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 µg/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±12%, mean±SD, P < 0.001) or antibody inhibition (−63±11%, P < 0.001). Extrahepatic LPL was not significantly changed by affinity column (−22±40%) or antibody inhibition (−3±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±164%) or in the tissue form of LPL measured in extracts of acetone-ether powders (+21±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 increase 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.

Received for publication 24 May 1976 and in revised form 7 December 1976.
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 postheparin 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%) of Metropolitan Life Insurance Company tables (Table 1). 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.

Antecubital 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 (4,500 U/M2) both before and during estrogen and blood drawn after 60 min for similar analyses.


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 = 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). 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

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

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## TABLE I
Changes in Triglyceride Lipolytic Activities with Estrogen

| Subject number | Age (yr) | Ideal body weight % | Triglyceride mg/100 ml | Total† (nmol FFA/ml/min) | Hepatic triglyceride lipase by affinity column | Extrahepatic lipoprotein lipase by affinity column | Total‡ (nmol FFA/ml/min) | Hepatic triglyceride lipase by antibody inhibition§ | Extrahepatic lipoprotein lipase by antibody inhibition§ | Adipose tissue lipoprotein lipase | Heparin-releasable nmol FFA/10^6 cells/min | Extracted acetonel ether powder |
|----------------|---------|---------------------|-----------------------|--------------------------|-----------------------------------------|-------------------------------------------|--------------------------|-----------------------------------------------|-----------------------------------------------|---------------------------------*|--------------------------------*|-----------------|
| 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                            | 1.4 2.0 0.8 0.9                          | 7.7 0.9 1.1 0.4 |                                |
| 2              | 22      | 114                 | 39 76                 | 102 58 48 18             | 22 15 148 117                         | 169 81                                    | 50 96                    |                                |                                |                                |                                |                                |
| 3              | 23      | 94                  | 38 119                | 188 87 83 37             | 21 31 326 114                         | 280 121                                   | 135 62                    |                                |                                |                                |                                |                                |
| 4              | 34      | 133                 | 50 111                | 188 79 84 37             | 23 10 437 209                         | 364 197                                   | 106 62                    |                                |                                |                                |                                |                                |
| 5              | 29      | 120                 | 70 95                 | 229 133 114 44           | 49 28 365 166                         | 304 128                                   | 90 96                     |                                |                                |                                |                                |                                |
| 6              | 34      | 195                 | 95 176                | 259 118 153 38           | 47 34 501 267                         | 369 112                                   | 196 141                   |                                |                                |                                |                                |                                |
| 7              | 24      | 125                 | 84 87                 | 97 46 37 9              | 14 13 147 55                         | 100 44                                    | 36 35                     |                                |                                |                                |                                |                                |
| 8              | 31      | 140                 | 104 151               | 88 28 32 4              | 18 4 92 72                           | 36 15                                    | 80 37                     |                                |                                |                                |                                |                                |
| 9              | 30      | 136                 | 103 131               | 252 77 269 50           | 67 44 388 82                         | 335 76                                    | 51 50                     |                                |                                |                                |                                |                                |
| 10             | 24      | 101                 | 46 73                 | 116 65 70 19            | 27 35 118 85                         | 87 17                                    | 56 78                     |                                |                                |                                |                                |                                |
| 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                     |                                |                                |                                |                                |                                |
| 13             | 28      | 81                  | 74 150                | 130 21                 | — — 138 40                           | 105 24                                    | 29 20                     |                                |                                |                                |                                |                                |
| Mean           | 28      | 120                 | 69 113                | 159 68 90 27            | 30 24 247 117                        | 300 76                                    | 76 66                     |                                |                                |                                |                                |                                |
| SD             | 24      | 35                  | 64 35                 | 70 16 17 15             | 140 64 118 53                        | 47 33                                    | 2.4 4.4 0.9 1.2           |                                |                                |                                |                                |                                |

* 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.
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- or glyceride-bound 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 μl so that the salt concentration in the 200-μl 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 mMHO, respectively; Peak II, 33.4±1.3 and 32.8±1.3 mMHO, respectively. Estimated recovery by summation of eluted lipolytic activity was 68±24% before and 65±24% during estrogen (n = 11).

**Statistical methods.** All results have been expressed as mean±SD. Statistical evaluation was performed both by parametric regression analysis (r), nonparametric Spearman’s rank correlation coefficient (r_s) and the Wilcoxon signed-rank 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±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±12%, P < 0.001; −48±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±6% of the activity eluted from heparin-Sepharose, Peak I (Fig. 2), and 70±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±12%, P < 0.001, n = 11) and antibody inhibition (−63±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 0.745, P < 0.01, n = 11).

Extrahepatic LPL (Table I, Fig. 1). Before estrogen, extrahepatic LPL represented 27±6% of the activity eluted from heparin-Sepharose, Peak II (Fig. 2), and 30±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±40%, n = 11, NS; −3±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/10⁶ cells per min and the LPL extracted from acetone-ether powders was 1.3±0.9 nmol FFA/10⁶ 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/10⁶ cells per min) and the ammonium buffer extracted activity from acetone-ether powders (−0.2±1.0 nmol FFA/10⁶ 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 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 = −0.600, P < 0.05, n = 13) or VLDL-TG (r = −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 = −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

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**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 (— —).
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 [14C]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.

ACKNOWLEDGMENTS

The authors would like to express their gratitude to Howard Beiter, Sue Coates, Karen Grams, Martha Kimura, Dr. Jean Leik, and Martha Pleasant for their assistance in this study. This research was supported by National Heart and Lung Institute contract HV 12157 (Northwest Lipid Research Clinic), HL 18291 (Estrogen Effects), and AM 06670 from the National Institutes of Health. Portions of this work were conducted through the University of Washington Clinical Research Center at University Hospital (National Institutes of Health grant FR-37) and Harborview Medical Center (National Institutes of Health grant RR-133).

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