Male hypogonadism: a review

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ABSTRACT

This article contains a systematic review of the main developments that have occurred in the area of male hypogonadism between the publication of the Endocrine Society Guidelines of 2010 and 2018 and after 2018.

INTRODUCTION

One of the first cases of hypogonadism successfully treated with testosterone (T) was published in 1937. The patient was a medical student aged 27 years with a history of measles before age 15 whose body was described as a '*prepuberal castrate with a feminine emphasis in the wide hips, genu valgum, girdle distribution of fat, protruding mammae and retarded development of larynx, genitalia and hair*'.[1] The patient was given T acetate 40 mg, 3 injections per week, for a period of 1 month. Within 60 hours from the first injection the patient experienced the first erection of his life. After 2 weeks, the scrotum became larger and rugose, after 3 weeks the areolar tissue became more pigmented and by the end of the month small hairs appeared on the chest and above the pubis, while hot flashes disappeared at once. This description exemplifies the remarkable response to T treatment (Tt) in an undervirilized man unable to produce T due to primary testicular pathology.

Over the years, the traditional policy of the US Food and Drug Administration (FDA) has been to require that T products raise low serum T concentrations into the normal range in order to be approved.[2] For this reason, clinical trials showing improvement in signs or symptoms of hypogonadism after Tt have been small and short,[3–6] and only recently larger studies[7 8] of men with T deficiency arising at an older age (eg, men affected by late-onset hypogonadism (LOH)) have been published. Nevertheless, based on clinical experience since the 1940s and small positive clinical studies,[3 4] there has been universal agreement that Tt is beneficial for men affected by pathological hypogonadism (PH), that is, the form of hypogonadism caused by pathological impairment of the hypothalamic-pituitary-testicular (HPT) axis that is associated with severe T deficiency and a constellation of typical signs and symptoms.[9] In March 2018, the Endocrine Society (ES) published new guidelines on Tt in men with androgen deficiency.[10] With a resurgence in publications related to this topic during the 8 years elapsed since the previous guidelines,[11] new important concepts and questions have been formulated. The 2010 and 2018 guidelines propose a rational approach supporting the use of Tt '*only in men with symptoms and signs of T deficiency, and unequivocally low serum T concentrations*'. However, a paper published in 2015 by the FDA reported that between 2010 and 2013 the number of men with a prescription claim for T from US retail pharmacies increased from 1 200 000 to 2 200 000,[2] indicating that at a time in which national guidelines were trying to rationalize the use of T and no new indications for its use had emerged, the number of T prescriptions increased out of proportion to clinical needs. Even more perplexing is the report that during this time 28%–40% of men receiving Tt never had a serum T measured before receiving a prescription,[2 12] implying that Tt was prescribed without a proper work-up, possibly to patients who were not hypogonadal. Additionally, >80% of T users reported by this paper were men between 40 and 74 years of age,[2] suggesting that the majority of these patients were affected by LOH, typically diagnosed in men older than 40 years, as opposed to PH, which is usually diagnosed earlier.

In the following pages, we aim to analyze the 2018 guidelines, and to offer a critical review of the most important developments that have occurred between 2010 and 2018 and after 2018. We, like others,[9] endorse the opinion that there are two distinct forms of hypogonadism, the first is PH, that is due to HPT disease, severe T deficiency and full-scale clinical presentation.[9] The second is LOH, a condition associated with advancing age, modest decrease in serum T and a subtler clinical presentation.[13–15] In this review, we also aim to discuss in detail the results of the Testosterone Trials,[7] the first large-scale study evaluating the effect of Tt in patients affected by LOH, and the never-ending controversies related to Tt, including those related to the risk of developing cardiovascular (CV) complications and prostate cancer (PCa).

CONTROL OF TESTOSTERONE PRODUCTION AND RELEASE DURING FETAL DEVELOPMENT, ADULT LIFE AND DISEASE STATUS

The HPT axis

Hypothalamic GnRH neurons are the key hierarchical element that integrates the reproductive hormone axis with cues related to peripheral sex

hormone levels and energy status. When these peripheral signals are optimally integrated, puberty initiates with the pulsatile secretion of GnRH into the hypothalamic-pituitary portal circulation. GnRH interacts with the G protein-coupled GnRH receptor located on the surface of the pituitary gonadotrophs and induces the pulsatile release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which in turn stimulate the gonads to secrete sex hormones and produce gametes. LH and FSH reach their cognate receptors (LH-R and FSH-R) located on the plasma membrane of testicular Leydig and Sertoli cells, respectively. Interestingly, LH-R is activated by both LH released by the pituitary and human chorionic gonadotropin (hCG), released by the placenta; however, hCG plays an active role only during the initial 3 months of fetal development. LH-R and FSH-R are G protein-coupled receptors triggering a cyclic AMP-protein kinase A-dependent signaling pathway. Leydig cells have the capacity to synthesize cholesterol from acetate or to take up this substrate for steroidogenesis from circulating lipoproteins, and, typical of any steroid secreting cell, they contain abundant smooth endoplasmic reticulum and mitochondria. Activation of LH-R on the surface of Leydig cells stimulates intracellular cholesterol translocation into the mitochondria by increasing production of the STAR protein[16] followed by its cleavage into pregnenolone by the enzyme CYP1A1. Pregnenolone translocates back into the cytoplasm and eventually is converted to T by the enzymes P450C17, 3β-HSD and 17β-HSD, whose upregulation is mediated by the activation of LH-R.[17] On the Sertoli cells, activation of FSH-R induces production of a number of proteins including CYP19A1, the glycoproteins inhibin A and B and numerous growth factor proteins that support spermatogenesis. Importantly, FSH does not support spermatogenesis directly, but through the release of these proteins from Sertoli cells and in collaboration with the high intratesticular concentrations of T generated by adjacent Leydig cells. In turn, sex hormones feed back to the higher structures of the HPG axis (eg, the hypothalamus and pituitary) and regulate their activity. It is clear that an additional regulatory pathway is required to link steroid hormone feedback mechanisms to GnRH secretion because GnRH neurons lack the prerequisite steroid hormone receptors.[18] Two papers published in 2003 demonstrated that the Kisspeptin-Kiss 1 receptor (Kp-Kiss1R) signaling pathway is a powerful proximal regulator of GnRH and gonadotropins secretion, as inactivating mutations of Kiss1R are associated with hypogonadotropic hypogonadism (HH) due to lack of GnRH secretion.[19 20] Successive studies revealed that Kp neurons are located in the infundibular nucleus and pre-optic area of the hypothalamus, they interact with Kiss1R-rich GnRH neurons by forming axo-somatic, axo-dendritic and axo-axonal points of contact,[21] and normal function of the Kp-Kiss1R axis is a necessary requirement to induce pulsatile release of GnRH and normal initiation of puberty. Approximately 75% of Kp neurons in the infundibular nucleus are called KNDy neurons because they coexpress kisspeptin (K), neurokinin B (N) and dynorphin A (Dy).[22] Neurokinin B (TAC3) stimulates while dynorphin A inhibits Kp release. These molecules are autocrine factors that interact synergistically on the surface of KNDy neurons with their own receptors known as neurokinin 3 and κ-opioid receptor (TACR3 and κ-OR), and induce coordinated and pulsatile GnRH secretion by regulating the release of Kp from KDNy cells. Patients with loss of function mutations in TAC3 or TAC3R fail to advance through puberty,[23] however Kp infusion in these patients restores GnRH pulsatility, suggesting that TAC3 is proximal to Kp in the reproductive neuroendocrine cascade regulating GnRH release, and acts as an autocrine modulator of its secretion.[24]

Sex steroid feedback on the hypothalamus and pituitary

The physiology of sex steroid feedback is challenging to decipher because of difficulty obtaining human pituitary and hypothalamic tissue for in vitro study and the multiple components of the pituitary-hypothalamic microenvironment: two sex steroids (T and E_2), at least two steroid hormone receptors (estrogen receptor (ER)α and androgen receptor (AR)), and the enzyme CYP19A1 that converts T into E_2. Studies in GnRH-deficient men treated with GnRH pulsatile therapy (to normalize the HPG axis) and ketoconazole (to achieve medical castration) show that the negative feedback of T at the level of pituitary gonadotrophs depends on its aromatization into E_2.[25] In agreement with this model, LH concentration is unaffected in a transgenic mouse line lacking the AR in the pituitary gland,[26] while it is elevated in E_2-depleted men treated with aromatase inhibitors[27] or affected by aromatase deficiency,[28] and in an estrogen-insensitive man affected by an inactivating mutation of ERα.[29] Pituitary feedback control of FSH secretion is mostly regulated by the glycoprotein inhibin B and by E_2, while T is active only after undergoing aromatization into E_2,[30] suggesting that at the pituitary level both gonadotropins are predominantly under the control of E_2. The situation is different in the hypothalamus, where KNDy neurons express both ERα and AR,[31 32] and both E_2 and T are able to exert negative feedback on Kp release by regulating neurokinin D and dynorphin A. Decreased availability of Kp is in turn followed by inhibition of GnRH production/release.[33]

Taken together, these data show that sex steroids-dependent control of the HPG axis takes place at two levels, the hypothalamus, where both E_2 and T are active through their receptors and modulate GnRH secretion through Kp, and the pituitary gland, where negative feedback on gonadotropin release is exerted principally by E_2, with T playing a secondary role.[25]

Testosterone during fetal development and postnatal life

Two generations of Leydig cells[34] have been described in eutherian mammals; a fetal Leydig cell population producing T during gestational life and an adult Leydig cell population responsible for the pubertal surge of T. Fetal Leydig cells differentiate in the testis from mesenchymal-like stem cells within the interstitial spaces at gestational week (GW) 8.[35] Between GW 8 and 12, T, which by this time has reached concentrations close to normal adult level,[36] drives the events associated with fetal virilization together with two other testicular hormones: the anti-Müllerian hormone, which causes regression of the Müllerian ducts, and insulin-like 3 (INSL3), which contributes to testicular descent into the scrotum. T production from fetal Leydig cells during this critical phase of development is dependent

on placental hCG and independent from LH, as demonstrated by the observations that hCG is maximally produced by the placenta between GW 8 and 12,[37 38] normal fetal sexual differentiation occurs in male carriers of inactivating mutations of the LHβ gene[39] or various forms of congenital isolated HH (table 1) but not in carriers of inactivating mutations of the LH receptor gene (LHR).[40] After week 12, fetal T production continues despite a drop in hCG[37] thanks to the gradual increase of pituitary-derived LH and FSH. Between GW 8 and 12, hCG-dependent T is responsible for the virilization of the epididymis, vasa deferentia and seminal vesicles from the Wolffian ducts, while the 5α reduced T metabolite dihydrotestosterone (DHT) is the ligand interacting with AR in the urogenital sinus to give rise to the prostate and prostatic urethra, and in the urogenital tubercle, swelling and folds to give rise to the glans, scrotum and shaft of the penis, respectively.[41] During the third trimester, T secreted under the control of LH is responsible for further phallic development and together with INSL3[42] for the final phases of inguino-scrotal testicular descent. T level remains high until late in the third trimester, when the fetus is exposed to high concentrations of estrogens from the placenta which inhibit serum LH, FSH and T level to prepubertal concentrations.[36] With the decline in estrogen levels after birth, the HPT axis is released from negative feedback suppression and this results in a postnatal surge of gonadotropin that stimulates the Leydig cells to produce T,[43] resulting in the phenomenon known as 'minipuberty,' which occurs during the first 6 months of life and has been associated with additional penile[44] and testicular growth.[45] Patients affected by congenital HH lack T during the third trimester of fetal development and the initial 6 months of postnatal life and as a consequence develop micropenis (defined as stretched penile length <0.75 in) with or without associated cryptorchidism.

Fractions of circulating testosterone

T circulates bound to sex hormone binding globulin (SHBG) and albumin, and in smaller fractions to cortisol binding protein (CBP) and orosomucoid.[46] The presence of binding globulins serves a number of physiological functions, including acting as transport proteins, functioning as T reservoir to minimize minute-to-minute fluctuations in concentration, prolonging T biological half-life and regulating tissue bioavailability.[46] SHBG circulates as a homodimer and each subunit contains one binding site for T. Contrary to what was assumed for many years, the interaction of T with the two homodimers is a complex, dynamic and non-linear process where binding of T to the first binding site causes a conformational change resulting in different binding affinity with the second binding site.[47] SHBG-bound T represents ~44% of circulating T. Due to the high affinity of binding between SHBG and T (association constant $\sim 1\times10^{9}$ L/mol), SHBG-bound T is not bioavailable (ie, does not reach AR in the target cell). Approximately 54% of T is low affinity bound to albumin (association constant of $2–4.1\times10^{4}$ L/mol). Due to this low affinity, albumin-bound T is thought to dissociate in the capillary bed of organs with long transit times and to become biologically active. Unbound T, also known as free T (FT), represents 1%–4% of circulating T. FT is

Table 1 Congenital causes of central hypogonadism (low Testosterone, low or inappropiately normal LH)

Isolated hypogonadotropic hypogonadism (IHH)	
Anosmic form of IHH (Kallmann syndrome [KS])	Mutated Gene
	ANOS1 formerly KAL1 (X-linked recessive)
	SEMA3A (Autosomal Dominant)
	SOX10 (Autosomal Dominant)
	IL17RD (Autosomal Dominant)
	FEZF1 (Autosomal Recessive)
Normosmic form of IHH	Mutated Gene
	KISS1R (Autosomal Recessive)
	KISS1 (Autosomal Recessive)
	GNRHR (Autosomal Recessive)
	GNRH1 (Autosomal Recessive)
	TAC3 and TAC3R (Autosomal Recessive)
Anosmic and Normosmic forms of IHH	Mutated Gene
	PROK2 and PROKR2 (Autosomal Recessive)
	FGF8 (Autosomal Dominant)
	FGFR1 (Autosomal Dominant)
	CHD7 (Autosomal Dominant)
	HS6ST1 (Oligogenic Inheritance together with FGFR1 and NSMF)
	FGF17 (Oligogenic Inheritance together with IL17RD, DUSP6, SPRY4, and FLRT3)
	NSMF (Oligogenic Inheritance together with FGFR1 and HS6ST1)
	DUSP6 (Oligogenic inheritance)
	SPRY4 (Oligogenic inheritance with DUSP6 and FGFR1)
	RFLT3 (Oligogenic inheritance with FGF17, HS6ST1, and FGFR1)
	WDR11(Oligogenic inheritance)
	AXL (Oligogenic inheritance)
Digenic Anosmic forms of IHH	Mutated genes
	Heterozygous mutation FGFR1 and Heterozygous deletion NSMF
Digenic Normosmic forms of IHH	Mutated genes
	Compound heterozygous mutation of GNRHR and heterozygous mutation FGFR1
IHH associated with obesity	Mutated genes
Leptin or leptin receptor mutations	LEP, LEPR
IHH associated with Mental Retardation	Genetic Abnormality
Prader Willi Syndrome (PWS)	Loss of paternal copy of the PWS "critical region" on chromosome 15q11.2-13
IHH associated with ß-subunit mutations	Genetic Abnormality
	ß-subunit of LH
	ß-subunit of FSH
Deficiencies of multiple pituitary hormones	Pituitary gland differentiation genes
	LHX3, LHX4, HESX1, and PROP-1
Acquired causes of secondary hypogonadism	
Suppression of gonadotropins release	Conditions leading to suppression of gonadotropin release
	Hyperprolactinemia
	Exogenous sex steroids
	Opioid induced hypogonadism
	Therapeutic use of GnRH agonists
	Acute critical illness
	Chronic illness (COPD, CHF, cirrhosis, AIDS, ESRD)
	T2DM
	Obesity
	Anorexia nervosa
	Chronic glucocorticoid treatment

Continued

Table 1 Continued

Damage to gonadotrophs	Conditions leading to damage of gonadotrophs
	Pituitary adenoma/cyst/carcinoma
	Infiltrative diseases: Langerhans cell histiocytosis, hemochromatosis, sarcoidosis
	Infectious: meningitis, tubercolosis
	Pituitary apoplexy
	Head trauma, traumatic brain injury
	Idiopathic

the fraction with direct access to the AR in the target cell that results in androgenic effects. The three T fractions (FT+TT+albumin-bound T) are measured together as 'total T' (TT). FT can be measured individually by equilibrium dialysis, or together with albumin-bound T as bioavailable T (BT).

The free hormone hypothesis

Multiple mechanisms have been hypothesized to explain the cellular uptake and downstream signaling of T. The most validated, the 'free hormone hypothesis', states that FT diffuses across the plasma membrane, binds AR and triggers its activation,[48] hence the intracellular concentration of T is related to the serum concentration of FT instead of TT. The free hormone hypothesis was complemented by the concept that, in addition to FT, albumin-bound T is also active in target tissues due to the low affinity of binding of albumin and T.[49] This model was confirmed in studies in which the presence of SHBG inhibited FT diffusion across the plasma membrane,[50–52] and clinically in a rare case of siblings with SHBG deficiency where the male sibling underwent normal puberty and development of secondary sexual characteristics and fertility while having low serum TT and normal FT.[53] Further observations backing the free hormone hypothesis and supporting the notion that FT is the most important fraction of T comes from epidemiological studies where androgen-dependent outcomes are better associated with FT or BT (a surrogate of FT) than TT. Such outcomes include bone mineral density, muscle strength and fat mass.[54] From the European Male Aging Study (EMAS), men with low FT and normal TT concentrations had higher LH levels, reported more sexual and physical symptoms and had lower hemoglobin values and bone mineral density than men with normal FT, regardless of whether the latter had TT that was normal or low.[55]

It is important to remember that several conditions alter SHBG concentrations and are associated with an increased (or decreased) serum level of TT while the biologically active fractions FT and BT remain unchanged (table 2). SHBG and TT increase with aging, hyperthyroidism, chronic liver disease and HIV. Drugs such as estrogens, phenytoin and tamoxifen increase SHBG.[56] Certain SHBG single nucleotide polymorphisms are associated with increased or decreased SHBG level.[57] SHBG and TT decrease in patients affected by obesity, type 2 diabetes mellitus (T2DM), insulin resistance, hypothyroidism, acromegaly, use of glucocorticoid, progestins and androgenic steroids and nephrotic syndrome (table 2).

Table 2 Conditions associated with changes in the concentration of SHBG

Increased	Decreased
Aging	Obesity/diabetes mellitus/metabolic syndrome
Hyperthyroidism	Nephrotic syndrome
Use of estrogens	Hypothyroidism
Chronic liver diseases	Acromegaly
HIV (+) status	Androgen, progestin, or glucocorticoid use
Thiazolidinedione use	Polymorphisms in the SHBG gene
Anticonvulsant use	
Smoking	
Polymorphisms in the SHBG gene	
↓	↓
TT, but not FT increased	TT, but not FT decreased

Production of T

T is produced by the Leydig cells located in the interstitial compartment between the seminiferous tubules. After puberty, the Leydig cells secrete 7 mg/day of T on average.[58] Approximately 5% of the T pool is of adrenal derivation. Studies in patients with PCa reveal that the adrenal glands release approximately 200 µg/day of T regardless of whether the patient has intact testes or is castrated,[59] and an additional 200 µg of T is formed in the periphery from the conversion of adrenal-derived androstenedione.[59] Lipophilic T passively diffuses across the plasma membrane from the general circulation into the target cell. Inside the target cell, T can be converted by the 5α reductase isoenzymes SRD5A1 or 2 into the more active androgen DHT, or, by CYP19, into E_2. Both T and DHT bind with a unique high affinity cytoplasmic AR protein. This interaction is highly specific, and is ensured by the fact that normal concentrations of circulating T are usually 10-fold above the equilibrium binding affinity for AR. When sufficient concentrations of T are not present, activation of AR can still take place in certain target tissues due to the conversion of T into DHT, a super-androgen with 4−10 times higher affinity for AR than T.[60 61] The presence of two ligands and one receptor is a question that has fascinated generations of endocrinologists. T is a weaker androgen than DHT by a factor of 10[62] due to a faster AR dissociation rate[63] and to differences in the way T interacts with the ligand binding pocket of AR. At the time of fetal development, T is responsible for the virilization of the Wolffian structures, while DHT causes the virilization of the anlagen responsible for the formation of the external genitals and prostate. Hence, SRD5A2 deficiency resulting in lack of DHT results at birth in a characteristic phenotype of undervirilized external genitalia and prostate.[41] Whether both T and DHT are required in adulthood is unclear. Pharmacological inhibition of DHT synthesis in men aged 18–50 years for 20 weeks demonstrated that all androgen-dependent functions of postpubertal males, including maintenance of muscle mass and strength, sexual function, erythropoiesis, prostate volume, prostate-specific antigen (PSA) levels and sebum production are interchangeably served by T and DHT.[64] Based on this, one could argue that DHT is needed only during fetal development, when SRD5A provides local amplification of an androgenic signal leading to virilization of local structures, for instance of the urogenital sinus, without inducing systemic hyperandrogenemia and virilization during critical

times of sexual differentiation. Further supporting the concept that T and DHT are interchangeable in adult individuals is the fact that the external genitalia of patients with SRD5A2 deficiency virilize at puberty, concomitant with maximal T production.[65]

Serum T levels with aging

Many investigators have attempted to establish if serum T level decreases as a function of the age-related changes occurring in the male genitourinary system. This association is complex, in part because serum T drops as a consequence of age and of certain comorbidities occurring more frequently in older individuals, in part because old males involved in healthy behavior or self-reporting excellent health maintain a relatively stable serum T level into the eighth decade.[66–68] Serum TT and FT reach maximum concentrations at around 25–30 years of age and then decline steadily by 0.4% and 1.2% per year, respectively, as shown by the cross-sectional EMAS[69] and Massachusetts Male Aging Study (MMAS),[70] while SHBG increases and accounts for the faster age-related decrease of FT compared with TT.[70] Age-dependent decrease in TT and FT is more significant in longitudinal compared with cross-sectional studies because the health of elderly individuals recruited to longitudinal studies is more likely to deteriorate during the years long course of these investigations. As an example, MMAS reported both cross-sectional[70] and longitudinal[71] results. The longitudinal data showed a steeper decline of TT and BT over 7–10 years than was seen in the cross-sectional data. The longitudinal data showed a decrease in TT of 1.6% per year, compared with 0.4% in the cross-sectional study, and a decrease in BT of 2%–3% per year, compared with 1.2% in the cross-sectional study. Another study, reporting centiles of serum TT levels (from the 2.5th to 97.5th percentile) in men aged 19–99 years from four large US/European epidemiological investigations showed that, between the 19–39 and 80–99 groups, serum TT concentrations dropped by 31% in the 2.5th percentile. This difference was only 11% at the 50th percentile, and there was no difference at the 97.5th percentile level. These data imply that the majority of age-related TT reduction occurs at the lowest percentile of TT concentrations.[72]

Age-related changes in the male reproductive system

Despite the maintenance of male fertility throughout adult life, aging is associated with well-established changes of the reproductive system. Testicular volume decreases from 16.5 cm^3 between age 20 and 30 years to approximately 14 cm^3 between age 80 and 90 years.[73] Another study reported a mean testicular size among young and older men of 29.7 vs 26.6 cm^3, respectively.[74] T is produced by the Leydig cells located in the interstitial compartment between the seminiferous tubules. In younger men there are 432 million Leydig cells, comprising 5% of testicular volume, while in older men the number of Leydig cells decreases by 44%.[75] The morphology of Leydig cells remains mostly normal during aging, but some undergo dedifferentiation and involution after acquisition of cytoplasmic or intranuclear inclusions. This involution is thought to be the consequence of decreased vascular supply deriving from atherosclerosis of the testicular arteries.[76] Aging has a number of well-known effects also on semen parameters; a study comparing men in the fourth versus sixth decade reported a 3%–22% decline in semen volume, 3%–37% in sperm motility and a 4%–18% in normal morphology.[77] Another investigation revealed that every 5 years semen volume decreases by 0.22 mL and sperm motility by 1.2%.[78] Some studies have reported an increase in sperm concentration[68 78] because semen volume decreases more than sperm count. Changes in morphology of the aging seminiferous tubule include narrowing, thickening of the basal membrane associated with fibrosis, reduction in the number of spermatogenetic and Sertoli cells.[79] Sertoli cells decrease from 500×10^6 at age 20–48 years to 300×10^6 at age 50–85 years.[80]

Serum T and chronic diseases

In addition to age, serum T is independently reduced by conditions such as diabetes,[81] obesity,[69] polypharmacy,[82] cardiac,[83] hepatic[84] or renal failure,[85] chronic obstructive lung disease,[86] rheumatological conditions,[87 88] cancer,[89] HIV positivity,[90] myocardial infarction (MI),[91] burns,[92] inflammatory bowel disease,[93] sepsis[94] and intensive care unit admission.[95] Medications more frequently prescribed to chronically ill patients such as opioids and glucocorticoids also contribute to low T level by interfering with the GnRH-LH axis.[96 97] In this context, T production is an indication of a man's health,[9] and therefore should not be measured in hospitalized or acutely sick patients.[98] Obesity is a frequent occurrence in men with LOH, and is the chronic condition associated with the most significant decline in serum T level. EMAS revealed that serum T level is 30% lower in obese versus normal weight men at any age, which is more than the whole age-dependent decrease occurring between 40 and 80 years of age,[99] and that obesity is associated with a 13-fold increased risk of LOH, whereas the presence of two or more other chronic comorbidities increased LOH risk by 5.2-fold. From a clinical point of view, identification of hypogonadism in men with chronic diseases may be difficult because they present symptoms and signs resulting from the underlying (non-reproductive) disorder that overlap with those of androgen deficiency. Interestingly, the form of LOH found in patients with obesity and chronic illnesses is not associated with an increase in gonadotropins.[14] An important observation with therapeutic implications is that serum T increases in individuals with obesity who lose weight, proportionally to the amount of weight loss,[100] indicating that the suppression of the HPG axis present in these individuals is functional and reversible.

The role of E_2 on serum T in LOH

Adipose tissue-expressed aromatase could play a role in the physiopathology of LOH in patients with obesity. An old theory states that as obesity is associated with an expansion of the adipose tissue, there is a concomitant increase in aromatase expression/activity in the pre-adipocytes resulting in amplification of the local aromatization of T to E_2. The increased serum E_2, in turn, is responsible for gonadotropin suppression and decreased serum T. This hypothesis was supported in early papers published in the 1980s.[101] However, more recent studies measuring serum

hormones by liquid chromatography-tandem mass spectrometry (LC/MS/MS) reveals a parallel decrease of E_2 and FT in hypogonadal men with[102 103] or without[104] diabetes, while other investigations have demonstrated that hypogonadal men express less aromatase mRNA in adipose tissue concomitant with decreased serum E_2 concentrations.[105]

CONNECTIONS BETWEEN THE REPRODUCTIVE SYSTEM AND OBESITY, DIABETES AND INFLAMMATORY STRESS

Obesity, diabetes (ie, the diabesity syndrome) and all conditions associated with insulin resistance and inflammation cause a decrease in SHBG and thus TT. Hence, the most useful test for the correct biochemical diagnosis of hypogonadism in these patients is FT, which is low in up to 40% of obese men,[81] 25%–40% of those affected by T2DM[106 107] and 50% of those affected by the two conditions simultaneously.[81] Low FT is also common in T2DM men between the ages of 18 and 35 years[108] and obese teenagers between 14 and 20 years.[109] In these patients, serum FT is indirectly correlated with markers of unfavorable metabolic health, such as increased inflammatory cytokines, HOMA-IR, triglycerides and obesity,[109 110] and LH and FSH are inappropriately low/normal revealing that this is a form of central HH. The physiopathology underlying HH in diabesity and chronic conditions is not related to elevated blood sugar, as FT levels are typically normal in patients with type 1 diabetes mellitus,[111] but rather to obesity and insulin resistance. Serum T increases when insulin resistance is reduced by diet-induced weight loss, bariatric surgery or pharmacological treatment with rosiglitazone.[100 112 113] The anatomical locus of the insulin resistance-induced impairment in patients with diabesity and LOH remained unidentified until five patients affected by this syndrome were treated with Kp, the proximal regulator of GnRH release.[114] These patients experienced an increase in LH and T as well as LH pulse frequency, confirming that the HPG axis is functionally intact in the diabesity syndrome, and that its reduced activity was caused by perturbed afferent inputs acting at or proximal to the Kp-producing neurons. This was confirmed by the observation that, in a diabetic rat model, low Kp mRNA expression was associated with low levels of gonadotropins and sex steroids, and administration of Kp corrects this defect.[115] Beyond obesity, inflammatory stress, present in all chronic conditions listed in the 'Serum T and chronic diseases' section, is associated with biochemical hypoandrogenemia and LOH. One of the hallmarks of inflammation is an increase in serum cytokines, and both inflammation[116] and cytokines[117] are known to induce insulin resistance and decrease serum T levels in human and animal models. Hence, insulin resistance could be the unifying mechanism responsible for LOH in males affected by the diabesity syndrome, inflammatory stress and various chronic conditions. In synthesis, the fine mechanisms causing LOH in obesity, diabetes and chronic diseases are not fully characterized, however endocrine, metabolic and inflammatory signals generated in the periphery of the body play an important role. In an attempt to understand the mechanisms involved, in the next sections we discuss how GnRH secretion is controlled by a variety of molecules regulating homeostasis, inflammation and metabolism.

Puberty and energy metabolism

The beginning of puberty and reproductive functions are very sensitive to insufficient or excessive body energy reserves, and sophisticated mechanisms have evolved to cause their inhibition when energetic conditions are unfavorable. Both calorically restricted states such as undernutrition, cachexia or strenuous exercise as well as states of excess body energy storage such as obesity and metabolic syndrome result in perturbations of puberty and fertility.[118–120] Based on the experiment showing reactivation of the HPG axis with Kp in patients affected by the diabesity syndrome,[114] it is reasonable to consider the hypothesis that the anatomical site that integrates peripheral signals of energy homeostasis and inflammation and modulates GnRH secretion is represented by Kp neurons, through the release of Kp. This possibility is supported by the fact that these neurons express receptors for many molecules important to energy homeostasis such as ghrelin,[121] leptin[122] and adiponectin,[122] inflammatory cytokines such as tumor necrosis factor-α (TNF-α)[123] and sex steroids such as E_2 and T.[31 32] The mechanism whereby these molecules affect Kp expression/release has not been formally elucidated but could very well be through the AMP-activated protein kinase AMPK. This protein is the master regulator of energy balance in both the peripheral organs as well as the central nervous system and the hypothalamus. Its activation occurs under conditions of perceived energy deficit, for instance, during fasting, while under conditions of feeding AMPK is inactive. Using a model of female puberty, Roa *et al* uncovered a pathway where conditions of energy deficit are associated with delayed puberty through the induction of hypothalamic AMPK, which in turn causes reduction of Kp expression in hypothalamic Kp neurons.[124] Because metabolic cues signaling energy deficit, for instance, the orexigenic hormone ghrelin, activate AMPK,[125] while refeeding or anorectic factors (for instance the anorexigenic hormone leptin) are associated with AMPK inactivation,[126] it is plausible to hypothesize that these hormones affect reproductive functions by directing changes in AMPK activation status, which in turn could modulate Kp release. In the next sections, we summarize available data on the impact of peripheral metabolic, endocrine and inflammatory cues on hypothalamic Kp neurons and on the HPG axis as a whole.

Leptin

Leptin is coded by the *lep* gene, which is expressed in adipocytes. Leptin acts via its receptor LepR, and signals the presence of body energy reserves to the brain, is involved in the regulation of appetite, in increasing energy expenditure and in the induction of puberty. Individuals with leptin deficiency due to energy deficit or inactivation of the leptin gene are affected by a form of HH that reverses on weight gain,[127] which increases serum leptin, or leptin administration,[128] respectively. Despite the fact that obesity is associated with increased serum leptin concentration, leptin is not anorexigenic in these subjects as they are affected by a status of leptin resistance. Leptin is known to stimulate the HPG axis by inducing expression of hypothalamic GnRH and LH pulsatility,[129] however this effect is not direct because LepR is not present in GnRH neurons.[129] One hypothesis

is that leptin stimulates GnRH through the mediation of Kp, as LepR is present in 40% of Kp neurons in the mouse arcuate nucleus,[130] and this process occurs after inactivation of AMPK.[131] This hypothesis is substantiated by the observation that intracerebroventricular leptin resumed Kp level in a model of hypogonadotropic ewe.[132] However, deletion of LepR specifically in Kp neurons had no effect on puberty or fertility in mice[133] and re-expression of leptin receptor in Kp neurons did not alleviate lack of pubertal development or infertility.[134] Hence, current knowledge does not support that the target of leptin associated with the initiation of puberty and reproductive functions is Kp, however, given that a direct loop connecting leptin and Kp has been described, it is still possible that leptin resistance may play a role in the physiopathology of low T present in individuals affected by obesity and chronic diseases.

Ghrelin

The orexigenic hormone ghrelin, which operates as a signal of energy insufficiency and is a functional antagonist of leptin, is produced in the stomach, increases during periods of fasting or negative energy balance such as starvation or anorexia and is decreased after eating, in obese individuals or in the presence of hyperglycemia. It interacts with the growth hormone secretagogue receptor expressed in various hypothalamic nuclei and in Kp-expressing neurons,[121] and influences food intake and reproductive functions, including the beginning of puberty.[135 136] Increased serum ghrelin occurring during fasting or after exogenous administration causes a reduction in the expression of Kp mRNA,[121] thereby inhibiting the HPG axis. In keeping with its proposed role as a signal for energy deficit, most of the reported actions of ghrelin on the reproductive axis are inhibitory, suggesting that it may mediate at least part of the suppressive effects of low body fuel stores and energy insufficiency on puberty and fertility. The mechanism used by ghrelin to reduce Kp expression may involve activation of AMPK in hypothalamic neurons.

Insulin

Because insulin (I) is a metabolic regulator of reproduction acting on both central and peripheral sections of the reproductive axis[137] and central and peripheral insulin resistance are essential features of diabesity and inflammation,[138 139] reproductive abnormalities observed in subjects affected by diabesity may result from insulin resistance-mediated abnormalities of the Insulin (I) insulin receptor (IR) (I-IR) axis. Indeed, mice with a neuron-specific disruption of the IR are a model of the diabesity syndrome because they are obese, have central insulin resistance and hypothalamic dysregulation of LH production and are infertile.[140] Identifying the population of neurons involved in the reproductive effects of insulin on GnRH secretion has not been easy. As an example, mice with IR deletion in Kp neurons have delayed onset of puberty without defects in adult reproductive capacity,[141] indicating that I-IR signaling in these neurons is important only for pubertal awakening of the HPG axis, but is not a critical factor for the achievement of other reproductive end points. Recent evidence suggests that astrocytic IR may mediate insulin signaling and reproductive abnormalities, as demonstrated by astrocytic IR ablation leading to delayed puberty and HH[142] and to decreased expression of astrocytic prostaglandin E synthase 2 (PGES2), the enzyme responsible for the conversion of prostaglandin H2 to prostaglandin E2 (PGE2). Because astrocytes deficient in PGE2 are unable to induce GnRH release,[143] PGE2 could very well be the candidate astrocytic neurotransmitter linking insulin resistance to HH.

Adiponectin

Adiponectin is produced by fat cells and released in large amounts in the general circulation. It regulates cellular homeostasis by inducing fatty acid oxidation, insulin sensitivity and energy expenditure. Conditions such as obesity and diabetes are associated with decreased serum adiponectin levels[144] while fasting or weight reduction are associated with increased serum adiponectin.[145] Due to its demonstrated ability, together with leptin, to reverse insulin resistance,[146] adiponectin may play a role in reproduction, as demonstrated by its ability to decrease LH and GnRH release[147] and by the presence of its receptor in hypothalamic GnRH neurons.[148] Although the precise role of adiponectin in linking energy metabolism and reproduction is not clearly understood, one possibility is that it is responsible for the suppression of the HPG axis during fasting.[122]

Inflammatory cytokines

It is logical to question whether inflammatory stress and in particular the increased concentration of serum cytokines associated with diabetes, obesity, metabolic syndrome and various chronic conditions plays a role in LOH. The importance of inflammation and inflammatory cytokines affecting the HPG axis is supported by a variety of experimental models,[149–151] the inverse relationship existing between markers of inflammation and serum LH and T levels,[152 153] studies showing that infusion of interleukin (IL)-2 in eugonadal men attenuates the feedforward effect of LH on T secretion,[154] and the observation that hypoandrogenic states resolve as inflammation improves.[154] The effect of cytokines on reproductive function may take place directly at the level of GnRH neurons, as they express various cytokine receptors[155] and injection of IL-6b and TNF-α or creation of a chronic inflammatory state by the induction of mild obesity results in impairment of GnRH mRNA expression and LH levels in various animal models.[156–158] In agreement with this is the observation that one of the most repressed genes in obesity, as established by genome-wide analysis, is GnRH.[159]

MALE HYPOGONADISM

There are two forms of hypogonadism, PH and LOH. The clinical presentation of hypogonadism is distinctive, and the correct diagnosis of its extreme forms was possible even before serum T measurement became available, for instance, in the introductory case report.[1] However, based on the 2010 and 2018 ES guidelines, there is general agreement that the diagnosis of PH or LOH should be based on a syndromic approach, that is, presence of typical clinical manifestations and biochemical tests showing unequivocally low T. When the syndromic approach is strictly applied, epidemiological studies have measured the prevalence of hypogonadism at 2%,[104] 5.6%[160] and 6%.[161] In contrast,

Table 3 Causes of primary hypogonadism in males (low testosterone, high LH)

Congenital	a. Klinefelter's syndrome and variants (47,XXY, 48XXXY, 49XXXXY, 46,XY and 47,XXY mosaicism) b. 46,XY/XO mosaicism c. Inactivating mutation of steroidogenic enzymes (STAR Protein, CYP11A1, HSD3B2, CYP17, HSD17B3, SRD5A2) d. LH receptor mutations e. Uncorrected Cryptorchidism f. Myotonic Dystrophy g. Bilateral Congenital Anorchia h. Testicular Adrenal Rest Tumors
Acquired	a. Orchitis (infection, radiation)- b. Testicular trauma/torsion c. Polyglandular Autoimmune disease d. Chemotherapy (suramin, alkylating agents) e. Drugs (Ketoconazole, Spironolactone, Abiraterone, Alcohol) f. Cryptorchidism g. Radiation h. Bilateral Orchiectomy i. Systemic Disorders (Cancer, Lymphoma, Amyloidosis)

when the diagnosis is (mistakenly) made using only the clinical or biochemical approach, the prevalence is as high as 24% and 29%,[160] respectively. For didactic purposes, the clinical presentation, diagnosis and treatment modalities of PH and LOH are discussed individually.

PATHOLOGICAL HYPOGONADISM

PH derives from testicular inability to produce physiological amount of T due to organic diseases of the HPG axis. Reduced circulating T that is present in patients affected by PH is associated with a constellation of symptoms and a spectrum of phenotypic abnormalities combined with decreased sperm production.

PH can be further classified according to the respective level of failure of the HPT axis. Primary hypogonadism results from a deficiency at the level of the testicles resulting in low T and consequent elevated gonadotropins (FSH and LH); central hypogonadism results from deficiency at the level of the pituitary gland (secondary) or hypothalamus (tertiary) resulting in low T and low or inappropriately normal gonadotropins. We present lists of known causes of primary and secondary PH in table 3 (causes of primary hypogonadism in males) and 1 (congenital and acquired causes of secondary hypogonadism). As a thorough review of all these different entities is beyond the scope of this paper, we will limit our discussion to the most frequent and refer the reader to the many excellent monographs available in the literature on each of these conditions

Presentation of primary hypogonadism

The clinical presentation of PH varies based on the time in which T deficiency develops (eg, prenatal, prepubertal or postpubertal) and whether the patient is affected by primary or central hypogonadism (table 4).

Prenatal hypogonadism

Lack of T occurring during the initial 3 months of fetal development in 46XY fetuses is associated with genital ambiguity proportional to the degree of T deficiency. The genital organs present within a spectrum of complete (complete lack of external male genitals, presence of gonadal dysgenesis), intermediate (posterior labial fusion, clitoromegaly, labial fusion, micropenis, perineoscrotal hypospadias, microphallus, or scrotal abnormalities such us bifid scrotum or cryptorchidism) or mild (micropenis, ectopic urethral meatus) undermasculinization. In many cases, ambiguous genitalia are the results of genetic abnormalities causing inactivation of gene responsible for T biosynthesis (table 3). Mutations of AR are associated with the hypogonadal phenotypes described in the context of the androgen insensitivity syndromes, but technically these patients produce normal quantities of T, hence are not affected by hypogonadism. T deficiency occurring during the third trimester of gestation or the first 6 months of postnatal life is usually due to congenital forms of HH (table 1). These subjects undergo normal fetal virilization as this phenomenon takes place during the first trimester of gestation under the control of placental hCG, but are affected by various developmental abnormalities such as micropenis, testicular hypotrophy (volume <2–4 mL) and/or cryptorchidism.

Prepubertal hypogonadism

For cases of hypogonadism occurring before puberty (table 4), the most revealing clinical signs are eunuchoid proportions with decreased peak bone mass, infantile genitalia, high pitched voice, increased subcutaneous fat, deficient muscle development, gynecomastia, lack of temporal recession of the hairline and lack of steroid dependent hair growth. Eunuchoid proportions, defined as lower body segment (floor to pubis) >2 cm longer than upper body segment (pubis to crown) and an arm span that is >5 cm longer than the vertical length, are due to delay in epiphysial closure from T and, more importantly, E_2 deficiency. These patients experience lack of libido, reduced spontaneous erections and are unable to ejaculate.

Postpubertal hypogonadism

If T deficiency occurs after puberty, the clinical presentation depends on the degree and duration of hypotestosteronemia (table 4). A classic scenario of abrupt lowering of T occurs in patients undergoing androgen deprivation therapy for PCa. Beside developing an immediate reduction of sexual function, these subjects also complain of low energy, hot flashes, insomnia, depression, changes in body composition with decreased lean and increased fat mass and decreased bone mineral mass associated with increased

Table 4 Signs and Symptoms of Hypogonadism: prenatal vs. pre-pubertal vs. post-pubertal, primary vs. secondary

	1st Trimester Gestational Life	3rd Trimester gestational life, mini-puberty	Prepuberty		Postpuberty	
			Primary Hypogonadism	Secondary Hypogonadism	Primary Hypogonadism	Secondary Hypogonadism
Ambiguous genitalia	+					
Micropenis, Small testes, cryptorchidism		+				
Small Phallus			+	+	–	–
Small Testes			++	++	+	+/-
High Pitch Voice			+	+	–	–
Eunuchoid Proportion			+	+	–	–
Recession Scalp Hairline			–	–	+	+
Chest/Facial Hair			–	–	+/-	+/-
Gynecomastia			+	+/-	+	+/-
Low Muscle Mass			+	+	+/-	+/-
Low Libido			+	+	+	+
Low Energy			+	+	+	+

risk of fracture. The majority of patients of this age group do not experience such a sudden and drastic decrease in serum T, hence they develop a gradual decrease in energy, libido and erectile function. Primary and secondary sexual characteristics do not regress to prepubertal level; hence, phallus, sexual hair, muscle mass, and bone mineral density do not diminish significantly for several months to years. Also, testicular size does not change significantly unless the patient is affected by primary hypogonadism. However, as T and its metabolite E_2 are essential for the acquisition and maintenance of bone mass, osteoporosis eventually develops and with it the risk of fragility fractures increases table 4. Another clinical sign of long-term hypogonadism is the appearance of fine facial wrinkling lateral to the mouth and eyes secondary to lack of sebum production. Gynecomastia is more frequent in patients with primary hypogonadism because high gonadotropins increase the expression of testicular aromatase. Other less specific symptoms include low motivation, depression and poor concentration. As T induces erythrocytosis via increased erythropoietin and suppressed hepcidin, men with androgen deficiency may have a mild hypoproliferative normocytic, normochromic anemia.

Clinical manifestations reported by hypogonadal men such as sarcopenia, low energy, depressed mood, fragility fractures and low libido overlap with those seen in elderly, obese or chronically ill patients, however, true hypogonadism is associated with distinctive signs and symptoms of androgen deficiency in the presence of low circulating T. A synthesis of the clinical features of hypogonadism presented according to age of appearance is available in table 4.

PH and fertility

The two physiological functions of the testes, T production and spermatogenesis, are intertwined, and in patients affected by primary hypogonadism, low T usually parallels low sperm count. This is not a certainty, however, as there are cases of infertility associated with normal Leydig cell function. Successful treatment of infertility may require procedures such as intracytoplasmic sperm injection in the context of in vitro fertilization (IVF). The degree of spermatogenesis impairment in men with secondary hypogonadism depends on the degree of T deficiency. In these patients, spermatogenesis can be achieved with medical therapies that are based on hCG, hCG+human menopausal gonadotropin (hMG) or GnRH (if the hypogonadism is of hypothalamic origin), possibly followed by IVF.

Klinefelter syndrome

Among forms of congenital primary hypogonadism associated with chromosomal abnormalities (table 3), Klinefelter syndrome (KS) is the most frequent and occurs prenatally and neonatally in 1 every 500–700 males, while its prevalence among adults is 1 every 2500. KS is not associated with increased risk of mortality, hence the lower prevalence among adults implies that this condition is often ignored or underdiagnosed.[162 163] KS is due to the presence of one or more extra X chromosomes due to maternal or paternal meiotic non-disjunction. Ninety per cent of men with KS have a 47,XXY karyotype. Of the remaining 10%, some are affected by mosaic KS (47,XXY/46,XY), and a minority

have more than one extra X chromosome (eg, 48,XXXY, 49,XXXXY). The phenotype of men affected by mosaicism is usually milder and spermatogenesis is normal if the testicular karyotype is 46,XY, while the presence of extra X chromosomes in 48,XXXY and 49,XXXY individuals is associated with more severe clinical presentations. Only a minority of boys, usually with the most severely affected phenotype, are correctly diagnosed before puberty. The abnormal phenotype is more easily recognized at or after puberty, as the testes remain small, usually ≤4 mL,[164] and undergo a progressive process of hyalinization/fibrosis, leading to the principal physical abnormality: presence of small, firm testes and infertility. As the process initially affects the seminiferous compartment and Sertoli cells, elevated FSH and undetectable inhibin B are early detectable hormonal abnormalities. T level increases at puberty, but less than in normal individuals, and serum T decreases over time to a clearly hypogonadal range. In mature KS individuals, serum T levels vary considerably and the degree of eunuchoidism, gynecomastia and penile size varies widely among patients. FSH is disproportionately elevated compared with LH, and both LH and FSH levels increase as a function of failing testicular function. In addition to phenotypic abnormalities of the reproductive system, KS causes a 20-fold increased risk of breast cancer compared with male controls,[165] and of hypogonadism-unrelated conditions such as increased risk for varicose veins, deep vein thrombosis, pulmonary embolism, systemic lupus erythematosus, rheumatoid arthritis, Sjögren's syndrome, non-Hodgkin's lymphoma, T2DM and psychiatric/neurocognitive conditions such as schizophrenia, depression, attention deficit disorder, language impairments and social dysfunction. The diagnosis is initially suspected on clinical grounds, and confirmed by the typical hormonal profile and karyotype.

Central hypogonadism

Central hypogonadism occurs in congenital and acquired conditions. Among congenital forms, isolated hypogonadotropic hypogonadism (IHH) is the consequence of inactivating mutations of genes controlling the GnRH pathway (normosmic forms of IHH, table 1). Mutations of this pathway have been described for KISS1R, KISS1, GNRHR, GNRH1, TAC3 and TAC3R. Mutations in genes responsible for the parallel migration of olfactory and GnRH-secreting neurons during embryogenesis have been described for ANOS1 (previously known as KAL1), SEMA3A, SOX10, IL17RD and FEZF1, and are typically associated with anosmia and IHH, also known as Kallmann syndrome. Other rarer forms of IHH can present with different degrees of anosmia or normosmia, and have autosomal dominant, autosomal recessive, oligogenic or digenic modes of inheritance (table 1). An important form of IHH associated with obesity and leptin deficiency led to the understanding of the critical role played by the leptin signaling pathway in the induction of puberty. Acquired forms of central hypogonadism are more frequent and are due to damage of the gonadotrophs occurring as a consequence of radiation therapy, compression (from pituitary masses, craniopharyngioma, meningioma or metastatic lesions), infiltration (hemochromatosis, sarcoidosis, Langerhans cell histiocytosis, hypophysitis), infectious disorders (tuberculosis, syphilis, fungal infections), infarction, trauma or apoplexy of the pituitary gland. Functional suppression of the HPT axis can occur with hyperprolactinemia, chronic use of exogenous androgenic steroids, glucocorticoids or opioids. Patients affected by chronic ailments and organ failures such as chronic obstructive pulmonary disease, chronic kidney disease, end-stage renal disease, end-stage liver disease and congestive heart failure are also frequently affected by central hypogonadism.

LATE-ONSET HYPOGONADISM

LOH is defined as a 'clinical and biochemical syndrome associated with advancing age and characterized by symptoms and a deficiency in serum T levels (below the young healthy adult male reference range)'.[13] Widespread interest for LOH derives from the fact that the US population is aging and there is a parallel between certain manifestations of aging and hypogonadism, such as changes in body composition (decreased lean body mass and increase fat mass), abnormal sexual function, anemia, decreased physical function and bone mineral density and increased fracture risk. The widespread interest for LOH among physicians and the public derives from the fact that Tt is known to improve body composition, sexual function, bone mineral density and hemoglobin in men affected by PH, hence it is reasonable to consider that this treatment may also benefit patients with LOH as long as these organ systems maintain T-responsiveness during older age.

Whether LOH should be treated with Tt is controversial, as reflected by divergent recommendations by professional societies. We agree with the 2018 US ES guidelines, where clinicians are recommended to offer Tt on an individualized basis after explicit discussion of the potential risks and benefits *'in men of 65 years or more who have symptoms or conditions suggestive of testosterone deficiency (such as low libido or unexplained anemia) and consistently and unequivocally low morning testosterone concentrations*)'.[10] A position statement by the ES of Australia does not recommend use of Tt for these patients based on the limited evidence showing an objective benefit.[9] In contrast, the Canadian Men Health's foundation has a more liberal approach and recommends a therapeutic trial of T supplementation (of perhaps 3 months) in the presence of a convincing clinical picture despite uncertain laboratory results.[166]

LOH is a spectrum of three hypogonadal conditions

A careful analysis of the relationship between TT, FT and LH in EMAS revealed that the majority of this cohort was eugonadal (76.7%) while 23.3% was affected by three different forms of LOH; 2% had primary hypogonadism (low T and high LH), 9.5% compensated hypogonadism (increased LH with normal T) and 11.8% secondary hypogonadism (low T and low or inappropriately normal or low LH).[104] Primary and compensated hypogonadism appeared to be a spectrum of the same entity. Adult-onset primary hypogonadism is attributed by EMAS to age-associated Leydig cells exhaustion, probably related to the age-related changes of the male urogenital system described in the 'Age-related changes in the male reproductive system' section. Compensated primary hypogonadism may represent an

earlier stage in which Leydig cells respond to elevated LH by producing physiological concentrations of T, and are believed to eventually develop full blown primary hypogonadism. Until additional longitudinal data are acquired, it will not be possible to understand with certainty what causes the transition from compensated to primary hypogonadism, and in particular if acquisition of additional chronic comorbidities plays a role. The EMAS groups with primary and compensated hypogonadism show the strongest direct association with age. Patients with primary hypogonadism show the most severely depleted level of serum T.[104] Patients affected by primary hypogonadism and secondary hypogonadism were more likely to report sexual symptoms while compensated hypogonadism was associated with physical symptoms; all three conditions correlated with the presence of chronic disease, although this association was stronger among patients with primary hypogonadism. Due to its unique biochemistry and clinical presentation consisting exclusively in physical symptoms, the authors of EMAS categorize compensated hypogonadism as an independent clinical entity. Among EMAS men with secondary hypogonadism, the single most powerful predictor of low T was obesity with a relative risk ratio (RRR) of 8.74 followed by the presence of comorbidities, while age did not play a role. As stated, these men endorsed the presence of sexual but not physical symptoms.

Symptoms of LOH

The most precise investigation of the connection between T levels and symptoms in middle age or elderly men was carried out by EMAS.[69] EMAS randomly sampled 3369 men, from 40 to 79 years of age, from eight European sites. The men underwent physical and cognitive tests, completed health questionnaires and had biochemical and hormonal assessments. One morning blood sample was used to measure TT by GC-MS and SHBG by immunoassay (IA) and FT was calculated with the equation by Vermeulen *et al*.[167] The symptoms selected from the questionnaires were dichotomized to define symptomatic and asymptomatic men. Of the 32 symptoms that were assessed, for 9 symptoms the free and/or total T was significantly different between the symptomatic and asymptomatic groups: 3 sexual symptoms, 3 physical symptoms and 3 psychological symptoms. These nine symptoms were further assessed to see if there was a definable threshold of free or total T below which the probability of the symptom increased above the background prevalence. All three assessed sexual symptoms had thresholds. Frequency of morning erections decreased as the measured TT fell below 317 ng/dL or FT 80.7 pg/mL. Erectile dysfunction increased below a TT of 244 ng/dL or FT 80.7 pg/mL. Frequency of sexual thoughts decreased below a TT of 231 ng/dL or FT of 46.1 pg/mL. About a third of the subjects had sexual symptoms. Of the variables assessing physical symptoms, a threshold was only found for decreased vigorous activity, with symptomatic men being defined as those who answered that they were limited (as opposed to limited, a little or not at all) in running, lifting heavy objects or participating in strenuous sport. The odds for this answer increased with a TT level below 375 ng/dL, but did not have any threshold with free T. Of the psychological symptoms, fatigue and sadness both had FT thresholds (below 46 pg/mL) but no total T threshold.

The group further analyzed whether the symptoms clustered with certain T parameters. The sexual symptoms clustered with low T and low FT, with the absence of symptoms clustering with normal T. This clustering suggests that the occurrence of low T and sexual symptoms define a distinct syndrome. No clustering was seen for the physical or psychological symptoms that were assessed.[69]

A study in the Chinese Han population assessed 1000 men between the ages of 40 and 79 years, of which 936 had measured morning TT by immunoassay (IA) and 766 additionally had measured SHBG and calculated FT. Responses to the Aging Males' Symptoms (AMS) questionnaire[167] were dichotomized into symptomatic (moderate, severe or extremely severe) or asymptomatic (none or mild). Of the 17 questions in the areas of physical, psychological and sexual symptoms, only the response to sexual symptoms had significant differences in T between symptomatic and asymptomatic subjects. More than a third of the subjects had sexual symptoms. Logistic regression identified a TT of 380 ng/dL or calculated FT of 77.5 pg/mL as the threshold below which all three sexual symptoms increased above baseline.[168]

These studies illustrate several important points: 1) low T is associated most specifically with an increase in sexual symptoms, and with physical and psychological symptoms and 2) sexual, physical and psychological symptoms are non-specific, may have a high background prevalence and do not, in isolation, identify the population with low T. Indeed, questionnaires that have been developed to assess symptoms of hypogonadism and aging have proven to have low specificity in the identification of LOH.[169 170] As a result, ES has recommended that symptoms alone are insufficient to make the diagnosis of hypogonadism, and laboratory assessment is also required.

LABORATORY DIAGNOSIS OF HYPOGONADISM (PH AND LOH)

Screening for hypogonadism

The ES does not recommend screening for hypogonadism in the general population, however, because available screening tools (eg measurement of TT or administration of questionnaires developed for androgen deficiency states) are substandard, there is low quality evidence in support of this statement. Epidemiological studies have demonstrated that in the general population the prevalence of low TT alone can be as high as 23.3%[104] or 24%,[160] of hypogonadal symptoms alone 16%[104] or 29%,[160] while in the same studies the percent of individuals with both biochemical and symptomatic hypogonadism was dramatically lower, at 2.1%[104] and 5.6%.[160] This suggests that screening protocols should not be based only on biochemistry or questionnaires, but rather on both, and, as recommended by ES, the diagnosis of hypogonadism should be based on the presence of unequivocal low T level and presence of classic symptoms.[10] ES does not advocate screening strategies also because it is not cost-effective and it is unclear whether prescribing Tt is beneficial to individuals who do not seek medical attention for this problem.[10] Yet, this statement is controversial, because some studies have identified a relationship between

Box 1 Conditions associated with high prevalence of hypogonadism

- Diseases of the sella turcica (mass lesions, surgery, radiation)
- Medication affecting testosterone production (high dose glucocorticoids, opioids, androgen ablation therapy for prostate cancer)
- HIV-associated weight loss
- End-stage renal disease and hemodialysis
- Moderate to severe COPD
- Infertility
- Osteoporosis
- Type 2 diabetes mellitus
- Low libido or erectile dysfunction
- Obesity

low T and increased all-cause mortality and death from CV causes,[171–173] but evidence showing that these outcomes are prevented by Tt is of low quality and is based on small[174] or retrospective[175] studies.

ES recommends that patients affected by certain clinical conditions where the prevalence of hypogonadism is high should be screened for hypogonadism by measuring TT, or FT if necessary. The conditions are listed in box 1.

Biochemistry of hypogonadism: measurement of serum T

Measurement of serum T presents several challenges due to hourly and daily variations in its concentration, inadequacy of measurement technology, variability related to age, health status, use of certain medications, body weight and SHBG concentrations. In young men, T is secreted according to a circadian pattern with a morning peak and should be measured at around 08:00 hours. Despite the fact that older men experience a blunting of the circadian rhythm,[176] there is evidence that T should be measured in the morning in this patient population.[177] Diagnosis of hypogonadism should not be entrusted to a single measurement due to day-to-day variations in serum T concentrations, and the observation that 30% of men with an initial T in the hypogonadal range have a normal level on repeat.[177] It is also important to be aware of other vagaries related to T measurement, for instance, interference may occur for the presence of other metabolites of steroid hormones with similar structure present in the bloodstream.

Testosterone assay methodology

Historically, IA platforms have been the technology of choice for the measurement of serum TT. IA produces reliable results for normal or elevated levels of TT but lacks sensitivity and specificity at lower concentrations, hence use of IA in women, children and hypogonadal men remains problematic.[178–180] LC/MS/MS is the benchmark technology for the measurement of T recommended by many experts.[10] Compared with IA, LC/MS/MS is impractical because it requires additional steps of extraction, is expensive, technically challenging and not widely available to hospital-based diagnostic labs. The Clinical Reference Laboratory of the Center for Disease Control (CDC) has made available a program to develop a harmonized TT reference range by measuring TT according to CDC gold standard procedures in 100 serum samples obtained from four epidemiological clinical trials.[69 181–183] The results were then compared with the original values obtained in the laboratories where the studies took place to develop normalizing equations, which were then applied to the entire cohort of patients of the four studies to establish harmonized reference ranges.[72] According to these, in healthy men with no obesity between 19 and 39 years of age, normal TT is between 264 and 916 ng/dL. This work has created a sense of urgency for clinical labs to obtain CDC certification for their TT assay, as clinicians can use the 264–916 ng/dL reference range for all CDC-certified TT measurements.

Measurement of FT

Based on the free hormone hypothesis the androgenic effect of T depends on its free fraction, so FT measurement has great clinical significance. The most precise assay is equilibrium dialysis, a technically challenging approach which involves two steps. FT measurement by IA platforms that do not involve a separation step is available but not recommended by professional societies, as it underestimates FT level when compared with equilibrium dialysis.[11 184] FT can be calculated (cFT) based on TT, SHBG and albumin level according to equations based on a linear model of T binding to SHBG,[185 186] however significant discrepancies were identified when these cFTs were compared with dialysis FT measurements, as the calculated values are derived from the superseded linear model of T and SHBG interactions. In contrast,[47] a computational algorithm based on the allosteric model of SHBG and T interaction reported similar FT levels to those obtained by equilibrium dialysis in two clinical trials in both men and women.[187 188]

Circulating T and target organ concentration

The question of whether measurement of circulating T correlates with target organ concentration is important because hormonal action takes place in the target organ, not in the bloodstream, and clinical trials have reported that changes in serum T or DHT concentration observed after testosterone replacement therapy, castration or DHT replacement were not associated with parallel changes in the prostate.[189–191] These data have important implications, and additional studies are required to understand if serum T levels are a reliable surrogate of tissue T levels.

Summary of testosterone measurement

The most important question in clinical andrology is what fraction of T should be measured to correctly diagnose hypogonadism in routine clinical practice. TT by IA is considered a good screening test, but LC/MS/MS is the gold standard and eventually should become available in every laboratory. IA can still be used provided that the assay is CDC-certified and manufactured by a reliable source. The reference range for CDC-certified assays is 264–916 ng/dL.

Use of FT instead of TT is recommended in patients affected by any of the conditions altering SHBG level, or when TT levels are close to the lower limit of normal. The most reliable method for the measurement of FT is equilibrium dialysis followed by LC/MS/MS or IA. The reference range for FT measured by equilibrium dialysis is not

established because the assay is not yet standardized, hence until more data become available one should use the reference range offered by the preferred laboratory.

Measurement of gonadotropins: primary versus secondary/tertiary hypogonadism

The distinction of primary versus central (secondary/tertiary) hypogonadism is important because gonadotropins or GnRH can be used to treat infertility in patients affected by the latter. Although the physiopathology of secondary versus tertiary hypogonadism is different (ie, in secondary hypogonadism the disease process affects the pituitary gland and in tertiary hypogonadism the hypothalamus), both respond to the same treatments with Tt, or gonadotropins. Secondary hypogonadism from an intrinsic problem of the pituitary gland does not respond to GnRH, whereas tertiary hypogonadism does.

Once a diagnosis of hypogonadism is obtained based on clinical and biochemical criteria, measurement of serum LH and FSH is necessary to understand if the patient is affected by primary or secondary/tertiary hypogonadism. LH and FSH are measured by IA and most of the commercially available assays have the ability to differentiate between low, normal and elevated levels. In most clinical laboratories, normal FSH is 2–7 mIU/mL and normal LH is 1–10 mIU/mL. The association of elevated gonadotropin with low/undetectable T is typical of primary gonadal failure, while low/inappropriately normal gonadotropin and low/undetectable T is typical of central hypogonadism. Rarely other combinations can be encountered, for instance, elevated FSH with normal T and LH is seen in cases of spermatogenic failure with preserved Leydig cell function. Reports where patients have normal T and LH but elevated FSH and normal spermatogenesis have been occasionally described, and represent compensated primary testicular disease with normal sperm counts and preserved fertility.[192] Normal T, FSH and LH in the presence of azoospermia and reduced ejaculate volume is typical of patients with obstructive azoospermia, whereas elevated T, E_2, LH and FSH is distinctive of androgen insensitivity syndromes. Following the distinction of primary versus central hypogonadism, the next step should be guided by the time in which hypogonadism started (before or after puberty) and the clinical presentation. Many cases of primary hypogonadism are due to KS, cryptorchidism or testicular trauma (table 3). Biochemistry suggestive of central hypogonadism should direct attention to the hypothalamic/pituitary unit as discussed in table 1. Men with this condition should be evaluated for hemochromatosis by requesting iron studies and questioned about chronic exposure to opioids, anabolic steroids or corticosteroids. The threshold to suspect the presence of other pituitary hormone deficiencies or excesses should be low, and ruled out by prolactin measurement, serum cortisol level at 08:00 AM, thyroid-stimulating hormone, T4 and, depending on clinical suspicion, insulin-like growth factor 1. All younger men with secondary/tertiary hypogonadism should receive a pituitary MRI. Older men should have a pituitary MRI if they have symptoms of tumor mass effect (ie, visual impairment or new-onset headache) or TT <150 ng/dL.[193]

TREATMENT

A formal discussion on the therapy of hypogonadism and on the different formulations of T available is beyond the scope of this review. As stated above, once the diagnosis of PH or LOH is made based on a careful assessment of symptoms and biochemical abnormalities, we recommend prescription of Tt to patients affected by PH to normalize and maintain virilization and sexual function. In theory, Tt in PH should be prescribed for years, targeting a serum TT concentration in the mid/upper normal range. In the case of LOH, we recommend prescription of Tt only to men whose diagnosis is based on a syndromic approach (eg, presence of symptoms and unequivocal low T). Because response to Tt varies among LOH men, it should be administered on a trial basis for approximately 6 months and continued only if the patient reports subjective improvement of his symptoms and no contraindications arise during treatment. Serum TT concentration to target is in the mid-normal range for patients with LOH.

Expected benefits from treatment of PH and LOH

A meta-analysis of randomized clinical trials of the effect of Tt versus placebo in men with T <432 ng/dL on health-related quality of life using the AMS scale reviewed four studies that showed a significant improvement in AMS total score with a mean difference of −2.96 (95% CI −4.21 to −7.71, lower score indicates fewer symptoms) in favor of treatment (range of possible points 17–85). Psychological, somatic and sexual subscale scores also improved (mean change in points, 95% CI and range of possible points: psychological: −0.89 (−1.41 to −0.37), range 5–25, somatic: −0.89 (−1.41 to −0.37), range 7–35, sexual: −1.29 (−1.75 to −0.83), range 5–25).[194] Another meta-analysis comprising four randomized controlled trials (RCTs) (including 1779 patients) at low risk of bias found that compared with placebo, Tt was associated with a significant increase in sexual desire or libido (standardized mean difference (SMD): 0.17; 95% CI 0.01 to 0.34; n=1383), erectile function (SMD: 0.16; 95% CI 0.06 to 0.27; n=1344) and sexual satisfaction (SMD: 0.16; 95% CI 0.01 to 0.31; n=676), but had no effect on energy or mood.[195]

The T trials

The landmark prospective trials evaluating the use of T supplementation in elderly men with hypogonadism were the Testosterone Trials. These were a set of 7 coordinated trials designed to establish the effects of Tt on men ≥65 years with unequivocally low T. Men were recruited on the basis of low morning values total T, with <275 ng/dL on the first screen, <300 ng/dL on the second and an average of <275 ng/dL. In addition, subjects were required to have one or more symptoms related to the main three trials (physical function, sexual function and vitality). Exclusion criteria included a history of PCa or prostatic intraepithelial neoplasia, a palpable prostate nodule by digital rectal examination and a risk of any PCa >35% or high-grade PCa >7% based on the PCa risk calculator.[196] Because PSA is lower in older men with low T, the PSA used in the risk calculator was adjusted to account for this. The PSA value used was: measured PSA (in ng/mL)+((460−serum testosterone concentration)×0.00128). Subjects with

an International Prostate Symptom Score (IPSS) of >19 were also excluded, as were those with a hemoglobin of >160 g/L and diagnosed but untreated sleep apnea. Subjects with a stroke or MI within the prior 3 months or systolic blood pressure >160 mm Hg or diastolic blood pressure >100 mm Hg were excluded. Recruits with a body mass index (BMI) >37 were excluded.[197] Seven hundred eighty-eight men were enrolled in the trial, and data were analyzed for 376 men in the Tt arm and 374 men in the placebo arm. The average age was 72 years, 88% were white, 5% black and 6% other. Average BMI was 31, with 63% having a BMI of >30 kg/m^2. Thirty-seven per cent had diabetes, 72% had hypertension, 15% had a history of MI, 4% had a history of stroke and 19% had a history of sleep apnea. Of the men who had a sufficiently low T at the initial screening visit, 31% did not meet the criteria for low T at the second screening visit.

The enrolled subjects had a baseline TT of 245±64 ng/dL and FT 67.0±24.5 pg/mL.[7] TT in the treatment arm increased to the mid-normal young male range, with a median TT of approximately 500 ng/dL and FT 150 pg/mL. E_2 also increased to the mid-normal young adult range, approximately 30 pg/mL, and DHT increased to approximately 100 ng/dL.[7]

Sexual function

The sexual function subtrial enrolled those men who met the general enrollment criteria and also self-reported a decrease in libido, had a score of ≤20 on the Derogatis Interview for Sexual Function (DISF)-II Assessment Sexual Desire Domain (DISF-M-II SR) questionnaire,[198] a total of 459 subjects. Subjects with severe peripheral vascular disease, any situation that would preclude sexual activity and autonomic neuropathy were excluded. Tt significantly increased sexual activity as measured by the Psychosexual Daily Questionnaire (PDQ), libido as measured by the DISF-M-II SR and erectile function as measured by the International Index of Erectile Function score (IIEF). The PDQ score also improved among all TT participants.

Question 4 of the PDQ asks for subjects to indicate which of the list of 12 activities a subject has experienced or is experiencing on the day of the questionnaire. The activities include sexual daydreams, anticipation of sex, flirting, spontaneous erections, masturbation and intercourse.[199] Although this result showed a statistically significant improvement with Tt compared with placebo (mean change from baseline of 0.58 (95% CI 0.38 to 0.78)), it was below the suggested cut-off for clinically meaningful change (≥0.6). Similarly, a clinically meaningful change in sexual desire, measured by the DISF-II-SDD, is ≥5.0 and the mean change from baseline of the treatment arm compared with the placebo for this outcome was 2.93 (95% CI 2.13 to 3.74).

Nonetheless, the OR for a subject to achieve a clinically significant improvement in sexual activity with T compared with placebo was 3.00, and for desire was 4.35.[200] T increased sexual acts of any type by 4 times a week. After adjustment for multiple comparisons, none of the baseline subject characteristics tested (which included BMI, smoking, diabetes, hypertension, history of MI, baseline total T) impacted the effect of T supplementation on the primary and secondary end points of the substudy.[201]

Physical function

The primary outcome of the physical function subtrial was the proportion of men whose 6 min walk distance increased by >50 m. The subtrial enrolled men who both self-reported difficulty walking a quarter mile or up a flight of stairs and had a gait speed of <1.2 m/s on the 6 min walk test. Three hundred eighty-seven men were enrolled in the subtrial. There were no significant differences in the treatment and placebo groups of the subtrial, but when all TT participants were assessed, the differences were significant (OR for a 50 m improvement in 6 min walk test was 1.77; p=0.003), possibly due to the increased power of the larger sample. When all participants were included, there was also a statistically significant increase in the treatment arm in 6 min walk distance and the proportion of participants who had an increase of at least 8 points on the 10-item Physical Functioning Scale.[7]

Vitality

Subjects were enrolled in the vitality subtrial if they self-reported decreased energy and had a Functional Assessment of Chronic Illness Therapy-Fatigue questionnaire (FACIT-Fatigue) scale score of <40. The study failed to show an improvement in its primary outcome, an increase of at least 4 points in the FACIT-Fatigue. There was a statistically significant change in the numerical measures of several of the scales used: the 36-Item Short Form Health Survey, the Vitality Scale and the Positive and Negative Affect Schedule (PANAS positive affect score and PANAS negative affect score) and the Patient Health Questionnaire-9. The numerical changes in these scores were generally less than the published minimally important differences.[196 202–204]

Cognitive function

The cognitive function trial enrolled 493 men (247 assigned to T, 246 to placebo) who were determined to have age-associated memory impairment based on subjective complaints and an impairment in delayed paragraph recall or visual memory as determined by the Wechsler Memory Scale, Revised, Logical Memory II scale. Cognitive function did not improve with T therapy.[7 205]

Anemia

The anemia trial included subjects whose baseline hemoglobin was 12.7 g/dL or less. The primary analysis was for the percentage of men with unexplained anemia whose hemoglobin improved by at least 1.0 g/dL. This occurred in 54% of the Tt group, significantly higher than the 15% rate in the placebo group. Even with known causes of anemia, the Tt arm had a greater proportion of hemoglobin improvement.[206]

Cardiovascular

The cardiovascular trial was designed to assess the effect of T therapy on coronary artery plaque progression as measured by coronary CT angiography, not on clinical outcomes. Tt was associated with a significantly greater increase in non-calcified plaque volume from baseline to 12

Box 2 Potential side effects of testosterone therapy

Erythrocytosis
Prostatic side effects
Thromboembolism
Gynecomastia
Cardiovascular
Secondary Exposure
Male Pattern Baldness
Sleep Apnea
Infertility
Decreased Testicular Size
Acne
Skin Reaction
Fluid Retention
Lipid profile

months compared with placebo. For the secondary outcome of coronary artery calcium score, there was no association of Tt with 12-month change.[207]

Bone

The bone trial excluded T-trial participants who were taking medications known to affect bone (with the exception of calcium and vitamin D, which all patients were instructed to take at a dose of 600 mg elemental calcium and 400 units of vitamin D_3, twice a day with meals), if they did not have any lumbar vertebra that were evaluable, and if they had a baseline Dual-energy X-ray absorptiometry (DEXA) T-score at any site less than −3.0. The results showed a significant increase in lumbar spine trabecular volumetric bone mineral density (vBMD). The change in vBMD was associated with the change in T in the treatment arm. Finite element analysis of quantitative CT data indicated an increase in estimated bone strength at the hip and spine in the Tt group.[208]

POTENTIAL ADVERSE EFFECTS OF TT

A list of potential side effects related to Tt is shown in box 2. Some of these are a consequence of the possible local aromatization of T in peripheral tissues (eg, gynecomastia and breast tenderness) or to inhibition of the HPG axis that occurs following administration of exogenous T (infertility, decreased testicular size). Other side effects are a consequence of a specific formulation of T, for instance, risk of secondary exposure among relatives of subject treated with transdermal gel formulations, or hepatotoxicity, which was described for methyltestosterone, an old oral formulation. Changes of the lipid profile (ie, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and total cholesterol) have been studied extensively. A meta-analysis that included data from 51 comparative trials showed a small decrease in HDL cholesterol with no significant effect on mortality or cardiovascular disease outcomes, while serum LDL and total cholesterol changes did not reach statistical significance.[209] Some of these complications, for instance, reduced fertility, smaller testicular size, gynecomastia or breast tenderness, are inherent to treatment with exogenous T and should be discussed beforehand with the patient.

Contraindications to the use of Tt

Tt is contraindicated in men affected by PCa, breast cancer, severe lower urinary tract symptoms (LUTS), erythrocytosis (hematocrit (HCT) >50%–52%) and congestive heart failure. Untreated obstructive sleep apnea (OSA) may be worsened by Tt,[10] hence patients with a diagnosis of OSA who are not receiving active treatment with continuous positive airway pressure therapy should not be started on Tt.

Prostatic diseases

During antenatal and postnatal life prostatic development and growth is quintessentially androgen dependent, as demonstrated by the fact that prostate development does not occur or is rudimental in various disease models with impaired androgen-AR axis, such as complete androgen insensitivity[210] and SRD5A2 deficiency,[211] prostate size regresses following long-term castration[212] and increases in hypogonadal men receiving Tt.[3] Based on this, it is logical to question whether Tt and the consequent increase in serum T concentration is responsible for an exacerbation of benign prostatic hyperplasia symptoms and/or rises the risk of PCa.

Lower urinary tract symptoms/benign prostatic hyperplasia

Prostate size and serum PSA increase in hypogonadal men treated with Tt, however this occurs only to the same extent as in age-related men with normal gonadal function[213] and is not associated with increased LUTS, as demonstrated by meta-analysis[195 209] and randomized placebo-controlled trials.[3 7]

Prostate cancer risk

There is no evidence that Tt is associated with increased PCa risk. In particular, the concept that serum T concentration is positively associated with PCa risk is unproven. If serum T level were to play a role and increase PCa risk, one would expect that higher T concentrations are associated with increased risk and rapid growth of PCa, while low T is protective and associated with its regression. In contrast to this, studies have identified positive,[214–216] negative[217–219] or no correlation at all[220 221] between serum T level and PCa risk. Additionally, there is no difference in serum TT of FT concentrations between black and white men despite the increased epidemiological risk of PCa in black compared with white men.[222] Based on these observations, on meta-analysis[209 223] and on insufficiently powered interventional studies,[7 8] no evidence demonstrates that Tt increases the risk of PCa in hypogonadal men. This important question will be conclusively answered only once the Testosterone ReplacementTherapy on the Incidence Of Major Adverse Cardiovascular Events and Efficacy Measures in Hypogonadal Men (TRAVERSE) trial, a large ongoing placebo-controlled prospective study, is completed. Data from a 2005 meta-analysis[223] established that men receiving Tt are at an increased risk of developing prostatic events (defined as PCa, PSA levels >4 ng/mL or PSA increment >1.5 ng/mL, prostate biopsy and increase in IPSS scores). The main contributor to this positive association was an increased number of prostatic biopsies in patients receiving Tt, however, none of the prostatic events considered was

individually different between the placebo versus T-treated groups. Despite the increased number of biopsies in men receiving Tt, two meta-analysis derived from 19 and 51 studies found no association between Tt and increased detection of PCa.[209 223]

Monitoring for prostatic diseases

In men of 50–69 years of age with at least 10 years of life expectancy, ES recommends engaging the patient in the decision making of monitoring for PCa. If monitoring is chosen, routine DRE and PSA measurements should take place at the onset of Tt and after 3 and 12 months. Patients considering Tt who are between 40 and 69 years with an increased risk of PCa because of African-Americans descent or a first-degree relative diagnosed with PCa should be offered monitoring options. Tt is not recommended without further urological evaluation in patients with a palpable prostate nodule or induration, or a PSA concentration of 4 ng/mL, or 3 ng/mL if at high risk. Furthermore, urological consultation should be requested when serum PSA concentration increases >1.4 ng/mL within any 12-month period of Tt, or PSA velocity is >0.4 ng/mL/year using the PSA level obtained after 6 months of T administration as the reference, or in the presence of American Urological Association (AUA)/IPSS prostate symptom score of >19. In men over the age of 55 years, it is recommended to calculate the PCa risk (http://deb.uthscsa.edu/URORiskCalc/Pages/calcs.jsp). After 1 year, monitoring should conform to standard guidelines for PCa screening.

Erythrocytosis

Erythrocytosis is the most frequent adverse event occurring in men receiving Tt compared with controls, as demonstrated by three meta-analysis where the risk to increase the HCT to >50%, >52% and >54% was increased to 3.69,[223] 3.18[209] and 8.14,[195] respectively. HCT increases in both young and old men, but more so in the latter.[224] The increase is seen in patients receiving higher doses of Tt through the intramuscular route and less in patients treated with transdermal T.[225] While the risk of venous thromboembolism (VTE) increases in relation to HCT according to epidemiological studies,[226] investigations evaluating the occurrence of VTE in men treated with Tt have demonstrated that increased risk is present only in men with pre-existent undiagnosed forms of thrombophilia-hypofibrinolysis.[227] When HCT exceeds 52%–54%, clinical options include decreasing the dose, changing to a transdermal formulation, discontinuing treatment or enrolling the patient in a program of blood donation. Increased HCT should also prompt evaluation for OSA.

CV complications

Whether Tt increases or not the risk of CV events is controversial. Despite a substantial number of epidemiological studies and meta-analysis which have reported an association of low T concentrations with higher all-cause mortality (particularly mortality due to CV disease),[171 172 228–230] we will be able to conclusively answer this question only after completion of the TRAVERSE trial, a randomized, blinded study of topical T versus placebo in men 45–80 years of age with low T, at least one symptom of hypogonadism and CV disease or increased risk of CV disease. The primary end point is the composite of CV death, non-fatal MI or non-fatal stroke, and the expected follow-up is 60 months (https://clinicaltrials.gov/ct2/show/NCT03518034). Available RCTs do not show an association between CV risk and Tt,[7 8] however they are short and not powered to conclusively address this issue.

One of the first papers that during the last decade raised concerns about Tt and CV complications was the 'Testosterone in Older Men with Mobility Limitations' trial,[231] which prospectively randomized men aged 65 years and older (mean age 74 years) with low TT or FT levels to placebo or T gel and achieved, in the treatment arm, a mean TT level of 574 ng/dL. The subjects had a high prevalence of chronic conditions such as coronary artery disease, diabetes, obesity and dyslipidemia. The study was stopped early because of the finding of excessive CV adverse events in the treatment group (23 out of 106 vs 5 out of 103 in the placebo arm).[231] These events included acute coronary syndromes and MI, and peripheral edema, elevated blood pressure and tachycardia that were considered to be CV in nature by the safety monitoring board. Subsequently, two retrospective trials raised additional concern about the risk of stroke, MI and death in men taking T. The first study was done in the Veterans Affairs healthcare system, which evaluated 8709 men who both had a T level checked and underwent coronary angiography.[232] The patients had a high burden of comorbid disease, including 20% with history of MI and 50% with diabetes. Fourteen per cent of patients were initiated on T therapy. The two groups were significantly different on multiple baseline characteristics, with the group receiving T having a lower prevalence of cardiac and cerebrovascular disease. The unadjusted rate of death, MI and stroke was lower in the group initiated on T therapy (21% in the no-T group vs 10% in the treated group).[233] Since the groups were not randomized, however, stabilized inverse probability of treatment weighting was used to adjust for treatment selection bias. After adjustment, the primary outcome, the time to all-cause mortality or to hospitalization for MI or ischemic stroke in the no-T versus T group was 19.9% vs 25.7%.[232] That the statistically derived event rate was so different from the absolute (unadjusted) event rate has been criticized. In addition, it has been claimed that 1132 men who had an MI or stroke and subsequently were treated with T were incorrectly excluded from analysis, whereas they should have been classified as not-treated patients, which would have increased the number of events in the non-T group by 71%, demonstrating a reduction in mortality with Tt.[234] The second trial was a retrospective cohort study using a database of commercial insurance and Medicare claims, which evaluated the relative rate of MI in the 90 days following an initial prescription for T compared with the 1 year prior to the prescription. These rates were compared with the same statistics preprescription and postprescription for sildenafil and tadalafil. The MI rate was increased after T prescription compared with sildenafil and tadalafil in men aged 65 years and older, and in those under the age of 65 years with pre-existing heart disease.[235] Important information was excluded: serum T level before and after initiation of Tt was not reported, so whether the subjects were indeed affected by hypogonadism and qualified for Tt could not be determined, nor could it be determined whether T normalized with treatment, and thus whether the reported CV events were associated with normal or abnormal level of

serum T. These two studies were cited by the FDA in their 31 January 2014 Safety Announcement indicating that the safety of T products would be re-evaluated. In March 2015, the FDA followed up with an update to the label of T products indicating that they possibly increase risk of heart attacks and strokes.[236] In contrast, the European Medicines Agency concluded that there is no consistent evidence of an increased risk of coronary heart disease associated with T therapy in hypogonadal men. The studies raising CV concern and the subsequent FDA communication raised controversy centered around the reliability of non-randomized retrospective studies in accurately determining the risk of Tt[233] and the high profile given to these studies despite the large number of subsequent trials showing no increased risk[237–241] or reduced risk.[242–247] Among the studies that did not find an association between Tt and CV risk, the most convincing included a series a retrospective cohort studies on the US veteran population by scientists at the Kansas City VA Medical Center and the University of Kansas Medical Center who evaluated outcomes in hypogonadal veterans in the VA healthcare system. In 83 010 veterans without history of MI or stroke, with documented low T, they identified the timing of T replacement, and categorized the included subjects based on whether follow-up T levels were normalized on therapy or not. In addition, propensity score inverse probability of treatment weighting was used to balance the measured covariates between the groups. The all-cause mortality, risk of MI and risk of stroke were all significantly lower in the group of hypogonadal veterans who received treatment with subsequent normalization of T compared with those who were not treated (HR, 95% CI, respectively: all cause mortality 0.44, 0.42 to 0.46; MI 0.76, 0.63 to 0.93; stroke 0.64, 0.43 to 0.96). The subjects who were treated but did not normalize serum T level saw no benefit.[237] When the group evaluated 18 055 hypogonadal veterans of known smoking status who received T therapy, the results showed that the current smokers were at increased risk of all-cause mortality, MI and stroke compared with the non-smokers, and all-cause mortality, but not MI or stroke, was reduced in the smokers whose T was normalized compared with those in whom the T was not normalized.[248] When the methodology was applied to 71 407 hypogonadal veterans without known history of prior DVT, cancer or hypercoagulable state, there was no significant difference in the DVT-free survival among the men who were treated with T and normalized, treated but not normalized or not treated.[249]

In summary, while it is fair to state that this topic is contentious, it also should be recognized that the evidence goes in both directions. Hence, during daily practice we advise to inform patients of the controversies existing on this topic, and we do endorse the 2018 ES guidelines recommending against Tt only in men with uncontrolled heart failure, MI or stroke within the last 6 months.[10]

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