sponses to acetylcholine. Although this reference group had normal coronary angiograms, referral due to chest pain sets these patients apart from a purely voluntary group with normal coronary arteries.

Much has been written about vasodilation of diseased and "control" human coronary arteries using an intravascular Doppler mounted catheter of the Millar Velocimeter (Houston, Texas) type.[11-14] Because of its catheter-based configuration, only proximal straight segments of the coronary arteries may be safely and easily accessed with this technique. This technique is also subject to a greater likelihood of artifact and poorer signal quality secondary to the size and relative inflexibility of the catheter and difficulty in ensuring coaxial positioning. With use of this technique, endothelium-dependent and endothelium-independent vasodilation has been reported in control subjects defined variously.[11-14] In some cases, mild coronary disease in the study vessel and/or severe disease in another vessel were allowed. Hypertension, hyperlipidemia, hyperglycemia, and current tobacco use are commonly seen in previously reported control subjects.

Our study describes coronary relaxation properties in a referral normal population, which we defined simply as normotensive nondiabetic subjects without angiographic coronary artery disease. Our study suggests that an optimal intracoronary adenosine bolus infusion is 16 μg. Similarly, an optimal intracoronary acetylcholine infusion rate appears to be 30 μg/min. We found that 39% of referral normal subjects had evidence of endothelial dysfunction, defined as reduced endothelium-dependent relaxation (<150% increase in coronary blood flow above baseline).

In a referral normal cardiac population, endothelium-independent coronary relaxation is nearly always normal, but endothelium-dependent relaxation may be depressed in a significant proportion of patients. Further study of the natural history of referral normal subjects with endothelial dysfunction is necessary to assess the potential cardiovascular risk of this finding in a presumed low-risk population.

1. Celermajer DS, Sorensen KE, Spiegelholter DJ, Georgakopoulos D, Robinson J, Deanfield JE. Aging is associated with endothelial dysfunction in healthy men years before the age-related decline in women. *J Am Coll Cardiol* 1994;24:471-476.
2. Treasure CB, Klein L, Vita JA, Manoukian SV, Renwick GH, Selwyn AP, Ganz P, Alexander RW. Hypertension and left ventricular hypertrophy are associated with impaired endothelium mediated relaxation in human coronary resistance vessels. *Circulation* 1993;87:86-93.
3. Zeiher A, Drexler H, Wollschlager H, Just H. Modulation of coronary vasomotor tone in humans: progressive endothelial dysfunction with different early stages of coronary atherosclerosis. *Circulation* 1991;83:391-401.
4. Drexler H, Zeiher AM. Endothelial function in human coronary arteries in vivo: focus on hypercholesterolemia. *Hypertension* 1991;18(suppl):II-90-II-99.
5. Herrington DM, Braden GA, Williams JK, Morgan TM. Endothelial dependent coronary vasomotor responsiveness in postmenopausal women with and without estrogen replacement therapy. *Am J Cardiol* 1994;73:951-952.
6. Quillen JE, Rossen JD, Oskarsson HJ, Minor RL Jr, Lopez AG, Winniford MD. Acute effect of cigarette smoking on the coronary circulation: constriction of epicardial and resistance vessels. *J Am Coll Cardiol* 1993;22:642-647.
7. Vita J, Treasure C, Nabel E. Coronary vasomotor response to acetylcholine relates to risk factors for coronary artery disease. *Circulation* 1990;81:491-497.
8. Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990's. *Nature* 1993; 362:801-809.
9. Brown BG, Zhao X-Q, Sacco DE, Albers JJ. Lipid lowering and plaque regression: new insights into prevention of plaque disruption and clinical events in coronary disease. *Circulation* 1993;87:1781-1791.
10. Haskell WL, Alderman EL, Fair JM, Maron DJ, Mackey SF, Superko R, Williams PT, Johnstone IM, Champagne MA, Krauss RM, Farquhar JW. Effects of intensive multiple risk factor reduction on coronary atherosclerosis and clinical cardiac events in men and women with coronary artery disease: the Stanford Coronary Risk Intervention Project (SCRIP). *Circulation* 1994;89:975-990.
11. Wilson RF, Laughlin DE, Ackell PH, Chilian WM, Holiday MD, Hartley CJ, Armstrong ML, Marcus ML, White CW. Transluminal, subselective measurement of coronary artery blood flow velocity and vasodilator reserve in man. *Circulation* 1985;72:82-92.
12. Houghton JL, Frank MJ, Carr AA, von Dohlen TW, Prisant LM. Relations among impaired coronary flow reserve, left ventricular hypertrophy and thallium perfusion defects in hypertensive patients without obstructive coronary artery disease. *J Am Coll Cardiol* 1990;15:43-51.
13. Drexler H, Zeiher AM, Meinzer K, Just H. Correction of endothelial dysfunction in coronary microcirculation of hypercholesterolemic patients by L-arginine. *Lancet* 1991;338:1546-1550.
14. Egashira K, Hirooka Y, Kai H, Sugimachi M, Suzuki S, Inou T, Tokeshita A. Reduction in serum cholesterol with pravastatin improves endothelium-dependent coronary vasomotion in patients with hypercholesterolemia. *Circulation* 1994;89:2519-2524.

Testosterone Decreases Lipoprotein(a) in Men

Joseph M. Zmuda, MS, Paul D. Thompson, MD, Roberta Dickenson, BS, Linda L. Bausserman, PhD

Lipoprotein(a) [Lp(a)] has recently emerged as an important risk factor for atherosclerotic cardiovascular disease.[1] Lp(a) levels are largely under genetic control and have proved remarkably resistant to therapeutic manipulations.[1] Of the commonly used lipid-lowering medications, only high-dose niacin appears to reduce Lp(a) levels.[2] Others have demonstrated that estrogen[3] and the anabolic-androgenic steroid stanozolol[4] reduce Lp(a) concentrations in postmenopausal women, but to our knowledge, the effect of androgenic hormone administration on Lp(a) in men has not been examined. Testosterone is normally aromatized to estradiol, and peripheral aromatization of testosterone is the major source of circulating estrogen in men.[5] We recently examined the effect of testosterone aromatization on serum lipid and lipoprotein levels in men by administering testosterone alone or in combination with the aromatase inhibitor testolactone.[6] Recent reports that

From the Divisions of Cardiology, The University of Pittsburgh, Pittsburgh, Pennsylvania and The Miriam Hospital and Brown University, Providence, Rhode Island.

This study was supported by National Institutes of Health Grant HL-28467 and gifts from the Miriam Foundation, the Haire family, William Jakober, and the McNulty family.

Dr. Thompson's address is: Preventive Cardiology, Suite 1212, Kaufmann Building, University of Pittsburgh Medical Center, 200 Lothrop Street, Pittsburgh, Pennsylvania.

Manuscript received November 29, 1995; revised manuscript received and accepted January 9, 1996.

©1996 by Excerpta Medica, Inc.
All rights reserved.

0002-9149/96/$15.00
PII S0002-9149(96)00174-9

estrogen decreases Lp(a)[3] prompted us to examine Lp(a) levels from our study to determine the effects of testosterone and its aromatization to estradiol on Lp(a) levels.

• • •

Fourteen healthy nonsmoking male weightlifters (aged 27 ± 7 years; mean ± SD) provided written informed consent and completed the study.[6] Subjects weighed 85.4 ± 21.0 kg before the study; body fat, estimated from the sum of 3 skinfold measurements, was 11.0 ± 6.0%. All of the men had been weightlifting for 5.7 ± 5.6 years and exercised ≥3 times per week. No subject averaged >1 alcoholic beverage daily or took regular medications, and all denied current and prior use of androgen. Baseline urinalysis confirmed that the subjects had not recently used either anabolic-androgenic steroids or testosterone.[6] The subjects were instructed to maintain their habitual level of physical activity and avoid altering their dietary habits during the study. Subjects were reimbursed for their participation as approved by the Miriam Hospital Clinical Research Review Board.

Subjects were randomly assigned to a counterbalanced cross-over design involving 3 treatments: testosterone enanthate (E.R. Squibb & Sons, Inc, Princeton, New Jersey), 200 mg/wk intramuscularly (IM); oral testolactone (E.R. Squibb & Sons, Inc), 250 mg 4 times daily (QID); and both testosterone enanthate, 200 mg/wk IM and testolactone, 250 mg QID. This testosterone dose has been recently studied as a male contraceptive[7] and is twice the minimum dose used to treat male hypogonadism.[8] Each treatment lasted 3 weeks, and treatments were separated by a 4-week washout period. Venous blood was obtained before and after each 3-week treatment, between 6 and 9 A.M., after a 12-hour fast, and before any testosterone injection. Samples were stored at −70°C until analysis.

Lp(a) levels were measured in duplicate with an enzyme-linked immunosorbent assay (MACRA, Strategic Diagnostics, Newark, Delaware). Measurement techniques for triglycerides and total low-density lipoprotein (LDL) cholesterol, and high-density lipoprotein (HDL) cholesterol have been detailed.[6] Interassay variation for all lipid and Lp(a) determinations was avoided by analyzing all samples for an individual subject in a single assay or autoanalyzer run. Intraassay coefficient of variation for Lp(a) was <5%. Serum testosterone and estradiol were assayed in duplicate with radioimmunoassay kits (Diagnostic Products Corporation, Los Angeles, California).

Data were analyzed with a treatment-by-time repeated measures analysis of variance. When interaction effects were significant, the effect of time for each treatment and the differences between treatments at each measurement point were tested statistically. A modified Bonferroni procedure was used to adjust for multiple comparisons.[9] Because Lp(a) demonstrated a skewed distribution, statistical analyses were performed on log-transformed values. Nontransformed Lp(a) values are presented in the report. Spearman correlation coefficients were used to examine the relation between initial Lp(a) values and its subsequent change. Results are presented as mean ± SD.

• • •

Pretreatment serum testosterone levels were in the normal physiologic range for young adult men. Serum testosterone levels increased by 39% when testosterone was given alone and by 105% when testosterone and testolactone were combined ($p < 0.01$ for both; Table I). Testosterone also produced a 47% increase ($p < 0.01$) in serum estradiol levels, an effect that was not observed when testosterone and testolactone were administered together. Testolactone alone did not significantly change either testosterone or estradiol levels. These results indicate that testolactone inhibited aromatase activity and blocked the conversion of exogenous testosterone to estradiol.

Average Lp(a) values decreased by 37% during treatment with testosterone, by 28% when testosterone and testolactone were combined ($p < 0.01$ for both), but did not change significantly during treatment with testolactone alone (Table I and Figure 1). Lp(a) levels were similar after the 2 testosterone treatments. These results suggest that most of the reduction in Lp(a) during testosterone treatment resulted from an androgenic effect and not from aromatization of testosterone to estradiol.

The absolute change in Lp(a) levels during testosterone and testosterone plus testolactone treatment was inversely related to initial Lp(a) concentrations ($r = -0.77$ and $r = -0.84$, respectively; $p < 0.01$ for both). Thus, men with the highest initial Lp(a) values experienced the greatest reduction in Lp(a) during testosterone treatment. Lp(a) concentrations were similar before each treatment, indicating that the 4-week washout period was sufficient to eliminate any residual drug effect.

As previously reported,[17] testosterone treatment also reduced HDL by 16%, an effect that was slightly but nonsignificantly greater when testosterone and testolactone were combined (−20%; $p < 0.01$ for

TABLE I Testosterone and Estradiol Levels During Three Drug Conditions

Treatment	Baseline	Week 3
Testosterone (nmol/L)		
Testosterone	18.7 ± 5.5	26.0 ± 4.9*
Testolactone	18.7 ± 5.2	22.9 ± 6.9
Testosterone + testolactone	18.4 ± 3.8	37.8 ± 11.1*†‡
Estradiol (pmol/L)		
Testosterone	133 ± 64	195 ± 75*
Testolactone	133 ± 32	118 ± 50†
Testosterone + testolactone	130 ± 44	113 ± 22†

*Significant ($p < 0.01$) difference from baseline within treatment.
†Significant ($p < 0.01$) difference from testosterone treatment.
‡Significant ($p < 0.01$) difference from testolactone treatment.
These results have been published previously[6] and are included to facilitate evaluation of Lp(a) changes.
Values are expressed as mean ± SD.

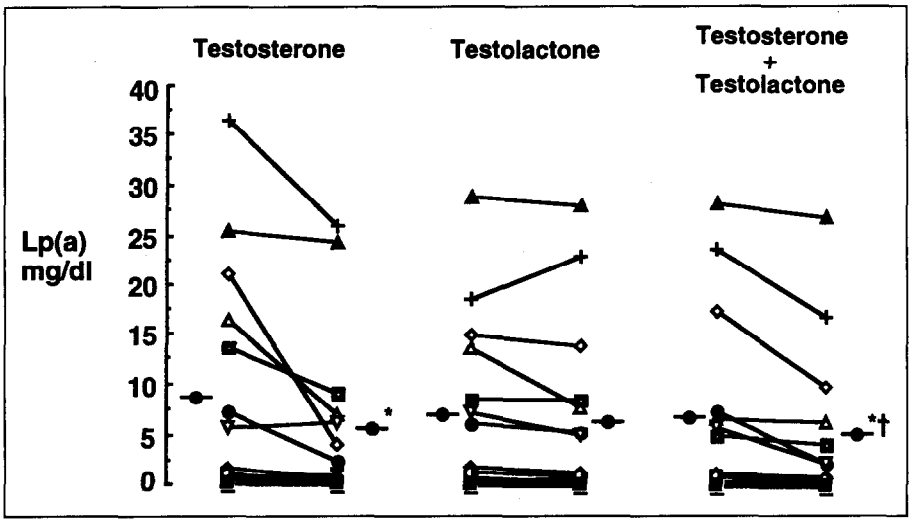

FIGURE 1. Mean and individual lipoprotein(a) (Lp[a]) values during the study. Statistical analyses were performed on log-transformed values. *Significant ($p < 0.01$) difference from baseline within treatment. †Significant ($p < 0.01$) difference from testolactone. Lp(a) values before the 3 treatments were not different.

TABLE II Lipid, Lipoprotein, and Lipoprotein(a) Concentrations During Three Drug Conditions

Treatment	Baseline	Week 3
Cholesterol (mg/dl)		
Testosterone	176 ± 33	173 ± 27
Testolactone	178 ± 29	171 ± 28
Testosterone + testolactone	187 ± 25	177 ± 28
LDL Cholesterol (mg/dl)		
Testosterone	108 ± 27	110 ± 21
Testolactone	108 ± 24	105 ± 22
Testosterone + testolactone	119 ± 22	118 ± 24
HDL Cholesterol (mg/dl)		
Testosterone	50 ± 15	42 ± 13*
Testolactone	48 ± 15	46 ± 15
Testosterone + testolactone	50 ± 14	40 ± 12*†
Lipoprotein(a) (mg/dl)		
Testosterone	9.4 ± 11.6	5.9 ± 8.7*
Testolactone	7.4 ± 8.8	6.9 ± 8.9
Testosterone + testolactone	7.2 ± 9.3	5.2 ± 7.9*†

*Significant ($p < 0.01$) difference from baseline within treatment.
†Significant ($p < 0.01$) difference from testolactone treatment.
Cholesterol, LDL, and HDL results have been published previously[6] and are included to allow evaluation of effect of testosterone on overall cardiac risk.
HDL = high-density lipoprotein; LDL = low-density lipoprotein.
Values are expressed as mean ± SD.

both; Table II). Total cholesterol, LDL cholesterol, and triglyceride levels did not change significantly during any of the drug conditions.

• • •

This report adds testosterone to the brief list of interventions known to affect Lp(a) levels. Neither hydroxymethylglutaryl coenzyme A reductase inhibitors,[10] bile acid sequestrants,[11] fibrates,[11] diet,[11] or vigorous exercise[12] appear to be effective in reducing Lp(a) levels. Recent reports suggest that estrogen[3] and high-dose niacin[2] lower Lp(a) concentrations. One prior study reported a 65% decrease in Lp(a) in postmenopausal women treated for 6 weeks with the nonaromatizable androgen stanozolol.[4] Consequently, it appears that androgens can reduce Lp(a) in both men and women.

The original aim of this study was to examine the effects of testosterone, with or without aromatization to estradiol, on HDL cholesterol levels.[6] The observation that estrogen decreases Lp(a)[3] prompted us to examine the effects of testosterone, testolactone, or the combination on Lp(a) levels. Because supraphysiologic doses of testosterone increase estrogen levels, the use of testolactone provided an opportunity to examine the androgenic effects of testosterone with and without its aromatization to estradiol. Testosterone alone increased serum estradiol levels by 47%, whereas estradiol levels did not change significantly when testosterone and testolactone were combined. Lp(a) decreased 37% during testosterone and 28% during testosterone and testolactone. These decreases were not significantly different, suggesting that most of the decrease in Lp(a) is mediated by testosterone and not by its conversion to estradiol.

The decrease in Lp(a) during testosterone and testosterone plus testolactone treatment was greatest in men with the highest initial Lp(a) levels and largely confined to the 7 men with baseline Lp(a) levels >5 mg/dl. These results suggest that the effect of testosterone may be most pronounced in subjects with the highest pretreatment Lp(a) levels.

The mechanisms by which testosterone treatment reduced Lp(a) concentrations are not clear. LDL cholesterol levels did not change in the present study, suggesting that the effect of testosterone on Lp(a) was independent of changes in LDL metabolism. Lp(a) synthesis, rather than catabolism, is thought to be the primary metabolic determinant of Lp(a) concentrations in humans,[13] and testosterone has recently been documented to reduce apo(a) gene expression in a transgenic mouse model expressing the human apo(a) gene.[14] Thus, it is possible that testosterone lowers Lp(a) levels by decreasing apo(a) synthesis.

Androgens have been assumed to increase atherosclerotic disease risk by reducing HDL-C.[6,15] The

present results suggest that the effect of testosterone on vascular disease risk factors is more complex, and that its deleterious effect on HDL cholesterol may be offset by potentially beneficial effects on Lp(a). Testosterone, like estrogen,[16] may also have favorable effects on coronary vasomotor function, because exogenous testosterone decreases exercise-induced ST segment depression in men with angina pectoris[17] and vasodilates rabbit[18] coronary arteries. Such results may have important implications for the prolonged use of testosterone in hypogonadism[19] and as a male contraceptive,[7] and to prevent frailty as men age.[20]

In summary, the results of the present study indicate that testosterone reduces Lp(a) concentrations in normal men primarily by an androgenic effect and not by its conversion to estradiol.

1. Scanu AM. Lipoprotein(a): a genetic risk factor for premature coronary heart disease. *JAMA* 1992;267:3326–3329.

2. Carlson LA, Hamsten A, Asplund A. Pronounced lowering of serum levels of lipoprotein Lp(a) in hyperlipidaemic subjects treated with nicotinic acid. *J Intern Med* 1989;226:271–276.

3. Sacks FM, McPherson R, Walsh BW. Effect of postmenopausal estrogen replacement on plasma Lp(a) lipoprotein concentrations. *Arch Intern Med* 1994;154:1106–1110.

4. Albers JJ, Taggart HM, Applebaum-Bowden D, Hafner S, Chestnut CH, Hazzard WR. Reduction of lecithin-cholesterol acyltransferase, apolipoprotein D and the Lp(a) lipoprotein with the anabolic steroid stanozolol. *Biochim Biophys Acta* 1984;795:293–296.

5. MacDonald PC, Madden JD, Brenner PF, Wilson JD, Siiteri PK. Origin of estrogen in normal men and in women with testicular feminization. *J Clin Endocrinol Metab* 1979;49:905–916.

6. Zmuda JM, Fahrenbach MC, Younkin BT, Bausserman LL, Terry RB, Catlin DH, Thompson PD. The effect of testosterone aromatization on high-density lipoprotein cholesterol level and postheparin lipolytic activity. *Metabolism.* 1993;42:446–450.

7. Handelsman DJ, Farley TMM, Waites GMH. Contraceptive efficacy of testosterone-induced azoospermia in normal men. *Lancet.* 1990;336:955–959.

8. Santen RJ. The testis. In: Felig P, Baxter JD, Broadus AE, Frohman LA, eds. Endocrinology and Metabolism. 2nd ed. New York: McGraw-Hill Book Co; 1987:821–905.

9. Glantz SA. Primer of Biostatistics. 3rd ed. New York: McGraw-Hill, Inc; 1992.

10. Kostner GM, Gavish D, Leopold B, Bolzano K, Weintraub MS, Breslow JL. HMG CoA reductase inhibitors lower LDL cholesterol without reducing Lp(a) levels. *Circulation.* 1989;80:1313–1319.

11. Brewer HH. Effectiveness of diet and drugs in the treatment of patients with elevated Lp(a) levels. In: Scanu AM, ed. Lipoprotein(a). San Diego, Calif: Academic Press, Inc; 1990:211–221.

12. Dufaux B, Order U, Muller R, Hollmann W. Delayed effects of prolonged exercise on serum lipoproteins. *Metabolism* 1986;35:105–109.

13. Rader DJ, Cain W, Zech LA, Usher D, Brewer HB. 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* 1993;91:443–447.

14. Frazer KA, Narla G, Zhang JL, Rubin EM. The apolipoprotein(a) gene is regulated by sex hormones and acute-phase inducers in YAC transgenic mice. *Nature Genetics* 1995;9:424–431.

15. Thompson PD, Cullinane EM, Sady SP, Chenevert C, Saritelli AL, Sady MA, Herbert PN. Contrasting effects of testosterone and stanozolol on serum lipoprotein levels. *JAMA* 1989;261:1165–1168.

16. Gilligan DM, Quyyumi AA, Cannon RO III. Effects of physiological levels of estrogen on coronary vasomotor function in postmenopausal women. *Circulation* 1994;89:2545–2551.

17. Jaffe MD. Effect of testosterone cypionate on postexercise ST segment depression. *Br Heart J* 1977;39:1217–1222.

18. Yue P, Chatterjee K, Beale C, Poole-Wilson PA, Collins P. Testosterone relaxes rabbit coronary arteries and aorta. *Circulation* 1995;91:1154–1160.

19. Bhasin S. Androgen treatment of hypogonadal men. *J Clin Endocrinol Metab* 1992;74:1221–1225.

20. Bardin CW, Swerdfoff RS, Santen RJ. Androgens: risks and benefits. *J Clin Endocrinol Metab* 1991;73:4–7.