

## A Dose-Escalating Study With the Fetal Estrogen Estetrol in Healthy Men

Herjan J. T. Coelingh Bennink,<sup>1</sup> Yvette Zimmerman,<sup>1</sup> Carole Verhoeven,<sup>1</sup> Alice E. Dutman,<sup>1</sup> Tjeert Mensinga,<sup>2</sup> Cornelis Klufft,<sup>3</sup> Yacov Reisman,<sup>4</sup> and Frans M. J. Debruyne<sup>5</sup>

<sup>1</sup>Pantarhei Oncology, 3700 AL Zeist, Netherlands; <sup>2</sup>QPS Netherlands BV, 9713 GZ Groningen, Netherlands; <sup>3</sup>Good Biomarker Sciences, 2333 CL Leiden, Netherlands; <sup>4</sup>Department of Urology, Amstelland Hospital, 1186 AM Amstelveen, Netherlands; and <sup>5</sup>Andros Men's Health Institutes, 6842 CV Arnhem, Netherlands

**Context:** Luteinizing hormone–releasing hormone (LHRH) agonists have replaced estrogens for endocrine treatment of advanced prostate cancer (PC) because of cardiovascular side effects. The fetal estrogen estetrol (E4) may be safer for PC treatment and is expected to decrease testosterone (T) and prevent estrogen deficiency.

**Objective:** To investigate the safety and T-suppressive effect of E4 in healthy men.

**Design:** Double-blind, randomized, placebo-controlled, dose-escalating study.

**Setting:** The study was conducted at a phase I clinical unit (QPS, Netherlands).

**Participants:** Healthy male volunteers aged 40 to 70 years.

**Intervention(s):** Three treatment cohorts of 15 volunteers with placebo (n = 5) and E4 (n = 10). Estetrol doses tested were 20, 40, and 60 mg/d. Subjects were treated for 4 weeks.

**Main Outcome Measures:** Subjective side effects, pharmacodynamic effects on hemostatic variables, lipids, glucose, bone parameters, and endocrine parameters related to T metabolism.

**Results:** Total and free T decreased dose-dependently and significantly. Nipple tenderness occurred in 40% and decrease of libido occurred in 30% of E4-treated men. The unwanted estrogenic effects on hemostasis were small, dose dependent, and in some cases significant. Lipid and bone parameters showed a favorable trend.

**Conclusion:** The effect of E4 on testosterone levels is insufficient for standalone PC treatment. Taking all clinical and pharmacodynamic variables into consideration, a daily dose of 40 mg E4 seems safe for further evaluation of endocrine PC treatment in combination with LHRH analogs. (*J Clin Endocrinol Metab* 103: 3239–3249, 2018)

The aim of the current study was to investigate the subjective and objective effects of the human fetal estrogen estetrol (E4) in healthy men to estimate its potential for the treatment of advanced prostate cancer (PC), defined as metastatic and/or locally infiltrating PC,

when surgical orchiectomy is insufficient or not the patient's choice.

In the past, estrogens, especially the synthetic estrogen diethylstilbestrol (DES), have been used effectively for the endocrine treatment of advanced PC (1). The objective

ISSN Print 0021-972X ISSN Online 1945-7197

Printed in USA

Copyright © 2018 Endocrine Society

Received 18 January 2018. Accepted 15 June 2018.

First Published Online 20 June 2018

Abbreviations: ADT, androgen deprivation therapy; APC, activated protein C; APCr, activated protein C resistance; COC, combined oral contraceptive; CTX-1, C-telopeptide; CV, coefficient of variation; DES, diethylstilbestrol; E2, estradiol; E4, estetrol; EE, ethinyl estradiol; F1+2, prothrombin fragment 1 + 2; HDL-C, high-density lipoprotein cholesterol; LC-MS/MS, liquid chromatography tandem-mass spectrometry; LDL-C, low-density lipoprotein cholesterol; LH, luteinizing hormone; LHRH, luteinizing hormone–releasing hormone; PC, prostate cancer; PK, pharmacokinetic; SHBG, sex hormone–binding globulin; T, testosterone; TFPI, tissue factor pathway inhibitor; TG, triglyceride.

of this therapy was to eliminate androgens, especially testosterone (T), because T, through its metabolite dihydrotestosterone, stimulates the growth of androgen-sensitive adenocarcinoma of the prostate, which comprise 90% of all PCs (2). This endocrine approach to PC treatment is known as androgen deprivation therapy (ADT), and estrogens were the first compounds used for this purpose (3). Estrogens suppress T synthesis through inhibition of luteinizing hormone (LH) by a negative feedback effect on the hypothalamic-pituitary axis (3–5). In addition, estrogens have a separate suppressive effect on free T levels by increasing the levels of the hepatic carrier protein sex hormone-binding globulin (SHBG) (4).

Although estrogens have been implicated as causal factor for PC (6, 7), they were efficacious for ADT of PC (3). However, the compounds used in the past, especially DES, had serious cardiovascular side effects, and estrogens were abandoned when the LH-releasing hormone (LHRH) agonists became available for ADT in the 1980s as a method for achieving medical orchiectomy (8).

Several papers suggest that the cardiovascular side effects of estrogen treatment are related to the route of administration and the type and dose of the estrogen (9–11). For example, parenteral estrogens, including transdermal application, prevent first-pass exposure of the liver and appear to reduce the risk of cardiovascular toxicity. Studies with the transdermal estradiol patch have demonstrated acceptable risk for cardiovascular events and less interference with hemostasis in patients with PC (10, 12).

With respect to the type of estrogen, estrogens that have been investigated for the treatment of PC are DES, ethinyl estradiol (EE), and estradiol (E2). Recently, the fetal estrogen E4 combined with drospirenone has shown reduced hemostatic effects as compared with EE/drospirenone combinations in women (13). These results suggest a lower cardiovascular risk for women taking E4 containing combined oral contraceptives (COCs) compared with women taking EE-containing COCs. This estrogen might be useful for ADT of advanced PC if E4 is also less harmful for the cardiovascular system in men. In addition to suppressing T, the use of a fetal estrogen for ADT prevents T and estrogen deficiency symptoms, such as hot flushes and sweating (14–16), fatigue (14, 17), and bone loss with an increased risk of fractures (18–20) as well as other symptoms that can occur such as arthralgia (14, 16, 21), mood changes (22), sleep disturbance (23), and cognition problems (22, 24, 25).

The objectives of the present dose-escalation study with E4 in healthy men 40 to 70 years old were to investigate the subjective effects of E4, its safety, and its effect on endocrine parameters, hemostasis, lipids, and bone biochemistry. In addition, the pharmacokinetic (PK) characteristics of E4 were assessed.

## Subjects and Methods

### Study design

This study was a phase Ib, double-blind, randomized, placebo-controlled, dose-escalation study in healthy male subjects conducted by QPS (Groningen, Netherlands). The study protocol was approved by the independent ethics committee Evaluation and Ethics in Biomedical Research (in Dutch: Stichting BEBO) and was performed in accordance with the Declaration of Helsinki and the International Conference on Harmonization Guidelines for Good Clinical Practice. All participants provided written informed consent prior to screening.

### Treatments

There were three consecutive 28-day treatment cohorts. In each cohort, 15 men participated; 10 received E4, and 5 received placebo (allocation ratio, 2:1). The E4 doses were 20, 40, and 60 mg in the first, second, and third cohort, respectively. After completion of the 20-mg and the 40-mg E4 dose cohort, safety and tolerance data were evaluated by the investigator and the sponsor and submitted to the independent ethics committee for approval to proceed to the next E4 dose cohort.

The study medication was supplied in tablets for oral administration and packed in blisters by Haupt Pharma (Münster, Germany). Tablets were taken once daily for 28 days between 8:00 and 10:00 AM. The pharmacist of QPS was responsible for the labeling of the study medication, which was done according to a randomization scheme. Personnel of the QPS pharmacy department were the only persons unblinded to the treatment assignment until database lock.

### Subjects

Subjects were nonsmoking, healthy men, 40 to 70 years old, with a body mass index between 18.5 and 30.0 kg/m<sup>2</sup> and a prostate-specific antigen value <3.0 ng/mL. Subjects were recruited by QPS. The main exclusion criteria were (1) a history or the presence of contraindications for the use of steroids, (2) prostate hyperplasia or micturition problems that suggested the presence of prostate hyperplasia (International Prostate Symptom Score >7), and (3) the presence of active acute or chronic infection. Subjects in cohort 3 were not included if they had a prothrombin and/or Factor V Leiden mutation at screening.

### Assessments

After screening, study visits were scheduled on days 1 (baseline), 2, 7, 14, and 28 (last day of study medication). Subjects in cohort 3 were contacted by telephone on day 21 for an additional check on well-being. After the treatment period, study visits were scheduled on days 29, 30, and 31. Subjects stayed overnight from days 27 to 29 for frequent blood sampling to assess PK variables of E4. A follow-up visit was scheduled for day 56. Blood samples were collected on days 1, 2, 7, 14, 28, and 56 to measure endocrine and hemostatic variables, lipids, fasting glucose, and bone turnover markers.

### Safety and tolerance

Safety and tolerance assessments included the recording of adverse events and physical examinations including palpation of the prostate, vital signs (blood pressure and heart rate), body

weight, ECGs, and safety laboratory parameters (hematology and biochemistry).

### Laboratory measurements

Hemostatic variables and angiotensinogen were analyzed at Good Biomarker Science (Leiden, Netherlands). Measurements of total T, free T, and E2 were performed by ABL (Assen, Netherlands). The E4 plasma levels were measured by QPS. All other laboratory measurements were performed under the responsibility of Stichting Certe KCL (Leeuwarden, Netherlands).

### Hemostasis variables

Hemostasis variables, measured at baseline and after 28 days, included endogenous thrombin potential-based activated protein C resistance (APCr), antithrombin, protein S activity, free tissue factor pathway inhibitor (TFPI), prothrombin fragment 1+2 (F1+2), and D-dimer.

Endogenous thrombin potential-based APCr was assessed as the ratio of thrombin generation with and without activated protein C (APC) using an end-point method with 30-minute incubation. APC was purchased as APC 1660 PAL, Enzyme Research Laboratories (South Bend, IN); tissue factor to start activation was from Dade Innovin (Miami, FL); phospholipid micelles (15  $\mu$ M), a mixture of DOPC/DOPE/DOPS (3:1:1), was from Avanti Polar Lipids (Alabaster, AL); Thrombin Substrate S2238 was purchased from Instrumentation Laboratory (Bedford, MA). The test plasma was defibrinated with Reptilase (Pentapharm, Basel, Switzerland) [reference range of the ratio <2.4; coefficient of variation (CV), <20%].

Prothrombin F1+2 was determined by ELISA (Enzygnost<sup>®</sup> F1+2 monoclonal; Siemens Health Care Diagnostics Products GmbH, The Hague, Netherlands) (CV, <15%; reference range, 69 to 229 pM). Antithrombin was determined on an STA<sup>®</sup> Compact Analyzer with the Coamatic<sup>®</sup> LR Antithrombin assay (Instrumentation Laboratory) (CV, <10%; reference range, 81% to 123%). Protein S activity was measured on an STA<sup>®</sup> Compact Analyzer with the STA<sup>®</sup> Protein S Clotting kit (Diagnostica Stago, Gennevilliers, France) (CV, <10%; reference range, 62% to 138%). D-dimer was quantified by ELISA (monoclonal 14: 7/8/7, Conjugate DD13/PO; Good Biomarker Science) (CV, <10%; reference range, 80 to 362 ng fibrinogen equivalent/mL). Free TFPI was determined by ELISA with the Asserachrom<sup>®</sup> Free TFPI kit (Diagnostica Stago) (CV, <10%; reference range, 3.0 to 19.8 ng/mL).

### Lipids and fasting glucose

Total cholesterol, high-density lipoprotein cholesterol (HDL-C), and triglycerides (TGs) were measured by enzymatic (colorimetric) methods (Roche Diagnostics, Almere, Netherlands). Total cholesterol (CV, <3%; assay range, 0.08 to 20.7 mmol/L); HDL-C (CV, <3.7%; assay range, 0.8 to 3.1 mmol/L); TG (CV, <4%; assay range, 0.05 to 11.3 mmol/L). Low-density lipoprotein cholesterol (LDL-C) was calculated as follows: total cholesterol – HDL-C – 0.45\*TG (CV, <2.7%; range, 0.1 to 25 mmol/L). Lipoprotein(a) was measured using nephelometry (Beckman Coulter, Woerden, Netherlands) (CV, <6%; assay range, 0.02 to 6.4 g/L). Fasting glucose was measured by enzymatic (colorimetric) methods (Roche Diagnostics) (CV, <3.9%; assay range, 0.11 to 41.6 mmol/L).

### Bone turnover markers

The biomarker for bone resorption C-telopeptide (CTX-1) was measured by electrochemiluminescence immunoassay (Roche Diagnostics) (CV%, <6%; assay range, 10 to 6000 pg/mL). The biomarker for bone formation osteocalcin was measured by immunoradiometric assay (MT-diagnostics, Etten-Leur, Netherlands) (CV%, <9%; assay range, 2.1 to 49  $\mu$ g/L).

### Endocrine parameters

Total T, free T, E2, LH, FSH, and SHBG were measured at baseline and after 7, 14, and 28 days of treatment. SHBG was measured on days 2 and 56. Total T, free T, and E2 were also measured on days 31 and 56. Angiotensinogen was measured at baseline and after 28 days of treatment.

Total T, free T, and E2 levels were measured using validated liquid chromatography tandem-mass spectrometry (LC-MS/MS) methods. To determine serum total T levels, samples were extracted using liquid-liquid extraction and subsequent derivatization prior to analysis with LC-MS/MS (CV,  $\leq$ 15%; assay range, 0.0500 to 20.0 ng/mL). Free T levels were measured by rapid equilibrium dialysis followed by LC-MS/MS (CV,  $\leq$ 15%; assay range, 1.00 to 100 pg/mL). E2 samples were extracted using solid phase extraction followed by derivatization. Derivatized products were extracted by liquid-liquid extraction and analyzed using LC-MS/MS (CV,  $\leq$ 15%; assay range, 5.00 to 100 pg/mL).

LH, FSH, and SHBG were measured by electrochemiluminescence immunoassay (Roche Diagnostics) (LH CV%, <11.5%; assay range, 0.1 to 200 IU/L; FSH CV%, <5.5%; assay range, 0.1 to 200 IU/L; SHBG CV%, <6.5%; assay range, 0.35 to 200 nM). Angiotensinogen was measured by ELISA (Human Total Angiotensinogen assay kit; IBL, Gunma, Japan) (CV, <15%; reference range, 0.31 to 20 ng/mL). The calibrator was replaced by purified plasma angiotensinogen obtained from Athens Research and Technology (Athens, GA).

### Pharmacokinetics

Blood samples for evaluating E4 PK parameters were collected on days 2, 7, 14, and 28 just prior to dosing with E4. On day 28, PK samples were collected at –30, 10, 20, 30, 45, 60, and 90 minutes and at 2, 4, 6, 8, 12, 16, 24, 36, 48, and 72 hours after the intake of E4. To measure E4, the plasma assay comprised sample preparation by liquid-liquid extraction and subsequent derivatization and analysis of the extract by LC-MS/MS (CV,  $\leq$ 15%; assay range for cohort 1 and cohort 2, 25 to 10,000 pg/mL; assay range for cohort 3, 2500 to 1,000,000 pg/mL). Samples were stored at –20°C until analysis.

### Statistical analysis

This was an exploratory study, and no formal hypothesis testing was performed. The total sample size of the study was 45 subjects, with 30 subjects using three different doses of E4 and 15 subjects randomized to placebo. The number of participants in this study was considered to be sufficient for a first impression of the safety and the pharmacodynamic effects of E4.

Observed values and changes from baseline were summarized using descriptive statistics (n, mean, median, SD, minimum, maximum) for each treatment. An analysis of covariance, including the baseline measurement as covariate, was performed to test the difference between the mean of the active treatment and the mean of the placebo treatment after the

28-day treatment period. Relevant covariates that may be potential confounding factors for hormonal endpoints, like age and obesity, have not been tested because the number of subjects in this phase I study was considered to be too small. All subjects who received treatment were included in the evaluation of the safety data. The per protocol population, defined as the group of subjects who completed the study without major protocol deviations, was used for the evaluation of efficacy and pharmacodynamic parameters.

The PK analysis set, which consisted of the All-Subjects-Treated population, was used for calculation of E4 plasma PK parameters. The E4 plasma PK parameters were calculated as appropriate using noncompartmental approaches. PK variables were computed using Phoenix WinNonlin, version 6.3 or higher (Pharsight Corporation, St. Louis, MO). Actual elapsed sampling times relative to E4 oral administration were used for the estimation of PK parameters. The 24-hour area under the curve was calculated using the linear trapezoidal method.

## Results

### Baseline characteristics and study completion

Of the 96 subjects screened, 51 were not invited for randomization. The most common reasons for screening failure were abnormal laboratory values, high blood pressure, and abnormal ECG values (Fig. 1). A total of 45 subjects were randomized, and 43 subjects completed all study-related visits and procedures. Two subjects in cohort 3 discontinued the study prematurely for reasons not related to the study medication. Baseline characteristics were similar among the groups (Table 1). At baseline, mean age ranged between 55 and 59 years, and mean body mass index was 25.8 kg/m<sup>2</sup>. Compliance

toward study medication was high (100% for 43 out of the 45 treated subjects).

### Safety and tolerance

E4 was well tolerated in all treatment groups. No clinically relevant changes in body weight, vital signs, ECG results, physical examinations, and safety laboratory parameters were recorded. No subject discontinued the study due to an adverse event during the intake of study medication. Side effects considered related to E4 were a decrease of libido in 30% (9 of 30) of subjects treated with E4 and nipple tenderness in 40% (12 of 30) of E4-treated subjects. Headache occurred equally in all groups. More than 90% of the side effects were classified as mild, and no serious side effects occurred.

### Pharmacodynamic parameters

Results of E4 treatment compared with placebo on the pharmacodynamic parameters are shown in Table 2.

### Hemostasis variables

The plasma levels of the two coagulation inhibitors (antithrombin and protein S activity) decreased over time in all three E4 dosing groups. Decreases in antithrombin activity up to 23% (all E4 groups vs placebo,  $P < 0.05$ ) and decreases in protein S activity up to 19% (40 mg E4 vs placebo,  $P < 0.01$ ; 60 mg E4 vs placebo,  $P < 0.05$ ) were observed. For antithrombin activity, the decreases showed a significant dose-response relationship (20 mg E4,  $P < 0.05$ ; 40 mg E4,  $P < 0.001$ ; 60 mg E4,  $P < 0.0001$ ). No changes were observed in the placebo group

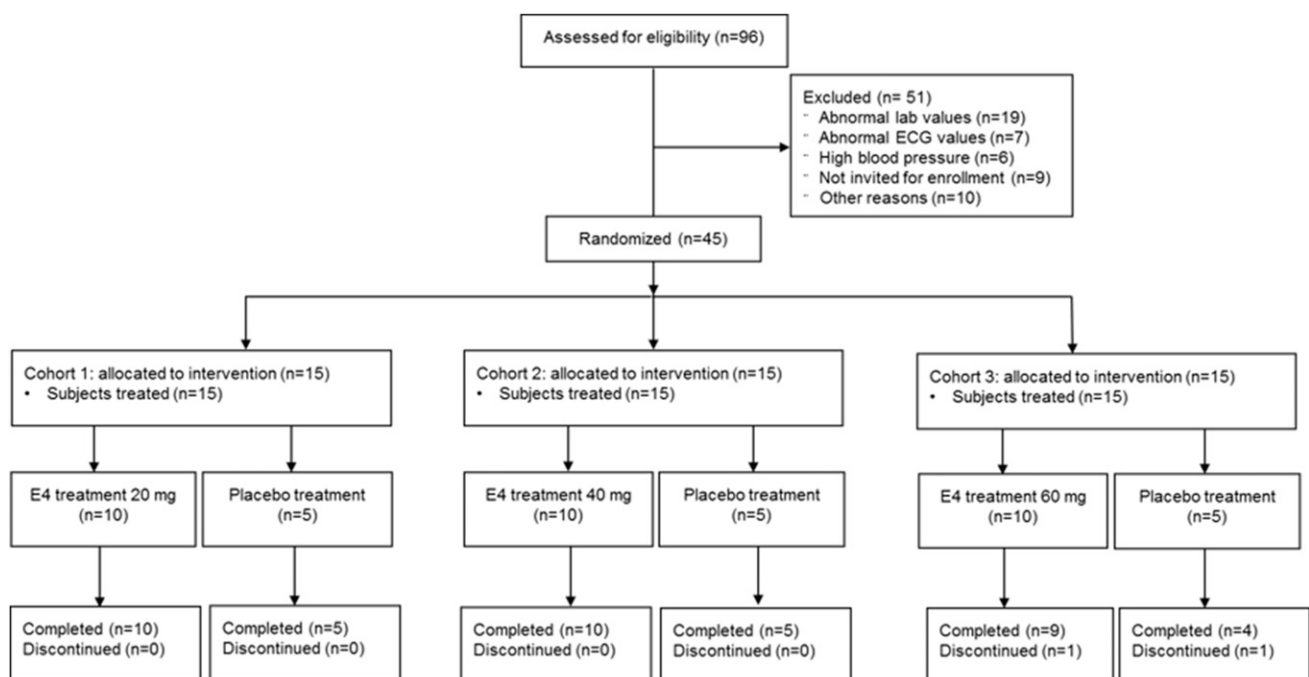


Figure 1. Subject allocation flow chart.

**Table 1. Baseline Characteristics of Study Subjects**

	Placebo (n = 15)	20 mg E4 (n = 10)	40 mg E4 (n = 10)	60 mg E4 (n = 10)
Age, y				
Mean $\pm$ SD	58.7 $\pm$ 6.7	59.9 $\pm$ 7.8	59.4 $\pm$ 5.2	55.2 $\pm$ 6.0
Range	47–66	49–70	50–69	42–61
Body weight, kg				
Mean $\pm$ SD	87.0 $\pm$ 13.0	86.0 $\pm$ 7.9	81.8 $\pm$ 6.7	84.6 $\pm$ 10.0
Range	63.7–111.3	73.0–97.5	73.7–94.5	72.6–102.2
BMI, kg/m <sup>2</sup>				
Mean $\pm$ SD	25.9 $\pm$ 2.9	25.2 $\pm$ 2.5	26.3 $\pm$ 2.3	25.9 $\pm$ 2.2
Range	19.9–30.0	20.0–28.9	22.7–29.3	23.8–29.7

Abbreviation: BMI, body mass index.

for these two parameters. For the 20-mg and 60-mg E4 groups, no changes were seen in the global coagulation inhibition test, which quantifies the ability of the anticoagulant protein APC to downregulate clotting (APCr). For APCr, the 40-mg and the placebo groups showed an increase in absolute change from baseline of 0.37 (40 mg E4 vs placebo,  $P < 0.05$ ) and 0.18, respectively. Free TFPI levels showed a decrease in all groups, including placebo. The placebo groups showed a decrease in free TFPI levels of 7%. For the 20-mg, 40-mg, and 60-mg E4 groups, the decrease was 17%, 24%, and 21%, respectively.

The coagulation activation markers D-dimer and prothrombin F1+2 showed different effects. D-dimer levels increased dose-dependently after E4 intake with 16%, 48%, and 99% for the 20, 40, and 60 mg E4 groups, respectively, whereas no relevant changes were observed in the placebo group. The prothrombin F1+2 levels showed a decrease in the placebo group and in the 20-mg and 40-mg E4 groups, whereas in the 60-mg E4 group the levels increased by 30% (60 mg E4 vs placebo,  $P < 0.05$ ).

### Lipids and fasting glucose

A decrease in total cholesterol and LDL-C levels was observed for all E4 groups (the decrease in total cholesterol ranged between 5% and 11%; the decrease in LDL-C ranged between 11% and 18%) and in the placebo group (7% and 8%, respectively). For all three E4 groups, small increases were seen for HDL-C (20 mg E4 vs placebo,  $P < 0.05$ ), whereas TG levels remained unchanged and did not differ from placebo. In the placebo group, a small decrease in HDL-C levels (5%) was observed. The lipoprotein(a) levels fluctuated between the E4 treatment groups. No significant changes were found compared with placebo for lipids, lipoproteins, and fasting glucose.

### Bone turnover markers

CTX-1 levels decreased in the 20-mg and 40-mg E4 groups by ~20% (20 mg vs placebo,  $P < 0.01$ ) and 27% (40 mg E4 vs placebo,  $P < 0.0001$ ). In the 60-mg E4 group, no decrease was observed. In the placebo group,

the levels of CTX-1 increased by 16%. Osteocalcin levels did not change during the treatment period in any of the treatment groups.

### Efficacy parameters

The effects of E4 compared with placebo on the endocrine parameters are shown in Table 3.

### Endocrine parameters

Figure 2 shows the results of total T, free T, SHBG, and angiotensinogen in whisker plots. A dose-dependent decrease was seen in total T levels, ranging from 28% in the 20-mg E4 group (vs. placebo,  $P < 0.05$ ) to 60% in the 40-mg E4 group (vs. placebo,  $P < 0.0001$ ) and 76% in the 60-mg E4 group (vs. placebo,  $P < 0.0001$ ), whereas no changes were observed for placebo. For free T levels, an even stronger dose-dependent decrease was observed, ranging from 43% in the 20-mg E4 group (vs. placebo,  $P < 0.01$ ) to 83% in the 40-mg E4 group (vs. placebo,  $P < 0.0001$ ) and 84% in the 60-mg E4 group (vs. placebo,  $P < 0.0001$ ). Free T levels in the placebo group did not change. SHBG levels increased significantly with E4 intake compared with placebo. By day 28, the increase in the 20-mg E4 group was 56% (20 mg E4 vs placebo,  $P < 0.01$ ); with the 40-mg and 60-mg E4 groups, the increases were 103% (40 mg E4 vs placebo,  $P < 0.0001$ ) and 121% (60 mg E4 vs placebo,  $P < 0.0001$ ), respectively. Mean angiotensinogen levels increased with E4 intake from 21% in the 20-mg E4 group to 75% in 40-mg E4 and 86% in the 60-mg E4 group. The angiotensinogen levels in the placebo group did not change. By day 56, mean total T, free T, and SHBG levels had returned to baseline levels in all dose groups.

FSH and E2 levels decreased significantly with E4 compared with placebo (for E2, 20 mg E4 vs placebo,  $P < 0.01$ ; all other comparisons,  $P < 0.0001$ ). The strongest decreases in FSH and E2 levels were observed in the 60-mg E4 group (90% and 71%, respectively).

LH levels in the placebo group increased by 20%; the increases with in the 20-mg and 40-mg E4 groups were

**Table 2. Actual Levels and Within-Subject Change ( $\Delta$ ) From Baseline of Pharmacodynamics Parameters Assessed at Baseline and at the End of the Study Treatment (Day 28)**

Parameter	Study Visit	Placebo (n = 13)	20 mg E4 (n = 10)	40 mg E4 (n = 10)	60 mg E4 (n = 9)
<b>Hemostasis</b>					
APCr (ratio)	Baseline	0.71 $\pm$ 0.42	1.33 $\pm$ 0.32	1.13 $\pm$ 0.55	0.88 $\pm$ 0.34
	D 28	0.89 $\pm$ 0.36	1.30 $\pm$ 0.35	1.50 $\pm$ 0.71 <sup>a</sup>	0.82 $\pm$ 0.23
	$\Delta$ from baseline	0.18 $\pm$ 0.36	-0.03 $\pm$ 0.36	0.37 $\pm$ 0.70	-0.06 $\pm$ 0.32
Antithrombin activity, %	Baseline	101.6 $\pm$ 9.1	103.1 $\pm$ 8.5	99.5 $\pm$ 9.9	108.8 $\pm$ 11.6
	D 28	99.6 $\pm$ 13.8	91.4 $\pm$ 9.0 <sup>a</sup>	82.5 $\pm$ 11.0 <sup>b</sup>	84.0 $\pm$ 10.3 <sup>c</sup>
	$\Delta$ from baseline	-2.0 $\pm$ 8.2	-11.7 $\pm$ 12.0	-17.0 $\pm$ 4.4	-24.8 $\pm$ 13.4
D-dimer, ng FE/mL	Baseline	150.1 $\pm$ 50.4	198.2 $\pm$ 60.1	144.1 $\pm$ 42.7	113.5 $\pm$ 52.4
	D 28	161.3 $\pm$ 64.0	228.9 $\pm$ 80.9	213.7 $\pm$ 119.5	226.3 $\pm$ 229.6
	$\Delta$ from baseline	11.2 $\pm$ 39.0	30.7 $\pm$ 69.3	69.6 $\pm$ 111.1	112.8 $\pm$ 213.8
Protein S activity, %	Baseline	112.3 $\pm$ 22.3	100.3 $\pm$ 15.1	100.7 $\pm$ 9.4	110.8 $\pm$ 20.8
	D 28	109.6 $\pm$ 22.6	90.0 $\pm$ 10.9	81.2 $\pm$ 9.9 <sup>a</sup>	93.6 $\pm$ 24.4 <sup>a</sup>
	$\Delta$ from baseline	-2.8 $\pm$ 16.3	-10.3 $\pm$ 14.5	-19.5 $\pm$ 6.0	-17.2 $\pm$ 34.7
F1+2, pmol/L	Baseline	137.0 $\pm$ 55.1	142.9 $\pm$ 80.7	106.6 $\pm$ 27.6	125.0 $\pm$ 43.2
	D 28	125.2 $\pm$ 61.8	122.3 $\pm$ 43.0	97.8 $\pm$ 33.4	162.4 $\pm$ 49.1 <sup>a</sup>
	$\Delta$ from baseline	-11.9 $\pm$ 36.8	-20.6 $\pm$ 47.0	-8.8 $\pm$ 37.1	41.4 $\pm$ 58.4
Free TFPI, ng/mL	Baseline	10.2 $\pm$ 2.3	9.8 $\pm$ 1.7	9.3 $\pm$ 1.7	10.1 $\pm$ 2.4
	D 28	9.4 $\pm$ 2.5	8.2 $\pm$ 0.9	7.1 $\pm$ 2.1	8.0 $\pm$ 2.2
	$\Delta$ from baseline	-0.8 $\pm$ 1.0	-1.6 $\pm$ 1.3	-2.2 $\pm$ 2.1	-2.1 $\pm$ 1.1
<b>Lipids, lipoprotein, and fasting glucose</b>					
Total C, mmol/L	Baseline	5.66 $\pm$ 1.08	5.44 $\pm$ 0.65	5.71 $\pm$ 0.65	5.64 $\pm$ 0.75
	D 28	5.27 $\pm$ 0.91	5.17 $\pm$ 0.63	5.16 $\pm$ 0.61	5.02 $\pm$ 0.89
	$\Delta$ from baseline	-0.38 $\pm$ 0.57	-0.27 $\pm$ 0.43	-0.55 $\pm$ 0.69	-0.64 $\pm$ 0.24
HDL-C, mmol/L	Baseline	1.50 $\pm$ 0.50	1.24 $\pm$ 0.33	1.60 $\pm$ 0.28	1.40 $\pm$ 0.27
	D 28	1.42 $\pm$ 0.54	1.36 $\pm$ 0.38 <sup>a</sup>	1.75 $\pm$ 0.29	1.41 $\pm$ 0.29
	$\Delta$ from baseline	-0.08 $\pm$ 0.12	0.11 $\pm$ 0.11	0.15 $\pm$ 0.22	0.01 $\pm$ 0.11
LDL-C, mmol/L	Baseline	3.59 $\pm$ 1.14	3.56 $\pm$ 0.55	3.65 $\pm$ 0.75	3.76 $\pm$ 0.71
	D 28	3.31 $\pm$ 0.93	3.18 $\pm$ 0.65	2.99 $\pm$ 0.65	3.09 $\pm$ 0.68
	$\Delta$ from baseline	-0.28 $\pm$ 0.46	-0.38 $\pm$ 0.38	-0.66 $\pm$ 0.55	-0.67 $\pm$ 0.28
TG, mmol/L	Baseline	1.18 $\pm$ 0.81	1.43 $\pm$ 0.37	1.01 $\pm$ 0.32	1.13 $\pm$ 0.49
	D 28	1.17 $\pm$ 0.71	1.41 $\pm$ 0.38	0.99 $\pm$ 0.25	1.17 $\pm$ 0.61
	$\Delta$ from baseline	-0.01 $\pm$ 0.23	-0.02 $\pm$ 0.30	-0.03 $\pm$ 0.28	0.05 $\pm$ 0.42
Lipoprotein(a), mmol/L	Baseline	0.269 $\pm$ 0.388	0.482 $\pm$ 0.416	0.338 $\pm$ 0.558	0.324 $\pm$ 0.350
	D 28	0.224 $\pm$ 0.302	0.478 $\pm$ 0.421	0.213 $\pm$ 0.352	0.388 $\pm$ 0.315
	$\Delta$ from baseline	-0.056 $\pm$ 0.174	-0.004 $\pm$ 0.084	-0.290 $\pm$ 0.267	0.064 $\pm$ 0.258
Fasting glucose, mmol/L	Baseline	5.55 $\pm$ 0.48	5.60 $\pm$ 0.23	5.42 $\pm$ 0.25	5.47 $\pm$ 0.38
	D 28	5.26 $\pm$ 0.37	5.20 $\pm$ 0.30	5.06 $\pm$ 0.18	5.06 $\pm$ 0.35
	$\Delta$ from baseline	-0.28 $\pm$ 0.33	-0.40 $\pm$ 0.17	-0.36 $\pm$ 0.27	-0.41 $\pm$ 0.55
<b>Bone turnover markers</b>					
Osteocalcin, $\mu$ g/L	Baseline	11.93 $\pm$ 4.53	10.96 $\pm$ 2.19	10.70 $\pm$ 3.84	9.83 $\pm$ 2.60
	D 28	12.35 $\pm$ 4.56	11.34 $\pm$ 2.25	10.09 $\pm$ 3.23	10.28 $\pm$ 2.88
	$\Delta$ from baseline	0.42 $\pm$ 1.02	0.38 $\pm$ 0.85	-0.61 $\pm$ 2.22	0.46 $\pm$ 3.38
CTX-1, pg/mL	Baseline	315.32 $\pm$ 146.59	386.62 $\pm$ 116.94	361.72 $\pm$ 174.52	248.00 $\pm$ 59.38
	D 28	366.60 $\pm$ 182.72	309.54 $\pm$ 102.34 <sup>a</sup>	264.44 $\pm$ 152.74 <sup>c</sup>	252.24 $\pm$ 80.36
	$\Delta$ from baseline	51.28 $\pm$ 59.90	-44.70 $\pm$ 53.72	-97.28 $\pm$ 76.94	4.24 $\pm$ 68.60

*P* values were derived using an analysis of covariance model to test the difference between the mean concentrations of the placebo group with the treatment groups, the baseline measurement was included as a covariate. Values are mean  $\pm$  SD.

Abbreviations: APCr, activated protein C resistance; FE, fibrinogen equivalent unit; ND, not determined.

<sup>a</sup>Statistically significantly different (*P* < 0.05) from placebo.

<sup>b</sup>Statistically significantly different (*P* < 0.001) from placebo.

<sup>c</sup>Statistically significantly different (*P* < 0.0001) from placebo.

4% and 17%, respectively. In the 60-mg E4 group, LH was 36% lower compared with baseline.

### PK parameters

The PK parameters of E4 are shown in Table 4. A total of 29 subjects receiving E4 treatment completed treatment and were included in the PK analysis. One subject in

the 60-mg E4 group was not included in the PK analysis because this subject discontinued the study early. The E4 plasma concentration profiles (results not shown) showed that, after multiple-dose oral administration, E4 becomes quickly available, with peak concentrations reached within 30 to 45 minutes, and then declines gradually. Trough plasma concentrations indicate that

**Table 3. Actual Levels and Within-Subject Change ( $\Delta$ ) From Baseline of Endocrine Parameters, Assessed at Baseline, at the End of the Study Treatment (Day 28), and at Follow-up (Day 56)**

Parameter	Study Visit	Placebo (n = 13)	20 mg E4 (n = 10)	40 mg E4 (n = 10)	60 mg E4 (n = 9)
Total T, nmol/L	Baseline	18.15 $\pm$ 5.24	13.28 $\pm$ 4.83	18.30 $\pm$ 7.16	18.25 $\pm$ 6.70
	D 28	17.97 $\pm$ 4.81	9.54 $\pm$ 6.39 <sup>a</sup>	7.30 $\pm$ 5.47 <sup>c</sup>	4.37 $\pm$ 3.26 <sup>c</sup>
	$\Delta$ from baseline	-0.18 $\pm$ 4.08	-3.73 $\pm$ 3.45	-11.00 $\pm$ 5.76	-13.89 $\pm$ 8.07
Free T, nmol/L	Baseline	17.13 $\pm$ 5.53	15.29 $\pm$ 6.36	20.96 $\pm$ 7.46	23.31 $\pm$ 4.40
	D 28	0.17 $\pm$ 0.04	0.14 $\pm$ 0.05	0.12 $\pm$ 0.03	0.19 $\pm$ 0.06
	$\Delta$ from baseline	0.01 $\pm$ 0.05	-0.06 $\pm$ 0.03	-0.10 $\pm$ 0.03	-0.16 $\pm$ 0.04
SHBG, nmol/L	Baseline	0.15 $\pm$ 0.04	0.15 $\pm$ 0.06	0.14 $\pm$ 0.03	0.29 $\pm$ 0.10
	D 28	43.14 $\pm$ 11.77	31.08 $\pm$ 6.88	48.01 $\pm$ 18.55	43.80 $\pm$ 16.74
	$\Delta$ from baseline	43.88 $\pm$ 13.46	48.61 $\pm$ 15.55 <sup>a</sup>	97.67 $\pm$ 30.24 <sup>c</sup>	96.77 $\pm$ 41.83 <sup>c</sup>
Angiotensinogen, ng/mL	Baseline	42.68 $\pm$ 13.23	33.45 $\pm$ 12.09	51.11 $\pm$ 23.08	48.52 $\pm$ 19.00
	D 28	28,641 $\pm$ 6272	34,007 $\pm$ 4805	27,683 $\pm$ 4693	33,525 $\pm$ 6290
	$\Delta$ from baseline	28,607 $\pm$ 7116	41,299 $\pm$ 9901	48,349 $\pm$ 13,996	62,359 $\pm$ 21,036
E2, pmol/L	Baseline	-34 $\pm$ 7201	7292 $\pm$ 11932	20,666 $\pm$ 11,133	28,834 $\pm$ 23,513
	D 28	ND	ND	ND	ND
	$\Delta$ from baseline	77.94 $\pm$ 16.94	64.57 $\pm$ 19.52	77.96 $\pm$ 17.65	80.78 $\pm$ 24.35
FSH, U/L	Baseline	83.99 $\pm$ 18.93	45.24 $\pm$ 29.65 <sup>a</sup>	29.39 $\pm$ 11.74 <sup>c</sup>	23.80 $\pm$ 8.33 <sup>c</sup>
	D 28	6.05 $\pm$ 8.26	-17.20 $\pm$ 14.08	-48.37 $\pm$ 12.63	-48.40 $\pm$ 25.17
	$\Delta$ from baseline	86.92 $\pm$ 29.17	74.36 $\pm$ 24.28	98.60 $\pm$ 37.15	103.51 $\pm$ 26.13
LH, U/L	Baseline	6.99 $\pm$ 2.99	5.92 $\pm$ 6.22	6.57 $\pm$ 4.44	6.24 $\pm$ 2.57
	D 28	7.19 $\pm$ 3.01	1.88 $\pm$ 1.14 <sup>c</sup>	1.08 $\pm$ 0.93 <sup>c</sup>	0.63 $\pm$ 0.37 <sup>c</sup>
	$\Delta$ from baseline	0.20 $\pm$ 0.69	-4.04 $\pm$ 5.69	-5.49 $\pm$ 3.67	-5.61 $\pm$ 2.52
LH, U/L	Baseline	ND	ND	ND	ND
	Day 28	4.82 $\pm$ 1.82	4.57 $\pm$ 2.25	4.06 $\pm$ 1.46	5.56 $\pm$ 2.22
	$\Delta$ from baseline	5.79 $\pm$ 1.63	4.77 $\pm$ 1.26	4.73 $\pm$ 1.66 <sup>b</sup>	3.56 $\pm$ 1.78
LH, U/L	Baseline	1.0 $\pm$ 1.3	0.2 $\pm$ 1.9	0.7 $\pm$ 1.4	-2.0 $\pm$ 2.7
	Follow-up	ND	ND	ND	ND

Values are mean  $\pm$  SD. *P* values were derived using an analysis of covariance model to test the difference between the mean concentrations of the placebo group with the treatment groups. Baseline measurement was included as a covariate.

Abbreviation: ND, not determined.

<sup>a</sup>Statistically significantly different (*P* < 0.05) from placebo.

<sup>b</sup>Statistically significantly different (*P* < 0.001) from placebo.

<sup>c</sup>Statistically significantly different (*P* < 0.0001) from placebo.

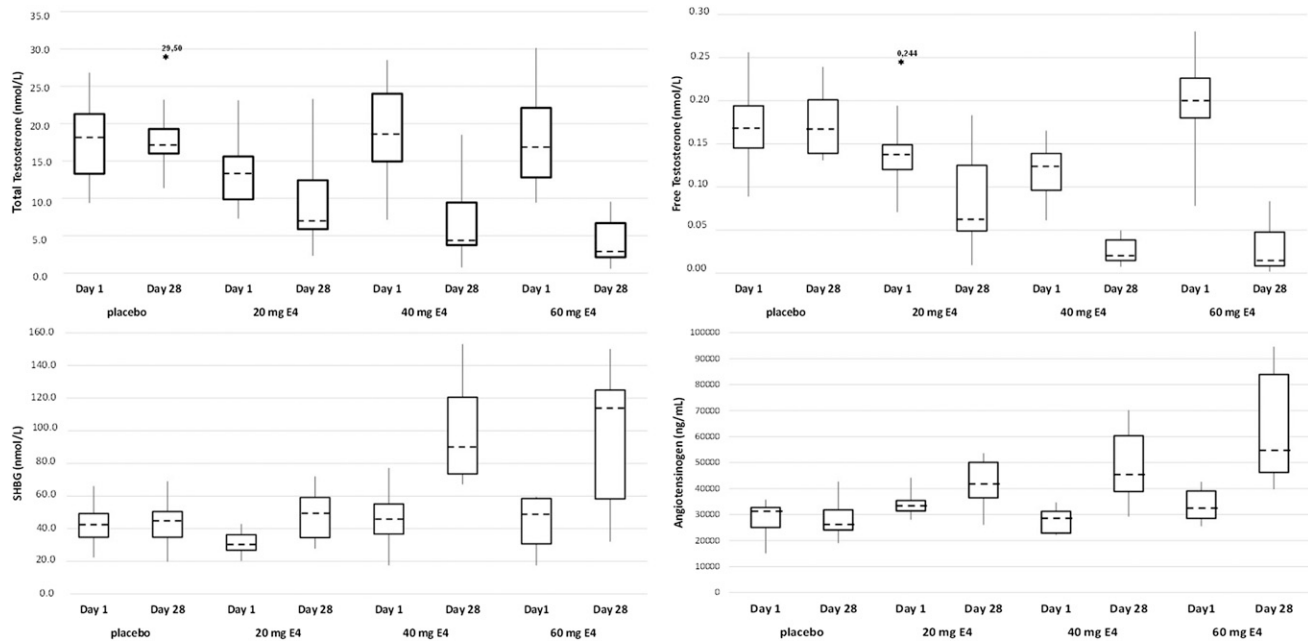
steady state was reached after 7 days of dosing. PK evaluation showed that the mean 24-hour area under the curve of E4 increased proportionately to dose when the dose was increased from 20 to 40 mg, but the increase was less than dose-proportional when the dose was increased to 60 mg. Mean  $C_{max}$  of E4 increased dose proportionally over the dose range of 20 to 60 mg. Median  $t_{max}$  was similar among the three dosing groups. Median values for  $t_{1/2}$  were similar for 20 mg and 40 mg E4 (~18 hours), whereas  $t_{1/2}$  was slightly higher for 60 mg E4. High variation in  $t_{1/2}$  was observed for 60 mg E4.

## Discussion

This dose-escalation study with E4 in healthy older men (40 to 70 years) was performed to estimate whether this fetal estrogen might be suitable as ADT for the treatment of men with advanced PC, defined as metastatic and/or locally infiltrating PC. E4 treatment would combine T suppression to inhibit tumor growth and estrogen

substitution to relieve the symptoms of estrogen deficiency, such as hot flashes and bone loss caused by the loss of the estrogen precursor T. More than 30 years ago, synthetic estrogens were abandoned for the treatment of PC because of the cardiovascular thromboembolic side effects (8). Because E4 has been identified as a potentially safer estrogen for the cardiovascular system in women (13), the question has arisen whether this estrogen would also be safer for the male cardiovascular system and be efficacious as ADT. In women, E4 is in phase III development as an estrogenic component of a COC with an E4 dose of 15 mg/d. This is about 10 times higher compared with available COCs containing natural estrogens, such as E2 (1.5 mg) (26) or estradiol-valerate (2 mg) (27). Based on this information from women, the doses of 20, 40, and 60 mg E4 used in this study are comparable to doses of 2, 4, and 6 mg E2.

The first and most important question is the safety of E4 in men because this is why estrogens failed in the past. In this relatively short study of 4 weeks of treatment, two



**Figure 2.** Total T, free T, SHBG, and angiotensinogen at baseline and day 28 by group. Whiskers represent minimum and maximum values; dashed lines indicate median values.

major subjective E4-related side effects occurred. First, nine men (30%) experienced loss of libido. This is an expected effect, confirming the suppression of T, which is the aim of ADT. Second, 12 men (40%) reported nipple tenderness. This is a known effect of estrogen treatment in men (28, 29) and could interfere with the acceptability of E4 treatment. The current study is too short to judge whether breast development (gynecomastia) (3, 12, 30, 31) will occur with long-term E4 treatment. However, it is known that nipple tenderness and gynecomastia can be prevented by a single dose of radiotherapy at the start of estrogen treatment (32). Both side effects observed were not dose dependent and were reversible. Headache occurred equally in all groups, including the placebo group.

The pharmacodynamic safety variables investigated in this study focused on cardiovascular risk factors. Most important are the hemostatic variables related to

venous thromboembolism because the occurrence of venous thromboembolism was the major reason to replace estrogens for PC treatment by LHRH analogs (8). With 5 mg DES, thromboembolic events occurred in 36% of the cases, and with 1 to 3 mg DES, this rate was still 10% to 17% (3). With E4, some hemostatic variables showed small procoagulant changes. The plasma levels of the anticoagulant proteins antithrombin, protein S, and free TFPI decreased; in the case of antithrombin, this decrease was dose dependent. The coagulation activation markers D-dimer and prothrombin F1+2 showed different effects. D-dimer levels increased dose-dependently after E4 intake, which may be related to the reduction in antithrombin. The prothrombin F1+2 levels showed a decrease in the 20-mg and 40-mg E4 groups, whereas in the 60-mg E4 group the levels increased. The limited effect of E4 on

**Table 4. Summary Pharmacokinetic Parameters of E4 on Day 28 After Multiple Dosing of 20, 40, or 60 mg E4 for 28 Days**

Parameters	20 mg E4 (n = 10)			40 mg E4 (n = 10)			60 mg E4 (n = 9)		
	Mean	SD	Median	Mean	SD	Median	Mean	SD	Median
C <sub>max</sub> , ng/mL	20.2	9.30	19.7	37.3	9.55	34.2	57.0	28.8	60.9
t <sub>max</sub> , h	0.98 <sup>a</sup>	1.77	0.43	0.88	0.38	0.75	0.99	0.52	0.75
AUC <sub>0-24</sub> , h/ng/mL	83	26.4	82	162	39.5	156	197	78.8	206
t <sub>1/2</sub> , h <sup>b</sup>	17.2	4.18	17.6	17.6	3.66	18.3	31.5	40.5	22.1

Abbreviations: AUC<sub>0-24</sub>, 24-h area under the curve; n, number of observations.

<sup>a</sup>One subject had an extremely high t<sub>max</sub> (6 h) after dosing with 20 mg E4.

<sup>b</sup>For four subjects, t<sub>1/2</sub> was not reportable due to adjusted R<sup>2</sup> value <0.80.



the plasma levels of F1+2 (20 mg and 40 mg E4 even decrease F1+2) suggests that E4 hardly, if at all, enhances the formation of thrombin (*i.e.*, E4 has virtually no effect on the activity of the coagulation cascade). The absence of an association between the D-dimer and F1+2 changes can be explained by the observation that antithrombin levels are decreased during E4 treatment. This may result in increased thrombin activity (fibrin formation/lysis and D-dimer formation) in the absence of enhanced thrombin generation (F1+2 formation).

Final judgment of the effects of E4 on hemostasis requires an extensive dedicated study measuring all relevant parameters for periods of at least 3 months.

Lipid changes related to the risk of arterial cardiovascular events were small and not significant, but the direction of the changes with E4 was favorable. The most relevant variable LDL-C decreased by 18% in the 40-mg and 60-mg E4 groups, whereas there was an 8% decrease in the placebo group. HDL-C, TGs, and fasting glucose did not change.

The two most important variables for bone metabolism were measured to judge whether E4 would be suitable to prevent bone loss and negate the increased fracture risk during ADT treatment. The results showed a decrease of bone resorption (CTX-1) with 20 mg and 40 mg E4 and no change of bone formation (osteocalcin), suggesting preservation of bone mass. However, better judgment of the effect of E4 on bone in men requires 12-month bone mass measurements, and final proof requires fracture rate studies.

Overall, the changes observed of hemostatic variables, lipid levels, and bone turnover markers were small. Taking all pharmacodynamic safety and efficacy variables into consideration, the 40-mg E4 dose seems safe for PC treatment. Relevant covariates that may be potential confounding factors for hormonal endpoints, like age and obesity, have not been tested because the number of subjects in this phase I study was considered to be too small. These aspects will be investigated in the ongoing PC study with E4. Based on data in women, 40 mg E4 would be comparable to 4 mg E2; in women, 2 mg E2 is sufficient to treat hypoestrogenicity. A dose of 40 mg E4 is therefore expected to be sufficient for the prevention of hypoestrogenic signs and symptoms in men. The dose-dependent increase of angiotensinogen confirms the estrogenicity of E4.

Would 40 mg E4 be sufficient as standalone estrogen ADT? Serum total T has always been the therapeutic target for ADT, and the main goal of ADT is to reduce total T levels to castration levels (<50 ng/dL). This target is not achieved with E4 in this study, although a dose-dependent decrease was seen in total T levels. However,

only ~1% to 2% of total T circulates as free T. Based on the free hormone hypothesis, free T is considered to be the biologically active form of T and is able to diffuse into cells and bind to the androgen receptor after conversion into dihydrotestosterone (2, 33). Therefore, measurement of free T levels has recently drawn attention as a more relevant target for ADT treatment than total T (4, 34). Levell *et al.* (35) have shown that lower free T levels correlate with improved survival of patients with PC. Because ~50% to 60% of total T is bound to SHBG (34, 36–38), a change in SHBG levels will directly affect the levels of free T. Estrogens are known to increase the levels of SHBG, and in this study a significant increase of SHBG was observed with all doses of E4. Altogether this leads to a strong suppression of free T with 40 mg E4, whereas 60 mg E4 does not further affect the SHBG and free T levels. However, because there is no generally accepted free T level as a target for ADT, the clinical effect of E4, based on free T suppression, remains to be established for standalone E4 ADT. Furthermore, because the free hormone hypothesis is currently under debate (39, 40), more research is needed regarding the clinical relevance of the suppressive effect of free T levels in PC. The gonadotropin inhibitory effect of E4 was confirmed by the dose-dependent suppression of FSH. LH levels did not change with 20 mg and 40 mg E4 compared with the placebo group and decreased after 4 weeks treatment with 60 mg E4. To judge the effect on LH adequately, more frequent blood sampling during a 24-hour period is necessary because of the short half-life of LH (41).

ADT of advanced PC starts with LHRH agonist therapy (42). Inhibition or antagonism of LHRH decreases LH levels and thereby decreases T levels. However, LHRH analogs suppress not only T but also suppress estrogens, which affects quality of life and causes multiple side effects, including hot flashes and sweating, fatigue, bone loss, and fractures (43, 44). Combining LHRH analogs with an estrogen such as E4 is expected to reduce these hypoestrogenic side effects and to have multiple additional treatment effects known to occur with estrogens, such as (1) further LH inhibition and thereby total T suppression, (2) additional free T inhibition through the increase of SHBG, (3) reduction of adrenal androgens, and (4) decreasing intratumoral androgen levels (3, 45, 46). By immediate T suppression, E4 may also suppress the tumor marker prostate-specific antigen faster after the start of treatment.

## Conclusion

The effect of E4 on testosterone levels is insufficient for standalone PC treatment. Taking all clinical and pharmacodynamic variables into consideration, a daily dose

of 40 mg E4 seems safe for further evaluation of endocrine PC treatment in combination with LHRH analogs.

## Acknowledgments

The authors thank Jan Rosing for expert advice on the hemostasis data.

**Clinical Trial Information:** ClinicalTrials.gov no. NCT02718378 (registered 9 February 2017).

**Correspondence and Reprint Requests:** Herjan J. T. Coelingh Bennink, MD, PhD, Pantarhei Oncology BV, P.O. Box 464, 3700 AL Zeist, Netherlands. E-mail: [hcb@pantarheibio.com](mailto:hcb@pantarheibio.com).

**Disclosure Summary:** H.J.T.C.B. is CEO and a shareholder and Y.Z., C.V., and A.E.D. are employees of Pantarhei Bioscience BV (Pantarhei Oncology BV is an affiliate of Pantarhei Bioscience BV). H.J.T.C.B., Y.Z., and C.V. are shareholders of Pantarhei Oncology BV. T.M. is an employee of QPS Netherlands BV. QPS received financial support from Pantarhei Oncology BV for the clinical part of the study. C.K. is CEO of Good Biomarker Sciences and received financial support from Pantarhei Oncology BV for the laboratory analysis and interpretation of hemostasis data. Y.R. has nothing to disclose. F.M.J.D. is a paid consultant for Pantarhei Oncology BV.

## References

- Presti JC Jr. Estrogen therapy for prostate carcinoma. *JAMA*. 1996; 275(15):1153.
- Stone L. Prostate cancer: The pathway to progression: DHT biosynthesis. *Nat Rev Urol*. 2017;14(9):516.
- Turo R, Smolski M, Esler R, Kujawa ML, Bromage SJ, Oakley N, Adeyoku A, Brown SC, Brough R, Sinclair A, Collins GN. Diethylstilboestrol for the treatment of prostate cancer: past, present and future. *Scand J Urol*. 2014;48(1):4–14.
- Rove KO, Crawford ED, Perachino M, Morote J, Klotz L, Lange PH, Andriole GL, Matsumoto AM, Taneja SS, Eisenberger MA, Reis LO. Maximal testosterone suppression in prostate cancer-free vs total testosterone. *Urology*. 2014;83(6):1217–1222.
- Langley RE, Godsland IF, Kynaston H, Clarke NW, Rosen SD, Morgan RC, Pollock P, Kockelbergh R, Lalani N, Dearnaley D, Parmar M, Abel PD. Early hormonal data from a multicentre phase II trial using transdermal oestrogen patches as first-line hormonal therapy in patients with locally advanced or metastatic prostate cancer. *BJU Int*. 2008;102(4):442–445.
- Bosland MC. A perspective on the role of estrogen in hormone-induced prostate carcinogenesis. *Cancer Lett*. 2013;334(1):28–33.
- Bonkhoff H. Estrogen receptor signaling in prostate cancer: Implications for carcinogenesis and tumor progression. *Prostate*. 2018;78(1):2–10.
- Leuprolide Study Group. Leuprolide versus diethylstilbestrol for metastatic prostate cancer. *N Engl J Med*. 1984;311(20):1281–1286.
- von Schoultz B, Carlström K, Collste L, Eriksson A, Henriksson P, Pousette A, Stege R. Estrogen therapy and liver function: metabolic effects of oral and parenteral administration. *Prostate*. 1989;14(4): 389–395.
- Ockrim JL, Lalani N, Kakkar AK, Abel PD. Transdermal estradiol therapy for prostate cancer reduces thrombophilic activation and protects against thromboembolism. *J Urol*. 2005;174(2):527–533, discussion 532–533.
- Bosset PO, Albiges L, Seisen T, de la Motte Rouge T, Phé V, Bitker MO, Roupêt M. Current role of diethylstilbestrol in the management of advanced prostate cancer. *BJU Int*. 2012;110(11 Pt C):E826–E829.
- Langley RE, Cafferty FH, Alhasso AA, Rosen SD, Sundaram SK, Freeman SC, Pollock P, Jinks RC, Godsland IF, Kockelbergh R, Clarke NW, Kynaston HG, Parmar MK, Abel PD. Cardiovascular outcomes in patients with locally advanced and metastatic prostate cancer treated with luteinising-hormone-releasing-hormone agonists or transdermal oestrogen: the randomised, phase 2 MRC PATCH trial (PR09). *Lancet Oncol*. 2013;14(4):306–316.
- Kluft C, Zimmerman Y, Mawet M, Klipping C, Duijkers IJ, Neuteboom J, Foidart JM, Bennink HC. Reduced hemostatic effects with drospirenone-based oral contraceptives containing estetrol vs. ethinyl estradiol. *Contraception*. 2017;95(2):140–147.
- Gravis G, Marino P, Joly F, Oudard S, Priou F, Esterni B, Latorzeff I, Delva R, Krakowski I, Laguerre B, Rolland F, Théodore C, Deplanque G, Ferrero JM, Pouessel D, Mourey L, Beuzebec P, Zanetta S, Habibian M, Berdah JF, Dauba J, Baciuchka M, Platini C, Linassier C, Labourey JL, Machiels JP, El Kouri C, Ravaud A, Suc E, Eymard JC, Hasbini A, Bousquet G, Soulie M, Fizazi K. Patients' self-assessment versus investigators' evaluation in a phase III trial in non-castrate metastatic prostate cancer (GETUG-AFU 15). *Eur J Cancer*. 2014;50(5):953–962.
- Karling P, Hammar M, Varenhorst E. Prevalence and duration of hot flushes after surgical or medical castration in men with prostatic carcinoma. *J Urol*. 1994;152(4):1170–1173.
- Sharifi R, Knoll LD, Smith J, Kramolowsky E. Leuprolide acetate (30-mg depot every four months) in the treatment of advanced prostate cancer. *Urology*. 1998;51(2):271–276.
- Storey DJ, McLaren DB, Atkinson MA, Butcher I, Frew LC, Smyth JF, Sharpe M. Clinically relevant fatigue in men with hormone-sensitive prostate cancer on long-term androgen deprivation therapy. *Ann Oncol*. 2012;23(6):1542–1549.
- Higano CS. Side effects of androgen deprivation therapy: monitoring and minimizing toxicity. *Urology*. 2003;61(2, Suppl 1):32–38.
- Berruti A, Dogliotti L, Terrone C, Cerutti S, Isaia G, Tarabuzzi R, Reimondo G, Mari M, Ardisson P, De Luca S, Fasolis G, Fontana D, Rossetti SR, Angeli A; Gruppo Onco Urologico Piemontese (G.O.U.P.), Rete Oncologica Piemontese. Changes in bone mineral density, lean body mass and fat content as measured by dual energy x-ray absorptiometry in patients with prostate cancer without apparent bone metastases given androgen deprivation therapy. *J Urol*. 2002;167(6):2361–2367, discussion 2367.
- Greenspan SL, Coates P, Sereika SM, Nelson JB, Trump DL, Resnick NM. Bone loss after initiation of androgen deprivation therapy in patients with prostate cancer. *J Clin Endocrinol Metab*. 2005;90(12):6410–6417.
- Ning YM, Pierce W, Maher VE, Karuri S, Tang SH, Chiu HJ, Palmbly T, Zirkelbach JF, Marathe D, Mehrotra N, Liu Q, Ghosh D, Cottrell CL, Leighton J, Sridhara R, Ibrahim A, Justice R, Pazdur R. Enzalutamide for treatment of patients with metastatic castration-resistant prostate cancer who have previously received docetaxel: U.S. Food and Drug Administration drug approval summary. *Clin Cancer Res*. 2013;19(22):6067–6073.
- Cherrier MM, Aubin S, Higano CS. Cognitive and mood changes in men undergoing intermittent combined androgen blockade for non-metastatic prostate cancer. *Psychooncology*. 2009;18(3): 237–247.
- Gonzalez BD, Small BJ, Cases MG, Williams NL, Fishman MN, Jacobsen PB, Jim HSL. Sleep disturbance in men receiving androgen deprivation therapy for prostate cancer: The role of hot flashes and nocturia. *Cancer*. 2018;124(3):499–506.
- Beer TM, Bland LB, Bussiere JR, Neiss MB, Wersinger EM, Garzotto M, Ryan CW, Janowsky JS. Testosterone loss and estradiol administration modify memory in men. *J Urol*. 2006; 175(1):130–135.
- Salminen EK, Portin RI, Koskinen AI, Helenius HY, Nurmi MJ. Estradiol and cognition during androgen deprivation in men with prostate carcinoma. *Cancer*. 2005;103(7):1381–1387.

26. Mansour D, Verhoeven C, Sommer W, Weisberg E, Taneepanichskul S, Melis GB, Sundström-Poromaa I, Korver T. Efficacy and tolerability of a monophasic combined oral contraceptive containing norgestrel acetate and 17 $\beta$ -oestradiol in a 24/4 regimen, in comparison to an oral contraceptive containing ethinylestradiol and drospirenone in a 21/7 regimen. *Eur J Contracept Reprod Health Care*. 2011;**16**(6):430–443.
27. Nelson A, Parke S, Mellinger U, Zampaglione E, Schmidt A. Efficacy and safety of a combined oral contraceptive containing estradiol valerate/dienogest: results from a clinical study conducted in North America. *J Womens Health (Larchmt)*. 2014;**23**(3):204–210.
28. Gerber GS, Zagaja GP, Ray PS, Rukstalis DB. Transdermal estrogen in the treatment of hot flashes in men with prostate cancer. *Urology*. 2000;**55**(1):97–101.
29. Oh WK. The evolving role of estrogen therapy in prostate cancer. *Clin Prostate Cancer*. 2002;**1**(2):81–89.
30. Langley RE, Kynaston HG, Alhasso AA, Duong T, Paez EM, Jovic G, Scrase CD, Robertson A, Cafferty F, Welland A, Carpenter R, Honeyfield L, Abel RL, Stone M, Parmar MK, Abel PD. A randomised comparison evaluating changes in bone mineral density in advanced prostate cancer: luteinising hormone-releasing hormone agonists versus transdermal oestradiol. *Eur Urol*. 2016;**69**(6):1016–1025.
31. Clemons J, Glodé LM, Gao D, Flaig TW. Low-dose diethylstilbestrol for the treatment of advanced prostate cancer. *Urol Oncol*. 2013;**31**(2):198–204.
32. Scherr DS, Pitts WR Jr. The nonsteroidal effects of diethylstilbestrol: the rationale for androgen deprivation therapy without estrogen deprivation in the treatment of prostate cancer. *J Urol*. 2003;**170**(5):1703–1708.
33. Mendel CM. The free hormone hypothesis: a physiologically based mathematical model. *Endocr Rev*. 1989;**10**(3):232–274.
34. Laurent MR, Hammond GL, Blokland M, Jardi F, Antonio L, Dubois V, Khalil R, Sterk SS, Gielen E, Decallonne B, Carmeliet G, Kaufman JM, Fiers T, Huhtaniemi IT, Vanderschueren D, Claessens F. Sex hormone-binding globulin regulation of androgen bioactivity in vivo: validation of the free hormone hypothesis. *Sci Rep*. 2016;**6**(1):35539.
35. Levell MJ, Siddall JK, Rowe E, Glashan RW, Robinson MR, Pidcock NB. Relationship of testosterone, sex hormone binding globulin, and calculated free testosterone to subsequent clinical progress in patients with carcinoma of the prostate treated with bilateral orchidectomy or estrogens. *Prostate*. 1987;**11**(1):17–21.
36. Sung J, Song YM. Genetic effects on serum testosterone and sex hormone-binding globulin in men: a Korean twin and family study. *Asian J Androl*. 2016;**18**(5):786–790.
37. Anderson DC. Sex-hormone-binding globulin. *Clin Endocrinol (Oxf)*. 1974;**3**(1):69–96.
38. Mody A, White D, Kanwal F, Garcia JM. Relevance of low testosterone to non-alcoholic fatty liver disease. *Cardiovasc Endocrinol*. 2015;**4**(3):83–89.
39. Goldman AL, Bhasin S, Wu FCW, Krishna M, Matsumoto AM, Jasuja R. A reappraisal of testosterone's binding in circulation: physiological and clinical implications. *Endocr Rev*. 2017;**38**(4):302–324.
40. Handelsman DJ. Free testosterone: pumping up the tires or ending the free ride? *Endocr Rev*. 2017;**38**(4):297–301.
41. Santen RJ, Bardin CW. Episodic luteinizing hormone secretion in man. Pulse analysis, clinical interpretation, physiologic mechanisms. *J Clin Invest*. 1973;**52**(10):2617–2628.
42. Mottet N, Bellmunt J, Bolla M, Briers E, Cumberbatch MG, De Santis M, Fossati N, Gross T, Henry AM, Joniau S, Lam TB, Mason MD, Matveev VB, Moldovan PC, van den Bergh RCN, Van den Broeck T, van der Poel HG, van der Kwast TH, Rouvière O, Schoots IG, Wiegel T, Cornford P. EAU-ESTRO-SIOG Guidelines on Prostate Cancer. Part 1: screening, diagnosis, and local treatment with curative intent. *Eur Urol*. 2017;**71**(4):618–629.
43. Freedland SJ, Eastham J, Shore N. Androgen deprivation therapy and estrogen deficiency induced adverse effects in the treatment of prostate cancer. *Prostate Cancer Prostatic Dis*. 2009;**12**(4):333–338.
44. Allan CA, Collins VR, Frydenberg M, McLachlan RI, Matthiesson KL. Androgen deprivation therapy complications. *Endocr Relat Cancer*. 2014;**21**(4):T119–T129.
45. Montgomery B, Nelson PS, Vessella R, Kalhorn T, Hess D, Corey E. Estradiol suppresses tissue androgens and prostate cancer growth in castration resistant prostate cancer. *BMC Cancer*. 2010;**10**(1):244.
46. Aggarwal R, Weinberg V, Small EJ, Oh W, Rushakoff R, Ryan CJ. The mechanism of action of estrogen in castration-resistant prostate cancer: clues from hormone levels. *Clin Genitourin Cancer*. 2009;**7**(3):E71–E76.