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Reduction of Serum Lipoprotein (a) by Estrogen in Men with Prostatic Cancer

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Abstract. The catabolism of lipoprotein(a), Lp(a), remains unclear. Very recently we observed that estrogen, a hormone known to increase low-density lipoprotein (LDL) receptor activity, reduced serum Lp(a) levels in a man with familial hypercholesterolemia (FH) (JAMA 267: 2328, 1992). In the present study, we attempted to further evaluate this Lp(a)-lowering action of estrogen in men without FH. Seven men, aged 61–84 yr, treated with estrogen for prostatic cancer were the subjects and seven men who underwent surgical treatment without estrogen therapy served as controls. Fasting blood was collected before and 1–3 months after estrogen therapy, and serum Lp(a) levels and lipoprotein profiles were determined. Estrogen treatment caused significant changes in serum lipoproteins, i.e., decreases in LDL-cholesterol, and increases in high-density lipoprotein (HDL)-cholesterol. Serum triglyceride levels tended to increase. Serum apo A-1 underwent a two-fold increase, while apo B did not change. Serum Lp(a) levels ranged from 8 to 62 mg/d*l*. After estrogen treatment serum Lp(a) was reduced markedly, with a mean reduction of 81% (71–95%). Serum lipids, lipoproteins and Lp(a) did not change significantly in the controls. The results demonstrated a regulating effect of estrogen on serum Lp(a) levels, and the findings further suggested that Lp(a) is removed via LDL receptors. However, previous studies have shown that maneuvers causing a decrease in LDL-cholesterol do not always cause a reduction in serum Lp(a). Thus, our findings suggested the possible presence of a receptor which is estrogen-inducible and different from the LDL receptor.

Key words: LDL-cholesterol, HDL-cholesterol, Lp(a), Estrogen, LDL receptor.

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LIPOPROTEIN (a) (Lp(a)) [1] is a low-density lipoprotein (LDL)-like particle to which the glycoprotein apolipoprotein (a) (apo(a)) is attached through disulfide binding [2, 3]. Because of its structural similarity to plasminogen [4], Lp(a) is believed to impair fibrinolysis and initiate atherogenesis [5]. The serum Lp(a) concentration is determined genetically and seems to be independent of age, sex and diet [2, 3]. A number of clinical studies have shown that high serum Lp(a) is associated with atherosclerosis of both coronary [6, 7] and cerebral arteries [8]. Moreover, the serum Lp(a) level is an indicator of the presence and severity of coronary artery disease [9]. Because of its clinical significance, it is very important to evaluate attempts to lower the serum Lp(a) concentration *in vivo*. The serum Lp(a) concentration is the result of a balance between synthesis and elimination. Lp(a) is mostly synthesized in the liver, however, the site of catabolism of Lp(a) remains unknown [2]. The LDL-receptor is a candidate for Lp(a) removal from the circulation. To investigate this possibility, several hypolipidemic agents, such as cholestyramine [10] and 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase inhibitors [11, 12], which induce LDL receptor expression, have been used to lower

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serum Lp(a), but most of these attempts have failed to demonstrate any reduction. The only exception is estrogen, a hormone which elevates LDL receptors and reduces LDL-cholesterol [13, 14]. Very recently, we observed a marked reduction in serum Lp(a) in a patient with familial hypercholesterolemia (FH) following estrogen therapy for prostatic cancer [15]. In the present study, we have attempted to further evaluate this intriguing Lp(a)-lowering action of estrogen in men without FH. A preliminary report of this work was presented at the Second International Conference on Lipoprotein [a], November 12–14, 1992.

Materials and Methods

Subjects

Fourteen men, aged 61–84 yr, who had prostatic cancer and were recruited from June, 1991 to April, 1992 at the urology outpatient clinic of Toranomon Hospital, served as the subjects (Table 1). The diagnosis was established histopathologically by needle biopsy of the prostate of all of the patients. Seven men had advanced prostatic cancer, either stage C or D [16], and received estrogen. Of these 7 men treated with estrogen, 5 underwent castration, 1 total prostatectomy, and 1 received no surgical treatment at all. Estrogen was administered as ethinyl estradiol, 1.5 mg per os daily. The remaining 7 patients, who were not treated with estrogen, served as controls. Of these 7 patients, 4 underwent total prostatectomy for localized disease, and 3 underwent castration alone for extensive disease. The two groups were well matched in terms of age and body mass index. Patients with overt signs of myocardial infarction, angina pectoris, and other types of arteriosclerotic disease were excluded from this study. None of these patients had been taking hypolipidemic agents or other drugs which affect serum lipid levels.

Determination of serum Lp(a) and lipoprotein profiles

Fasting blood was collected before and 1–3 months after estrogen treatment, and the serum Lp(a) and the lipoprotein profiles were determined. Blood samples were drawn via venipuncture after at least 12 h of fasting. Blood was allowed to clot for 1 h at room temperature, and the serum was obtained by low-speed centrifugation. Fresh serum samples were used to determine serum lipids, Lp(a) and apolipoproteins. Serum

Table 1. Profiles of study subjects

Patient	Age (yr)	BMI (kg/m^2)	Basal lipids and Lp(a) (mg/dl)				Surgical Treatment*
			TC	TG	HDL-C	Lp(a)	
a) Estrogen-treated subjects							
1	61	21.1	222	78	40	62	P
2	84	19.4	168	57	63	12	(-)
3	66	16.8	109	86	26	19	C
4	84	17.2	277	187	51	11	C
5	69	24.0	181	102	43	16	C
6	61	25.5	196	126	44	8	C
7	67	33.0	162	118	55	20	C
b) Controls							
1	70	24.6	173	112	53	20	P
2	75	17.9	180	57	63	48	P
3	66	24.5	285	177	53	55	P
4	76	21.5	227	43	51	28	P
5	82	22.7	206	188	43	6	C
6	83	18.3	240	55	77	7	C
7	80	20.7	173	61	58	3	C

*P, prostatectomy; C, castration.
BMI, body mass index; TC, total cholesterol; TG, triglyceride; HDL-C, HDL-cholesterol.

cholesterol [17] and triglyceride [18] were determined enzymatically, and HDL-cholesterol was measured by the heparin-Mn^{2+} precipitation method [19]. Serum LDL-cholesterol levels were calculated by means of the Friedewald equation (LDL-cholesterol = total cholesterol − HDL-cholesterol − triglyceride/5) [20]. Serum apolipoproteins (apo) A-1, B and E were measured by a single radial immunodiffusion method [21]. Portions of the serum samples were stored at −70°C until the time of the Lp(a) assay. Serum Lp(a) concentrations were determined by enzyme-linked immunosorbent assay (ELISA) with a commercial kit (Tint Eliza, Lp(a), Biopool, Sweden) which detects serum concentrations in the range of 1 to 60 mg/d*l*. When serum was found to contain relatively high levels of Lp(a), samples were diluted and the Lp(a) concentration was measured again. Intra- and interassay coefficients of variation were 2.5% (n=14) and 6.7% (n=8), respectively [9].

Statistical analysis

All results are expressed as the mean ± SEM. Student's *t*-test for paired and unpaired samples was performed to compare baseline levels and changes after 1–3 months of treatment. Because of the skewed distribution of the Lp(a) concentrations, we used a non-parametric test to compare Lp(a) levels before and after treatment.

Results

Serum lipids and apoproteins

The serum lipid and apoprotein data before and after estrogen therapy are summarized in Table 2. Among the 7 subjects treated with estrogen, 2 had hypercholesterolemia and 5 had normal serum lipids before estrogen. All measurements, including age, body mass index, serum lipids and apolipoproteins, were similar in both groups (Table 1).

Among the estrogen-treated subjects, serum LDL-cholesterol decreased by 48%, whereas serum HDL-cholesterol increased by 57%. Serum triglyceride tended to increase (Fig. 1). Estrogen administration caused a two-fold increase in serum apo A-1, but there were no changes in either apo B or apo E (Fig. 2). In the control group, there were no significant changes in serum lipids or apoproteins during the study period (Table 2).

Serum Lp(a)

The serum Lp(a) concentration varied greatly from subject to subject, widely ranging from 8 to 62 mg/d*l*. The serum Lp(a) concentration was not correlated with the serum cholesterol level (r=0.334) or LDL-cholesterol (r=0.374). In the estrogen-treated subjects, the absolute serum Lp(a) level decreased significantly ($P<0.05$) (Fig.

Table 2. Changes in serum lipids and lipoprotein profile

	Estrogen (+) (n=7)		Estrogen (−) (n=7)	
	before	after	before	after
(a) Lipids (mg/d*l*)				
Triglyceride	108±16	174±30	99±23	90±18
Cholesterol	189±20	168±11	212±16	206±20
LDL-cholesterol	120±17	62±9**	135±14	128±17
HDL-cholesterol	46±4	72±6**	57±4	60±6
(b) apolipoproteins (mg/d*l*)				
apo A-1	116±9	204±9**	141±8	144±13
apo B	82±8	77±10	92±10	92±8
apo E	4.7±0.5	3.5±0.3	5.2±0.6	5.5±0.5
(c) Lp(a) (mg/d*l*)	21±7	4±2*	24±8	28±9

Data were shown as the mean±SEM. *, $P<0.05$; **, $P<0.01$.

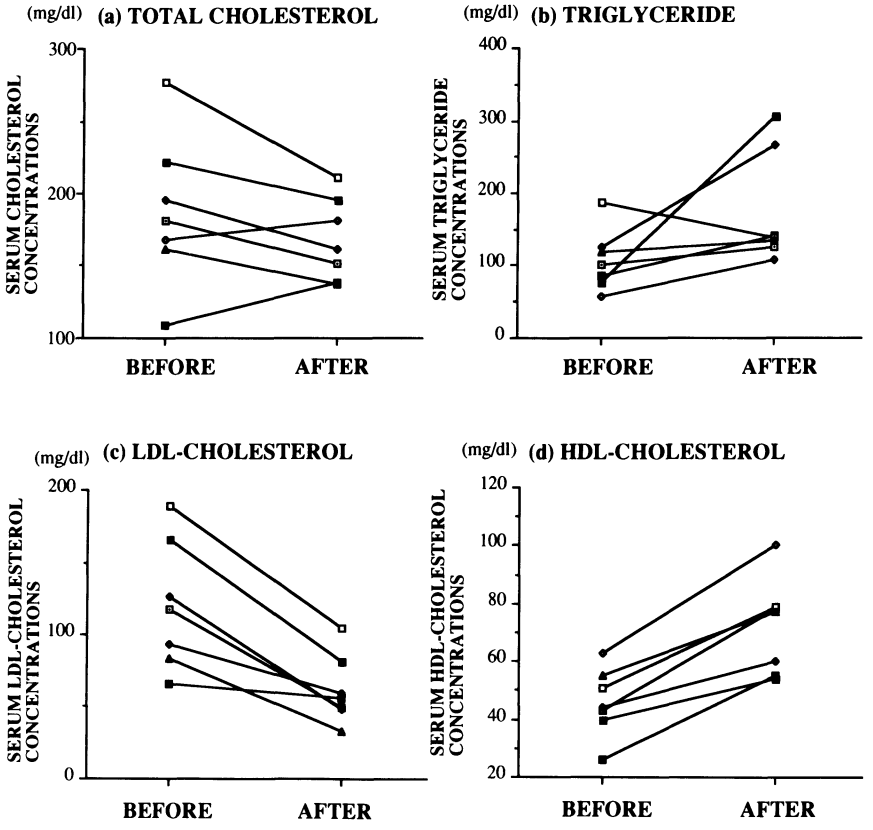

Fig. 1. Changes in serum lipid concentrations in estrogen-treated subjects. (a) Total cholesterol, (b) Triglyceride, (c) LDL-cholesterol, (d) HDL-cholesterol.

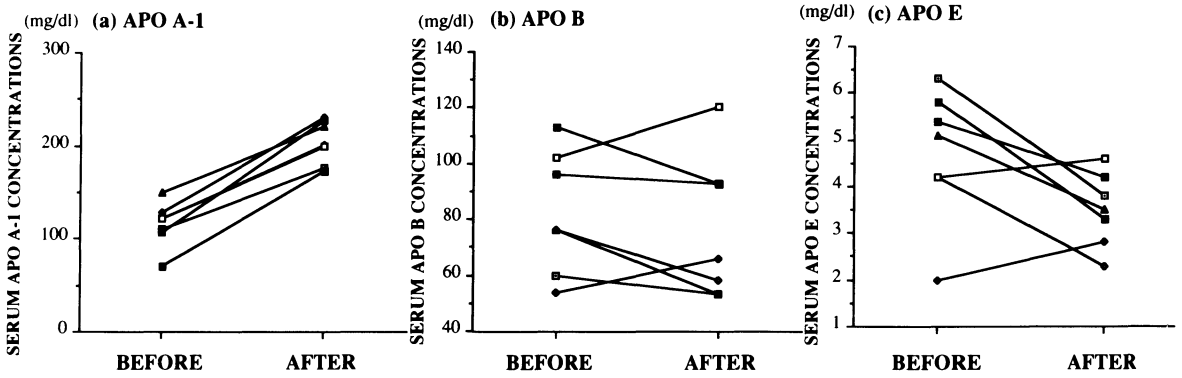

Fig. 2. Changes in serum apolipoprotein concentrations in estrogen-treated subjects. (a) apo A-1, (b) apo B, (c) apo E.

3). When the percent reduction was calculated, because initial values differed greatly, it was highly significant (% reduction: 71–95%, $P<0.001$). There was no correlation between the change in LDL-cholesterol and the reduction in Lp(a) ($r=0.034$). There was no change in serum Lp(a) in the control group (Fig. 3).

Discussion

Although Lp(a) has attracted attention as a new risk factor for atherosclerosis of both the coronary [6, 7, 9] and cerebral [8] arteries, many aspects of its metabolism remain unclear. Previous studies have indicated that the metabolic behavior of this specific lipoprotein differs from that of other lipoproteins, such as LDL, and the serum Lp(a)

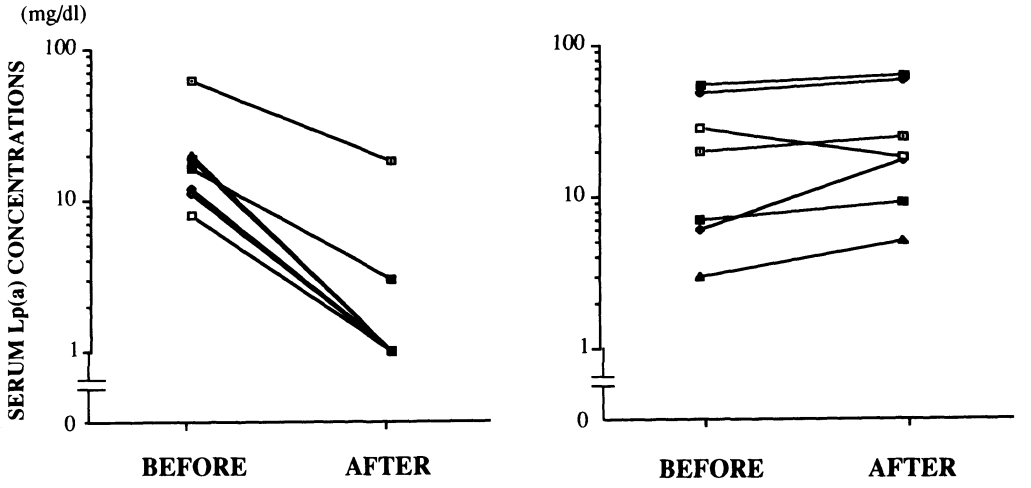

Fig. 3. Changes in serum Lp (a) concentrations in estrogen-treated subjects (left) and controls (right).

level is believed to be mostly under genetic control [2, 3]. The present study demonstrated a regulatory role of estrogen on the serum Lp(a) level, and the findings also provided a clue to the clarification of serum Lp(a) metabolism.

Previously we unexpectedly found that estrogen therapy for prostatic cancer caused a marked reduction in serum Lp(a) in parallel with a reduction in LDL in a man with FH [15]. Our observation was limited to only one man with a particular disease, and we attempted to further evaluate this intriguing action of estrogen in a significant number of subjects without any particular lipoprotein disorders. In the present study, we demonstrated that estrogen therapy for male subjects with prostatic cancer caused a significant reduction of serum Lp(a), and the results were the same as observed in the man with FH. Preliminary evidence for this estrogen action was presented by Soma *et al.* [22] in postmenopausal women. Very recently, Henriksson *et al.* [23] reported a decrease in serum Lp(a) during estrogen treatment in men with prostatic cancer. Our observations are essentially the same as theirs.

Although the exact mechanism of estrogen's action was unknown, we previously proposed [15] that enhanced Lp(a) removal via LDL receptors might be responsible for the reduction in serum Lp(a), because the reduction in serum Lp(a) occurred in parallel with that of LDL-cholesterol, and estrogen is known to cause a significant decrease in LDL by increasing LDL-receptor expression, and hence LDL uptake in the liver [13, 14]. Certainly the LDL receptor is a possible candidate, but our recent observations [24] as well as those of others [10–12], indicate that intervention trials with hypolipidemic agents, such as cholestyramine and HMG CoA reductase inhibitors, which induce LDL-receptor expression, do not cause a reduction in serum Lp(a), while estrogen does. In more detail, we observed that the serum Lp(a) level in FH subjects without medication did not differ significantly from that in FH subjects treated with either or both cholestyramine and HMG CoA reductase inhibitor (31.2±27.7, n=11 *vs.* 27.9±20.5 mg/d*l*, n=15, respectively) [24]. At present we have no explanation for this different effect of maneuvers which increase LDL receptor activity. Anyway, this led us to speculate that there may be another receptor, which is estrogen-inducible and specific for Lp(a), although no such receptor has yet been demonstrated. Recently it has been reported that cholesteryl ester-loaded macrophages can internalize and degrade large amount of ^{125}I-Lp(a) [25], however, no evidence has been available showing that the receptors can be induced by estrogen. Thus, the possible existence of an Lp(a) receptor remains to be assessed in further investigations.

It has been reported that the serum Lp(a) level was high in subjects with leukemia, and after remission the level decrease [26]. In our present study, the serum Lp(a) level did not change after total prostatectomy in subjects without estrogen. This indicates that the situation in prostatic cancer is different from that in leukemia.

Estrogen administration causes profound

changes in serum lipoproteins in both males [23] and females [27]. As reported earlier [15], in the present study we observed that, in addition to a significant reduction in both Lp(a) and LDL-cholesterol, estrogen administration caused a remarkable increase in both HDL-cholesterol and apo A-1, a major constituent of HDL-particles. Serum triglyceride tended to increase after estrogen. It is known that estrogen enhances VLDL production by the liver [27], and this might be the cause of the increase in serum triglyceride in our patients. Although VLDL production was enhanced, LDL removal was also augmented by increased LDL receptor activity. The total number of VLDL and LDL particles probably did not change, and hence the amount of serum apo B remained unchanged.

There have been no reports stating that estrogen increase Lp(a) production by the liver, but this possibility remains to be assessed in further studies.

In conclusion, estrogen treatment caused a marked reduction in serum Lp(a) in male subjects with prostatic cancer. The study demonstrated a regulating effect of estrogen on serum Lp(a) and our findings also suggested the possible presence of a receptor which is estrogen-inducible and different from the LDL receptor.

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