RESEARCH

# Pituitary-testis axis dysfunction following adjuvant androgen deprivation therapy

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## Abstract

Men with high-risk, non-metastatic prostate cancer receive adjuvant androgen deprivation therapy (ADT) for at least 2 years according to Danish guidelines. It remains unclarified if patients regain the function of the pituitary–testis axis after cessation of ADT. Thus, we aimed to investigate the function of the pituitary–testis axis following adjuvant ADT. In this study, we included men who underwent external beam radiation therapy and ADT for high-risk prostate cancer. All patients underwent assessment of testosterone deficiency (TD) symptoms, full biochemical assessment of the pituitary–testis axis, and dynamic stimulatory tests of gonadotropin (gonadotropin-releasing hormone (GnRH) test) and testosterone production (human chorionic gonadotrophin (hCG) test). Patients were diagnosed with TD based on a combination of TD symptoms and testosterone below age-specific reference ranges. TD was characterized as primary, secondary, or mixed based on serum gonadotropins and stimulatory tests. We found that among the 51 patients included in the study, the median time on ADT was 3.2 years and median time since ADT cessation was 3.8 years. Twenty-eight patients were diagnosed with TD; 10 had primary TD (testicular dysfunction), 11 secondary TD (pituitary dysfunction), and 7 mixed TD (combined pituitary and testicular dysfunction). An inadequate testosterone response to hCG stimulation was shown in 42 patients, whereas only 11 patients had a subnormal gonadotropin response to GnRH. We conclude that persistent TD is a common long-term consequence of adjuvant ADT in prostate cancer survivors, equally distributed between pituitary and testicular dysfunction. The study emphasizes the necessity for systematic follow-up of full pituitary–testis axis function in patients receiving adjuvant ADT.

**Key Words**

- pituitary–testis axis
- testosterone deficiency
- androgen deprivation therapy
- prostate cancer

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## Introduction

Androgen deprivation therapy (ADT), provided as gonadotropin-releasing hormone (GnRH) agonists or antagonists, remains a backbone in the treatment of patients with high-risk prostate cancer undergoing curatively intended radiation therapy (Kishan *et al.* 2022). GnRH therapy suppresses the pituitary gonadotropin

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release (Faure *et al.* 1982). The lack of testicular stimulation from gonadotropins leads to deficient testosterone production whereby medical castration is obtained. When ADT is discontinued, the pituitary–testis axis is expected to regain its previous function. However, in men treated with ADT for 6 months or shorter, testosterone concentrations normalize in 80–100% of cases within 12–18 months (Murthy *et al.* 2006, Murthy *et al.* 2007, Kato *et al.* 2020, Roy *et al.* 2020), whereas treatment beyond 2 years may lead to irreversibly low serum testosterone in up to 60% of the patients (Planas *et al.* 2016, Takei *et al.* 2018, Spiegel *et al.* 2019).

Guidelines recommend that testosterone deficiency (TD) is diagnosed based on a combination of the presence of clinical symptoms and low serum testosterone. Furthermore, one should consider measuring serum free testosterone (FT) instead of total testosterone in conditions that might alter sex hormone-binding globulin (SHBG) or when total testosterone is in the lower end of normal range (Corona *et al.* 2020). Thus, the focus of previous studies on serum total testosterone alone is not necessarily translatable to the incidence of TD. Additionally, it remains to be elucidated whether TD following ADT is caused by dysfunction of the pituitary gland, testicles, or a combination of the two.

This study was conducted to investigate how ADT affects the risk of persistent TD in prostate cancer patients treated with radiation therapy and adjuvant ADT. Furthermore, we aimed to understand the pathophysiology of the TD induced by adjuvant ADT through a detailed assessment of the pituitary–testis axis function in all included patients.

## Materials and methods

### Study population and study flow

Patients with high-risk prostate cancer who received adjuvant ADT for at least 2 years in combination with radiation therapy (78 Gy with 1.8–2 Gy/fraction with 5 fractions/weeks) were considered eligible for study inclusion. We did not define any inclusion criteria related to minimum time since finalized ADT. Exclusion criteria were clinical or biochemical relapse and/or general disease burden at risk of affecting pituitary–testis axis function, corresponding to a Charlson comorbidity score ≥5. Patients were referred to participation in relation to follow-up visits at the Department of Urology, Rigshospitalet, Copenhagen, Denmark. The invited patients were consecutive patients attending the outpatient clinic at the Department of Urology for routine clinical follow-up following prostate cancer in the period August 2020 to April 2021. Patients were invited for participation, regardless of complaints of TD symptoms. A flowchart is shown in Fig. 1. The study design was cross-sectional. Information of, for example, body mass index from before ADT initiation was retrieved from medical records. Furthermore, a subset of patients had reproductive hormones tested prior to ADT initiation used for evaluation of changes in reproductive hormone production across ADT.

Patients who were deemed eligible for study inclusion were invited for a full andrological workup, including clinical evaluation, testicular ultrasound, blood tests, reproductive hormone testing, full pituitary screen including blood tests of all axes and an adrenocorticotropic hormone (ACTH) test, dual-energy X-ray absorption (DXA, regional bone scan and full body scan), and systematic assessment of TD symptoms through clinical evaluation and a questionnaire (O'Connor *et al.* 2008). The study was approved by the ethical committees of the Capital Region of Denmark (H-20050918) and the Data Protection Agency of the Capital Region (P-2021-157) and performed according to the Declaration of Helsinki. Individual consent was obtained verbally and in writing from all participants.

### Characterization of testosterone deficiency

If patients had low serum testosterone or FT, sex hormones were measured in an additional set of blood tests to secure

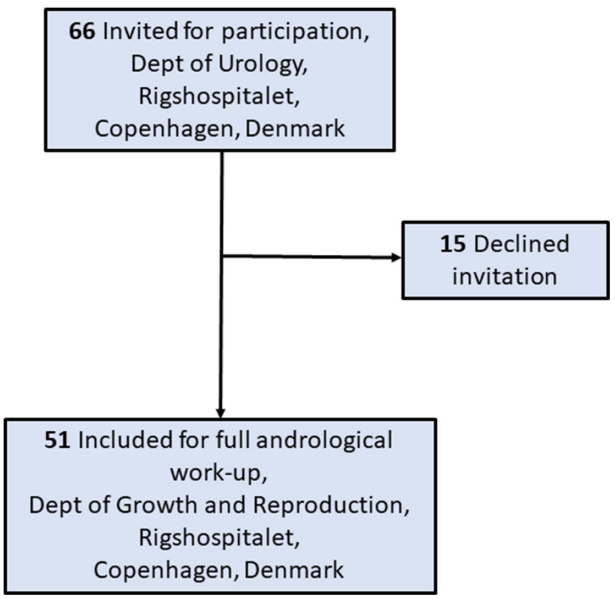


**Figure 1**
Flow diagram of study inclusion of patients.

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the correct diagnosis in accordance with clinical guidelines (Corona *et al.* 2020). However, the initial set of test results were used for further analyses to secure standardized handling of test results across groups. At follow-up, patients were diagnosed with TD based on a combination of clinical symptoms of TD, testosterone, and FT values below –2 S.D.. Two trained andrologists separately assessed all patients and characterized each patient with either TD or no TD. Patients with TD were further divided into primary (pTD), secondary (sTD), or mixed TD (mTD). pTD was characterized as symptoms of TD, low FT, and high luteinizing hormone (LH) and sTD as symptoms of TD, low FT, and low LH. mTD was defined as symptoms of TD, low FT, and LH within the normal range, thus insufficiently lower than expected but with a GnRH-stimulated LH response in the lower normal range.

### Assessment of testosterone deficiency symptoms

Patients responded to the European Male Aging Study (EMAS) sexual function questionnaire (O'Connor *et al.* 2008) and systematically reported presence of symptoms compatible with TD to the doctor. The EMAS sexual function questionnaire was only used to quantify symptoms of TD and was not used for the diagnosis of TD.

### Reproductive hormone analyses and additional blood tests

Serum concentrations of testosterone, LH, follicle-stimulating hormone (FSH), and SHBG were determined using a time-resolved fluoroimmunoassay (Delfia, Wallac, Turku, Finland). Inter- and intraassay coefficients of variation (CVs) for measurements of the hormones were FSH (3% and 2%), LH (2% and 3%), SHBG (5% and 4%), and testosterone (10% and 6%). FT was calculated using the Vermeulen formula with fixed albumin (43.8 g/L) (Vermeulen *et al.* 1999). Presented reference ranges are age adjusted and laboratory specific based on healthy men between 60 and 80 years of age. Inhibin B was determined using a specific two-sided enzyme immunometric assay (Serotec, Oxford, UK) and inter- and intraassay CVs were <11%. Serum insulin-like factor 3 (INSL3) was quantified by liquid chromatography tandem mass spectrometry as described previously and intra- and interassay CVs were <5% and 10% (Albrethsen *et al.* 2018). Serum insulin-like growth factor-1 (IGF-1) was measured using an IDS-iSYS IGF-1 assay (Immuno-diagnostic Systems LTD, Bolton, UK) and inter- and intraassay CVs were 6% and 2%. In a subset of patients ($N=33$), plasma samples from before ADT were retrieved from a previous biobank, and plasma testosterone, SHBG, FT, LH, and INSL3 were compared before initiation of ADT and at follow-up. Reproductive hormone analyses were performed at the Department of Growth and Reproduction, Rigshospitalet. Additional analyses were performed at the Department of Clinical Biochemistry, Rigshospitalet. All analyses were validated and accredited by the Danish Accreditation Fund (DANAK, https://www.danak.dk/).

### Reproductive hormone testing

Reproductive hormone testing was performed as described previously (Bang *et al.* 2017). All GnRH tests were initiated between 08:00 and 12:00 h. Prior to the tests, a baseline blood sample was drawn.

#### GnRH stimulation test

An i.v. injection of 100 µg GnRH (Relefact, Sanofi-Aventis, Frankfurt, Germany) was given, and a blood sample was collected after 30 min.

#### Human chorionic gonadotrophin (hCG) stimulation test

An injection of 5000 IU hCG (Pregnyl, Oragnon, Amsterdam, Netherlands) was given. The follow-up blood sample was taken 72 h later.

#### ACTH test

An i.v. injection of 250 µg synacthen was given, and a blood sample was collected after 30 min.

### Body composition

Fat and lean body masses and bone mineral density were assessed through a DXA scan (Lunar Prodigy Advance; GE Medical Systems Lunar, Milwaukee, WI, USA). Software (Prodigy, enCORE 2004, version 8.8; GE Lunar Corp., Madison, WI, USA) was used to estimate regional and total fat and fat-free tissue masses. CVs were 2% for total body fat, <5% for regional body fat, and <2% for bone.

### Statistical analyses

Continuous variables are displayed as medians with interquartile ranges (IQR) and categorical variables as number with percentages (%). Differences in variables between groups were compared using a chi-square test for categorical variables and an independent samples *t*-test for continuous variables. Data were $\log_{10}$-transformed

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when not normally distributed. Histograms and Q–Q plots were used to assess normal distribution (data not shown). Analyses of the association between sex hormone concentrations and time since ADT were performed using a linear regression model. Analyses were corrected for age and time of ADT. Models were checked for assumptions of the linear model, including normal distribution of the residuals, homogeneity of variance, linearity, and independent observations. Data with repeated measures (sex hormone concentrations before and after ADT) were handled using a mixed model for each outcome variable (LH, testosterone, FT, and INSL3), with the covariates group (no TD and TD) and time adjusted for the interaction between the two covariates. A random effect accounting for an individual specific concentration was included. A Tukey *post-hoc* test was performed to assess between- and within-group differences. Mixed model analyses were performed in SAS Enterprise Guide version 7.1. Other statistical analyses were performed using IBM SPSS statistics version 25. A $P$ value < 0.05 was considered statistically significant.

## Results

Patient characteristics are shown in Table 1. The study population consisted of 51 patients with a median age of 74 years (IQR: 69–76). Median time of ADT was 3.2 years (IQR: 3.0–3.4) and time since ADT cessation was 3.8 years (IQR: 2.6–5.1). Of the 51 patients included, 12 sought medical advice concerning sustained symptoms of TD prior to inclusion, whereas 39 did not. However, upon questioning, all men characterized as having TD had symptoms compatible with TD. None of the patients were treated with testosterone therapy prior to inclusion.

### Characterization of testosterone deficiency

Out of the 51 patients, 28 were diagnosed with TD. Among these patients, 10 were characterized as having pTD, 7 as having mTD, and 11 as having sTD. A reduced testosterone/LH ratio was shown in 40 patients, compared to age-specific normal ranges, 31 had a reduced FT/LH ratio, and 43 patients an inadequate testosterone response to hCG stimulation, all together indicating an impaired Leydig cell function in the majority of patients (Fig. 2A–C). Inhibin B/FSH ratio was reduced in 38 patients, suggesting impaired spermatogenesis (Fig. 2D). Patients with mTD and sTD ($N = 18$) had inadequate serum gonadotropin concentrations relative to their testicular hormone production, corresponding to left-shifted testosterone/LH, FT/LH, and inhibin B/FSH ratios (Fig. 2A, B and D). Only patients with sTD ($N = 11$) had an inadequate response to GnRH stimulation; however, the mTD patients had responses in the low-normal range (Fig. 2E).

### Symptoms of testosterone deficiency

Patients with TD reported a lower overall sexual function compared with patients with no TD (difference: 7 points, 95% CI: 4–12 points, $P = 0.001$) as well as a higher frequency of erectile dysfunction (27 of 28 patients with TD vs 17 of 23 with no TD, $P = 0.020$), lack of libido (19 of 28 with TD

**Table 1** Subject characteristics.

| Characteristics | Cohort |
|---|---|
| | $N = 51$ |
| Age at treatment initiation, years, median (IQR) | 67 (62–69) |
| Age at follow-up, years, median (IQR) | 74 (69–76) |
| Length of ADT, years, median (IQR) | 3.2 (3.0–3.4) |
| Time since ADT cessation, years, median (IQR) | 3.8 (2.6–5.1) |
| Gleason score, *N* (%) | |
| 7 | 32 (63) |
| 8 | 9 (18) |
| 9 | 10 (19) |
| Clinical tumor stage, *N* (%) | |
| T1 | 3 (6) |
| T2 | 8 (15) |
| T3 | 37 (73) |
| T4 | 3 (6) |
| D'Amico risk classification, *N* (%) | |
| Low | 0 (0) |
| Intermediate | 5 (10) |
| High | 46 (90) |

ADT, androgen deprivation therapy; IQR, interquartile range.

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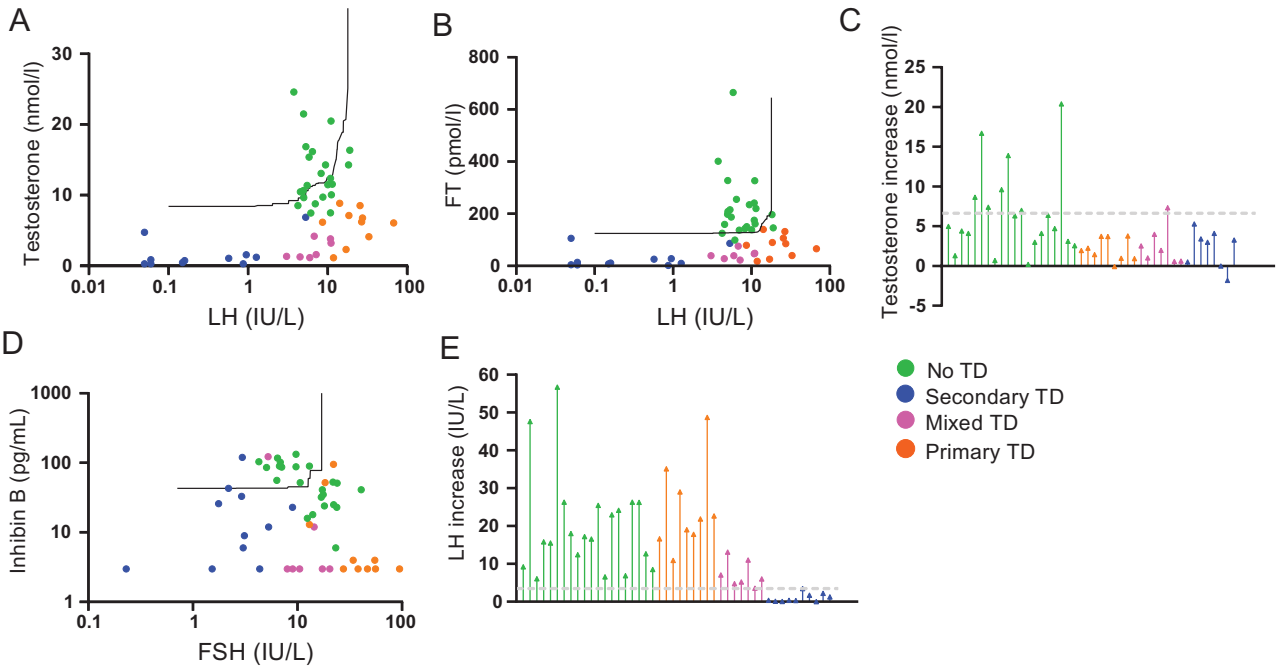


**Figure 2**
Basal and stimulated serum concentrations of pituitary–testis hormones on age- and assay-specific reference curves. (A and B) Testosterone and free testosterone (FT) in relation to luteinizing hormone (LH). Each dot represents a single patient. The black line represents the reference limit of healthy Danish adult men (60–80 years old), with 95% having high testosterone and low LH. (C) Increase in testosterone in response to human chorionic gonadotropin, 5000 IU. Patients are aligned on the x-axis according to type of testosterone deficiency (TD). Each arrow represents a single patient. Ninety-five per cent of healthy Danish adult men are on the upper side of the dashed grey line. (D) Inhibin B in relation to follicle-stimulating hormone (FSH). Ninety-five per cent of healthy Danish adult men are on the left side of the black line. (E) LH increase in response to gonadotropin-releasing hormone stimulation, Relefact 100 µg. Patients are aligned on the x-axis according to type of TD. Each arrow represents a single patient. Ninety-five per cent of healthy Danish adult men are on the upper side of the dashed grey line. Green colours represent the men with no TD, blue with secondary TD, magenta with mixed TD, and orange with primary TD, all colours are maintained throughout the different graphs. $N = 51$ for all figures.

vs 8 of 23 with no TD, $P = 0.019$), fatigue (15 of 28 with TD vs 6 of 23 with no TD, $P = 0.047$), heat flushes (18 of 28 with TD vs 4 of 23 with no TD, $P = 0.001$), and loss of muscle strength (15 of 28 with TD vs 4 of 23 with no TD, $P = 0.008$). In the no TD group, 17 patients had at least one symptom compatible with TD and two patients had testosterone or FT below normal range but no symptoms of TD.

## Testosterone deficiency and time since androgen deprivation therapy

For each year since end of ADT, testosterone increased by 2.5 nmol/L (95% CI: 1.4 – 3.6 nmol/L, Fig. 3A) and hCG stimulated testosterone by 1.08 nmol/L (95% CI: 1.01–1.14 nmol/L, Fig. 3B). For each year since the end of ADT, LH increased by 1.7 IU/L (95% CI: 1.3–2.2 IU/L, Fig. 3C) and stimulated LH by 1.2 IU/L (95% CI: 1.1–1.3 IU/L, Fig. 3D).

## Changes in pituitary–testis function with androgen deprivation therapy

There were no statistically significant differences in serum testosterone and FT from prior to ADT to follow-up among patients with no TD (testosterone: –4%, 95% CI: –24 to 15%, and FT: –8%, 95% CI: -30 to 15%). Testosterone decreased by 66% (95 % CI: 25–87%) and FT by 66% (95% CI: 42–89%) in patients who were subsequently classified as having TD (Fig. 4A and B). Both TD and no TD patients experienced an increase in LH (37%, 95% CI: 5–68%), with no significant differences between groups (TD: 17%, 95% CI: –21 to 54%) as well as an increase in LH to FT ratio, indicating a decreasing Leydig cell capacity in both TD and no TD patients (Fig. 4C and D). Serum INSL3 was 44% (95% CI: 11–77%) lower in patients who developed TD, and ADT was associated with a 64% (95% CI: 43–85%) decline in serum INSL3 in both groups (Fig. 4E).

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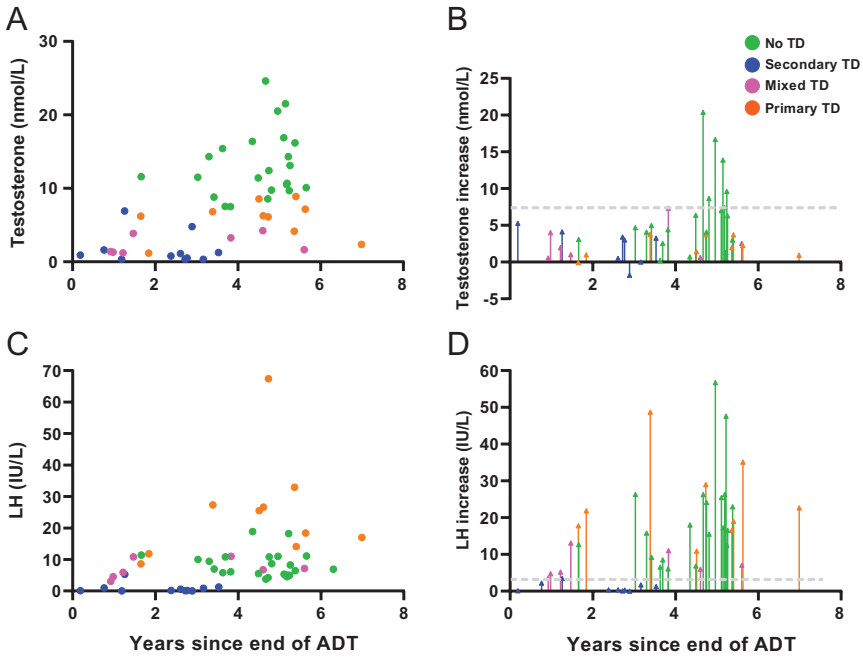


**Figure 3**
Temporal trends in pituitary–testis hormones and years since the end of androgen deprivation therapy. (A) Testosterone concentrations over time since the end of androgen deprivation therapy (ADT). (B) Human chorionic gonadotropin (5000 IU) stimulated testosterone increase over time since the end of ADT. (C) Lutenizing hormone (LH) concentrations over time since the end of ADT. (D) Gonadotropin-releasing hormone stimulated (Relefact 100 µg) LH increase over time since the end of ADT. Each dot and triangle represent a single patient. Green colours represent the men with no testosterone deficiency (TD), blue with secondary TD, magenta with mixed TD, and orange with primary TD, all colours are maintained throughout the different graphs. *N* = 46 for all figures.

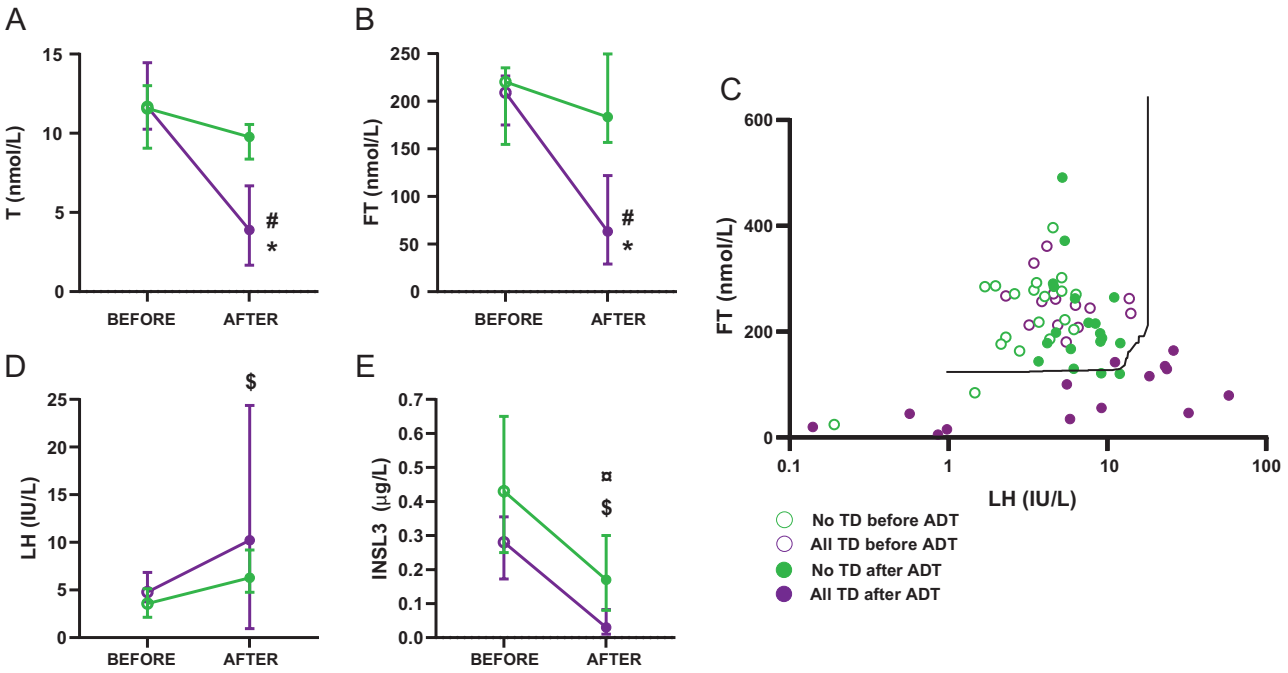


**Figure 4**
Changes in pituitary–testis hormone concentrations from before to after androgen deprivation therapy. (A) Testosterone (T) concentrations before and after androgen deprivation therapy (ADT) in patients without testosterone deficiency (no TD) and patients with TD at follow up. *Significantly different from before ADT in TD group, P < 0.001. #Significantly different from no TD after ADT, P < 0.001. (B) Free testosterone (FT) concentrations before and after ADT in patients with no TD and patients with TD at follow-up. *Significantly different from before ADT in TD group, P < 0.001. #Significantly different from no TD, after ADT, P < 0.001. (C) FT in relation to luteinizing hormone (LH). Each patient is represented twice: as an open circle before ADT and as a closed circle after ADT. The black line represents the reference limit of healthy Danish adult men (60–80 years old), with 95% having high testosterone and low LH. (D) LH before and after ADT. $Significantly different from before ADT, both groups, P = 0.023. (E) Insulin-like factor 3 (INSL3) before and after ADT. $Significantly different from before ADT, both groups, P < 0.001. ¤Significant difference between TD and no TD, both timepoints, P = 0.010. Analyses were performed using a mixed model with outcome variable (sex hormones) and group (no TD and TD) and time adjusted for the interaction between the two covariates. A Tukey post-hoc test was performed to assess between- and within-group differences. Green colours represent the patients with no TD, purple colours are patients with all types of TD. Open circles represent patients before ADT and closed circles patients after ADT. *N* = 33 for all figures.

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### Metabolic function following ADT

Patients gained 2.0 kg/m$^2$ in BMI from initiation of ADT to follow-up with no significant differences between groups (Table 2). Fat mass, fat-free mass, and bone mineral density did not differ between TD and no TD patients. HbA1c values, reflecting mean blood glucose concentrations, were higher (4.2 mmol/mol, 95% CI: 0.7–7.8 mmol/mol) in patients with TD compared with patients with no TD. Blood lipids did not differ significantly between patients with and without TD. Patients with mTD generally had a poorer metabolic profile with higher fat mass and blood glucose compared to patients with other types of TD and no TD (Table 2).

## Discussion

Castration-based therapy as an adjuvant to curatively intended radiation is the standard of care in high-risk prostate cancer. However, ADT results in a significant decrease in quality of life (Wei *et al.* 2002, Penson *et al.* 2003), loss of sexual function (Donovan *et al.* 2018), and increases in risk of osteoporosis (Shahinian *et al.* 2005), anaemia (Strum *et al.* 1997), and metabolic disease (Keating *et al.* 2006, Wang *et al.* 2016). A substantial number of prostate cancer patients receiving ADT are willing to trade up to 10% in 5-year survival to maintain sexual function and quality of life (Singer *et al.* 1991, Wilke *et al.* 2010). Thus, focus on the long-term consequences and potential irreparable dysfunction on the pituitary–testis axis due to ADT is of considerable importance to the patients.

The study importantly shows that a substantial number of patients receiving adjuvant ADT in combination with radiation therapy experienced lasting pituitary–testis axis dysfunction, several years after cessation of ADT. Testicular dysfunction (pTD), pituitary dysfunction (sTD), and a combination of the two (mTD) were equally frequent causes of TD.

Short-term ADT has been shown to cause Leydig cell atrophy (Giberti *et al.* 1988, Johansen *et al.* 1990), but it has not been elucidated if these changes are irreversible. Our data suggest a persistent decline in Leydig cell function (increase in LH, increase in LH to FT ratio, and insufficient response to HCG) even in patients without TD. This was supported by a general decrease in serum INSL3 – a secretory product of the Leydig cells (Ivell *et al.* 1997, Kawamura *et al.* 2004). INSL3 has been described to decline with age and around 0.014 ng/mL each year in this age group and could account for up to half of the observed INSL3 decline. The contribution from radiation therapy of the prostate on Leydig cell insufficiency is expected to be sparse (Midzak *et al.* 2009, Farhood *et al.* 2019). As to our knowledge, no previous studies have described the long-term consequences of ADT on pituitary function. However, in this study, 35% of all patients showed an insufficient gonadotropin secretion following ADT. Thus, ADT also exerts a long-lasting suppression of pituitary gonadotropin secretion.

We found that both testosterone and LH increased with time since ADT cessation, indicating some degree of restoration of pituitary–testis function even several years following treatment. Previous larger studies show that the likelihood of reaching serum testosterone concentrations within the normal range, after both short- and long-term ADT (6–36 months), drastically decreases if testosterone is not normalized within the first 2 years after cessation of ADT (Tsumura *et al.* 2015, Nam *et al.* 2018, Roy *et al.* 2020). The fact that sTD was more common the first years following cessation of ADT, and pTD later in the follow-up period, suggests that the ADT-induced pituitary dysfunction tends to be reversible, whereas testicular dysfunction is more persistent.

GnRH analogues are used to treat a variety of conditions including endometriosis, central precocious puberty, and gender dysphoria (Kennedy *et al.* 2005, Carel & Leger 2008, Hembree *et al.* 2017). Whether the persisting dysfunction of the pituitary–testis axis observed in this study is transferrable to other patient groups is unknown but should be a subject of future studies.

ADT is associated with an increased risk of metabolic diseases, including diabetes and cardiovascular disease (Keating *et al.* 2006, Wang *et al.* 2016). It remains to be elucidated if the patients with persisting TD are at higher risk of metabolic disease compared with the patients with a fully recovered pituitary–testis axis. In this study, we found no substantial differences in metabolic profile, including body composition and blood lipids, between patients with and without TD. Patients with mTD generally had a poorer metabolic profile compared to the other groups but also tended to have a higher BMI before ADT initiation, which could explain the differences.

The use of testosterone treatment in patients with previous prostate cancer is highly debated as testosterone has been suggested to increase prostate cancer risk and disease progression (Morgentaler & Traish 2009). In contrast, a short time to recovery of endogenous serum testosterone is not considered a risk factor for relapse (Roy *et al.* 2020, Zapatero *et al.* 2021). Randomized controlled trials of testosterone treatment for the recovery of serum

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**Table 2** Subject characteristics based on type of testosterone deficiency.

| | **No TD** | **TD** (primary) | **TD** (mixed) | **TD** (secondary) |
|---|---|---|---|---|
| | *N* = 23 | *N* = 10 | *N* = 7 | *N* = 11 |
| **General characeristics, median (IQR)** | | | | |
| Age at treatment initiation, years | 67 (62–70) | 68 (60–69) | 67 (63–71) | 66 (57–68) |
| Age at follow-up, years | 75 (70–78) | 76 (68–77) | 72 (69–77) | 71 (61–74) |
| Length of ADT, years | 3.2 (3.0–3.3) | 3.2 (3.1–3.5) | 3.0 (2.6–3.3) | 3.3 (3.0–3.5) |
| Time since ADT cessation, years | 4.8 (3.7–5.2) | 4.7 (3.4–5.4) | 1.5 (1.0–4.6) | 2.6 (1.2–2.9) |
| Overall sexual function score[a] | 10 (7–17) | 4 (2–10) | 2 (1–3) | 1 (1–4) |
| **Anthropometrics at follow-up, median (IQR)** | | | | |
| BMI, kg/m$^2$ | 28 (24–32) | 27 (25–33) | 33 (31–35) | 31 (26–34) |
| BMI increase[b], kg/m$^2$ | 2.2 (0.8–4.3) | 2.0 (–1.4 to 2.6) | 1.6 (0.0–3.0) | 2.2 (1.0–5.3) |
| Fat mass, kg | 28 (21–32) | 24 (22–34) | 50 (35–55) | 35 (23–44) |
| Fat % | 36 (29–39) | 34 (32–38) | 48 (38–49) | 37 (27–40) |
| Fat-free mass, kg | 50 (48–57) | 52 (47–58) | 56 (52–58) | 61 (53–64) |
| Android:gynoid fat ratio | 1.32 (1.17–1.45) | 1.13 (1.11–1.17) | 1.24 (1.11–1.27) | 1.23 (1.12–1.36) |
| T-score spine | –0.5 (–1.5 to 0.6) | –0.4 (–0.9 to 0.0) | –1.0 (–1.2 to 0.3) | –0.4 (–0.8 to 0.9) |
| T-score hip | –1.1 (–2.0 to 0.4) | –1.2 (–1.8 to 0.1) | –0.8 (–3.1 to 0.8) | –0.4 (–1.0 to 0.3) |
| Z-score spine | –0.2 (–0.8 to 0.8) | –0.1 (–0.4 to 1.1) | –0.9 (–1.3 to 0.7) | –0.1 (–0.9 to 0.9) |
| Z-score hip | –0.4 (–1.1 to 0.4) | –0.7 (–0.9 to 0.1) | –0.6 (–2.6 to 0.1) | –0.3 (–0.8 to 0.5) |
| **Sex hormones at follow-up, median (IQR)** | | | | |
| LH, IU/L *(1.2 – 12.3)*[c] | 7.0 (5.0–10.8) | 22.0 (14.1–27.3) | 6.8 (4.5–10.8) | 0.2 (0.1–1.0) |
| FSH, IU/L *(2.0 – 27.5)*[c] | 13.0 (6.9–21.8) | 36.1 (22.1–54.8) | 10.5 (8.0–17.3) | 3.0 (1.8–4.4) |
| Testosterone, nmol/L *(8.0–26.0)*[c] | 11.6 (9.8–16.2) | 6.2 (4.2–7.2) | 1.6 (1.3–3.9) | 0.9 (0.4–1.6) |
| FT, pmol/L *(170–590)*[c] | 197 (146–242) | 83 (41–107) | 40 (28–49) | 12 (5.1–28) |
| Inhibin B, µg/L *(8.7–363)*[c] | 52 (25–90) | 4 (3–13) | 3 (3–12) | 12 (3–33) |
| **Pituitary function at follow-up, median (IQR)** | | | | |
| Synacthen test, 30 min cortisol, nmol/L *(> 420)* | 588 (501–626) | 537 (508–587) | 660 (600–791) | 601 (577–637) |
| IGF-1, µg/L *(50–200)* | 107 (87–138) | 127 (99–157) | 112 (85–121) | 98 (86–145) |
| TSH, IU/L *(0.4–4.8)* | 1.4 (1.0–2.1) | 2.0 (1.5–3.3) | 2.4 (1.7–3.3) | 1.5 (1.0–1.9) |
| Free T4, pmol/L *(12.0–22.0)* | 16 (15–17) | 15 (14–16) | 15 (13–16) | 14 (13–16) |
| Prolactin, 10-3 IU/L *(69–266)* | 126 (101–161) | 145 (119–199) | 160 (125–189) | 117 (66–165) |
| **Additional andrological measures at follow-up, median (IQR)** | | | | |
| Testicular size, mL | 7.8 (5.4–10.2) | 5.7 (3.5–6.5) | 6.0 (4.7–7.1) | 5.1 (3.2–5.9) |
| Hemoglobin, mmol/L *(8.3–10.5)* | 9.3 (8.2–9.7) | 8.3 (7.6–9.0) | 8.3 (7.5–8.9) | 8.3 (7.9–8.7) |
| Hematocrit *(0.4–0.5)* | 0.44 (0.40–0.47) | 0.41 (0.39–0.43) | 0.42 (0.40–0.43) | 0.40 (0.38–0.41) |
| PSA *(<4.0 µg/L)* | 0.2 (0.1–0.4) | 0.2 (0.1–0.4) | 0.1 (0.1–0.2) | 0.1 (0.1–0.2) |
| **Metabolic parameters at follow-up, median (IQR)** | | | | |
| HbA1c, mmol/mol *(<48)* | 38 (36–43) | 40 (38–50) | 50 (38–50) | 40 (37–43) |
| Cholesterol, total, mmol/L *(<5.0)* | 5.1 (4.7–5.4) | 5.1 (4.9–5.1) | 5.1 (5.1–5.2) | 5.1 (4.5–5.4) |
| Cholesterol, LDL, mmol/L *(<3.0)* | 3.1 (2.8–3.3) | 3.1 (2.7–3.1) | 3.1 (3.1–3.5) | 3.1 (2.8–3.5) |
| Cholesterol, HDL, mmol/L *(>1.0)* | 1.48 (1.07–1.72) | 1.43 (1.35–1.87) | 1.19 (0.90–1.44) | 1.65 (1.23–1.98) |
| Triglycerides, mmol/L *(<2.0)* | 1.14 (1.07–1.67) | 0.99 (0.71–1.85) | 2.99 (1.10–3.62) | 1.44 (1.06–1.73) |
| **Comorbidity at time of follow-up, *N* (%)** | | | | |
| Hypertension | 7 (30) | 3 (30) | 3 (43) | 2 (18) |
| Hypercholesterolemia | 9 (39) | 7 (70) | 4 (57) | 3 (27) |
| Type 2 diabetes | 2 (9) | 3 (30) | 3 (43) | 2 (18) |
| Osteoporosis | 1 (4) | 2 (20) | 1 (14) | 1 (9) |
| Cardiovascular disease | 5 (22) | 4 (40) | 3 (43) | 5 (45) |
| Pulmonary disease | 2 (9) | 0 (0) | 1 (14) | 1 (9) |
| Hematologic disease | 2 (9) | 0 (0) | 0 (0) | 1 (9) |
| **Type of ADT, *N* (%)** | | | | |
| Leuprorelin | 4 (17) | 4 (40) | 5 (71) | 11 (100) |
| Histreline acetate | 10 (44) | 2 (20) | 2 (29) | 0 (0) |
| Goserelin | 6 (26) | 3 (30) | 0 (0) | 0 (0) |
| Triptorelin | 3 (13) | 1 (10) | 0 (0) | 0 (0) |

(*Continued*)

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**Table 2** Continued.

| | No TD | TD (primary) | TD (mixed) | TD (secondary) |
|---|---|---|---|---|
| | *N* = 23 | *N* = 10 | *N* = 7 | *N* = 11 |
| **Testosterone deficiency symptoms at time of follow-up, *N* (%)** | | | | |
| Erectile dysfunction | 17 (74) | 9 (90) | 7 (100) | 11 (100) |
| Lack of libido | 8 (35) | 5 (50) | 5 (71) | 9 (82) |
| Fatigue | 6 (26) | 4 (40) | 4 (57) | 7 (64) |
| Heat flushes | 4 (17) | 4 (40) | 5 (71) | 9 (82) |
| Loss of muscle strength | 4 (17) | 5 (50) | 3 (43) | 7 (64) |
| Gynecomastia | 4 (17) | 2 (20) | 1 (14) | 2 (18) |
| No symptoms | 5 (22) | 0 (0) | 0 (0) | 0 (0) |

ADT, androgen deprivation therapy; BMI, body mass index; FSH, follicle-stimulating hormone; FT, free testosterone; IGF-1; insulin-like growth factor; IQR, interquartile range; LH, luteinizing hormone; TD, testosterone deficiency; TSH, thyroid-stimulating hormone.
[a]Assessed from European Male Aging Study sexual function questionnaire.
[b]From before ADT to time of follow-up.
Reference ranges for blood tests are shown in italics.
[c]Reference ranges were age specific but are given here for 70 years of age.

testosterone levels are missing. Since trends in survival rates are improving over time in this group of patients, potential treatment options should be considered for future research to reduce long-term consequences of persistent TD in men otherwise cured (Orrason *et al.* 2020).

In this study, 55% of patients were diagnosed with TD. A precise estimate of prevalence of TD in this group could not be made due to the limited sample size as well as the fact that 12 of 51 patients were included with known symptoms of TD. Furthermore, 15 of the 66 invited patients declined their initial invitation introducing some degree of selection bias in the study, which is a limitation. Due to the observational nature of the study, causal conclusions could not be drawn. We did not have clinical andrological assessment of patients prior to ADT. Thus, we cannot rule out that patients with preceding TD were included. The risk of late-onset TD increases with age (Wu *et al.* 2010). We based comparisons in the cross-sectional data on a laboratory-specific age-matched control cohort but were unable to draw comparisons on our longitudinal data. Our study is limited by inclusion of a restricted number of patients. We did not have significant power to evaluate changes in pituitary–testis hormones over time, based on the type of TD. Consequently, all TDs were pooled in these analyses.

## Conclusions

In conclusion, persistent TD is a very common long-term consequence of adjuvant ADT in prostate cancer survivors. Causes of TD are equally distributed between pituitary and testicular dysfunction. The study emphasizes the necessity for systematic follow-up of pituitary–testis function following adjuvant ADT as well as the need for randomized controlled trials investigating the safety and feasibility of testosterone treatment in prostate cancer survivors following adjuvant ADT.

**Declaration of interest**
The authors declare no conflicts of interest that could be perceived as prejudicing the impartiality of the research reported.

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