

EFFECT OF ESTROGEN ON TRIACYLGLYCEROL METABOLISM: INHIBITION OF POST-HEPARIN PLASMA LIPOPROTEIN LIPASE BY PHOSVITIN, AN ESTROGEN-INDUCED PROTEIN

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1. Introduction

Estrogen administration in human subjects and the changes which occur in female turkeys at the onset of egg production in response to estrogen are characterized by a marked increase in the plasma TG concentration [1–3]. Several investigations in human subjects [1,4,5] as well as in birds [5] have shown an increase in synthesis of TG by the liver in response to estrogens. The post-heparin plasma lipolytic activities in human subjects have been shown to decrease during estrogen treatment [4,5,7]. The purpose of this study was to determine if the PHLA of laying turkeys were decreased, when compared to non-laying birds, and what factors were involved in the regulation of these lipolytic activities.

The activities of LPL and TGL from laying turkey post-heparin plasma were decreased relative to the non-laying birds and administration of estrogen to male turkeys caused a similar decrease in PHLA. Furthermore, phosvitin, an estrogen induced protein caused a marked inhibition of LPL's from human and turkey post-heparin plasma, and LPL from chicken adipose tissue.

Abbreviations and definitions: PHLA, post-heparin plasma lipolytic activities; TG, triacylglycerol; LPL, lipoprotein lipase, a TG hydrolase which is inhibited by NaCl and protamine sulfate and requires serum for activity; TGL, a TG hydrolase which is not inhibited by NaCl or protamine sulfate and does not require serum for activity; DES, diethylstilbestrol; Pv, phosvitin, an estrogen induced phosphoprotein; Lv, lipovitellin, an estrogen-induced lipoprotein.

2. Materials and methods

2.1. Source of plasma

Plasma was obtained from the wing vein of large Broad Breasted White male and female turkeys of the Nicholas strain. Post-heparin plasma was obtained 30 min after injection of heparin (Upjohn Co., Kalamazoo, MI) at a concentration of 100 units/kg. The plasma was recovered by low speed centrifugation and stored at -15°C until further use. In experiments where male turkeys were treated with estrogen, 60 mg DES were dissolved in 1 ml of corn oil and injected subcutaneously. The dietary regimen consisted of a breeder ration which was formulated to provide 20% protein and a balance of vitamins and minerals. Turkey breeder hens were forced out of egg production by withholding water for one day and feed for three days.

2.2. Isolation and assay of lipases

LPL and TGL were separated from turkey and human post-heparin plasma by heparin-Sepharose 4B affinity chromatography as described by Boberg et al. [8] and Ganesan and Bass [9]. Chicken adipose tissue LPL was purified as described by Korn and Quigley [10]. Lipolytic activity was determined by measuring the release of ^{14}C -labeled fatty acids from a stabilized triolein emulsion as described by Ganesan et al. [11].

2.3. Activation and inhibition of lipases

Human or turkey serum (0.05 ml) were used as

source of activators of post-heparin plasma and adipose tissue LPL's. Sodium chloride and protamine sulfate were used as inhibitors of lipolytic activity in final concentrations of 1 M and 3 mg per ml of enzyme solution, respectively.

Phosvitin and Lipovitellin were purified from turkey egg yolk as described by Burley and Cook [12]. Pv or Lv (0–60 mg) were incubated with the lipases for 10 min at 27°C prior to incubation with the substrate for 60 min at 37°C. In experiments with anti-Pv serum (Calbiochem, Los Angeles, CA) the Pv, anti-Pv serum and lipase were preincubated with periodic shaking for 4 h at 4°C prior to incubation with the triolein substrate for 60 min at 37°C.

2.4. Analytical methods

Protein was determined by the method of Lowry et al. [13] and plasma TG concentrations were measured by the method of Kessler and Lederer [14].

3. Results

3.1. Purification of TG lipases

Two TG lipases were partially purified from turkey and human post-heparin plasma by affinity chromatography on columns of heparin-Sepharose 4B. A TG hydrolase which was not inhibited by protamine sulfate and which did not require serum for activity was eluted with 0.72 M NaCl. This lipase was

designated as TGL. Another TG hydrolase which was inhibited by protamine sulfate and NaCl which required serum for activity was eluted with 1.5 M NaCl. This lipase was designated LPL since the inhibition and activation characteristics were similar to LPL from human post-heparin plasma [11]. LPL was prepared from chicken adipose tissue by the method of Korn and Quigley [10]. This LPL was activated by human and turkey serum and inhibited by NaCl and protamine sulfate.

3.2. Effect of egg production and estrogen administration on partially purified lipases

Assay of the lipolytic activities of turkey post-heparin plasma and partially purified lipases is shown in table 1. PHLA of male turkeys, sexually immature female turkeys (non-laying) and laying turkeys which were forced out of egg production (un-laying) are very similar. At the onset of egg production, the total PHLA as well as the activities of LPL and TGL were decreased 2 to 3-fold and the plasma TG increased 24-fold. The LPL was decreased to a greater extent than the TGL (71% and 52% respectively). When the female turkeys were forced out of egg production, the PHLA and plasma TG levels returned to non-laying levels.

To explore the possibility that decreased PHLA in laying birds was due to the effect of estrogen, we injected male turkeys with a single dose of DES (table 1). Four days after estrogen injection, PHLA

Table 1
Assay of turkey post-heparin plasma TG lipases^a

	Age (weeks)	Number of birds	Plasma TG mg/100 ml	Total PHLA	TGL	LPL
Turkeys						
Non-laying females	19	15	81	52.8	15.6	37.2
Laying females	30	15	1974	18.5	7.6	10.9
Un-laying females (4 weeks out of production)	34	15	59	44.8	13.5	31.3
Males	30	10	60	58.5	12.8	45.7
Estrogen treated males (4 days after injection with DES)	30	10	1417	12.0	6.6	5.4

^aAll values are expressed as μ moles free fatty acids released/ml of post-heparin plasma/h and represent the mean of duplicate analyses of plasma pooled from the number of birds indicated.

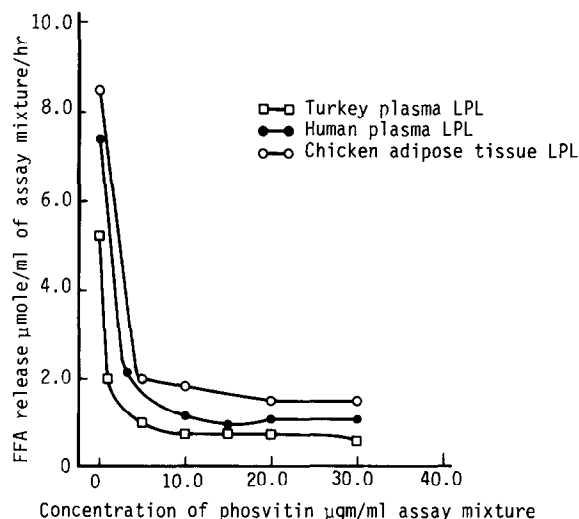


Fig.1. Effect of phosvitin on LPL's from turkey and human post-heparin plasma and chicken adipose tissue. LPL's and phosvitin were incubated for 10 min prior to the addition of the triolein substrate mixture. Turkey plasma LPL protein conc. 2.5 μ g/ml assay mixture. Human plasma LPL protein conc. 2.0 μ g/ml assay mixture. Chicken adipose tissue LPL protein conc. 4.0 mg/ml assay mixture.

were decreased. The decrease in the activities of LPL and TGL and the increase in plasma TG levels were quite similar to those in laying birds.

3.3. Effect of phosvitin and lipovitellin on partially purified post-heparin plasma LPL, TGL and adipose tissue LPL

Lv and Pv are two proteins, unique to laying birds or estrogenized male birds [15,16] which are

synthesized by the liver and after appearing in the systemic circulation are deposited in the egg. Lv and Pv were purified from egg yolk and their effect on partially purified lipases was measured. Lv at concentrations up to 30 μ g protein/ml of assay mixture had no effect on either TGL or LPL. In contrast, Pv, at a level of 3 μ g protein/ml assay mixture, had a strong inhibitory effect on partially purified LPL's from human and turkey post-heparin plasma (fig.1). Incubation of Pv with anti-Pv serum caused this inhibitory effect of Pv to be reversed (table 2). Chicken adipose tissue LPL was strongly inhibited by Pv (fig.1) but the protamine insensitive TGL was not affected by Pv (table 2).

4. Discussion

Estrogens have a dramatic effect on TG metabolism in the avian species. At the onset of egg production, the plasma TG levels increase several fold. Elevation of plasma TG can be a result of either an increased rate of production of TG-rich lipoproteins by the liver or decreased removal of TG from the plasma or a combination of both of these mechanisms. The major mechanism for the removal of TG from plasma is believed to be by the catabolic action of LPL present in adipose tissue, heart, skeletal muscle and other tissues. Some or all of these enzymes can be released into the circulation by the intravenous injection of heparin.

Our results show that turkey post-heparin plasma contains two TG lipases (1) a serum stimulated and protamine sulfate inhibited LPL and (2) a protamine

Table 2
Effect of phosvitin on post-heparin TG lipases from human plasma, turkey plasma and chicken adipose tissue^a

Additives	Human post-heparin plasma LPL (4 μ g protein)	Turkey post-heparin plasma LPL (4 μ g protein)	Turkey post-heparin plasma TGL (0.2 mg protein)	Chicken adipose tissue LPL (4 mg protein)
0.05 ml human serum (control)	7.4	5.2	4.8	8.3
30 μ g Pv + 0.05 ml human serum	1.6	1.2	5.1	0.3
30 μ g Pv + Antiserum to Pv to precipitate an excess of 30 μ g + 0.05 ml human plasma	5.9	4.4	4.3	4.8

^aLipolytic activities are expressed as μ moles free fatty acids released/ml of assay mixture/h.

insensitive TGL which does not require serum for activity. Both of these lipolytic activities are decreased in the post-heparin plasma of laying birds or estrogen-treated male birds. Furthermore, Pv, an estrogen-induced protein, was shown to be a potent inhibitor of post-heparin plasma LPL and adipose tissue LPL in an in vitro assay system. These results indicate that phosvitin, by indirect action of estrogens, may play a role in the regulation of plasma and adipose tissue TG levels in laying birds.

Since human LPL was inhibited as strongly as turkey LPL by Pv, it would be of considerable interest to examine the possibility of an estrogen-induced protein analogous to Pv in the plasma of women who are being treated with estrogens.

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