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## Platinum Priority – Prostate Cancer

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# Serum Testosterone and Dihydrotestosterone and Prostate Cancer Risk in the Placebo Arm of the Reduction by Dutasteride of Prostate Cancer Events Trial

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### Abstract

**Background:** Findings of studies on the association between androgens and prostate cancer (PCa) are mixed. Androgens may affect prostate-specific antigen (PSA) levels, thereby influencing biopsy recommendations. Also, androgens may stimulate prostate growth at very low levels with no additional effects at higher levels (saturation model). **Objective:** To test whether androgens were associated with PCa risk in the placebo arm of a prospective study in which biopsies were performed regardless of PSA level.

**Design, setting, and participants:** Of 8122 men in the Reduction by Dutasteride of Prostate Cancer Events (REDUCE) trial, 4073 men (50.1%) received placebo. Key entry criteria were PSA 2.5–10 ng/ml and one prior negative biopsy.

**Intervention:** Per-protocol biopsies at 2 and 4 yr; for-cause biopsies at physician discretion. **Outcome measurements and statistical analysis:** Multivariable logistic regression was used to test the association between baseline log-transformed testosterone and dihydrotestosterone (DHT) levels and the risk of detecting either PCa or low-grade PCa (Gleason score <6) compared with high-grade PCa (Gleason score >7). In secondary analysis, we stratified the analysis by low baseline androgen levels (testosterone <10 nmol/l; DHT <0.76 nmol/l) compared with normal baseline androgen levels.

**Results and limitations:** Of 4073 men, 3255 (79.9%) had at least one biopsy after randomization and were analyzed. Androgen levels tested continuously or by quintiles were generally unrelated to PCa detection or grade. PCa detection was similar among men with low compared with normal baseline testosterone levels (25.5% and 25.1%;  $p = 0.831$ ). In secondary analysis, higher testosterone levels at baseline were associated with higher PCa detection (odds ratio: 1.23; 95% confidence interval, 1.06–1.43;  $p = 0.006$ ) only if men had low baseline testosterone (<10 nmol/l). For men with normal baseline testosterone ( $\geq 10$  nmol/l), higher testosterone levels at baseline were unrelated to PCa risk ( $p = 0.33$ ). No association was found for DHT and PCa (all  $p > 0.85$ ).

**Conclusions:** Baseline serum testosterone and DHT levels were unrelated to PCa detection or grade. Our findings of the lowest testosterone levels being associated with the lowest PCa risk with no further changes with higher testosterone support a saturation model but must be confirmed in future studies using an a priori defined hypothesis. **ClinicalTrials.gov identifier:** NCT00056407.

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## 1. Introduction

Based on the dramatic response of advanced prostate cancer (PCa) to castration, it was presumed that androgens were associated with PCa development (androgen hypothesis) [1]. However, this hypothesis was not confirmed in many observational studies [2–4]. In 2008, a large study found no association between serum androgens and PCa risk [5]. Surprisingly, other studies found high PCa prevalence among men with low testosterone, contrary to the androgen hypothesis [6,7]. Yet other studies found that low testosterone levels were associated with high-grade PCa [8,9], worse presentation [10], lymph node [11] and seminal vesicle involvement [9] after prostatectomy, and worse responses to hormonal therapy [12]. Those findings suggest that low androgens may actually promote PCa risk, especially aggressive PCa [13]. However, not all studies agree, with other studies finding that among men with PCa, there is no association between androgen levels and PCa aggressiveness [5,14,15].

Mixed results from prior studies may have multiple reasons. First, as prostate biopsy is recommended for men with elevated prostate-specific antigen (PSA), and androgens can influence PSA production [16], androgens may influence PCa detection without truly affecting PCa risk. For example, more androgens could increase serum PSA, thereby increasing biopsy recommendations and resulting in unequal opportunities to detect PCa. Second, recent experimental [17] and clinical studies suggested that androgens stimulate prostate growth at very low levels but have no additional effect at higher levels (saturation model) [18]. In a placebo-controlled trial of 44 hypogonadal men with baseline testosterone levels  $<10.4$  nmol/l, testosterone replacement increased serum testosterone levels without increasing intraprostatic androgens or biomarkers levels, gene expression, or PCa incidence [19]. Thus, a saturation point is suggested around or below those levels. Third, many studies tested androgens continuously or grouping by tertiles or quartiles, regardless of the clinical threshold for hypogonadism. Consequently, depending on the percentage with “low” androgens, the lowest tertile or quartile may have included men with “normal” testosterone levels (albeit at the lower end). Thus, the association between androgens and PCa risk among men with low compared with normal androgen levels remains untested.

The Reduction by Dutasteride of Prostate Cancer Events (REDUCE) trial is unique in addressing the association between serum androgens and prostate outcomes such as PSA, prostate volume (PV), and PCa risk because of its design characteristics. REDUCE was a large, prospective, 4-yr, double-blind, placebo-controlled, randomized clinical trial of dutasteride for risk reduction of biopsy-detectable PCa. Importantly, REDUCE minimized detection bias by requiring 2- and 4-yr biopsies for all participants, regardless of PSA values. In the placebo arm of REDUCE, we performed a post hoc hypothesis-generating epidemiologic study to test whether higher serum testosterone and dihydrotestosterone (DHT) levels at baseline were associated with higher PCa detection during the 4-yr study. In secondary analysis,

we retested this hypothesis among men with low compared with normal androgen levels at baseline.

## 2. Patients and methods

### 2.1. Study population and procedures

Local institutional review boards approved the protocol, and participants gave consent before enrollment. An independent committee oversaw the REDUCE trial.

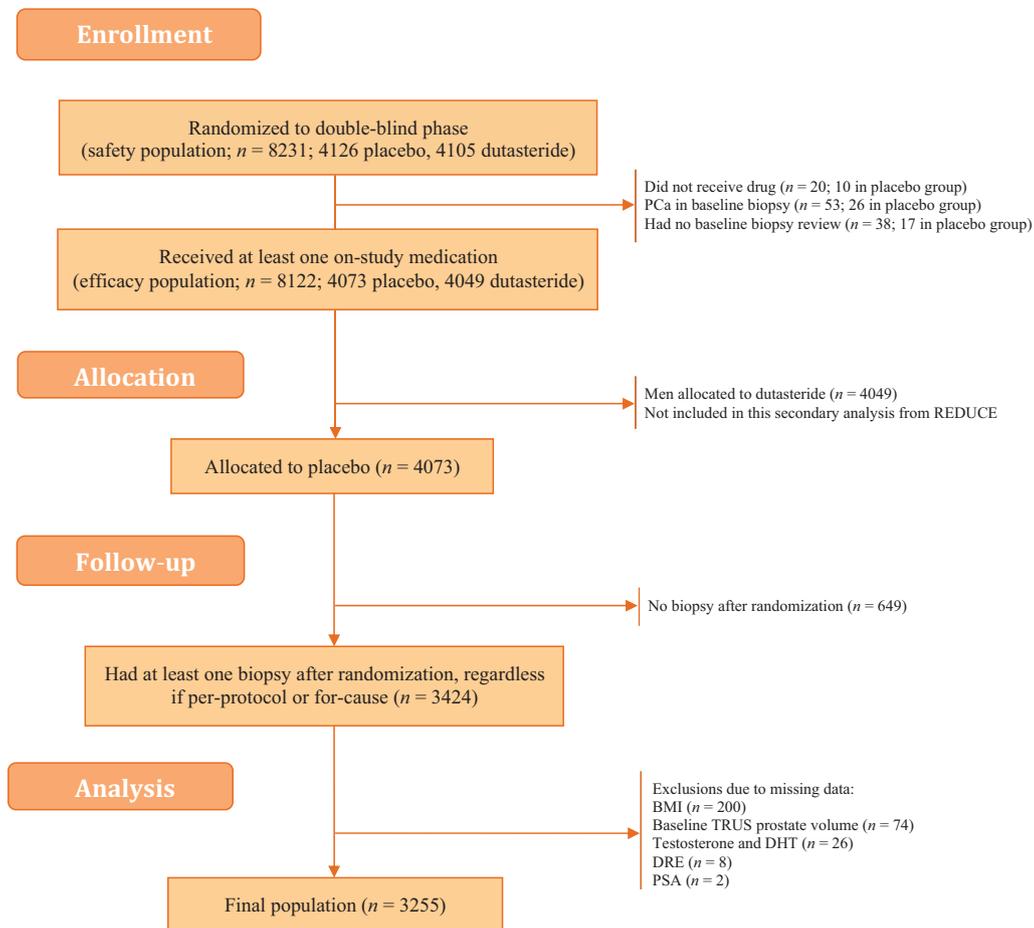
The REDUCE design was published previously [20]. Briefly, men were aged 50–75 yr and had serum PSA  $\geq 2.5$  or 3.0 ng/ml, depending on age (50–60 or 60–75 yr, respectively), but had PSA  $\leq 10$  ng/ml, one negative biopsy  $\leq 6$  mo before the study, PV  $<80$  ml, and International Prostate Symptom Score  $<25$  or  $<20$  on  $\alpha$ -blockers. Double-blind randomization was performed to dutasteride 0.5 mg or placebo daily for 4 yr, with similar study arms [21]. Of 8122 men in the efficacy population (those men who used on-study medication), 4073 men (50.1%) received placebo. Ten-core transrectal ultrasound (TRUS)-guided biopsies were required per protocol at 2 and 4 yr regardless of PSA levels; for-cause biopsies were allowed anytime at a physician's discretion. An experienced uropathologist analyzed all biopsies. Testosterone and DHT were measured at baseline using high-turbulent-flow liquid chromatography tandem mass spectrometry [22] (Quest Diagnostics, Madison, NJ, USA) (see Appendix for details) and PSA was tested at each semiannual visit. Timing of blood collection was not available.

### 2.2. Statistical analysis and outcomes

Of 4073 men, 3424 (84.1%) had at least one per-protocol or for-cause biopsy after randomization and were analyzed. Population characteristics were published before [23]. Testosterone and DHT levels were similar regardless of receiving biopsy after randomization (Mann-Whitney test,  $p \geq 0.15$ ). Of 3424 men, after excluding cases with missing testosterone or DHT ( $n = 26$ , 0.7%), PSA ( $n = 2$ , 0.05%), digital rectal examination (DRE;  $n = 8$ , 0.2%), prestudy TRUS PV ( $n = 74$ , 2.2%), and body mass index (BMI;  $n = 200$ , 5.8%), we obtained 3255 men (Fig. 1).

Baseline characteristics by quintiles of androgens were tested for trends using a nonparametric test (Cuzick [24]). In men in whom PCa was detected, we tested for differences in serum testosterone and DHT levels by Gleason scores using the Kruskal-Wallis test. We examined PCa detection rates over the range of baseline androgen levels using locally weighted scatterplot smoothing (LOWESS). LOWESS uses local subsets of data to fit smoothed regression lines. More weight is given to data closer to the point at which outcome is being estimated compared with more distant data. Our outcome was PCa detected at any time during the 4-yr REDUCE study and coded as 0 (no PCa) or 1 (PCa detected). LOWESS allowed us to explore whether the association between androgen levels and PCa risk was nonlinear.

We used multinomial logistic regression to test whether serum baseline testosterone and DHT levels, continuously or by quintiles, were associated with the following outcomes: no PCa (base), low-grade PCa (Gleason score 2–6), or high-grade PCa (Gleason score 7–10). We adjusted for age (continuous), race (white, black, other), and baseline data on DRE findings (suspicious compared with not suspicious for PCa), PSA (continuous), TRUS PV (continuous), BMI (continuous), and family history of PCa (yes/no). We also tested for trends using the median value of each quintile as a continuous variable. We used log-transformed values of serum testosterone and DHT levels, PSA, BMI, and TRUS PV because of their nonnormal distribution. However, because using either untransformed or log-transformed androgen levels did not affect the statistical significance of our analysis, we reported the main results of our regression models using untransformed androgen values, as those estimates are more clinically meaningful.



**Fig. 1 – Assembly of our study population.** PCa = prostate cancer; REDUCE = Reduction by Dutasteride of Prostate Cancer Events; BMI = body mass index; TRUS = transrectal ultrasound; DHT = dihydrotestosterone; DRE = digital rectal examination; PSA = prostate-specific antigen.

In secondary analysis, we used binomial logistic regression and the previous covariates to test the association between log-transformed baseline testosterone and DHT levels and PCa detection by strata of low testosterone levels ( $\leq 10$  nmol/l or  $\leq 288.4$  ng/dl) compared with normal testosterone levels ( $> 10$  nmol/l or  $> 288.4$  ng/dl). Men were also divided by low DHT levels ( $\leq 0.76$  nmol/l or  $\leq 22.1$  ng/dl) compared with normal DHT levels ( $> 0.76$  nmol/l or  $> 22.1$  ng/dl). The cutoff for testosterone was based upon usual ranges in healthy young men [25]. As no clinical cutoff exists for DHT, we defined a cut-off to create a “low” DHT group with equal size compared with the “low” testosterone group (at the 18th percentile of testosterone distribution). We used Stata v.11.0 (StataCorp, College Station, TX, USA) with a significance level ( $\alpha$ ) of 0.05.

### 3. Results

At baseline, men in higher testosterone quintiles were more likely to be nonwhite ( $p = 0.024$ ) and had lower BMIs ( $p < 0.001$ ) and higher DHT levels ( $p < 0.001$ ) (Table 1) compared with men in lower quintiles. No trend was observed for age ( $p = 0.093$ ), positive family history of PCa ( $p = 0.46$ ), PSA ( $p = 0.17$ ), DRE findings ( $p = 0.34$ ), or PV ( $p = 0.21$ ) across testosterone quintiles.

In higher compared with lower DHT quintiles, men were younger ( $p < 0.001$ ) and had lower BMIs ( $p < 0.001$ ) and higher testosterone levels ( $p < 0.001$ ) (Table 2). Also, men

in higher compared with lower DHT quintiles had an almost significant trend to be nonwhite ( $p = 0.06$ ), but they were similar regarding family history of PCa ( $p = 0.78$ ), PSA ( $p = 0.13$ ), DRE findings ( $p = 0.42$ ), and PV ( $p = 0.20$ ).

Of the men, 25.2% (819 of 3255) had PCa detected, with Gleason score 6 in 71.6% of the PCa cases ( $n = 586$ ), Gleason score 7 in 25.5% of the cases ( $n = 209$ ), and Gleason score  $> 8$  in 2.3% of the cases ( $n = 19$ ). Among men with PCa, serum testosterone and DHT levels were statistically similar regardless of Gleason scores (all  $p \geq 0.52$ ) (Table 3). In multivariable analysis, no association was found between quintiles of androgens with risk of low- or high-grade PCa (all  $p \geq 0.11$ ), except for the second quintile of DHT, which was associated with lower risk of low-grade PCa (odds ratio [OR]: 0.74; 95% confidence interval [CI], 0.55–0.98;  $p = 0.04$ ) (Table 4). Androgens were also unrelated to low- and high-grade PCa when tested continuously or as a  $p$  trend across quintiles (all  $p \geq 0.28$ ).

The LOWESS plot suggested that PCa risk was unrelated to testosterone and DHT levels across most of the range (Fig. 2). However, among men with low baseline testosterone levels ( $n = 596$ ; 18%), those with the lowest baseline testosterone had the lowest PCa risk. This risk increased as baseline testosterone levels approached

**Table 1 – Baseline characteristics of men in the placebo arm of the REDUCE trial by first, third, and fifth quintile of serum testosterone levels at baseline**

	First quintile, 2.7–10.3 nmol/l	Third quintile, 13.3–16.3 nmol/l	Fifth quintile, 20.6–45.7 nmol/l	p trend <sup>†</sup>
Observations, no.	654	654	646	
Age, yr				0.093
Mean (SD)	62.2 (6.1)	62.7 (6.0)	62.9 (6.2)	
Range	50.0–75.0	49.0–76.0	50.0–76.0	
Race, no. (%)				0.024
White	611 (93.4)	600 (91.7)	583 (90.2)	
Black	11 (1.7)	12 (1.8)	12 (1.9)	
Other	32 (4.9)	42 (6.4)	51 (7.9)	
Positive family history of PCa, no. (%)	86 (13.1)	76 (11.6)	76 (11.6)	0.46
DRE suspicious for PCa, no. (%)	21 (3.2)	24 (3.7)	20 (3.1)	0.34
BMI, kg/m <sup>2</sup>				<0.001
Median (IQR)	28.2 (25.8–31.0)	27.0 (24.9–29.4)	25.7 (23.9–27.8)	
Range	18.0–51.1	19.8–41.6	16.5–49.0	
PSA, ng/ml				0.17
Median (IQR)	5.6 (4.3–7.3)	5.5 (4.4–7.2)	5.8 (4.4–7.3)	
Range	2.0–10.0	2.4–14.2	2.5–11.3	
Testosterone, nmol/l				<0.001
Median (IQR)	8.5 (7.3–9.4)	14.7 (14.0–15.5)	24.2 (22.0–27.1)	
Range	2.7–10.3	13.3–16.3	20.6–45.7	
DHT, nmol/l				<0.001
Median (IQR)	0.8 (0.6–1.0)	1.2 (0.9–1.5)	1.8 (1.4–2.4)	
Range	0.1–5.6	0.1–7.4	0.2–10.2	
Prostate volume, cc				0.21
Median (IQR)	44.7 (33.1–57.4)	43.7 (34.6–55.7)	43.1 (32.4–56.5)	
Range	7.0–256.8	7.7–161.8	5.8–105.6	

SD = standard deviation; PCa = prostate cancer; DRE = digital rectal examination; BMI = body mass index; IQR = interquartile range; PSA = prostate-specific antigen; DHT = dihydrotestosterone.

<sup>†</sup> Nonparametric test for trends derived from Wilcoxon rank-sum test (Cuzick [24]).

**Table 2 – Baseline characteristics of men in the placebo arm of the REDUCE trial by first, third, and fifth quintile of serum dihydrotestosterone levels at baseline**

	First quintile, 0.1–0.8 nmol/l	Third quintile, 1.1–1.4 nmol/l	Fifth quintile, 1.9–10.2 nmol/l	p trend <sup>†</sup>
Observations, no.	678	622	648	
Age, yr				<0.001
Mean (SD)	63.5 (6.0)	63.0 (6.1)	61.9 (6.2)	
Range	50.0–76.0	49.0–75.0	50.0–76.0	
Race, no. (%)				0.06
White	630 (92.9)	570 (91.6)	588 (90.7)	
Black	13 (1.9)	13 (2.1)	11 (1.7)	
Other	35 (5.2)	39 (6.3)	49 (7.6)	
Positive family history of PCa, no. (%)	83 (12.2)	69 (11.1)	85 (13.1)	0.78
DRE suspicious for PCa, no. (%)	31 (4.6)	19 (3.1)	24 (3.7)	0.42
BMI, kg/m <sup>2</sup>				<0.001
Median (IQR)	28.6 (26.1–31.3)	26.8 (24.9–28.9)	25.9 (23.9–28.1)	
Range	18.0–51.1	17.2–45.6	16.5–43.4	
PSA, ng/ml				0.13
Median (IQR)	5.6 (4.2–7.2)	5.7 (4.4–7.2)	5.8 (4.4–7.5)	
Range	2.0–23.2	1.8–10.0	2.4–11.3	
Testosterone, nmol/l				<0.001
Median (IQR)	10.6 (8.3–13.2)	15.3 (12.3–18.6)	20.2 (15.5–25.0)	
Range	2.8–36.0	2.7–35.4	5.6–45.7	
DHT, nmol/l				<0.001
Median (IQR)	0.6 (0.5–0.7)	1.2 (1.1–1.3)	2.4 (2.1–3.3)	
Range	0.1–0.8	1.1–1.4	1.9–10.2	
Prostate volume, cc				0.20
Median (IQR)	44.5 (33.4–57.5)	42.4 (33.1–55.3)	43.8 (33.1–56.2)	
Range	7.0–264.9	5.8–126.8	10.0–178.3	

SD = standard deviation; PCa = prostate cancer; DRE = digital rectal examination; BMI = body mass index; IQR = interquartile range; PSA = prostate-specific antigen; DHT = dihydrotestosterone.

<sup>†</sup> Nonparametric test for trends derived from Wilcoxon rank-sum test (Cuzick [24]).

**Table 3 – Summary of serum testosterone and dihydrotestosterone levels in men detected with prostate cancer in the placebo arm of the REDUCE trial, by groups of Gleason score**

	Gleason 2–6 (n = 591)	Gleason 7 (n = 209)	Gleason 8–10 (n = 19)	p <sup>*</sup>
Testosterone, nmol/l				0.72
Median (IQR)	15.0 (11.1–20.1)	15.1 (11.1–19.1)	16.0 (11.7–21.0)	
Range	4.2–36.0	4.8–40.2	8.3–26.1	
DHT, nmol/l				0.52
Median (IQR)	1.2 (0.8–1.6)	1.1 (0.8–1.6)	1.2 (1.0–1.6)	
Range	0.2–9.8	0.1–7.1	0.7–2.9	

IQR = interquartile range; DHT = dihydrotestosterone.  
<sup>\*</sup> Kruskal-Wallis test.

**Table 4 – Estimates of the association between baseline testosterone and dihydrotestosterone levels and the risk of prostate cancer among 3255 men in the placebo arm of the REDUCE trial, using multinomial regression models adjusted for confounders<sup>†</sup> and testing androgens as either categorical or continuous variables**

	Gleason 2–6			Gleason 7–10		
	OR	95% CI	p	OR	95% CI	p
Testosterone						
As a categorical variable						
By groups of quintiles						
First (reference)	1			1		
Second	0.79	0.59–1.05	0.11	0.71	0.45–1.13	0.15
Third	0.90	0.68–1.20	0.48	0.99	0.64–1.53	0.96
Fourth	0.91	0.68–1.22	0.53	1.06	0.69–1.64	0.77
Fifth	1.06	0.80–1.43	0.64	0.92	0.58–1.45	0.70
As a continuous variable						
Untransformed	1.00	0.99–1.02	0.59	1.00	0.98–1.03	0.72
Log transformed	1.10	0.87–1.40	0.41	1.14	0.80–1.65	0.47
Median value of each quintile <sup>§</sup>	1.01	0.99–1.03	0.28	1.00	0.98–1.03	0.75
DHT						
As a categorical variable						
By groups of quintiles						
First (reference)	1			1		
Second	0.74	0.55–0.98	0.04	0.97	0.63–1.50	0.90
Third	0.98	0.74–1.30	0.90	1.22	0.79–1.86	0.37
Fourth	0.94	0.70–1.25	0.66	0.83	0.52–1.33	0.44
Fifth	0.90	0.67–1.20	0.47	0.94	0.59–1.49	0.79
As a continuous variable						
Untransformed	1.03	0.94–1.12	0.57	1.00	0.87–1.16	0.97
Log transformed	1.04	0.89–1.22	0.63	1.01	0.79–1.29	0.93
Median value of each quintile <sup>§</sup>	1.01	0.87–1.16	0.98	0.95	0.75–1.19	0.63

OR = odds ratio; CI = confidence interval; DHT = dihydrotestosterone.

<sup>†</sup> Base outcome for all models was the absence of cancer detected in all biopsies performed after randomization during the REDUCE trial. Models were adjusted for age, prostate-specific antigen (log-transformed values), prostate volume (log-transformed values), digital rectal examination, ethnicity, body mass index (log-transformed values), and family history of prostate cancer.

<sup>§</sup> The median value of each quintile of testosterone and DHT was attributed to all observations of that particular quintile.

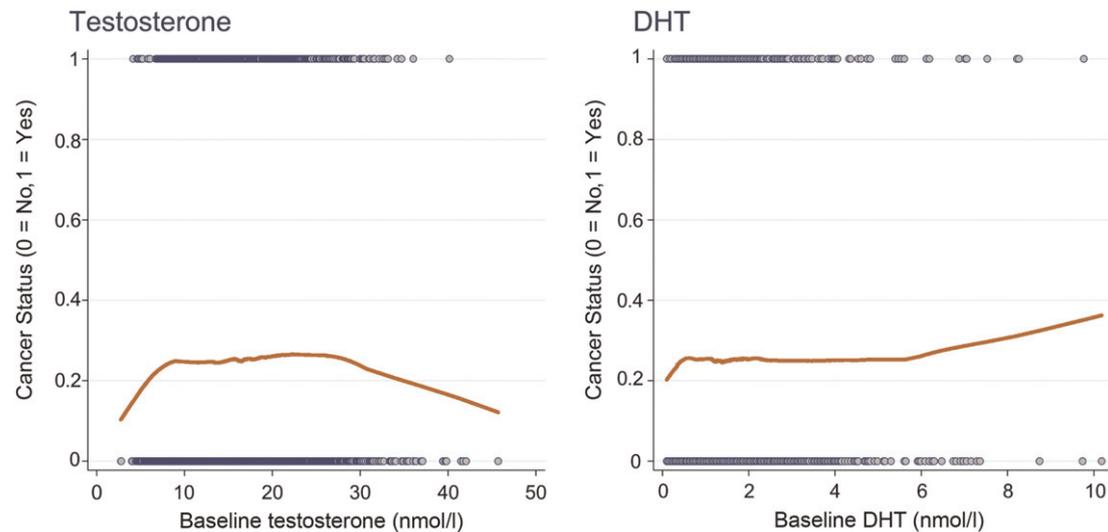
normal levels; thereafter, PCa risk stabilized regardless of higher testosterone levels. At the higher end of baseline testosterone levels, PCa detection decreased, but the reduced number of observations prevented any interpretation. PCa detection was similar among men with low compared with normal baseline testosterone levels (25.5% and 25.1%, respectively;  $p = 0.831$ ).

In secondary analysis, among men with low baseline testosterone, higher testosterone levels were associated with increased PCa risk (OR: 1.23; 95% CI, 1.06–1.43;  $p = 0.006$ ). Conversely, among men with normal testosterone levels, testosterone was unrelated to PCa risk ( $p = 0.33$ ). Serum DHT levels were unrelated to PCa risk, regardless of low or normal DHT levels (all  $p > 0.85$ ).

#### 4. Discussion

We found that serum baseline testosterone and DHT levels were unrelated to PCa risk in the placebo arm during 4 yr of the REDUCE trial. However, in secondary analysis, among men with low baseline testosterone values ( $\leq 10$  nmol/l), those with the lowest baseline testosterone levels had the lowest PCa risk, but among men with normal testosterone ( $> 10$  nmol/l), baseline testosterone levels were unrelated to PCa risk.

Serum testosterone and DHT levels, whether as quintiles or continuous, were unrelated to detection of low- or high-grade PCa, except for a marginally significant association in the second quintile of DHT. Despite different study designs



**Fig. 2 – Locally weighted scatterplot smoothing of serum levels of testosterone and dihydrotestosterone (DHT) at baseline and final cancer status after considering all biopsies during 4 yr of the REDUCE trial. The overlapping circles on the top and bottom of the chart represent each individual case. Men detected with prostate cancer were coded as 1, whereas men with no prostate cancer detected were coded as 0.**

(ie, all men were biopsied in our study, whereas prior studies used clinical diagnoses), these results agree with a meta-analysis of 8 prospective studies [2] and a large pooled analysis of 18 prospective studies, which found no association between either testosterone or DHT and PCa [5]. Thus, our results, in which all men received at least one biopsy after randomization, agree that across the full range of androgen levels, serum androgen levels are unrelated to PCa risk. We did not find any evidence to support earlier studies that suggest men with low androgen levels may have increased PCa risk [7] or more aggressive PCa [8–12]. Possible explanations for our lack of agreement with those prior studies may be due to distinct study populations or the fact that men with positive biopsies were removed from the REDUCE trial.

Despite negative results, in secondary analysis we found that the association between serum testosterone and PCa risk may be “saturable,” perhaps similar to the association between androgens and prostatic growth proposed in the saturation model [18]. Among men with low baseline testosterone levels, those men with the lowest baseline testosterone levels had the lowest PCa risk. However, among men with normal baseline testosterone levels, there was no association between testosterone and PCa risk. If the saturation model is also valid for the effect of serum testosterone on PCa risk, then PCa risk would be lowest in men with the lowest baseline testosterone levels but increase up to a “saturation point.” Thereafter, additional PCa risk would be independent of further increases in testosterone levels. Our results also showed that there were no differences in PSA or PV across quintiles of testosterone and DHT at baseline, further supporting the saturation model. Also, our findings suggest that men with high serum testosterone and DHT levels are not at greater risk of developing PCa, higher PSA levels, or larger prostates. Unfortunately, as no men had testosterone  $<1.7$  nmol/l, we could not address the link between castration and PCa risk.

The null association between testosterone and PCa risk in most of the REDUCE population with normal testosterone levels is supported by prior studies [2]. A saturation model is supported by an animal study showing that intraprostatic androgen levels and prostate mass are extremely sensitive to serum testosterone around near-castrated levels, with further tapering off as levels increase [17]. A small trial in men with a median baseline testosterone level of 9.8 nmol/l showed that testosterone replacement increased serum levels but had no effect on intraprostatic androgen and biomarker levels, gene expression, or PCa incidence [19]. Collectively, our study and prior evidence support a saturation model with maximal sensitivity to testosterone presumably below levels of 10 nmol/l.

Our secondary findings must be confirmed. Of note, PCa detection between men with low compared with normal baseline testosterone was similar, perhaps because the optimal threshold above which testosterone becomes saturated may be  $\leq 10$  nmol/l. Indeed, Figure 2 suggests an optimal threshold around 7 nmol/l. Thus, men at the upper end of “low” testosterone had similar PCa risk to men with normal testosterone. Also, lower PCa risk among men with very high testosterone balanced the lower risk of men with low testosterone. We avoided exploring alternative testosterone thresholds to prevent multiple testing issues and rather relied on an a priori–defined cutoff. If further studies validate the saturation model, then more work is needed to accurately estimate the saturation point. Although our findings related to testosterone levels agree with a saturation model, no association was found for DHT levels. While Figure 2 suggested slightly lower PCa risk among men with very low DHT levels, this risk was not statistically significant. Finally, the clinical relevance of this null secondary finding for DHT is unclear. The idea that most serum DHT is presumably derived from intracellular conversion of testosterone in the prostate might explain why serum DHT was unrelated to PCa risk.

REDUCE has many strengths: prospective data collection; a large, international, and multicentric population; and per-protocol biopsies regardless of PSA. Also, many of our results were largely consistent with prior studies (ie, obesity linked with low androgens, null associations between androgens and PCa risk). Some limitations are that men were at increased risk for PCa and may not represent the general population. Also, as all participants had one prior negative biopsy, we could not test the association between serum androgens and PCa risk on initial biopsy. However, our negative results between androgen levels and PCa risk agree with prior studies that were not limited to prior negative biopsy cohorts. Also, some men included in REDUCE likely had PCa undetected at baseline biopsy, which might have affected baseline androgens and biased our analysis through reverse causation; thus, longer prospective studies are still needed. Additionally, we analyzed serum androgens only at baseline, which may not reflect either intraprostatic levels [26,27] or the impact of androgens on the prostate, which is likely a lifelong process. No data on free testosterone levels were available. Clinically, some biopsy-detectable PCa may also be nonsignificant. From a statistical standpoint, we had insufficient numbers of men among low-androgen groups to use Gleason score as an outcome in secondary analysis. Lastly, the limited number of non-Caucasian men prevented us from exploring whether race influenced the association between androgens and PCa risk.

## 5. Conclusions

In the placebo arm of REDUCE, baseline testosterone and DHT levels were unrelated to PCa risk. Our secondary analysis showed no association between serum DHT levels and PCa. However, the results of our secondary analysis—suggesting that among men with low baseline testosterone levels, those men with the lowest testosterone levels at baseline had the lowest PCa risk—require confirmation in studies using an a priori defined hypothesis.

**Author contributions:** Roberto L. Muller had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

**Study concept and design:** Muller, Freedland.

**Acquisition of data:** Muller, Freedland, REDUCE Study Team.

**Analysis and interpretation of data:** Muller, Freedland.

**Drafting of the manuscript:** Muller, Freedland.

**Critical revision of the manuscript for important intellectual content:** Muller, Gerber, Moreira, Castro-Santamaria, Andriole, Freedland.

**Statistical analysis:** Muller, Freedland.

**Obtaining funding:** Andriole, Castro-Santamaria, Freedland.

**Administrative, technical, or material support:** Andriole, Castro-Santamaria.

**Supervision:** Freedland.

**Other (specify):** None.

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## Appendix – Results from intra- and inter-run precision studies of high-turbulent-flow liquid chromatography tandem mass spectrometry used for measuring serum testosterone and dihydrotestosterone\*

### Intrarun precision study

- Four levels of spiked serum were analyzed after a calibration run. Ten aliquots from each level were injected *in a single run*.
- The precision is defined as relative standard deviation (RSD). The criteria for acceptance for accuracy are  $100 \pm 15\%$  and precision of  $\leq 15\%$ . The following precision values were found:

	Level I	Level II	Level III	Level IV
Target value, ng/dl	10.0	50.0	250.0	1200.0
Mean, ng/dl	9.6	43.7	218.7	1125.8
RSD, %	7.1	10.5	10.8	9.1
Accuracy, %	96	87	109	94

### Inter-run studies

- Four levels of spiked serum were analyzed after a calibration run. Ten aliquots from each level were analyzed *on five different days*.
- The precision is defined as RSD. The criteria for acceptance for accuracy are  $100 \pm 15\%$  and precision of  $\leq 15\%$ . The following precision values were found:

	Level I	Level II	Level III	Level IV
Target value, ng/dl	10.0	50.0	250.0	1200.0
Mean, ng/dl	9.7	52.2	254.3	1218.1
RSD, %	9.8	13.4	11.0	12.6
Accuracy, %	97	104	102	105

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\* Information provided by the central laboratory used in the Reduction by Dutasteride of Prostate Cancer Events trial (Quest Diagnostics, Madison, NJ, USA).

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