

Effect of Estrogen on Post-Heparin Lipolytic Activity

SELECTIVE DECLINE IN HEPATIC TRIGLYCERIDE LIPASE

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ABSTRACT The rise in plasma triglyceride (TG) levels associated with estrogen administration has been thought to arise from impaired clearance because of the uniform suppression of post-heparin lipolytic activity (PHLA). Recently PHLA has been shown to consist of two activities: hepatic TG lipase and extrahepatic lipoprotein lipase (LPL). To determine whether estrogen might induce a selective decline in one of these activities, both hepatic TG lipase and extrahepatic LPL were measured in post-heparin plasma from 13 normal women before and after 2 wk of treatment with ethinyl estradiol (1 μ g/kg per day). Hepatic TG lipase and extrahepatic LPL were determined by two techniques: (a) separation by heparin-Sepharose column chromatography, and (b) selective inhibition with specific antibodies to post-heparin hepatic TG lipase and milk LPL. Estrogen uniformly depressed hepatic TG lipase as measured by affinity column ($-68 \pm 12\%$, mean \pm SD, $P < 0.001$) or antibody inhibition ($-63 \pm 11\%$, $P < 0.001$). Extrahepatic LPL was not significantly changed by affinity column ($-22 \pm 40\%$) or antibody inhibition ($-3 \pm 42\%$). Direct measurement of adipose tissue LPL from buttock fat biopsies also showed no systematic change in the activated form of LPL measured as heparin-elutable LPL ($+64 \pm 164\%$) or in the tissue form of LPL measured in extracts of acetone-ether powders ($+21 \pm 77\%$). The change in hepatic TG lipase correlated with the change in PHLA ($r = 0.969$, $P < 0.01$). However, neither the change in PHLA nor hepatic TG lipase correlated with the in-

crease in TG during estrogen. The decrease in PHLA during estrogen thus results from a selective decline in hepatic TG lipase.

INTRODUCTION

Estrogens and estrogen-containing oral contraceptives elevate triglyceride (TG)¹ levels in normal women (1) and may also aggravate pre-existing hypertriglyceridemia, sometimes producing marked hyperlipidemia (2) and, occasionally, a complication of hypertriglyceridemia such as pancreatitis (3-5).

The mechanism of this effect may relate to impaired TG removal, increased TG synthesis, or both acting synergistically. Studying TG turnover in humans, Kekki and Nikkilä (6), Glueck et al. (7), Kissebah et al. (8), and Hazzard et al. (9) have independently suggested that the hypertriglyceridemia associated with estrogen therapy is due to increased TG production. However, studies in rats by Hamosh and Hamosh (10) and Kim and Kalkhoff (11) have demonstrated decreased adipose tissue lipoprotein lipase (LPL) activity with estrogen, inferring an impairment in LPL-related TG removal. Studies in humans have consistently demonstrated a decrease in plasma post-heparin lipolytic activity (PHLA) during estrogen (9) and oral contraceptive (12) therapy, which would be consistent with the findings in the rat studies. The observation that LPL, thought to mediate removal of circulating lipoprotein TG, is released into plasma by heparin led to the use of PHLA as an index of tissue LPL activity (13). However, despite the depressed PHLA during estrogen treatment, oral (14) and intravenous (15) fat tolerance remain normal.

¹Abbreviations used in this paper: LPL, lipoprotein lipase; PHLA, post-heparin lipolytic activity; TG, triglyceride; VLDL, very low density lipoprotein.

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These discrepancies have led to further investigation to explain the decrease in PHLA associated with estrogens. Since it has recently been shown that heparin releases into plasma not only extrahepatic LPL but also hepatic TG lipase, both of which are measured in the PHLA assay (16), one possibility might be that there is a differential suppression of one of the triglyceride lipases within total PHLA. It has been shown that hepatic TG lipase and extrahepatic LPL are distinguishable by the differential requirement of extrahepatic LPL for the C-II apolipoprotein co-factor (17), its inhibition by sodium chloride and protamine sulfate (16), their differing salt elution patterns from heparin-Sepharose affinity columns (18), and their selective inhibition by specific antibodies (19, 20). Changes in PHLA can thus be evaluated more specifically by the separate measurement of hepatic TG lipase and extrahepatic LPL in post-heparin plasma. Furthermore, LPL can be measured directly in adipose tissue biopsies as the activated form of LPL released from adipose tissue by heparin and the tissue form of LPL in extracts of acetone ether powders (21).

Therefore, to determine whether the decrease in PHLA associated with estrogen administration might be due to a selective decline in either hepatic TG lipase or extrahepatic LPL or both, these lipase activities were measured by heparin-Sepharose chromatography and a specific antibody inhibition technique in normal women before and during estrogen therapy. Adipose tissue LPL was measured directly both as the ammonium buffer extracted activity from acetone ether powders and as the heparin-elutable activity. These studies demonstrate that the decline in PHLA during estrogen therapy is wholly attributable to a selective suppression in hepatic TG lipase.

METHODS

13 healthy volunteer women were studied as outpatients at the Veterans Administration Hospital Metabolic Ward before and after 14 days of ethinyl estradiol, 1 µg/kg per day (mean dose 68±18 µg/day, mean±SD, compared to 50 µg/day in routine oral contraceptives). Their mean age was 28 yr (range 22–34 yr) and relative body weight 120% (range 81–195%) from Metropolitan Life Insurance Company tables (Table I). Identical studies were performed on day 5 of their menstrual cycles (i.e., the day menses ended) and on day 19 (after 14 days of estrogen), each subject thus serving as her own control. The subjects were on ad libitum diets during the study. 12 of the 13 subjects experienced a slight weight gain (mean change of 1.2±1 kg) during estrogen treatment, similar to previous short-term studies with oral contraceptives (12). No subject had taken estrogens or been pregnant for at least 1 yr before participation in this study.

Antecutibal venous blood was drawn for TG analysis (22) of whole plasma and of ultracentrifugally isolated $d < 1.006$, very low density lipoproteins (VLDL) (23) after an overnight, 12-h fast. An adipose tissue biopsy was

then aspirated from the buttock for LPL determination and adipose cell measurement (see below). Intravenous heparin (The Upjohn Co., Kalamazoo, Mich.) (380 U/M₂) was administered and blood drawn after 10 min for determination of total PHLA and separation of post-heparin lipolytic activities. Since it has been suggested (20) that the release of the post-heparin lipolytic activities is a function of the heparin dose, three subjects were also given second heparin injections within two days at 12-fold the initial dose (4,560 U/M₂) both before and during estrogen and blood drawn after 60 min for similar analyses.

Materials. Bovine serum albumin (fatty acid free) was purchased from Miles Laboratories Inc., Elkhart, Ind.; triolein and heparin (for the affinity chromatography) from Sigma Chemical Co., St. Louis, Mo.; glyceryl tri[1-¹⁴C]oleate from Amersham/Searle Corp., Arlington Heights, Ill.; Sepharose 4B from Pharmacia Fine Chemicals Inc., Piscataway, N. J., and gum arabic from Fisher Scientific Co., Pittsburgh, Pa. Purified egg lecithin was a gift of William C. Vogel.

Analytical methods

All manipulations were performed at 4°C unless otherwise indicated.

Plasma PHLA. Blood samples were collected in chilled tubes containing EDTA (1 mg/ml plasma), centrifuged at 4°C, and plasma stored at -18°C until analyzed. The variability of hepatic TG lipase and extrahepatic LPL was not related to the length of storage of samples. Total PHLA was measured in two ways. Post-heparin plasma was incubated directly with the substrate-serum mixture (for comparison with the activity eluted from heparin-Sepharose) and after preincubation for 2 h at 4°C with an equal volume of normal rabbit serum (for comparison with the antibody inhibition measurements). Each assay contained 1 µmol triolein (0.05 µCi glyceryl tri [1-¹⁴C]oleate), 3 mg gum arabic, 2 mg bovine albumin (fatty acid free), 40 nmol Tris HCl (pH 8.2), 20 µl normal human fasting serum, and appropriate amounts of sample in a total volume of 200 µl. The substrate-serum mixture was incubated 30 min at 28°C before the addition of sample. After 30 min of sample incubation fatty acids were extracted by the method of Belfrage and Vaughan (24). The two measurements of total PHLA were highly intercorrelated (Spearman's rank correlation coefficient, $r_s = 0.816$, $n = 26$, $P < 0.001$).

Antibody inhibition studies. Selective measurement of post-heparin extrahepatic LPL was determined by the method of Huttunen et al. (20).² Post-heparin plasma (10 µl) and hepatic TG lipase antibody (diluted 1:2 with normal rabbit serum) were incubated for 2 h at 4°C and the remaining extrahepatic LPL activity then measured in the usual PHLA assay system. Similarly, selective measurement of post-heparin hepatic TG lipase was determined in the PHLA assay after incubation with 10 µl milk LPL antibody (19). All post-heparin plasma activities from each subject before and during estrogen were assayed with the same preparation of substrate. Incubation of post-heparin plasma with both hepatic TG lipase antibody and milk LPL antibody inhibited 95–100% of the total lipolytic activity.

Adipose tissue LPL determination. Subcutaneous buttock adipose tissue biopsy was performed by the method of Hirsch and Goldrick (25) and fat cell diameter was measured from a fixed frozen section of adipose tissue by

² Using specific antisera kindly given by Doctors Christian Ehnholm, Jussi Huttunen, and Esko Nikkilä.

TABLE I
Changes in Triglyceride Lipolytic Activities with Estrogen

Subject number	Age	Ideal body weight	Post-heparin plasma lipolytic activities*																		Adipose tissue lipoprotein lipase				
			Triglyceride				Total†		Hepatic triglyceride lipase by affinity column†		Extrahepatic lipoprotein lipase by affinity column†		Total‡		Hepatic triglyceride lipase by anti-body inhibition§		Extrahepatic lipoprotein lipase by anti-body inhibition§		Heparin-releasable		Extracted acetone ether powder				
			Before	During	Before	During	Before	During	Before	During	Before	During	Before	During	Before	During	Before	During	Before	During	Before	During			
yr	%	mg/100 ml		nmol FFA/ml/min																		nmol FFA/10 ⁶ cells/min			
1	30	105	72	140	90	25	33	7	11	4	269	136	221	81	67	62	2.4	1.6	0.3	0.4					
2	22	114	39	76	102	58	48	18	22	15	148	117	169	81	50	96	1.4	2.0	0.8	0.9					
3	23	94	38	119	188 (410)	87 (242)	83 (295)	37 (99)	21 (113)	31 (115)	326 (314)	114 (216)	280 (291)	121 (143)	135 (175)	62 (186)	7.7	0.9	1.1	0.4					
4	34	133	50	111	188	79	84	37	23	10	437	209	364	197	106	62	1.4	5.3	1.1	1.1					
5	29	120	70	95	229	133	114	44	49	28	365	166	304	128	90	96	2.9	5.6	0.8	1.2					
6	34	195	95	176	259	118	153	38	47	34	501	267	369	112	196	141	5.2	1.7	1.4	1.3					
7	24	125	84	87	97	46	37	9	14	13	147	55	100	44	36	35	5.7	1.5	1.8	1.8					
8	31	140	104	151	88	28	32	4	18	4	92	72	36	15	80	37	4.9	3.6	2.5	3.0					
9	30	136	103	131	252 (309)	77 (160)	269 (328)	50 (68)	67 (169)	44 (104)	388 (508)	82 (309)	335 (363)	76 (159)	51 (305)	50 (113)	7.5	8.4	3.4	1.9					
10	24	101	46	73	116	65	70	19	27	35	118	85	87	17	56	78	2.7	15.1	1.6	4.0					
11	27	84	55	121	208	98	67	35	34	41	161	98	121	41	64	87	—	—	—	—					
12	28	129	73	57	124	51	—	—	—	—	126	79	107	49	31	35	3.9	9.1	0.9	2.6					
13	28	81	74	150	130	21	—	—	—	—	138	40	105	24	29	20	0.4	0.5	0.2	0.1					
Mean	28	120	69	113	159	68	90	27	30	24	247	117	300	76	76	66	3.8	4.6	1.3	1.6					
SD			24	35	64	35	70	16	17	15	140	64	118	53	47	33	2.4	4.4	0.9	1.2					
P			<0.001		<0.001		<0.005		NS		<0.001		<0.001		NS		NS			NS	NS				

* Values in parentheses were obtained from plasma collected 60 min after 12 times the standard heparin dose.

† Assayed without sample preincubation with normal rabbit serum.

§ Assayed with sample preincubation with normal rabbit serum.

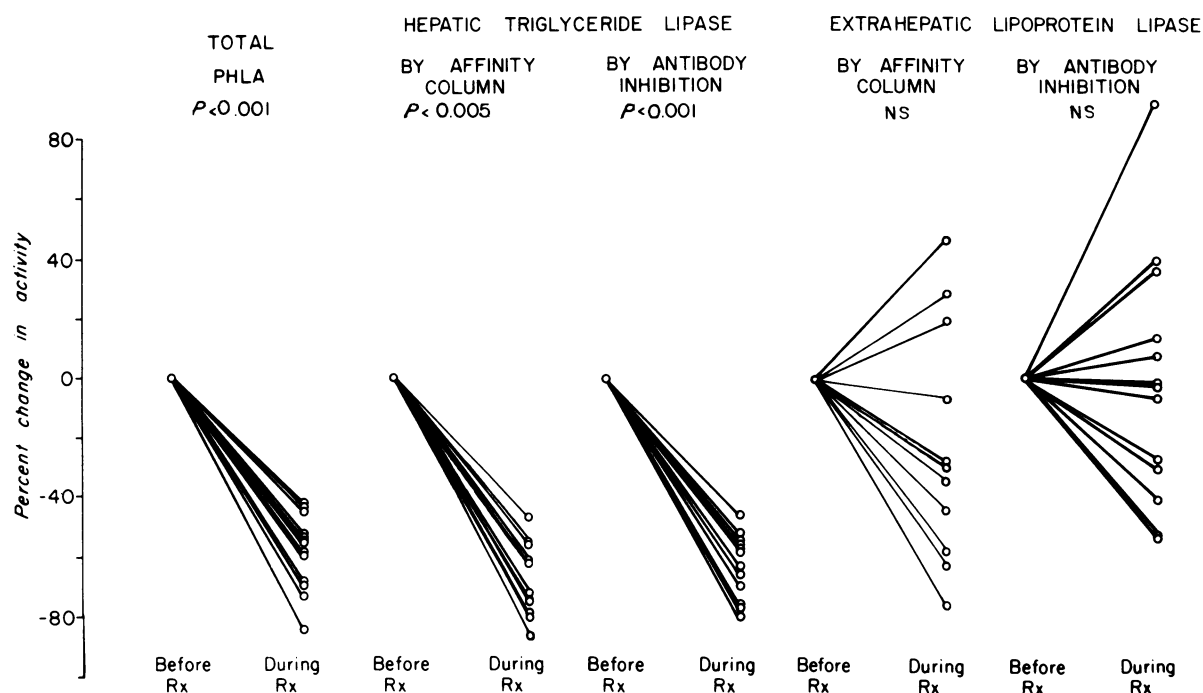


FIGURE 1 Percent change in plasma post-heparin lipolytic activities during estrogen: total PHLA, hepatic TG lipase (by affinity column and by antibody inhibition), and extrahepatic LPL (by affinity column and by antibody inhibition). The total PHLA shown was measured without incubation with normal rabbit serum. These values were closely correlated to those obtained for total PHLA measured after incubation with normal rabbit serum ($r_s = 0.816$).

the method of Sjöström et al. (26). Fat cell volume was calculated from the mean fat cell diameter according to Goldrick (27) and fat cell weight was obtained by multiplying the cell volume by the specific gravity of triglyceride. (26). Measurement of LPL activity as the heparin-elutable LPL and acetone ether-extracted LPL was performed as previously described (28).

Heparin-Sepharose chromatography. Heparin was covalently bound to Sepharose 4B by the method of Iverius (29). Each post-heparin plasma sample (4 ml) was applied to heparin-Sepharose columns (diameter = 1 cm, height = 4 cm) previously equilibrated with 5 mM sodium barbital buffer (pH 7.4) containing 0.15 M NaCl. The heparin-Sepharose columns were washed with 15 ml of the equilibration buffer and developed in 2–3 h with a linear gradient (80 ml) from 0.15 to 1.7 M NaCl in 5 mM sodium barbital, pH 7.4. The gradient was monitored by measuring reciprocal ohms, MHOs, with a Radiometer conductivity meter type CDM₂ (Radiometer A/S, Copenhagen, Denmark). The post-heparin plasma samples collected from each subject before and during estrogen treatment were run simultaneously and immediately assayed with the same preparation of substrate. Enzyme sample size was 20 μ l so that the salt concentration in the 200- μ l assay would not exceed 0.2 M NaCl. Post-heparin plasma was separated into two well-defined activity peaks by heparin-Sepharose column chromatography. Each peak eluted at the same conductivity before and during estrogen therapy: Peak I, 22.1 ± 1.0 and 21.7 ± 0.9 mMH, respectively; Peak II, 33.4 ± 1.3 and 32.8 ± 1.3 mMH, respectively. Estimated recovery by summation of eluted lipolytic activity was $68 \pm 24\%$ before and $65 \pm 24\%$ during estrogen ($n = 11$).

Statistical methods. All results have been expressed as mean \pm SD. Statistical evaluation was performed both by parametric regression analysis (r), nonparametric Spearman's rank correlation coefficient (r_s) and the Wilcoxon ranked-sign test for paired samples (30).

RESULTS

TG levels and PHLA (Table I). During estrogen administration fasting plasma TG levels increased in 12 of the 13 subjects; the mean increase for the group was $76 \pm 12\%$ ($P < 0.001$). Before estrogen treatment total PHLA varied from 88 to 259 nmol FFA/ml per min (mean 159 ± 64 nmol FFA/ml per min) without and from 92 to 501 nmol FFA/ml per min (mean 247 ± 140 nmol FFA/ml per min) with incubation with normal rabbit serum. During estrogen treatment total PHLA decreased in all subjects regardless of assay method ($-58 \pm 12\%$, $P < 0.001$; $-48 \pm 18\%$, $P < 0.001$, respectively) (Fig. 1). There was no significant correlation between the increase in TG and the decrease in PHLA.

Hepatic TG lipase (Table I, Fig. 1). Before estrogen treatment hepatic TG lipase represented $73 \pm 6\%$ of the activity eluted from heparin-Sepharose, Peak I (Fig. 2), and $70 \pm 14\%$ of total PHLA when

measured during antibody inhibition of extrahepatic LPL. During estrogen treatment hepatic TG lipase decreased in all subjects both measured by heparin-Sepharose column chromatography ($-68 \pm 12\%$, $P < 0.001$, $n = 11$) and antibody inhibition ($-63 \pm 11\%$, $P < 0.001$, $n = 13$). The decrease in PHLA during estrogen therapy was strongly correlated with the decline in hepatic TG lipase measured either by heparin-Sepharose column chromatography ($r = 0.834$, $P < 0.01$, $n = 11$) or antibody inhibition ($r = 0.939$, $P < 0.01$, $n = 13$) (Fig. 3). The decrease in hepatic TG lipase measured by the affinity column correlated with the decrease in hepatic TG lipase determined by antibody inhibition ($r_s = 0.745$, $P < 0.01$, $n = 11$).

Extrahepatic LPL (Table I, Fig. 1). Before estrogen, extrahepatic LPL represented $27 \pm 6\%$ of the activity eluted from heparin-Sepharose, Peak II (Fig. 2), and $30 \pm 14\%$ of the total PHLA when measured during antibody inhibition of hepatic TG lipase. Extrahepatic LPL responded variably to estrogen when measured either by heparin-Sepharose column chromatography ($+29$ to -59%) or by antibody inhibition ($+92$ to -54%) with no significant net change ($-22 \pm 40\%$, $n = 11$, NS; $-3 \pm 43\%$, $n = 13$, NS, respectively). The decrease in PHLA was not correlated with the changes in extrahepatic LPL measured by either technique (affinity column: $r = 0.429$, $n = 11$; antibody inhibition: $r = 0.455$, $n = 13$) (Fig. 3). The change in extrahepatic LPL measured by the affinity column was not significantly correlated with the change measured by antibody inhibition.

Adipose tissue LPL. The change in adipose tissue LPL during estrogen administration was studied in 12 subjects. Before estrogen adipose tissue, heparin-elutable LPL was 3.8 ± 2.4 nmol FFA/ 10^6 cells per min and the LPL extracted from acetone-ether

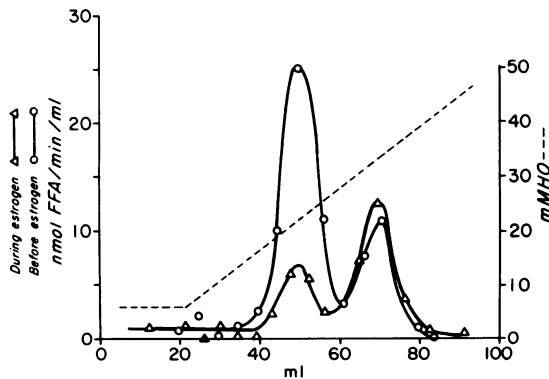


FIGURE 2 Elution patterns of triglyceride lipolytic activity after heparin-Sepharose chromatography of post-heparin plasma from subject 10 before (O—O) and during (Δ — Δ) estrogen. The NaCl gradients were measured by conductivity in mMHO (---).

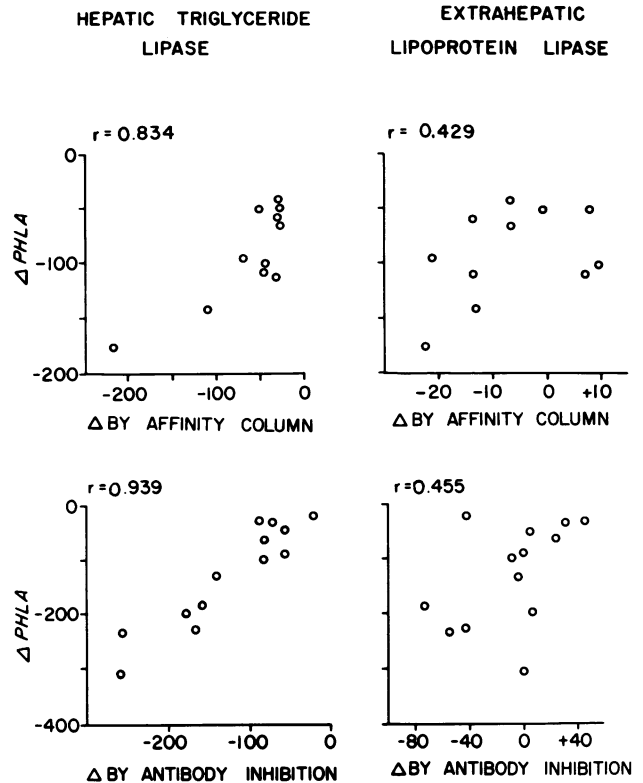


FIGURE 3 Linear regressions of the decrease in PHLA vs. changes in post-heparin hepatic TG lipase (by affinity column and by antibody inhibition) and extrahepatic LPL (by affinity column and by antibody inhibition).

powders was 1.3 ± 0.9 nmol FFA/ 10^6 cells per min. Treatment with estrogen was associated with a variable response in adipose tissue LPL levels (Fig. 4). The changes in adipose tissue heparin-elutable LPL (-0.8 ± 5.0 nmol FFA/ 10^6 cells per min) and the ammonium buffer extracted activity from acetone-ether powders (-0.2 ± 1.0 nmol FFA/ 10^6 cells per min) were not significant. The changes in adipose tissue heparin-elutable LPL were significantly correlated with the changes in post-heparin extrahepatic LPL by antibody inhibition ($r_s = 0.622$, $P < 0.05$, $n = 12$) but not by affinity column.

TG level and TG lipases. A significant correlation was found between the changes in extrahepatic LPL as measured by antibody inhibition and the increase in plasma TG ($r_s = -0.600$, $P < 0.05$, $n = 13$) or VLDL-TG ($r_s = -0.626$, $P < 0.05$, $n = 13$). The changes in adipose tissue heparin elutable LPL were weakly correlated with the increase in plasma TG ($r_s = -0.530$, $0.05 < P < 0.1$, $n = 12$). The increase in plasma TG or VLDL-TG was not significantly correlated with the decrease in post-heparin hepatic TG lipase or extrahepatic LPL by affinity column.

Effect of heparin dosage (Table I). The effect

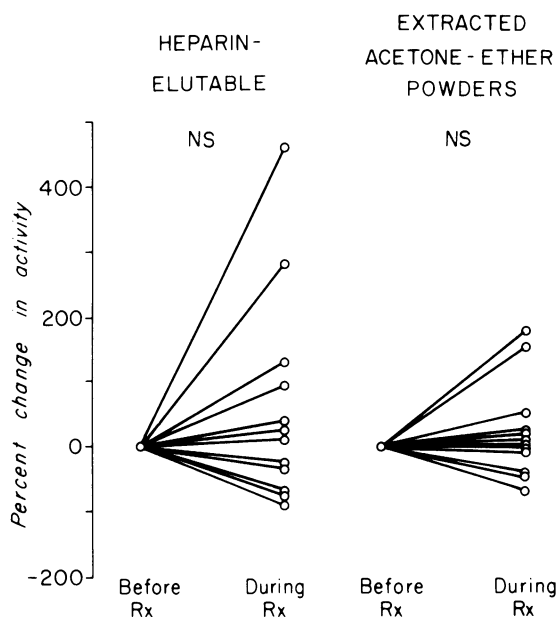


FIGURE 4 Percent change in adipose tissue LPL during estrogen: heparin-elutable LPL and the LPL extracted from acetone-ether powders.

of estrogen upon hepatic TG lipase, and extrahepatic LPL was measured in three subjects (nos. 3, 9, and 11, Table 1) both 10 min after the standard dose of heparin and 60 min after 12 times that dose (14). In all three subjects the decrease in PHLA observed during estrogen was evident at both low and high doses as was the decline in hepatic TG lipase and the lack of a consistent change in extrahepatic LPL.

DISCUSSION

The elevation in TG induced by oral contraceptives and estrogens has presented a paradox. Whereas PHLA, an indirect but traditional measure of TG removal capacity, was dramatically and consistently depressed, oral (14) and intravenous (15) fat tolerance remain unchanged. Moreover, independent studies of TG turnover with three different techniques: [^3H]-glycerol (6, 7) and [^{14}C]palmitate incorporation into circulating TG (8), and lipolysis of endogenous TG during heparin infusion (9), do not reveal impaired TG removal during estrogen (7, 9) or oral contraceptive (6, 8) therapy. The present studies examine the possibility of a selective suppression in one of the TG lipases within total PHLA and resolve this paradox.

The decline in PHLA observed in this group of women during estrogen therapy was strongly correlated with a selective decrease in hepatic TG lipase. When measured as Peak I by heparin-Sepharose affinity chromatography, the decrease in PHLA vs.

hepatic TG lipase in each subject accounted for 70% of the variance about the line derived from least-squares analysis for the group as a whole (i.e., r^2). After selective antibody inhibition of extrahepatic LPL, this relationship accounted for 88% of the variance. Studies by Glueck et al. (31) that indicate an estrogen-associated depression in protamine-resistant hepatic TG lipase (32) in women with familial hypertriglyceridemia complement the present report with normal premenopausal women, and infer a similar effect of estrogens over a broad range of TG levels.

Estrogens appear to have no consistent effect upon post-heparin plasma extrahepatic LPL among different subjects and no net effect in the group as a whole. The response of different subjects varied widely regarding extrahepatic LPL both by affinity chromatography and antibody inhibition. Nevertheless, it remains conceivable that small changes in an extrahepatic LPL might occur during estrogen therapy. That this variable response might have physiological importance is suggested by the significant inverse relationship between extrahepatic LPL changes and changes in plasma TG.

Adipose tissue LPL is not depressed by estrogen in any consistent manner. This was the case for both total adipose tissue LPL (measured in acetone-ether powders of the buttock biopsies) and that eluted during incubation with heparin, thought to represent an activated, releasable form of the enzyme (21). There was also considerable variation in these responses among different individuals. This could either reflect the variance of the assays per se (including the biopsies and tissue handling) or a true, significant difference among subjects as suggested by the weak correlation between changes in adipose tissue heparin-elutable LPL and increases in plasma TG. The variable and, as a group, absent response of adipose tissue LPL to estrogen therapy contrasts markedly with the uniform depression in this enzyme activity observed in rats by Hamosh and Hamosh (10), Kim and Kalkhoff (11), and Wilson et al. (33). This contrasting response may be due to differences between species as suggested by low adipose tissue LPL in uremic (34) and hypothyroid (35) man as compared to normal levels in uremic (36) or hypothyroid (37, 38) rats. It is clear from the present studies, however, that premenopausal women do not respond to the usual doses of estrogens employed in oral contraceptive or postmenopausal treatment with a major and consistent decrease in adipose tissue LPL.

Despite the marked decline in plasma hepatic TG lipase during estrogen demonstrated in the present studies, it is important to emphasize that estrogen might induce a change in the hepatic binding of TG lipase vis-à-vis heparin rather than a true

decrease in tissue enzyme. The same qualification is required in interpreting the selective increase in post-heparin hepatic TG lipase during treatment with oxandrolone, a 19-nortestosterone derivative, reported by Ehnholm et al. (39) with the same antibody technique (and antibody) employed in the present studies. Only direct analysis of hepatic tissue TG lipase will permit definitive conclusions regarding the selective effects of sex steroids upon this enzyme. Nevertheless, the contrast between the 70% reduction in hepatic TG lipase activity in post-heparin plasma and the maintenance of normal TG clearance during estrogen therapy is impressive. The results are consistent with the suggestion by Ehnholm et al. (39) that hepatic TG lipase probably does not play a major physiological role in triglyceride removal. The present studies demonstrate that the decline in PHLA during estrogen therapy is due to a selective suppression in hepatic TG lipase without any consistent change in extrahepatic LPL. Thus, increased TG synthesis would appear to represent the leading hypothesis regarding the mechanism of hypertriglyceridemia induced by estrogens and oral contraceptives.

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