Identification of an adipose tissue-like lipoprotein lipase in perfusates of chicken liver

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Abstract The nature of the lipolytic activity released from chicken livers perfused with Krebs-Ringer buffer (pH 7.0) containing heparin (50 or 10 U/ml), fraction V albumin (3%), and glycerol (20%) was investigated. The nonrecirculating perfusates contained both the previously described NaCl-resistant "liver lipase" as well as an apoLp-Glu-activated lipoprotein lipase (LPL). Crude perfusate lipolytic activity was separated on heparin–Sepharose columns into two enzymatic peaks which were eluted at mean NaCl molarities of 0.75 M (liver lipase) and 1.2 M (LPL). The liver LPL activity was stimulated 7-fold by human apoLp-Glu (half maximal activity at 1.5 μg/ml) and inhibited by apoLp-Ala, apoLp-Ser, apoLp-GlnI, and apoLP-GlnII. Liver LPL was fully inhibited by anti-adipose LPL immunoglobulins. The "liver lipase" was not affected by apoLp-Glu (3–34 μg/ml) or anti-adipose LPL immunoglobulins. The data demonstrate the presence in liver perfusates of a LPL with properties similar to adipose tissue lipoprotein lipase.

Supplementary key words apolipoproteins

Intravenous injections of heparin in the rat, pig, and man cause release of two triacylglycerol hydrolases into the plasma. The two lipases differ in their sensitivity to NaCl, protamine sulfate, and diethyl-*p*-nitrophenyl phosphate, and in their requirement for a lipoprotein polypeptide (1–5). One of these lipases, lipoprotein lipase (LPL, glycerol ester hydrolase, EC 3.1.1.3) is claimed to originate solely in extrahepatic tissues and requires apoLp-Glu for maximal activity; the second, resistant to high NaCl molarities and protamine sulfate, is believed to come from the liver.

In this report, evidence is presented demonstrating that chicken livers perfused with heparin-containing buffers release into perfusates the previously described NaCl-resistant "liver lipase" and a lipoprotein lipase that is immunologically identical to adipose tissue lipoprotein lipase.

MATERIALS AND METHODS

Liver perfusion

Animal donors for liver perfusions were male white Leghorn chickens, 3–15 months old; they were fasted overnight before use. Animals were killed by decapitation. Following laparotomy, the right portal vein was ligated and the liver excised. Perfusion was conducted through the left portal vein with a nonrecirculating buffer at a rate of 20 ml/min for a maximum period of 5 min. The perfusion solution was an avian Krebs bicarbonate buffer (6) gassed with 95% CO_2–5% O_2. To increase the stability of the displaced lipolytic enzymes (7), the Krebs bicarbonate buffer was modified by the inclusion of 20% glycerol (v/v) and 3% bovine serum albumin (fraction V, Sigma Chemical Co., St. Louis, MO). In addition, the pH of the solution was adjusted to 7.0 at 37°C, the perfusate temperature. Heparin from porcine intestinal mucosa was obtained from Sigma Chemical Co. Its concentration when included in the perfusion fluid was either 10 or 50 U/ml. The heparin contained 160 U/mg.

Lipase assays

Lipolytic activity was assayed with a synthetic [^{14}C]triolein substrate emulsified in the presence of gum arabic. Triolein containing [1-^{14}C]oleate in all three positions was purchased from DHOM Products, North Hollywood, CA. Fifty μmol of triolein was

Abbreviations: LPL, lipoprotein lipase. The nomenclature of apolipoproteins employed is based on their COOH-terminal amino acids. In the terminology suggested by Alaupovic (17), they are defined as follows: apoLp-Ser, apoC-I; apoLp-Glu, apoC-II; apoLp-Ala, apoC-III; apoLp-GlnI, apoA-I; apoLp-GlnII, apoA-II.

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sonicated in the presence of 200 mg of gum arabic in a total volume of 2 ml with the microprobe of a Bronwill Biosionik IV sonicator. The sample was subjected to six 15-sec sonication bursts at room temperature alternating with 15-sec cooling periods in ice. Two assay systems were employed to measure the two types of lipolytic activities present in liver perfusates. The first system (low NaCl molarity, pH 8.6) contained the following components in a total volume of 0.5 ml: 1.25 μmol of [^{14}C]triolein (0.133 μCi per μmol of triolein); 2.5 mg of gum arabic; 5 mg of albumin; 0.02 ml of heated rat serum; 0.1 mmol of Tris buffer, pH 8.6; 0.05 mmol of NaCl; 5 μmol of $CaCl_2$; and 0–0.1 ml of enzyme preparation. In the second assay system, the NaCl molarity was 1.0 M, the pH 9.0, and the serum was omitted.

Enzyme purification

Lipolytic activities in liver perfusates were separated by affinity chromatography on heparin–Sepharose 4B columns by methods previously published (7). All buffers used during purification contained 30% glycerol (v/v). Pooled liver perfusates were adjusted to 0.3 M NaCl and 30% glycerol and were diluted sufficiently to contain less than 8 U heparin/ml before application to heparin–Sepharose 4B columns. The columns were equilibrated with 0.3 M NaCl in 0.005 M sodium barbital buffer, pH 7.0. Elution was conducted with a linear NaCl gradient from 0.5 to 1.5 M NaCl in 0.005 M sodium barbital, pH 7.0, using a total volume of 260 ml.

NaCl molarities of samples eluted from chromatographic columns were estimated by measuring their conductivities (conductivity meter, Radiometer Copenhagen). Standard curves were prepared with solutions containing varying molarities of NaCl in 30% glycerol (v/v), 0.005 M sodium barbital, pH 7.0. The volume of samples assayed was calculated so that the NaCl molarity in the 0.5 ml assay was 0.1 M NaCl in the first system (0.1 M NaCl, pH 8.6,

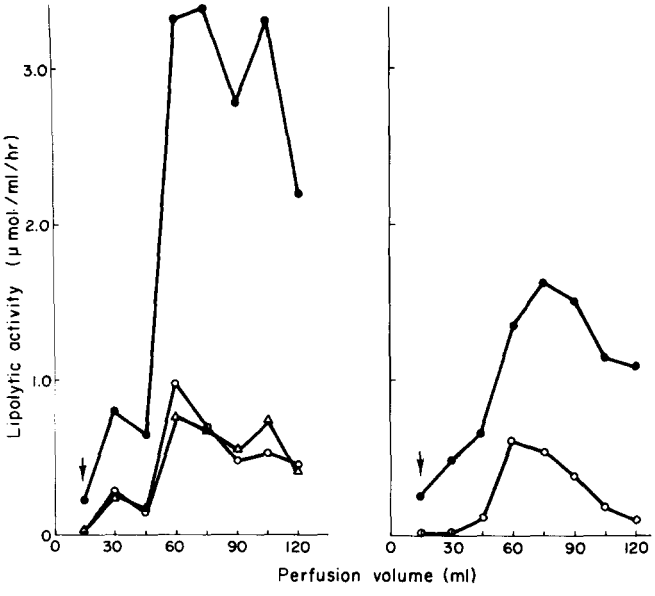

Fig. 1. Release of lipolytic activities in avian liver perfusates. Livers excised from two 15-month-old roosters were perfused with 0.15 M NaCl (15 ml) and then (arrow) with modified avian Krebs bicarbonate buffer containing 50 U heparin/ml. Data are presented for two representative perfusions. Conditions of assay are 0.1 M NaCl, serum 0.02 ml/0.5 ml, pH 8.6, ● —— ●; 1 M NaCl, no serum, pH 9.0, ○ —— ○; 0.1 M NaCl, no serum, pH 8.6, △ —— △.

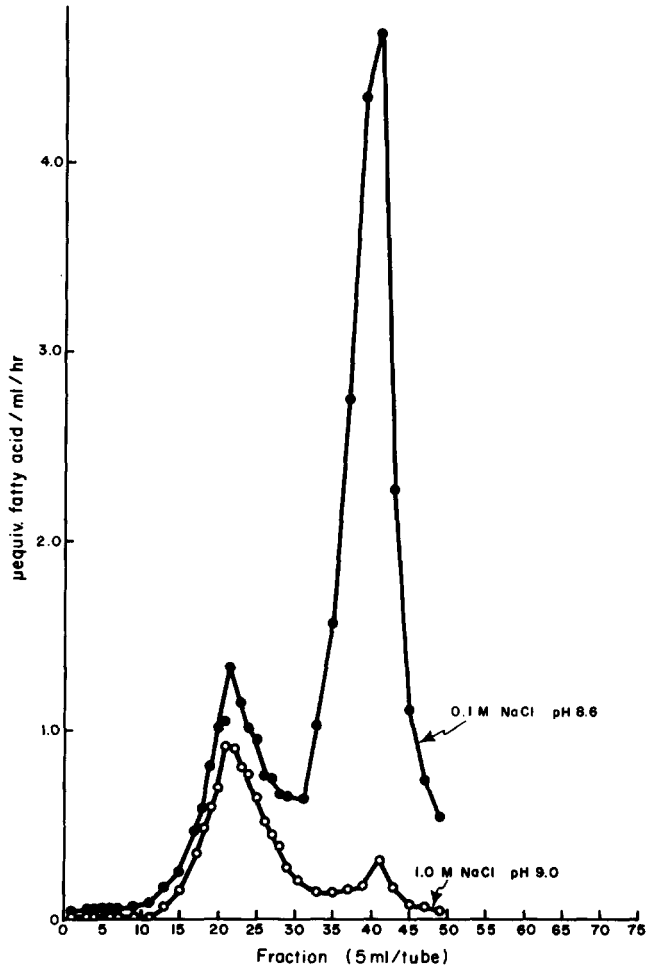

Fig. 2. Heparin–Sepharose chromatography of 15-month-old rooster liver perfusates. Liver perfusates were pooled, adjusted to a NaCl molarity of 0.25 M and heparin concentration of 8 U/ml and applied to a 1.5 × 15 cm heparin–Sepharose column. The column was developed with a linear NaCl gradient between 0.5 and 1.5 M NaCl in 30% glycerol, 0.005 M sodium barbital, pH 7.0, using a total volume of 260 ml. Lipolytic activity was measured at 0.1 M NaCl, pH 8.6, with 0.02 ml of rat serum per 0.5 ml assay volume and at 1.0 M NaCl, pH 9.0, in the absence of serum.

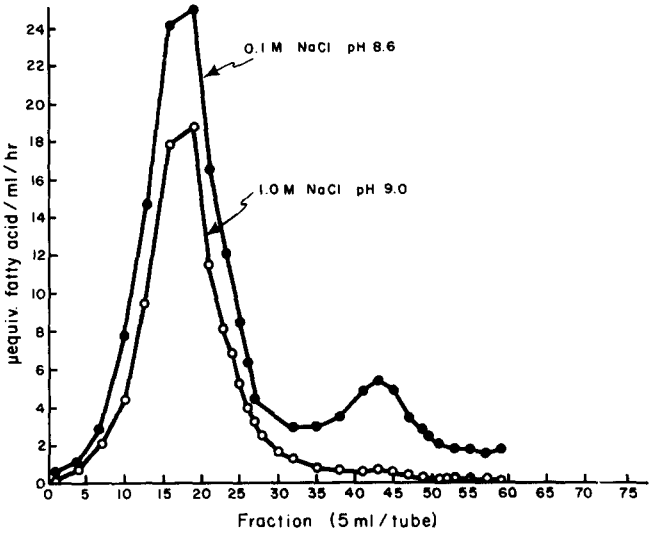

Fig. 3. Heparin–Sepharose chromatography of 3-month-old rooster liver perfusates. Conditions were the same as those described for Fig. 2.

serum included). In this assay, sample size varied between 0.026 ml and 0.078 ml. A volume of 0.1 ml of enzyme solution was employed in the second system (1 M NaCl, no serum).

Other procedures

Protein was determined by the procedure of Bensadoun and Weinstein (8).

Anti-adipose tissue LPL sera were successfully prepared in rabbits by methods recently published (9). Serum from a single rabbit was employed in this study. A partially purified immunoglobulin fraction was prepared by five sequential precipitations in the presence of 50% ammonium sulfate at 20°C (10). The final precipitate was dissolved in 0.05 M ammonium bicarbonate and desalted by gel filtration on Sephadex G 100. Inhibition of lipolytic activities by this anti-LPL immunoglobulin fraction was studied as follows.

Constant amounts of a given enzyme preparation were incubated with increasing amounts (0–100 μg) of anti-LPL immunoglobulins in a series of centrifuge tubes for 1 hr at 20°C. The incubation medium (0.4 ml) was adjusted to 0.2 M NaCl, 30% glycerol, and 0.005 M sodium veronal buffer pH 7.0. Parallel incubations were carried out with a control rabbit immunoglobulin fraction prepared by ammonium sulfate precipitation and gel filtration. After incubation, the tubes were centrifuged at 28,000 *g* for 30 min at 4°C. Enzymatic activity in the supernatant was determined as described above.

Inhibition of lipolytic activities by the same partially purified immunoglobulin fraction was also studied by the following alternate method. Constant amounts of a given enzyme preparation were incubated with increasing ratios of immune to control immunoglobulin, the total amount of immunoglobulin protein remaining constant.

Human apolipoproteins were prepared in the laboratory of Dr. W. Virgil Brown at the University of California, San Diego, using gel filtration and diethylaminoethylcellulose chromatography (11).

RESULTS

Lipolytic activities in liver perfusates

Fig. 1 presents the results of two representative liver perfusions. Lipolytic activity was released in the perfusate when heparin was introduced in the perfusion fluid. Lipolytic activity measured in the presence of serum and 0.1 M NaCl at pH 8.6 was reduced to less than 25% when serum was omitted in the assay system. Similar inhibition was observed when the activity was assayed at pH 9.0 in the presence of 1.0 M NaCl. These early results suggested that liver perfusates contained a significant portion of serum-dependent, NaCl-sensitive lipolytic activity.

Heparin–Sepharose chromatography of liver perfusate lipolytic activity

Lipolytic activity in liver perfusates was resolved by chromatography on heparin–Sepharose columns into two clearly separated peaks. Typical chromatograms and recovery of activities are presented for two age groups, 3- and 15-month-old animals, in **Figs. 2** and **3**, and Table 1. Seventy to eighty percent

TABLE 1. Heparin-Sepharose chromatography of pooled liver perfusates

Step	3-month-old[a]	15-month-old[b]
	μmol/hr	
Applied	1970	271
Unadsorbed	487[c]	58[c]
Recovered		
Tube 0–30	1579	81
Tube 31–60	385	176

[a] Liver perfusates from six livers (3-month-old roosters) were pooled, adjusted to a NaCl molarity of 0.25 M and a heparin concentration of 8 U/ml and applied to a 1.5 × 15 cm heparin–Sepharose column. Lipolytic activity was measured at 0.1 M NaCl, pH 8.6, in the presence of serum (first assay system described in Materials and Methods). Elution profile is shown in Fig. 3.

[b] Liver perfusates from five livers (15-month-old roosters) treated as in footnote *a*. Elution profile in Fig. 2.

[c] The lipolytic activities of the unadsorbed protein fractions measured at 1 M NaCl (second assay systems described in Materials and Methods) were 125 and 0 μmol/hr, respectively, for the liver perfusates of the 3-month-old and 15-month-old roosters.

of the applied activity was retained on the columns. Elution of the heparin–Sepharose columns with a NaCl gradient yielded two clearly resolved activity peaks. The first peak was eluted at 0.75 M NaCl. Its enzymatic activity was not inhibited by 1 M NaCl; 31 fractions in the ascending portion of the first peak from five purifications gave a mean recovery of activity in the presence of 1 M NaCl of 80.1 ± 16% (relative to activity at 0.1 M NaCl). In contrast, the second peak, eluted at 1.15 M NaCl, was essentially inhibited when assayed at pH 9.0 in the presence of 1.0 M NaCl; 19 fractions from four purifications sampled in the descending portion of the second peak yielded a mean recovery of 6.9 ± 4%.

In all purifications performed, the sum of recovered activities in the first and second peaks was higher than that adsorbed to affinity columns, suggesting that the crude perfusates contained lipase inhibitors. The adsorbed activity is defined as the activity applied on the column minus the unadsorbed activity. In the two purifications summarized in **Table 1**, the recoveries of enzyme activities were 130 and 120% (relative to adsorbed activities), respectively, for liver perfusates of 3- and 15-month-old roosters.

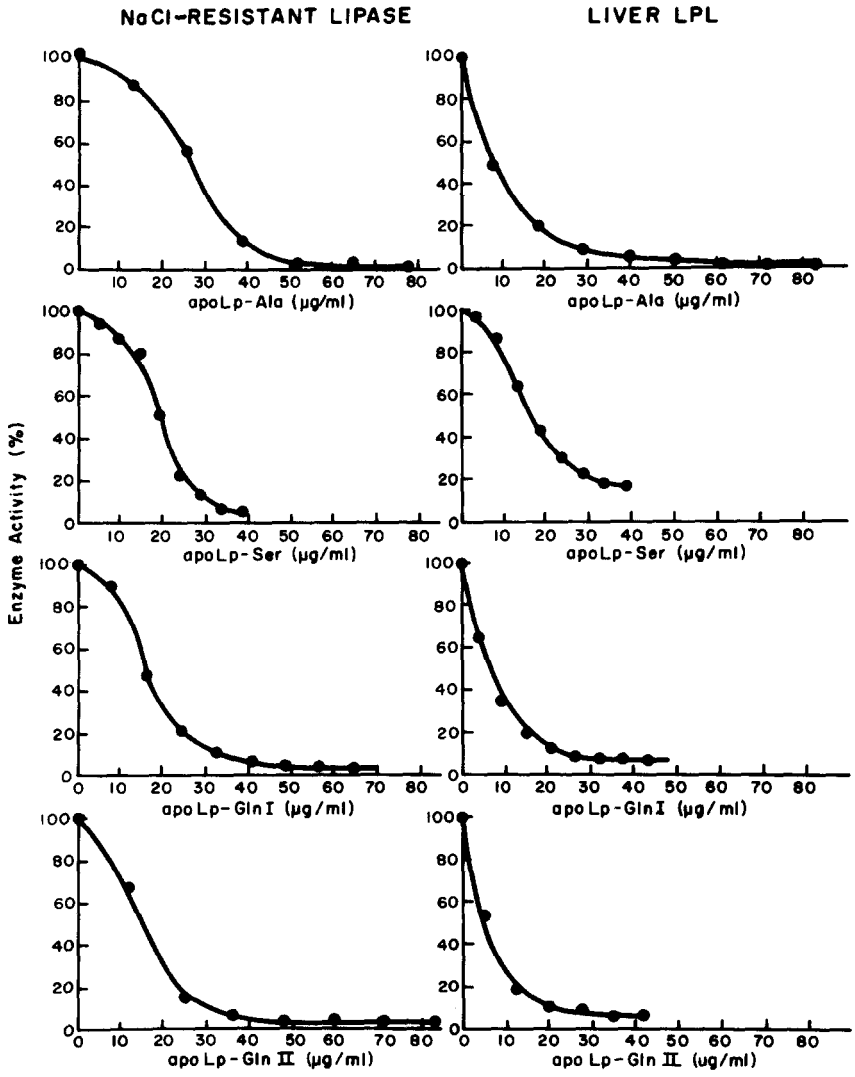

Fig. 4. Effect of apoLp-Ala, apoLp-Ser, apoLp-GlnI and apoLp-GlnII on NaCl-resistant liver lipase and liver lipoprotein lipase. Enzyme preparations employed were those obtained by heparin–Sepharose chromatography. For the liver LPL studies the apolipoproteins were added to complete assay systems containing 0.02 ml of rat serum, 0.1 M NaCl, 0.2 M Tris buffer, pH 8.6. In the experiments with the NaCl-resistant liver lipase, serum was omitted and the assays were conducted in the presence of 1.0 M NaCl, 0.2 M Tris buffer, pH 9.0. Enzyme activities are expressed as percentages of that present in the absence of the apolipoprotein of interest. Enzyme preparations employed had the following activities: NaCl-resistant lipase, 4.20 μmol/ml per hr; liver LPL, 3.97 μmol/ml per hr. The enzyme volumes employed for each assay tube were 0.1 and 0.04 ml, respectively, for the NaCl-resistant lipase and the liver LPL.

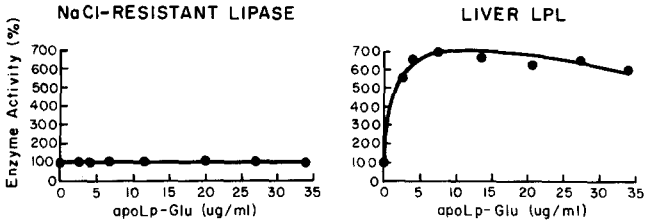

Fig. 5. Effect of apoLp-Glu on NaCl-resistant liver lipase and liver lipoprotein lipase. Conditions are the same as those outlined in Fig. 4 except that serum was omitted in the assay of the liver LPL. Enzyme preparations employed had the following activities in the absence of apoLp-Glu: NaCl resistant lipase, 9.20 μmol/ml per hr; liver LPL, 0.366 μmol/ml per hr. The enzyme volume employed for each assay tube was 0.04 ml for both lipases.

Characterization of two lipolytic enzymes in liver perfusates

Figs. 1 and 2 illustrate the differences in sensitivity to NaCl of the first and second peaks. On the basis of this property and the NaCl molarities at which these two activity peaks were eluted, it became apparent that the first peak was very similar to the previously described liver lipase and the second activity peak to the adipose tissue LPL. This provisional identification was confirmed by studying the effects of human apolipoproteins on these two partially purified lipolytic activities. ApoLp-Ala, apoLp-Ser, apoLp-GlnI, and apoLp-GlnII inhibited both activities (**Fig. 4**); apoLp-Glu stimulated the LPL and had no effect on the liver lipase (**Fig. 5**). The pH optimum of the liver LPL measured with Tris buffers (0.2 M in the assay) was 9.0. A partially purified immunoglobulin fraction prepared from anti-adipose tissue LPL sera inhibited the liver LPL activity but did not alter the liver lipase. Similar results were obtained when increasing amounts of anti-LPL immunoglobulins were incubated with constant amounts of enzyme (**Fig. 6**) and when the amount of immunoglobulins (immune plus control) was maintained constant during preincubation (data not shown).

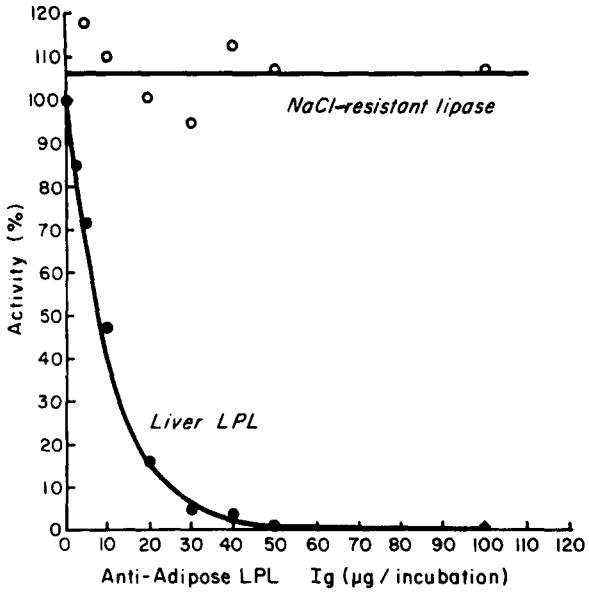

Fig. 6. Effect of an anti-adipose LPL immunoglobulin on NaCl-resistant lipase and liver LPL. Constant amounts of enzyme (NaCl-resistant lipase, 0.069 ml, 0.462 μmol/ml per hr; liver LPL, 0.102 ml, 0.262 μmol/ml per hr) were incubated in centrifugal tubes for 1 hr at 20°C with increasing amounts of rabbit anti-adipose LPL immunoglobulin or control rabbit immunoglobulin in a total incubation volume of 0.4 ml. After incubation, the tubes were centrifuged at 28,000 *g* for 30 min at 4°C. Enzymatic activities in 0.2 ml of the supernatants were determined in the presence of 0.1 M NaCl for LPL and 1.0 M NaCl for the liver lipase. Activity measured in the presence of immune immunoglobulin was expressed as a percentage of that present in the presence of control immunoglobulin.

TABLE 2. Effect of age on lipolytic activity of liver perfusates

Age	Total Lipolytic Activity[a]	Liver Weight	$\frac{\text{Activity at 1.0 M NaCl}}{\text{Activity at 0.1 M NaCl}} \times 100$[b]
months	*μmol/hr*	*g*	*%*
2.0 (6)	138 ± 33		77 ± 5
2.5 (13)	191 ± 47	16.7 ± 0.6[c]	80 ± 4
3.0 (12)	310 ± 33		86 ± 5
4.0 (3)	153	19.5	61
5.5 (9)	179 ± 51	25.1 ± 1.4	48 ± 5
15.0 (8)	98 ± 37		31 ± 19
22.0 (4)	14 ± 5	24.6 ± 1.2	13 ± 1

[a] Total lipolytic activity represents the lipolytic activity measured at 0.1 M NaCl, pH 8.6, in the perfusate of a single liver. Perfusion buffer contained 10 U/ml heparin. Perfusion volume was limited arbitrarily to 100 ml. Numbers of livers perfused are indicated in parentheses. Results are means ± standard errors.

[b] Lipolytic activity measured in the presence of 1 M NaCl expressed as a percentage of that measured in the presence of 0.1 M NaCl.

[c] Mean ± standard errors of seven livers.

Effect of age on liver lipolytic activity

Age had a marked effect on both the total lipolytic activity and the ratio of the two lipolytic activities in liver perfusates. In mature animals (15 months and older) total lipolytic activity appearing in 100 ml of perfusate was lower than in the young animals (2–5 months) studied (**Table 2**). These differences are further accentuated when the data are expressed per g of liver weight. Assay of the lipolytic activity in the presence of 1 M NaCl suggests that the liver lipoprotein lipase is a minor component in the perfusate of young roosters but accounts for most of the lipolytic activity in the mature animals. These effects of age were also confirmed when the two lipolytic activities were resolved by chromatography on heparin–Sepharose columns (see Figs. 2 and 3).

DISCUSSION

This study documents the presence in the rooster liver perfusates of two distinct lipolytic enzymes. One of these resembles the previously described NaCl-resistant "liver lipase" (1–3); the second exhibits properties similar to those of avian adipose tissue LPL (9). The two lipolytic activities were clearly resolved by chromatography on heparin–Sepharose 4B columns. The identification of the second activity peak as a lipoprotein lipase was established by its stimulation by apoLp-Glu and its inhibition by 1 M NaCl and specific anti-adipose LPL immunoglobulins.

Liver LPL has been detected immunologically by Yasuoka and Fujii (12) in the rat. The antiserum employed was prepared against postheparin rat LPL prepared by the method of Fielding (13). Their data indicate that, in 150–200 g rats, approximately half of the lipolytic activity extracted from fresh livers with 0.067 M phosphate buffer at pH 7.4 is a lipase immunologically identical to plasma LPL. Ganesan, Ganesan, and Bradford (14) have reported the presence in dog liver perfusates of a C-I (apoLp-Ser)-activated lipoprotein lipase. This lipolytic activity was not stimulated by C-II (apoLp-Glu). Failure to identify such a lipolytic activity in avian liver perfusates may reflect species or experimental differences.

Kelley et al. (15) have reported that the avian hepatic NaCl-resistant lipase released by heparin in the plasma decreased in females with egg laying or in males injected with diethylstilbestrol. In the present study with male chickens a dramatic decrease in salt-resistant liver lipase was observed with age. The hormonal status of these animals was not determined and it is therefore not possible to correlate this decrease with specific alterations in hormone titers.

The site of synthesis as well as the functional roles of the liver LPL and of the salt-resistant liver lipase are still unknown. Both enzymes may act in concert in the transfer of remnant triglyceride fatty acids into the parenchymal cells. Alternatively, the liver LPL might represent enzyme protein originating in extrahepatic tissues. The identification in the liver of a LPL immunologically identical to adipose tissue LPL is consistent with the theory of Felts, Itakura, and Crane (16) that LPL associated with VLDL and chylomicron remnants is taken up by the liver.

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