Plasma lipoprotein metabolism in lean and in fat chickens produced by divergent selection for plasma very low density lipoprotein concentration

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Abstract Plasma lipoprotein metabolism was studied in vivo in two lines of chickens produced by selection for high and low plasma very low density lipoprotein (VLDL) concentration. Rates of VLDL secretion were measured by determining the rate of accumulation of triglyceride in the plasma after intravenous injection of anti-lipoprotein lipase antibody. The clearance of VLDL-triglyceride and its uptake into liver and adipose tissue was examined using radioactively labeled VLDL synthesized in vivo. The rate of VLDL secretion was about threefold higher in the high-VLDL line as compared to the leaner, low VLDL-line (6.7 vs 2.1 μmol VLDL triglyceride/h per ml of plasma). The clearance of VLDL from the circulation of the low VLDL line was much faster than that of the high VLDL line ($t_{1/2}$ of 3.7 and 13.6 min, respectively). The proportion of administered radiolabel taken up by the abdominal fat pad was substantially greater in the fat line than in the lean line (11.9 vs 4.8%, respectively). Lipoprotein lipase acitivities in leg muscle and heart were consistently greater in the low-VLDL line and β-hydroxybutyrate concentrations in the plasma of the low-VLDL line were significantly greater than those in the high-VLDL line (0.86 vs 0.48 μmol/ml). The results show that the approximately tenfold difference in plasma VLDL concentration between lines is primarily due to markedly different rates of hepatic VLDL production and that selection has made a major effect on partitioning of VLDL triglyceride between adipose and other tissues. We propose that a direction of fatty acids to oxidation rather than VLDL synthesis in the liver of birds of the low-VLDL line is a major cause of their low rate of VLDL secretion and makes an important contribution to improved efficiency of protein utilization. In addition, preferential use of VLDL-triglyceride and β-hydroxybutyrate may reduce amino acid oxidation by muscle.— **Griffin, H., F. Acamovic, K. Guo, and J. Peddie.** Lipoprotein metabolism in lean and in fat chickens produced by divergent selection for plasma very low density lipoprotein concentration. *J. Lipid Res.* 1989. **30:** 1243–1250.

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Adipocytes in birds have a limited capacity for lipogenesis and much of the fat that accumulates in avian adipose tissue is synthesized in the liver or derived from the diet. Plasma lipid transport in immature birds (1) is similar to that in mammals, although dietary fatty acids are absorbed directly into the portal vein (as nonesterified fatty acids and portomicrons) rather than through the lymphatic system (2, 3).

Previous studies have shown that the concentration of very low density lipoproteins in the plasma of fully fed broiler (meat-type) chickens is sufficiently well correlated (r up to 0.7) with their body fat content to act as an indirect means of estimating fatness in live birds (4, 5). Divergent genetic selection of a commercial broiler grandparent line for plasma VLDL concentration for seven generations (6, 7) has produced lean and fat lines of chicken with a greater than sixfold difference in plasma VLDL concentration. Birds of the leaner, low VLDL lines are much more efficient (+27%) than those of the fatter, high-VLDL line at converting dietary protein into body protein and this suggests that selection has had a major impact on protein and/or amino acid metabolism, as well as on fat deposition.

The low- and high-VLDL lines provide a potentially very useful model for studying both the role of plasma lipoprotein metabolism in regulating adipose tissue growth and the biochemical basis of the relationships between body composition and feed efficiency. This report describes an investigation of the origin of the large differences in plasma VLDL concentration between the lines and examines the effects of selection on tissue lipoprotein lipase activities and partition of lipoprotein triglyceride between tissues.

Abbreviations: VLDL, very low density lipoprotein; LPL, lipoprotein lipase; TLC, thin-layer chromatography.

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MATERIALS AND METHODS

Materials

Heparin (from porcine pancreas and containing 176 U/mg) and bovine serum albumin (Cohn fraction V) were bought from Sigma Chemical Co., Poole, UK. Fatty acids were removed from albumin using charcoal (8). Intralipid (20%) was obtained from Kabi Vitrum, Stockholm, Sweden. [1-^{14}C]Palmitic acid and [9,10(n)-^{3}H]palmitic acid were bought from Amersham International and used at the specific activity supplied (2 MBq/μmol).

Chickens, diets, and husbandry conditions

Day-old chicks were obtained from the low- and high-VLDL lines at the 6th, 7th, or 8th generation of selection and reared to 3 weeks of age in brooder cages on a standard chick starter diet. The chicks were then transferred to individual cages and fed the same low-fat diet used during selection (9). This contained 25 g ether-extractable fat/kg. They were kept on a 14-h light: 10-h dark photoperiod with lights switched on at 0600 h. Feed and water were provided ad libitum. Broiler chickens reared under such conditions eat continually throughout the light period with increases in feed intake immediately after lights on and before lights out (10). Experiments were performed between 1000 and 1600 h when birds would be expected to be fully fed and feeding regularly every 15–20 min. Normal (commercial) broiler chicks were used in some experiments and these were reared under the same conditions. Except when stated, comparisons between lines were made at 6–7 weeks of age (i.e., the same age at which the selection has been performed in creating the lines).

Rates of lipoprotein secretion

The rate of secretion of VLDL into the plasma was measured by determining the rate of accumulation of lipoprotein triglyceride in the plasma after intravenous injection of anti-LPL antibody, as initially described by Kompiang, Bensadoun, and Yang (11). The antiserum used in the present study was raised in sheep by intramuscular injection of 50–100 μg of chicken LPL at 2–3 week intervals. The LPL was purified from adipose tissue by heparin-affinity and concanavalin A-affinity chromatography, using methods similar to those used by Kompiang et al. (11). Freund's complete adjuvant was used for the initial immunization and Freund's incomplete adjuvant thereafter.

Antibody titres were assessed by determining the ability of sera to precipitate LPL purified from adipose tissue and to inhibit LPL activity in vitro. Immunoprecipitation was performed by incubating 100 μl of 10 mM sodium phosphate, pH 6.5, containing 20% glycerol and increasing amounts of partially purified LPL for 30 min at 4°C with 100 μl of antiserum diluted 50-fold with 0.9% NaCl. Ten μl of donkey anti-sheep antiserum (Scottish Antibody Production Unit, Carluke, Scotland) was added to each incubation which was continued for a further 30 min at 4°C. Precipitated antibody and antibody-enzyme complexes were removed by centrifugation at 3000 *g* for 20 min. Lipase activity was determined by incubating aliquots of the supernatant with 10 μmol Intralipid-triglyceride in 50 mM Tris-HCl, pH 8.0, containing 2 mM $CaCl_2$ and 2% (w/v) fatty acid-depleted bovine serum albumin in a total volume of 250 μl. Intralipid had previously been activated by incubating with recalcified chicken plasma at 37°C for 30 min (3 ml plasma/ml of Intralipid). Incubations were performed at 37°C for 30 min and terminated by addition of 2.4 ml of 0.1 M glycine, pH 2.7. Fatty acids released were determined as described by Bowyer, Cridland, and King (12). The inhibitory activity of antisera was assessed by adding enzyme and diluted antiserum directly to the same assay system.

Antibodies capable of precipitating LPL were present in sheep serum after the third immunization, but serum with a high inhibitory titre was obtained only after a final immunization with an LPL preparation that had been incubated in 10 mg glutaraldehyde/ml for 30 min at 20°C immediately prior to injection. Immunotitration of this antiserum against LPL as described by Kompiang et al. (11), but testing its ability to inhibit rather than immunoprecipitate, indicate a titre equivalent to about 1600 μmol fatty acid released/h per ml. Antibody was partially purified from this serum by precipitation with 40% ammonium sulfate and dialyzed exhaustively against 0.9% NaCl. The equivalent of 4 ml of crude antiserum was injected intravenously via the wing vein into each bird. Blood samples were removed from the contralateral wing vein immediately before injection of antibody and at 10-min intervals thereafter for determination of plasma VLDL concentration.

Analysis of the LPL preparation used for immunization by SDS-PAGE showed it contained a single major component with a molecular weight of about 60,000. The specificity of the antiserum was not examined in detail. Use of the antiserum for immunoblotting of a post-heparin plasma sample that had been partially purified by heparin-affinity chromatography and then separated by SDS-PAGE showed that most of the reactivity was towards LPL. Antibodies to smaller molecular weight components were also present, but whether these were smaller molecular weight forms of LPL or contaminants was not determined.

Preparation of labeled VLDL

Biologically labeled VLDL were prepared by intravenous injection of fully fed 6-week-old broiler chickens with 6.4 MBq of [^{14}C]- or [^{3}H]palmitic acid (2 MBq/μmol; Amersham International, England) in 2 ml of recalcified chicken plasma. Two ml of antilipoprotein lipase antibody

preparation was injected intravenously after 10 min and the bird was killed 40 min later by intravenous injection of sodium pentobarbitone (Expiral, Abbott Laboratories, Queenborough, Kent). Blood was collected from the jugular vein and EDTA was added to a final concentration of 2 mg/ml. Plasma was prepared by centrifugation at 1000 *g* for 10 min at 10°C. VLDL were isolated by centrifuging plasma at 100,000 *g* for 20 h at 10°C after overlaying with 0.9% NaCl. The labeled VLDL were used within 6 h.

The distribution of label between the lipid classes in the labeled lipoprotein was determined by TLC. About 97% of the radioactivity in VLDL was present in triglyceride with about 1.5% in both the nonesterified fatty acid and phospholipid fractions.

Lipoprotein clearance and uptake into tissues

Labeled VLDL (containing about 6×10^5 dpm [^{14}C]- or 2×10^6 dpm [^{3}H]palmitate) were injected into the wing vein and blood was sampled from the contralateral vein after 1 min and at intervals thereafter. Birds were killed by intravenous injection of sodium pentobarbitone, and abdominal fat (the retroperitoneal depot and fat surrounding the gizzard), heart, liver, and leg (gastrocnemius) muscles were rapidly removed, washed in ice-cold saline, frozen in liquid nitrogen, and stored at −70°C. Frozen tissues were homogenized by crushing, and duplicate samples of 1 g were extracted according to the method of Folch, Lees, and Sloane Stanley (13). Solvent was evaporated under a stream of nitrogen and extracted lipid was dissolved in scintillation fluid (Optiphase X, Fisons, Loughborough, UK) for determination of radioactivity.

Measurement of lipoprotein lipase activity

Frozen adipose tissue was crushed and 2-g samples were homogenized in 20 ml water containing 20 μg heparin/ml at 0°C using a Polytron homogenizer (Kinematica, Lucerne, Switzerland). After 30 min at 0°C and occasional stirring, the infranatant was decanted from beneath the floating fat. Two ml of infranatant was mixed with 10 ml acetone at 0°C and 8 mg bovine serum albumin was added as carrier. After 30 min at 0°C, precipitated protein was recovered by centrifuging at 1000 *g* for 10 min at 0°C. The protein pellet was washed once with 10 ml of acetone and once with 10 ml of diethyl ether, both at 0°C. Residual ether was removed under nitrogen and the resulting acetone-ether powders were stored at −70°C. Assay of LPL activity in acetone-ether powders from the infranatant and floating fat fractions indicated that homogenizing adipose tissue in water containing heparin released greater than 90% of total activity into the infranatant.

Acetone-ether powders were prepared from whole homogenates of heart and leg muscle using essentially the same procedure.

LPL activity was measured by homogenizing acetone-ether powders (derived from 0.2 g of tissue) in 4 ml of 20 mM Tris-HCl, pH 8.2, containing 20 μg heparin/ml, at 0°C. Duplicate 20-, 40-, and 60-μl aliquots of extract were incubated for 30 min at 37°C with 10 μmol activated Intralipid-triglyceride in 250 μl of 50 mM Tris-HCl, pH 8.0, containing 2% (w/v) fatty acid-depleted bovine serum albumin, 2 mM $CaCl_2$, and a final concentration of 10 μg heparin/ml. At this concentration, heparin slightly stimulated activity. Reactions were stopped by addition of 2.4 ml of 0.1 M glycine, pH 2.7, and fatty acids were determined as described above. Assays of replicate acetone-ether powders from the same crushed abdominal fat pad demonstrated a high reproducibility for this procedure, with a coefficient of variation of 6% (n = 12).

Post-heparin plasma was prepared from blood samples withdrawn from the wing vein 2 min after intravenous injection of 500 U heparin/kg body weight in saline. Lipase activity was determined using the assay system described above. LPL accounts for greater than 98% of lipolytic activity in post-heparin plasma of broiler chickens (14).

Other methods

Plasma VLDL concentration was routinely measured using a turbidimetric assay (5). Absorbance units were converted to μmol VLDL-triglyceride using a standard curve derived by isolating VLDL from plasma by centrifuging for 18 h at 100,000 *g* and 10°C. The triglyceride content of isolated VLDL and plasma, and plasma concentrations of β-hydroxybutyrate were measured enzymatically using kits from BCL, Lewes, England and Sigma Diagnostics, St. Louis, MO, respectively. Statistical comparisons were made using Student's *t*-test.

RESULTS

Measurement of rate of VLDL secretion

The results of a preliminary experiment to test the validity of using anti-LPL antibody to measure rate of VLDL secretion in fully fed broiler chickens are shown in **Fig. 1**. Intravenous injection of 2 ml of anti-LPL antibody preparation caused a rapid rise in plasma VLDL concentration. The rate of accumulation was initially linear but began to decrease after about 20 min. Both ^{14}C-labeled VLDL injected 1 min after the antibody and ^{3}H-labeled VLDL injected after 20 min were cleared very slowly from the circulation. This suggests that the decline in rate of VLDL accumulation was due to a decrease in rate of VLDL secretion rather than a reduction in the effectiveness of the antibody with time. VLDL synthesis and secretion in isolated chick hepatocytes are very sensitive to glucagon (15) and the decline in rate of accumulation of

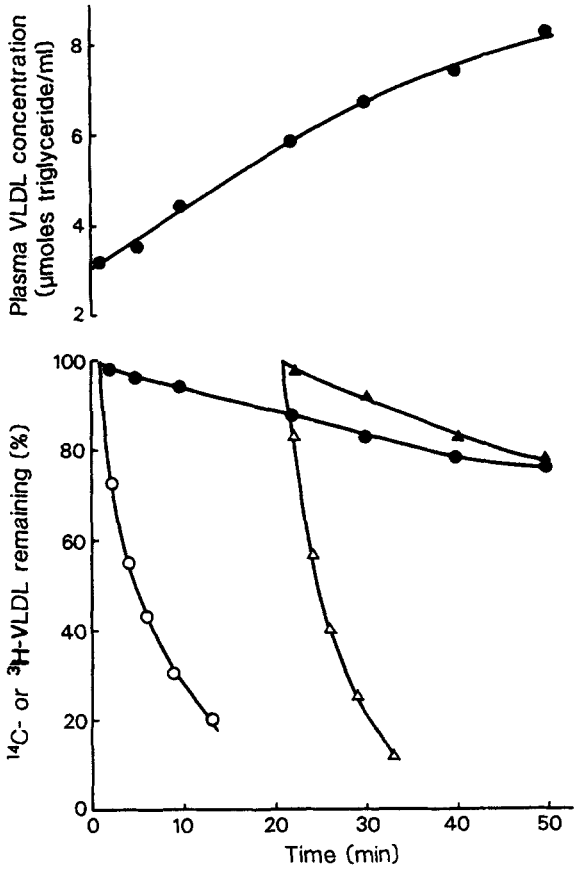

Fig. 1. Blockage of lipoprotein clearance by anti-lipoprotein lipase antibodies. Two ml of anti-LPL antibody preparation was injected at zero time into a 6-week-old broiler chicken via the wing vein. ^{14}C-labeled VLDL was injected intravenously 1 min later and ^{3}H-labeled VLDL injected after 20 min. The upper part of the figure shows the effect on plasma VLDL concentration. The lower part shows the clearance of [^{14}C]- (●) and [^{3}H]VLDL (▲). Plasma radioactivity is expressed as a percentage of the initial concentration at time of injection which was determined by extrapolation. Curves describing the clearance of the same labeled VLDL from the circulation of a second bird that was not treated with antibody are superimposed for comparison (○, △).

VLDL in this particular experiment may be due to an increase in circulating glucagon caused by the stress of frequent handling. To minimize stress in subsequent experiments, blood samples were taken only every 10 min and birds were returned to their cages between samplings. Under these conditions, VLDL accumulation was usually linear for at least 40 min after injection of antibody. Injection of 0.25 ml of antibody at 10-min intervals did not produce a progressive increase in rate of VLDL accumulation and this suggests that the amount of antibody routinely used (2 ml) provided a substantial excess.

A slow clearance of labeled VLDL from the circulation of chickens injected with anti-LPL antibody (Fig. 1) was also observed by Kompiang et al. (11) using antisera raised in rabbit and goat. It may reflect removal of labeled-VLDL damaged during isolation; we have found that [^{3}H]VLDL that had been frozen and thawed once before injection were rapidly and completely removed from the circulation even in the presence of antibody (data not shown). Alternatively it may be due to the equilibration of labeled-VLDL with VLDL bound to the vascular system or to VLDL clearance via lipoprotein lipase-independent pathways, e.g., by receptor-mediated endocytosis.

Blockage of lipoprotein metabolism using an anti-lipoprotein lipase antibody causes an accumulation of both portomicrons and VLDL. Portomicrons are considerably larger than VLDL and they can be recovered by centrifuging plasma at 100,000 *g* for 30 min (16). Less than 10% of total plasma triglyceride was present in a portomicron fraction prepared from the plasma of birds injected with anti-LPL antibody, and the rate of accumulation of plasma triglyceride following antibody injection into broilers on a low fat diet appears, therefore, to largely reflect the rate of VLDL secretion by the liver.

VLDL synthesis and clearance in high- and low-VLDL lines

The accumulation of VLDL in the plasma of birds from the high- and low-VLDL lines following injection of antibody is shown in **Fig. 2**. The rate of triglyceride secretion into the plasma of high-VLDL line birds was about threefold greater than that in birds of the low-VLDL line (**Table 1**) but the relationship between initial VLDL concentration and rate of VLDL secretion was not linear (**Fig. 3**).

Removal of VLDL labeled with [^{14}C]- or [^{3}H]palmitate from the circulation of birds from both lines appeared to

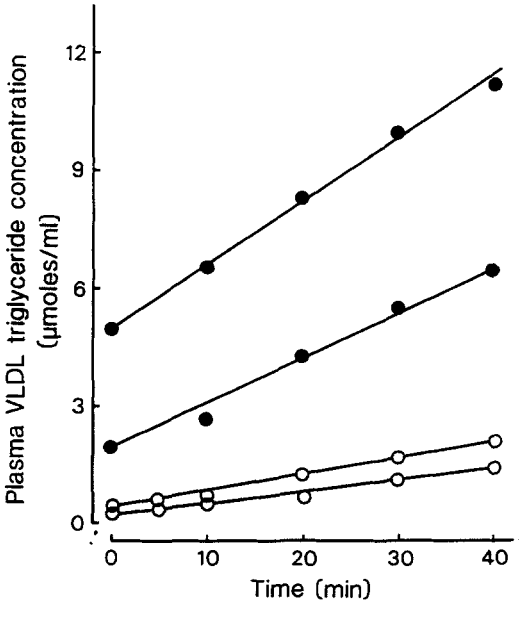

Fig. 2. Lipoprotein accumulation in the plasma of low- (○) and high-VLDL (●) line birds after injection of anti-lipoprotein lipase antibody. The results from two birds/line are shown.

TABLE 1. Plasma VLDL metabolism in high- and low-VLDL lines

	High-VLDL	Low-VLDL
VLDL production (μmol triglyceride/h per ml of plasma)	6.74 ± 1.74	2.14 ± 0.45[a]
$t_{1/2}$ for VLDL clearance (min)	13.6 ± 4.1	3.7 ± 0.7[a]
VLDL concentration (μmol triglyceride/ml of plasma)	3.2 ± 2.0[a]	0.28 ± 0.18
VLDL clearance (μmol triglyceride/h per ml of plasma)	7.6 ± 4.01[b]	2.1 ± 1.21

Rates of VLDL clearance were calculated from the $t_{1/2}$ of [^{14}C]VLDL clearance and the initial VLDL concentration in the plasma. Results are means ± SD of eight birds/line.
[a]Significant difference between lines: $P < 0.001$.
[b]Significant difference between lines: $P < 0.01$.

obey first order kinetics until at least 70% of the label had been removed. Clearance was much more rapid in birds of the low-VLDL line (Table 1) and calculation of the mean rates of VLDL turnover from the initial VLDL concentration and the $t_{1/2}$ for VLDL clearance in each bird produced values very similar to those determined using antibody (Table 1). Labeled VLDL prepared from a bird of the high-VLDL line was cleared from the circulation of a normal broiler with a half-life of 3.5 min, indicating that the slow clearance of labeled VLDL from the circulation of birds of the high-VLDL line was not due to any property of the lipoproteins themselves.

Fate of radioactively labeled VLDL

The uptake of radioactivity by tissues after intravenous injection of [^{14}C]VLDL was investigated in low- and high-VLDL birds killed 20 and 40 min after administration of label. Different time intervals were chosen to compensate for the different rates of clearance expected and to minimize possible re-entry into the plasma of radioactivity taken up by the liver. Release of label taken up by adipose tissue in fully fed birds was assumed to be negligible over the periods used.

Greater than 80% of the labeled VLDL had been removed from the plasma by 20 and 40 min (**Table 2**) and radioactivity in adipose tissue was therefore probably approaching a maximum. The proportion of label taken up by the abdominal fat of high-VLDL birds was over 2.5-fold greater than that in low-VLDL birds, although there was no significant difference in the total adipose tissue LPL activity in the abdominal fat pad of the same birds (see Table 3).

Changes in tissue lipoprotein lipase activities in response to selection for VLDL concentration

LPL activities in post-heparin plasma, adipose tissue, heart, and leg muscle in low- and high-VLDL birds at the 6th, 7th, or 8th generation of selection are shown in **Table 3**. Post-heparin lipase activity in the plasma of high-VLDL birds tended to be lower than that in the low-VLDL line, but differences were statistically significant only after 8 generations of selection. The total activity of lipoprotein lipase in the abdominal fat pad of the two lines was similar at the 6th generation of selection, but significantly greater in the lean line at the 7th generation. A more consistent response to selection was observed in heart and leg muscle: the specific activity of LPL in these tissues was significantly higher in the low-VLDL line at both the 6th and 7th generation of selection.

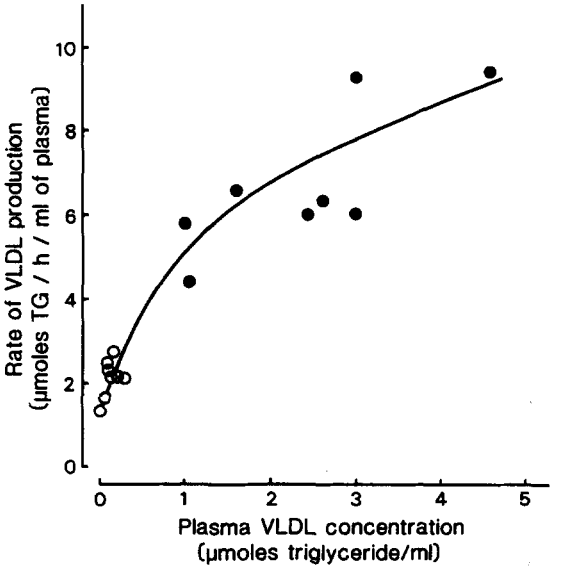

Fig. 3. Relationships between rate of lipoprotein production and plasma VLDL concentration. Rates of VLDL secretion into the plasma were determined using the anti-LPL antibody approach and compared with the VLDL concentrations present in the plasma immediately before injection of antibody; (●) high-VLDL line; (○) low-VLDL line.

TABLE 2. Fate of ^{14}C-labeled VLDL in high- and low-VLDL chickens

Tissue	% of Injected Radioactivity Recovered	
	High-VLDL	Low-VLDL
Abdominal fat pad	11.9 ± 4.0	4.8 ± 1.1[a]
Liver	12.1 ± 2.3	19.9 ± 3.7[b]
Plasma	17.7 ± 9.1	8.0 ± 2.0[b]

Labeled VLDL containing 6.89 × 10^5 dpm of [^{14}C]palmitate were injected intravenously into each 7-week-old chicken. Blood samples were taken from birds of the low-VLDL line after 20 min and from those of the high-VLDL line after 40 min. Birds were killed immediately after blood sampling and the radioactivity present in tissue lipids was determined as described in Materials and Methods. Plasma volume was assumed to be the same as in normal broilers at the same age (4.7% of body weight; H. Griffin, unpublished data). Radioactivity in tissues has not been corrected for the contribution of trapped blood. Values are the mean ± SD of results from eight birds/line. Birds were from the 7th generation of selection. Tissue LPL activities and carcass characteristics of the same birds are described in Table 3.

[a]Significant difference between lines, $P < 0.01$.

[b]Significant difference between lines, $P < 0.05$.

β-Hydroxybutyrate concentration in the plasma of low-VLDL birds was substantially greater than in those of the high-VLDL line after 7 generations of selection: 0.86 ± 0.18 versus 0.48 ± 0.16 μmol/ml, respectively ($P < 0.001$). β-Hydroxybutyrate concentration in normal broilers varied from 0.42 ± 0.18 μmol/ml in the fully fed state to 3.11 ± 0.74 μmol/ml after an 18-h fast (means ± SD of eight birds/group in each case).

DISCUSSION

Hypertriglyceridemia can arise through an increase in rate of VLDL secretion, an inhibition of VLDL clearance, or a combination of the two mechanisms. The results of the present study suggest that differences in plasma VLDL concentration produced by divergent genetic selection for plasma VLDL in broiler chickens are primarily the result of differences in rate of hepatic VLDL secretion. Previous studies on the hydrolysis of chicken VLDL by lipoprotein lipase in vitro (17) have shown that substrate concentrations of 5–10 μmol triglyceride/ml are needed to produce maximum activity. Plasma VLDL concentrations in normal broilers range from 0.5 to 2 μmol triglyceride/ml and this suggests that the rate of hydrolysis of lipoprotein triglyceride by lipoprotein lipase in chickens in vivo is severely limited by substrate concentration. This would account for the observation in the present study that relatively large differences in rates of VLDL secretion in the low-VLDL line produce little variation in plasma VLDL concentration (Fig. 3). Presumably any increase in VLDL secretion in these birds tends to be offset by an increase in rate of VLDL clearance due to small increase in substrate concentration. In the high-VLDL line, differences in rates of VLDL secretion had a much greater effect on plasma VLDL concentrations, suggesting that the mechanisms involved in clearance of VLDL from chicken plasma can be saturated. Total lipoprotein lipase activities in adipose tissue, heart, and leg muscle tended to be lower in the high-VLDL line. There was no clear evidence at the 6th generation of selection that hypertriglyceridemia in the high-VLDL line was a

TABLE 3. Response of tissue lipoprotein lipase activities to divergent selection for plasma VLDL concentration

	6th Generation					
	Age: 3 Weeks		Age: 7 Weeks		7th or 8th Generation	
	Low-VLDL	High-VLDL	Low-VLDL	High-VLDL	Low-VLDL	High-VLDL
Number of birds	10	10	10	10	8 or 12[a]	8 or 12[a]
Body weight (g)	671 ± 22	641 ± 65	2043 ± 171	2032 ± 98	1910 ± 102[b]	2086 ± 178
Abdominal fat (g/kg)	6.3 ± 2.2[c]	12.9 ± 5.7	11.0 ± 2.8[c]	29.8 ± 3.8	14.0 ± 2.5[c]	35.1 ± 10.8
LPL activity[d]						
Post-heparin plasma	52.2 ± 18.8	53.2 ± 19.3	65.2 ± 20	49.4 ± 33	98.0 ± 4.5[a,b]	65.5 ± 20.5[a]
Leg muscle	11.7 ± 0.9[c]	9.9 ± 1.3	9.4 ± 2.3[b]	7.4 ± 1.0	13.0 ± 4.5[b]	7.9 ± 1.4
Heart	76.2 ± 11.3[c]	59.2 ± 7.3	72.5 ± 15.2[b]	54.5 ± 13.9	110 ± 8.0[b]	80.0 ± 26.6
Abdominal fat pad	998 ± 420	1172 ± 550	4036 ± 1743	3840 ± 848	6997 ± 617[b]	5515 ± 1324

[a]Data are for twelve 8th generation birds.

[b,c]Values for birds of the low-VLDL line that are significantly different (*t*-test) from values for birds of the high-VLDL line are indicated by: [b], $P < 0.05$; [c], $P < 0.01$.

[d]Lipoprotein lipase activity is expressed as μmol fatty acid released/h per g of tissue, per ml of plasma or per whole fat pad. Values are the means ± SD of results from 8–12 birds per line.

result of a reduced capacity to hydrolyze circulating VLDL. However, the significantly lower LPL activity in post-heparin plasma from birds of the high-VLDL line after 8 generations of selection suggests that a reduction in total functional LPL activity may be making a significant contribution to the continuing increase in plasma VLDL concentration in the high-VLDL line over later generations (7). The possibility that VLDL clearance by receptor-mediated endocytosis has been influenced by selection was not examined directly. The very slow removal of labeled VLDL from birds injected with anti-LPL antiserum (Fig. 1) indicates that clearance of newly synthesized VLDL by mechanisms other than LPL has a quantitatively minor role in normal broilers.

The magnitude of the difference in rates of VLDL production in the low- and high-VLDL lines was somewhat surprising, since earlier studies (18) using tritiated water had shown no significant differences in rates of hepatic lipogenesis between the lines. Studies on perfused rat liver (19) have shown that a high proportion of fatty acids synthesized within the liver or derived from exogenous sources are oxidized to CO_2 or converted to ketone bodies rather than exported as VLDL. Plasma concentrations of β-hydroxybutyrate in normal broilers are relatively high and this appears to indicate a substantial rate of fatty acid oxidation in chicken liver, even in the fully fed state. The observation that plasma β-hydroxybutyrate concentrations are substantially greater in the low-VLDL line than in the high-VLDL line (0.86 vs 0.48 μmol/ml) suggests that direction of fatty acids to oxidation rather than VLDL synthesis makes a major contribution to the low rate of VLDL synthesis in the low-VDL line.

The high rate of VLDL production in the fatter, high-VLDL line makes much more triglyceride available for deposition in adipose tissue, but selection for higher VLDL concentration has also increased the proportion of VLDL triglyceride directed towards fat deposition (Table 2). The combination of these effects means that the abdominal fat pad depot in birds of the high VLDL line takes up lipoprotein-borne fatty acids at 6–8 times the rate of that in the low-VLDL line. The between-line difference in abdominal fat pad weight is only about 2.5-fold and differences in total body fat are much smaller (6). These comparisons indicate that there are important influences on fat deposition in these birds in addition to those involved in regulating plasma lipoprotein metabolism. It may be, for example, that the contribution of adipose tissue lipogenesis has been underestimated, as argued elsewhere (1).

The mechanism by which selection for high VLDL concentrations has directed VLDL triglyceride to adipose tissue is not clear. Total LPL activity in the abdominal fat pad of the fatter, high-VLDL line was similar or slightly less than in the low-VLDL line and this suggests that changes in functional LPL activity in adipose tissue are not responsible. The greater LPL activity in muscle in the low-VLDL line may increase its use of circulating VLDL triglyceride at the expense of adipose tissue. The differences in total LPL activity in muscle produced by selection were small but they may mask much larger changes in LPL activity at the capillary bed (20). Alternatively, studies by Fielding (21) and Fielding, Shore, and Fielding (22) indicate that VLDL concentrations per se could have a major influence on the fate of circulating VLDL. These authors found that the apparent K_m of LPL for VLDL in perfused rat heart was substantially lower than that in perfused adipose tissue and suggested that this would favor uptake of VLDL triglyceride by muscle when plasma VLDL concentrations were low, and by adipose tissue when plasma VLDL concentrations were high. The results of the present study are consistent with this proposal, although preliminary studies have shown no differences in apparent K_m for LPL isolated from chicken heart and adipose tissue towards either VLDL or portomicrons (H. Griffin, unpublished results).

The liver is thought to be the major site of oxidation of amino acids in birds, as in mammals (23). Preferential oxidation of fatty acids rather than amino acids in the livers of the birds of the low-VLDL line provides a simple explanation for their substantially better efficiency of protein utilization. However, a higher rate of ketone body formation may also have a sparing effect on amino acid oxidation in peripheral tissues, since both β-hydroxybutyrate and acetoacetate have been shown to inhibit leucine oxidation in chicken skeletal muscle (24). A lower rate of fatty acid oxidation has been shown to be a major cause of the high rate of VLDL synthesis in the obese Zucker rat (25) and this has been attributed to a greater sensitivity of carnitine acyl transferase to inhibition by malonyl CoA (26) and to elevated concentrations of malonyl coA (27). It should prove interesting to determine the underlying cause of differences in rates of hepatic VLDL synthesis in chickens because of its apparently key influence on feed and protein conversion efficiencies in a commercially important species.

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