Hens of the "Restricted Ovulator" (R/O) chicken strain are characterized by the absence of egg-laying and concomitant severe hyperlipidemia due to a single gene defect (Ho, K.-J., Lawrence, W. D., Lewis, L. A., Liu, L. B., and Taylor, C. B. (1974) Arch. Pathol. 98, 161–172). However, the underlying biochemical defect has not been identified. Previous studies on receptor-mediated growth of chicken oocytes have led to the characterization of a 95-kDa oocyte plasma membrane receptor that binds very low density lipoproteins (VLDL) (George, R., Barber, D. L., and Schneider, W. J. (1987) J. Biol. Chem. 262, 16838–16847). The current experiments demonstrate the absence of this receptor from R/O oocytes. Ligand binding experiments showed that ovarian proteins from mutant hens failed to display high affinity, saturable, and specific binding of $^{125}$I-VLDL. Ligand blotting with $^{125}$I-VLDL and Western blotting with polyclonal anti-receptor antibodies visualized the 95-kDa receptor in normal oocytes, but R/O ovarian membranes were devoid of any cross-reactive protein. Finally, plasma clearance of intravenously injected $^{125}$I-VLDL was dramatically impaired in R/O in comparison to normal hens, with a concomitant decrease in the radioactivity accumulating in R/O oocytes. These data strongly suggest that the absence of the 95-kDa receptor for VLDL from oocytes is responsible for the R/O phenotype, and that the receptor not only binds VLDL, but also mediates its uptake. This animal model provides a powerful tool for investigations of receptor-mediated endocytosis in oocytes. This animal model was first described in 1974 (2) as a strain of chickens with hereditary hyperlipidemia and absence of egg laying. Subsequent breeding studies strongly suggested that this phenotype was due to a single, sex-linked gene defect (3, 4). Morphologically, the ovaries of mutant hens are grossly abnormal in that follicles never reach mature size (maximum weight about one-sixth of that of normal) (Ref. 2), the follicles are often dark (greenish) or of dull yellow color and frequently show wrinkled surfaces, and no discharged follicles are observed. Schjeide et al. (3, 5) named these non-laying hens "Restricted Ovulator" (R/O) and determined that their oocytes fail to incorporate normal amounts of "low density lipoproteins" and "lipovitellins," yolk proteins now characterized as derived from plasma VLDL and vitellogenin, respectively. As a consequence of this lack of yolk deposition and the failure to suppress hepatic lipoprotein synthesis and secretion, massive amounts of lipids accumulate in the blood plasma. Triglyceride levels are elevated 4–5-fold, total cholesterol 6-fold, and phospholipid 3-fold (2) compared to laying sisters; at 18 months, R/O hens develop striking aortic atherosclerosis (6). Heterozygous carrier roosters are normolipidemic and phenotypically normal (2). It had been suggested (5) that the biochemical defect in R/O hens may be a failure to express cell surface sites involved in entry of plasma precursors into growing oocytes. In the current study, we have obtained direct experimental evidence for the lack of normal receptors for VLDL in R/O oocytes and thus for the crucial role of this receptor in the normal process of receptor-mediated oocyte growth.

Hepatically synthesized macromolecules, such as very low density lipoprotein (VLDL), are thought to be taken up via receptor-mediated endocytosis into growing oocytes of avian species. Recently, the chicken oocyte receptor for VLDL has been characterized in our laboratory (1). However, a direct involvement of this oocyte membrane protein in the endocytosis of VLDL has not yet been demonstrated. For this purpose, we have initiated studies with what we believe presents a powerful experimental model for the study of receptor-mediated endocytosis in oocytes. This animal model was first described in 1974 (2) as a strain of chickens with hereditary hyperlipidemia and absence of egg laying. Subsequent breeding studies strongly suggested that this phenotype was due to a single, sex-linked gene defect (3, 4). Morphologically, the ovaries of mutant hens are grossly abnormal in that follicles never reach mature size (maximum weight about one-sixth of that of normal) (Ref. 2), the follicles are often dark (greenish) or of dull yellow color and frequently show wrinkled surfaces, and no discharged follicles are observed. Schjeide et al. (3, 5) named these non-laying hens "Restricted Ovulator" (R/O) and determined that their oocytes fail to incorporate normal amounts of "low density lipoproteins" and "lipovitellins," yolk proteins now characterized as derived from plasma VLDL and vitellogenin, respectively. As a consequence of this lack of yolk deposition and the failure to suppress hepatic lipoprotein synthesis and secretion, massive amounts of lipids accumulate in the blood plasma. Triglyceride levels are elevated 4–5-fold, total cholesterol 6-fold, and phospholipid 3-fold (2) compared to laying sisters; at 18 months, R/O hens develop striking aortic atherosclerosis (6). Heterozygous carrier roosters are normolipidemic and phenotypically normal (2). It had been suggested (5) that the biochemical defect in R/O hens may be a failure to express cell surface sites involved in entry of plasma precursors into growing oocytes. In the current study, we have obtained direct experimental evidence for the lack of normal receptors for VLDL in R/O oocytes and thus for the crucial role of this receptor in the normal process of receptor-mediated oocyte growth.

**EXPERIMENTAL PROCEDURES**

**Materials**—We obtained Sephadex G-25 columns (PD-10) from Pharmacia LKB Biotechnologies Inc.; octyl-$eta$-D-glucoside, phenylmethylsulfonyl fluoride, leupeptin, Triton X-100, and bovine serum albumin from Sigma; $M_m$ standards from Bethesda Research Laboratories; nitrocellulose paper BA 85 from Schleicher & Schuell; N-flow cellulose acetate membrane filters N 25/45 from Oxo Ltd., Basingstoke, England; sodium $^{125}$Iiodide (11–17 mCi/µg) from Edmonton Radiopharmaceutical Centre, Edmonton, Alberta, Canada; potassium bromide from BDH Chemicals; and goat anti-rabbit IgG

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Oocyte Receptor Defect

Animals—White Leghorn hens and roosters were provided by Dr. Frank Robinson, Department of Animal Science, The University of Alberta. Hatchlings from the R/O breeding colony at the University of Wisconsin, Madison, WI, were kindly provided by Dr. J. James Bitgood, Poultry Science Department. Of the 50 animals originally received, 24 were hens. These were housed at the University of Alberta Research Farm, separated into individual cages at 22 weeks of age, and their ability to lay eggs was monitored. At 30 weeks, nine hens had failed to lay, while 13 had laid several eggs each. Blood was obtained from all hens at this point, and severe hyperlipidemia confirmed in eight of the nine animals lacking oviposition; these eight had not laid for 7 months, consistently showing 4-fold elevated triglyceride levels, and are designated R/O. In the current study, three R/O hens and four of their normal sisters (laying hens) have been used. They were maintained on layer’s mash, with a light period of 12 h and free access to water and feed.

Preparation of Membrane Fractions—All operations were performed at 0–4 °C. Oocyte membranes were prepared from 3–15-mm diameter follicles as previously described (1). For reasons described under “Results,” in some experiments normal and R/O ovarian membranes were obtained after removal of all laying hen follicles larger than 8 mm in diameter, and washing the remaining tissue in buffer containing 20 mM Tris-HCl (pH 8), 1 mM CaCl₂, 150 mM NaCl, 1 mM phenylmethylsulfonyl fluoride, and 2 μM leupeptin (buffer A). The tissue was minced with scissors and subjected to homogenization in 20 volumes of buffer A containing octylglucoside (9) and detergent-free samples (10) were measured with the Mini Trans-blot system from Bio-Rad as described (1). 

Lipoproteins—Plasma VLDL from normal and R/O hens, and Rooster HDL, which is devoid of apolipoprotein B, did not compete for receptor binding of either of the labeled ligands; however, unlabeled VLDL from both laying hen and R/O contained the high molecular weight apolipoprotein B and apolipoprotein VLDL-II, a disulfide-linked homodimer of 9.5-kDa subunits which is highly resistant to reduction (16). While these data suggested a lack of gross differences in the structure of VLDL apolipoproteins, we were interested in the ability of R/O VLDL to interact with the previously characterized apolipoprotein B–specific chicken oocyte receptor for triglyceride-rich lipoproteins (1, 7). In order to test this, competition binding experiments with membranes derived from normal chicken oocytes were performed. As can be seen from Fig. 2, both 125I-labeled R/O VLDL and 125I-labeled laying hen VLDL bound to octylglucoside extracts from oocyte membranes. At a concentration of 13 μg/ml (corresponding to the Kₐ (1)), receptor-binding of 125I-labeled laying hen VLDL amounted to 28.8 μg/mg protein, while that of 125I-labeled R/O VLDL was 28.4 μg/mg (average of two different experiments with two different radiolabeled VLDL preparations from R/O and laying hen, respectively). Rooster HDL, which is devoid of apolipoprotein B (1), did not compete for receptor binding of either of the labeled ligands; however, unlabeled VLDL from both laying hen and R/O hens.

RESULTS

R/O VLDL Is Functionally Identical to Laying Hen VLDL—Fig. 1 shows the results of analysis by SDS-polyacrylamide gel electrophoresis of the apolipoprotein contents of VLDL from laying hen and R/O, respectively. The protein patterns obtained were identical; both lipoproteins contained the high molecular weight apolipoprotein B and apolipoprotein VLDL-II, a disulfide-linked homodimer of 9.5-kDa subunits which is highly resistant to reduction (16). While these data suggested a lack of gross differences in the structure of VLDL apolipoproteins, we were interested in the ability of R/O VLDL to interact with the previously characterized apolipoprotein B–specific chicken oocyte receptor for triglyceride-rich lipoproteins (1, 7). In order to test this, competition binding experiments with membranes derived from normal chicken oocytes were performed. As can be seen from Fig. 2, both 125I-labeled R/O VLDL and 125I-labeled laying hen VLDL bound to octylglucoside extracts from oocyte membranes. At a concentration of 13 μg/ml (corresponding to the Kₐ (1)), receptor-binding of 125I-labeled laying hen VLDL amounted to 28.8 μg/mg protein, while that of 125I-labeled R/O VLDL was 28.4 μg/mg (average of two different experiments with two different radiolabeled VLDL preparations from R/O and laying hen, respectively). Rooster HDL, which is devoid of apolipoprotein B (1), did not compete for receptor binding of either of the labeled ligands; however, unlabeled VLDL from both laying hen and

µl of blood were removed at the indicated times and immediately mixed with 25 µl of 0.5 M EDTA, plasma obtained after brief centrifugation, and radioactivity determined in 100-µl aliquots. At all time points, greater than 97% of the plasma-125I radioactivity was precipitable by 10% (w/v) trichloroacetic acid. At the end of the study, the birds were killed by cervical dislocation and the ovary and liver removed. The ovaries were rinsed extensively in 0.15 M NaCl, and follicles of 6–10 mm diameter were dissected to remove theca externa and most of the theca interna, and the remaining tissue containing the intact oocyte subjected to scintillation counting for 125I radioactivity. Lobes of liver were removed, perfused with 0.15 M NaCl through central veins while gently squeezing the tissue until the perfusate was clear, and the radioactivity in slices weighing 0.5–1 g was determined by scintillation counting.

In Vivo Studies—Hens had free access to food and water and were not anesthetized at any time during the experiment. They were on a 12-h light cycle (off at 8 p.m.) and housed in individual cages. At 10 a.m., they received 125I-VLDL prepared from White Leghorn layers as described above; the radiolabeled lipoprotein was mixed with 0.15 M NaCl and sterilized by filtration through 0.45-µm cellulose acetate membrane filters. One ml of the solution, containing 1 mg of 125I-VLDL (specific radioactivity, 177 cpm/ng) was injected through the left vena cutanea ulnaris (wing vein), and 2 min later a blood sample was drawn from the right wing vein (zero time point). Five hundred
Oocyte Receptor Defect

Ovarian Membranes from R/O Hens Lack Functional Receptor for VLDL—Next, we wanted to test whether the R/O phenotype was due to an absence of receptor-mediated binding of VLDL to oocyte membranes. Since the largest R/O oocytes are much smaller than normal oocytes and often discolored and irregular in shape (see Introduction), it was difficult to obtain R/O oocytes that matched laying hen oocytes in appearance and quantity. Thus, as an alternative to the use of oocyte membranes, we prepared membrane fractions from R/O and laying hen ovaries. From laying hen ovaries, all follicles larger than those in R/O ovaries (about 8 mm in diameter) were removed, while R/O ovaries were used as a whole. Hence, the membranes used in the experiments described below were different from those used previously to characterize the oocyte receptor (1) and in the experiments described above in that they contained components of the mesovarian ligament, the follicular stalks, and theca externae (17). Previously, we had used larger individually removed follicles (average 10 mm diameter) from which theca externa and most of theca interna had been dissected away.

Fig. 4A demonstrates that even such crude membrane preparations from a laying hen ovary showed high affinity, saturable binding of $^{125}$I-VLDL. As expected, however, nonspecific binding as determined in the presence of excess unlabeled VLDL or 10 mM EDTA (not shown) was significantly higher than observed previously with oocyte membranes (1). Moreover, rooster HDL, which does not inhibit binding of VLDL...
to its receptor (Ref. 1, and Fig. 2), interfered with the interaction of \( ^{125}\text{I}-\text{VLDL} \) with ovarian membranes (Fig. 4A). Namely, in the presence of 1 mg/ml of HDL, binding was suppressed, but a saturable binding component was maintained. In experiments not shown, increasing concentrations of HDL progressively inhibited \( ^{125}\text{I}-\text{VLDL} \) binding to ovarian membranes, and maximal inhibition was observed at concentrations of unlabelled HDL that were 10-fold higher than that of \( ^{125}\text{I}-\text{VLDL} \) in the assay mixtures. In the experiments of Fig. 4, the concentration of unlabelled HDL was 20-fold higher than the highest concentration of \( ^{125}\text{I}-\text{VLDL} \) tested. Unlabelled VLDL at the same level as HDL completely abolished the saturable binding component and only a linear component representing nonspecific binding was observed. The amount of receptor-mediated binding was obtained from the difference between \( ^{125}\text{I}-\text{VLDL} \) binding in the presence of HDL minus that in the presence of VLDL (Fig. 4C, closed symbols). The binding of \( ^{125}\text{I}-\text{VLDL} \) to ovarian membranes from laying hen displayed a \( K_d \) of \(-10 \mu\text{g/ml} \), a value very close to that reported previously for the receptor in oocyte membrane extracts (13 \( \mu\text{g/ml} \); Ref. 1). As expected, the specific binding activity (micrograms of \( ^{125}\text{I}-\text{VLDL} \) bound per mg protein) was much lower in ovarian membranes (average \( B_{\text{max}} \), 0.84 \( \mu\text{g/mg} \)) than in pure oocyte membranes (average \( B_{\text{max}} \), 65 \( \mu\text{g/mg} \); Refs. 1 and 7).

When ovarian membranes from a R/O hen (a sister of the laying hen used above) were tested for their ability to bind \( ^{125}\text{I}-\text{VLDL} \) in the same fashion, a small, apparently saturable binding component was observed, which was abolished by excess unlabelled VLDL. However, in contrast to membranes from laying hen, HDL inhibited \( ^{125}\text{I}-\text{VLDL} \) binding to R/O ovarian membranes to exactly the same extent as VLDL (Fig. 4B). Consequently, only linear components representing nonspecific binding were obtained with R/O membrane extracts, indicating a lack of receptor-mediated binding of \( ^{125}\text{I}-\text{VLDL} \) to R/O ovarian membranes. Fig. 4C shows a comparison of specific (i.e. receptor-mediated) \( ^{125}\text{I}-\text{VLDL} \) binding to laying hen versus R/O ovarian membrane extracts, demonstrating a total lack of high affinity, specific sites in R/O ovaries.

In order to test directly for the presence or absence of the previously characterized 95-kDa oocyte VLDL receptor in ovarian membranes from laying hens and R/O, we applied ligand and immunoblotting to membrane extracts. As shown in Fig. 5, left panel, \( ^{125}\text{I}-\text{VLDL} \) bound to the 95-kDa receptor in laying hen oocyte membranes as well as in laying hen ovarian membranes, but failed to bind to any protein of ovarian membrane extracts obtained from two different R/O hens. All three animals used in this comparative study were siblings, and were 35 weeks of age at killing, well beyond the age for onset of egg laying in normal hens; the normal hen had layed an egg every 1–2 days, while the two R/O siblings had not produced any eggs.

We also used polyclonal rabbit IgG directed against the bovine LDL receptor which we have previously shown to cross-react with the 95-kDa receptor in oocyte membrane extracts (1) in immunoblotting experiments. As shown in Fig. 5, right panel, the anti-receptor antibody bound to a 95-kDa protein in oocyte and ovarian membrane extracts from the laying hen, while no specific reaction was observed in her R/O siblings. A faint, low M, band was present in all ovarian laying hen VLDL (257 cpn/ng) and 1 mg/ml of unlabelled rooster HDL (○), 1 mg/ml of unlabelled laying hen VLDL (●), or no addition (○). The amount of bound radiolabelled ligand was determined by filtration as described under “Experimental Procedures.” In panel C, the receptor-bound \( ^{125}\text{I}-\text{VLDL} \) was calculated as the amount of binding in the presence of excess unlabelled VLDL (●) from that in the presence of excess unlabelled HDL (○), laying hen (LH); ◊, R/O ovarian membrane extract.

![Graph](image-url)
FIG. 5. Ligand and Western blotting of ovarian membranes. Oocyte membrane proteins (6 μg of protein/lane; lanes A and E) and ovarian membrane extracts (210 μg of protein/lane; lanes B–D and F–H) were subjected to electrophoresis and transfer to nitrocellulose as described in the legend to Fig. 3. Lanes A–D were incubated with 125I-labeled laying hen VLDL (5 μg/ml, 212 cpm/ng), and lanes E–H with 5 μg/ml of rabbit anti-receptor IgG, followed by 125I-labeled goat anti-rabbit IgG (1 μg/ml; 870 cpm/ng). Exposure to Kodak XAR-5 film was for 22 h at room temperature. M, standards are indicated.

FIG. 6. Plasma clearance of 125I-VLDL following intravenous injection. 125I-Labeled laying hen VLDL (1 mg of protein, 177 cpm/ng) was injected via the left wing vein and samples withdrawn and processed for scintillation counting at the indicated time points as described under “Experimental Procedures.” The data are from single, 37-week-old animals (■, laying hen; □, R/O). The 100% values were: laying hen (LH), 1.14 × 108 cpm/ml plasma; R/O, 0.89 × 108 cpm/ml plasma.

samples, but was absent in oocyte membrane extracts; this band reacted also with nonimmune rabbit IgG (not shown).

Plasma Clearance of 125I-VLDL Is Delayed in R/O—When 125I-labeled laying hen VLDL was intravenously administered to laying and R/O hens, there was a dramatic difference in disappearance of radioactivity from the plasma (Fig. 6). In the laying hen, 50% of the injected material was cleared in 5 h, and after 30 h, the remaining radioactivity in laying hen plasma was only 2%, whereas it was 67% in the R/O’s plasma. At each time point, greater than 97% of the plasma radioactivity was precipitable with 10% (w/v) trichloroacetic acid. At termination of the experiment, the laying hen ovary showed a 6-fold greater accumulation of radioactivity than the R/O ovary upon inspection of the rinsed organs by a scintillation monitor (Type 42B, Mini Instruments Ltd., Essex, United Kingdom). The radioactivity in large laying hen oocytes could not be determined because of size limitations set by our scintillation counter. In addition, since newly endocytosed 125I-VLDL is not evenly distributed throughout the yolk, determination of 125I radioactivity in yolk samples would be misleading. However, laying hen follicles of 6–10 mm diameter, prepared as described under “Experimental Procedures,” could be counted intact; they contained an average of 2.2-fold more radioactivity than comparable R/O follicles; e.g. two 8-mm diameter follicles had accumulated a total of 1.16 × 106 cpm in the laying hen, but only 0.52 × 106 cpm in the R/O. Perfused liver slices, in contrast, contained about 7 × 104 cpm/g wet weight in laying hen and 1.2 × 105 cpm/g wet weight in R/O.

DISCUSSION

The current studies establish that the previously characterized 95-kDa chicken oocyte receptor for low and very low density lipoproteins is absent from oocytes of the non-laying R/O hen. We believe that this failure to express VLDL receptors in the plasma membrane of the oocyte constitutes the biochemical defect in R/O hens that leads to retarded oocyte growth and severe hyperlipidemia, for the following reasons.

First, the failure to accumulate normal amounts of VLDL in R/O oocytes is not due to an inability of R/O VLDL to interact with functional VLDL receptors, as shown in Figs. 2 and 3. In both filtration binding assays and ligand blotting experiments, the recognition of laying hen VLDL and R/O VLDL by specific receptor sites on normal oocyte membranes were indistinguishable. Furthermore, there was no apparent difference in any structural aspects of the VLDL particles were indistinguishable. Furthermore, there was no apparent difference in any structural aspects of the VLDL particles from laying hen versus R/O plasma. The apolipoprotein pattern (Fig. 1), chemical composition, and morphological appearance in the electron microscope (not shown) were identical. Ligand blotting revealed that 125I-VLDL from both laying hen and R/O bound to the 95-kDa receptor in normal oocyte membranes, and that both unlabeled lipoproteins were equally effective in competing for binding of either labeled ligand (Fig. 3), confirming the binding data (Fig. 2). These findings exclude a lack of receptor recognition of R/O VLDL.

Second, ovarian membranes from laying hen, but not from R/O hens, bind 125I-labeled laying hen VLDL, as well as 125I-labeled R/O VLDL (not shown) in specific fashion (Fig. 4). The specific activity (125I-VLDL bound per mg of protein) of normal ovarian membranes prepared as described under “Experimental Procedures” was, as expected for a preparation contaminated with large amounts of extra-oocytic membranes, much lower than that of oocyte membranes (Bmax, 0.84 versus 65 μg/mg). There was also a HDL-inhibitable component of 125I-VLDL binding to ovarian membranes. Inasmuch as we have previously shown that rooster HDL, which does not contain apolipoprotein B, is unable to bind to the oocyte receptor (1), and the receptor recognizes exclusively apolipoprotein B (7), the HDL-inhibitable component cannot represent binding to the receptor, but most likely is elicted by other uncharacterized components present in ovarian membrane extracts. The HDL-resistant portion of 125I-VLDL binding to laying hen membranes was abolished by an excess of unlabeled VLDL, and thus represented receptor-mediated

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binding; this conclusion was further supported by the K, for this interaction being identical to that for 125I-VLDL binding to pure oocyte membranes (10 versus 15 μg/ml (Ref. 1)). In contrast, R/O membranes displayed no HDL-resistant component, and only nonspecific binding was observed. We conclude that laying hen oocyte and ovarian membranes express specific receptors for the binding of VLDL, whereas those of R/O are devoid of functional receptors.

Third, both ligand- and immunoblotting revealed the absence of the 95-kDa receptor from R/O ovarian tissue. Both blotting procedures, however, demonstrated that the receptor can be visualized not only in normal oocyte membranes as previously reported (1), but also in extracts of membranes prepared from normal ovaries from which large oocytes had been removed (Fig. 5). While the ligand blotting showed that the 95-kDa VLDL receptor was absent from the R/O sample, the absence of any specific immunoreactive band by Western blotting with polyclonal anti-receptor IgG might suggest that the 95-kDa receptor present in R/O membranes might have a different (i.e., truncated) amino acid sequence. These observations are consistent with the possibility that the 95-kDa VLDL receptor is absent from the R/O sample, an observation that has important implications for understanding the mechanism of R/O egg production (2).

Fourth, 125I-VLDL clearance from plasma following intravenous injection was dramatically retarded in R/O (Fig. 6). Concomitantly, the accumulation of radioactivity in R/O oocytes was significantly reduced in comparison to laying hen oocytes of identical size despite the much higher plasma 125I-VLDL levels in R/O throughout the experiment. Due to the limited number of R/O hens available to us, the turnover study could be performed only in a single mutant animal; plasma clearance studies in laying hens were performed in three different animals, and exactly the same result as shown in Fig. 6 was obtained. Thus, the differences between laying hen and R/O in plasma decay curves and oocyte accumulation are likely significant.

One obvious explanation, and the one favored by us in light of the biochemical findings reported here, is that these differences are due to the absence in R/O hens of oocyte receptors that mediate the endocytosis of VLDL. However, there may be other reasons for slower plasma clearance of 125I-VLDL in R/O. For example, the high endogenous levels of circulating VLDL in the hyperlipidemic animals may compete for binding of 125I-VLDL to receptors in tissues of high catabolic capacity, thereby delaying removal of the tracer from the plasma. However, in another hyperlipidemic animal model for receptor deficiency, namely the WHHL rabbit which is devoid of LDL receptors, in vivo studies have shown that retarded plasma clearance of radiolabeled lipoproteins is not due to the high circulating LDL levels, but can exclusively be accounted for by the receptor defect (18, 19). Another possibility is that the high endogenous levels of VLDL in R/O lead to a reduced number of receptors via metabolic regulation in extra-oocytic tissues, particularly the liver, in turn causing delayed plasma clearance of 125I-VLDL. However, hepatic 125I radioactivity following perfusion was not reduced in R/O versus laying hens. We have also searched directly (by ligand and Western blotting) for the presence of receptors for VLDL in the liver and other organs of normal laying hens, and failed to detect such sites. Presumably, in the laying hen, the 95-kDa receptor may serve to target most of the circulating VLDL to oocytes before catabolism in other tissues. Thus, the biochemical evidence for absence of specific oocyte receptors for VLDL, together with the current in vivo experiments, strongly suggests that (i) the 95-kDa receptor normally functions in uptake of VLDL into oocytes and (ii) its absence from oocytes elicits the R/O phenotype. Finally, these findings raise the question whether the chicken expresses apolipoprotein B-specific receptors in somatic cells, possibly different from the oocyte receptor. This question is of particular interest in the light of the normal phenotype of R/O carrier roosters (2). Studies in cultured normal and mutant fibroblasts to address this question are now in progress.

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