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Testosterone-induced suppression of lipoprotein(a) in normal men; relation to basal lipoprotein(a) level

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Abstract

The concentration of lipoprotein(a) [Lp(a)] in human plasma is largely genetically determined and is inversely correlated to the size of apolipoprotein(a) [apo(a)]. Additionally, Lp(a) values are relatively stable within individuals and are only marginally susceptible to therapeutic treatment. The aim of our study was to evaluate the effect of exogenous testosterone on plasma Lp(a) concentration. The study was carried out on 19 healthy men who were receiving weekly intramuscular injections of 200 mg testosterone enanthate. Lp(a) values were determined at multiple time-points by a double monoclonal antibody-based enzyme immunoassay. This method is not sensitive to variation in Lp(a) size and the values are expressed in nmol/l. Apo(a) size isoforms were determined by agarose gel electrophoresis followed by immunoblotting. No correlation was found between the baseline Lp(a) values and the baseline values of testosterone or estradiol. The Lp(a) response to testosterone treatment varied widely among subjects and was dependent upon the pretreatment Lp(a) concentration. For 10 subjects with low Lp(a) values (< 25 nmol/l), no significant decrease in Lp(a) was observed while, for the nine individuals with Lp(a) values > 25 nmol/l, there was a significant and consistent reduction in Lp(a) ranging from 25 to 59%. Lp(a) levels returned to baseline values following cessation of testosterone administration. Apo(a) size polymorphism did not appear to play a role in the determination of Lp(a) response to testosterone.

Keywords: Lipoprotein(a) concentration; Apolipoprotein(a) isoform; Exogenous testosterone

1. Introduction

Lipoprotein(a) [Lp(a)] has been implicated in both thrombogenesis and atherogenesis and is considered an important risk factor for atherosclerotic cardiovascular disease [1]. Lp(a) constitutes a class of lipoprotein particles structurally related to low-density lipoprotein; both

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lipoprotein classes have similar lipid compositions and contain one molecule of apolipoprotein B-100 per particle. In addition, Lp(a) contains a highly glycosylated protein, designated apo(a), covalently linked to apo B-100 [1]. The length of the apo(a) polypeptide varies within and among individuals and this variation is responsible for the large number of apo(a) size isoforms detected in human plasma [2]. Lp(a) concentrations are predominantly genetically determined with approximately 90% of the variation determined by the apo(a) gene [3]. Although Lp(a) appears to be resistant to most of the dietary and drug interventions known to affect lipid and lipoprotein concentrations, Lp(a) can be modulated by a variety of hormones including growth hormone, thyroid hormone, estrogen and progesterone. A low dose infusion of growth hormone increased Lp(a) to 142% of pre-treatment concentration [4]. Lp(a) values were significantly increased in subjects with hyperthyroidism whereas treatment of hyperthyroidism was shown to reduce Lp(a) values [5,6]. Furthermore, numerous studies have confirmed that estrogen or progesterone, alone or in combination, decrease plasma Lp(a) concentrations both in women [7–9], or in men [10].

Androgens are 19 carbon steroids that maintain the development of male reproductive organs, sexual characteristics and reproductive function. In human males, testosterone is the most abundant circulating androgen. Administration of exogenous testosterone in healthy men has been shown to decrease HDL cholesterol levels [11]. Earlier studies have shown that the 17 α-acylated derivatives of testosterone, stanozolol and danazol significantly reduced Lp(a) values [12,13]. However, both these studies were performed in women only and the effect of testosterone administration on plasma Lp(a) concentrations has not yet been examined. Because testosterone is commonly used as replacement therapy for hypogonadal males and additionally, is being considered as a potential hormonal male contraceptive agent, we have examined the effect of exogenous testosterone on plasma Lp(a) values in healthy males.

2. Materials and methods

2.1. Study subjects

Nineteen healthy men, ranging in age from 19 to 42, completed the study. These men were participating in a study using exogenous testosterone as a male contraceptive [11]. All the participants signed informed consents and had normal medical histories and physical examinations. This study consisted of a 3- to 4-month baseline period, a 20-week treatment period and a 4- to 6-month post-treatment period. During the treatment period, each subject received weekly intramuscular injections of 200 mg testosterone enanthate. In each of the three phases, fasting blood samples were drawn monthly for analysis of lipids, lipoproteins and Lp(a). In the treatment phase, all monthly blood samples were drawn 7 days after the preceding testosterone injection. Plasma samples were stored at $-80°C$ for the entire duration of the study.

2.2. Determination of hormones and lipids

Serum testosterone was measured by radioimmunoassay using reagents from the World Health Organization Matched Reagent Program [14]. Estradiol was measured by a radioimmunoassay kit (ICN Biomedicals Inc., Carson, CA). Lipid measurements have been reported previously [11].

2.3. Lp(a) concentration

Lp(a) was measured by a double monoclonal antibody-based ELISA as described [15]. In this assay, the detection monoclonal antibody, a-40, is directed to an epitope that is present only once per apo(a) particle. Therefore, this assay is not affected by apo(a) size polymorphism and Lp(a) can be accurately measured with values expressed in nmol/l [15]. To minimize the analytical variation, all the samples for each subject were analyzed in duplicate in the same ELISA plate at the end of the study.

Table 1
Characteristics of study participants at baseline

Subject[a] ID	Age	Apo(a) isoform[b]		Lp(a) nmol/l	Testosterone nmol/l	Estradiol pmol/l
		1	2			
NG4-05	22	26		0.6 ± 0.0	19.2	0.12
NG4-25	43	33		8.1 ± 4.6	11.6	0.14
NG4-07	37	28	35	9.4 ± 2.9	16.1	0.12
NG4-31	40	21	24	10.2 ± 1.0	23.4	0.21
NG4-03	30	26	31	13.1 ± 1.7	17.8	0.14
NG4-24	20	27	36	16.1 ± 2.7	21.4	0.15
NG4-22	39	28		19.7 ± 6.2	16.5	0.13
NG4-29	38	19		22.7 ± 4.5	14.0	0.12
NG4-21	26	21	32	24.2 ± 6.3	15.0	0.20
NG4-27	31	30		24.2 ± 5.0	22.2	0.18
NG4-01	27	19	28	27.5 ± 2.3	25.6	0.22
NG4-32	20	28	21	38.1 ± 5.1	14.9	0.14
NG4-30	26	24	28	39.2 ± 7.5	13.4	0.15
NG4-20	26	28	32	42.3 ± 2.4	18.0	0.17
NG4-10	20	27		43.1 ± 1.3	11.3	0.14
NG4-04	29	28	35	53.3 ± 5.3	11.1	0.17
NG4-09	24	26		53.6 ± 5.3	19.6	0.25
NG4-23	41	21	38	85.2 ± 9.8	13.4	0.13
NG4-28	24	21	27	111.1 ± 5.6	14.2	0.14

[a]Subjects have been sorted by initial Lp(a) value.
[b]Column one refers to the major apo(a) isoform and column two refers to the secondary isoform. Isoform size is expressed in terms of Kringle 4 number.

2.4. Apo(a) size isoforms

Apo(a) phenotyping was performed by a high-resolution SDS-agarose gel electrophoresis method, followed by detection of the bands by immunoblotting [2]. The apo(a) size isoforms are designated in terms of the number of kringle 4 repeats contained in each apo(a) isoform [15].

2.5. Statistical analysis

The pretreatment, treatment, and post-treatment Lp(a) values for each man, were calculated as the mean of the 3–5 values obtained at each time period. The biological variation (CV_b) of Lp(a) during the pre-treatment was computed as described [16]. Specifically, $CV_b = (CV_t^2 - CV_a^2)^{1/2}$, where CV_b is the coefficient of biological variation, CV_t is the total intraindividual Lp(a) variability and CV_a is the coefficient of analytical variation which, based on the inter-assay CV of the quality control samples, was calculated to be 4%. Statistical differences between pretreatment, treatment, and post-treatment Lp(a) values were determined by the Wilcoxon Match Pairs Test.

3. Results

Information on the participants' age, apo(a) size isoforms and baseline values of testosterone, estradiol and Lp(a) is presented in Table 1. Mean testosterone levels before treatment were 17.7 ± 1.8 nmol/l, range 11.1–25.6 nmol/l and, during treatment, increased 65% to 27.1 ± 4.1 nmol/l, range 20.1–32.4 nmol/l. Post-treatment testosterone levels (16.4 ± 2.0 nmol/l) were not significantly different from pretreatment levels. Estradiol levels increased from 0.15 ± 0.01 nmol/l to 0.31 ± 0.03 nmol/l during testosterone treatment and returned to baseline levels during the recovery period. The observed increase in estradiol is due to the fact that testosterone is normally aromatized to estradiol in vivo. Complete lipid

Table 2
Lipid and lipoprotein concentration (mmol/l)

	Cholesterol			Triglyceride			HDL-cholesterol			LDL-cholesterol		
	B	T	P	B	T	P	B	T	P	B	T	P
NG4-05	4.94	4.59	4.74	0.78	0.80	0.64	1.51	1.34	1.51	3.07	2.87	2.93
NG4-25	5.70	5.56	5.97	1.26	1.73	1.77	1.05	0.85	1.00	4.06	3.91	4.15
NG4-07	4.30	3.79	4.02	0.68	0.91	0.79	1.54	1.31	1.39	2.44	2.05	2.26
NG4-31	4.28	3.78	4.35	1.28	1.90	1.37	1.01	0.80	0.98	2.67	2.10	2.73
NG4-03	3.52	3.01	3.34	0.42	0.46	0.37	1.58	1.33	1.58	1.74	1.45	1.58
NG4-24	4.46	4.34	4.42	0.53	0.58	0.60	1.36	1.19	1.32	2.86	2.87	2.82
NG4-22	4.81	4.70	4.82	0.58	0.62	0.81	1.69	1.48	1.57	2.84	2.92	2.88
NG4-29	4.01	3.95	4.25	1.07	1.30	1.21	1.08	0.95	1.05	2.42	2.39	2.63
NG4-27	4.35	4.19	3.89	1.26	0.72	0.82	1.34	1.15	1.33	2.42	2.71	2.18
NG4-21	4.86	4.35	4.78	1.20	1.18	0.90	1.25	1.07	1.21	3.05	2.74	3.15
NG4-01	6.48	5.94	6.51	1.07	1.19	1.33	1.09	1.05	1.18	4.88	4.34	4.71
NG4-32	4.41	4.41	4.85	1.11	1.55	1.45	1.01	0.99	1.11	2.87	2.70	3.06
NG4-30	6.66	5.64	6.34	1.00	0.93	0.95	1.91	1.46	1.89	4.28	3.74	4.00
NG4-20	6.68	4.34	4.45	1.49	2.45	2.11	1.11	0.93	1.09	2.88	2.28	2.38
NG4-10	4.87	4.59	4.99	1.37	1.80	1.85	1.14	0.94	1.03	3.08	2.83	3.09
NG4-04	6.73	5.65	6.03	1.16	0.97	1.02	2.21	1.75	1.98	3.98	3.45	3.57
NG4-09	4.07	3.81	4.15	0.87	0.93	0.70	1.53	1.35	1.60	2.14	2.03	2.22
NG4-23	4.30	4.21	4.52	1.62	1.17	0.96	0.93	0.90	0.96	2.62	2.76	3.11
NG4-28	4.46	3.95	4.41	0.63	0.60	0.69	1.13	1.01	1.14	3.04	2.65	2.95
Mean	4.84	4.46*	4.78	1.02	1.15	1.07	1.34	1.15*	1.31	3.02	2.78	2.97
SEM	0.21	0.17	0.20	0.08	0.12	0.11	0.08	0.06	0.07	0.18	0.16	0.17

B, mean value of the pretreatment phase; T, mean value of the treatment phase; P, mean value of the post-treatment phase.
*$P < 0.05$ compared with pretreatment and post-treatment values.

and lipoprotein data for each study participant at baseline, treatment and post-treatment period, are presented in Table 2. Both HDL cholesterol and total cholesterol values decreased significantly during the treatment period while no significant difference was observed in LDL cholesterol and triglyceride concentrations. No significant difference was found between the baseline and the post-treatment period for any of the evaluated lipid values.

As expected [16], pretreatment Lp(a) varied widely among the study subjects from 0.6 to 111 nmol/l. There was no relationship between Lp(a) levels at baseline and basal testosterone or estradiol levels. After testosterone treatment, median Lp(a) concentrations were reduced significantly from 24.2 to 18.9 nmol/l ($P = 0.004$). However, the Lp(a) response to testosterone was not uniform but varied widely among subjects. Based on the Lp(a) response to the treatment, the participants were divided into two groups (Fig. 1). As apparent, among individuals with pretreatment Lp(a) levels below 25 nmol/l, there was no consistent change of Lp(a) in response to testosterone treatment (Fig. 1). Approximately half of the men in this group experienced an increase in Lp(a) values and the remaining a decrease. However, none of these changes were statistically significant. In contrast, the nine subjects with baseline Lp(a) levels greater than 25 nmol/l had a significant reduction in Lp(a) concentrations after testosterone therapy ($P = 0.008$). The median Lp(a) decreased from 43.1 nmol/l to 27.6 nmol/l. The average decrease of Lp(a) for this group of men was 37 $\pm$ 11%. The rate of decrease was greater in the first month of treatment and no statistically significant correlation was found between the absolute or percent change in Lp(a) values and the change in testosterone levels. Additionally, no significant difference was found in the subjects

between pre- and post-treatment Lp(a) concentrations.

The biological variation (CV_b) of Lp(a) was computed for each subject from the Lp(a) values obtained on plasma samples (3–5 per subject) collected on a monthly basis before the initiation of the treatment. Among study participants, there was a very wide variation in the Lp(a) CV_b ranging from 0 to 57%. For the men with low Lp(a) values, the CV_b was usually high (22 ± 16%). In contrast, for the men with pretreatment Lp(a) levels > 25 nmol/l, the CV_b was relatively low (8 ± 6%). These findings are consistent with our previous observations on Lp(a) biological variation [16].

Based on the phenotyping results, 12 subjects (63%) appeared to have two apo(a) size isoforms (Table 1). This number is lower than that obtained in a large White population in which 76% of the subjects were heterozygous for apo(a) isoforms (Marcovina et al., unpublished data). However, in agreement with this finding, seven of the subjects (78%) with Lp(a) levels > 25 nmol/l were heterozygous. Even though there was a trend toward small apo(a) size isoforms in subjects with Lp(a) levels > 25 nmol/l, no statistically significant difference was found in apo(a) isoform distribution between the two groups. The reduction of Lp(a) with testosterone treatment among the men with the major isoform ≤24 kringles was 34 ± 6%, not statistically different than that for the men with the major apo(a) isoform >24 kringles, 40 ± 14%.

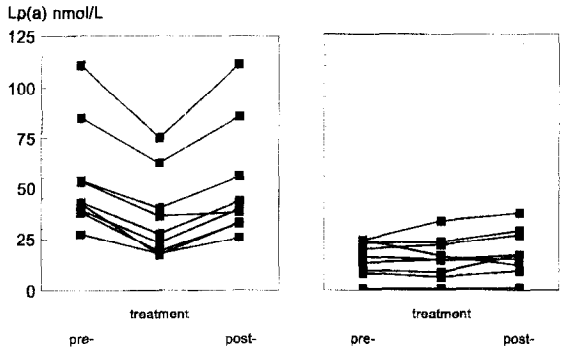

Fig. 1. Effect of testosterone administration on Lp(a) concentrations. On the left side are depicted the treatment and post-treatment Lp(a) values in nine men with baseline Lp(a) concentrations > 25 nmol/l. On the right side are indicated the treatment and post-treatment Lp(a) values in 10 men with baseline Lp(a) concentrations < 25 nmol/l.

4. Discussion

Lp(a) plays a role both in atherogenicity and thrombogenicity and high Lp(a) values are considered a risk factor for coronary artery disease [1]. While plasma Lp(a) levels are largely genetically determined, the results of various studies have indicated that Lp(a) concentrations can be modulated by a variety of hormones [4–10]. Contrasting results have been reported on the correlation between endogenous testosterone levels and plasma Lp(a) levels [17,18]. In a study performed in two populations from Texas and Finland, no statistically significant association was found between endogenous testosterone levels and Lp(a) concentrations [17] while a significant positive correlation between Lp(a) and the Lp(a) to testosterone ratio was found in a group of 114 men from Greece [18]. No information is available thus far on the effect of exogenous testosterone on Lp(a) levels. Two studies [12,13] using oral androgen therapy of osteoporosis or endometriosis in women have reported a suppressive effect on Lp(a) levels. However, interpretation of this work is difficult because oral androgens exert larger effects on the liver (because of high concentrations in the portal circulation) than the parenterally administered testosterone used in our study. The compounds used in the earlier studies [12,13] are both 17 α-acylated, leading to even greater direct liver effects. Furthermore, in one of the earlier studies [13], danazol was administered to women in the reproductive age, leading to suppressed endogenous estrogen and progesterone levels as well as increased androgens. No information prior to our study was available on the effects of the physiological androgen, testosterone and no information on any androgen was available in men.

Confirming a previous report [17], no relation was found between baseline Lp(a) levels and baseline testosterone and estradiol concentrations in a group of 19 healthy individuals participating in a male contraceptive study. Weekly intramuscular

injections of 200 mg testosterone enanthate resulted in a significant decrease of median Lp(a) concentrations from 24.2 to 18.9 nmol/l ($P = 0.004$). However, it was evident that the Lp(a) response to testosterone, unlike HDL cholesterol [11], was not uniform but varied widely among the subjects. No consistent change in Lp(a) values was found in 10 subjects with initial low Lp(a) levels (< 25 nmol/l) while in the nine men with high Lp(a) values (> 25 nmol/l) there was a consistent reduction of Lp(a) ranging from 25 to 59%. We further evaluated whether apo(a) size polymorphism may explain the response of Lp(a) to testosterone. No significant correlation was found between apo(a) size isoform and the changes in Lp(a) concentration induced by testosterone administration. Thus, among this group of men there was no indication that apo(a) size plays a major role in the determination of the Lp(a) response. In contrast, initial Lp(a) concentration appears to be the significant determinant of Lp(a) response to the treatment.

The potential health risks or benefits of long-term exogenous testosterone administration are difficult to predict. A lowering of HDL concentrations, particularly in individuals with low baseline levels, may be expected to increase the risk of coronary artery disease. Our current findings of reduction of high Lp(a) levels with testosterone treatment, could potentially reduce the risk. However, studies have yet to be performed to assess if Lp(a) reduction is beneficial, particularly considering that elevated Lp(a) is not universally accepted as a reliable predictor of either coronary or cerebrovascular diseases.

This is the first evidence in humans that exogenous testosterone affects Lp(a) values and that this effect is only apparent in subjects whose initial Lp(a) values are above a defined threshold. The results of a recent study provide evidence that testosterone plays a major regulatory role in determining plasma levels of human apo(a) in transgenic mice [19]. Additionally, the results indicated that in these animals, plasma levels of apo(a) are regulated by testosterone at the mRNA level. The evidence so far available strongly suggests that Lp(a) production rate is the major determinant of Lp(a) concentrations [20]. Therefore, it can be hypothesized that testosterone also acts by reducing apo(a) synthesis in the liver in humans. However, the evidence provided by our study that testosterone does not appear to be able to further reduce low Lp(a) values, poses intriguing questions on the possible physiological reasons for maintaining these values and the mechanism(s) by which this homeostatic effect is achieved.

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