Apolipoprotein VLDL-II inhibits lipolysis of triglyceride-rich lipoproteins in the laying hen

Wolfgang J. Schneider,† Rogayah Carroll,* David L. Severson,* and Johannes Nimpf†

Department of Pharmacology and Therapeutics,* Faculty of Medicine, University of Calgary, Calgary, Alberta T2N 4N1, Canada, and Department of Biochemistry and Lipid and Lipoprotein Research Group,† The University of Alberta, Edmonton, Alberta T6G 2S2, Canada

Abstract In the laying hen, very low density lipoprotein (VLDL) particles contain large amounts of apolipoprotein (apo)-VLDL-II in addition to apoB. These triglyceride-rich lipoproteins are transported from the liver primarily to growing oocytes. Since no appreciable hydrolysis of triglyceride occurs during this transport, we have investigated the possibility that apoVLDL-II functions as an inhibitor of lipoprotein lipase (LPL). The presence of LPL in chicken follicular granulosa cells was demonstrated by immunoblotting, and LPL activity with the usual in vitro characteristics could be measured in cultured granulosa cell extracts. ApoVLDL-II inhibited LPL activity in these extracts as well as in the post-heparin medium of rat cardiac myocytes. Half-maximal inhibition in both systems occurred at 40 μg/ml, a concentration that is one-tenth of the circulating apoVLDL-II in the laying hen. Much less inhibition was observed with reduced and alkylated apoVLDL-II and with apoA-I. We conclude that the presence of apoVLDL-II on laying hen VLDL ensures efficient delivery of triglyceride to the oocyte for subsequent use as energy source by the embryo. — **Schneider, W. J., R. Carroll, D. L. Severson, and J. Nimpf.** Apolipoprotein VLDL-II inhibits lipolysis of triglyceride-rich lipoproteins in the laying hen. *J. Lipid Res.* 1990. **31:** 507–513.

Supplementary key words chicken • oocyte • lipoprotein lipase • granulosa

Lipoprotein lipase (LPL) is one of the pivotal enzymes for metabolism of the triglyceride component of chylomicrons and very low density lipoproteins (VLDL). Functional LPL is found on the surface of a variety of cells (reviewed in ref. 1). A portion of LPL is present on the luminal surface of capillary endothelial cells; however, these cells do not synthesize the enzyme. LPL synthesis has been demonstrated in adipocytes (2, 3), cardiac myocytes (4–6), granulosa cells (7, 8), mammary acinar cells, and tumor cells as well as macrophages (9). The cDNA sequences of several mammalian adipose lipoprotein lipases (10–13) as well as of avian adipose lipoprotein lipase (14) have revealed that this enzyme belongs to a gene family that includes hepatic lipase and pancreatic lipase (10–15). While recent studies (16) have shed light on the developmental and genetic control of tissue-specific expression of LPL, the regulation of LPL activity by affector proteins has been well studied only in respect to the known LPL activator, human apoC-II. Inhibition of LPL by other proteins (17) and by circulating apolipoproteins, although of particular interest because of its relevance to lipoprotein metabolism, has been less extensively investigated, with the exception of apoC-III (18–21).

Recently, we have initiated studies into the elucidation of VLDL transport from the plasma compartment into the growing oocyte of the chicken (22–24). In this pathway, following their release from thecal capillaries, the triglyceride-rich VLDL particles first cross the basal lamina, then pass through intercellular gaps in the granulosa cell monolayer, and finally reach the oocyte plasma membrane where they bind to an apoB-specific surface receptor (22–24). In addition to apoB, laying hen VLDL contain apoVLDL-II, a disulfide-bonded homodimer of 9.5-kDa subunits (25), the hepatic synthesis of which is under strict estrogen control (26). After receptor-mediated endocytosis, the apoB of the VLDL particle undergoes specific proteolysis, while the lipid composition of the particles found in the egg yolk is indistinguishable from that in the plasma (23). Thus, the major portion of endocytosed plasma VLDL does not undergo any appreciable lipolysis along this route. This creates a discrepancy in that Bensadoun and colleagues (7, 27, 28) have demonstrated LPL synthesis in the granulosa cell layer of the chicken follicle. Such LPL could be active on the surface of granulosa cells per se, or alternatively, be transported to the capillary endothelium in the thecal envelopes (29) where it would be expected to act on the traversing VLDL. In the current study, we resolve this apparent discrepancy by demonstrating that apoVLDL-II, an apolipo-

Abbreviations: apo, apolipoprotein; LPL, lipoprotein lipase; HDL, high density lipoprotein; VLDL, very low density lipoprotein; IDL, intermediate density lipoprotein; DTT, dithiothreitol.

protein present on VLDL particles in laying hens—but not in roosters or immature hens—is an effective inhibitor of LPL. This suggests an important role of apoVLDL-II in assurance of lipid transport into the growing oocyte, i.e., in the reproductive effort of the hen.

MATERIALS AND METHODS

The isolation and purification of apoVLDL-II from the plasma of estrogen-treated roosters, as well as reductive alkylation of apoVLDL-II, was described previously (24). Chicken apoA-I was obtained from the high density lipoprotein fraction of rooster plasma prepared as described (23). Rooster HDL contained only apoA-I (24); the apolipoprotein was purified from the lyophylized lipoprotein by extraction with buffer containing 10 mM Tris-HCl, 60 mM octyglucoside, pH 7.4, exactly as described for the isolation and purification of apoVLDL-II (24). Human apoC-II was kindly provided by Dr. M. Huff (London, Ontario) and Dr. P. Connelly (Toronto, Ontario). Ca^{2+}-tolerant cardiac myocytes were isolated from the hearts of adult male rats as described by Kryski, Kenno, and Severson (30). The medium from myocyte incubations was collected by centrifugation 10 min after the addition of 5 U/ml heparin (6), and heparin-releasable LPL activity in this post-heparin medium was measured as previously described (5). Briefly, an albumin-stabilized [^{3}H]triolein (glyceroltri[9,10(n)-^{3}H]oleate) substrate emulsion was prepared by sonication (5). Assays routinely contained 0.6 mM [^{3}H]triolein (1 μCi/μmol), 25 mM PIPES (pH 7.5), 0.5% (w/v) albumin, 50 mM $MgCl_2$, appropriate quantities of post-heparin medium, and either 1% (v/v) chicken serum or 1 μg/ml apoC-II as LPL activators. Chicken serum was obtained from young non-laying hens or roosters and was heat-treated (50–55°C for 60 min) in order to inactivate any lipases present in the sample. All incubations were performed in duplicate. LPL activity in the post-heparin medium was determined by measuring the release of radiolabeled oleate after a 30-min incubation at 30°C (5) and is routinely expressed as nmol oleate released /h per 10^6 cells. When LPL activity was determined in the presence of apoVLDL-II or apoA-I, control assay incubations contained the appropriate quantities of the octyglucoside detergent; concentrations of octylglucoside up to 2 mM had little or no effect on LPL activity. Chicken granulosa cells were isolated and cultured as described previously (7). Frozen cell pellets from cultured chicken granulosa cells were sonicated (6 × 30 sec) in a buffer containing 0.25 M sucrose, 1 mM EDTA, 1 mM dithiothreitol, 10 mM HEPES, pH 7.4. LPL activity in aliquots (50–100 μg protein) of cell sonicates was determined as described above, and is routinely expressed as nmol oleate released per hour per mg protein.

Western blotting was performed as described (31) with rabbit antiserum to human milk LPL kindly provided by Dr. R. Zechner, Department of Medical Biochemistry, The University of Graz, Austria. For visualization of bound antibody we used ^{125}I-labeled Protein A.

RESULTS

When LPL activity released by heparin into the incubation medium of rat cardiac myocytes was measured as a function of the concentration of human apoC-II, we determined that a concentration of 1 μg/ml resulted in optimal activation of the enzyme under our experimental conditions (**Fig. 1**, panel A). The dramatic effect of apoVLDL-II on LPL activity in the presence of maximally stimulating concentrations of apoC-II is shown in Fig. 1, panel B. There was a precipitous drop in LPL activity above a concentration of 40 μg/ml of apoVLDL-II, and at 80 μg/ml, the residual activity was only 2% of that in the absence of apoVLDL-II (Fig. 1, B). We routinely use heat-treated chicken serum for activation of lipoprotein lipase in cardiac myocytes; this serum is obtained from young, non-laying hens or roosters and thus does not contain apoVLDL-II, the synthesis of which is under strict estrogen control (24, 26). **Fig. 2** shows the activation of LPL in the post-heparin medium of cardiac myocytes by increasing concentrations of chicken serum (panel A), and the inhibition of such stimulated LPL activity by apoVLDL-II (panel B). Clearly, with either 0.5 or 1.0% chicken serum as activator, apoVLDL-II shows the same inhibitory effect on chicken serum-stimulated LPL (Fig. 2, B) as on that stimulated by human apoC-II (Fig. 1, B).

Although these findings indicated that apoVLDL-II-free chicken serum had no inhibitory effects on LPL, we tested

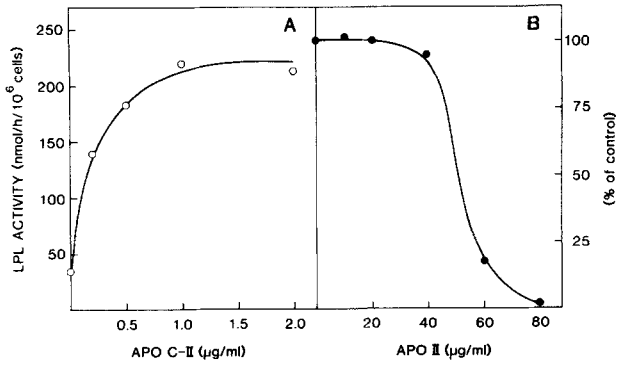

Fig. 1. Effect of human apoC-II and apoVLDL-II (apo II) on LPL activity in the post-heparin medium of cardiac myocytes. Panel A: LPL activity was measured in the presence of the indicated concentrations of apoC-II. Panel B: LPL activity was determined in the presence of 1 μg/ml apoC-II (100% = 303 nmol/h per 10^6 cells) and the indicated concentrations of apo II; results are expressed as percent of control activity, and are from a single experiment; similar results were obtained in three additional experiments.

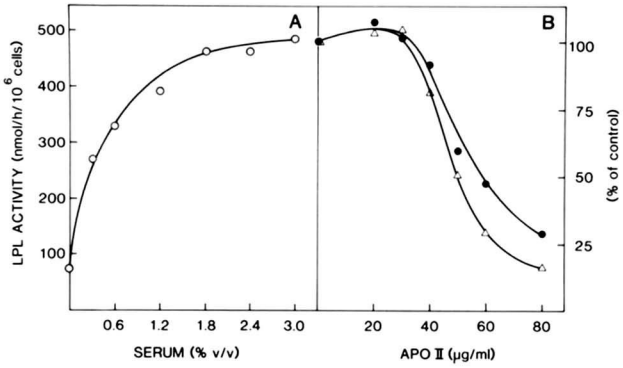

Fig. 2. Effect of apoVLDL-II on serum-stimulated LPL activity in the post-heparin medium of cardiac myocytes. Panel A: LPL activity was measured in the presence of the indicated concentrations of heat-treated chicken serum. Panel B: LPL activity was measured in the presence of 0.5% (△; 100% = 648 nmol/h per 10^6 cells) or 1% chicken serum (●; 100% = 793 nmol/h per 10^6 cells) and the indicated concentrations of apo II; results are expressed as percent of control and are from a single experiment.

directly whether the inhibition by apoVLDL-II was a specific or a general effect of chicken apolipoproteins on cardiac myocyte LPL. To this end, we determined the effect of chicken apoA-I, apoVLDL-II, apoVLDL-II that had been subjected to reductive alkylation on LPL activity in post-heparin medium of cardiac myocytes (**Fig. 3**). ApoVLDL-II showed the typical inhibitory effect, while chicken apoA-I only had a negligible effect on LPL activity. Even at high concentrations of apoA-I, the activity did not decrease to below 80% of control, while apoVLDL-II inhibited strongly. Higher concentrations of apoVLDL-II were not tested because substantial inhibition of LPL activity by the octylglucoside detergent would have resulted.

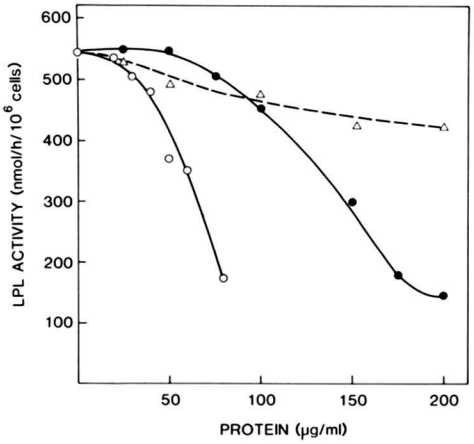

Fig. 3. Effect of chicken apolipoproteins on LPL activity in the post-heparin medium of cardiac myocytes. LPL activity was measured in the presence of 1% (v/v) serum and the indicated concentrations of apoVLDL-II (○), reduced and alkylated apoVLDL-II (●), and apoA-I (△--△). Results are from a single representative experiment; similar results were obtained in two additional experiments.

Reduced and alkylated apoVLDL-II, which represents the monomeric form of this disulfide-bonded dimeric protein (24) was much less effective in inhibiting LPL activity than native apoVLDL-II (Fig. 3).

After having characterized apoVLDL-II as inhibitor of LPL on rat cardiac myocytes, we wanted to investigate the expression and properties of the enzyme synthesized by chicken granulosa cells. **Fig. 4** demonstrates by Western blotting with a rabbit antiserum to human milk LPL the presence of a cross-reactive protein in chicken granulosa cell extracts. In the absence of sulfhydryl reducing agents, the granulosa cell protein migrated with an apparent M_r of 56,000, whereas in the presence of sulfhydryl reagents its apparent M_r was 62,000. This finding is in excellent agreement with previously reported molecular weights for lipoprotein lipases from a variety of sources (9), as well as with the molecular mass deduced from the recently reported cDNA sequence for avian adipose lipoprotein lipase (14). Different rates of mobility of this protein,

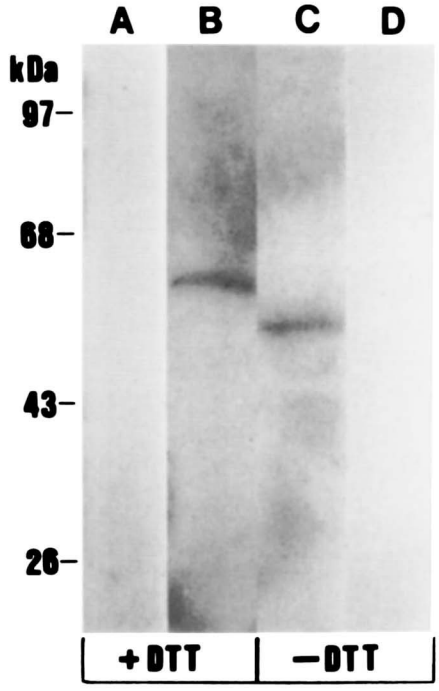

Fig. 4. Immunoblot analysis of lipoprotein lipase of cultured chicken follicular granulosa cells. The cells from 30 dishes were collected by scraping and centrifugation; the cell pellet was sonicated in 600 μl of buffer containing 0.25 M sucrose, 1 mM EDTA, 10 mM HEPES, pH 7.4, as described in Materials and Methods, and a soluble fraction was obtained by centrifugation at 10,000 *g* for 1 h at 4°C. Equal aliquots of the supernatant (230 μg of protein per lane) were subjected to SDS-gradient gel electrophoresis (4.5–18% polyacrylamide) in the presence (lanes A, B) or absence (C, D) of 20 mM dithiothreitol (DTT), followed by transfer to nitrocellulose. The strips were incubated with nonimmune rabbit serum (lanes A, D) or rabbit antiserum to human milk LPL (lanes B, C), both at a dilution of 1:25. Bound antibody was detected with 0.5 μg/ml of ^{125}I-labeled Protein A(~1,000 cpm/ng) and autoradiography. The positions of migration of M_r standards are indicated on the left.

dependent on its reduction state, are consistent with its high content of cysteine residues (14), and have previously been observed for LPL from bovine milk (32). Cleavage of intramolecular disulfide bonds by reduction may lead to a more unfolded conformation and retardation in SDS-polyacrylamide gel electrophoresis. These data demonstrate for the first time the presence of LPL on cultured chicken ovarian granulosa cells through identification by Western blotting.

In preliminary experiments, triacylglycerol lipase activity in granulosa cell sonicates was characterized. The addition of serum resulted in a 6.3-fold increase in lipase activity; this serum-stimulated activity was further enhanced (1.6-fold) by the addition of heparin (2 U/ml) to the assay. Increasing the ionic strength of the assay to 1 M by addition of $MgCl_2$ reduced serum-stimulated lipase activity to less than 1% of the control. These in vitro characteristics all indicated that LPL activity can be determined in chicken granulosa cells.

Fig. 5 (panel A) shows that human apoC-II clearly stimulated this enzyme; LPL activity measured with 1.5 μg/ml apoC-II was very similar to activity stimulated with 2% chicken serum. Fig. 5 (panel B) demonstrates that apoVLDL-II was able to completely suppress the apoC-II-stimulated LPL activity at a concentration of 80 μg/ml, with a half-maximal inhibitory effect at 40 μg/ml, very similar to that needed for inhibition of LPL activity from cardiac myocytes (Fig. 1).

DISCUSSION

The current studies were performed in order to shed further light on the mechanisms for the uptake of VLDL particles from plasma into the growing oocyte of the laying hen. In particular, these experiments were aimed at explaining why the triglyceride portion of the VLDL fraction in the laying hen remains intact while in the circulation and during the final stages of this transport, which involves passage of the lipoprotein through thecal layers and the follicular granulosa cell monolayer, both of which express lipoprotein lipase activity (7, 27). Since size and triglyceride content of plasma VLDL and yolk lipoprotein particles are indistinguishable (23), we hypothesized that a protein component of the VLDL particles in laying hens protected the triglyceride moiety from lipolytic attack. Here, we have obtained experimental evidence that apoVLDL-II, an apolipoprotein induced by estrogens at the onset of egg laying, is able to inhibit lipoprotein lipase in at least two cell lines, namely rat cardiac myocytes as well as chicken ovarian granulosa cells.

The use of post-heparin medium of cardiac myocytes has the advantage of being a well-characterized source of LPL (6) without the presence of other lipases that may be present in cell extracts. LPL activity with characteristic in vitro properties was detectable in chicken granulosa cell sonicates, in confirmation of previous results (28, 29). In addition, for the first time the presence of LPL in cultured chicken ovarian granulosa cells was identified by Western blotting (Fig. 4).

The addition of detergent-soluble pure apoVLDL-II caused a dramatic reduction of measurable LPL activity in both cell lines with both apoVLDL-II-free chicken serum and human apoC-II as activators (Figs. 1, 2 and Fig. 5). This inhibition is specific in that apoA-I and reduced and alkylated apoVLDL-II had a much smaller inhibitory effect on the enzyme (Fig. 3). Half-maximal inhibition of LPL was observed at a concentration of approximately 40 μg/ml of apoVLDL-II with the enzyme of both cell types. Laying hen serum contains an average of 1.5 mg/ml of VLDL protein (23, 33), 40–60% of which consists of apoVLDL-II (34), and thus the serum concentration of apoVLDL-II in a laying hen ranges from 600 to 900 μg/ml, at least 10-fold higher than that found effective in inhibition of LPL in the current study. Although the substrate in our assay is a triolein emulsion which likely behaves differently from VLDL in terms of activator displacement by apoVLDL-II, these results strongly suggest that the triglyceride moiety of VLDL is well protected from lipolysis during transport to the oocyte.

A previous study (17) has addressed the effects of proteins isolated from egg yolk lipoproteins on bovine milk lipoprotein lipase. Two yolk protein fractions were shown to possess cofactor activity, and another fraction displayed inhibitory activity towards bovine milk LPL. The latter fraction, termed S2, was shown to contain yolk apoVLDL-II among other components (35). Upon addition of 25 μg/ml of S2 protein, purified bovine milk LPL was inhibited by 75–90% of control (17). While these studies suggested that

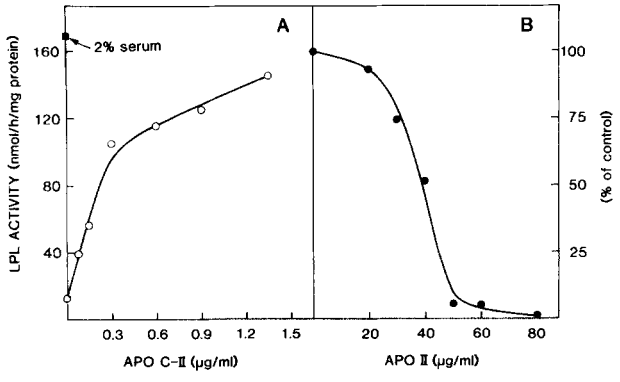

Fig. 5. Effect of apoC-II and apoVLDL-II on LPL activity in chicken granulosa cells. Panel A: LPL activity was measured in the presence of the indicated concentrations of apoC-II. For comparison, activity measured in the presence of 2% chicken serum is also indicated (■). Panel B: LPL activity was determined in the presence of 1.5 μg/ml apoC-II (100% = 87.3 nmol/h per mg protein) and the indicated concentrations of apo II; results are expressed as the percentage of control. A similar inhibition curve was obtained when the apoC-II concentration was reduced to 0.3 μg/ml.

S2 contained an inhibitor of LPL, it was not directly shown that apoVLDL-II, and not another component of the S2 yolk fraction, was responsible for this effect. Furthermore, plasma proteins imported into the oocyte undergo postendocytic modification (23), and this process cannot be excluded for apoVLDL-II in yolk. In the present study we have used pure apoVLDL-II isolated from plasma, the compartment in which relevant interactions between LPL and its effectors occur.

As discussed by Bensadoun and coworkers (28, 29), LPL synthesized by granulosa cells may be active on the granulosa cells themselves, or be the source of enzyme residing on the capillary endothelium of the thecal envelope. In either case, VLDL in the laying hen would be exposed to LPL on the capillary endothelium during its residence in plasma and shortly before entering the oocyte via receptor-mediated endocytosis. Tissue lipoprotein lipase would be active in vivo, because chicken serum is a rich source for lipoprotein lipase activator protein, as shown by the present as well as previous studies (29). In fact, LPL of both cell types used in our experiments was stimulated to similar extents by apoC-II from human plasma and heat-treated chicken serum, respectively (Figs. 1A, 2A, and 5A).

Previous studies by Griffin, Grant, and Perry (36) have addressed the question of substrate specificity of chicken lipoprotein lipase. In particular, these investigators studied the hydrolysis of VLDL particles from immature and laying hens. Two of their observations are relevant to the current discussion: first, VLDL particles from laying hens were much smaller than those from immature hens (30 vs 58 nm on average); second, hydrolysis of VLDL from immature hens proceeded much more rapidly than that of VLDL from laying hens; prolonged lipolysis resulted in 40% of the substrate being used in the case of immature hen VLDL, but only 1–15% of laying hen VLDL triacylglycerol was hydrolyzed. This lends further support to our notion that apoVLDL-II is the protective component. The particle sizes reported by Griffin et al. (36) and Burley, Sleigh, and Shenstone (37) correlate well with the one reported by us (38 nm for VLDL particles from both plasma and yolk; ref. 23). Griffin et al. (36) and Perry and Gilbert (38) have speculated that the small size of laying hen VLDL facilitates the passage out of the ovarian follicular capillaries and through connective tissue layers in the follicle, before finally passing through the basal lamina and intercellular gaps in the granulosa cell layer. However, it is clear that the smaller size of laying hen VLDL particles is not due to a lower triglyceride content compared to that of VLDL from immature hens (36).

We have previously suggested that the presence of apoVLDL-II on oocyte-destined VLDL particles might protect them from the action of lipoprotein lipase (24). We have also proposed an alternative or additional role of apoVLDL-II as a structural stabilizer for triglyceride-rich lipoprotein particles in laying hens (24). The findings in the present study, combined with those previously reported by Griffin et al. (36) and Perry and Gilbert (38) could indeed be interpreted as apoVLDL-II serving a dual function. Namely, since its induction by estrogen at onset of egg-laying in the hen coincides with the disappearance of the larger VLDL particles and the appearance of smaller VLDL particles that can penetrate the follicle layers as described above, apoVLDL-II might be responsible for this decrease in size. At the same time, it is the apoVLDL-II moiety of the lipoprotein particle that inhibits lipoprotein lipase action, as shown herein. Future studies will address the mechanism underlying this dual action of apoVLDL-II; possibly, the smaller surface curvature of laying hen VLDL favors the displacement of activator protein, and enzyme access to the lipoprotein surface is blocked by occupation with apoVLDL-II.

Bensadoun and Kompiang (28) reported that infusion of anti-LPL serum inhibited not only plasma triglyceride removal in roosters, but also the accumulation of triglyceride in the growing follicles of laying hens. They concluded that LPL normally mediates triglyceride fatty acid transport into the follicle. However, their studies did not address the accumulation of VLDL triglyceride in the ooctye per se, since radiolabeled triglyceride was quantitated in follicles from which only the thecal tissue had been removed (28). Indeed, such preparations contain, in addition to the oocyte, theca interna and granulosa cells, exactly the sites of follicular LPL activity. Therefore, the inhibition of triglyceride fatty acid uptake by anti-LPL antiserum observed by Bensadoun and Kompiang (28) could simply reflect the systemic decrease of LPL activity under these conditions. Alternatively, or in addition to this effect, there may be a significant local inhibition of lipolysis in thecal and granulosa cells. These results, then, are not incompatible with our notion that LPL action on triglyceride-rich lipoproteins is subject to inhibition by apoVLDL-II, a requirement for efficient triglyceride transport to the oocyte for subsequent utilization by the developing embryo. Our findings also explain, at least in part, why laying hens have high levels of VLDL, but minimal amounts of IDL and LDL. On the other hand, the main triglyceride-carrying lipoprotein class in roosters and immature hens, which do not synthesize apoVLDL-II, is LDL.

This work was supported by grants from The Heart and Stroke Foundation of Alberta (WJS), Medical Research Council of Canada (DLS), and the Alberta Heritage Foundation for Medical Research (AHFMR). D. L. S. and W. J. S. are Heritage Medical Scholars, and J. N. is a Postdoctoral Fellow of the AHFMR. We thank our colleague Shinji Yokoyama for many helpful suggestions

and critical reading of this manuscript. We appreciate the excellent technical assistance by Grace Ozimek and Calla Shank-Hogue, and the expert help of Yolanda Gillam in preparation of this manuscript.

Manuscript received 24 August 1989 and in revised form 6 November 1989.

REFERENCES

1. Cryer, A. 1981. Tissue lipoprotein lipase activity and its action in lipoprotein metabolism. *Int. J. Biochem.* **13:** 525–541.
2. Rodbell, M. 1964. Localization of lipoprotein lipase in fat cells of adipose tissue. *J. Biol. Chem.* **239:** 753–755.
3. Arnaud, J. O., O. Nobili, and J. Boyer. 1979. Differential properties of lipases active as membrane-bound enzymes in isolated fat cells. *Biochim. Biophys. Acta.* **572:** 193–200.
4. Bagby, G. J., M. S. Liu, and J. A. Spitzer. 1977. Lipoprotein lipase activity in rat heart myocytes. *Life Sci.* **21:** 467–474.
5. Ramirez, I., A. Kryski, O. Ben-Zeev, M. C. Schotz, and D. L. Severson. 1985. Characterization of triacylglycerol hydrolase activities in isolated myocardial cells from rat heart. *Biochem. J.* **232:** 229–236.
6. Severson, D. L., M. Lee, and R. Carrol. 1988. Secretion of lipoprotein lipase from myocardial cells isolated from adult rat hearts. *Mol. Cell. Biochem.* **79:** 17–24.
7. Brannon, P. M., A. H. Cheung, and A. Bensadoun. 1978. Synthesis of lipoprotein lipase in cultured avian granulosa cells. *Biochim. Biophys. Acta.* **531:** 96–108.
8. Brannon, P.M., and A. Bensadoun. 1980. Determination of the half-life of lipoprotein lipase in cultured granulosa cells. *Biochim. Biophys. Acta.* **618:** 173–182.
9. Garfinkel, A. S., and M. C. Schotz. 1987. Lipoprotein lipase. Chapter 11. *In* Plasma Lipoproteins A. M. Gotto, Jr., editor. Elsevier Publishing Co., New York 335–357.
10. Wion, K. L., T. G. Kirchgessner, A. J. Lusis, M. C. Schotz, and R. M. Lawn. 1987. Human lipoprotein lipase complementary DNA sequence. *Science.* **235:** 1638–1641.
11. Kirchgessner, T. G., K. L. Svenson, A. J. Lusis, and M. C. Schotz. 1987. The sequence of cDNA encoding lipoprotein lipase: a member of a lipase gene family. *J. Biol. Chem.* **262:** 8463–8466.
12. Senda, M., K. Oka, V. Brown, P. K. Quasba, and Y. Furuichi. 1987. Molecular cloning and sequence of a cDNA coding for bovine lipoprotein lipase. *Proc. Natl. Acad. Sci. USA.* **84:** 4369–4373.
13. Enerbäck, S., H. Semb, G. Bengtsson-Olivecrona, P. Carlsson, M. L. Hermansson, T. Olivecrona, and G. Bjursell. 1987. Molecular cloning and sequence analysis of cDNA encoding lipoprotein lipase of guinea pig. *Gene.* **58:** 1–12.
14. Cooper, D. A., J. C. Stein, P. J. Strielemann, and A. Bensadoun. 1989. Avian adipose lipoprotein lipase: cDNA sequence and reciprocal regulation of mRNA levels in adipose and heart. *Biochim. Biophys. Acta.* **1008:** 92–101.
15. Ben-Zeev, O., D. M. Ben-Avram, H. Wong, J. Nikazy, J. E. Shively, and M. C. Schotz. 1987. Hepatic lipase: a member of a family of structurally related lipases. *Biochim. Biophys. Acta.* **919:** 13–20.
16. Kirchgessner, T. G., R. C. LeBoeuf, C. A. Langner, S. Zollmann, C. H. Chang, B. A. Taylor, M. C. Schotz, J. I. Gordon, and J. A. Lusis. 1989. Genetic and developmental regulation of the lipoprotein lipase gene: loci both distal and proximal to the lipoprotein lipase structural gene control enzyme expression. *J. Biol. Chem.* **264:** 1473–1482.
17. Bengtsson, G., and T. Olivecrona. 1977. Stimulation and inhibition of milk (lipoprotein) lipase by proteins from egg yolk lipoproteins. *Eur. J. Biochem.* **79:** 225–231.
18. Jackson, R. L., S. Tajima, T. Yamamura, S. Yokoyama, and A. Yamamoto. 1986. Comparison of apolipoprotein C-II-deficient triacylglycerol-rich lipoproteins and trioleoglycerol/phosphatidylcholine-stabilized particles as substrates for lipoprotein lipase. *Biochim. Biophys. Acta.* **875:** 211–219.
19. Bensadoun, A., C. Ehnholm, D. Steinberg, and W. V. Brown. 1974. Purification and characterization of lipoprotein lipase from pig adipose tissue. *J. Biol. Chem.* **249:** 2220–2227.
20. Havel, R. J., C. J. Fielding, T. Olivecrona, V. Shore, P. E. Fielding, and T. Egelrud. 1973. Cofactor activity of protein components of human very low density lipoproteins in the hydrolysis of triglycerides by lipoprotein lipase from different sources. *Biochemistry.* **12:** 1828–1833.
21. Wang, C-S., W. J. McConathy, H. U. Kloer, and P. Alaupovic. 1985. Modulation of lipoprotein lipase activity by apolipoproteins. Effect of apolipoprotein C-III. *J. Clin. Invest.* **75:** 384–390.
22. George, R., D. L. Barber, and W. J. Schneider. 1987. Characterization of the chicken oocyte receptor for low and very low density lipoproteins. *J. Biol. Chem.* **262:** 16838–16847.
23. Nimpf, J., M. Radosavljevic, and W. J. Schneider. 1989. Specific postendocytic proteolysis of apolipoprotein-B in oocytes does not abolish receptor recognition. *Proc. Natl. Acad. Sci. USA.* **86:** 906–910.
24. Nimpf, J., R. George, and W. J. Schneider. 1988. Apolipoprotein specificity of the chicken oocyte receptor for low and very low density lipoproteins. *J. Lipid. Res.* **29:** 657–667.
25. Dugaiczyk, A., A. S. Inglis, P. M. Strike, R. W. Burley, W. G. Beattie, and L. Chan. 1981. Comparison of the nucleotide sequence of cloned DNA coding for an apolipoprotein (apoVLDL-II) from avian blood and the amino acid sequence of an egg-yolk protein (apovitellenin I): equivalence of the two sequences. *Gene.* **14:** 175–182.
26. Wiskocil, R., P. Bensky, W. Dower, R. G. Goldberger, J. I. Gordon, and R. G. Deeley. 1980. Coordinate regulation of two estrogen-dependent genes in avian liver. *Proc. Natl. Acad. Sci. USA.* **77:** 4474–4478.
27. Benson, J. D., A. Bensadoun, and D. Cohen. 1975. Lipoprotein lipase of ovarian follicles in the domestic chicken (*Gallus domesticus*). *Proc. Soc. Exp. Biol. Med.* **148:** 347–350.
28. Bensadoun, A., and I. P. Kompiang. 1979. Role of lipoprotein lipase in plasma triglyceride removal. *Fed. Proc.* **38:** 2622–2626.
29. Bensadoun, A., and C. Clifford. 1971. Mechanism of uptake of plasma lipoprotein triglyceride by avian ovarian follicles. *In* XIX Annu. Colloq. Protides Biol. Fluids. Pergamon Press, Brugge, Belgium. 227–231.
30. Kryski, A., K. A. Kenno, and D. L. Severson. 1985. Stimulation of lipolysis in rat heart myocytes by isoproterenol. *Am. J. Physiol.* **248:** H208–216.
31. Russell, D. W., W. J. Schneider, T. Yamamoto, K. L. Luskey, M. S. Brown, and J. L. Goldstein. 1984. Domain map of the LDL receptor: sequence homology with the epidermal growth factor precursor. *Cell.* **37:** 577–585.
32. Olivecrona, T., and G. Bengtsson-Olivecrona. 1987. Lipoprotein lipase from milk—the model enzyme in lipoprotein lipase research. *In* Lipoprotein Lipase. J. Borensztajn, editor. Evener Publishers, Chicago. 15–58.

33. Ho, K-J., W. D. Lawrence, L. A. Lewis, L. B. Liu, and C. B. Taylor. 1974. Hereditary hyperlipidemia in nonlaying chickens. *Arch. Pathol.* **98:** 161–172.
34. Chan, L., R. L. Jackson, B. O'Malley, and A. R. Means. 1976. Synthesis of very low density lipoproteins in the cockerel. Effects of estrogen. *J. Clin. Invest.* **58:** 368–369.
35. Bengtsson, G., S-E. Marklund, and T. Olivecrona. 1977. Protein components of very low density lipoproteins form hen's egg yolk. *Eur. J. Biochem.* **79:** 211–223.
36. Griffin, H., G. Grant, and M. Perry. 1982. Hydrolysis of plasma triacylglycerol-rich lipoproteins from immature and laying hens (*Gallus domesticus*) by lipoprotein lipase in vitro. *Biochem. J.* **206:** 647–654.
37. Burley, R. W., R. W. Sleigh, and F. S. Shenstone. 1984. Lipoproteins from the blood and egg yolk of the hen. *Eur. J. Biochem.* **142:** 171–176.
38. Perry, M. M., and A. B. Gilbert. 1979. Yolk transport in the ovarian follicle of the hen (*Gallus domesticus*): lipoprotein-like particles at the periphery of the oocyte in the rapid growth phase. *J. Cell Sci.* **39:** 257–262.