Corticotropin-Induced Reduction of Plasma Lipoprotein(a) Concentrations in Healthy Individuals and Hemodialysis Patients: Relation to Apolipoprotein(a) Size Polymorphism

Margret Arnadottir, Anna-Lena Berg, Florian Kronenberg, Arno Lingenhel, Therése Hugosson, Jörgen Hegbrant, and Peter Nilsson-Ehle

Lipoprotein(a) [Lp(a)], a strong independent cardiovascular risk factor, consists of the unique apolipoprotein(a) [apo(a)] covalently linked to a low-density lipoprotein particle. Apo(a) contains a widely differing number of the plasminogen-like kringle IV, a size polymorphism that is codominantly inherited. In addition to powerful genetic control, renal failure is known to influence the plasma Lp(a) concentration. There is still a lot to be learned about the mode and site of catabolism of Lp(a), and there is no readily applicable Lp(a)-lowering treatment available. Therefore, it was of interest to study further the Lp(a)-lowering effect of corticotropin (ACTH) that has been demonstrated in small studies. The main purpose of the present study was to investigate the influence of ACTH on different apo(a) isoforms. Short-term treatment with ACTH decreased the plasma Lp(a) concentration in all 26 study participants. The two study groups (12 healthy individuals and 14 hemodialysis patients) responded similarly, with a median decrease in plasma Lp(a) of 39% and 49%, respectively. In subjects with two clearly separable apo(a) bands, apo(a) phenotyping and densitometric scanning of the bands before and after treatment with ACTH revealed a change in the proportion of apo(a) isoforms, ie, a shift toward the isoform with lower molecular weight. This was observed in seven of nine investigated subjects (four of five healthy individuals and three of four hemodialysis patients).

AN INCREASED PLASMA concentration of lipoprotein(a) [Lp(a)] is a strong independent risk factor for the development of atherosclerosis.[1-3] Accordingly, much interest is focused on the factors that determine the plasma Lp(a) concentration, ie, genetic control, metabolic pathways, and therapeutic agents. Several important aspects of the genetic control mechanism have been elucidated, but the catabolic pathways are largely unknown and no readily applicable treatment is available.[4]

Lp(a) consists of a low-density lipoprotein particle linked by a disulfide bridge to apolipoprotein(a) [apo(a)], a glycoprotein that varies considerably in size among individuals.[5] There is a high degree of homology between plasminogen and apo(a).[6] The former protein contains five subunits called kringles (I to V), whereas the latter is characterized by repetition of a widely differing number of the plasminogen-like kringle IV, explaining the interindividual size variation.[6] This size polymorphism of apo(a) is determined by the apo(a) gene on chromosome 6, which contains a corresponding number of repeats of the kringle IV encoding sequence.[7] The plasma Lp(a) concentration correlates inversely with the molecular weight of Lp(a),[7] and consequently also with the size of the apo(a) gene.[8] In fact, the apo(a) gene locus has been shown to account for more than 90% of the variation in plasma Lp(a) concentrations in healthy individuals, roughly half of which can be ascribed to the size polymorphism.[9,10] The pattern of inheritance is codominant.

Safe and effective means to decrease plasma Lp(a) have been sought, hitherto not successfully. Recently, we reported that short-term treatment with corticotropin (ACTH) induces a Lp(a)-lowering effect in healthy individuals,[11] steroid-treated patients with kidney disease,[12] and hemodialysis patients.[13]

The aim of the present study was to investigate further the Lp(a)-lowering effect of ACTH, taking the size polymorphism of apo(a) into consideration. Two groups were studied, healthy individuals and hemodialysis patients. In both groups, the apo(a) gene locus would be expected to be a major determinant of the Lp(a) concentration, whereas in hemodialysis patients, uremia exerts an additional influence that increases Lp(a).

SUBJECTS AND METHODS

Subjects

Twelve healthy individuals and 14 hemodialysis patients were included in the study after provision of informed consent and approval by the local ethics committees. The healthy subjects were male medical students with a median age of 25 years (range, 22 to 29). They were selected to represent a wide range of plasma Lp(a) concentrations. Hemodialysis patients were recruited at the Dialysis Department, National University Hospital, Reykjavik, and at Park Dialys, Lund. These 10 male and four female patients had a median age of 62 years (range, 49 to 72). They received low-flux hemodialysis for 4 to 5 hours three times per week.

All study participants had normal results on liver function tests and were nondiabetic and euthyroid. The healthy subjects had serum creatinine concentrations within the reference range. None of the study participants were under treatment with a lipid-lowering agent or steroid hormone.

Procedure

The healthy individuals underwent the following procedure: day 1, blood samples were collected at 8 AM, and thereafter, 1 mg Synacthen Depot, a synthetic *N*-terminal fragment (1-24) of ACTH (Ciba-Geigy, Basel, Switzerland), was administered; days 2, 3, and 4, Synacthen Depot 1 mg was injected intramuscularly at 8 AM; and day 5, blood

From the Department of Medicine, National University Hospital, Reykjavik, Iceland; Departments of Nephrology and Clinical Chemistry, University Hospital, Lund, Sweden; Institute of Medical Biology and Human Genetics, University of Innsbruck, Innsbruck, Austria; and Park Dialys, Gambro Group Renal Care, Lund, Sweden.

Submitted April 7, 1998; accepted August 26, 1998.

Supported by grants from the Research Foundation of National University Hospital, Reykjavik, the Austrian Program of Advanced Research and Technology of the Austrian Academy of Science, Austrian Nationalbank (5553), the Swedish Medical Research Council (04966), the Medical Faculty, University of Lund, and the Påhlssons Foundation.

Address reprint requests to Margret Arnadottir, MD, Department of Medicine, National University Hospital, IS-101 Reykjavik, Iceland.

0026-0495/99/4803-0013$10.00/0

samples were drawn at 8 AM. Hemodialysis patients underwent the following scheme: day 1, blood samples were collected before a hemodialysis session performed in the morning. Directly after treatment, Synacthen Depot was injected intramuscularly at a dose of 1 mg; days 3, 4, 5, and 6, Synacthen Depot 1 mg was injected intramuscularly, always at noon; and day 8, blood samples were drawn before dialysis.

Blood Samples

Blood samples were collected after a 12-hour fast for analysis of plasma Lp(a) concentrations and determination of apo(a) phenotypes and genotypes. Plasma and whole-blood samples were stored frozen at −20°C to allow analysis in one series.

Analyses

Lp(a) was quantified with a double-antibody enzyme-linked immunosorbent assay with an affinity-purified polyclonal rabbit anti-apo(a) for coating and the horseradish peroxidase–conjugated monoclonal antibody 1A2 for detection.[14] Each sample was analyzed in triplicate.

Apo(a) phenotyping and genotyping was performed on samples obtained before administration of ACTH. Phenotyping was performed with sodium dodecyl sulfate–agarose gel electrophoresis of plasma under reducing conditions, followed by immunoblotting using the monoclonal antibody 1A2 for detection of apo(a) isoforms.[15]

Phenotyping was performed on samples from nine subjects (five healthy individuals and four hemodialysis patients) obtained both before and after ACTH treatment, and the relative proportions of apo(a) isoforms were quantified by densitometric scanning using a Personal Densitometer SI (Molecular Dynamics, Sunnyvale, CA). These nine heterozygotic subjects were selected on the basis of clearly separable electrophoretic bands.

The apo(a) genotype was determined by *Kpn*I digestion of genomic DNA followed by pulsed-field gel electrophoresis.[8,9]

All analyses were performed at the Institute of Medical Biology and Human Genetics, University of Innsbruck.

Statistical Methods

Data are presented as the median and range and, when indicated, as the mean ± SD. Wilcoxon's rank-order test for the paired case was used to compare variables before and after ACTH treatment. The relationship between variables was estimated by Spearman's rank correlation test.

RESULTS

The number of kringle IV repeats (the average of the genotypic isoforms within an individual) correlated inversely with the initial plasma Lp(a) concentration in both the hemodialysis patients ($r = -.37$, $P < .05$) and the healthy individuals ($r = -.42$, $P < .05$).

ACTH treatment was well tolerated by both study groups. After treatment, all subjects manifested a decrease in plasma Lp(a) (Table 1). In healthy individuals, the median relative change was −39% (−56% to −12%, $P < .01$), and in hemodialysis patients, it was −49% (−79% to −22%, $P = .001$). The mean change was −37% ± 13% and −49% ± 17%, respectively.

Both in healthy individuals and in hemodialysis patients, there was a strong correlation between the initial level and the absolute reduction of plasma Lp(a) ($r = .94$, $P < .01$, and $r = .91$, $P < .01$, respectively; Fig 1). In healthy individuals, there was an inverse correlation between the number of kringle IV repeats and the absolute reduction ($r = -.47$, $P < .05$). This relationship could not be demonstrated in hemodialysis patients.

Table 1. Plasma Lp(a) Concentration Before and After Short-Term ACTH Treatment in 12 Healthy Individuals and 14 Hemodialysis Patients

Subject No.	Lp(a) (mg/L)			
	Healthy Individuals		Hemodialysis Patients	
	Before	After	Before	After
1	881	678	576	437
2	319	224	486	338
3	173	103	476	225
4	163	103	253	149
5	137	73	175	90
6	121	107	151	31
7	108	79	87	46
8	75	35	66	29
9	57	33	57	13
10	33	20	51	40
11	23	14	48	26
12	9	4	46	23
13		—	35	15
14		—	32	13

NOTE. In the combined material, the median change in plasma Lp(a) induced by ACTH was −44% (−79% to −12%) and the mean change was −43% ± 16%.

In both groups, the relative Lp(a) reduction tended to correlate inversely with the initial Lp(a) concentration and directly with the number of kringle IV repeats. Analysis of the combined material showed that both relationships were significant ($r = -.39$, $P < .05$, and $r = .36$, $P < .05$, respectively; Fig 2).

Nine subjects who showed two clearly separable apo(a) bands on sodium dodecyl sulfate–agarose gel electrophoresis were selected for densitometric scanning of the apo(a) bands in samples collected both before and after treatment with ACTH. The results of all tests are shown in Table 2. At baseline, the isoform of higher molecular weight prevailed in all four hemodialysis patients, whereas the relationship between iso-

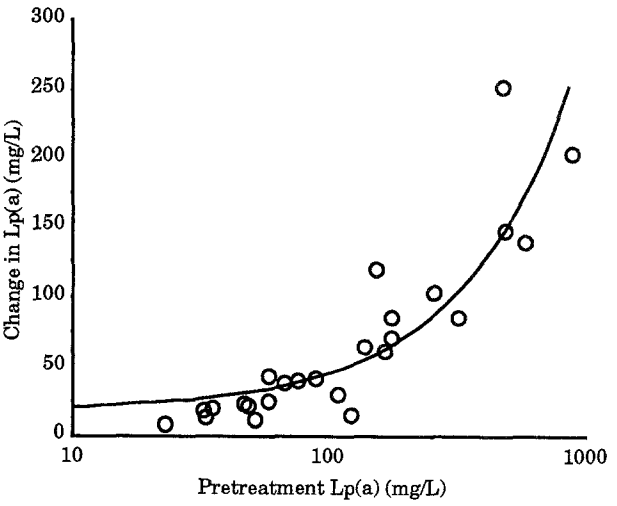

Fig 1. Relationship between the plasma Lp(a) concentration before ACTH treatment and the absolute change in plasma Lp(a) induced by ACTH treatment in the combined patient material (12 healthy individuals and 14 hemodialysis patients). Pretreatment Lp(a) is presented on a logarithmic scale.

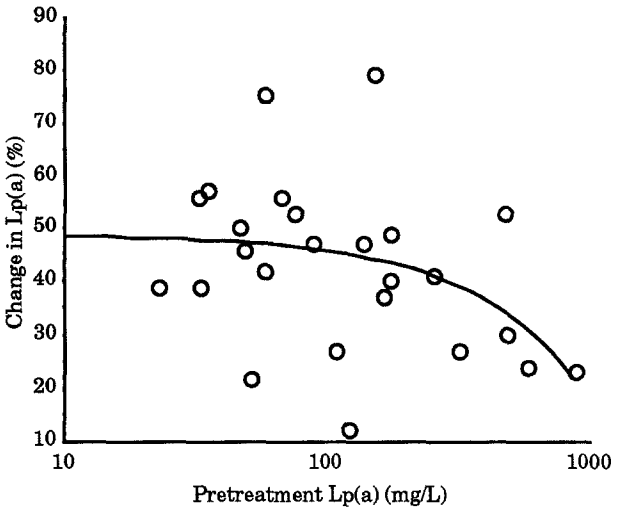

Fig 2. Relationship between the plasma Lp(a) concentration before ACTH treatment and the relative change in plasma Lp(a) induced by ACTH treatment in the combined patient material (12 healthy individuals and 14 hemodialysis patients). Pretreatment Lp(a) is presented on a logarithmic scale.

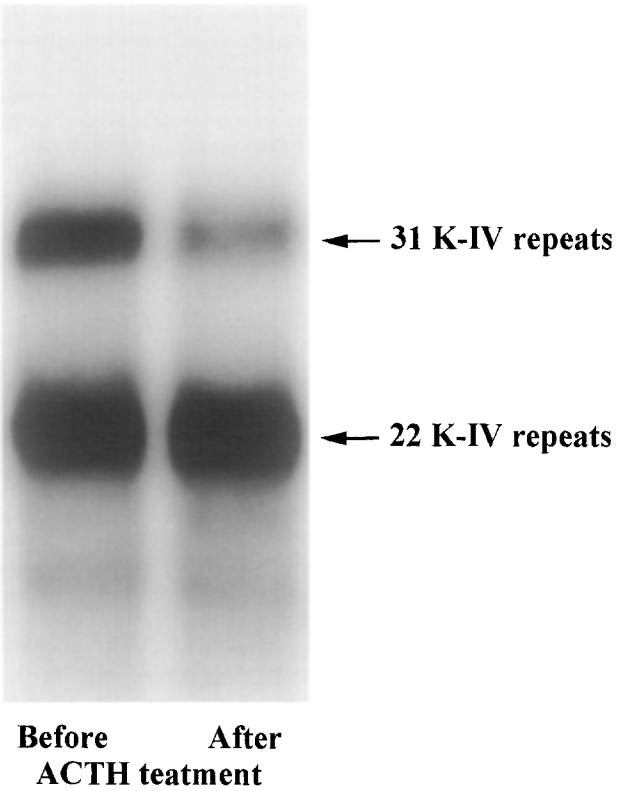

Fig 3. Example of the change in the relative proportion of apo(a) isoforms after treatment with ACTH. After immunoblotting, apo(a) isoforms were densitometrically scanned. The ratio of the lower-molecular weight (22 K-IV repeats) and higher-molecular weight (31 K-IV repeats) apo(a) bands increased from 1.04 to 1.52.

forms varied in healthy individuals. ACTH treatment induced a shift toward the isoform of lower molecular weight in seven of nine subjects (four of five healthy individuals and three of four hemodialysis patients) (Fig 3). After treatment, the median increase in the ratio of the isoforms (lower molecular weight to higher molecular weight) was 27% (−13% to 65%, $P = .0506$). The change in the ratio of the isoforms did not correlate with the initial ratio of the isoforms, initial Lp(a) concentration, absolute or relative change in Lp(a) concentration, or number of kringle IV repeats.

DISCUSSION

This study investigated the effect of short-term ACTH treatment on the plasma Lp(a) concentration and the ratio of lower- and higher-molecular weight isoforms of apo(a). Healthy individuals and hemodialysis patients responded similarly to ACTH, which caused a relative decrease in serum Lp(a) of approximately 40% to 50% and generally induced a shift toward the apo(a) isoform of lower molecular weight.

It is well known that ACTH regulates the receptors and lipolytic enzymes involved in the handling of cholesterol for steroid production in the adrenal gland.[16] In short-term treatment studies, we have recently demonstrated that ACTH also has strong effects to decrease serum lipoproteins.[11-13] These treatment studies found significant reductions in serum concentrations of low-density lipoprotein cholesterol, triglycerides, and apolipoprotein B.[11-13] The hypothesis that the effect of ACTH on lipoprotein metabolism is of a direct nature was supported by in vitro findings in HepG2 cell culture indicating facilitated uptake of apolipoprotein B–containing lipoproteins by the hepatic low-density lipoprotein receptor[11,17] and inhibited secretion of hepatic lipase[18] in response to the addition of ACTH to the medium. Furthermore, short-term treatment with ACTH decreased Lp(a) and increased high-density lipoprotein cholesterol, changes that were also found in subjects treated with dexamethasone.[11] It is not known whether the Lp(a)-lowering effect of ACTH treatment is solely mediated through an increased glucocorticoid influence or is to some extent dependent on the direct action of ACTH.

The present study was performed to expand the knowledge of the effect of ACTH on Lp(a). Two groups representing different influences on Lp(a) metabolism were included. The participants

Table 2. Effect of Short-Term Treatment With ACTH on the Relationship Between Apo(a) Isoforms (lower molecular weight/higher molecular weight) in Four Dialysis Patients and Five Healthy Individuals

	Patients				Healthy Subjects				
Parameter	P	P	P	P	H	H	H	H	H
Apo(a) isoforms (no. of K-IV repeats)	23/35	28/33	32/38	24/35	23/32	22/31	23/32	23/33	28/34
Before treatment	0.49	0.57	0.62	0.46	3.56	1.04	1.05	0.61	1.22
After treatment	0.80	0.82	0.55	0.76	3.65	1.52	1.25	0.53	1.55

were chosen to represent a wide range of initial Lp(a) concentrations. Since direct comparison between the groups was not intended, identical study protocols were not necessary.

In healthy individuals, Lp(a) concentrations can be assumed mainly to reflect variations in the apo(a) gene locus on chromosome 6. On the other hand, in renal failure, unknown mechanisms influence Lp(a) metabolism at a relatively early stage.[19] In one study, the mean Lp(a) concentration of more than 500 hemodialysis patients was found to be 27% higher than in control subjects, whereas their apo(a) phenotype distribution largely coincided with that of the general population.[20] Moreover, only patients with high–molecular weight phenotypes of Lp(a) manifested increased plasma concentrations as compared with controls.[20,21] These results are supported by the finding that after renal transplantation, a decrease in plasma Lp(a) was found only in patients with high–molecular weight apo(a) phenotypes.[22] Therefore, renal failure seems to influence Lp(a) metabolism. Recent findings are in accordance with this notion: it has been reported that the healthy human kidney is able to remove a large amount of Lp(a) from the renal circulation.[23]

The present study confirms the results of smaller studies indicating that ACTH has a strong effect of decrease on the plasma Lp(a) concentration. Such a response was demonstrated in all 26 study subjects. In absolute terms, this effect was most marked in subjects with a high initial plasma Lp(a) concentration. On the other hand, there was an inverse correlation between the magnitude of the relative change and the initial Lp(a) concentration. However, these relationships seemed to be at least partly the result of a skewed distribution of plasma Lp(a) concentrations (Figs 1 and 2). In individuals with two clearly separable apo(a) isoforms, expression of these isoforms was estimated by densitometric scanning before and after treatment with ACTH. Interestingly, a shift toward the apo(a) isoform of lower molecular weight was found in seven of nine subjects after ACTH treatment.

The shift toward the apo(a) isoform of lower molecular weight indicates that ACTH induces a decrease in the high–molecular weight isoforms of apo(a) via inhibited production or accelerated removal. Disturbed production is the more likely mechanism considering that, in general, the Lp(a) concentration seems to be controlled by variations in production.[24,25] The response to ACTH was similar in both study groups despite the different influences acting on their Lp(a) metabolism.

In addition to ACTH, other hormones such as glucocorticoids,[11] estrogen,[26] and anabolic steroids[27] are known to reduce plasma Lp(a) concentrations. For practical purposes, ACTH has the advantage of a marked effect to decrease other apolipoprotein B–containing lipoproteins as well.[11-13] At present, we do not know the therapeutic potential of the lipid-lowering effects of ACTH. Possibly, ACTH can replace glucocorticoids as a treatment in certain inflammatory diseases, acting not only as an antiinflammatory/immunosuppressive agent but also as a lipid-lowering drug. Prospective studies on this issue are in progress. Regarding future therapeutic possibilities, it is of importance to know that the lipid-lowering effect of ACTH lasted at least during 1 year of treatment and was well tolerated according to unpublished results of a treatment trial in nephrotic patients (Berg AL, Arnadottir M, Nilsson-Ehle P, May 1997).

In conclusion, the present study demonstrates a strong Lp(a)-lowering effect of short-term ACTH treatment in both healthy individuals and hemodialysis patients. Furthermore, it demonstrates that in most cases, ACTH induces a shift toward expression of the apo(a) isoform of lower molecular weight. This probably reflects an inhibited production of high–molecular weight isoforms.

ACKNOWLEDGMENT

We thank Evi Trenkwalder, Margret Stefansdottir, and Siv Svensson for excellent technical assistance. We are indebted to Professor Gerd Utermann for valuable discussions.

REFERENCES

1. Rader DJ, Hoeg JM, Brewer HB Jr: Quantitation of plasma apolipoproteins in the primary and secondary prevention of coronary artery disease. Ann Intern Med 120:1012-1025, 1994

2. Kronenberg F, Steinmetz A, Kostner GM, et al: Lipoprotein(a) in health and disease. Crit Rev Clin Lab Sci 33:495-543, 1996

3. Wild SH, Fortmann SP, Marcovina SM: A prospective case-control study of lipoprotein(a) levels and apo(a) size and risk of coronary heart disease in Stanford Five-City Project participants. Arterioscler Thromb Vasc Biol 17:239-245, 1997

4. Kronenberg F, Utermann G, Dieplinger H: Lipoprotein(a) in renal disease. Am J Kidney Dis 27:1-25, 1996

5. Utermann G: The mysteries of lipoprotein (a). Science 426:904-910, 1989

6. McLean JW, Tomlinson JE, Kuang WJ, et al: cDNA sequence of human apolipoprotein(a) is homologous to plasminogen. Nature 300:132-137, 1987

7. Utermann G, Menzel HJ, Kraft HG, et al: Lp(a) glycoprotein phenotypes: Inheritance and relation to Lp(a)-lipoprotein concentrations in plasma. J Clin Invest 80:458-465, 1987

8. Lackner C, Boerwinkle E, Leffert CC, et al: Molecular basis of apolipoprotein (a) isoform size heterogeneity as revealed by pulsed-field gel electrophoresis. J Clin Invest 87:2153-2161, 1991

9. Kraft HG, Köchl S, Menzel HJ, et al: The apolipoprotein (a) gene: A transcribed hypervariable locus controlling plasma lipoprotein (a) concentration. Hum Genet 90:220-230, 1992

10. Boerwinkle E, Leffert CC, Lin J, et al: Apolipoprotein (a) gene accounts for greater than 90% of the variation in plasma lipoprotein (a) concentrations. J Clin Invest 90:52-60, 1992

11. Berg A-L, Nilsson-Ehle P: Direct effects of ACTH on plasma lipoprotein metabolism in man: Studies in vivo and in vitro. Metabolism 43:90-97, 1994

12. Berg A-L, Nilsson-Ehle P: ACTH lowers serum lipids in steroid treated hyperlipidemic patients with kidney disease. Kidney Int 50:538-542, 1996

13. Arnadottir M, Berg AL, Dallongeville J, et al: Adrenocorticotrophic hormone lowers serum Lp(a) and LDL cholesterol concentrations but does not influence triglyceride metabolism in hemodialysis patients. Kidney Int 52:1651-1655, 1997

14. Kronenberg F, Lobentanz E-M, König P, et al: Effect of sample storage on the measurements of lipoprotein (a), apolipoproteins B and A-IV, total and high-density lipoprotein cholesterol and triglycerides. J Lipid Res 35:1318-1328, 1994

15. Kraft HG, Lingenhel A, Bader G, et al: The relative electrophoretic mobility of apo(a) isoforms depends on the gel system: Proposal of a nomenclature for apo(a) phenotypes. Atherosclerosis 125:53-61, 1996

16. Pedersen RC, Brownie AC: The mechanism of action of

adrenocorticotrophic hormone on cholesterol metabolism in the adrenal cortex, in Litwack G (ed): Biochemical Actions of Hormones, vol 21. New York, NY, Academic, 1986, pp 129-166

17. Berg AL, Nilsson-Ehle P: Regulation of hepatic lipase secretion from Hep G2 cells by ACTH and corticosteroids. Horm Metab Res 29:475-476, 1997

18. Bartens W, Krämer-Guth A, Wanner C: Corticotropin increases the receptor-specific uptake of native low-density lipoprotein (LDL)—but not oxidized LDL and native or oxidized lipoprotein(a) [Lp(a)]—in HEPG2 cells: No evidence for Lp(a) catabolism via the LDL receptor. Metabolism 46:726-729, 1997

19. Arnadottir M, Thysell H, Nilsson-Ehle P: Serum concentration of Lp(a) is increased in moderate renal failure. Nephron 72:712-713, 1996

20. Kronenberg F, König P, Neyer U, et al: Multicenter study of lipoprotein(a) and apolipoprotein(a) phenotypes in patients with end-stage renal disease treated by hemodialysis or continuous ambulatory peritoneal dialysis. J Am Soc Nephrol 6:110-120, 1995

21. Dieplinger H, Lackner C, Kronenberg F, et al: Elevated plasma concentrations of lipoprotein(a) in patients with end-stage renal disease are not related to the size polymorphism of apolipoprotein(a). J Clin Invest 91:397-401, 1993

22. Kronenberg F, König P, Lhotta K, et al: Apolipoprotein(a) phenotype associated decrease in lipoprotein(a) plasma concentrations after renal transplantation. Arterioscler Thromb 14:1399-1404, 1994

23. Kronenberg F, Trenkwalder E, Lingenhel A, et al: Renovascular arteriovenous differences in Lp(a) plasma concentrations suggest removal of Lp(a) from the renal circulation. J Lipid Res 38:1755-1763, 1997

24. Krempler F, Kostner GM, Bolzano K, et al: Turnover of lipoprotein(a) in man. J Clin Invest 65:1483-1490, 1980

25. Rader DJ, Cain W, Zech LA, et al: Variation in lipoprotein(a) concentrations among individuals with the same apolipoprotein(a) isoform is determined by the rate of lipoprotein(a) production. J Clin Invest 91:443-447, 1993

26. Tuck CH, Holleran S, Berglund L: Hormonal regulation of lipoprotein(a) levels: Effects of estrogen replacement therapy on lipoprotein (a) and acute phase reactants in postmenopausal women. Arterioscler Thromb Vasc Biol 9:1822-1829, 1997

27. Crook D, Sidhu M, Seed M, et al: Lipoprotein Lp(a) levels are reduced by danazol, an anabolic steroid. Atherosclerosis 92:41-47, 1992