Estrogen Induces Hyperlipidemia in Fasted Chicks (43009)

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Abstract. **To determine whether the estrogen-induced hyperlipidemia is affected by fasting, male growing chicks were administered subcutaneously a single dose of 17β-estradiol (25 mg/kg body wt), and the hormone treatment lasted for 2 days with or wiithout feed (Experiment 1). In the second experiment, chicks were initially fasted for 1 or 3 days, and then treated with the same dosage of 17β-estradiol as in Experiment 1 for 2 days without feed. Plasma and liver lipids, and the activities of hepatic malic enzyme, glucose-6-phosphate dehydrogenase, and hormone-sensitive lipase in the adipose tissue were determined. Compared with fed control chicks, estrogen treatment in fed birds resulted in a marked elevation of plasma lipids, especially triglyceride during the 2-day period (137 vs 2263 mg/dl). In fasted chicks, the present finding that estrogen also induced a marked hyperlipidemia is noteworthy. Upon estrogen treatment (Experiment 1), the level of plasma triglyceride in fasted birds increased about 16 times over that of the fasted control group (133 vs 2093 mg/dl). Even in chicks fasted for 5 days (Experiment 2), estrogen treatment resulted in a persistent hypertriglyceridemia (75 vs 1369 mg/dl). In fed chicks, estrogen treatment also induced a fatty liver with massive accumulation of triglyceride, but the liver of estrogen-treated/fasted chicks appeared to be normal. In both fed and fasted chicks, malic enzyme was found to be the major NADPH producing enzyme in the liver. Upon fasting, both malic enzyme and glucose-6-phosphate dehydrogenase activities decreased significantly ($P < 0.05$). In fed chicks, the total activities of both enzymes increased with estrogen treatment, whereas the effect of hormone on these enzymes was less obvious in fasted chicks. The hormone-sensitive lipase activity in the adipose tissue was much lower in fed chicks compared with that of fasted birds (0.15 vs 0.33 nmol of oleic acid released/min/mg protein). Estrogen treatment in fed chicks had no effect on the hormone-sensitive lipase activity, but its activity was enhanced by the hormone treatment in fasted chicks. The present finding that hyperlipidemia persisted in estrogenized chicks during the fasting seems to indicate the complex nature of this hormonal influence on lipid metabolism.**

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The administration of estrogen in avian species causes a remarkable hyperlipidemia (1, 2). The estrogen-induced hyperlipidemia is characterized by a marked elevation in triglyceride and also, to a lesser extent, in phospholipid and cholesterol (1–3). The metabolic basis for hypertriglyceridemia has been attributed to the enhanced hepatic triglyceride synthesis and oversecretion of very low-density lipoprotein (VLDL) under the estrogenic influence (4–6).

Since nutritional status is an important regulatory factor for hepatic lipogenesis, this study examined the effect of fasting on the estrogen-induced hyperlipidemia. NADPH is an essential cofactor for lipogenesis, and the activities of NADPH-producing enzymes, such as malic enzyme (ME) and glucose-6-phosphate dehydrogenase (G6PDH), in the liver are dependent upon the nutritional and hormonal status of the animal (7, 8). However, there is a lack of information on whether the fasting in combination with estrogen treatment is related to the activities of these enzymes. Therefore, in this study we investigated the effect of estrogen on the activities of hepatic ME and G6PDH in fed and fasted chicks. Since the fasting could induce the fatty acid mobilization, the activity of hormone-sensitive lipase in the adipose tissue was also examined in relation to estrogen treatment and nutritional state. The changes in levels of plasma and liver lipids and other metabolites in the plasma were also monitored in both fed and fasted chicks with and without estrogen treatment.

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Materials and Methods

Animals and Treatment. Male crossbred chicks (New Hampshire male × Colombian female) were raised in wire cages under a constant lighting cycle (7:00–21:00 hr) with a regular grain diet as described previously (9). The grain diet consisted of 23% crude protein, 3% fat, 5% crude fiber, over 65% carbohydrate, and a mineral and vitamin premix. In the first experiment, chicks (5-week old) were randomly divided into four groups: fed control (DC), fed-estrogenized (DE), fasted control (TC), and fasted estrogenized (TE). A single dose of 17β-estradiol (25 mg/kg body wt) dissolved in propylene glycol (or the carrier alone for control groups) was injected subcutaneously to each animal, and the hormone treatment lasted for 2 days with or without feed. In the second experiment, chicks (12-week old) were initially fasted for 1 or 3 days and then injected with the same dosage of estrogen as in Experiment 1. After the estrogen treatment, the fasting was maintained for 2 additional days, then the birds were sacrificed. In the preliminary study, a single injection of estradiol (25 mg/kg body wt) resulted in a linear increase in plasma triglyceride for up to 2.5 days followed by a decrease thereafter (Fig. 1).

Plasma and Liver Lipid Analysis. After 2 days of estrogen treatment, blood samples were drawn from the wing vein into tubes containing EDTA (1.0 mg/ml). The plasma was separated by centrifugation at 1500g for 20 min at 4°C. After bleeding, the birds were sacrificed by cervical dislocation. The liver and adipose tissue were immediately taken and prepared for appropriate enzyme assays as described below. The levels of total plasma and liver cholesterol were determined enzymatically (10, 11) using A-Gent cholesterol reagent (Abbott Laboratories, Inc., Chicago, IL). The triglyceride values were measured according to the colorimetric method of Foster and Dunn (12), and the total phospholipid contents were determined according to the method of Eng and Noble (13). The free fatty acid values were measured according to the method of Brunk and Swanson (14). The concentrations of plasma glucose and albumin were measured using commercial diagnostic kits (Sigma Chemical Co., St. Louis, MO). The protein content was determined according to the method of Lowry et al. (15).

Assay of ME and G6PDH. A small portion (1 g) of liver tissue was homogenized with 9 ml of 0.05 M Tris-HCl buffer (pH 7.4) containing 0.15 M KCl in ice using a tissue homogenizer equipped with glass-Teflon pestle. The homogenate was centrifuged at 1500g for 15 min, and the resulting supernatant was recentrifuged at 20,000g for 30 min at 4°C. The supernatant containing cytosol fraction was assayed for the activities of NADPH-producing enzymes. Malic enzyme (16) and G6PDH (17, 18) were assayed spectrophotometrically by monitoring the formation of NADPH during the

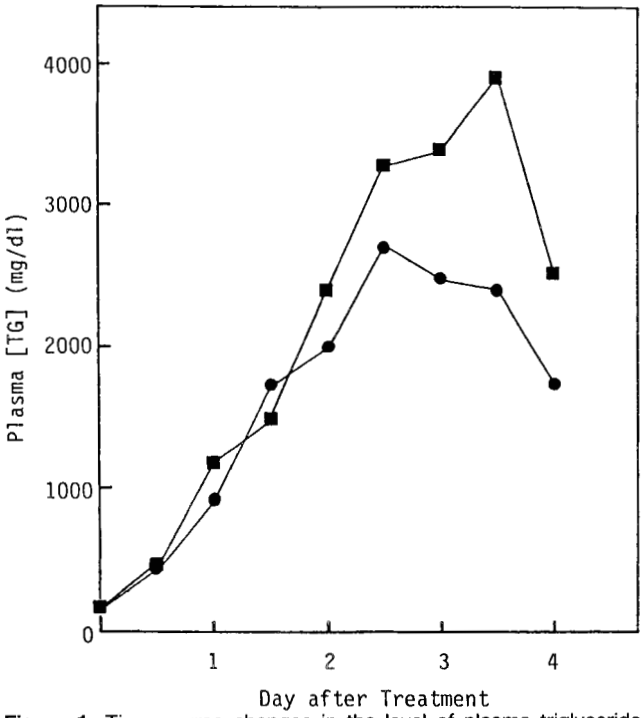

Figure 1. Time course changes in the level of plasma triglyceride after the subcutaneous injection of a single dose (25 mg/kg body wt) of 17β-estradiol (●——●) or 17β-estradiol dipropionate (■——■) into chicks.

conversion of malate to pyruvate and of G6P to 6-phosphogluconate, respectively.

Hormone-Sensitive Lipase (HSL) Assay. Adipose tissue surrounding the gizzard of chicks (Experiment 1) was removed and fat cells were prepared by digestion with collagenase (19, 20). The isolated adipocytes were homogenized with 9 volumes of 10 mM Tris-HCl buffer (pH 7.4) containing 0.25 M sucrose and 1 mM EDTA. The homogenate was centrifuged at 1500g for 15 min and the supernatant was used as a source of the enzyme. HSL was assayed according to the method of Khoo and Steinberg (21). A stock solution of 12 μmol of unlabeled substrate/ml containing about 50 μCi of [^{3}H]triolein was prepared and stored at 4°C in absolute ethanol. The incubation was carried out for 30 min at 30°C in a final volume of 0.8 ml containing 0.15 mM triolein ([^{3}H]triolein, 0.5 μCi), 5 mM phosphate buffer (pH 7.0), 20 mg of fatty acid-free bovine serum albumin, 1.25% ethanol, and 0.1 mg of protein. The reaction was stopped by the addition of 3 ml of chloroform-methanol-benzene (1:2.4:2) containing 0.3 μmol of oleic acid as carrier, followed by the addition of 0.1 ml of 1 N NaOH (22). The mixture was vortexed vigorously and centrifuged for 10 min at 1000g. An aliquot of 1 ml from the upper aqueous phase was then transferred to 10 ml of scintillation fluid (toluene base containing one third of Triton X-100 detergent). Radioactivity was counted in a Beckman LS 2800 liquid scintillation spectrophotometer and the quench was corrected by a preestablished external standardization.

Statistical Analysis. Data were analyzed by the

analysis of variance procedure, and the means between each group were compared with Student's t test by using SAS progrma (SAS Institute Inc., Cary, NC).

Results and Discussion

Data on weight gain, liver weight, and levels of plasma glucose and albumin in fed (DC and DE) and fasted (TC and TE) chicks with or without estrogen treatment are shown in Table I. Two days of estrogen treatment resulted in a significant increase in the weight gain in the fed group (53 vs 81 g), whereas fasting resulted in significant weight losses in both the TC (−73 g) and TE groups (−80 g). In the DE group, the liver weight nearly doubled with a massive fatty infiltration. The liver of the TE group weighed slightly more than that of the TC group, but it had no sign of fatty liver. In both fed and fasted birds, estrogen treatment decreased the plasma glucose content and the plasma albumin level was elevated only in the DE group.

Changes in the levels of plasma lipids in fed and fasted chicks with or without estrogen treatment are summarized in Table II. The levels of plasma triglyceride (TG), phospholipid (PL), and free fatty acid (FFA) were similar between the DC and TC groups, but plasma cholesterol (CH) content was elevated significantly ($P < 0.05$) by 2 days of fasting (DC, 116.6 vs TC, 161.0 mg/dl). The present finding is consistent with the results of others showing that plasma CH content increased in fasted rabbits (23, 24). Although the mechanism of the rise in CH was not investigated in the present study, it may have resulted from the rise in low-density lipoprotein due to the down-regulation of low-density lipoprotein receptor in the fasted state (24). During the 2-day period, estrogen treatment in fed chicks showed marked increases in all plasma lipids, especially the level of TG (DC, 137.4 vs DE, 2262.7 mg/dl). Plasma CH, PL, and FFA in the DE group increased to about three, six, and five times those of the DC group, respectively. However, the present finding that estrogen also induced hyperlipidemia in fasted chicks is noteworthy. Upon estrogen treatment, plasma TG of the TE group increased to about 16 times that of the TC group (132.6 vs 2093.2 mg/dl). Plasma levels of CH, PL, and FFA in the TE group were 280.9, 937.7, and 16.4 mg/dl compared with 161.0, 187.5, and 6.0 mg/dl in the TC group, respectively. Estrogen has been known to stimulate the hepatic lipogenesis and VLDL secretion (4, 6). However, the development of hyperlipidemia by estrogen in fasted chicks has never been reported. Normally, the fasting would result in a decrease in the hepatic lipid synthesis due to the lack of precursors and reduced enzyme activities. Therefore, it is quite unusual for estrogen treated birds to maintain such a high level of plasma lipid during fasting. It would be of interest to find the cause of hyperlipidemia in fasted chicks under the estrogenic influence.

Figure 2 shows the effects of estrogen treatment on plasma lipids at different days of fasting. In fasted chicks, fasting up to 3 days resulted in no significant changes in the levels of plasma TG, CH, and PL compared with those of the fed control group. However, fasting for 5 days resulted in decreases in the levels of plasma TG and PL and an increase in the level of CH. Although the extent of hyperlipidemia was not as great as that of the DE group, the hyperlipidemic effect of estrogen was consistent in fasted chicks as seen in Experiment 1. Even chicks fasted for 5 days had a marked elevation of TG as well as CH and PL with 2days of estrogen treatment. With prolonged fasting, the substrates for lipid synthesis in the liver would have been almost depleted. Therefore, if hepatic overproduction of TG and oversecretion of VLDL are the only mechanisms responsible for estrogen-induced hyperlipidemia, it is rather difficult to explain the persistent hyperlipidemia in estrogen-treated chicks fasted for 5 days.

Effects of estrogen on levels of liver lipids in fed and fasted chicks are shown in Table II. In contrast to the DC group, 2 days of fasting resulted in a significant decrease in liver TG (DC, 7.4 vs TC, 2.9 mg/g wet tissue). In fed chicks, estrogen treatment resulted in the development of a fatty liver due to a large accumulation of TG (DC, 7.4 vs DE, 91.9 mg/g wet tissue). Present results are comparable to those of others (25). The pronounced growth of liver as induced by estrogen appears to be associated with both hyperplasia and

Table I. Effects of Estrogen on Weight Gain, Liver Weight, and the Levels of Plasma Glucose and Albumin in Fed and Fasted Chicks

	Fed	Fed + estrogen	Fasted	Fasted + estrogen
Body weight				
Initial weight (g)	528.5 ± 17.0	520.0 ± 14.7	523.3 ± 8.4	510.0 ± 15.0
Final weight (g)	581.8 ± 17.7	601.3 ± 11.5	450.7 ± 8.0	430.2 ± 10.3
Gain (g/2 days)	53.3 ± 2.7	81.3 ± 4.6a	−72.7 ± 1.1b	−79.7 ± 7.8d
Liver weight (g)	14.5 ± 0.3	27.9 ± 1.1a	9.5 ± 0.3b	11.9 ± 0.6c, d
Plasma (mg/dl)				
Glucose	214.3 ± 17.7	192.9 ± 18.0	152.5 ± 7.5b	139.3 ± 11.1d
Albumin	1.4 ± 0.1	1.8 ± 0.1a	1.7 ± 0.1b	1.7 ± 0.1

Note. Values are mean ± SE ($n = 6$). Values with letters a (fed versus fed + estrogen), b (fed versus fasted), c (fasted versus fasted + estrogen), and d (fed + estrogen versus fasted + estrogen) are significantly different ($P < 0.05$).

Table II. Effects of Estrogen on Levels of Plasma and Liver Lipids in Fed and Fasted Chicks

	Fed	Fed + estrogen	Fasted	Fasted + estrogen
Plasma lipids (mg/dl)				
TG	137.4 ± 7.2	2662.7 ± 125.0a	132.6 ± 6.5	2093.2 ± 268.4c
CH	116.6 ± 8.1	351.3 ± 34.5a	161.0 ± 8.0b	280.9 ± 25.1c
PL	194.5 ± 8.8	1176.5 ± 68.3a	187.5 ± 7.1	937.7 ± 67.3c, d
FFA	5.4 ± 0.9	25.5 ± 1.7a	6.0 ± 0.9	16.4 ± 2.7c, d
Liver lipids (mg/g wet tissue)				
TG	7.4 ± 0.7	91.9 ± 10.4a	2.9 ± 0.2b	6.6 ± 1.2c, d
CH	2.5 ± 0.1	5.6 ± 0.8a	2.9 ± 0.1b	2.7 ± 0.2d
PL	22.3 ± 0.7	21.3 ± 0.6	25.0 ± 0.8b	26.7 ± 0.4d
FFA	6.1 ± 0.2	5.4 ± 0.1a	5.8 ± 0.2	6.4 ± 0.2d

Note. Values are mean ± SE (n = 6). Values with letters a (fed versus fed + estrogen), b (fed versus fasted), c (fasted versus fasted + estrogen), and d (fed + estrogen versus fasted + estrogen) are significantly different ($P < 0.05$).

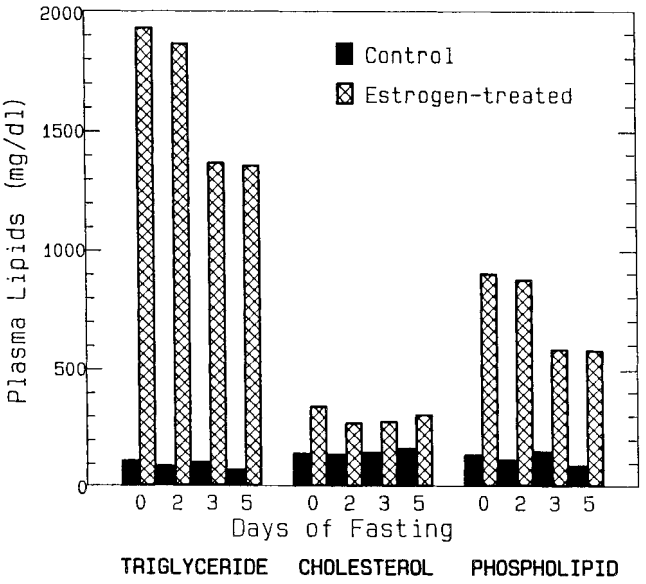

Figure 2. Changes in the levels of plasma triglyceride, cholesterol, and phospholipid in chicks at different days of fasting with and without estrogen treatment (Experiment 2).

cellular hypertropy with an excess accumulation of TG (24). Although VLDL secretion has shown to be greatly enhanced in estrogen-treated animals (6), the massive TG deposit in the liver seems to indicate that there is an imbalance between TG synthesis and its secretion in VLDL. Estrogen treatment in fasted chicks also increased the liver TG content about twice that of the TC control group (2.9 vs 6.6 mg/g wet tissue), but the TG content of the TE group was less than that of the fed control group. Estrogen treatment also increased the liver CH content in the fed chicks but it had no effect in the fasted group. Based on the present results, it appears that estrogen treatment of chicks results in hyperlipidemia as well as fatty liver in the fed state, whereas it results in only hyperlipidemia in the fasted state.

The changes in activities of hepatic ME and G6PDH in fed and fasted chicks with or without estrogen treatment (Experiment 1) are shown in Table III. In all experimental groups, ME was found to be the major NADPH-producing enzyme in the chick liver. Upon fasting, both ME and G6PDH activities decreased significantly ($P < 0.05$). In fed chicks, the specific activities of both enzymes remained unchanged by estrogen treatment, while total activities of these enzymes increased significantly. In fasted chicks, however, the effect of hormone on these enzymes was less apparent than in their fed counterparts.

Figure 3 shows the effects of estrogen administration at different fasting stages on the activities of hepatic NADPH-producing enzymes. The predominance of ME over G6PDH as the NADPH-producing enzyme was consistent with the findings of Experiment 1. In fasted groups, the activities of both enzymes decreased gradually as the fasting continued. Estrogen treatment resulted in increased activities of ME and G6PDH in both fed and fasted chicks. The enhancement of both ME and G6PDH activities appears to be consistent with the stimulation of hepatic acetyl-CoA carboxylase and fatty acid synthetase by estrogen (4, 5), which will increase the demand for reducing equivalents for *de novo* synthesis of fatty acids. In addition, the increased hepatic production of mRNA for apoVLDL-II, an apoprotein for VLDL (26), further demonstrates the concerted effect of estrogen for an enhanced secretion of TG-rich VLDL as well as hepatic lipogenesis.

However, the present finding that estrogen induces the hyperlipidemia in chicks fasted for 5 days suggests that factors other than the increased hepatic lipogenesis might be involved. It is possible that estrogen could exert through fatty acid mobilization from the adipose tissue by hormone-sensitive lipase (27). The elevation of plasma FFA level in fasted/estrogen-treated chicks to more than 2.5 times that of fasted control birds supports that possibility. In the present study, the HSL activity was much lower in fed chicks compared with that of fasted chicks (0.15 vs 0.33 nmol of oleic acid released/min/mg protein) (Table IV). Estrogen treatment in fed chicks had no apparent effect on the HSL activity, but estrogen treatment in fasted chicks appeared to enhance the HSL activity, although statistically not significant. By visual observation, the depletion of adipose tissue was more obvious in estrogen-treated chicks than in untreated birds.

Table III. Effects of Estrogen on Activities of Hepatic Malic Enzyme and Glucose-6-Phosphate Dehydrogenase in Fed and Fasted Chicks

	Malic enzyme		G6PDH	
	mU[a]	Total[b]	mU[a]	Total[b]
Fed[c]	57.0 ± 2.2	60.2 ± 2.3	9.0 ± 0.9	9.4 ± 0.8
Fed + Estrogen	61.7 ± 7.1	121.1 ± 12.5a	11.5 ± 1.4	22.7 ± 2.8a
Fasted	31.6 ± 2.6b	26.9 ± 1.7b	5.8 ± 0.5b	5.0 ± 0.5b
Fasted + estrogen	30.5 ± 1.6d	30.0 ± 2.4d	4.6 ± 0.4d	4.5 ± 0.3d

[a] U = μmol NADPH/min/mg protein.
[b] Total enzyme activity (μmol NADPH produced/min/liver).
[c] Values are mean ± SE (n = 6). Values with letters a (fed versus fed + estrogen), b (fed versus fasted), c (fasted versus fasted + estrogen), and d (fed + estrogen versus fasted + estrogen) are significantly different ($P < 0.05$).

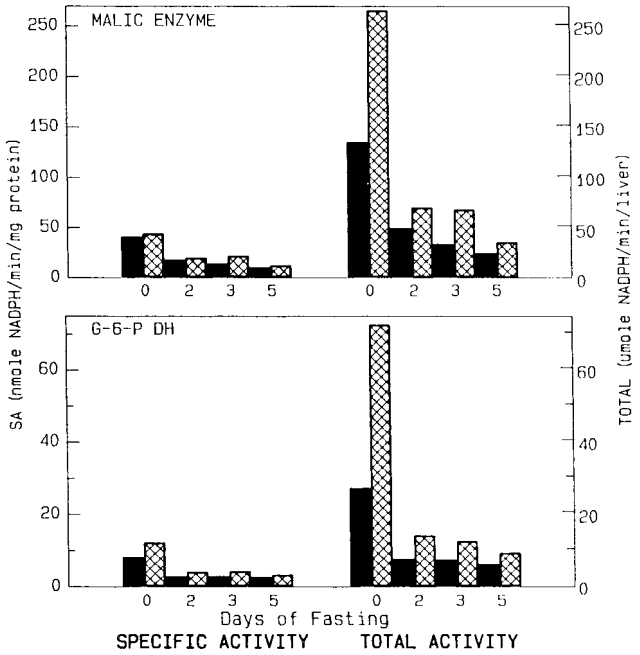

Figure 3. Changes in the activities of malic enzyme and glucose-6-phosphate dehydrogenase in the liver of chicks at different days of fasting with (▩) and without (■) estrogen treatment (Experiment 2).

Table IV. Effect of Estrogen on the Activity of Adipose Tissue Hormone-Sensitive Lipase in Fed and Fasted Chicks

	Hormone-sensitive lipase[a]
Fed	0.15 ± 0.04
Fed + estrogen	0.15 ± 0.04
Fasted	0.33 ± 0.06b
Fasted + estrogen	0.46 ± 0.06d

[a] Values are mean (nmol oleic acid/min/mg protein) ± SE (n = 6). Values with letters a (fed versus fed + estrogen), b (fed versus fasted), c (fasted versus fasted + estrogen), and d (fed + estrogen versus fasted + estrogen) are significantly different ($P < 0.05$).

An increased lipolytic action in the fat bodies and the elevation of the serum FFA level also have been reported in lizards following estrogen administration (28, 29). The FFA released into the circulation is transported to the liver and extrahepatic tissues. In the fasted state, FFA is utilized for energy. With estrogen treatment, however, FFA transported to the liver may be utilized for TG formation. If this is the case, VLDL secretion continues in the fasting state under estrogenic influence. Consequently, VLDL may accumulate due to a combination of continued secretion and a decrease in lipoprotein lipase activity during fasting (30).

In addition, it can be speculated that VLDL of estrogen-treated chicks may be catabolized differently from VLDL of untreated birds, thereby contributing to the development of hyperlipidemia in fasted chicks. The findings of kinetic studies in our laboratory that the fractional catabolic rate of VLDL from estrogen-treated chicks was significantly ($P < 0.05$) lower than that of VLDL from untreated birds indicates that possibility (unpublished results). Whether the estrogen causes the production of abnormal VLDL particles which are resistant to the action of lipoprotein lipase in the plasma remains to be investigated.

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