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Research Article

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Effects of Estrogen on Low Density Lipoprotein Metabolism in Males

Short-term and Long-term Studies during Hormonal Treatment of Prostatic Carcinoma

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Abstract

To characterize the effects of estrogen treatment on the metabolism of LDL we studied six males with metastatic prostatic carcinoma before and during the initiation of therapy; a repeated study was performed in five participants after 3–6 mo of treatment. The fractional catabolic rate (FCR) of autologous ^{125}I-LDL was calculated both from elimination curves of plasma radioactivity and from urine/plasma (U/P) radioactivity ratios.

Within 1–2 d of onset of estrogen therapy a more rapid decay of plasma radioactivity occurred, and FCR measured from U/P ratios increased by 20%. Concomitantly, LDL cholesterol levels decreased by 16%. After 3–6 mo of treatment FCR determined by both techniques was almost doubled, and LDL cholesterol was reduced by 34%. This occurred despite a 29% increase in the calculated synthesis rate of LDL. Tissue culture studies demonstrated that the receptor affinity of LDL isolated from patients on long-term estrogen therapy was reduced.

We conclude that a profound increase in LDL catabolism is induced through administration of pharmacological doses of estrogen in males, and hypothesize that this is the consequence of an increased expression of hepatic LDL receptors. This enhanced catabolism of LDL leaves LDL particles in plasma with lower affinity for the LDL receptor.

Introduction

Increased plasma levels of LDL are associated with an increased risk for cardiovascular disease in both males and females (1, 2). Fertile women have lower levels of LDL than men of similar age, whereas this sex difference is diminished after menopause (3). When estrogens are administered to males, reduced plasma levels of LDL are seen (4–6), but very little information on the mechanism behind this change is presently available.

The concentration of LDL in plasma is determined by the balance between their formation and elimination (7, 8). The synthesis of LDL is complex and not yet completely understood. VLDL are secreted from the liver and processed to VLDL remnants (or intermediate density lipoproteins [IDL][1]) by the action of lipoprotein lipase. IDL are cleared by receptors in the liver or transformed into LDL. Thus, the formation of LDL depends on both the initial secretion of VLDL and the efficiency of the hydrolysis and receptor interaction of the VLDL-IDL particles before the subsequent conversion into LDL. The elimination of LDL from plasma, which is mainly mediated via specific receptors for LDL (9–11), occurs for the major part in the liver, which is a key organ in the regulation of LDL plasma concentrations. The expression of hepatic LDL receptors has been shown to be regulated by various hormonal and metabolic stimuli in several animal species (11–13). Of particular interest is the observation that pharmacological doses of estrogens markedly increase LDL receptor expression and LDL uptake in the liver (14–16).

In a previous paper (6) we demonstrated that the biliary secretion of cholesterol was increased during estrogen therapy, and that the resulting change in biliary cholesterol was related to the degree of reduction in serum LDL cholesterol levels. In the present study we have investigated the effect on LDL kinetics of both short-term (acute) and long-term estrogen administration to males with prostatic carcinoma (6). The aim was to clarify whether the reduction in LDL concentration was the consequence of a reduced synthesis of LDL, an enhanced elimination rate of the lipoprotein, or both. We were able to demonstrate that the lowering of plasma LDL levels in these patients was obtained through increased catabolism of LDL.

Methods

Patients. The study comprised six male patients in whom estrogen therapy was to be initiated because of metastatic cancer of the prostate (6). The clinical data of the individual patients are shown in Table I. None of the patients was markedly obese, their relative body weight (RBW) varying between 90 and 125%. Three of the patients were healthy except for the carcinoma, while three were treated for benign hypertension. Their ongoing medication with beta blockers or thiazides was kept unchanged during the studies. One patient had evidence of mild ischemic heart disease. There were no plasma lipid abnormalities, and none of the patients had clinical or laboratory evidence of thyroid, hepatic, or renal disease. Therapy with estrogens had been decided on clinical grounds by their urologist, and informed consent was obtained from each subject. The ethical aspects of the study were approved by the Ethical Committee of Karolinska Institute (5 November 1984).

Experimental procedure. The patients were hospitalized at the metabolic ward or followed closely as outpatients. During the study they were given a standardized diet of natural type (17). About 35% of the energy content was supplied as fat, most of which contained saturated fatty acids. The major part of the carbohydrates, which accounted for 45% of the calories, was supplied as starch. The energy intake, calculated from standard foodstuff tables, was adjusted to keep the body weight constant. The daily intake of cholesterol was ~ 0.5 mmol (200 mg). Potassium iodide, 200 mg daily, was given orally 5 d before and during the studies to suppress uptake of radioiodine by the thyroid.

After an overnight fast ~ 100 ml blood was drawn into EDTA-containing vacuum tubes, and plasma was obtained after low speed centrifugation in the cold. LDL were prepared by sequential ultracen-

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1. *Abbreviations used in this paper:* FCR, fractional catabolic rate; IDL, intermediate density lipoproteins; LPDS, lipoprotein-deficient serum; RBW, relative body weight; U/P, urine/plasma.

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Table I. Basal Data on the Patients

Patient	Age	Weight	Relative body weight*	Plasma lipids		Clinical remarks‡
				Cholesterol	Triglyceride	
	yr	*kg*	*%*	*mmol/liter*		
I.H.	68	75	107	6.6	2.0	
A.E.	78	65	90	5.6	1.1	HT
B.L.	73	79	125	6.1	1.7	HT
F.S.	66	85	125	5.4	1.8	
G.R.	72	76	110	4.8	1.5	HT, IHD
G.F.	67	75	99	4.2	1.0	
Mean±SEM	71±1	76±2	109±5	5.5±0.4	1.5±0.2	

To convert values in millimoles/liter to milligrams/deciliter, multiply cholesterol by 38.7 and triglycerides by 88.5.

* Calculated as body weight (kilograms)/[height (centimeters) − 100].

‡ *HT*, hypertension; *IHD*, ischemic heart disease.

trifugation (see below), labeled with ^{125}I, and reinjected into the patient within 5 d of initial sampling (cf. references 18 and 19). Blood samples were collected in EDTA-containing tubes at 10 min and 2, 4, 6, 8, 10, 12, 24, and 36 h after the injection. Daily fasting blood samples were then collected (at 8 a.m.) until a few days before the administration of estrogens, when samples were collected every 12 h. The urine was collected in 24-h portions during the whole study.

Treatment with estrogens was started when the plasma ^{125}I-radioactivity curve was in the constant log-linear phase (between 9 and 13 d after the injection of ^{125}I-LDL). The patients were given 160 mg polyestradiol phosphate in a single intramuscular injection and started on oral ethinyl estradiol, 1 mg daily (6). Blood sampling and urine collections were continued for 1 wk after the initiation of estrogen treatment.

After 1 mo the dose of oral ethinyl estradiol was changed to 150 μg daily, and monthly intramuscular injections of 80 mg of polyestradiol phosphate were given (6). The ^{125}I-LDL turnover study was repeated after 3–6 mo of therapy. A similar protocol was used, except that blood samples were drawn only for 12–14 d. Five of the patients participated in the study during long-term treatment. All of the patients responded favorably to the hormonal therapy. Body weights remained constant within 2 kg, and there were no abnormalities of liver function tests or other routine laboratory investigations.

Lipoprotein preparation and radiolabeling. To plasma was added solid KBr to a density of 1.019 g/ml. This solution was centrifuged in an ultracentrifuge (L5-75; Beckman Instruments, Inc., Palo Alto, CA) for 18 h at 50,000 rpm using a 60 Ti rotor (Beckman Instruments, Inc.). The top fraction was removed by slicing the tube with a tube slicer (Beckman Instruments, Inc.). The density of the infranatant was adjusted to 1.063 g/ml by adding solid KBr and thereafter centrifuged for 18 h at 59,000 rpm in the same rotor. The top fraction containing LDL was washed once by ultracentrifugation at $d = 1.063$ g/ml for 18 h at 59,000 rpm. The isolated LDL were dialyzed extensively against 0.15 M NaCl with 0.01% EDTA, pH 7.0. The entire isolation procedure was performed at 4°C.

The isolated LDL were iodinated with ^{125}I-Na (obtained from the Radiochemical Centre, Amersham, UK) using the method of McFarlane (20) as modified for lipoproteins (21). Unbound ^{125}I was removed by chromatography on a Sephadex G-25 column (Pharmacia, Uppsala, Sweden) followed by extensive dialysis against 0.15 M NaCl with 0.01% EDTA, pH 7.0, overnight. The sp act of the LDL was 150–350 cpm/ng protein; < 2% of the radioactivity was soluble in 10% TCA, and < 10% could be extracted with chloroform/methanol (2:1, vol/vol). The labeled ^{125}I-LDL were sterilized by passage through two 0.45-μm filters (cat. No. SLHA 025 BS; Millipore/Continental Water Systems, Bedford, MA). Approximately 30–60 μCi (1.1–2.2 MBq) of LDL (0.5–2 mg) were diluted with 5% human albumin and 0.15 M NaCl to a total vol of 5 ml and reinjected into the patient.

Lipoprotein quantitation and characterization. Lipoprotein quantitation using a standardized procedure (22) was performed on at least two occasions before as well as within 1 wk of the initiation of therapy. An additional 2–4 quantitative lipoprotein analyses were obtained during long-term estrogen treatment. Cholesterol and triglyceride levels were determined using enzymatic techniques (Boehringer Mannheim Biochemicals, Indianapolis, IN). The level of LDL apoprotein was determined by multiplying the LDL cholesterol level by the protein/cholesterol ratio in the isolated LDL (see below). During initiation of therapy, the lipoprotein electrophoretic pattern of plasma was followed every 12 h in one subject using an agarose gel procedure (23).

The concentration of protein in isolated LDL was determined according to Lowry et al. (24), and that of cholesterol as described above. The size distribution of LDL particles was determined by electron microscopy (25). The isolated LDL were sprayed onto grids, contrasted by negative staining with phosphotungstic acid, and examined in a JEOL 100 C electron microscope. Size distribution was determined from micrographs with the use of a particle size analyzer (model TGZ 3; Carl Zeiss, Inc., Thornwood, NY). A total of 200 particles were analyzed in each sample.

Lipoprotein turnover. The fractional catabolic rate (FCR) of ^{125}I-LDL was calculated both from the slope of the plasma radioactivity decay curve and from the urine/plasma (U/P) ratio. When calculated from the plasma radioactivity curve, the two-compartment model of Matthews (26) was used as described (18, 19). In this model the radioactivity decay curve is deconvoluted into the sum of two exponentials, one fast and one slow. The model assumes steady-state conditions and also permits the calculation of the distribution of the label between the intravascular and the extravascular body pools.

FCR from U/P ratio was calculated by dividing the total amount of ^{125}I radioactivity in the urine during a 24-h period with the total plasma ^{125}I-radioactivity at 8 a.m. on the same day. Plasma volume was calculated as 4.5% of body weight and corrected for overweight with factor $b = [(4{,}500 + ac)/(100 + c)]/45$, where a represents the plasma volume per kilogram adipose tissue (9.7 ml for males), c is the excess of relative body weight (RBW − 100), and RBW was calculated as body weight (kilograms)/[height (centimeters) − 100] × 100 (27).

The absolute catabolic rate of LDL apoprotein was estimated according to Langer et al. as described (18, 19). This equals the synthetic rate of apo LDL under steady-state conditions. The FCR was multiplied by total plasma concentration of apo LDL and this synthetic rate was expressed as milligrams of apoprotein synthesized per day normalized for body weight. The data presented are the synthesis rates calculated with the use of FCR values derived from plasma radioactivity curves. Similar findings were obtained using FCR values from U/P ratios (not shown).

Fibroblast assay of LDL degradation. Human fibroblasts, derived from a skin biopsy from a healthy 22-yr-old female, were used before their 15th passage. They were grown in RPMI-1640 medium supplemented with 1% L-glutamine, penicillin (100 IU/ml), streptomycin (100 μg/ml), and 10% FCS or 10% of human lipoprotein-deficient serum (LPDS-medium). LDL were isolated by sequential ultracentrifugation (see above) of plasma from four of the patients on long-term estrogen therapy and from eight age-matched male controls. Before the experiment all LDL preparations were filtered through 0.45-μm filters and diluted with 0.15 M NaCl/0.01% EDTA solution to a final protein concentration of 490 μg/ml as determined with the Lowry procedure (24).

On day 0 fibroblasts (45,000 cells in 3 ml of FCS-medium) were seeded to 20-cm^2 Petri dishes (Nunc, Roskilde, Denmark). On day 3, when cells were 50% confluent, the medium was removed and replaced with 3 ml of fresh FCS-medium. The following day the medium was aspirated and replaced with 3 ml of LPDS-medium after one wash with 4 ml of plain RPMI-1640 medium. After incubation in LPDS-medium for 48 h the cells were incubated with 1.4 ml of fresh LPDS-medium containing ^{125}I-LDL (16 μg/ml). 100 μl of NaCl/EDTA solution containing 490 μg/ml of LDL protein was added to each dish, giving a final

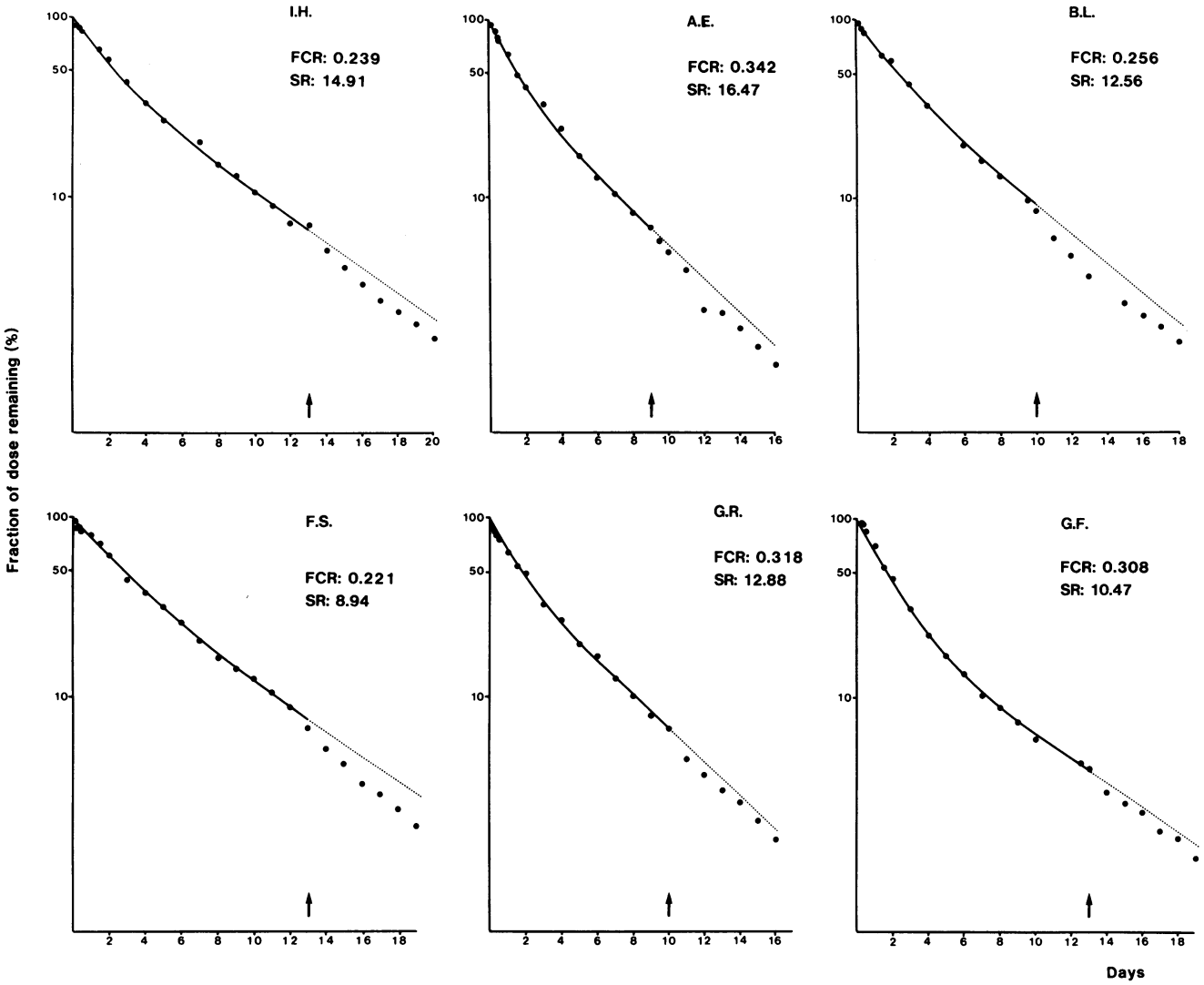

Figure 1. Plasma radioactivity curves (% of dose remaining) in the individual patients after injection of ^{125}I-LDL. When the curve was in the log-linear phase, estrogen treatment was instituted (*arrow*). Values for FCR and synthetic rate (*SR*) obtained from the curve during the pretreatment phase are shown for each individual.

concentration of ^{125}I-LDL and unlabeled LDL of 15 and 33 μg/ml, respectively. All incubations were performed in duplicate. After incubation at 37°C for 6 h in a humidified incubator (5% CO_2/95% air) the degradation of ^{125}I-LDL was determined (nanograms of ^{125}I-LDL protein degraded per hour) after precipitation of 1.2 ml of cell medium with TCA as described (28). Blank values (obtained from identical incubations without cells) have been subtracted from the data presented.

Statistical calculations. Data are presented as means±SEM. The significance of differences was tested by two-tailed *t* test. Correlations were evaluated with the Spearman rank correlation test.

Results

The fasting concentrations of LDL cholesterol in the six patients studied under basal conditions averaged 4.13±0.46 mmol/liter (160±18 mg/dl) (range, 2.82–5.48 mmol/liter [109–212 mg/dl]). The corresponding levels of apo LDL ranged between 75 and 146 mg/dl with a mean of 110±9 mg/dl. These values, which remained stable during the period of the basal turnover determinations, were in all cases within the normal range observed in an age-matched Swedish male population.[2]

After injection of ^{125}I-labeled autologous LDL, the plasma radioactivity dieaway curves could in all cases be fitted by a two-compartment model. The FCR values derived by this procedure were between 0.221 and 0.342 d^{-1} (mean, 0.281±0.018) in the six patients (Fig. 1). The estimated synthesis rate of apo LDL varied from 8.9 to 16.5 $mg \cdot kg^{-1} \cdot d^{-1}$ (mean, 12.7±1.0 $mg \cdot kg^{-1} \cdot d^{-1}$). The corresponding FCR values calculated from the U/P ratios (Table II) were between 0.237 and 0.368 d^{-1} (mean, 0.314±0.017 d^{-1}). There was good agreement between the two independent ways of calculating the FCR in the individual patients (R_s = 0.89, $P < 0.05$). Again, these values were within the range observed in healthy male controls of similar age.[2]

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Table II. FCR of LDL Estimated from U/P Ratios before (B) and within 6–8 d of Initiation of Estrogen Therapy (A) in Patients with Prostatic Cancer

Patient	FCR calculated from U/P ratio	
	B	A
	d^{-1}	
I.H.	0.237	0.278
A.E.	0.368	0.496
B.L.	0.320	0.402
F.S.	0.300	0.345
G.R.	0.353	0.425
G.F.	0.303	0.315
Mean±SEM	0.314±0.017	0.377±0.030*

* Significantly different from *B*, $P < 0.01$ (paired *t* test).

When estrogen therapy at doses recommended for the treatment of prostatic cancer was initiated, clear changes in the plasma lipoprotein pattern occurred very rapidly (Table III). From two repeated plasma samples obtained from the patients during the first 6–8 d of therapy a mean decrease in LDL cholesterol of 16% (range, 4–43%) could be demonstrated. Within the same time frame a slight increase in HDL cholesterol levels (~ 10%) could be seen; again, there was considerable variation (range, −6–39%). There was a clear tendency for plasma triglycerides to increase on initiation of therapy, and this increase was observed in all lipoprotein fractions (Table III). An example of the drastic change in lipoprotein profile that was frequently observed is seen in Fig. 2, which depicts the results of analyses performed twice daily in patient G.R. It is evident that the increase in plasma triglycerides was a very early event in response to estrogen therapy. There was an acute lowering of plasma total cholesterol, which was normalized within 6–8 d, however. In contrast, the triglyceride levels remained increased. Analysis of the lipoprotein pattern by agarose gel electrophoresis (Fig. 2, *insert*) clearly reveals that the initial event was an increase in the amount of pre-β-lipoproteins (VLDL), and a simultaneous decrease in the lipoproteins with β-mobility (LDL). There was also a gradual increase in α-lipoproteins (HDL), which contributed to the "normalization" of plasma total cholesterol in spite of the decrease in LDL cholesterol levels.

Table III. Changes in Lipoprotein Levels within 6–8 d of Initiation of Therapy with Estrogen in Six Patients with Prostatic Cancer (Means±SEM)

	Basal	Acute estrogen therapy	Change	Significance of change*
	mmol/liter		%	
Cholesterol				
Total	5.45±0.36	4.83±0.39	−11	NS
VLDL	0.39±0.06	0.42±0.08	+8	NS
LDL	4.11±0.34	3.44±0.36	−16	$P < 0.02$
HDL	0.95±0.10	1.05±0.05	+11	NS
Triglycerides				
Total	1.51±0.16	1.87±0.25	+24	NS
VLDL	0.84±0.14	0.95±0.18	+13	NS
LDL	0.44±0.05	0.60±0.08	+36	$P < 0.05$
HDL	0.24±0.03	0.33±0.04	+38	NS

To convert values in millimoles/liter to milligrams/deciliter, multiply cholesterol by 38.7 and triglycerides by 88.5.
* Analyzed by *t* test.

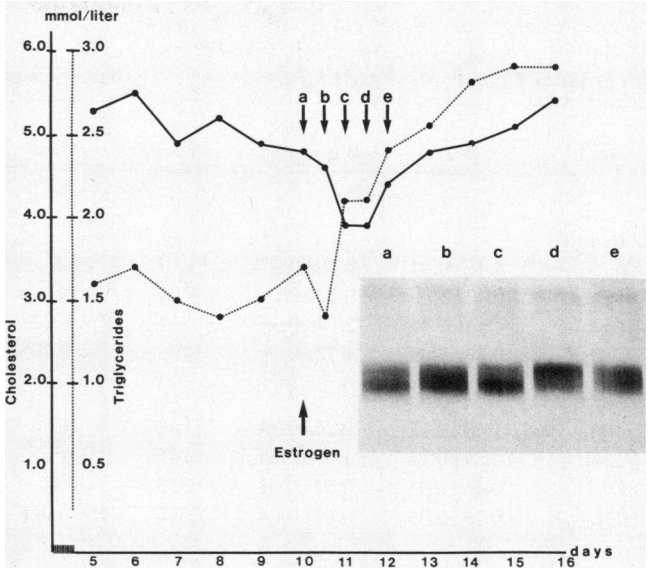

Figure 2. Plasma lipid levels in patient G. R. during the initiation of estrogen therapy (*arrow*). *Solid line,* cholesterol; *dotted line,* triglycerides. To convert values in millimoles/liter to milligrams/deciliter, multiply cholesterol by 38.7 and triglycerides by 88.5. The insert demonstrates lipoprotein analysis by agarose gel electrophoresis at the indicated time points (lanes *a–e*).

When the plasma ^{125}I-LDL clearance curves of the six patients were followed during the early phase of estrogen therapy, a clear downward shift of the plasma radioactivity curve was evident in every patient within 1–2 d after initiation of estrogen therapy (Fig. 1). This indicated that the radiolabeled LDL particles were cleared from plasma at a higher rate than before therapy. To assess whether the changes in LDL concentration and apparent elimination were the result of a change in plasma volume distribution, we determined the plasma levels of albumin and IgG twice daily during the total procedure in four of the subjects. However, no changes in the levels of these proteins during the initiation of estrogen therapy were noted (data not shown), arguing against such a possibility.

Analysis of the U/P ratios and LDL-FCR calculated from those (Table II) provided further strong evidence that the LDL elimination was stimulated acutely by the initiation of estrogen therapy. Thus, the U/P ratio increased distinctly in all patients in response to therapy, with a mean increase of 20% during the first week. Again, there was a marked interindividual variation, the range of increase varying from 4 to 35%.

The observed changes in lipoprotein pattern in the short-term experiments could be demonstrated even more drastically when five of the patients were restudied during long-term estrogen therapy (Tables IV and V). Thus, after a treatment period of 3–6 mo, with the estrogen doses reduced to 80 mg/mo of intramuscular polyestradiol phosphate and 150 μg

Table IV. Changes in Lipoprotein Levels in Response to 3–6 mo of Therapy with Estrogen in Five Patients with Prostatic Cancer (Means±SEM)

Analysis	Basal	Long-term estrogen therapy	Change	Significance of change*
	mmol/liter		%	
Cholesterol				
Total	5.42±0.44	4.90±0.37	−10	NS
VLDL	0.42±0.06	0.52±0.14	+24	NS
LDL	4.13±0.46	2.74±0.34	−34	$P<0.02$
HDL	0.88±0.09	1.72±0.19	+96	$P<0.005$
Triglycerides				
Total	1.59±0.17	2.26±0.34	+42	NS
VLDL	0.89±0.15	1.29±0.24	+45	NS
LDL	0.46±0.05	0.52±0.08	+13	NS
HDL	0.25±0.03	0.49±0.04	+96	$P<0.025$

To convert values in millimoles/liter to milligrams/deciliter, multiply cholesterol by 38.7 and triglycerides by 88.5.
* Analyzed by paired *t* test.

daily of ethinyl estradiol, there was a marked reduction (~ 34%) of the LDL cholesterol levels. Apo LDL concentrations were reduced by 26%, whereas HDL cholesterol concentrations were almost doubled. Plasma total triglycerides were increased by ~ 50% due to increases in all the lipoprotein fractions. The HDL triglycerides were almost doubled (Table IV).

The in vivo clearance of autologous ^{125}I-LDL was reexamined during the chronic estrogen therapy phase. Analysis of the plasma radioactivity curves revealed that the radiolabeled lipoproteins were removed considerably faster from plasma than before therapy (illustrated for patient G.F. in Fig. 3). The FCR values calculated from plasma curve analysis were increased in all patients with a mean value of 81% (range, 59–160%). Concomitant calculations of FCR values from U/P radioactivity ratios demonstrated a mean increase of 99% (Table V). The estimated absolute catabolic rate of LDL, which under steady-state conditions equals the synthetic rate, was 29% higher during chronic estrogen treatment, in spite of a decrease in the intravascular apo LDL pool of 26% (Table V). Thus, the decrease in LDL levels could be completely explained by the marked increase in FCR.

No indications of a change in plasma volume were found during short-term estrogen treatment. It could not be excluded that such changes might be present during long-term therapy, however. Thus, in addition to plasma volume calculations from analysis of the 10-min ^{125}I-LDL plasma radioactivity measurement, we performed independent measurements of plasma volume using ^{131}I-albumin in three of the patients before and during long-term administration of estrogen. In both sets of experiments, no evidence of a change in plasma volume during hormone therapy were found (data not shown).

During the short-term (acute) experiments the LDL fraction used for radiolabeling was harvested before estrogen therapy. Thus, possible changes in composition and/or conformation induced by estrogen therapy should not have effected the tracer in the first set of experiments. During the long-term studies, however, the possibility must be considered that such changes might affect the affinity of the LDL particle for the LDL-receptor. To address this issue, LDL were isolated from five patients on long-term estrogen treatment and eight age-matched male controls. A reduced cholesterol to protein ratio in LDL from estrogen-treated subjects was observed, but there was no difference in size of the particles, as measured by electron microscopy of negatively stained LDL (Table VI). When the ability to compete with normal LDL for binding to the

Table V. Kinetics of ^{125}I-LDL in Five Patients with Prostatic Cancer before (B) and after 3–6 mo of Estrogen Therapy (E)

Patient	Period	LDL apo B	LDL-FCR: Calculated from plasma data	LDL-FCR: Calculated from U/P ratios	Absolute catabolic rate of LDL
		mg/dl	d^{-1}		$mg \cdot kg^{-1} \cdot d^{-1}$
I.H.	B	146	0.239	0.237	14.91
	E	111	0.394	0.451	18.22
B.L.	B	129	0.256	0.320	12.56
	E	70	0.666	0.955	17.97
F.S.	B	107	0.221	0.300	8.94
	E	76	0.352	0.439	10.09
G.R.	B	97	0.318	0.353	12.88
	E	99	0.448	0.597	17.11
G.F.	B	75	0.308	0.303	10.47
	E	54	0.571	0.572	13.93
	B	111±11	0.268±0.017	0.303±0.017	11.95±1.03
	E	82±9	0.486±0.052	0.603±0.084	15.44±1.55
Change (%)		−26%	+81%	+99%	+29%
Significance of difference*		$P<0.05$	$P<0.02$	$P<0.05$	$P<0.02$

* Analyzed by paired *t* test.

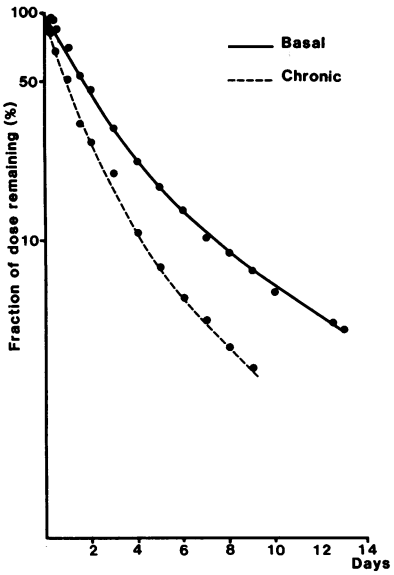

Figure 3. Plasma radioactivity curves (% of dose remaining) after injection of ^{125}I-LDL before (*basal*) and during long-term (*chronic*) treatment with estrogens in patient G.F.

LDL receptor was determined in LDL isolated from four patients on estrogen treatment, it was evident that the affinity to the LDL receptor was actually lower than that of controls (Table VII). This finding thus clearly establishes that the stimulation of ^{125}I-LDL FCR seen during long-term estrogen therapy is not the consequence of induced changes in the properties of LDL.

Finally, a further indication that the lowering of plasma LDL during estrogen treatment occurred as a consequence of an increased catabolism of the LDL particle was the fact that the LDL cholesterol concentration was negatively correlated ($R_s = -0.79$, $P < 0.01$) to the FCR value (Fig. 4). No correlation between the estimated synthesis rate and the concentration of LDL could be obtained.

Discussion

The present study was undertaken to determine whether the decrease in plasma levels of LDL cholesterol observed during treatment with estrogen is the result of decreased synthesis or stimulated catabolism. The results give conclusive evidence that pharmacological treatment with estrogen for 3–6 mo in males with prostatic carcinoma results in drastic changes in the FCR of plasma LDL. The reduction of the pool of circulating LDL occurs in spite of an accelerated rate of synthesis of this lipoprotein. This fact argues strongly against the alternative hypothesis, namely, that the increased FCR is secondary to the decrease in LDL pool size (cf. reference 29).

The most plausible explanation for the drastic increase in LDL catabolism is induction of LDL receptor activity, presumably in the liver. Such a mechanism is known to exist in

Table VI. Properties of LDL Particles Isolated Simultaneously from Eight Normal Subjects (Mean Age, 72±1 yr) and the Five Patients on Chronic Treatment with Estrogen (Mean±SEM)

	Controls	Estrogen-treated patients	Significance of difference*
Cholesterol/protein ratio (*mg/mg*)	1.45±0.02	1.30±0.04	$P < 0.01$
Diameter (*nm*)‡	21.1±1.7	20.8±0.6	NS

* Analyzed by *t* test.
‡ Determined by electron microscopy after negative staining.

Table VII. Comparison of the Inhibitory Effect of Isolated LDL Preparations on the Degradation of ^{125}I-LDL in Cultured Fibroblasts

Source of added LDL	Degradation of ^{125}I-LDL	Inhibition of ^{125}I-LDL degradation
	$ng \cdot mg^{-1} \cdot h^{-1}$	%
No addition	1,285	0
Control subjects (n = 8)		
Mean±SEM	425±21	67±2
Range	(282–470)	(63–78)
Estrogen-treated patients		
I.H.	583	55
F.S.	604	53
G.R.	534	58
G.F.	563	56
Mean±SEM	571±13*	56±1*

Human fibroblasts were seeded, preincubated, and supplemented with LPDS-medium containing 15 μg/ml of ^{125}I-LDL as described in Methods. Unlabeled LDL, isolated from four subjects on long-term estrogen therapy and from eight age-matched controls were thereafter added to a final concentration of 30 μg/ml. After 6 h of incubation the degradation of ^{125}I-LDL was determined as described elsewhere. Each value presented is the average from duplicate incubations.
* $P < 0.002$ (*t* test).

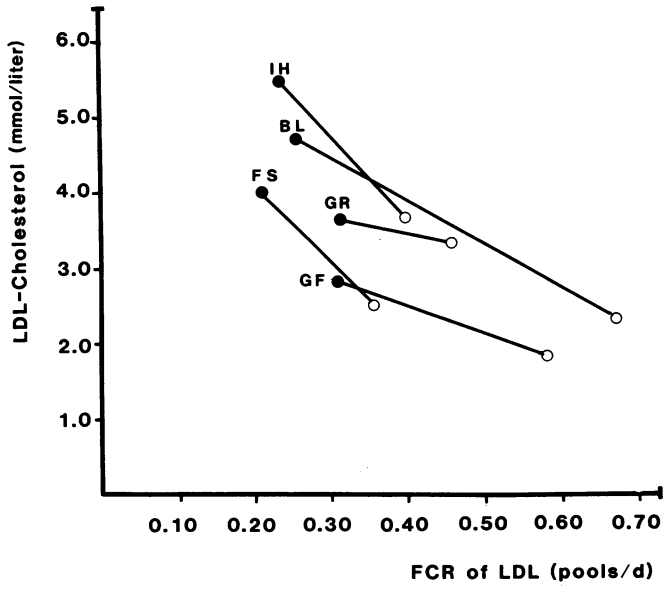

Figure 4. Correlation between LDL cholesterol concentration and LDL-FCR. LDL-FCR was calculated from the plasma radioactivity curves after injection of ^{125}I-LDL before (●) and during (○) long-term treatment with estrogen. $R_s = -0.79$ ($P < 0.01$). To convert LDL cholesterol values in millimoles/liter to milligrams/deciliter, multiply by 38.7.

the rat and in the rabbit (14–16). From the results of the present study it cannot be totally excluded that non–receptor-mediated LDL catabolism is also stimulated by estrogen. Definite confirmation of our hypothesis must therefore await direct determinations of LDL receptor binding activity in the human liver during estrogen treatment. It is of interest to note that studies of ^{125}I-LDL binding, internalization, and degradation in human hepatoma cells (Hep G2) have indicated an increased LDL receptor activity in response to incubation with high doses of estrogen (30).

The possibility that estrogen treatment affected the LDL particles so that their elimination was stimulated was refuted in two ways. First, the acute studies indicated an enhanced catabolism using a radiolabeled tracer representing untreated LDL (Fig. 1). Second, the affinity for interaction with the LDL receptor was actually lower when LDL particles were isolated during estrogen therapy (Table VII). The latter observation is most easily explained if it is assumed that in a situation with increased LDL receptor expression, particles with higher affinity are most avidly taken up by the receptors. A similar situation has recently been reported during cholestyramine treatment in the guinea pig (31). Indeed, the degree of stimulation of LDL catabolism observed during chronic estrogen administration in the present work could tend to underestimate the change in receptor activity.

Of particular interest was the rapidity of the onset of the changes in LDL catabolism in response to initiation of therapy (Fig. 1). A clear change in the radioactivity curve was observed within 1–2 d, and this was associated with a reduced plasma level of LDL cholesterol. It should be pointed out that the estimations of LDL-FCR from U/P radioactivity determinations in this situation only represent minimum approximations, however, as the steady state is obviously perturbed by treatment. Results of a similar nature have been reported from studies of ^{125}I-LDL turnover during the initiation of parenteral nutrition (32) and in response to an insulin-glucose clamp perfusion (33). The changes in those studies, although not of such impressive magnitude as those seen in the present work, have been interpreted as demonstrating stimulation of LDL catabolism. Since insulin has been shown to enhance LDL receptor expression in vitro (34), it has been suggested that a rapid increase in LDL receptors may occur also in vivo. The present finding of a rapid increase in LDL catabolism during the initial (acute) administration of estrogen would be in agreement with these results, and may indicate that the human hepatic LDL receptors can be regulated very rapidly in vivo, similar to what has been shown for the dog (35). Also in support of this concept is the fact that most of the estrogen-induced effect on LDL receptor expression in Hep G2 cells in vitro was observed within 24 h (30).

Another important new finding of the present study is the fact that high FCR values of ^{125}I-LDL can be induced in these relatively old males by estrogen treatment. With increasing age there is a gradual reduction of LDL-FCR in normal males (36),[2] and the basal FCR values observed in the present work are in good agreement with those observed in healthy age-matched men.[2] The increase of LDL-FCR during estrogen therapy also indicates that at a high age the liver can express significant amounts of LDL receptors when adequate stimulation is provided. Thus, the reduction of LDL catabolism that occurs with advancing age[2] is not likely to be the consequence of a reduced capacity for receptor synthesis.

Several other drastic effects on the plasma lipoprotein pattern could also be established in response to estrogen therapy with the present experimental model. The estimated production rate of LDL was clearly increased during long-term estrogen treatment (Table V), which may explain why the reduction of the pool size of plasma LDL was not as large as predicted from the increase in LDL-FCR. It is generally thought that considerable amounts of IDL are cleared from plasma by the hepatic LDL (apo B, E) receptors (7). A stimulation of LDL receptor expression may thus lead to a reduced amount of IDL being converted to LDL, resulting in a reduced apparent synthesis of LDL (7, 29). Since this was not observed in the present study, estrogen treatment must either have stimulated VLDL synthesis profoundly, thereby overloading the VLDL remnant (IDL) clearance capacity, or it must have directly or indirectly stimulated the conversion of IDL to LDL particles in some way. Although the question was not specifically addressed in the present work, it is well known that estrogen treatment reduces the activity of hepatic triglyceride lipase (37). Since suppression of the hepatic lipase has actually been shown to decrease the conversion of IDL to LDL in monkeys (38), it is tempting to speculate that the rise in apparent LDL production rate during estrogen treatment is the result of an increased synthesis of VLDL. Obviously, the possibility of direct secretion of LDL in this situation cannot be excluded presently.

An increased production rate of VLDL and apo B has been reported in response to high doses of estrogen in several animal models (39–41). The rapidity of the onset of the increase in VLDL (Fig. 2) also makes the concept of an enhanced VLDL secretion during estrogen therapy in man very likely. Turnover studies of apo B-VLDL in estrogen-treated premenopausal females have indicated an increased production of VLDL apo B in this situation (42). The slower accumulation of HDL may instead indicate that the HDL increase is a secondary effect, related to a more efficient metabolism of VLDL and/or a reduced catabolism of HDL (37). An increased ratio between apo CII and CIII has been reported during estrogen stimulation of Hep G2 cells (40, 43), and such a change will be expected to enhance the catabolism of VLDL by lipoprotein lipase (44). The activity of lipoprotein lipase itself may to some extent be negatively influenced by estrogen, however (45). As mentioned, a profound suppression of hepatic lipase activity is well established during treatment with this hormone (37). This effect on hepatic lipase has been postulated to be of major importance for the increase in HDL levels, and particularly for the increase of the HDL_2 fraction. An increased production rate of apo A-I, the major apolipoprotein of HDL, has recently been demonstrated in response to estrogen using cultured Hep G2 cells (40, 43). A moderate rise in HDL apo A-I production was also observed during estrogen therapy in premenopausal females (42).

Finally, it is of major interest to consider the possible mechanism(s) responsible for the apparently parallel changes in LDL catabolism and biliary cholesterol secretion in response to estrogen (6). We are still not able to conclude whether the stimulation of cholesterol secretion into bile is secondary to an enhanced load of cholesterol to the liver via primarily induced LDL receptors, or if a primary increase in cholesterol secretion leads to a depletion of hepatic cholesterol with a secondary (compensatory) effect on hepatic LDL receptor expression. Considering the physiological role of estro-

genic hormones, it is possible that its primary function in the regulation of lipid metabolism may be to stimulate triglyceride (and cholesterol) export as VLDL from the liver. The effect on hepatic LDL receptors and LDL catabolism may well be associated with such an increased VLDL triglyceride transport, secondarily resulting in an increased load of cholesterol in the liver. This in turn may stimulate direct biliary excretion of free cholesterol, whereas it is not yet known if cholesterol elimination as bile acids is enhanced or not.

In conclusion, we have demonstrated the induction of a rapid and profound increase in LDL elimination from plasma by pharmacological doses of estrogen given to elderly males. Hypothetically, this reflects an increase in the number of specific LDL receptors in the liver. The regulation of hepatic LDL receptor activity by various hormonal and pharmacological procedures should merit further study.

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