for their low activator content include their low phospholipid/cholesterol ratio (Table 1), small size and the presence of apo-VLDL-II.

The differences between plasma VLD lipoproteins from immature and laying hens are substantial and it seems likely they are primarily the

result of oestrogen-induced changes in the nature of VLD lipoproteins synthesized by the liver rather than a consequence of altered VLD lipoprotein catabolism. Hepatic synthesis of apo-VLDL-II, which accounts for about 50% of laying-hen plasma VLD lipoprotein apoprotein, is completely oestrogen-dependent (Wiskocil *et al.*, 1980) and the differences in size and lipid composition of immature- and laying-hen plasma VLD lipoprotein could also be due to the effects of oestrogens on the pattern of hepatic lipid and/or apoprotein synthesis. The variation in susceptibility of VLD lipoproteins from different laying hens could be due to more subtle hormonal and/or genetic effects on lipoprotein synthesis. Alternatively it may be a reflection of the metabolic history of the lipoproteins: those lipoproteins showing the least susceptibility *in vitro* may have been hydrolysed substantially *in vivo*.

The synthesis by laying hens of VLD lipoproteins with a limited susceptibility to hydrolysis by lipoprotein lipase would allow the transfer of largely intact lipoproteins from liver to yolk. Lipoprotein lipase activity in the adipose tissue and heart of laying hens is much lower than that in immature hens (Husbands, 1972) and this must also contribute to the direction of VLD lipoprotein triacylglycerol to the ovary rather than to other tissues. The small size of laying-hen VLD lipoproteins is thought to facilitate their passage out of the capillaries in the ovary and through the various connective-tissue layers in the ovarian follicle (Perry & Gilbert, 1979). Lipoprotein lipase is present in the granulosa cells of the ovarian follicle and Bensadoun & Kompiang (1979) have claimed that lipoprotein lipase mediates uptake of triacylglycerol fatty acids into the oocyte. However, the specific activity of the granulosa cell lipoprotein lipase is about one-tenth that in rooster adipose tissue (Bensadoun & Kompiang, 1979) and it is unlikely that lipoprotein lipase is directly involved in transporting lipids into yolk.

Portomicrons are rapidly cleared from the circulation (Bensadoun & Kompiang, 1979) and this probably explains their absence from the plasma of hens fed on diets relatively low in fat. The accumulation of portomicrons in hens fed on diets containing 15% (w/w) added corn oil, however, indicates that the mechanisms involved in their removal from the circulation can be overloaded. Normal clearance of portomicrons is dependent on their metabolism by lipoprotein lipase (Bensadoun & Kompiang, 1979). Although portomicrons from immature and laying hens are similarly susceptible to hydrolysis by lipoprotein lipase, the lower tissue activity of lipoprotein lipase in laying hens could well account for the greater accumulation of portomicrons in laying-hen plasma compared with that in immature hens (Fig. 1).

Although the physiological importance of lipoprotein lipase activator apoprotein to lipoprotein metabolism is well established, at least in mammals, the mechanism by which activator stimulates lipoprotein lipase is not fully understood. The initial rates of hydrolysis of plasma VLD lipoproteins from immature and laying hens were very similar despite the considerable difference in their activator content and this suggests high activator content is not necessary for initial association of chicken lipoprotein lipase with lipoprotein substrates. The limited susceptibility of laying-hen plasma VLD lipoproteins is, however, consistent with proposals (Fitzharris *et al.*, 1981) that the primary role of activator is to facilitate interaction between enzyme and triacylglycerol in the face of changes in lipoprotein surface structure that occur as hydrolysis proceeds.

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