

Testosterone enanthate at a dose of 200 mg/week decreases HDL-cholesterol levels in healthy men

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Summary

The concept that androgen alone can provide an effective male contraceptive has been tested in a multicentre, multiphase trial by the World Health Organization. Results from this trial showed that an ester of testosterone, testosterone enanthate (TE), administered at a dose of 200 mg/week, has a very high contraceptive efficacy, and suggested that, at least in some populations, androgen alone might provide a viable option for the control of male fertility. It has been claimed that testosterone represents one of the gender-related risk factors for coronary artery disease (CAD) in men. Epidemiological and interventional studies have failed to establish a convincing relationship between testosterone and high density lipoprotein cholesterol (HDL-C). Therefore, there is concern about possible negative effects on lipoprotein asset of an androgen-alone male contraceptive. In this study we analysed the effects of long-term (12 months) administration of TE (200 mg/week) in normal healthy men. Blood samples (six men >10 h fast = Group 1; 30 men >4 h fast = Group 2) were drawn from 36 men, monthly before the beginning of the injections (control), every 3 months throughout the study period (treatment), and 1 month after stopping TE injections (recovery). Total cholesterol (chol), triglycerides, HDL-C and LDL-C levels were measured in these samples. Biochemical parameters were also monitored. TE administration induced a significant decrease (15–20%) in HDL-C levels that was of comparable magnitude in men from both groups (fasting and non-fasting) and occurred regardless of basal HDL-C levels. No statistically significant effect on other lipoproteins was detected. Considering all men together, HDL-C levels were decreased in 78% of the men by month 3, 83% by month 6, 94% by month 9 and 97% by month 12 of treatment. In all men the HDL-C decrease was reversible within 1 month of stopping TE administration. It is concluded that: (1) injection of 200 mg TE/week causes a 15–20% decrease in HDL-C in normal men with no effect on other lipoproteins, (2) the suppressive effect of TE is maintained throughout the 1-year-injection period, and a direct relationship between the duration of TE administration and the proportion of men showing decreased HDL-C levels, was observed. (3) The HDL-C decrease was reversible within 1 month of stopping TE administration. These data will be important in designing further studies on male

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contraception, and in interpreting the relationship between testosterone levels, HDL-C levels and potential cardiovascular risk.

Keywords: coronary artery disease, lipids, male contraception, risk factor, testosterone

Introduction

The basic principle of the hormonal approach to male contraception is to achieve suppression of spermatogenesis through a decrease in blood LH and FSH levels, while maintaining normal androgenization. The 200 mg weekly administration of an ester of testosterone, testosterone enanthate (TE), has been chosen as the easiest and most feasible means to test this concept by the World Health Organization (WHO, 1990) in a multicentre-multiphase trial. Results from the first phase of this trial have shown that azoospermia, achieved in approximately 60% of the Caucasian volunteers and in about 90% of the Chinese population, can provide high contraceptive effectiveness, comparable to oral female contraceptives (WHO, 1990). These results are very promising and suggest that an androgen-alone regimen might represent, at least in some populations, a viable option for male contraception. Although risks and benefits of testosterone administration have been established in studies in hypogonadal men and in short-term studies in normal healthy men (Bagatell *et al.*, 1994; Cunningham *et al.*, 1978; Friedl *et al.*, 1990; Morley *et al.*, 1993; Mooradian *et al.*, 1987; Snyder & Lawrence, 1980; Swerdloff *et al.*, 1977; Tenover, 1992), there is a lack of data on the effects of testosterone administered to a large number of normal healthy men over an extended period of time which would resemble the target population of a male contraceptive.

It has been claimed that testosterone represents one of the gender-related risk factors for coronary artery disease in men (Barrett-Connor & Khaw, 1988; Chute *et al.*, 1987; Kirkland *et al.*, 1987; Kalin & Zumoff, 1990; Seed, 1990). Therefore, concerns about potential long-term side effects of an androgen-alone contraceptive regimen include the possible increased risk of cardiovascular disease due to alteration of the lipid profile. Although epidemiological studies have failed to establish a definitive association between high density lipoprotein cholesterol (HDL-C) and testosterone, most of the authors report a positive correlation between these two factors (Barrett-Connor & Khaw, 1988; Dai *et al.*, 1984; Haffner *et al.*, 1993; Khaw & Barrett-Connor, 1991; Lichtenstein *et al.*, 1987; Phillips *et al.*, 1994), a finding inconsistent with the association of coronary artery disease (CAD), low HDL-C levels and male gender (Duell PB & Bierman ED, 1990; Freedman *et al.*, 1991). On the contrary, we and others have demonstrated recently that weekly administration of 200 mg TE causes a significant decrease in HDL-C levels in normal healthy men over a short period of administration (Bagatell *et al.*, 1994; Thompson *et al.*, 1989; Zmunda *et al.*, 1993). It has been suggested that a solution

to these inconsistencies may be that the suppressive effect of testosterone is transient. Therefore, the purpose of the present study was to extend previous results by evaluating the effects of 200 mg of TE on the lipid profile over a 1-year period of injections in 36 healthy men. We also evaluated biochemical parameters throughout the injection period.

Materials and methods

Subjects

Thirty-six healthy men, aged 20–37 years, were studied in this protocol. All men were healthy by medical history and clinical examination. They had a normal blood count, electrolytes, liver tests, lipid profile and urinalysis. Subjects were asked to maintain stable diet habits and not to undergo major changes of lifestyle throughout the study period.

Protocol

The study consisted of a control period that lasted at least 2 months, a 12-month treatment period and a recovery period that lasted until the subjects had at least three sperm counts within the baseline range. During the treatment phase each subject received weekly injections of 200 mg TE intramuscularly (Bristol-Meyers-Squibb Inc., Princeton, NJ, diluted in 1 ml). Six men had blood samples drawn after at least 10 h of fasting (fasting men). The other 30 men had their blood drawn after >4 h of fasting (non-fasting men). Blood samples were drawn monthly in the control period, every 3 months during TE administration, and monthly for 3 months following the end of treatment. Total cholesterol, triglycerides, LDL cholesterol, HDL cholesterol, GOT, GPT, blood urea nitrogen (BUN), creatinine, alkaline phosphate and total bilirubin were measured in these samples.

Assays

Lipoproteins: High density lipoproteins (HDL) were separated from plasma by chemical precipitation with dextran sulphate-magnesium (m.w. 50,000) (Bachorik *et al.*, 1986; Friedewald *et al.*, 1972; Godsland *et al.*, 1987; Warnick *et al.*, 1990). Cholesterol and triglycerides in plasma, and cholesterol in the HDL fraction, were quantified enzymically on an Abbott Spectrum Multichromatic Analyzer. Cholesterol determination involves a Trindertype method monitored at 500/604 nm; triglyceride determination was performed by a UV method monitored at 340/380 nm and involving a free glycerol blank (Albers *et al.*, 1986). VLDL cholesterol was estimated as triglycerides

divided by five, and LDL cholesterol was calculated as total cholesterol minus HDL cholesterol, minus estimated VLDL cholesterol.

Chemistry: Laboratory tests were performed according to previously well validated methodologies (Searcy, 1969).

Hormones: Serum levels of testosterone were measured by radioimmunoassay (Matsumoto *et al.*, 1983).

Statistical analysis

Plasma concentrations of lipoproteins were analysed separately for the fasting and non-fasting group. In each group analysis of variance with repeated measures (ANOVA) was used to determine differences throughout the study period.

Results

Hormone levels

TE administration caused a significant increase ($p < 0.0001$) in serum testosterone levels from 13.24 ± 0.44 nmol/l (control levels) to 26.41 ± 1.46 nmol/l (month 12 of treatment). One month after stopping TE administration, serum testosterone levels had returned to levels (12.4 ± 1.1 nmol/l) which were not significantly different from baseline.

Plasma lipoproteins

TE administration induced a significant decrease in plasma HDL-C levels ($p < 0.05$) (Fig. 1). The HDL-C decrease was detectable both in men who fasted for at least 10 h before the blood sample (Group 1) and in the men who did not fast (Group 2) (Fig. 1 and Table 1). HDL-C levels were decreased significantly below basal levels by month 3 (88% of baseline in both groups) and were maintained at reduced levels throughout the entire length of TE administration in both groups (Fig. 1 and Table 1). Considering all the men together, HDL-C levels were decreased in 28/36 (78%) of the men by month 3, 30/36 (83%) by month 6, 34/36 (94%) by month 9 and 35/36 (97%) by month 12 of treatment. Decrease of HDL-C levels occurred regardless of basal levels (Fig. 2). In all of the men the decrease in HDL-C levels was reversible within 1 month of stopping TE administration (Fig. 1 and Table 1). No statistically significant effect on total cholesterol, LDL-cholesterol and triglycerides could be detected in either group (Table 1).

Chemistry

The biochemical parameters analysed showed no change throughout 1 year of TE administration (Table 2).

Discussion

In this study we have assessed the effects on lipoprotein levels and biochemical parameters of a contraceptive regimen consisting of weekly injections of 200 mg TE in

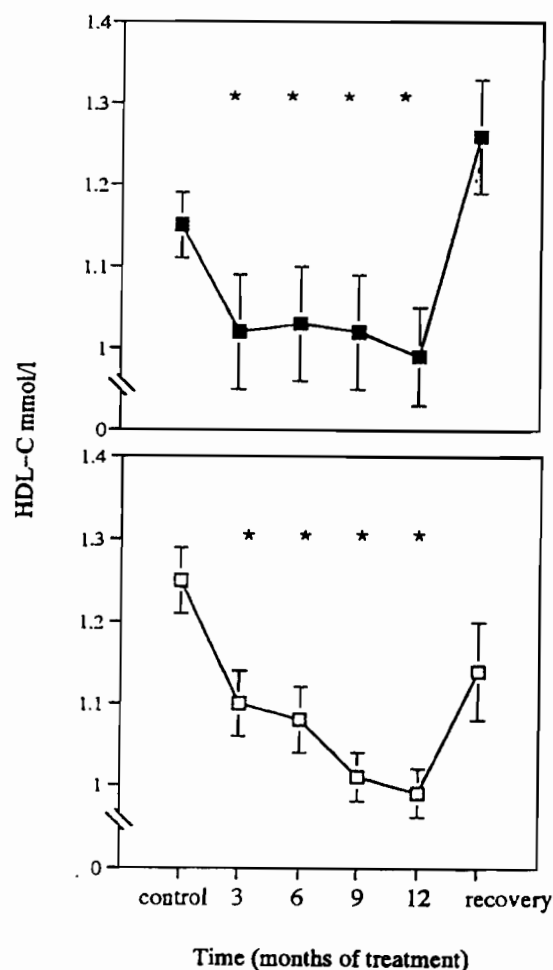


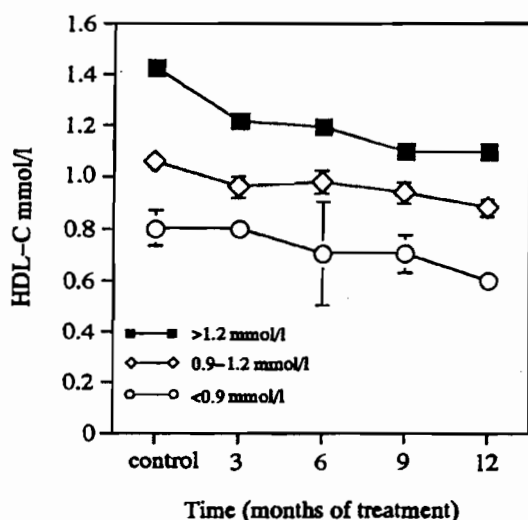
Figure 1. Mean (\pm SEM) plasma HDL-C levels in normal healthy men undergoing TE injections, before (control), during, and the next month (recovery) after stopping injections. In the upper panel, men fasting for at least 10 h before blood sampling (Group 1) are represented, while in the lower panel men fasting >4 h are represented (Group 2). TE administration caused a significant decrease ($p > 0.05$) in HDL-C levels in both groups of men.

normal healthy men for 1 year. This regimen caused a significant decrease in HDL-C concentrations, regardless of basal levels, with no significant effect on other lipoproteins. The decrease in HDL-C was evident within 3 months of beginning TE injections, and was maintained throughout the period of TE treatment. With increasing duration of TE administration, a greater proportion of men demonstrated suppressed HDL-C levels. In all of the men the HDL-C decrease was reversible within 1 month of stopping TE administration.

In this study we provide clear evidence that prolonged administration of TE at a dose which results in supraphysiological serum levels of testosterone (Sokol *et al.*, 1982; Weinbauer *et al.*, 1990), suppresses HDL-C levels. These

Table 1. Mean (\pm SEM) plasma levels (mmol/l) of lipoproteins in fasting (Group 1; $n=6$) and non-fasting men (Group 2; $n=30$) before (control = mean of two samples for each subject), monthly throughout TE administration (TE-Treatment) and the next month after stopping injections (recovery)

	Control	TE-Treatment				Recovery
		Month 3	Month 6	Month 9	Month 12	
Group 1 ($n=6$)						
Total cholesterol	5.13 \pm 0.1	4.66 \pm 0.19	4.93 \pm 0.11	4.80 \pm 0.23	4.71 \pm 0.23	5.17 \pm 0.20
HDL-C	1.15 \pm 0.04	1.02 \pm 0.07*	1.03 \pm 0.07*	1.02 \pm 0.07*	0.09 \pm 0.06*	1.22 \pm 0.05
LDL-C	3.54 \pm 0.09	3.21 \pm 0.16	3.47 \pm 0.16	3.32 \pm 0.21	3.03 \pm 0.30	3.23 \pm 0.30
Group 2 ($n=30$)						
Total cholesterol	4.94 \pm 0.11	4.73 \pm 0.18	4.88 \pm 0.18	4.65 \pm 0.16	4.69 \pm 0.17	5.14 \pm 0.25
HDL-C	1.25 \pm 0.04	1.10 \pm 0.04*	1.08 \pm 0.04*	1.01 \pm 0.03*	0.99 \pm 0.03*	1.14 \pm 0.06
LDL-C	3.03 \pm 0.10	3.09 \pm 0.18	3.15 \pm 0.17	2.90 \pm 0.16	2.96 \pm 0.16	3.32 \pm 0.25

* $p<0.05$, compared with respective control values.

results confirm and extend previous data on shorter periods of TE administration (Bagatell *et al.*, 1994; Thompson *et al.*, 1989; Zmunda *et al.*, 1993). These data are also in agreement with most of the interventional studies reported in the literature, based on parenteral as well as oral administration of androgens to athletes, hypogonadal men and transsexuals, which show a suppressive effect of androgens on HDL-C (Cunningham *et al.*, 1978; Mooradian *et al.*, 1987; Snyder, 1984; Swerdloff *et al.*, 1977). In contrast, epidemiological studies have failed to establish a conclusive relationship

Figure 2. Blood levels of HDL-C in men with differing baseline HDL-C levels (control) throughout TE administration. <0.9 mmol/l, $n=2$; 0.9-1.2 mmol/l, $n=14$; >1.2 mmol/l, $n=20$. The decrease in HDL-C levels caused by TE administration occurred regardless of basal levels.**Table 2.** Biochemical parameters (mean \pm SEM) in 36 men throughout 1 year of injections with 200 mg TE/week (TE-Treatment) before (Control) and after (Recovery) the end of the injections. TE administration did not cause any significant change in the biochemical parameters that were analysed.

Parameter	Control	TE-Treatment				Recovery
		Month 3	Month 6	Month 9	Month 12	
GOT (U/l)	24 \pm 0.9	26 \pm 1.8	26 \pm 1.3	27 \pm 1.8	28 \pm 1.7	27 \pm 2.5
GPT (U/l)	25 \pm 1.5	25 \pm 2.7	27 \pm 1.8	28 \pm 1.9	31 \pm 2.7	32 \pm 3.3
GGT (U/l)	19 \pm 0.9	17 \pm 1.3	20 \pm 1.6	19 \pm 1.5	20 \pm 1.9	24 \pm 2.2
Total bilirubin (μ mol/l)	11 \pm 0.9	11 \pm 1.2	12 \pm 1.4	12 \pm 1.0	15 \pm 7.4	11 \pm 1.0
Alkaline phosphatase (U/l)	60 \pm 1.6	62 \pm 2.2	60 \pm 2.3	58 \pm 2.3	58 \pm 2.3	61 \pm 2.7
BUN (mmol/l)	5.4 \pm 0.2	4.9 \pm 0.2	5.1 \pm 0.2	5.0 \pm 0.2	5.1 \pm 0.2	6.0 \pm 0.3
Creatinine (μ mol/l)	96 \pm 0.9	107 \pm 1.8	103 \pm 1.8	103 \pm 1.8	104 \pm 1.8	103 \pm 1.8

between the blood levels of testosterone and HDL-C, reporting either a positive, a negative or no correlation (Barrett-Connor, 1992; Dai *et al.*, 1984; Haffner *et al.*, 1993; Khaw & Barrett-Connor, 1991; Lichtenstein *et al.*, 1987; Phillips *et al.*, 1994). There are numerous problems with epidemiological studies that correlate analysis of HDL-C levels with testosterone levels retrospectively often based on measurements in frozen sera. Most of the studies that have performed multivariate analysis, correcting for different variables that can affect lipids, may have missed real biological associations between testosterone and lipoproteins. To explain the discrepancy between epidemiological studies and interventional studies, it has been suggested that the effects of TE are transient. Our data do not confirm this hypothesis. On the contrary, it is of interest to note that, in our study, the percentage of men showing decreased HDL-C levels increased throughout the length of the study. While 22% of the men did not show any decrease by month 3 of treatment, by the end of 1 year of treatment 97% of the men showed a decrease in HDL-C levels. We may speculate that, if these men were followed for a longer period, HDL-C would decrease in all of them. The reason(s) why some men are more resistant to the negative effects of TE on HDL-C is unclear, as are the mechanism(s) by which androgens decrease HDL-C. Kinetics studies reported an enhanced catabolism of HDL-C (Hazzard *et al.*, 1984) following androgen intake, and other studies have shown increased activity of hepatic triglyceride lipase. Whether these or other factors that regulate lipoprotein metabolism may have a different sensitivity to androgens in different individuals is presently unknown.

The decrease of HDL-C levels by a contraceptive is an undesirable effect because of the possible increased risk of cardiovascular disease. Whether the 15–20% decrease in HDL-C, detected in our study, may result in increased risk of CAD in men, remains a matter of speculation, and only

long-term observations will establish the relative risk of this treatment (Gordon *et al.*, 1977; Gordon *et al.*, 1989; Kannel *et al.*, 1979; Jacobs *et al.*, 1990; Manninen *et al.*, 1988). However, these data should be taken into consideration when designing further studies on hormonal male contraceptives. Because of its kinetic properties, testosterone enanthate at the dose of 200 mg/week causes supraphysiological testosterone peaks between injections (Snyder, 1984; Sokol *et al.*, 1982; Weinbauer *et al.*, 1990). These supraphysiological testosterone levels are likely to be responsible for the decreased HDL-cholesterol. We may speculate that the use of androgens in a formulation system, and at a dose that maintains serum levels of testosterone within the physiological range, either alone or in association with non-androgenic progestins or GnRH antagonists, might avoid negative effects on plasma HDL-C levels and, by inference, represent safer contraceptive tools.

In agreement with previous observations on testosterone esters, testosterone enanthate did not significantly affect biochemical parameters, including those reflecting liver function.

In conclusion, we report the metabolic effects of testosterone enanthate at the dose that has been used most commonly for the control of male fertility. This dose of TE causes a significant decrease in plasma levels of HDL-cholesterol with no change in other lipoproteins. The suppressive effect of exogenous testosterone is not transient, but is maintained throughout the hormone administration. These data also suggest that the dosage of testosterone used in these studies may be excessive for contraception.

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