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Lipoprotein Lipase of Ovarian Follicles in the Domestic Chicken (*Gallus Domesticus*) (38537)

JOHN D. BENSON, ANDRE BENSADOUN, AND DEBBIE COHEN

Poultry Science Department & Division of Biological Sciences, Cornell University, Ithaca, New York 14850

The transport of triglyceride-fatty acids (TGFA) from the circulation to the developing oocyte of the chicken is an excellent model system for the study of mechanisms of lipoprotein transport. The laying hen synthesizes fatty acids in the liver, incorporates them into triglycerides and transports them, primarily as part of very low density lipoproteins (VLDL), to the ovary. The mechanism of transfer of plasma VLDL triglyceride to the ooplasm is not well understood. It is not clear whether triglycerides are hydrolyzed and resynthesized prior to deposition in the yolk (1, 2) or transported into the oocyte with minor or no alterations (3–5). The latter concept has been supported by the failure to find appreciable lipoprotein lipase activity in ovarian tissues (2, 4, 6).

In preliminary studies we noted that during the separation of yolk from follicular membranes, a thin membraneous layer of cells, the granulosa cells, could be dissected free of the thecal cells and yolk. Since these cells are strategically located near follicular capillary beds (7) and adjoining the yolk, we decided to investigate whether these cells, contained a lipase that may hydrolyze plasma triglycerides en route to the oocyte.

Materials and Methods. Follicles varying in weight from 0.05 to 18 g were removed from laying White Leghorn hens and the granulosa cells dissected free of adjoining yolk and thecal tissues in 0.15 *M* NaCl at 37°. The identification of the granulosa cells was confirmed by light microscopy. If the dissection is conducted carefully the integrity of the granulosa cell layer can be maintained and separated quantitatively from other follicular elements. A more complete anatomical description of the preparation will be published elsewhere. Acetone–ether powders of the cells were made as described by Patten and Hollenberg (8) for adipose tissues. Preliminary studies revealed that the yield of enzyme was greater if the cells were not homogenized; consequently, homogenization was omitted.

In studies where lipase activity of different size follicles were compared, acetone–ether powders were made by dispersing the cells with a glass rod in 7 ml of cold acetone, centrifuging for 5 min at 2° and 1500 *g*. This procedure was repeated with 7 ml of acetone (25°) and 7 ml of ether (25°). The ether was aspirated and the samples dried *in vacuo*.

Lipoprotein lipase was determined in duplicate with a synthetic [^{14}C]triolein substrate emulsified in the presence of gum arabic (9).

TABLE I. LIPOPROTEIN LIPASE ACTIVITIES OF CHICKEN TISSUES.[a]

Tissue	Activity: μmoles fatty acid/hr/mg prot.
Granulosa	1.62
Theca	0.13
Yolk	0.02
Adipose	3.30

[a] Tissues from six laying hens were pooled. Enzyme activity was determined on extracts of acetone powder (5 mg/ml) as described in Materials and Methods. Each value is the mean for four determinations.

In the previously described assay system (9) porcine serum was replaced by 10 μl of rat serum in a total assay volume of 0.5 ml. [^{14}C]triolein, 99.5% pure at reception from manufacturer, was further purified by thin layer chromatography. The triolein molarity in the assay system was 2.5 m*M* with a specific activity of 20,250 dpm/μmole triolein. NH_4OH—NH_4Cl buffer (25 m*M*, pH 8.6) was as effective an extractant of lipoprotein lipase as heparin (50 units/ml H_2O). This is in contrast with observations made with porcine adipose tissue acetone powders

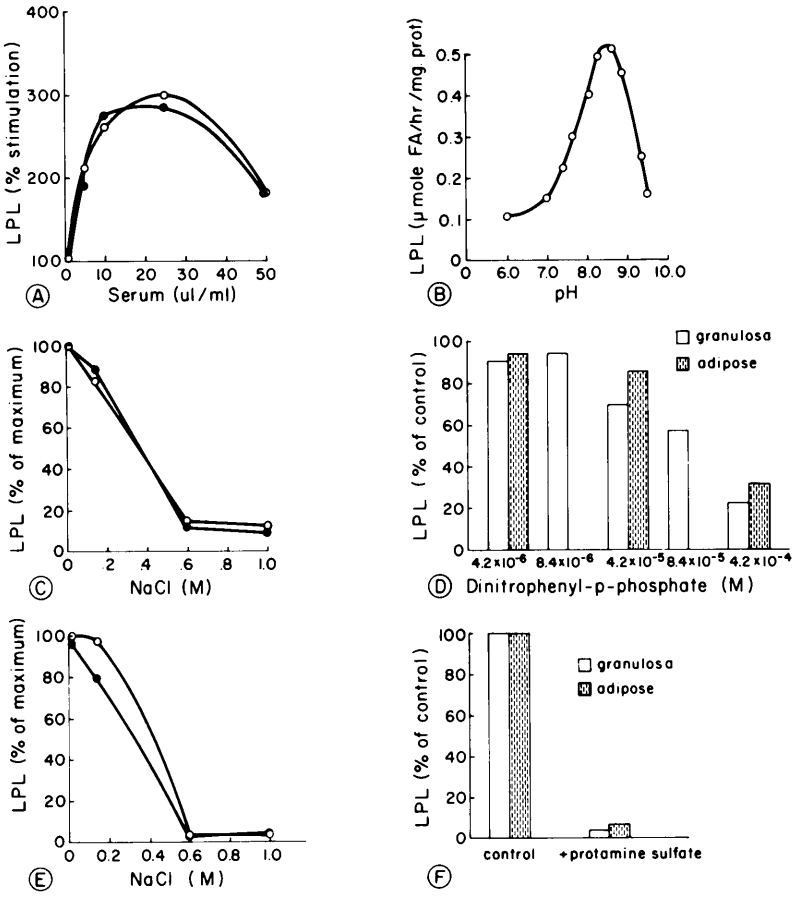

FIG. 1. Comparison of granulosa and adipose tissue lipases. (a) Activity in the presence of varying volumes of serum from a fed rat. The activity (μmole FA/hr/mg protein) in the absence of serum was 0.28 for granulosa (O——O) and 1.66 for adipose tissue (●——●). Appropriate blanks were conducted to correct for the lipase activity of rat serum. (b) Activity at different pH's. (c) Activity in the presence of NaCl. Extracts of granulosa (O——O) and adipose tissue (●——●) were assayed with varying concentrations of NaCl. Activity (μmoles FA/hr/mg protein) in the absence of NaCl were: granulosa, 0.79; adipose tissue, 4.30. (d) Activity in presence of dinitrophenyl-*p*-phosphate. In the absence of DNPP the activity (μmole FA/hr/mg protein) was: granulosa, □, 0.47; adipose tissue, ■, 2.6. (e) Activity following preincubation in buffered NaCl. NH_4OH-NH_4Cl extracts of acetone powder (10 mg/ml) were preincubated in 0.25 *M* Tris–HCl, pH 8.6 containing the indicated molarities of NaCl for 30 min at 27°. Seventy microliter of this mixture were then assayed for lipase activity. All lipase assays were conducted at 0.15 *M* NaCl. The activities of granulosa and adipose tissue in the absence of added NaCl were (μmole FA/hr/mg protein) 0.29 and 1.26, respectively. (f) Activity following preincubation with protamine sulfate. Fifty microliter of tissue extract were preincubated with 50 μl of 2 *M* Tris–HCl with or without 1.25 mg protamine sulfate (control). After preincubating for 10 min at 27°, 0.4 ml of of incubation mixture was added and incubation was continued for 60 min at 30°. Values are the mean of 2 experiments. Control enzyme activity (μmoles FA/hr/ml extract): granulosa, □, 0.69; adipose, ■, 2.65.

(9) where eight to tenfold more enzyme is extracted with heparin in water.

Protein, using crystallized albumin as standard was determined by the procedure of Lowry *et al.* (10).

Triolein (Sigma grade I), crystallized albumin, and heparin (sodium salt, from hog intestinal mucosa, 170 mg/ml) were from Sigma Chemical Co., St. Louis. Glycerol trioleate-1-^{4}C was from Dhom Products, North Hollywood, CA.

Results and Discussion. The specific activ-

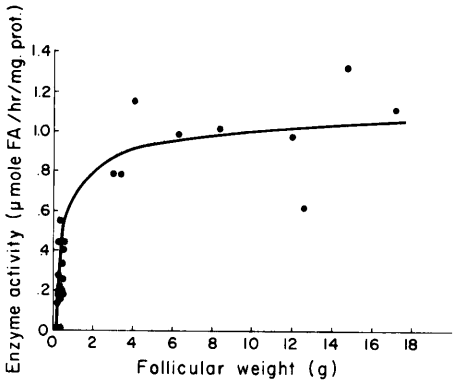

FIG. 2. Granulosa lipase from different size follicles. Granulosa cells were quantitatively removed from five to nine follicles of each of four hens. Following acetone powder preparations, lipase activity was determined.

ity (enzymic activity/mg protein) of granulosa lipase was 0.5 of that of adipose tissue and 10 times that of the adjoining thecal cells (Table I). Yolk powders did not contain significant lipase activity. Earlier reports (2, 4, 6) of lack of significant lipase activity in chicken ovaries can probably be traced to the fact that tissue elements analyzed did not contain the granulosa cell layer or included granulosa cells associated with resting oocytes prior to their period of rapid maturation. The finding of significant lipase activity in granulosa cells provides this tissue with the requisite enzyme for the hydrolysis of lipoprotein triglyceride.

Characteristics of ovarian granulosa cell lipoprotein lipase. Since lipase activity in granulosa cells had not been reported previously, we performed several studies to characterize the enzyme and compared it to lipoprotein lipase activity of chicken adipose tissue. Both granulosa and adipose tissue lipoprotein lipase were stimulated 300% by rat serum (Fig. 1). High density lipoproteins (20 μg/ml) purified from chicken plasma could replace rat serum in the assay system. The similar stimulatory effects of rat sera on granulosa and adipose tissue lipoprotein lipase and the stimulation of granulosa lipase by purified high density lipoproteins suggest that the granulosa lipase is a lipoprotein lipase.

The pH optimum for the granulosa lipase was 8.3–8.6 (Fig. 1b) which closely resembles that of adipose tissue lipoprotein lipase (11).

Granulosa and adipose tissue lipases were markedly inhibited when assayed with increasing ionic strengths in the form of NaCl (Fig. 1c). Similar inhibition curves were observed (Fig. 1e) when enzyme sources were preincubated in NaCl and assayed at 0.15 *M* NaCl. Lipases from the two tissues were similarly affected by increasing concentrations of dinitrophenyl-*p*-phosphate (Fig. 1d), a compound which inhibits lipoprotein lipase (12, 13). Inhibition by protamine sulfate (Fig. 1f) was also observed for enzyme preparations extracted from both tissues.

Like lipoprotein lipase from chicken adipose tissue, granulosa lipase was unaffected by heparin (0.17–1,700 units/ml).

Thus, the characteristics of granulosa lipase are nearly identical to adipose tissue lipoprotein lipase and would support the contention that the granulosa lipase is a lipoprotein lipase (LPL).

Relationship of LPL activity with follicular size. Granulosa cell lipase activity was very low in follicles weighing less than 0.5 g (Fig. 2); LPL activity increased dramatically as follicular size increased and leveled off above 2 g.

To insure that differences in activity due to follicular size were not a function of the protein concentration in the assay, we diluted all the extracts to a constant protein concentration (60 μg/ml) and then determined lipase activity. Results identical to those presented in Fig. 1 were obtained. The activity of granulosa LPL thus begins to increase at a time when lipid is being deposited into the follicle.

We feel that the identification of lipoprotein lipase in granulosa cells supports the view (1, 2) that hydrolysis or at least partial hydrolysis may be occurring during TGFA transport into the oocyte. Failure of others (2, 4, 6) to find significant lipoprotein lipase in ovaries detracted from this concept. On the other hand, we cannot be sure whether this level of activity is associated with transport into the developing oocyte or merely transport into granulosa cells. Further it cannot be stated categorically that LPL associated with granulosa cells is directly

responsible, *in vivo*, for the hydrolysis of plasma very low density triglyceride. Analogous to the hypothesis (15) of transfer of LPL from adipocytes to endothelial cell surface it could be hypothesized that the granulosa cells synthesize LPL and export it to the capillary endothelial cell surfaces in the thecal envelopes.

Summary. A lipase, bearing the characteristics of adipose tissue lipoprotein lipase (LPL) has been characterized in avian ovarian granulosa cells. The activity is low in cells from follicles weighing less than 0.5 g; in heavier follicles which have entered the rapid growth phase, significant activity (1 μmole fatty acid/mg protein/hr) could be identified. Granulosa LPL provides follicular tissues with the requisite enzyme system to hydrolyze very low density lipoprotein triglyceride en route to the oocyte.

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