

Hormonal Regulation of Serum Lp (a) Levels

Opposite Effects after Estrogen Treatment and Orchidectomy in Males with Prostatic Carcinoma

Peter Henriksson, Bo Angelin, and Lars Berglund

Metabolism Unit, Departments of Medicine and Clinical Chemistry, Karolinska Institutet at Huddinge University Hospital, S-141 86 Huddinge, Sweden

Abstract

Serum concentrations of lipoprotein (a) [Lp (a)] were determined in two groups of elderly males suffering from prostatic carcinoma, who were randomized to treatment with estrogen ($n = 15$) or orchidectomy ($n = 16$). Estrogen was given as oral ethinylestradiol, 150 μg daily, combined with intramuscular polyestradiol phosphate, 80 mg/mo. The baseline levels were similar in both groups, but 6 mo after initiation of therapy, serum Lp (a) levels were decreased $\sim 50\%$ in the estrogen-treated group ($P < 0.001$) in contrast to a 20% increase ($P < 0.01$) in the orchidectomized group. Concomitantly, LDL cholesterol decreased by 30% and HDL cholesterol increased by almost 60% in the estrogen-treated patients. There was no relationship between the change in LDL cholesterol and Lp (a) reduction. In conclusion, Lp (a) levels in males were found to drastically decrease upon estrogen treatment and to increase after orchidectomy, suggesting that sex hormones, and particularly estrogens, exert a regulatory role on the serum Lp (a) level in man. (*J. Clin. Invest.* 1992; 89:1166–1171.) Key words: Lp (a) lipoprotein • LDL receptors • estrogen • orchidectomy • coronary heart disease

Introduction

Lipoprotein (a) [Lp (a)]¹ is a complex between low density lipoprotein and apolipoprotein (a), the level of which is a strong independent risk factor for premature ischemic heart disease (1–12). Apo (a) is a glycoprotein which is linked to the structural protein of LDL, apo B-100, by a disulphide linkage, and which shows considerable size variation (from ≈ 300 to ≈ 800 kD) among individuals owing to different numbers of cysteine-rich sequences that are homologous to kringle 4 of plasminogen (2, 3, 13–17). The size variation is inversely related to the serum concentration; thus, the lower molecular weight isoforms are present in higher concentrations (2, 3, 15–21).

The mechanisms for Lp (a) synthesis and clearance are largely unknown, and no clear evidence of variation with age, sex, or diet has been described (1–3, 22–24). In familial hypercholesterolemia, the serum concentration of Lp (a) is fre-

quently increased (12, 25–27). The role of the LDL receptor in the clearance of Lp (a) is still debated, however. Thus, no reduction in Lp (a) levels has been observed during treatment with drugs known to stimulate the elimination of LDL from plasma via induction of LDL receptors, such as cholestyramine and cholesterol synthesis inhibitors (12, 28–30). On the other hand, overexpression of the human LDL receptor in transgenic mice clearly increases the plasma clearance of Lp (a) (31). The only hypolipidemic agent reported to lower Lp (a) levels is nicotinic acid (32), particularly when combined with neomycin (33). This has been taken as evidence for the concept that changes in Lp (a) synthesis rate may be more important than changes in clearance in the determination of serum Lp (a) levels (1–3).

An interesting model for studying the regulation of serum Lp (a) levels in man emerges from our studies on lipoprotein metabolism in males treated with estrogen or orchidectomy due to prostatic carcinoma (34, 35). During estrogen treatment there is a profound stimulation of LDL clearance, accompanied by a distinctly increased production of LDL particles (35). Also, preliminary evidence for a reduced Lp (a) concentration during combined treatment with estrogen and progestagen has recently been presented (36). In the present study, we have therefore compared the effects of estrogen therapy and orchidectomy on serum Lp (a) concentrations, and found evidence for an important hormonal regulation of this lipoprotein fraction.

Methods

Subjects, experimental procedure. We studied 31 elderly males with recently diagnosed prostatic cancer suitable for hormonal treatment as judged by a senior urologist. Patients with a history or signs of previous thromboembolism, cerebrovascular lesion, myocardial infarction, severe angina pectoris, severe intermittent claudication, or congestive heart failure were excluded from the study. Altogether 23 of the patients have been included in our previous report on gallstone incidence (34).

After informed consent, the patients were randomly allocated either to orchidectomy ($n = 16$) or estrogen therapy ($n = 15$). Estrogen was given as polyestradiol phosphate, 160 mg intramuscularly monthly for the first 3 mo, followed by 80 mg intramuscularly monthly. In addition, the patients were given ethinyl estradiol, 1 mg per os daily for the first 2 wk and then 150 μg daily. This dosage is the lowest one recommended in Sweden for the treatment of prostatic cancer. Serum samples were obtained in the morning between 8 and 9 a.m. after an overnight fast and stored at -70°C until analyzed.

The ethical aspects of the study were approved by the ethical committee at Huddinge University Hospital, Sweden.

Materials. Acrylamide, (*N,N'*-methylene) bisacrylamide, and SDS were from Bio-Rad Laboratories (Richmond, CA). Monoclonal ¹²⁵I-antihuman Lp (a) antibodies were supplied as a kit (Pharmacia LKB, Uppsala, Sweden). Bovine serum albumin (fraction V) was purchased from Sigma Chemical Co. (St. Louis, MO).

Address correspondence to Dr. Bo Angelin, Department of Medicine, Huddinge University Hospital, S-141 86 Huddinge, Sweden.

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1. Abbreviations used in this paper: FSH, follicle-stimulating hormone; GH, growth hormone; Lp (a), lipoprotein (a).

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Serum hormone analysis. Serum concentrations of testosterone, follicle stimulating hormone (FSH), luteinizing hormone, and growth hormone (GH) were determined by radioimmunoassay using kits from Diagnostic Products Corp., Los Angeles, CA; Farnos Diagnostica OY, Turku, Finland; and Pharmacia LKB, Uppsala, Sweden.

Serum lipoprotein and apolipoprotein analysis. Total serum cholesterol and triglyceride were determined with standard enzymatic techniques (Boehringer Mannheim GmbH, Mannheim, Germany). HDL cholesterol was determined after precipitation with heparin-Mn Cl₂, and the concentration of LDL cholesterol was calculated according to the formula of Friedewald (37).

The serum concentration of Lp (a) was determined in duplicate with a two-site immunoradiometric assay (Pharmacia Diagnostics AB, Uppsala, Sweden). The method uses two monoclonal antibodies to different epitopes of human apo (a), one labeled with ¹²⁵I and one coupled to microsepharose. The detection limit of the assay was 12 mg/liter. The coefficient of variation was 3.8% at a standard concentration of 364 mg/liter and 7.2% at a standard concentration of 137 mg/liter. The Lp (a) serum standard was obtained from Immuno AG (Vienna, Austria; lot no. 2900-090).

SDS-polyacrylamide gel electrophoresis. Apo (a) isoform size was determined by SDS-PAGE of whole serum with subsequent Western blotting (38). Briefly, 20 μl of serum was mixed with 200 μl sample buffer (1% SDS, 20% glycerol, 0.1% bromophenol blue, 100 mM Tris-HCl, pH 6.8) and 8.8 μl β-mercaptoethanol and the solution was boiled for 10 min. Aliquots were used for electrophoresis which was performed according to Laemmli (39) in a 3–8% gradient gel using a vertical electrophoresis apparatus (Bio-Rad Laboratories). The electrophoresis was run for 4 h at 45 mA. The transfer of proteins to nitrocellulose filters was performed overnight at 4°C according to Towbin et al. (40) at 300–500 mA in a cell blotting chamber (Bio-Rad Laboratories). Immunoblotting was performed using the ¹²⁵I-labeled monoclonal anti-Lp (a) antibody described above. The filters were blocked by incubation for 1 h at room temperature with 5% BSA, 0.01% antifoam A, 0.2% NP40, 80 mM NaCl, 2 mM CaCl₂, 50 mM Tris-HCl, pH 8.0. Incubation then continued for 1 h in the same buffer at room temperature with added ¹²⁵I-anti-Lp (a) antibody (3–4 × 10⁶ cpm/30 ml). The filters were then washed in the incubation buffer, twice with and once without 0.5% BSA. Apparent molecular weight of apo B was estimated from the migration of human LDL standards.

Statistical analysis. Data are presented as means±SEM or, for Lp (a), as means (range). Student's *t* test for paired and unpaired samples was used to compare baseline characteristics and changes after six months of therapy. Due to the skewed distribution of apo (a) levels, they were compared using Mann-Whitney's U test or Wilcoxon's signed rank test. The relative change in Lp (a) was found to be normally distributed. Consequently, regression was performed with the relative change in Lp (a) as the dependent variable and the relative changes in the other lipids as tentative explanatory variables. Multiple stepwise regression was performed using a critical value of the F-distribution (*F* value) of 4.0 to include and 3.996 to exclude independent variables.

Results

The two groups of male patients with prostatic cancer were well matched as regards baseline characteristics, including age (estrogen treatment, 68±1 yr; orchidectomy group 67±1 yr) and body weight (estrogen-treated group 68±3 kg; orchidectomy 70±2 kg). There was no significant change in weight after six months of therapy (estrogen-treated group, 69±2 kg; orchidectomy group, 71±3 kg). As expected, both groups displayed a drastic reduction of serum testosterone levels in response to therapy (Table I). The FSH and luteinizing hormone concentrations were distinctly increased in the orchidectomy group. In contrast, the serum GH levels were increased by estrogen therapy, and tended to be lower after orchidectomy (Table I).

As seen in Table II, the baseline lipoprotein values were similar in the two groups. The baseline Lp (a) levels in both groups were similar to the level in a corresponding normal population when assayed under identical conditions (mean level, 140 mg/liter, *n* = 33). In agreement with our previous results (34, 35), estrogen therapy resulted in a drastic lowering of LDL cholesterol and a marked increase in HDL cholesterol levels after six months of therapy, whereas no consistent changes in lipoprotein levels were observed after orchidectomy.

Distinct changes in Lp (a) levels were induced by both forms of treatment (Table II; Fig. 1). Thus, estrogen treatment markedly reduced serum Lp (a) levels, with a mean decrease of 51% (*P* < 0.001). Of the 15 patients in the estrogen-treated group, a decrease in Lp (a) was found in 13, and in many cases the decrease was pronounced (Fig. 1). In contrast, Lp (a) levels were increased by a mean of 20% in the orchidectomized group (*P* < 0.01).

Since there is a wide variation in serum Lp (a) levels, and this variation to a major extent is determined by the specific apo (a) phenotype, it was of particular importance to study the individual response in subjects with varying apo (a) phenotypes. As exemplified in Fig. 2, the changes in Lp (a) concentrations could be demonstrated, regardless of protein size isoform. No relationship was observed between apo (a) phenotype and the magnitude of change in Lp (a) level in this limited series of patients. Furthermore, there were no identifiable differences in the clinical characteristics of the individuals who did not display the general pattern of response to treatment.

Additional support for the concept of opposite effects of estrogen treatment and orchidectomy on serum Lp (a) levels could be gained from the data obtained in three patients with

Table I. Effects on Serum Hormone Levels in Patients with Prostatic Cancer Treated with Estrogen or Orchidectomy

	Estrogen (<i>n</i> = 15)		Orchidectomy (<i>n</i> = 16)	
	Baseline	6 mo	Baseline	6 mo
Testosterone (nmol/liter)	17.8±1.9	0.9±0.1*	22.7±2.0	0.7±0.1*
Follicle-stimulating hormone (arbitrary U/liter)	6.4±0.6	6.5±2.6 [‡]	5.7±0.7	24.6±2.8*
Luteinizing hormone (arbitrary U/liter)	8.9±1.1	5.7±1.9 [‡]	7.8±0.6	25.4±3.6*
Growth hormone (μg/liter)	1.6±0.5	6.6±1.7 ^{§†}	2.1±0.8	0.5±0.2

* Significantly different from baseline, *P* < 0.001; [‡] *P* < 0.01.

[†] Significantly different from orchidectomy, *P* < 0.01; [‡] *P* < 0.001.

Means±SEM.

Table II. Effects on Serum Lipid and Lipoprotein Levels in Patients with Prostatic Cancer Treated with Estrogen or Orchidectomy

	Estrogen		Orchidectomy	
	(n = 15)		(n = 16)	
	Baseline	6 mo	Baseline	6 mo
Cholesterol (mmol/liter)	5.2±0.2	5.0±0.2	5.4±0.2	5.4±0.3
Triglycerides (mmol/liter)	1.4±0.1	1.7±0.2	1.5±0.2	1.6±0.2
HDL cholesterol (mmol/liter)	1.2±0.1	1.9±0.1 ^{‡§}	1.3±0.1	1.4±0.1
LDL cholesterol (mmol/liter)	3.4±0.2	2.3±0.2 [‡]	3.3±0.2	3.4±0.2
Lipoprotein (a) (mg/liter)*	134 (<12-434)	66 (<12-193) [‡]	141 (<12-577)	168 (<12-588) [‡]

* Mean (range).

[‡] P < 0.01; [§] P < 0.001 compared to baseline.

[§] P < 0.01; ^{||} P < 0.001 compared to orchidectomy.

Means±SEM.

To convert mmol/liter to mg/dl, multiply cholesterol by 38.7 and triglycerides by 88.5.

prostatic cancer who first received estrogen therapy for six months, and subsequently were switched to treatment with orchidectomy due to lack of clinical improvement. From analysis of these patients (Table III), it is very clear that the pronounced effect of estrogen was reversed in response to orchidectomy.

When the change in Lp (a) level in the 31 patients was analyzed in relation to the other lipoprotein changes, there was a significant relation to the relative change in HDL cholesterol

($r = 0.62, P = 0.0004$) but not to that in LDL cholesterol. Furthermore, no relations between the relative changes in Lp (a) and those in hormone levels were observed in the treated groups. When multiple regression analysis was performed the relative change in Lp (a) concentration could in part be explained by the relative changes in HDL cholesterol and triglyceride levels (relative change in Lp [a] = $[1.1 - 0.7 \times \text{relative change in HDL cholesterol} - 0.2 \times \text{relative change in triglyceride}]$).

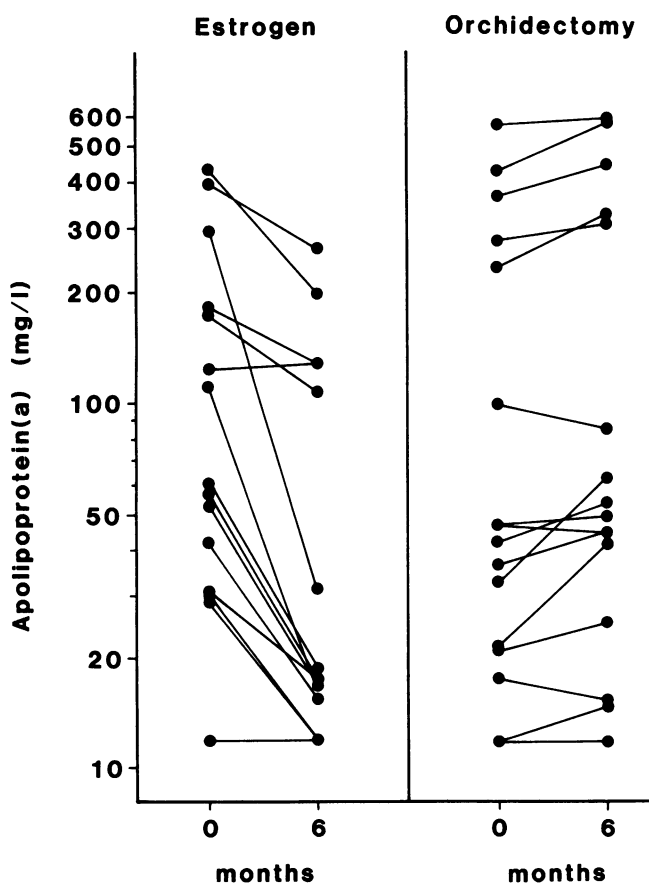


Figure 1. Individual serum Lp (a) levels at baseline and after six months treatment with estrogen or orchidectomy. Values below the detection limit of the assay (12 mg/liter) are displayed as 12 mg/liter.

Discussion

The serum concentration of Lp (a) is under strict genetic control (1-3). However, in addition to variation of the number of kringle 4 repeats, the level of Lp (a) is clearly influenced by other factors, both genetic and nongenetic. The pronounced polymorphism of apo (a) in the population has considerably hampered studies of potentially important regulatory mechanisms, such as hormonal influences. It is of some interest that in one animal model, the European hedgehog, in which Lp (a) is the major apo B-containing lipoprotein (41), there is a profound seasonal variation of Lp (a) concentration which appears to be related to serum testosterone levels (42). In previous human studies, no significant differences between sexes in Lp (a) levels have been reported (23, 24). However, higher serum levels of Lp (a) have been reported in postmenopausal compared to premenopausal females (23). Except for two brief reports (36, 43), there is very little information on the effects of hormonal treatment on Lp (a) levels in man.

In the present study, we could clearly demonstrate significant and contrasting effects of two hormonal interventions in elderly males with prostatic cancer. In response to orchidectomy, resulting in lowered serum testosterone levels, there was a 20% mean increase in serum Lp (a) levels, regardless of apo (a) phenotype. In marked contrast, estrogen treatment resulted in drastic reductions in serum Lp (a) levels, again regardless of size isoform. Thus, it would seem that the presence of sex hormone suppresses Lp (a) levels, and the fact that both male and female hormones appear to have a lowering effect may be relevant for the lack of observed sex differences (23, 24). The estrogen doses used in the present study were, however, pharmacological, and the results may thus not be comparable to those

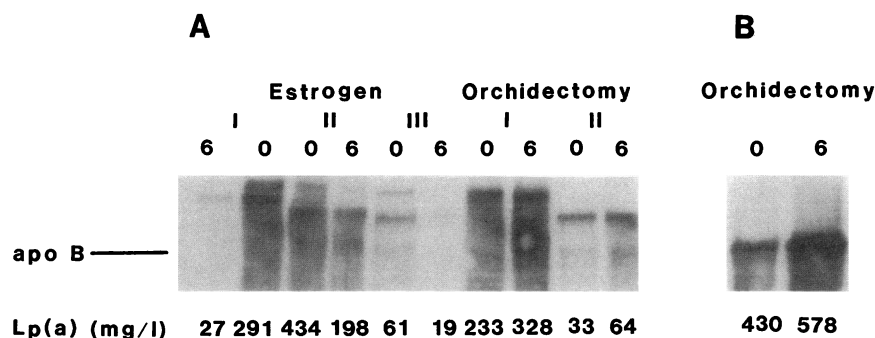


Figure 2. Immunoblot analysis of the apo (a) protein in patients with prostatic carcinoma before (0) and after (6) six months estrogen treatment or orchidectomy. Measured values for Lp (a) concentrations are given below. According to the nomenclature of Utermann (18), the phenotypes of the patients correspond to (from left to right) S3/S4; S2/S4; S1/S3; S3; S1; B.

obtained in response to the hormone levels observed under physiological conditions. In this context, it is nevertheless of interest to note that a lowering of Lp (a) levels has been observed during treatment with the anabolic steroid stanozolol in postmenopausal females (43).

Although the results of the present study were distinct, it is difficult to fully explain the mechanisms through which estrogen treatment or orchidectomy regulate Lp (a) levels. According to current view, the synthesis of Lp (a) is more important than its clearance in determining the final serum concentration (1–3). Thus, turnover studies have failed to explain the increased Lp (a) levels in familial hypercholesterolemia by a reduced fractional catabolic rate (44, 45) and pharmacological treatment aimed at reducing lipoprotein synthesis, such as nicotinic acid therapy, appears to be more efficient in reducing Lp (a) levels (32, 33). In males treated with estrogen, the formation of LDL is increased by almost 30%, however (35), which clearly contrasts against the 50% reduction in Lp (a) levels observed in the present study during an identical regime. It is of course possible that sex hormones, and particularly estrogen, influence the assembly of apo (a) to apo B which is believed to occur through a disulphide linkage in the hepatocyte (2, 3). Indirect evidence that such a mechanism may be of importance emerges from the recent finding of a reduced Lp (a) concentration in individuals with high Lp (a) levels during treatment with the reducing agent acetylcysteine (46).

Table III. Effects on Serum Cholesterol and Lp (a) Levels in Three Patients with Prostatic Carcinoma before and after Treatment with Estrogen for Six Months, and Six Months after Subsequent Orchidectomy and Cessation of Estrogen Therapy

	Basal	Estrogen treatment	Orchidectomy
Patient 1 (70 yr; 72 kg)			
Total cholesterol (mmol/liter)	8.1	5.7	8.9
HDL cholesterol (mmol/liter)	0.9	1.6	1.2
Lp (a) (mg/liter)	423	219	473
Patient 2 (69 yr; 85 kg)			
Total cholesterol (mmol/liter)	6.3	5.1	7.6
HDL cholesterol (mmol/liter)	1.1	2.2	1.3
Lp (a) (mg/liter)	112	<12	165
Patient 3 (71 yr; 63 kg)			
Total cholesterol (mmol/liter)	5.6	4.5	6.4
HDL cholesterol (mmol/liter)	1.2	2.0	1.0
Lp (a) (mg/liter)	18	<12	25

To convert mmol/liter to mg/dl, multiply cholesterol by 38.7.

During estrogen treatment in males, the fractional catabolic rate of LDL apo B is increased by ~ 80% (35) due to a threefold induction of hepatic LDL receptors (Angelin, B., H. Olivecrona, M. Rudling, E. Reihner, S. Ewerth, P. Henriksson, and K. Einarsson, submitted for publication). This results in clear reductions of LDL cholesterol levels, as also observed in the present study. It is now evident that Lp (a) can be cleared through LDL receptor-mediated mechanisms, although the catabolism may be somewhat reduced compared to that of LDL (31, 44, 47–49). In fact, treatment with drugs such as cholestyramine and cholesterol synthesis inhibitors, which are known to induce hepatic LDL receptor expression in man (50, 51), do not reduce, and sometimes may even increase, Lp (a) levels (12, 28–30). However, recent studies using a drastic induction of human LDL receptors in transgenic mice have demonstrated the possibility for an enhanced clearance of Lp (a) particles during stimulation of the LDL receptor pathway (31). Thus, it is still possible that at least part of the estrogen-mediated effect on Lp (a) serum concentration observed in our study is mediated through an increased clearance. However, no correlation between the change in Lp (a) concentration and the change in LDL cholesterol level was observed in the estrogen-treated group. Thus, studies using kinetic analysis of Lp (a) turnover are needed to establish the relative importance of Lp (a) clearance and synthesis for the effects of estrogen. The importance of testosterone, and “normal” levels of estrogen, may well be exerted through effects on Lp (a) synthesis, although this cannot be decided presently. Finally, although we could not find any statistical correlation to the changes in the concentrations of other hormones induced by estrogen treatment or orchidectomy, further studies are needed to ascertain whether or not some treatment effects may be mediated by secondary hormonal changes.

The distinct effects of sex hormones, and particularly exogenous estrogen, on serum Lp (a) levels may have some clinical relevance. Thus, in addition to the effects on LDL and HDL cholesterol levels, a reduced level of Lp (a) may have an important role in explaining the apparently reduced risk for ischemic heart disease and stroke in estrogen-treated postmenopausal females (52–54). Preliminary evidence for a reduced Lp (a) concentration during combined treatment with estrogen and progestagen has recently been presented (36). However, the complexity of the metabolic response to estrogen should be evident from the fact that estrogen-treated males with prostatic cancer actually have a higher risk for subsequent cardiovascular events when compared to those treated by orchidectomy (55, 56).

In conclusion, Lp (a) levels in males were found to decrease drastically upon estrogen treatment and to increase after orchidectomy.

dectomy, suggesting that sex hormones, particularly estrogen, exert a regulatory role of the serum Lp (a) level in man.

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