Circulating steroid hormone variations throughout different stages of prostate cancer

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Abstract

Steroid hormones play a central role in the maintenance and progression of prostate cancer. The androgen receptor is the primary driver of tumor cell proliferation and is activated by the androgens testosterone and 5α-dihydrotestosterone. Inhibition of this pathway through medical or surgical castration improves survival in the majority of advanced prostate cancer patients. However, conversion of adrenal androgen precursors and alternative steroidogenic pathways have been found to contribute to tumor progression and resistance to treatment. The emergence of highly accurate detection methods allows us to study steroidogenic mechanisms in more detail, even after treatment with potent steroidogenic inhibitors such as the CYP17A1 inhibitor abiraterone. A clear overview of steroid hormone levels in patients throughout the local, metastatic and castration-resistant stages of prostate cancer and treatment modalities is key toward a better understanding of their role in tumor progression and treatment resistance. In this review, we summarize the currently available data on steroid hormones that have been implicated in the various stages of prostate cancer. Additionally, this review addresses the implications of these findings, highlights important studies in this field and identifies current gaps in literature.

Key Words
- androgens
- prostate cancer
- circulating markers

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Introduction

Prostate cancer (PC) is the most prevalent form of cancer, with the exception of non-melanoma skin cancers, in men in Western countries with an estimated 677.473 new cases in Europe and North America in 2014 (Forman & Ferlay 2014). In addition, it is a major contributor to cancer-related mortality in these regions with an estimated 126.430 prostate cancer-related deaths, ranking third in men after lung and colorectal cancers (Forman & Ferlay 2014). PC presents relatively late in life, after a median of 68 years and is often discovered during routine examinations or upon examination of urogenital discomfort. Early stage, localized PC can be treated with curative intent by prostatectomy or localized radiotherapy (Attard *et al*. 2016). Active surveillance may be employed in some cases if immediate treatment is not deemed necessary or beneficial, for example in patients with low risk (Klotz & Emberton 2014, Hamdy *et al*. 2016). Metastatic prostate cancer is very difficult to treat due to its tendency to metastasize to the bone (Ye *et al*. 2007). As such, only palliative treatment options exist.

The androgen receptor (AR) is the main driver of prostate cancer proliferation and is primarily activated by the androgenic steroid hormones testosterone and 5α-dihydrotestosterone (DHT). Androgens are derived

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from cholesterol in a multi-step process (Fig. 1) that primarily involves the testes, although androgens precursors are also secreted from the zona reticularis of the adrenal cortex. In the prostate, circulating testosterone is converted by 5α-reductase (SRD5A1 and 2) into DHT, which has higher affinity and improved retention at the AR compared to testosterone (Askew *et al.* 2007). Upon activation, the AR dissociates from its chaperone heat

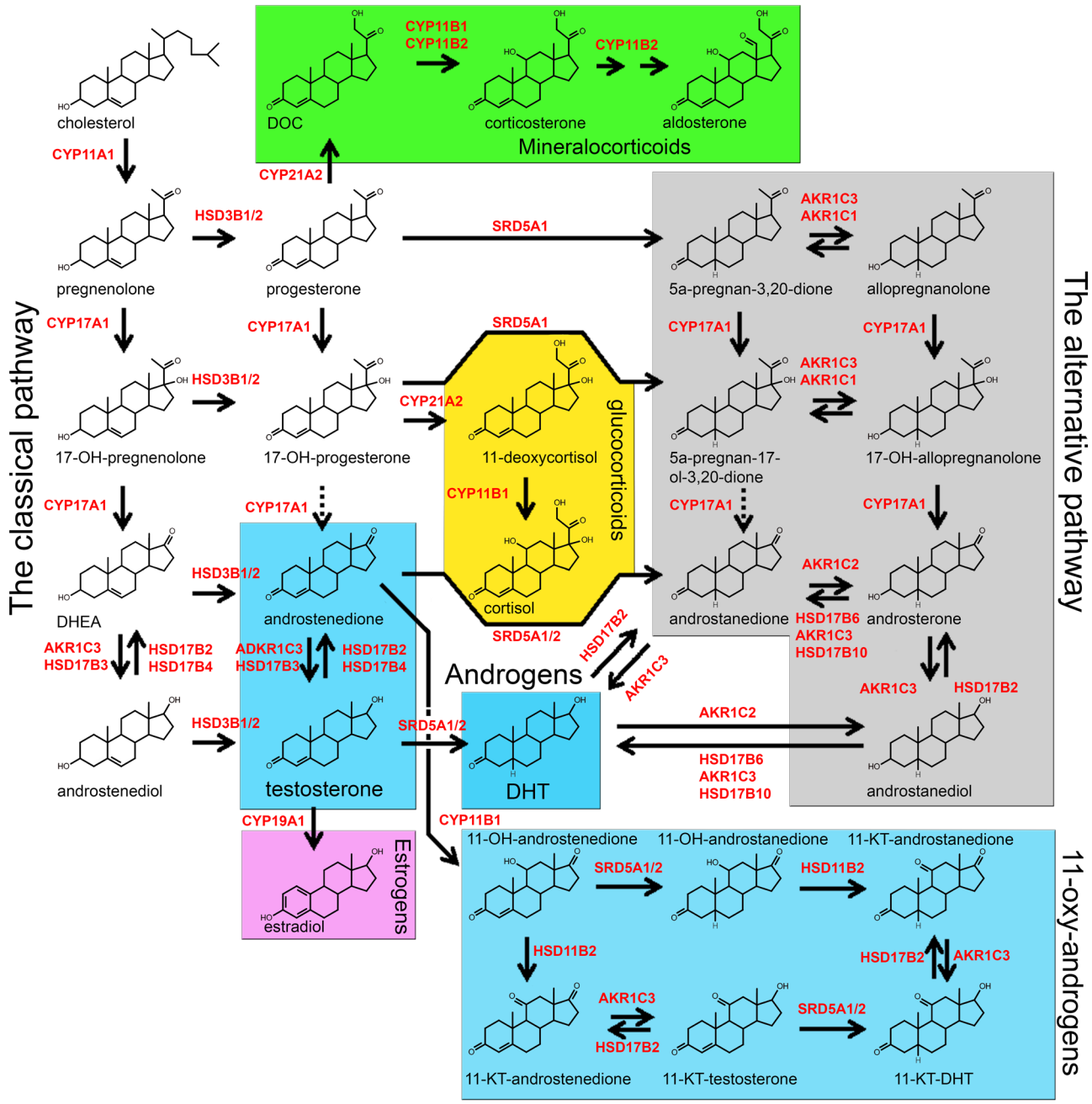

Figure 1
Overview of steroidogenesis leading to the production of androgens, estrogens, mineralocorticoids and glucocorticoids. The classical pathway produces DHT through conversion of DHEA, androstenedione and testosterone. The backdoor pathway of DHT synthesis completely bypasses canonical androgen synthesis and instead involves 5α-reduced conversion products ultimately leading to the production of androsterone or androstanediol as precursors for DHT. An alternative pathway produces DHT with androstanedione as intermediary rather than testosterone. Black arrows depict conversions steps with the responsible enzymes listed in red. The 11-oxygenated androgen synthesis pathway is listed, starting with the conversion of androstenedione to 11-hydroxy-androstenedione. DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone-sulfate; DHT, 5α-dihydrotestosterone; DOC, deoxycorticosterone.

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shock protein 90 (HSP90) and translocates to the nucleus where it acts as a transcription factor (Trepel *et al*. 2010). The AR subsequently drives the expression of oncogenes causing proliferation of PC cells.

Androgen deprivation therapy (ADT) through medical castration with or without anti-androgens is the mainstay therapy for advanced prostate cancer (Perlmutter & Lepor 2007). First-line ADT typically consists of treatment with gonadotropin-releasing hormone (GnRH) agonists or antagonists reducing the secretion of luteinizing hormone (LH) and consequently preventing the production of testosterone in testicular Leydig cells. GnRH agonists initially cause an LH surge followed by downregulation of the GnRH receptor and sustained suppression of LH levels, while GnRH antagonists cause an immediate reduction of LH secretion. This treatment improves survival in most men, but resistance to treatment typically occurs within 2–3 years. This next stage of the disease is termed castration-resistant prostate cancer (CRPC) and is accompanied by a poor overall survival of 16–18 months on average (Harris *et al*. 2009).

CRPC can be treated with docetaxel chemotherapy (Tannock *et al*. 2004), the second-line anti-androgen axis drugs abiraterone and enzalutamide, but resistance to these drugs typically occurs within 6–18 months (Ryan *et al*. 2013*b*, Beer *et al*. 2014). Compared to first-line ADT, abiraterone causes a more complete suppression of androgen synthesis by inhibiting cytochrome P450 (CYP) 17-hydroxylase/17,20-lyase (CYP17A1), a protein that catalyzes key steps in the production of androgens (Fig. 1). As a result, the production of the androgen precursors dehydroepiandrosterone (DHEA) and androstenedione and subsequently that of potent androgens is inhibited. Hence, abiraterone also suppresses adrenal androgen precursor synthesis, while first-line ADT only suppresses gonadal androgen synthesis. Abiraterone has to be co-administered with glucocorticoids as CYP17A1 inhibition results in enhanced ACTH-stimulation of the adrenal, which, in combination with the CYP17A1 block, causes significant accumulation of steroids with mineralocorticoid activity and consequently hypokalemia and hypertension (Pia *et al*. 2013). Interestingly, abiraterone and its metabolite Δ4-abiraterone also act as inhibitors of 3β-hydroxysteroid dehydrogenase (3β-HSD) and as AR antagonists, respectively (Li *et al*. 2012, 2015).

Enzalutamide is a recently developed and potent antagonist of the androgen receptor. It displaces DHT at lower concentrations than earlier anti-androgens such as biculatamide (Tran *et al*. 2009). Compared to flutamide and biculatamide, it shows less agonistic properties in AR-mutated or AR-overexpressing settings (Tran *et al*. 2009, Korpal *et al*. 2013), which is critical as one of the mechanisms sustaining PC growth is through continuously evolving AR mutations in the cancer cells. The clinical benefits shown in phase III randomized clinical trials with enzalutamide and abiraterone suggest that AR activation is still an essential component of CRPC growth and progression, both before as well as after docetaxel chemotherapy (de Bono *et al*. 2011, Scher *et al*. 2012, Ryan *et al*. 2013*b*, Beer *et al*. 2014). Continued expression of AR-regulated genes such as prostate-specific antigen (PSA) during second-line anti-androgen axis treatment supports this hypothesis (Conteduca *et al*. 2016).

Several mechanisms for continued AR activation in the presence of low circulating levels of androgenic steroids have been proposed. Key observations have come from studies looking at continued relevance of the AR (Chen *et al*. 2004*a*) and residual androgen presence in PC tissues (Mohler *et al*. 2004). Conversion of circulating adrenal androgens androstenedione, DHEA and its sulfated form (DHEAS) into testosterone and DHT through elevation of 17β-hydroxysteroid dehydrogenase (HSD) has been shown to occur in CRPC (Stanbrough *et al*. 2006, Hofland *et al*. 2010, Kumagai *et al*. 2013). It has been suggested that, in the absence of testis-derived testosterone, expression of steroidogenic enzymes may allow tumor cells to generate androgens themselves (Locke *et al*. 2008, Montgomery *et al*. 2008, Ishizaki *et al*. 2013). However, in what way *de novo* synthesis of androgens contributes to the CRPC resistance phenotype in the clinical setting has not yet been determined.

Alternatively, additional DHT synthesis pathways have been proposed that completely bypass the classic androgen synthesis pathway via testosterone (Fig. 1). Other mechanisms involved in resistance to castration include AR ligand promiscuity due to mutations, allowing the AR to become activated by a variety of steroid hormones (Duff & McEwan 2005). As such, steroid hormones other than testosterone and DHT may be of great clinical interest considering their suspected involvement in resistance mechanisms. Also, the role of steroidal ligands for activation of (hetero)dimers of various splice variants of the AR constitutes an expanding field of interest (Cao *et al*. 2016).

Given the crucial role of androgenic hormones during the disease evolution, the purpose of this review is to create an overview of variations in circulating steroid concentrations throughout different stages and treatment

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modalities of PC. While efforts have been undertaken to include data on all stages and modalities, most findings in literature report serum steroids levels in the treatment-naïve stage of prostate cancer, after treatment with androgen deprivation therapy and/or abiraterone with prednisone. Thus, we will primarily discuss those stages and treatment modalities.

This overview summarizes our understanding of steroid fluxes and how they may relate to prostate cancer progression. As the AR continues to constitute a key driving factor of PC growth, detailed knowledge of all relevant ligands are of crucial importance. This could also help to identify circulating steroid levels for clinical use, i.e. for prediction of response to anti-hormonal therapy. Finally, gaps in currently available data and future prospects will be identified.

Steroidogenesis

Steroid hormones are derived from cholesterol in a sequential process involving several steroidogenic enzymes (Fig. 1). First, the cholesterol side chain is cleaved by the mitochondrial protein cytochrome P450 side chain cleavage enzyme CYP11A1 to generate the common steroid precursor pregnenolone (Miller & Auchus 2011, Chien *et al*. 2017). This reaction occurs primarily in the gonads and adrenal cortex after stimulation with gonadotrophins and adrenocorticotrophic hormone (ACTH), respectively (Payne 1990, Sewer & Waterman 2003). Pregnenolone can be converted by 3β-HSD type 1 or 2 (encoded by *HSD3B1* and *HSD3B2*) to progesterone, which serves an important function in female reproduction. Progesterone in turn can by catalyzed by steroid 21-hydroxylase (CYP21A2) and 11β-hydroxylase (CYP11B1 and CYP11B2) to generate mineralocorticoids (Ryan & Engel 1957, Miller & Auchus 2011). Alternatively, both pregnenolone and progesterone can be hydroxylated by CYP17A1 to generate 17OH-pregnenolone and 17OH-progesterone, respectively (Miller & Auchus 2011). The glucocorticoid cortisol can be synthesized from 17OH-progesterone catalyzed by CYP21A2 and CYP11B1. DHEA can be produced from 17OH-pregnenolone through the 17,20-lyase activity of CYP17A1 in conjunction with cytochrome b5 (CYB5A) (Kok *et al*. 2010). In the adrenal gland, DHEA is sulfated by DHEA sulfotransferase (SULT2A1) to DHEAS, and impairment of DHEA sulfation causes increased generation of active androgens (Noordam *et al*. 2009, Oostdijk *et al*. 2015). Dehydrogenization and isomerization of DHEA by 3β-HSD produces the androgen and estrogen precursor androstenedione. The production of testosterone from androstenedione is catalyzed by HSD17B3 in the testes (Lin *et al*. 1997, Miller & Auchus 2011) and aldo-ketoreductase family 1 member C3 (AKR1C3, also known as HSD17B5) in other tissues. Increased expression of AKR1C3 has been detected in advanced CRPC (Hofland *et al*. 2010), and recently, it has been shown to confer resistance to androgen pathway-targeting therapies (Liu *et al*. 2015, 2017). A final conversion step catalyzed by the two 5-alpha reductase isozymes (SRD5A1 and SRD5A2) in the prostate generates DHT, which has the highest AR-binding affinity of all endogenous androgens (Gao *et al*. 2005). Alternatively, androstenedione and testosterone can be aromatized by CYP19A1 to form the estrogenic steroid hormones estrone and estradiol, respectively (Rahman *et al*. 2016).

In recent years, alternative DHT synthesis pathways have been proposed to contribute to intratumoral DHT while bypassing testosterone (Chang *et al*. 2011, Penning 2014). Especially in patients treated with the CYP17A1 inhibitor abiraterone, it is thought that accumulation of steroids upstream of CYP17A1 (Attard *et al*. 2008) may contribute to the production of alternative pathway steroids such as 5α-progesterone and allopregnanolone. Thus, changes in steroid serum levels may have clinical consequences for treatment resistance that we are not fully aware of yet.

Steroid hormone levels throughout the different stages of prostate cancer

Several different units of measurements are used in literature to report serum steroid concentrations (e.g. ng/mL, ng/dL, nM). To facilitate the comparison of findings from different publications and between different steroids we have chosen to summarize all data in this article in molar concentrations. An overview of conversion factors can be found in Table 1. Unless specified, steroid concentrations of controls were obtained from healthy individuals in the relevant age range that resembles those at risk or suffering from prostate cancer (>50 years old), since steroid levels can vary significantly by age (Belanger *et al*. 1994). Data on circulating levels of the relevant steroid hormones have been summarized in Fig. 2 and Supplementary Table 1 (see section on supplementary data given at the end of this article).

The modality used to measure steroid levels also differs between studies. The recent advance of novel liquid chromatography tandem mass spectrometry (LC–MS/MS)

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Table 1 An overview of steroid hormones that have been associated with the prostate cancer, including steroids of the classical, alternative and backdoor pathways of DHT synthesis.

Steroid hormone	Alternative names	Molecular weight	Conversion factor
11-deoxycorticosterone	DOC	330.46	3.03
11-deoxycortisol	S	346.46	2.89
17-hydroxy-allopregnanolone		334.49	2.99
17-hydroxy-pregnenolone	17OH-Preg	332.48	3.01
17-hydroxy-progesterone	17OH-Prog	330.46	3.03
5α-dihydrotestosterone	DHT	290.44	3.44
5α-pregnan-17-ol-3,20-dione		332.48	3.01
5α-pregnane-3,20-dione	5α-progesterone	316.48	3.16
Androstanediol-glucoronide	ADG	468.59	2.13
Aldosterone		360.45	2.77
Allopregnanolone		318.49	3.14
Androstanediol	3α-androstanediol	292.46	3.42
Androstanedione	5α-dione	288.42	3.47
Androstenediol		290.44	3.44
Androstenedione	Δ4-dione, adione	286.4	3.49
Androsterone		290.44	3.44
Corticosterone	B	346.47	2.89
Cortisol	hydrocortisone	362.46	2.76
Dehydroepiandrosterone	DHEA	288.42	3.47
Dehydroepiandrosterone-sulfate	DHEAS	368.49	2.71
17b-estradiol	E_2	272.39	3.67
Pregnenolone		316.48	3.16
Progesterone		314.46	3.18
Testosterone	T	288.42	3.47

techniques in the last decade has significantly improved the field of steroid hormone estimations. Compared to radioimmunoassays (RIA) and chemiluminescence immunoassays, LC–MS/MS can reach a lower limit of quantification, lacks cross-reactivity and can perform multi-steroid measurements in a single run. This review includes results from both mass spectrometry- and antibody-based assays to give a broad overview of the available data, although LC–MS/MS data are generally preferred.

Testosterone

Targeting testosterone synthesis has been central to prostate cancer treatment ever since the discovery of the effects of castration on prostate cancer by Huggins, more than 70 years ago, was awarded with the Nobel Prize. As such, many studies have evaluated testosterone levels in order to determine treatment efficacy, predict cancer or progression risk or to study molecular pathways related to AR signaling. Importantly, testosterone shows a diurnal rhythm with peak levels in the morning and nadir at night, although the circadian amplitude dissipates with increasing age (Bremner *et al*. 1983, Diver *et al*. 2003).

Serum testosterone concentrations in healthy controls are reported with high consistency throughout multiple larger studies, which included >1000 subjects (Belanger *et al*. 1994, Severi *et al*. 2006, Crawford *et al*. 2007, Daniels *et al*. 2010, Mondul *et al*. 2010, Tsilidis *et al*. 2015, Schenk *et al*. 2016) as well as in a large meta-analysis of 18 studies by the Endogenous Hormones and Prostate Cancer Collaborative group (EHPCCG) (Endogenous Hormones and Prostate Cancer Collaborative Group 2008). These data are in line with data obtained from LC–MS/MS measurements in another study (Yamashita *et al*. 2009). The reported median testosterone concentrations in these studies lie between 10 and 24 nM and interquartile range (IQR) values varied from 8.7 to 29.9 nM for the control populations (Endogenous Hormones and Prostate Cancer Collaborative Group 2008). Several smaller studies reported serum testosterone mean values that fall securely within this 'normal' range (Gann *et al*. 1996, Chen *et al*. 2003, Trifiro *et al*. 2010).

Relevant confounders of serum testosterone levels are age, body mass index (BMI) and chronic illness (Wu *et al*. 2008). Total serum testosterone declines by approximately 1 nM per decade (Harman *et al*. 2001). Taking the concomitant rise of sex hormone-binding globulin (SHBG) into account, the decline in circulating levels of unbound or free testosterone is even more pronounced with age. Through multifactorial causes obesity is also associated with lower levels of total testosterone. However, free

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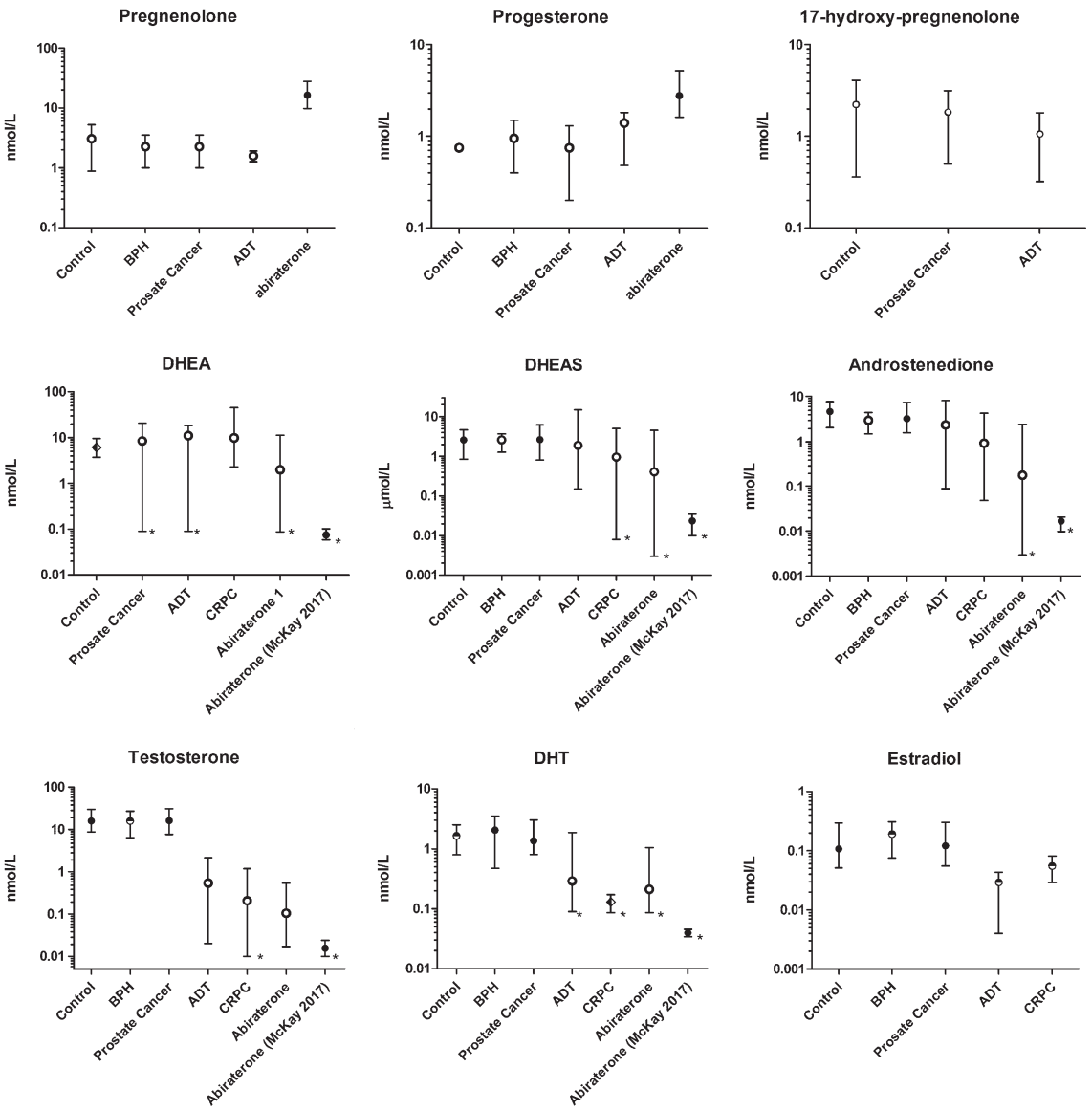

Figure 2

Serum steroid concentrations in healthy men and patients with prostate cancer during different modalities of treatment. The symbols depict the median of reported values from literature. A complete overview of papers reporting serum values for these steroids is available in Supplementary Table 1. Interquartile range data are presented when a full circle symbol is used. A semi-full circle depicts 95% confidence interval data. An open circle depicts range data. A semi-full diamond depicts a range of mean values from literature. Values from the recent McKay *et al.* (2017) study are listed independently because they achieved lower limits of quantification than other studies. *levels at or below the lower limit of quantification in the original study. The *y*-axis steroid values are spaced logarithmically. ADT, androgen deprivation therapy; BPH, benign prostatic hyperplasia; CRPC, castration-resistant prostate cancer; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone-sulfate; DHT, 5α-dihydrotestosterone.

testosterone concentrations remain normal in moderately obese subjects (BMI<35) due to the concurrent decline in SHBG (Giagulli *et al.* 1994, Saboor Aftab *et al.* 2013). Importantly, the elderly male population displays a high prevalence of chronic diseases. The presence of comorbidity is also accompanied by lower testosterone levels (Ahern *et al.* 2016).

Several studies comparing healthy to benign prostatic hyperplasia (BPH) subjects have reported equal testosterone levels, with mean values varying around 15 nM (Hammond *et al.* 1978, Heracek *et al.* 2007*a*, Grosman *et al.* 2010). Similarly, multiple studies report serum testosterone levels in subjects with localized PC that are on par with healthy controls (Gann *et al.* 1996,

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Endogenous Hormones and Prostate Cancer Collaborative Group 2008, Schenk *et al*. 2016). Serum testosterone values did not appear to predict the incidence of prostate cancer (Daniels *et al*. 2010, Schenk *et al*. 2016). In contrast, no large differences were observed between aggressive (Gleason ≥ 7) and indolent tumors (Gleason < 7) (Severi *et al*. 2006, Muller *et al*. 2012).

After orchiectomy or ADT, intended to lower serum testosterone below 'castrate levels', serum testosterone levels change dramatically. The established cut-off for castrate serum testosterone levels is currently 1.73 nM (50 ng/dL). However, many studies report mean levels far below this limit using both immunoassays and LC–MS/MS, usually ≤0.5 nM (Rohl & Beuke 1992, Ishizaki *et al*. 2012, Hara *et al*. 2012, 2013, Mostaghel *et al*. 2014). This is slightly lower than the EC_{50} for AR activation of 0.63 nM reported in literature (Sonneveld *et al*. 2005) and near the significant effect threshold (0.3 nM) observed in another study (Campana *et al*. 2016). Testosterone levels of orchiectomized and ADT-treated subjects were comparable, although a statistically significant difference was observed in a study employing sensitive isotope dilution LC–MS/MS in 66 subjects (orchiectomy: 0.319 nM; ADT: 0.138 nM) (van der Sluis *et al*. 2012). Another study compared serum testosterone levels in ADT-responsive, ADT-nonresponsive and CRPC patients after treatment with combined androgen block but did not observe significant differences with mean levels of 0.52, 0.38 and 0.31 nM, respectively. Despite the clear effect of ADT on serum testosterone levels, intratumoral testosterone levels in CRPC tissues are similar (2.78 nM vs 3.26 nM, respectively) (Mohler *et al*. 2004, Titus *et al*. 2005) or even higher (Montgomery *et al*. 2008) compared to pre-castrate levels. Intratumoral testosterone levels are moderately reduced shortly after the initiation of LHRH antagonist treatment, as measured by LC–MS/MS (Shaw *et al*. 2016), suggesting intratumoral compensatory mechanisms during long-term ADT.

Finally, serum testosterone levels can be decreased to a greater extent, in a range that can only be measured reliably with LC–MS/MS, by abiraterone treatment. In one trial, median serum testosterone in CRPC patients declined from 0.35 nM at baseline to 0.02 nM after 12 weeks treatment with 1000 mg abiraterone and 5 mg prednisone daily (McKay *et al*. 2017). This is lower than the *in vitro* activation thresholds observed in literature, although intracellular levels may differ (Sonneveld *et al*. 2005, Campana *et al*. 2016). Equally low serum testosterone levels were detected in two other studies (Attard *et al*. 2008, Ryan *et al*. 2014) while a milder reduction was observed in a study that compared ketoconazole-progressive patients with abiraterone treatment (Kim *et al*. 2014).

A single study reported on post-enzalutamide levels in the neo-adjuvant setting. After 180 days of enzalutamide-only treatment, serum and intratumoral testosterone levels were significantly increased when compared to both baseline and combination treatment consisting of enzalutamide, dutasteride and androgen deprivation therapy (Montgomery *et al*. 2017). This is likely due to augmented hypothalamic–pituitary–gonadal axis activity following ablation of negative feedback by enzalutamide. The additive effects of enzalutamide on local or circulating androgen levels including testosterone in the castrate setting are unknown.

5α-dihydrotestosterone (DHT)

As the most potent natural androgen, DHT constitutes an essential target for the treatment of prostate cancer. DHT is synthesized within prostate cells from testosterone by the 5α-reductases, but is also present in serum. In control subjects, mean and median values between 1.2 and 2.0 nM have been reported by several studies, including one study employing LC–MS/MS (Hammond *et al*. 1978, Gann *et al*. 1996, Yamashita *et al*. 2009, Trifiro *et al*. 2010, Stanczyk *et al*. 2013). Similar values were observed in control subjects in the meta-study of the EHPCCG (Endogenous Hormones and Prostate Cancer Collaborative Group 2008), with interquartile ranges varying between 0.91 and 2.52 nM across 7 reviewed studies. A slightly higher mean serum DHT concentration of 3.22 nM was observed by Belanger and coworkers, which could reflect the occurrence of cross-reactivity in their radioimmunoassay (Belanger *et al*. 1994, Yarrow *et al*. 2013, Krasowski *et al*. 2014).

In BPH subjects, reported values were quite similar (Hammond *et al*. 1978, Heracek *et al*. 2007*a*) although a small but statistically significant difference was detected in a follow-up study comparing men who did not develop BPH (1.44 nM) compared to men who did (1.65 nM) (Parsons *et al*. 2010). DHT levels can be strongly reduced with the 5α-reductase inhibitors finasteride and dutasteride. This has been tested in BPH patients, resulting in serum DHT levels below 0.03 nM, measured by mass spectrometry, after 24 weeks of treatment with 2.5–5 mg of dutasteride per day (Clark *et al*. 2004).

Determined by both RIA and LC–MS/MS, mean serum DHT levels in PC patients before castration are similar

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to healthy controls (Hammond *et al*. 1978, Endogenous Hormones and Prostate Cancer Collaborative Group 2008, Miyoshi *et al*. 2014) but can be reduced to 0.1–0.5 nM (mean) by either orchiectomy (Rohl & Beuke 1992) or ADT (Hara *et al*. 2013, Mostaghel *et al*. 2014, Taplin *et al*. 2014). Even lower levels (<0.086 nM and <0.44 nM) were achieved in two studies using LC–MS/MS (Kim *et al*. 2014, McKay *et al*. 2017) in which CRPC patients were treated with abiraterone and prednisone. DHT concentrations necessary to activate the AR *in vitro* are estimated to be between 0.05 and 0.5 nM, and in some mammalian-cell-based luciferase assays EC_{50} values as low as 0.01 nM were measured (Sonneveld *et al*. 2005, Dennis *et al*. 2008, Campana *et al*. 2016, Lallous *et al*. 2016). As such, values detected in castrated patients appear to approximate the EC_{50} values. DHT values in some abiraterone-treated patients were lower than 0.086 nM in one study but could not be assessed more accurately because they reached the lower limits of quantification (Kim *et al*. 2014). Therefore, it remains unclear whether abiraterone lowers serum DHT concentration below the AR activation limits. In contrast to abiraterone, enzalutamide in the neoadjuvant setting increased circulating DHT levels compared to baseline (Montgomery *et al*. 2017).

Androstenedione

Serum concentrations of the androgen precursor androstenedione are generally lower than testosterone levels in men, with several studies reporting mean values between 2.95 and 4.7 nM (Belanger *et al*. 1994, Severi *et al*. 2006, Stanczyk *et al*. 2013, Tsilidis *et al*. 2015). This is in line with the findings of the EHPCCG, who report IQR values between 2.07 and 7.80 nM (Endogenous Hormones and Prostate Cancer Collaborative Group 2008).

Circulating androstenedione levels appear to be relatively similar in both BPH (Hammond *et al*. 1978) and PC subjects (Hammond *et al*. 1978, Chen *et al*. 2003, Severi *et al*. 2006, Endogenous Hormones and Prostate Cancer Collaborative Group 2008). Unlike testosterone and DHT, serum androstenedione levels are not dramatically affected by either orchiectomy or ADT (Ayub & Levell 1990, van der Sluis *et al*. 2012, Mostaghel *et al*. 2014). One study detected a mild albeit statistically significant decrease in androstenedione levels (5.58–2.89 nM after ADT), although still within the ranges reported by other studies (Hara *et al*. 2013). This is in agreement with the adrenal cortex being the predominant source of androstenedione in men and with the adrenal cortex contributing to ongoing AR activation within PC cells after castration. Similar to testosterone and DHT, neoadjuvant enzalutamide treatment also increased serum androstenedione levels (Montgomery *et al*. 2017).

In contrast, abiraterone dramatically lowers serum androstenedione levels in CRPC patients, since CYP17A1 catalyzes key steps in the synthesis of androstenedione. Mean values between 0.011 and 0.27 nM have been reported using LC–MS/MS in CRPC patients after 12–24 weeks of abiraterone and prednisone treatment (Attard *et al*. 2012, Mostaghel *et al*. 2014, Taplin *et al*. 2014). Circulating androstenedione levels in untreated men are around or below the EC_{50} values for AR activation observed *in vitro*, which vary from 5.01 nM to 70 nM (Chen *et al*. 2004*b*, Sonneveld *et al*. 2005). As such, it is unlikely that androstenedione in itself is a major contributor to AR activation in prostate cancer, especially after suppression by abiraterone.

DHEA

DHEA is an important precursor of androstenedione and testosterone, although much of it circulates in the form of its inactive sulfate ester, DHEAS. Serum DHEA levels decline strongly with age and mean concentrations are 4.03–9.07 nM in subjects aged 50–80 years (Belanger *et al*. 1994), a process commonly referred to as adrenopause, which is slightly inaccurate as glucocorticoids and mineralocorticoids do not decline with age. *In vitro* data suggest that the required DHEA concentration to activate the AR is above 100 nM (Mizokami *et al*. 2004), meaning that DHEA is unlikely to contribute to AR activation at any stage *in vivo*. DHEA levels appear normal in subjects with localized PC (Nishiyama *et al*. 2007, Taplin *et al*. 2014), although this is difficult to judge because of small sample sizes and the large differences between studies, as well as the high intra-individual variability of serum levels, despite the use of LC–MS/MS (Taplin *et al*. 2014; range: 0.08–20.57 nM). DHEA levels were also not significantly affected by ADT or ADT in combination with other anti-hormonal agents (Mostaghel *et al*. 2014). Again, treatment with enzalutamide in the neoadjuvant setting significantly stimulated serum DHEA levels (Montgomery *et al*. 2017).

Like androstenedione, serum DHEA levels are reduced upon treatment with abiraterone with reported values between 0.08 and 2.7 nM in several LC–MS/MS studies (Attard *et al*. 2008, Taplin *et al*. 2014, McKay *et al*. 2017). A possible explanation for circulating DHEA levels not

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being reduced as strongly as testosterone may be the continued presence of high (albeit diminished) levels of DHEAS after abiraterone treatment.

DHEAS

Serum DHEAS levels are higher than those of all other androgenic steroids combined and it is the only one that circulates in the micromolar range. DHEAS is a not an AR agonist (Bjerregaard-Olesen *et al.* 2016), but it can be converted into more potent androgens after removal of the sulfate group by the enzyme steroid sulfatase (STS) and conversion by 3β-HSD and 17β-HSD. STS activity has no relevant impact on circulating DHEAS levels (Hammer *et al.* 2005) but desulfation of DHEAS can occur in prostate cells (Purohit & Foster 2012). In control subjects, serum DHEAS mean and median values typically fall between 1.2 and 3.2 µM (Belanger *et al.* 1994, Severi *et al.* 2006, Endogenous Hormones and Prostate Cancer Collaborative Group 2008), with reported IQRs between 0.8 and 4.68 µM by the EHPCCG.

Slightly lower DHEAS values were observed in BPH (2.6 µM) and PC subjects (1.9 µM) compared to control subjects (4.3 µM) in one study, but sample size was limited (Mitamura *et al.* 2003). Additionally, lower DHEAS levels were associated with an increased risk for aggressive PC (Severi *et al.* 2006). The EHPCCG meta-study did not detect the differences between control and PC subjects (Endogenous Hormones and Prostate Cancer Collaborative Group 2008).

Serum DHEAS levels appeared unaffected by orchiectomy and ADT in two LC–MS/MS studies (van der Sluis *et al.* 2012, Taplin *et al.* 2014), although Hara and coworkers observed a statistically significant 38% decrease in patients with localized PC after 6 months of ADT (Hara *et al.* 2013). Reported DHEAS levels in CRPC subjects were lower in some studies, with mean values before abiraterone treatment around 0.55–1.0 µM (Attard *et al.* 2008, Ryan *et al.* 2014), although values in CRPC patients can vary wildly (Matsubara *et al.* 2014). In contrast to the studies of Attard and coworkers and Ryan and coworkers much higher pretreatment mean values (5.2–6.2 µM) were reported in another study employing LC–MS/MS (Taplin *et al.* 2014, Attard *et al.* 2008, Ryan *et al.* 2014). This may be due to population differences between these studies. The latter study included younger patients (median 55 years) with localized disease, good performance status and no prior prostate cancer targeting treatment while the former studies included older (median for both studies: 69 years) patients previously treated with – and progressed on – ADT and/or chemotherapy and were in some cases prescribed corticosteroids. In each of these studies, however, abiraterone treatment strongly reduced DHEAS levels by 80–90%, with on-treatment values between 0.14 and 0.4 µM. Even lower post-abiraterone treatment levels (median <0.03 µM) have been reported using highly sensitive LC-ESI-MS/MS by McKay and coworkers after 24 weeks of treatment (McKay *et al.* 2017). Importantly, not only abiraterone but also administration of prednisone contributes to this effect through attenuation of ACTH levels. As markers of adrenocortical function, pretreatment levels of DHEA, DHEAS and androstenedione are all predictive biomarkers for abiraterone efficacy in CRPC patients (Attard *et al.* 2009). Serum testosterone, androstenedione and DHEAS also proved to be prognostic for overall survival in a cohort treated with abiraterone (Ryan *et al.* 2013*a*).

Estradiol

Although initially employed solely as treatment to reduce serum testosterone levels, interest in the role of endogenous estradiol (E_2) has risen in recent years, especially as a possible factor in the development of prostate cancer. A shift from apoptosis-inducing estrogen receptor (ER)-β signaling to the growth-stimulatory effects of ER-α during PC evolution suggests a proliferative role of estrogens in advanced disease stages (Rahman *et al.* 2016). E_2 levels are very low in male subjects, with reported mean concentrations for healthy control groups in the 82–234 pM range (Hammond *et al.* 1978, Hsing & Comstock 1993, Belanger *et al.* 1994, Chen *et al.* 2003, Severi *et al.* 2006, Endogenous Hormones and Prostate Cancer Collaborative Group 2008, Grosman *et al.* 2010). Findings in the meta-study of the EHPCCG show that there is a high level of disparity between different studies, with IQR values ranging from 51–84 pM to 173–296 pM. No differences in serum E_2 levels were detected between control and BPH subjects (Hammond *et al.* 1978, Grosman *et al.* 2010).

Most studies comparing E_2 concentrations in control and PC subjects did not find differences between these groups (Hsing & Comstock 1993, Severi *et al.* 2006, Endogenous Hormones and Prostate Cancer Collaborative Group 2008, Daniels *et al.* 2010), although a single study reported higher levels in the PC group (200.1 pM) compared to controls (156.4 pM) (Grosman *et al.* 2010). Testosterone is converted into E_2 by aromatase and,

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consequently, targeting testosterone synthesis with ADT will also reduce serum E_2 levels. Several studies observed this effect, where ADT was able to reduce mean serum E_2 to levels in the range of 4–33 pM (Kitahara *et al*. 1999, Basaria *et al*. 2002, Qin *et al*. 2013). Qin and coworkers noted a small non-significant increase in E_2 levels in PC patients who progressed on complete androgen block (CAB) therapy, which combines regular ADT with anti-androgens and in some cases 5α-reductase inhibitors (Qin *et al*. 2013). Only one study reported serum E_2 levels in abiraterone-treated CRPC patients. After 4 weeks of treatment, the median E_2 level was further reduced from 7.2 pM to 2.9 pM (Attard *et al*. 2008). Patients with higher pretreatment E_2 levels were also more likely to experience a ≥50% PSA decline following abiraterone treatment (Attard *et al*. 2009).

Pregnenolone and progesterone

Pregnenolone is the common steroid hormone precursor and is synthesized from cholesterol by CYP11A1. Pregnenolone is not an attractive target for the treatment of prostate cancer because of potential side effects caused by glucocorticoid and mineralocorticoid deficiencies. Serum pregnenolone levels have not been investigated in great detail. The reported values in subjects with BPH or PC appear to be similar to those in healthy controls (1.5–2.5 nM) (Hammond *et al*. 1977, 1978, Belanger *et al*. 1994). Elevated pregnenolone levels were observed in abiraterone-treated CRPC patients (16.3 nM, IQR 9.85–27.77 nM) using LC–MS/MS, and it was also noted that patients with high serum abiraterone levels (>35 ng/mL) had higher pregnenolone levels than patients with low abiraterone concentrations (McKay *et al*. 2017). This can be explained by impaired conversion of pregnenolone to 17OH-pregnenolone because of CYP17A1 inhibition by abiraterone, resulting in the accumulation of the former steroid.

Progesterone is not often considered in the context of androgens and prostate cancer, despite its well-characterized activation of AR mutants and the assumed protective role of the stromal progesterone receptor in BPH and PC development (Chen *et al*. 2017). Serum concentrations in healthy male controls are slightly lower than those for pregnenolone, with reported means around 0.5–0.75 nM (Hammond *et al*. 1978, Belanger *et al*. 1994).

Hammond and coworkers observed no clear differences in progesterone levels between controls and BPH or PC subjects, and Ayub and Levell observed no change in progesterone levels in PC patients treated with orchiectomy or ADT (Hammond *et al*. 1978). However, they did observe higher mean values at baseline (1.53–1.83 nM) than Hammond and coworkers (Ayub & Levell 1990, Hammond *et al*. 1978). Since progesterone is subject to hydrolysis by CYP17A1, LC–MS/MS-measured serum progesterone levels were mildly increased in abiraterone-treated subjects (median 2.77 nM, IQR 1.62–5.18 nM) (McKay *et al*. 2017). Both pregnenolone and progesterone serum levels were increased by enzalutamide treatment in eugonadal setting, again suggesting increased hypothalamic–pituitary–gonadal axis activity. However, adjuvant combination treatment with enzalutamide, dutasteride and ADT does not result in an increase in pregnenolone and significantly lowers progesterone (Montgomery *et al*. 2017).

17OH-pregnenolone and 17OH-progesterone

Derived from pregnenolone, 17OH-pregnenolone is an intermediate in the production of androgens as it can be converted into DHEA by the 17,20-lyase activity of CYP17A1. Belanger and coworkers reported a mean serum 17OH-pregnenolone of 2.01 nM in healthy controls, and a total range of 1.08–12.3 nM is reported elsewhere (Kushnir *et al*. 2006, Belanger *et al*. 1994).

17OH-pregnenolone levels are rarely reported in human subjects in the context of prostate cancer. Two small studies reported values for PC patients before and after treatment with estrogens, but 17OH-pregnenolone levels appeared to be unaffected (Hammond *et al*. 1977, Kitahara *et al*. 1999). Since abiraterone inhibits CYP17A1 activity, it is likely that serum 17OH-pregnenolone levels will be impaired in patients taking this drug.

17OH-progesterone is the product of hydrolysis of progesterone by CYP17A1 and is a glucocorticoid precursor. Serum concentrations decline with age (Belanger *et al*. 1994) and reported concentrations are in the 0.75–4.2 nM range for healthy controls (Hammond *et al*. 1978, Kushnir *et al*. 2006). 17OH-progesterone levels are similar in BPH and PC subjects and appear to be unaffected by orchiectomy or ADT (Hammond *et al*. 1978, Ayub & Levell 1990). Unfortunately, data in abiraterone-treated patients are not available.

Androsterone

Androsterone is an inactive metabolite of DHT, but can also be converted back to DHT through the actions

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of 17β-HSD types 5 (AKR1C3), 6 (HSD17B6) and 10 (HSD17B10). It is also an intermediate of the backdoor DHT synthesis pathway (Arlt *et al*. 2004, Auchus 2004). Mean serum values for healthy controls were found to be between 1.4 and 2.87 nM (Belanger *et al*. 1994), although a slightly lower range of 1–1.5 nM was observed elsewhere (Hammond *et al*. 1978).

This latter study also did not observe a difference between control and BPH or PC subjects. Two other recent studies using LC–MS/MS (Mostaghel *et al*. 2014, Taplin *et al*. 2014) report mean values for PC patients (0.49 and 0.28–0.34 nM, respectively) that are below the range reported by Hammond and coworkers (0.5–2.2 nM) (Hammond *et al*. 1978). This difference is likely due to the use of liquid chromatography/tandem mass spectrometry in the former studies, preventing assay cross-reactivity among steroid hormone measurements. ADT treatment reduced serum androsterone levels by approximately 50% in both of these studies, reaching levels between 0.16 and 0.28 nM (Mostaghel *et al*. 2014, Taplin *et al*. 2014). The abiraterone-treated subjects in the study of Taplin and coworkers reached serum androsterone concentrations below their LC–MS/MS assay detection limit of 0.03 nM (Taplin *et al*. 2014).

3α-androstanediol

3α-androstanediol (5α-androstane-3α,17β-diol), a metabolite of DHT, is usually measured in its inactive glucuronidized metabolite, 3α-androstanediol-glucuronide. Studies analyzed by the EHPCCG report median values between 5.7 and 14.5 nM, with IQRs ranging from 3.9 to 19.8 nM (Endogenous Hormones and Prostate Cancer Collaborative Group 2008). These levels have been confirmed in more recent studies (Wiren *et al*. 2007, Schenk *et al*. 2016).

Glucocorticoids and precursors

This class of steroid hormones is not known for its causative role in prostate cancer, but the glucocorticoid receptor has been implicated in advanced stages of disease (Arora *et al*. 2013).

Recently, altered metabolism of cortisol by intratumoral 11β-hydroxysteroid dehydrogenase type 2 loss has been discovered as a resistance mechanism to enzalutamide treatment (Li *et al*. 2017). Levels of cortisol, the main effector glucocorticoid, adhere to a circadian rhythm with a peak during the morning (138–635 nM) and a steady decline until a nadir is reached during the night (Auchus *et al*. 2014). Cortisol and its precursor 11-deoxycortisol are conversion products of 17OH-progesterone and are thus dependent on CYP17A1 activity. It is not expected that cortisol levels are aberrant in BPH or PC patients. Indeed, mean morning cortisol concentrations of 536 and 509 nM were observed in patient with local and advanced prostate cancer, respectively (Heracek *et al*. 2007*b*).

Inhibition of cortisol production following abiraterone treatment without addition of glucocorticoids causes withdrawal of negative feedback at the hypothalamic and pituitary level and subsequently increases ACTH levels up to 6-fold. Hence, cortisol levels remain relatively constant at baseline, but responses to ACTH following a short synacthen test were invariably insufficient. Since ACTH subsequently drives the production of mineralocorticoids with hypertensive capabilities and possibly also adrenal androgens, abiraterone is co-administered with the glucocorticoid prednisone, which is now the standard care treatment (O'Donnell *et al*. 2004, Attard *et al*. 2008). Indeed, mean cortisol levels declined from 303.6–358.8 nM to 124.2–276 nM after 21 days of abiraterone and corticosteroid co-treatment. Interestingly, 11-deoxycortisol levels were increased as a consequence of abiraterone treatment, from 0.867–2.60 nM to 2.89–9.39 nM (Ryan *et al*. 2010), confirming inhibition of 11β-hydroxylase *in vivo* (Yin & Hu 2014).

Glucocorticoids may also impose more direct effects on prostate cancer proliferation upon mutation of the androgen receptor. Treatment with hormonal therapy in CRPC patients positively selects for mutations in the AR ligand-binding domain, several of which can be activated by glucocorticoids (Carreira *et al*. 2014, Romanel *et al*. 2015). This is very relevant to abiraterone-treated patients for two reasons. Firstly, abiraterone is commonly co-administered with the GR agonist prednisone and, secondly, abiraterone-treated patients have elevated corticosterone levels, which also has GR activity (discussed below).

Mineralocorticoids and precursors

Mineralocorticoids are not known to be effectors of prostate cancer signaling and proliferation. These steroids are involved in fluid and electrolyte homeostasis and thus do not appear to play a major role in the development of prostate cancer. However, abiraterone inhibits synthesis of downstream steroids, causing pregnenolone and progesterone levels to accumulate. Similar to

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glucocorticoids, no fluctuations in mineralocorticoids have been described in BPH or ADT-treated PC patients.

In abiraterone-treated patients, a strong increase was observed in deoxycorticosterone (DOC) from 0.196 to 2.07 nM (median) and in corticosterone from 3.83 to 188.0 nM (median) (Attard *et al*. 2008). Although cortisol is the main glucocorticoid in humans, corticosterone also has glucocorticoid activity. Several papers have shown that the mineralocorticoid excess can be treated by addition of prednisone (5 mg daily) or dexamethasone (0.5 mg daily), which is why abiraterone is always co-administered with exogenous glucocorticoids (Attard *et al*. 2008, Ryan *et al*. 2010, Pia *et al*. 2013). Additional inhibition of the mineralocorticoid receptor can be accomplished by eplerenone, whereas spironolactone should be avoided due to its capability of AR activation (Richards *et al*. 2012). Although strong elevation of DOC and corticosterone was observed in the study by Ryan and coworkers aldosterone levels remained relatively stable (Ryan *et al*. 2010). This is likely due to effects of ACTH, which primarily stimulates the zona fasciculata rather than the zona glomerulosa, which produces aldosterone and contains aldosterone synthase (CYP11B2). Thus, the mineralocorticoid excess symptoms in abiraterone-treated patients are caused by the mineralocorticoid DOC.

Alternative pathways of androgen synthesis

In addition to the canonical pathway of DHT synthesis, involving gonadal-derived testosterone, alternative pathways have been proposed recently (Auchus 2004, Chang *et al*. 2011). It has been shown that androstenedione can be preferentially converted into 5α-androstanedione by SRD5A1 (Chang *et al*. 2011), the isozyme that is upregulated in CRPC tissue (Titus *et al*. 2005). To our knowledge, there are no studies reporting circulating 5α-androstanedione levels in male subjects or patients with prostate cancer.

Alternatively, another mechanism involving 5α-reduction of progesterone and synthesis of allopregnanolone may lead to the production DHT without involvement of canonical androgen pathway intermediates (Fukami *et al*. 2013). In this pathway, allopregnanolone is metabolized with high efficiency into androsterone by CYP17A1 (Auchus 2004). Since abiraterone reduces levels of canonical androgens and leads to accumulation of progesterone metabolites, this pathway may be of particular interest in patients that progress on abiraterone. Indeed urinary steroid metabolite analysis in PC patients treated with abiraterone revealed augmented concentrations of backdoor pathway metabolites (Attard *et al*. 2012). However, serum values of backdoor pathway intermediates in this group or other PC patients in general are missing.

Finally, there is another class of 11-oxygenated C19 steroids that may play an important role in prostate cancer that has previously been overlooked. After DHEAS, 11OH-androstenedione is the most prevalent androgen precursor produced by the adrenal cortex. It can be produced from androstenedione by CYP11B1, and although this steroid itself does not activate the AR, it can be converted into 11-keto variants of testosterone and DHT that are equally potent agonists of the AR (Swart & Storbeck 2015, Pretorius *et al*. 2016). Plasma levels of 11OH-androstenedione and 11keto-testosterone have recently been investigated and shown to reach a high baseline and augmented post-ADT levels of above 100 nM (du Toit *et al*. 2017). Notably, 11keto-DHT levels were in the 10–20 nM range before treatment and showed no decrease after ADT, suggesting significant AR activation potential. This is also reflected by prominent tissue levels of these 11-oxygenated C19 steroids (du Toit *et al*. 2017). Until recently, these steroids and their physiological role has been overlooked in the literature and an increasing number of studies now confirm these steroids as interesting targets for androgen-dependent diseases, including PC.

Discussion and future prospects

Improving our understanding of steroid hormones, their homeostasis and their involvement in prostate cancer pathogenesis has been a central feature of prostate cancer research for decades. Research has focused predominantly on the canonical AR ligands testosterone and DHT. Limited data are available on the adrenal derived androgens DHEA and androstenedione, but little is known about possible variations in upstream steroids. Similarly, data on steroids downstream of testosterone and DHT are scarce.

The recent paradigm shift in treatment of PC with novel second-line hormonal therapy also necessitates accurate studies into the effects of these drugs on circulating and intratumoral hormone levels. This should also include the effects of enzalutamide. Aberrant hormonal pathways have been identified, but it is clear that we still do not fully understand how resistance occurs in many patients. It is therefore important that more data are obtained from these subjects. Making use of LC–MS/MS technological advancement to detect very small changes and measure

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steroids at increasingly lower limits of quantification will be key to progressing our knowledge.

Inter-study variation is a relevant drawback complicating comparisons between different studies. Within the EHPCCG meta-study alone, median and IQR values vary drastically on a study-to-study basis. A value fitting securely within the normal range of one study may be considered aberrant compared to the normal range of another study. Without access to all the potential confounders within each individual study, it is necessary to consider a broader 'normal' range. Then, however, it becomes important not to lose track of potential small differences between two distinct populations, even if these values both fit within the broader normal range. For example, considering the small difference in estradiol levels between ADT-sensitive and CRPC subjects observed within the study of Qin and coworkers, it is possible that both values fall within a broader range of 'normal' estradiol levels, but a subtle shift may still be informative (Qin *et al* 2013). Those subtle changes, rather than more drastic shifts, may play an important role in the occurrence of resistance pathways.

The exact relevance of such subtle changes becomes apparent in the context of *in vitro* AR activation assays (Mizokami *et al*. 2004, Sonneveld *et al*. 2005, Dennis *et al*. 2008, Campana *et al*. 2016). Serum testosterone and DHT levels in healthy subjects greatly surpass the EC_{50} values required for AR activation. However, ADT lowers serum testosterone and DHT to levels near or below the EC_{50} values, and this is accompanied by a PSA decline in most castrate patients. Abiraterone is capable of further lowering testosterone levels below the activation threshold and lowering DHT levels to the EC_{50} threshold, subsequently accompanied by an additional decline in serum PSA (McKay *et al*. 2017). Small changes within this range may therefore greatly affect activation of the androgen receptor.

Levels of androstenedione and DHEA do not appear to reach sufficient levels *in vivo* to significantly contribute to AR activation under castrate conditions. However, these steroids may contribute to an important phenomenon observed in patient biopsies: high intracellular concentrations of DHT and testosterone despite castration serum testosterone levels (Montgomery *et al*. 2008, Mostaghel 2014, Taplin *et al*. 2014). These concentrations are sufficient to fully activate the AR and consequently intracellular steroidogenesis constitutes an important factor in cancer progression after castration. Elucidating this process of intracellular steroidogenesis is an important aim for further investigations, but unfortunately, CRPC tissue samples are difficult to obtain.

Abiraterone strongly reduces intraprostatic DHEA, androstenedione and DHT levels (Taplin *et al*. 2014) and may thus impair intracellular steroid hormone conversion. However, it is important to consider serum DHEAS levels, which are in the >100 nM range in abiraterone-treated patients (Taplin *et al*. 2014). DHEAS remains an important potential depot for downstream androgens synthesis (Tamae *et al*. 2015) after abiraterone treatment, particularly since organic anion transporting polypeptides (OATPs) associated with DHEAS uptake appear to be upregulated after androgen deprivation, at least *in vitro* (Arakawa *et al*. 2012).

While the clinical benefits of enzalutamide in different settings are being studied thoroughly, the effects of enzalutamide on steroid metabolism have been rarely reported. Only a single study reported serum steroid levels after enzalutamide treatment in the neoadjuvant setting for patients with high-risk localized prostate cancer who are scheduled to undergo prostatectomy (Montgomery *et al*. 2017). This study shows that while enzalutamide is a direct AR antagonist, it does significantly affect serum steroid levels, including elevated levels of testosterone and DHT. As far as we know, serum steroid levels after enzalutamide in the CRPC setting have not been reported in literature thus far, which represents an obvious omission in our understanding of resistance in the CRPC patients on enzalutamide therapy.

Finally, the alternative DHT synthesis pathways and the 11-oxygenated C19 steroids are also important considerations for further investigations, especially in CRPC patients. Hopefully, future studies will be able to elucidate the mechanisms of intracellular steroidogenesis and reveal its contribution in the castrate, abiraterone and post-abiraterone setting. Currently, little is known about the effects of the hormonal therapies on the levels of 11-oxygenated C19 steroids.

For a long time CRPC has been considered androgen-independent (Nelson *et al*. 2003). Recent preclinical developments and the successful introduction of 2nd-line hormonal treatment after castration have firmly established the relevance of residual presence of intratumoral androgens. Even following abiraterone treatment, steroid hormones can still significantly affect AR activation. Consequently, circulating steroid hormone levels continue to require clinical consideration and offer relevant targets for optimization or improvement of treatment of patients with advanced PC.

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Supplementary data
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Declaration of interest
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