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.

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
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...
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. 2013b, 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 bicalutamide (Tran et al. 2009). Compared to flutamide and bicalutamide, 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. 2013b, 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. 2004a) 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
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 adrenocorticotropic 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 be 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 17OHPregnenolone and 17OHProgesterone, respectively (Miller & Auchus 2011). The glucocorticoid cortisol can be synthesized from 17OHProgesterone catalyzed by CYP21A2 and CYP11B1. DHEA can be produced from 17OHPregnenolone 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 S-alpha reductase isoforms (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 estrogens 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-progestosterone 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)
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.

<table>
<thead>
<tr>
<th>Steroid hormone</th>
<th>Alternative names</th>
<th>Molecular weight</th>
<th>Conversion factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>11-deoxy cortisol</td>
<td>DOC</td>
<td>330.46</td>
<td>3.03</td>
</tr>
<tr>
<td>11-deoxy corticosterone</td>
<td>S</td>
<td>346.46</td>
<td>2.89</td>
</tr>
<tr>
<td>17-hydroxy-allopregnanolone</td>
<td>17OH-Preg</td>
<td>334.49</td>
<td>2.99</td>
</tr>
<tr>
<td>17-hydroxy-pregnenolone</td>
<td>17OH-Preg</td>
<td>330.46</td>
<td>3.03</td>
</tr>
<tr>
<td>5α-dihydrotestosterone</td>
<td>DHT</td>
<td>290.44</td>
<td>3.44</td>
</tr>
<tr>
<td>5α-pregn-17-ol-3,20-dione</td>
<td></td>
<td>332.48</td>
<td>3.01</td>
</tr>
<tr>
<td>5α-pregnan-3,20-dione</td>
<td>5α-progesterone</td>
<td>316.48</td>
<td>3.16</td>
</tr>
<tr>
<td>Androstenediol-glucoronide</td>
<td>ADG</td>
<td>468.59</td>
<td>2.13</td>
</tr>
<tr>
<td>Aldosterone</td>
<td></td>
<td>360.45</td>
<td>2.77</td>
</tr>
<tr>
<td>Allopregnanolone</td>
<td></td>
<td>318.49</td>
<td>1.34</td>
</tr>
<tr>
<td>Androstenediol</td>
<td>3α-androstenediol</td>
<td>292.46</td>
<td>3.42</td>
</tr>
<tr>
<td>Androstenedione</td>
<td>5α-dione</td>
<td>288.42</td>
<td>3.47</td>
</tr>
<tr>
<td>Androstenedioli</td>
<td></td>
<td>290.44</td>
<td>3.44</td>
</tr>
<tr>
<td>Androstenedione</td>
<td>Δ4-dione, adione</td>
<td>286.4</td>
<td>3.49</td>
</tr>
<tr>
<td>Androsterone</td>
<td></td>
<td>290.44</td>
<td>3.44</td>
</tr>
<tr>
<td>Corticosterone</td>
<td>B</td>
<td>346.47</td>
<td>2.89</td>
</tr>
<tr>
<td>Cortisol</td>
<td>hydrocortisone</td>
<td>362.46</td>
<td>2.76</td>
</tr>
<tr>
<td>Dehydriepiandrosterone</td>
<td>DHEA</td>
<td>288.42</td>
<td>3.47</td>
</tr>
<tr>
<td>Dehydriepiandrosterone-sulfate</td>
<td>DHEAS</td>
<td>368.49</td>
<td>2.71</td>
</tr>
<tr>
<td>17b-estradiol</td>
<td>E2</td>
<td>272.39</td>
<td>3.67</td>
</tr>
<tr>
<td>Pregnenolone</td>
<td></td>
<td>316.48</td>
<td>3.16</td>
</tr>
<tr>
<td>Progesterone</td>
<td></td>
<td>314.46</td>
<td>3.18</td>
</tr>
<tr>
<td>Testosterone</td>
<td>T</td>
<td>288.42</td>
<td>3.47</td>
</tr>
</tbody>
</table>

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...
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. 2007a, 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,
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 EC50 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 orchietomized 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-negative 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. 2007a) 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...
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 EC₅₀ values as low as 0.01 nM were measured (Sonneveld et al. 2005, Dennis et al. 2008, Campana et al. 2016, Lalouss et al. 2016). As such, values detected in castrated patients appear to approximate the EC₅₀ 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, Stonczek et al. 2013, Tislidis 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 EC₅₀ values for AR activation observed in vitro, which vary from 5.01 nM to 70 nM (Chen et al. 2004b, 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
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 orchietomy 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. 2013a).

Estradiol

Although initially employed solely as treatment to reduce serum testosterone levels, interest in the role of endogenous estradiol (E2) 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). E2 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 E2 levels were detected between control and BPH subjects (Hammond et al. 1978, Grosman et al. 2010).

Most studies comparing E2 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 E2 by aromatase and,
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 4–33pM (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 7.2pM to 2.9pM (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 (1.5–2.5nM) (Hammond et al. 1977, 1978, Belanger et al. 1994). Elevated pregnenolone levels were observed in abiraterone-treated CRPC patients (16.3nM, IQR 9.85–27.77nM) using LC–MS/MS, and it was also noted that patients with high serum abiraterone levels (>35ng/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 (1.53–1.83nM) 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 2.77nM, IQR 1.62–5.18nM) (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 2.01nM in healthy controls, and a total range of 1.08–12.3nM 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 0.75–4.2nM 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...
of 17β-HSD types 5 (AKR1C3), 6 (HSD17B6) and 10 (HSD17B10). It is also an intermediate of the backdoor
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-androstane-3α,17β-diol
3α-androstane-3α,17β-diol, a metabolite of DHT, is usually measured in its inactive
glucuronidized metabolite, 3α-androstane-3α,17β-diol-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.

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. 2007b).

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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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-androstenedione 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-androstenedione 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
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 EUPCCG 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 >100nM 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

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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