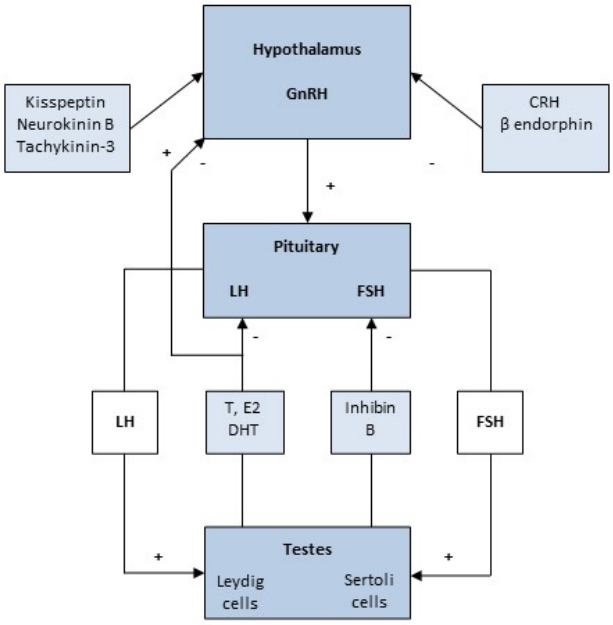
Appendix 1

Physiology of testosterone production

The pituitary gland regulates testicular activity through secretion of LH, which regulates testosterone production in Leydig cells and follicle-stimulating hormone (FSH), which mainly controls sperm production in the seminiferous tubules [1, 2]. The production and secretion of gonadotropins is stimulated by hypothalamic gonadotropin releasing hormone (GnRH) and inhibited by negative feedback mediated by the central action of sex steroids and inhibin B (Figure 1) [1, 2]. Gonadotropin releasing hormone is secreted in a pulsatile manner and negatively controlled by the activity of hypothalamic neurons, including corticotrophin-releasing hormone (CRH) and β endorphin neurons [1, 2]. Conversely, kisspeptin-1 (Kiss-1) neurons, neurokinin-B and tachykinin-3 are involved in GnRH stimulation. Leptin is involved in activation of Kiss-1 signalling [3]. About 25 mg of testosterone is present in the normal testes, and, on average, 5-10 mg of testosterone are secreted daily [1, 2]. The testes also produce lesser amounts of other androgens, such as androstenedione and dihydrotestosterone (DHT). A small amount of extra-gonadal testosterone is derived from the circulating weak adrenal androgen precursor dehydroepiandrosterone (DHEA), although its specific contribution to daily testosterone production is limited in men [4, 5]. In physiological terms, DHT formation accounts for 6-8% of testosterone metabolism, and the ratio of plasma testosterone/DHT is approximately 1:20 [1, 2]. Finally, testosterone and its precursor, Δ4 androstenedione, can be aromatised through P450 aromatase to other bioactive metabolites, such as oestrone (E_1) and 17-β-oestradiol (E_2), with a daily production of ~45 µg [1, 2]. Leydig cells can also directly produce and release into the bloodstream small amounts of oestrogens, with a daily production rate of 5-10 µg (up to 20% of circulating oestrogens) [6].

Figure 1: Physiology of testosterone production



GnRH = gonadotropin releasing hormone; LH = luteinising hormone; FSH = follicle-stimulating hormone; T = testosterone; E2 = 17-β-oestradiol; DHT = dehydroepiandrosterone; CRH = corticotrophin releasing hormone.

- ***Circulation and transport of testosterone***

In healthy men, 60-70% of circulating testosterone is bound to the high-affinity sex-hormone-binding globulin (SHBG), a protein produced by the liver, which prevents its bound testosterone sub-fraction from biological action. The remaining circulating testosterone binds to lower affinity, high-capacity binding proteins, (albumin, α-1 acid glycoprotein and corticosteroid-binding protein), and only 1-2% of testosterone remains non-protein bound [7]. There is a general agreement that testosterone bound to lower-affinity proteins can easily dissociate in the capillary bed of many organs, accounting for so-called 'bioavailable' testosterone [7]. It is important to recognise that several clinical conditions and ageing itself can modify SHBG levels, thus altering circulating total testosterone levels (Table 2). If not recognised, these factors could lead to an incorrect estimation of male androgen status; therefore, when indicated SHBG should be tested and free testosterone calculated [8].

- ***Androgen receptor***

Testosterone and DHT exert their biological action through activation of a specific nuclear receptor. The androgen receptor (AR) gene is localised on the X chromosome (Xq11–12), encoded in eight exons [9]. Exon 1 includes two polymorphic trinucleotide repeat segments encoding polyglutamine (CAG) and polyglycine (GGN) tracts in the N-terminal transactivation domain of its protein. Activity of the AR is inversely associated with the length of the CAG repeat chains [9]. However, the specific role of AR CAG repeat number in relation to hypogonadal symptoms or to clinical management of testosterone deficiency remains unclear [10, 11]. A RCT has shown that a higher CAG repeat number is positively associated with a change in fasting insulin, triglyceride and diastolic blood pressure, demonstrating the more sensitive the receptor, the greater the benefit [12].

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