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Physiological Levels of Estradiol Stimulate Plasma High Density Lipoprotein$_2$ Cholesterol Levels in Normal Men*

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ABSTRACT

Premenopausal women have a lower risk of coronary artery disease than men or postmenopausal women; estrogens are thought to contribute to this lower risk. Administration of exogenous estrogen to postmenopausal women increases plasma high density lipoprotein (HDL) cholesterol and may reduce mortality from coronary disease in users. Although many investigations have examined the roles of estrogen in the regulation of lipoproteins in women, little attention has been directed to estrogen regulation of lipids in men. We designed a paradigm to study the role of physiological levels of estradiol (E_2) on plasma lipoproteins in healthy men. We used a GnRH antagonist, Nal-Glu, to suppress endogenous steroid hormones in healthy men. We then administered testosterone (T) enanthate (100 mg, im, weekly) to restore T levels to the baseline range, and we administered an aromatase inhibitor, testolactone (Teslac), to prevent the normal conversion of T to E_2, thereby producing a selective estrogen deficiency state in normal young men. As controls, we administered Nal-Glu and T along with placebo Teslac to a separate group of men; a third group of men received all placebo medications.

We found that in men who received Nal-Glu plus T plus Teslac, E_2 levels were profoundly suppressed during treatment, whereas T levels remained in the baseline range. Plasma HDL cholesterol, particularly, the HDL_2 fraction, decreased significantly in response to the low serum E_2 level. Plasma apoprotein-AI levels also decreased significantly. Plasma LDL and triglyceride levels did not change. All hormone and lipoprotein parameters returned to baseline within 4 weeks after treatment ended. In men who received Nal-Glu plus T, plasma HDL and apoprotein-AI decreased, but these decreases did not achieve statistical significance. Only a small decrease in HDL_2 cholesterol was seen in these men. There were no hormonal or lipid changes in the placebo group. We conclude that in men, physiological levels of E_2 are important in maintaining plasma levels of HDL cholesterol, especially the HDL_2 fraction. These observations suggest that estrogen, in the amount normally produced in men, may offer some degree of protection against cardiovascular disease in males, as they do in women. (*J Clin Endocrinol Metab* **78**: 855–861, 1994)

IN GENERAL, the risk of coronary artery disease (CAD) in premenopausal women is lower than that in men; however, risk of CAD increases considerably in women after menopause (1, 2). As menopause is associated with loss of ovarian function and, consequently, a dramatic decrease in serum estradiol (E_2), estrogens have been postulated to confer a protective effect against the development of atherosclerosis. This protection is probably mediated both directly, via effects on arterial walls, and indirectly, via effects on a variety of circulating factors, including plasma lipoproteins. In particular, estrogens are associated with increased levels of high density lipoprotein (HDL) cholesterol, which, in turn, are associated with decreased cardiovascular risk (3, 4). Some, but not all, studies show a decrease in HDL in postmenopausal E_2-deficient women compared to levels in premenopausal women (5–7); however, administration of exogenous estrogen to women results in increased plasma HDL cholesterol (2, 8–10), and decreased cardiovascular mortality may be associated with the use of postmenopausal estrogen replacement (9, 11, 12).

Although the effects of exogenous and endogenous estrogens on plasma lipids in women have been well studied, the role of estrogens in regulating lipids in men has received considerably less attention. The administration of supraphysiological doses of estrogen to men with prostate cancer increases HDL cholesterol and triglycerides and decreases low density lipoprotein (LDL) cholesterol (13, 14); in this setting, a deficiency of testosterone (T) is also induced. Friedl *et al.* (15) showed that the administration of high dose T to normal men did not significantly suppress plasma HDL, but when the normal conversion of T to E_2 was blocked by administration of an aromatase inhibitor, HDL levels did decrease significantly. These data demonstrate that estrogens can play a role in the regulation of plasma lipids in men as well as in women; however, E_2 levels in these studies were greatly supraphysiological. We hypothesized that the small quantity of E_2 that circulates in normal men also regulates

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plasma HDL cholesterol levels. To test this hypothesis, we required a model in which we could induce a selective deficiency of plasma E_2 in healthy men while at the same time serum T levels remained unchanged. We used an antagonist of GnRH, which causes immediate and sustained inhibition of gonadotropin and gonadal steroid secretion and thereby induces profound reversible hypogonadism, to create such a model. We have previously studied the GnRH antagonist, Nal-Glu ([Ac-D2Nal[1],D4ClPhe[2],D3Pal[3],Arg[5],DGlu[6]-(AA),DAla[10]]GnRH), in healthy men (16–18) and have confirmed its effectiveness in suppressing gonadotropin and gonadal steroid levels. We administered this agent in combination with physiological amounts of T replacement to one group of healthy men; in a separate group of men, we administered Nal-Glu together with T and testolactone (Teslac), an oral inhibitor of the aromatase enzyme complex that normally converts T to E_2. A third group of men served as placebo controls. Using this paradigm, we have demonstrated that physiological levels of E_2 in men play a role in the regulation of HDL cholesterol, particularly in regulation of the HDL_2 density subfraction.

Materials and Methods

Subjects

Thirty healthy men, aged 20–40 yr, were recruited by advertisement in the community. All were of normal body weight and had normal blood chemistries and fasting lipid profiles (*i.e.* HDL cholesterol, ≥0.91 mmol/L; LDL cholesterol, ≤4.16 mmol/L; triglycerides, <2.82 mmol/L). None of the men took regular medications, smoked, or abused alcohol. Several men exercised regularly, but none was an elite athlete. All men signed a consent form approved by the University of Washington Human Subjects Committee.

Experimental protocol

It was necessary to administer the GnRH antagonist Nal-Glu and T enanthate together with Teslac to create a selective deficiency of E_2. Had we administered only Teslac to some of the men, a selective E_2 deficiency would have resulted temporarily. However, in a man with an intact pituitary-gonadal axis, a decrease in E_2 would have led to an increase in gonadotropins, resulting in an increase in serum T. Thus, a new equilibrium, with increased T and decreased E_2 levels, would have occurred, and it would have been impossible to distinguish the effects of decreased E_2 from the effects of increased T. By administering the GnRH antagonist, we prevented a compensatory increase in serum gonadotropins and were able to maintain T levels relatively constant by administering a dose of T that kept serum T levels very close to the baseline range.

The study consisted of a 4-week pretreatment phase, a 6-week treatment period, and a 4-week recovery period. During the treatment period, each man was randomly assigned to one of the following experimental regimens (n = 10 in each group): 1) Nal-Glu plus T plus Teslac: 75 μg/kg Nal-Glu, sc, daily plus T enanthate (100 mg in 1 mL sesame oil, im, weekly; 1 mL, im, weekly; Schoen Pharmaceuticals, Port Washington, NY), plus Teslac (250 mg, orally, four times daily; Bristol-Meyers-Squibb, New Brunswick, NJ); these men were selectively E_2 deficient during the treatment period; 2) Nal-Glu plus T: 75 μg/kg, Nal-Glu, sc, daily plus T enanthate (100 mg in 1 mL sesame oil, im, weekly, plus placebo Teslac capsules four times daily); these men had normal levels of T and E_2 during the treatment period; and 3) placebo control: NaCl vehicle (0.9 mL; 150 mmol/L, sc, daily), sesame oil placebo (1 mL, im, weekly), plus placebo Teslac capsules four times daily. Intramuscular injections were administered by the nursing staff at the University of Washington Clinical Research Center. Each subject self-administered his sc injection daily during the treatment period. Teslac capsules were dispensed weekly during the treatment period; to ensure compliance, empty bottles were returned at each weekly visit.

The study was double blind. Serum T and E_2 were measured twice during the pretreatment period, on day 3 of treatment, at the end of each week of treatment, and weekly during the posttreatment period. Total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides were measured twice during the pretreatment period, at weeks 1, 3, 5, and 6 of the treatment period, and weekly during the posttreatment period. HDL_2 and HDL_3 subfractions and apoprotein-AI (Apo-AI) were measured once during the pretreatment phase, on weeks 3 and 6 of the treatment period, and on the last day of the posttreatment period. Blood samples were collected between 0700–1000 h, after a minimum of 10 h of fasting. All samples were drawn with the subjects in a seated position. During the treatment period, blood samples were collected immediately before the daily and weekly injections were administered.

All subjects were weighed weekly and completed a 3-day food record during each phase of the study. The intake records were analyzed for calories, nutrients, and minerals using version 13 of the University of Minnesota Nutrition Coordinating Center data base (19). Subjects were asked to maintain their usual diet and exercise habits throughout the course of the study. Each subject was interviewed weekly by one of the investigators. Changes in diet, exercise habits, physical health, and side-effects were assessed during these interviews. Subjects were examined at the end of each phase of the study.

GnRH antagonist preparation

The antagonist was supplied by the NICHHD Contraceptive Development Branch. It was dissolved in bacteriostatic water containing 4% mannitol, diluted to a concentration of 10 mg/mL, and then, under sterile conditions, passed through a 0.2-μm filter into sterile vials and stored at −20 C until use. Subjects received a new vial of antagonist each week and refrigerated each vial between injections.

Lipoprotein and hormone assays

HDL cholesterol and HDL subclasses were measured by dextran sulfate-magnesium precipitation using the method of Warnick *et al.* (20, 21). Total cholesterol and triglycerides were measured enzymatically (22) using an ABA 200 biochromatic instrument (Abbott Laboratories, North Chicago, IL). LDL cholesterol was calculated indirectly, using the formula, LDL cholesterol = total cholesterol − (HDL cholesterol + triglycerides/5) (23). These calculations were made before the data were converted to System International units. Apo-AI was measured by nephelometry using a Behring Nephelometer Analyzer (Behring Diagnostics, Marburg, Germany). T was measured by RIA, as described previously (24). E_2 was measured by RIA using a kit purchased from ICN Biomedicals, Inc. (Diagnostics Division, Carson, CA). In our laboratory, the limit of detectability of the assay is 18.3 pmol/L.

Statistical analysis

Total cholesterol, HDL cholesterol, and triglycerides are known to exhibit day to day variability (25, 26). Therefore, in the analysis of these lipid parameters, we determined the means of pretreatment values, treatment week 5 and 6 values, and posttreatment week 3 and 4 values for each subject and used these means for statistical analyses. Gonadal steroids were measured weekly, and we used the means of pretreatment values, treatment week 5 and 6 values, and posttreatment week 3 and 4 values for each subject and used these means for statistical analyses as well. Single samples of HDL_2, HDL_3, and Apo-AI from the end of the pretreatment, treatment, and posttreatment periods were compared statistically. To facilitate comparison of changes in total plasma HDL in relation to changes in the HDL_2 and HDL_3 subfractions, mean levels of plasma HDL in samples in which HDL_2 and HDL_3 were measured are also presented. For each parameter, between-group differences were assessed using two-way analysis of variance with time and treatment as dependent variables, followed by paired Student's *t* tests where appropriate. Within each experimental group, the mean pretreatment, end of treatment, and posttreatment values of each parameter were compared using one-way analysis of variance with repeated measures along with

Dunnett's multiple range test. All data are expressed as the mean and associated 95% confidence interval; $P < 0.05$ was considered significant.

One man in the placebo group gained more than 7 kg during the study. This man's HDL cholesterol levels fluctuated considerably during the study as well. For these reasons, data from this man were excluded from the analysis.

Results

Steroid hormones (Fig. 1)

In the men who received Nal-Glu plus T plus Teslac, E_2 levels were profoundly suppressed during the treatment period; mean serum E_2 decreased from 125 ± 21 to 43 ± 13 pmol/L ($P < 0.05$). After Teslac administration ended, normal E_2 production resumed, and serum E_2 levels were at their baseline level by the end of the posttreatment period. Nadir serum E_2 levels were slightly, but not significantly, decreased in men receiving Nal-Glu plus T; E_2 levels did not change in the placebo group. In men who received Nal-Glu, T, and Teslac or Nal-Glu plus T, mean serum T levels 3 days after the first T injection were 2–3 nmol/L greater than the respective baseline levels. At the end of each week of treatment, serum T levels in these men were slightly, but not significantly, below the respective baseline levels. In men who received placebo, serum T levels remained constant throughout the study.

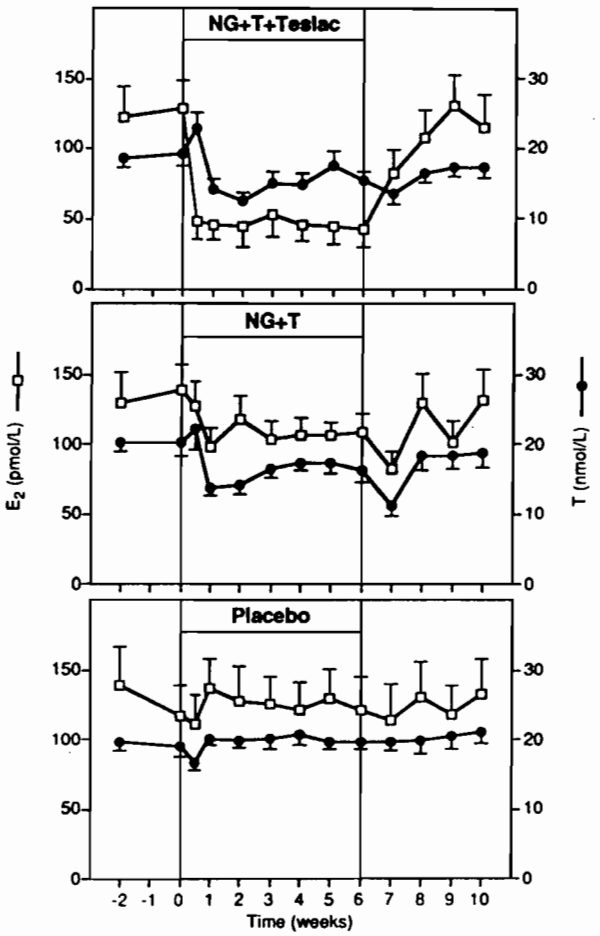

FIG. 1. Mean levels of serum E_2 and T before, during, and after treatment in men who received Nal-Glu (NG) plus T plus Teslac (n = 10), Nal-Glu plus T (n = 10), or placebo (n = 9). During the treatment period, blood samples were always drawn immediately before injections, except during week 1, when a sample was also obtained on day 3.

Lipoproteins

HDL cholesterol (Figs. 2 and 3 and Table 1). In men receiving Nal-Glu plus T plus Teslac, the mean plasma HDL cholesterol level decreased by 8 ± 3% ($P < 0.05$) by the end of the treatment period. This decrease was due primarily to the decrease in plasma HDL_2 cholesterol levels, which fell from 0.36 ± 0.03 to 0.19 ± 0.02 mmol/L (76 ± 5% of baseline; $P < 0.05$) by week 6 of treatment. The decrease in HDL_2 was generally greater in men whose baseline HDL_2 level was 0.26 mmol/L or higher. HDL_3 cholesterol levels did not change during the study in these subjects (mean decrease, 0.02 ± 0.05 mmol/L). When the treatment period ended, total HDL cholesterol and HDL_2 cholesterol levels returned to baseline.

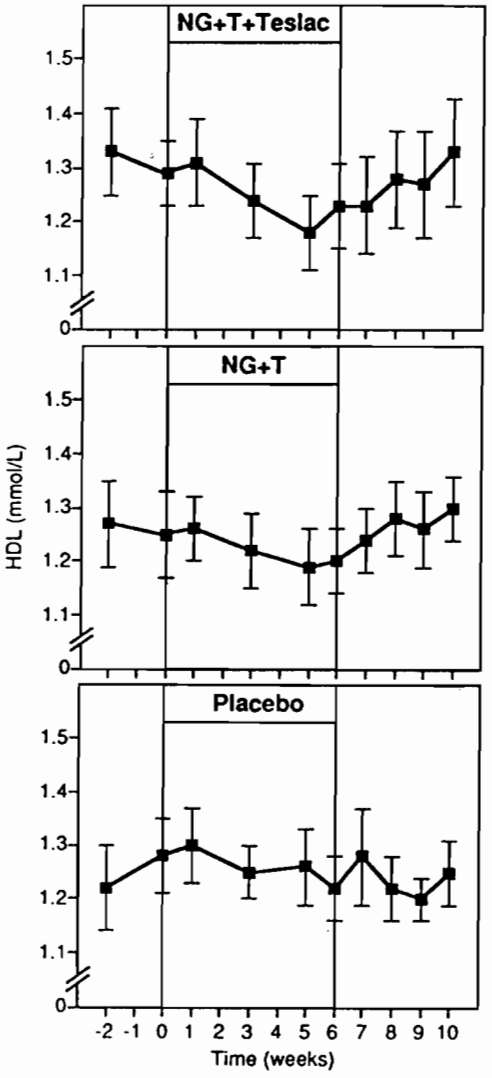

FIG. 2. Mean levels of HDL cholesterol during the study in men who received Nal-Glu (NG) plus T plus Teslac (*top panel*; n = 10), Nal-Glu plus T (*middle panel*; n = 10), or placebo (*bottom panel*; n = 9).

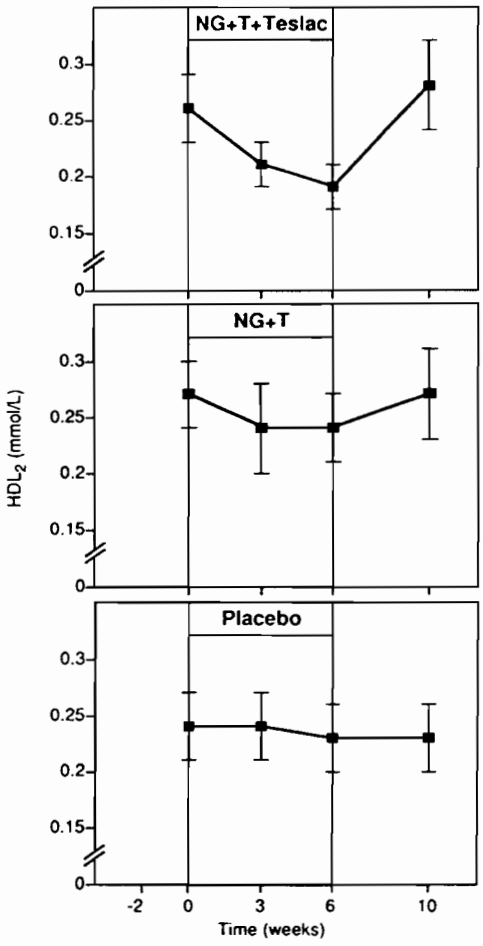

FIG. 3. Mean levels of HDL_2 cholesterol during the study in men who received Nal-Glu (NG) plus T plus Teslac (*top panel*; n = 10), Nal-Glu plus T (*middle panel*; n = 10), or placebo (*bottom panel*; n = 9).

In men who received Nal-Glu plus T, mean plasma HDL decreased by 5 ± 3% by the end of the treatment period (P = NS). Mean plasma HDL_2 decreased by 9 ± 5% (P = NS), whereas mean plasma HDL_3 decreased only very slightly (mean decrease, 0.04 ± 0.06 mmol/L). There were no changes in HDL cholesterol or in the HDL_2 or HDL_3 subfractions during the course of the study in men who received placebo.

Apo-AI (Table 1). Mean Apo-AI decreased by 7 ± 3% (range, 19% decrease to 6% increase) during the treatment period in men who received Nal-Glu plus T plus Teslac ($P < 0.05$); baseline values were reestablished by the end of the posttreatment period. Mean Apo-AI levels decreased by 5 ± 4% (range, 22% decrease to 25% increase) in men receiving Nal-Glu plus T, but this decrease did not reach statistical significance. Apo-AI levels did not change in the placebo group.

Total cholesterol, LDL cholesterol, and triglycerides (Table 1). Mean levels of total cholesterol, LDL cholesterol, and triglycerides did not change during the treatment period in any of the groups. During the posttreatment period, mean levels of total cholesterol, LDL, and triglycerides were unchanged in men who had received Nal-Glu plus T or placebo. In the group that had received Nal-Glu plus T plus Teslac, plasma LDL increased in 7 of the 10 men, and the mean plasma LDL level increased slightly, although significance was not achieved. Two men in this group had substantial increases in triglycerides during the posttreatment period (81% and 215%); dietary cholesterol, but not total caloric intake or body weight, also increased in these men. Because of the values in these 2 men, mean plasma triglycerides increased by 40 ± 24% (P = NS) during the posttreatment period. As a consequence of the increases in plasma levels of LDL cholesterol and triglycerides, mean total cholesterol in men who had received Teslac increased by 4.0 ± 2.0% above the baseline value ($P < 0.05$ compared to the end of treatment value; P = NS compared to pretreatment value).

Body weights and caloric intake (Table 2). Body weights did not change significantly in any of the groups. There was a trend toward consumption of fewer calories in all of the groups during the course of the study, but these decreases did not reach statistical significance. Daily consumption of

TABLE 1. Mean levels of HDL cholesterol, HDL subfractions, Apo-AI, total and LDL cholesterol, and triglycerides (mean ± SE) during the baseline period, the last 2 weeks of treatment, and the last 2 weeks of the posttreatment period

	Baseline	Treatment	Post-treatment
HDL cholesterol (mmol/L)			
Nal-Glu + T + Teslac (n = 10)	1.31 ± 0.06	1.21 ± 0.09[a]	1.29 ± 0.09
	(1.33 ± 0.07)	(1.23 ± 0.08)[a]	(1.33 ± 1.0)
Nal-Glu + T (n = 10)	1.26 ± 0.07	1.19 ± 0.06	1.28 ± 0.06
	(1.27 ± 0.08)	(1.20 ± 0.06)	(1.30 ± 0.06)
Placebo (n = 9)	1.25 ± 0.07	1.26 ± 0.06	1.22 ± 0.04
	(1.22 ± 0.07)	(1.25 ± 0.06)	(1.24 ± 0.06)
HDL_2 cholesterol (mmol/L)			
Nal-Glu + T + Teslac	0.26 ± 0.03	0.19 ± 0.02[a]	0.28 ± 0.04
Nal-Glu + T	0.27 ± 0.03	0.24 ± 0.03	0.27 ± 0.04
Placebo	0.24 ± 0.03	0.23 ± 0.03	0.21 ± 0.03
HDL_3 cholesterol (mmol/L)			
Nal-Glu + T + Teslac	1.07 ± 0.05	1.04 ± 0.06	1.03 ± 0.06
Nal-Glu + T	1.02 ± 0.05	0.97 ± 0.04	1.03 ± 0.04
Placebo	1.00 ± 0.05	1.03 ± 0.05	1.02 ± 0.04
Apo-AI (mg/dL)			
Nal-Glu + T + Teslac	138.6 ± 5.3	130.4 ± 7.2[a]	141.6 ± 7.2
Nal-Glu + T	140.6 ± 6.9	130.7 ± 4.4	137.8 ± 5.0
Placebo	135.2 ± 7.9	133.9 ± 6.2	137.6 ± 6.3
Total cholesterol (mmol/L)			
Nal-Glu + T + Teslac	4.41 ± 0.19	4.37 ± 0.18	4.60 ± 0.19[b]
Nal-Glu + T	4.33 ± 0.23	4.22 ± 0.20	4.28 ± 0.13
Placebo	4.29 ± 0.18	4.27 ± 0.17	4.15 ± 0.20
LDL cholesterol (mmol/L)			
Nal-Glu + T + Teslac	2.71 ± 0.16	2.64 ± 0.24	2.84 ± 0.13
Nal-Glu + T	2.52 ± 0.19	2.51 ± 0.16	2.47 ± 0.17
Placebo	2.55 ± 0.15	2.44 ± 0.13	2.36 ± 0.16
Triglycerides (mmol/L)			
Nal-Glu + T + Teslac	0.82 ± 0.11	0.88 ± 0.12	1.20 ± 0.22
Nal-Glu + T	1.11 ± 0.02	1.06 ± 0.17	1.14 ± 0.18
Placebo	0.84 ± 0.07	0.97 ± 0.13	0.92 ± 0.12

During each phase of the study, the mean plasma levels of HDL cholesterol in the sample analyzed for HDL_2 and HDL_3 are shown in parentheses. To convert to milligrams per dL, multiply cholesterol by 38.7 and triglycerides by 88.5.

[a] $P < 0.05$ compared with baseline and posttreatment values.

[b] $P < 0.05$ compared with end of treatment value.

TABLE 2. Mean body weights and caloric intakes in the study subjects (mean ± SE) during the baseline, treatment, and posttreatment periods

	Baseline	Treatment	Posttreatment
BW (kg)			
Nal-Glu + T + Teslac	74.6 ± 3.1	75.1 ± 3.0	75.7 ± 3.0
Nal-Glu + T	78.2 ± 2.5	79.2 ± 2.2	78.6 ± 2.3
Placebo	83.1 ± 5.5	83.5 ± 5.5	83.5 ± 5.6
Calories (Cal/day)			
Nal-Glu + T + Teslac	2829 ± 234	2409 ± 161	2234 ± 155
Nal-Glu + T	2967 ± 342	2708 ± 178	2424 ± 188
Placebo	3054 ± 224	2598 ± 140	2607 ± 214

cholesterol and percentage of calories consumed as fat did not change in any group throughout the study.

Side-effects

All men were able to self-administer the daily injections without difficulty. Most of the men receiving Nal-Glu experienced some local irritation and/or erythema at the site of injection; these effects disappeared within a few hours. A nontender sc nodule developed at the injection site in most of these men and was most apparent in the leaner men. These nodules regressed within 2 weeks after injection and did not hinder the daily activities of any of the men. None of the men reported major changes in libido or behavior during the course of the study.

Discussion

We created selective deficiency of estradiol in healthy men for 6 weeks by administering a GnRH antagonist together with T replacement and Teslac, an inhibitor of aromatization. During the treatment period, these men were selectively E_2 deficient, and they had significantly lower levels of HDL cholesterol than in the baseline and posttreatment phases of the study. This decrease was manifested primarily in the HDL_2 density fraction. Plasma levels of Apo-AI decreased in parallel to the decrease in plasma HDL. In contrast, in men who received antagonist and T replacement, but no Teslac (and whose E_2 levels did not change), there were no significant changes in plasma lipids. These data demonstrate that the small quantity of E_2 that normally circulates in men has a role in the regulation of plasma HDL cholesterol in men, especially the HDL_2 fraction.

In previous studies of the role of estrogens in regulating lipoproteins in men, E_2 levels were greatly supraphysiological, and in some cases, T deficiency was present as well. In these studies, estrogens caused an increase in HDL cholesterol (13–14, 27) and Apo-AI (27); the effects of the treatment on the HDL density subfractions were not reported. Our data are consistent with these reports. They are also consistent with the work of Friedl *et al.* (15), who showed that aromatization of exogenous T to E_2 is important in limiting the decrease in HDL cholesterol observed when exogenous T enanthate is administered, and with the recent data of Zmuda *et al.* (28), who showed that the combination of high dose T plus Teslac produced a greater decrease in the HDL_2 density fraction than in the HDL_3 fraction. Our finding of a selective effect of gonadal steroids on the HDL_2 density fraction is also in agreement with studies of estrogen administration to women; in many of these studies, HDL_2 increased to a greater degree than did HDL_3 (8–10).

Hepatic lipase hydrolyzes HDL cholesterol particles, particularly particles in the HDL_2 density fraction (29, 30); this enzyme is stimulated by androgens and suppressed by estrogens (29, 30). We did not measure hepatic lipase activity in this study, but our data are consistent with the hypothesis that selective suppression of E_2 in the men who received Teslac led to an increase in hepatic lipase activity and a resultant decrease in plasma HDL_2. This hypothesis is supported by the data of Friedl *et al.* (15) and Zmuda *et al.* (28); in these studies, hepatic lipase activity rose more in men who received T plus Teslac than in men who received only T.

We found no change in calculated LDL levels in men who received Teslac, whereas orally administered estrogens have been shown to decrease plasma LDL in men (13, 14, 31), primarily through enhanced catabolism (31). The postulated mechanism of increased LDL catabolism is through increased expression of hepatic LDL receptors; it is likely that the first pass effect of oral estrogens accounts for the majority of the stimulation of LDL receptor synthesis. Our data suggest that small changes in circulating E_2 in men have little effect on the LDL receptor. The significance of the small increase in mean plasma LDL during the posttreatment period is not clear; it occurred in 7 of the 10 men, but was unrelated to changes in serum E_2 levels or changes in body weight or dietary habits. Similarly, although oral estrogen administration frequently results in an increase in plasma triglycerides, we observed essentially no change in triglycerides during the E_2-deficient period in the men who received Teslac. The increase in mean plasma triglyceride levels during the posttreatment period in these men resulted primarily from marked increases in plasma triglycerides in two of the men; in these two men, plasma E_2 levels during the study were no different from those in the other men, whereas their dietary cholesterol intakes increased after the completion of treatment. Thus, in these men, dietary, rather than hormonal, factors may have caused their higher triglyceride levels.

Mean baseline T and E_2 levels were not different among the three groups in the baseline state. Based on T levels achieved on day 3 of treatment, serum T levels in both groups were most likely a few nanomoles per L greater than baseline on the first few days after each T injection, whereas at the end of each week, T levels were slightly, but not significantly, lower than baseline. In the men who received Nal-Glu plus T, serum E_2 levels were at baseline on day 3 of treatment. At the end of each week of treatment, mean E_2 levels in these men were slightly below the mean baseline level, but the decrease was not statistically significant. In contrast, men who received Nal-Glu plus T plus Teslac had E_2 levels that were in the hypogonadal range on day 3 of treatment and at the end of each treatment week, demonstrating the effectiveness of Teslac as an inhibitor of peripheral aromatization.

In men who received Nal-Glu, T, and Teslac, total HDL cholesterol and Apo-AI levels fell significantly during the

treatment period. In the men who received Nal-Glu and T (but no Teslac), there was a trend toward a decrease in these parameters as well, although the decreases were not statistically significant. In this group of subjects, the observed decreases in total HDL and in Apo-AI may have resulted from the slight elevations in serum T levels that occurred in the first few days after each T injection. Although we did not measure T levels in the first few days after injection during weeks 2–6 of the treatment period, we found that in men receiving Nal-Glu in combination with exogenous T, peak serum T levels after T injection were similar from week to week (unpublished data). Thus, serum T levels were most likely 2–3 mmol/L above the baseline level during the first part of each treatment week.

At the end of the treatment period, mean HDL_3 levels were suppressed by similar amounts in both groups of men who received Nal-Glu. At the same time, mean HDL_2 in men who received Nal-Glu plus T were slightly, but not significantly, lower than the baseline level, again presumably as a result of the small elevation in serum T levels that occurred during part of each week. However, the data show that in men who received Nal-Glu plus T plus Teslac, lowering of serum E_2 resulted in significant suppression of HDL_2, an effect that can clearly be distinguished from the effects of T. Thus, at normal male levels, the primary effect of E_2 appears to be to maintain circulating levels of the HDL_2 density subfraction.

Several studies (32–34) have demonstrated that low levels of HDL cholesterol independently predict increased mortality from coronary artery disease. Jacobs *et al.* (32) found that cardiovascular mortality resulting from a decrease in HDL cholesterol of 0.26 mmol/L was approximately equal to an increase in LDL cholesterol of 0.78 mmol/L. In other studies (35, 36), coronary risk decreased by 2–3% for each increment in plasma HDL of 0.026 mmol/L. In our study, plasma HDL in men who received Teslac decreased by 0.10 mmol/L during the period of E_2 deficiency. By extrapolation, if suppression of E_2 were maintained over a period of years, the risk of CAD in these men might increase by 10–12%. In addition, HDL_2 has been shown to have a greater inverse correlation with risk of CAD than does HDL_3 (37–39). Taken together, the epidemiological and interventional data suggest that in males, physiological levels of E_2 offer some degree of coronary protection. However, supraphysiological E_2 levels in men are unlikely to be cardioprotective. Several studies in which high doses of oral estrogens were administered to men have showed increased morbidity and/or mortality in treated patients compared to controls (40–42), presumably due to an increased thrombotic tendency.

In summary, we created a model of selective E_2 deficiency in healthy men by administering a GnRH antagonist in combination with a physiological replacement dose of T and an inhibitor of aromatization. After 6 weeks, men receiving this regimen showed significant decreases in total HDL cholesterol, especially the HDL_2 density fraction, and in Apo-AI. There was only a small decrease in HDL_2 cholesterol in men who received antagonist and T along with nonsignificant decreases in HDL and Apo-AI. These data suggest that the levels of E_2 present in serum of normal men may protect against cardiovascular disease, analogous to the protective effects of estrogen in women.

Acknowledgments

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References

1. **Castelli WP.** 1984 Epidemiology of coronary heart disease: the Framingham study. Am J Med. 76A:4–12.
2. **Knopp RH.** 1988 The effects of postmenopausal estrogen therapy on the incidence of arteriosclerotic vascular disease in women. Obstet Gynecol. 72:295–305.
3. **Jacobs DR, Mebane IL, Bangdiwala SI, Criqui MH, Tyroler HA.** 1990 High density lipoprotein cholesterol as a predictor of cardiovascular disease mortality in men and women: the follow-up study of the lipid research clinics prevalence study. Am J Epidemiol. 131:32–47.
4. **Gordon T, Castelli WP, Hjortland MC, Kannel WB, Dawber TR.** 1977 High density lipoprotein as a protective factor against coronary heart disease. Am J Med. 62:707–714.
5. **00000** 1980 The Lipid Research Clinics Population Studies Data Book, vol 1. Washington DC: DHHS, NIH Publication 80–1527.
6. **Stevenson JC, Crook D, Gosland IF.** 1993 Influence of age and menopause on serum lipids and lipoproteins in healthy women. Atherosclerosis. 98:83–90.
7. **Matthews KA, Meilahn E, Kuller LH, Kelsey SF, Caggiula AW, Wing RR.** 1989 Menopause and risk factors for coronary heart disease. N Engl J Med. 321:641–646.
8. **Fahreus L.** 1988 The effects of estradiol on blood lipids and lipoproteins in postmenopausal women. Obstet Gynecol. 72:18S–22S.
9. **Knopp RH.** 1991 Estrogen replacement therapy for reduction of cardiovascular risk in women. Curr Opin Lipidol. 2:240–247.
10. **Walsh BW, Schiff I, Rosner B, Greenberg L, Ravnikar V, Sacks FM.** 1991 Effects of postmenopausal estrogen replacement on the concentration and metabolism of plasma lipoproteins. N Engl J Med. 325:1196–1204.
11. **Henderson BE, Paginini-Hill A, Ross RK.** 1991 Decreased mortality in users of estrogen replacement therapy. Arch Intern Med. 151:75–78.
12. **Bush TL, Barrett-Connor E, Cowan LD, et al.** 1987 Cardiovascular mortality and noncontraceptive use of estrogen in women: results from the Lipid Research Clinics Program Follow-up Study. Circulation. 75:1102–1109.
13. **Bulusu NV, Lewis SP, Das A, Clayton WE.** 1982 Serum lipid changes after estrogen therapy in prostatic carcinoma. Urology. 20:147–150.
14. **Henriksson P, Einarsson K, Eriksson A, Kelter U, Angelin B.** 1989 Estrogen induced gallstone formaltion in males. J Clin Invest. 84:811–816.
15. **Friedl KE, Hannan CJ, Jones RE, Kettler TM, Plymate SR.** 1990 High-density lipoprotein is not decreased if an aromatizable androgen is administered. Metabolism. 39:69–77.
16. **Bagatell CJ, McLachlan RI, deKretser DM, Burger HG, Vale WW, Rivier JE, Bremner WJ.** 1989 A Comparison of the suppressive effects of testosterone, a potent new gonadotropin-releasing hormone antagonist on gonadotropin and inhibin levels in normal men. J Clin Endocrinol Metab. 69:43–48.
17. **Tenover JS, Dahl KD, Vale WW, Rivier JE, Bremner WJ.** 1990 Hormonal responses to a potent gonadotropin hormone-releasing hormone antagonist in normal elderly men. J Clin Endocrinol Metab.

71:881–888.
18. **Bagatell CJ, Knopp, RH, Vale WW, Rivier JE, Bremner WJ.** 1992 Physiologic levels of testosterone suppress HDL cholesterol levels in normal men. Ann Intern Med. 116:967–973.
19. **Dennis B, Ernst N, Hjortland M, Tillotson J, Grambisch B.** 1980 The NHLBI Nutition Data System. J Am Diet Assoc. 77:641–647.
20. **Bachorik PS, Albers JJ.** 1986 Precipitation methods for quantification of lipoproteins. In: Albers, JJ, Segrest, JP eds. Methods in enzymology. Orlando: Academic Press; vol 129:78–100.
21. **Warnick GR, Benderson J, Albers JJ.** 1982 Dextran sulfate Mg^{2+} precipitation procedure for quantitation of high-density-lipoprotein cholesterol. Clin Chem. 28:1379–1388.
22. **Warnick GR.** 1986 Enzymatic methods for quantification of lipoprotein lipids. In: Albers JJ, Segrest JP, eds. Methods in enzymology. Orlando: Academic Press; vol 129:101–123.
23. **Friedwald WT, Levy RI, Fredrickson DS.** 1972 Estimation of the concentration of low-density lipoprotein cholesterol in plasma without the use of the preparative centrifuge. Clin Chem. 18:499–501.
24. **Matsumoto AM, Paulsen CA, Hopper BR, Rebar RW, Bremner WJ.** 1983 Human chorionic gonadotropin and testicular function: stimulation of testosterone, testosterone precursors, and sperm production despite high estradiol levels. J Clin Endocrinol Metab. 56:720–728.
25. **Morgadam M, Ahmed SW, Mensh AH, Godwin ID.** 1990 Within-person fluctuations of serum cholesterol and lipoproteins. Arch Intern Med. 150:1645–1648.
26. **Bookstein L, Gidding SS, Donovan M, Smith FA.** 1990 Day to day variability of serum cholesterol, triglyceride, and high-density lipoprotein cholesterol levels. Arch Intern Med. 150:1653–1657.
27. **Moorjani S, Dupont A, Labrie F, et al.** 1988 Changes in plasma lipoproteins during various androgen suppression therapies in men with prostatic carcinoma: effects of orchiectomy, estrogen, and combination treatment with luteinizing hormone-releasing hormone agonist and flutamide. J Clin Endocrinol Metab. 66:614–621.
28. **Zmuda JM, Fahrenbachm MC, Younkin BT, et al.** 1993 The effect of testosterone aromatization on high density lipoprotein cholesterol levels and post-heparin lipolytic activity. Metabolism. 42:446–450.
29. **Applebaum-Bowden D, Goldberg AP, Pyalisto OJ, Brunzell JD, Hazzard WR.** 1977 Effect of estrogen on post-heparin hepatic lipase. J Clin Invest. 59:601–608.
30. **Tikkanen MJ, Nikkila EA.** 1987 Regulation of hepatic lipase and serum lipoproteins by sex steroids. Am Heart J. 113:562–567.
31. **Erikkson M, Berglund L, Rudling M, Henriksson P, Angelin B.** 1989 Effects of estrogen on low density lipoprotein metabolism in males. J Clin Invest. 84:802–810.
32. **Jacobs DR, Mebane IL, Bangdiwala SI, Criqui MH, Tyroler HA.** 1990 High density lipoprotein cholesterol as a predictor of cardiovascular disease mortality in men and women: the follow-up study of the lipid research clinics prevalence study. Am J Epidemiol. 131:32–47.
33. **Goldbourt U, Holtzman E, Neufield HN.** 1985 Total and high density lipoprotein cholesterol in the serum and risk of mortality: evidence of a threshold effect. Br Med J. 290:1239–12343.
34. **Castelli WP, Garrison RJ, Wilson PWF, Abbott GD, Kalsousdian S, Kannel WB.** 1986 Incidence of coronary heart disease and lipoprotein cholesterol levels. JAMA. 256:2835–2838.
35. **Manninen V, Elo MO, Frick MH, et al.** 1988 Lipid alterations and the decline in the incidence of coronary heart disease in the Helsinki Heart Study. J Am Med Assoc. 260:641–650.
36. **Gordon DL, Probstfield JL, Garrison RJ, et al.** 1989 High-density lipoprotein cholesterol and cardiovascular disease: four prospective American studies. Circulation. 79:8–15.
37. **Naito HK.** 1985 The association of serum lipids, lipoproteins, and apolipoproteins with coronary artery disease assessed by coronary arteriography. Ann NY Acad Sci. 454:230–237.
38. **Miller NE.** 1987 Associations of high density lipoprotein subclasses with ischemic heart disaease and coronary atherosclerosis. Am Heart J. 113:589–597.
39. **Salonen JT.** 1990 Epidemiology of high density lipoproteins and atherosclerosis. In: Carson LA, ed. Proceedings of the Karolinska Hospital Symposium on Disorders of HDL. London: Smith Gordon; 145–154.
40. **Blackard CE, Doe RP, Mellinger GT, Byar DP.** 1970 Incidence of cardiovascular disease and death in patients receiving diethylstilbestrol for carcinoma of the prostate. Cancer. 26:249–252.
41. **VA Cooperative Urological Research Group.** 1967 Treatment and survival of patients with cancer of the prostate. Surg Gynecol Obstet. 124:1011–1018.
42. **Coronary Drug Project Reserch group.** 1973 Findings leading to the discontinuation of the 2.5 mg/day estrogen group. JAMA. 226:652–657.