Chapter 3
Physiology of Testosterone Production

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In 1889, Charles Brown-Séquard reported before the Sociéte de Biologie in Paris the improved vitality, strength, and mental facility he had experienced after self-administering the testicular extracts of dogs and guinea pigs [1]. Although his work was not rigorously reproduced, it marked the start of a long effort to isolate this unknown male sex hormone, an accomplishment for which Ruzicka and Butenandt were awarded the Nobel Prize in Chemistry in 1939 [2]. Today, the effects of testosterone are better understood but no less impressive. Androgens play an essential role in the development of male reproductive organs, the maintenance of male fertility, and the preservation of secondary male sexual characteristics.

This chapter explores the physiology of testosterone production, beginning with its intracellular synthesis and steroidogenic conversion, and then following testosterone's transport across the cell membrane and into circulation. Regulation of this system via the hypothalamic–pituitary–gonadal (HPG) axis is reviewed. Finally, this discussion concludes with two increasingly prevalent clinical circumstances in which testosterone physiology is altered: the metabolic syndrome and the aging male.

Testosterone Production

Testosterone is the predominant circulating androgen in men, with roughly 6–7 mg produced per day. Over 95 % of testosterone originates from within the testes, where 400 million Leydig cells process cholesterol through the steroidogenic pathway [3]. The adrenal glands contribute the remainder of circulating testosterone. As a result of this synthetic effort from both sites, the relatively inert

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cholesterol molecule is oxidized into a variety of biochemically active androgens, including testosterone.

Both functionally and anatomically the testis has two compartments: (1) the avascular seminiferous tubules that are home to Sertoli cells and spermatogenesis, and (2) the interstitium that is composed of blood vessels, lymphatics, immune cells, nerves, connective tissue, and Leydig cells. Three periods of Leydig cell development and function have been proposed after birth: neonatal, prepubertal, and pubertal [4]. During the neonatal period, Leydig cell numbers increase in response to gonadotropins secreted by the pituitary gland. The number of cells peaks at 2–3 months of age with a concomitant peak in testosterone levels. Immediately thereafter, however, Leydig regression ensues and, by 1 year of age, the prepubertal period starts, with a lifetime testosterone nadir that persists until the onset of puberty [4, 5]. At puberty, the Leydig cells are primed for steroid biosynthesis and conversion. Cytologically this is made apparent by the so-called organelle association of large, anastomosing tubules of smooth endoplasmic reticulum with many mitochondria and well-developed Golgi bodies [6]. Often, intracellular inclusions called Reinke's crystals may be visualized, but their nature and possible function is not well understood.

With pubertal maturation, Leydig cells increase their enzymatic oxidation of cholesterol to testosterone. Given the greatly limited storage potential of these cells, this process must occur on a continuous basis with rapid mobilization of this cholesterol [7]. For this reason, Leydig cells have developed great functional capacity for endogenous cholesterol synthesis from acetate. In addition, they are able to mobilize intracellular stores of cholesterol as needed from lipid droplets that contain cholesterol esters and to obtain extracellular sources of cholesterol via endocytosis of intravascular lipoproteins.

Testosterone Biosynthesis

From the most basic perspective, the biosynthesis of testosterone consists of several oxidative steps that result in the cleavage of a 27 carbon (C_{27}) substrate, cholesterol, down to the 19-carbon (C_{19}) product, testosterone (Fig. 3.1). Cytochrome $P450_{SCC}$ (CYP11A) catalyzes the first step in this process—two separate hydroxylations that result in the biochemically inactive, C_{21} steroid pregnenolone [8]. This is the only enzymatic reaction that occurs within the inner membrane of the mitochondria, and as such, it allows for a means of regulating testosterone biosynthesis. The aqueous intermembrane space of the mitochondria proves a major barrier for cholesterol transport to the CYP11A enzyme, which is located in the inner membrane. However, a lipophilic environment is created by a 30 kDa steroidogenic acute regulatory protein (StAR), which actively facilitates translocation of cholesterol [9]. While the precise mechanisms involved in its expression and function are not fully understood, it is now accepted that StAR regulates the rate-limiting step in steroid biosynthesis and represents the point of convergence for several hormonal and

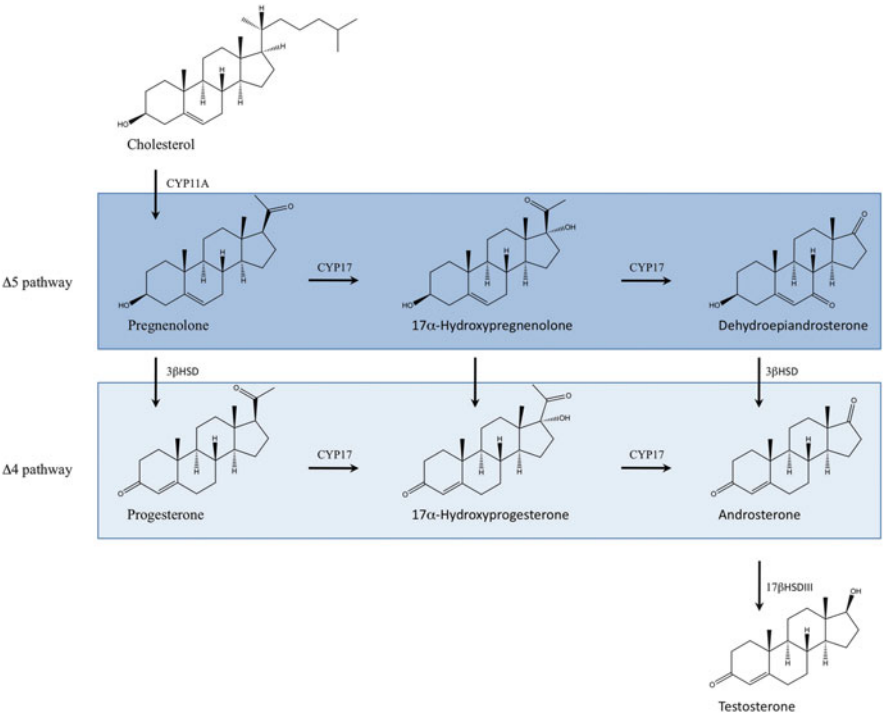

Fig. 3.1 The steroidogenic pathway

signaling pathways within the cell [10]. This is underscored by patients suffering from lipoid congenital adrenal hyperplasia, an autosomal recessive disorder that results in inactivation of the StAR gene and consequent severe impairment in steroid biosynthesis [11].

After this first side chain cleavage of cholesterol, pregnenolone is transported to the endoplasmic reticulum, where it is further metabolized by a variety of enzymes to make additional C_{19} and C_{21} steroids. Two of these pathways potentially lead to the ultimate production of testosterone: the Δ4 pathway and the Δ5 pathway; the notations Δ4 and Δ5 correspond to the location of the double bond on the steroid. Regardless of which pathway is utilized, two reactions must be completed including: (1) a 17α-hydroxylation of the C_{21} steroid, and (2) a cleavage of the $C_{17–20}$ bond to produce a C_{19} steroid [8]. In the Δ5 pathway these steps are accomplished using pregnenolone as a direct substrate, which is transformed to 17 α-hydroxypregnenolone and then to dehydroepiandrosterone (DHEA). The Δ4 pathway requires an additional step, as pregnenolone is first converted to progesterone by 3β-hydroxysteroid dehydrogenase/isomerase. This steroid, in turn, undergoes similar hydroxylation and conversion by lyase to produce 17 α-hydroxyprogesterone and androstenedione, respectively. Contrary to what has previously been believed, both the Δ4 and Δ5 pathways are catalyzed by a

single enzyme: cytochrome $P450_{C17}$ (CYP17) [12]. Furthermore, the human and bovine forms of this enzyme show a preference for the substrate 17 α-hydroxypregnenolone—the Δ5 pathway—and ultimately end up producing DHEA primarily [13]. In the end, DHEA is still converted to androstenedione, again by 3-β-hydroxysteroid dehydrogenase/isomerase.

The final step in the biosynthesis of testosterone is the reduction of androstenedione to testosterone by 17β-hydroxysteroid dehydrogenase. Several isoenzymes are found in humans; however, type III is expressed exclusively in the testes [8]. Ultimately, while Leydig cells secrete primarily testosterone, any of the aforementioned intermediaries may leak out as well.

Testosterone Transport, Conversion, and Degradation

After synthesis, testosterone diffuses from the interstitial space that bathes Leydig cells into the venous system via the pampiniform plexus, at which point it is available to affect target tissues. More precisely, the biologically available testosterone is available to affect target tissues. Of the total testosterone in circulation, 2 % remains free, or unbound, and 98 % is associated with plasma proteins. This includes the ~54 % bound to albumin and ~44 % bound to sex hormone binding globulin (SHBG). According to the free hormone hypothesis, it is only the unbound, free testosterone that is able to passively diffuse across cell membranes and cause downstream effects. Therefore in order for bound testosterone to contribute an appreciable effect, it must first dissociate from its binding protein [14]. In the case of testosterone bound to SHBG, this quantity is negligible as the binding affinity is too high to dissociate a significant amount of hormone. Conversely in the case of albumin, which has a dissociation constant many orders of magnitude lower, this does impact the total amount of testosterone available to cells. Thus, bioavailable testosterone is typically defined as unbound hormone *plus* albumin-bound hormone.

From a laboratory perspective, total testosterone can be easily assayed and is the initial step for the evaluation of androgen insufficiency. Assessment of free testosterone is more expensive and time consuming, generally requiring the use of equilibrium dialysis or precipitation assays [15]. Despite the well-established hypotheses regarding bioavailable testosterone, there is now some evidence suggesting the existence of endocytotic cellular uptake pathways for carrier-bound steroids; however, these are likely to play a role only in limited tissues under specific physiologic conditions [16].

The total level of testosterone is dependent upon the relative rates of its production and metabolism. In terms of metabolism, testosterone may follow one of three enzymatic pathways (Fig. 3.2). In the first, cytochrome $P450_{arom}$ (CYP19) aromatizes testosterone to create the C_{18} estrogen estradiol. While this conversion primarily takes place in adipose tissues, CYP19 is expressed in a number of tissues, including Leydig cells [8].

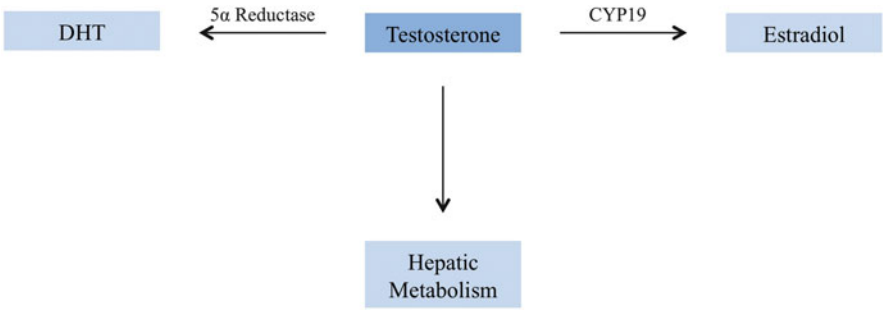

Fig. 3.2 Testosterone conversion and metabolism

Alternatively, testosterone may be reduced to 5α-dihydrotestosterone (DHT) by 5α reductase. Both testosterone and 5α-dihydrotestosterone bind to the same androgen receptor; however, DHT, as the most potent natural androgen, binds with much greater affinity [17]. Both molecules are essential to embryologic and pubertal development of the male genitourinary system, with testosterone responsible for maturation of the Wolffian ducts and DHT responsible for external virilization both in utero and during puberty. Thus far, two forms of 5α reductase have been identified: isoenzymes I and II. Type I 5α reductase is predominantly expressed in the liver and somatic tissue, whereas type II 5α reductase is predominantly found in the prostate, epididymis, seminal vesicles, and genital skin [18]. Pharmacologic inhibitors of 5α reductase have been used to relieve prostatic obstruction and consequently alleviate lower urinary tract symptoms in some patients. Two examples of such medications are finasteride, which is selective for the type II isoenzyme, and dutasteride, which is nonselective and inhibits both forms of 5α reductase.

Lastly, both testosterone and DHT can be degraded in the liver by a number of enzymes that will not be elaborated upon here. The end result of this process is to generate steroids conjugated with a glucuronide or sulfate group that are ultimately excreted by the skin or the kidneys.

Hormonal Regulation of Testosterone Production

The regulation of testosterone synthesis by the Leydig cells of the testis falls into two broad categories. The first includes endocrine signaling by the gonadotropins, primarily luteinizing hormone, which are secreted by the anterior pituitary gland and are the key mediators of the HPG axis. The second includes more discrete paracrine signaling by locally produced factors within the interstitial compartment of the testis.

Endocrine Signaling

The gonadotropins luteinizing hormone (LH) and follicle stimulating hormone (FSH) belong to a larger family of heterodimeric proteins known as glycoprotein hormones. As heterodimers, these molecules are composed of two different subunits. The α subunit is common to every member of the glycoprotein family; however, depending upon the β subunit that is chosen for dimerization, a variety of related hormones may be created: LH, FSH, chorionic gonadotropin (hCG), and thyroid stimulating hormone (TSH). Functionally, LH exerts its effects on testosterone production by binding to the G protein-coupled receptors on Leydig cells, and FSH exerts its effects on spermatogenesis by binding to the G protein-coupled receptors on Sertoli cells. This entire axis is tightly regulated by a negative feedback system in which testosterone plays a pivotal role (Fig. 3.3).

Gonadotropin-releasing hormone (GnRH), produced by the neurons of the hypothalamus, empties into the hypothalamic hypophyseal portal system to make its way to the anterior pituitary, where it acts to stimulate gonadotropin release. This process occurs in a pulsatile, coupled manner: GnRH pulses are followed by shorter-lived pulses of LH [19]. This episodic secretion is essential to the male reproductive axis, and continuous GnRH administration fails to achieve the same effect [20]. Yet the pulsatility of GnRH secretion is not constant throughout life. Recall that during the neonatal period, Leydig cell numbers increase and testosterone levels peak in response to GnRH and LH release at 2–3 months of life [4]. Thereafter, LH and GnRH levels drop and the neuronal GnRH generator goes dormant for the rest of prepuberty. With the onset of puberty, pulsatile GnRH secretions resume, and LH levels begin to rise as well—initially only at night but eventually throughout the day, as well [21].

After its pulsatile secretion, LH travels to the testes where it induces steroid synthesis in both an acute and a chronic manner. Binding of LH to the LH receptor, a G protein coupled receptor (GPCR), results in activation of adenylyl cyclase with a subsequent rise in cAMP and concomitant activation of the protein kinase A (PKA) pathway. There is evidence that multiple other pathways may be upregulated by this GPCR; however, in Leydig cells most investigators agree that these steroidogenic effects are primarily regulated by the GPCR/adenylyl cyclase/cAMP/PKA pathway [22]. Activated PKA in turn results in two major downstream effects. The first consists of increased translocation of cholesterol to the mitochondrial inner membrane secondary to upregulation of the acute regulatory protein (StAR) [11]. The second includes increased activation of the cholesterol side-chain cleavage system, mediated both by CYP11A and CYP17 [23].

While LH is accepted as being primarily responsible for the endocrine control of testosterone production by Leydig cells, there is some evidence FSH may play a role as well, albeit on a more limited scale. This likely occurs via the direct effect of Sertoli cells (triggered by FSH) on Leydig cells [4].

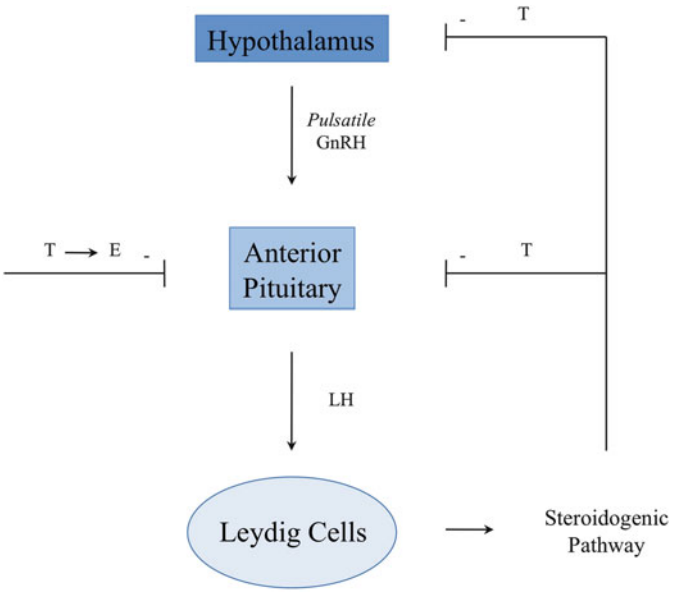

Fig. 3.3 The hypothalamic–pituitary–testis axis

Paracrine Signaling

Several non-pituitary, locally produced factors are identified as further regulators of Leydig cell function. Insulin-like growth factor 1 (IGF-1) and tumor growth factor beta (TGFβ) have well-established influences in this regard [4]. Both in vitro and in vivo studies demonstrate the important role IGF-1 plays in Leydig cell development and function [24, 25]. Furthermore, while the in vivo evidence is not as cogent, animal studies suggest that TGFβ modulates both steroidogenesis and Leydig cell proliferation [26]. A myriad of other potential local regulators are identified in vitro in rat models; however, studies on these molecules are often difficult to definitively translate to human Leydig cells [4].

More recently, additional regulators are generating increased attention including insulin-like factor 3 (INSL3), ghrelin, and leptin [27]. INSL3 is a peptide that belongs to the insulin-like growth factor (IGF) and relaxin family of hormones. It previously had an established role in testicular descent with uncertain biological significance in adults [28]; however, more recent human studies have shown a correlation between INSL3 levels and Leydig cell functional status [29, 30]. While the autocrine and paracrine effects of this peptide remain to be more fully elucidated, in data collected from adult men it appears that INSL3 operates outside of the HPG axis, reflecting both differentiation status and absolute number of Leydig cells [31].

Leptin and ghrelin have more firmly established roles in testosterone production and physiology than does INSL3. While the primary function of leptin and ghrelin

is to operate as a coordinated system regulating energy homeostasis, there is increasing evidence that they play a combined role in modulating testosterone levels as well [32]. In one model, rats fed a restricted diet with repeated administration of ghrelin showed decreased LH and testosterone with reduced testis weight [32, 33]. Conversely, ablation of ghrelin in leptin-deficient *ob*/*ob* mice resulted in increased steroidogenesis and reduction of testicular apoptosis [34]. Thus, it appears that unopposed ghrelin may exert an inhibitory effect on testosterone production. This occurs centrally, as demonstrated by decreased LH levels in rats given daily doses of ghrelin, and peripherally at the level of the Leydig cell, as shown by in vitro models showing a ghrelin-mediated inhibition of testosterone secretion in a dose-dependent fashion [34–36]. Further substantiating this hypothesis are data collected from human testicular samples showing an inverse correlation between ghrelin expression by Leydig cells and peripheral testosterone levels [37].

Negative Feedback Control

LH levels are maintained within a narrow physiologic range. Therefore, the pulsatile, stimulatory effects of GnRH must be balanced by a refined set of negative feedback mechanisms. These mechanisms act at both the level of the anterior pituitary and the hypothalamus, and they primarily are the result of circulating testosterone and estrogen (Fig. 3.3). Note that this discussion regarding negative feedback control only pertains to LH physiology. Control of FSH and Sertoli cells occurs in an analogous manner and, however, involves additional factors—primarily activin and inhibin B—which do not primarily affect LH secretion and which will not be discussed here.

One well-designed human study illustrates the interplay between testosterone, estrogen, and the aromatization of testosterone to estrogen in the negative feedback of LH secretion [38]. Prior to completion of this study, the respective contributions of these sex steroids to negative feedback control of LH were unclear. Furthermore, the precise sites of inhibition via testosterone and estrogen were uncertain. Thirteen men with idiopathic hypogonadotropic hypogonadism (IHH), who do not produce GnRH at baseline but who may be normalized with exogenous pulsatile GnRH administration, allowed the study authors to better answer these remaining questions. By measuring the peripheral LH levels of these individuals in response to exogenous sex steroids and then comparing these values to healthy volunteers, a site of action as well as the relative contributions of testosterone and estrogen could be inferred.

From the work of Pitteloud et al. several conclusions can be made: (1) Testosterone and estrogen independently inhibit LH secretion, (2) Testosterone does require aromatization to estrogen to inhibit LH secretion at the pituitary but not the hypothalamus, and (3) While estrogen can act at either location it predominantly functions at the level of the hypothalamus [38]. While these discoveries do not hold

immediate clinical applications, they do clarify a long-standing source of confusion and better elucidate the contributions of testosterone and estrogen to negative feedback control.

Altered Testosterone Physiology

Previous discussion has focused on the physiology of testosterone production in the normal male. There are two instances of altered physiology that warrant particular attention because of their increasing prevalence: the metabolic syndrome and the aging male.

Metabolic Syndrome

Changes in diet and reductions in physical activity have resulted in a rising wave of global obesity, not only within developed countries but now around the world [39]. Not only does one's total body fat predict important comorbidities such as coronary artery disease, stroke, and diabetes, but the distribution of body fat also makes a difference as individuals with a greater percentage of visceral fat appear to have an increased risk of metabolic consequences, as in the metabolic syndrome [40]. Hypogonadism is often seen in this picture, and it has been suggested that testosterone replacement may improve lipid profiles and insulin resistance in men with the metabolic syndrome [41]. The hypogonadal-obesity cycle attempts to explain this relationship: increased adipose tissues lead to greater testosterone deficiency through increased conversion of testosterone to estradiol by aromatase. This relative deficiency of testosterone and excess of estradiol, in turn, leads to even greater fat deposition and subsequent further declines in testosterone [42]. In addition, overall abdominal obesity may lead to increased glucocorticoid turnover and production with disruption of the HPG/adrenal axis, thereby leading to mild hypogonadism [43].

Of interest, two of the paracrine signals discussed earlier—leptin and ghrelin—have been studied in some detail with regard to the metabolic syndrome. Leptin has been demonstrated to increase in obese individuals with an attendant fall in serum testosterone [42, 44]. Administration of exogenous testosterone appears to suppress leptin levels, but this effect is short-lived and leptin levels return to the pre-therapy range after cessation of testosterone [45]. The data regarding ghrelin is more limited; however, there is evidence that suggests that testosterone replacement raises ghrelin levels back to their normal range [42].

The Aging Male

After the age of 40, serum testosterone declines at a rate 0.4–2.6 % per year with an associated decrease in muscle mass, strength, sexual function, and bone mass [46]. This decline in testosterone independently predicts disturbances in insulin and glucose metabolism, potentially leading to the metabolic syndrome as discussed previously [47]. However, not all men will exhibit clinically significant symptoms associated with this decline in testosterone levels [48]. Using data from the Boston Area Community Health Survey (BACH), Araujo et al. found 24 % of their 1,475 subjects aged 30–79 to have low total testosterone (<300 ng/mL) and 5.6 % of the 1,475 patients to be symptomatic [46]. Prevalence of low testosterone increases with age. This is reflected by estimates obtained from the Massachusetts Male Aging Study (MMAS), an observational cohort of 1,709 subjects aged 40–70 and enrolled between 1987 and 1989 with two separate follow-up phases. While the initial crude prevalence of androgen deficiency at baseline was 6.0 %, this increased to 12.3 % during the first follow-up phase of the study between 1995 and 1997 [48].

Why there is a range in testosterone decline and its associated symptomatology remains an area of interest. For instance, in contradiction to the typical aging male with a diminishing testosterone and minimally elevated LH, there is a population of men who mount a large enough rise in their LH to maintain a normal serum testosterone [49]. An increased amount of attention is being paid to these men and to the androgen receptor itself, with particular research being devoted to the number of CAG trinucleotide repeats present in the transactivation exon of this gene. There is a demonstrated inverse correlation between the number of CAG repeats and both the transcriptional activity of the androgen-dependent genes and their downstream effects [50–52]. Furthermore, men with normal total testosterone concentrations and longer CAG repeats run a greater risk of developing andropausal symptoms [52].

Summary

Testosterone production is a finely balanced process with many points of potential regulation starting with the translocation of cholesterol across the mitochondrial membrane and ending with the ultimate negative feedback of testosterone in the HPG axis. Two scenarios in which this physiology is altered are becoming increasingly, clinically relevant: metabolic syndrome and the aging male.

References

1. Brown-Sequard CE. The effects produced on man by subcutaneous injections of liquid obtained from the testicles of animals. Lancet. 1889;134(3438):3.
2. Freeman ER, Bloom DA, McGuire EJ. A brief history of testosterone. J Urol. 2001;165(2): 371–3.
3. Petersen PM, Pakkenberg B. Stereological quantification of Leydig and Sertoli cells in the testis of old and young men. Image Anal Stereol. 2000;19(3):215–8.
4. Lejeune H, Habert R, Saez JM. Origin, proliferation and differentiation of Leydig cells. J Mol Endocrinol. 1998;20(1):1–25.
5. Nistal M, Paniagua R, Regadera J, et al. A quantitative morphological study of human Leydig cells from birth to adulthood. Cell Tissue Res. 1986;246(2):229–36.
6. Prince FP. Ultrastructural evidence of mature Leydig cells and Leydig cell regression in the neonatal human testis. Anat Rec. 1990;228(4):405–17.
7. Weinbauer G, Gromoll J, Simoni M, et al. Physiology of Testicular Function. In: Nieschlag E, Behre HM, Ahlen HV, editors. Andrology: male reproductive health and dysfunction. 2nd ed. New York: Springer; 2001.
8. Payne AH, Hales DB. Overview of steroidogenic enzymes in the pathway from cholesterol to active steroid hormones. Endocr Rev. 2004;25(6):947–70.
9. Clark BJ, Stocco DM. Steroidogenic acute regulatory protein: the StAR still shines brightly. Mol Cell Endocrinol. 1997;134(1):1–8.
10. Manna PR, Dyson MT, Stocco DM. Regulation of the steroidogenic acute regulatory protein gene expression: present and future perspectives. Mol Hum Reprod. 2009;15(6):321–33.
11. Lin D, Sugawara T, Strauss III JF, et al. Role of steroidogenic acute regulatory protein in adrenal and gonadal steroidogenesis. Science. 1995;267(5205):1828–31.
12. Nakajin S, Shively JE, Yuan PM, et al. Microsomal cytochrome P-450 from neonatal pig testis: two enzymatic activities (17 alpha-hydroxylase and c17,20-lyase) associated with one protein. Biochemistry. 1981;20(14):4037–42.
13. Brock BJ, Waterman MR. Biochemical differences between rat and human cytochrome P450c17 support the different steroidogenic needs of these two species. Biochemistry. 1999;38(5):1598–606.
14. Mendel CM. The free hormone hypothesis. Distinction from the free hormone transport hypothesis. J Androl. 1992;13(2):107–16.
15. Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. J Clin Endocrinol Metab. 1999;84(10):3666–72.
16. Willnow TE, Nykjaer A. Cellular uptake of steroid carrier proteins—mechanisms and implications. Mol Cell Endocrinol. 2010;316(1):93–102.
17. Jin Y, Penning TM. Steroid 5alpha-reductases and 3alpha-hydroxysteroid dehydrogenases: key enzymes in androgen metabolism. Best Pract Res Clin Endocrinol Metab. 2001;15(1):79–94.
18. El-Awady MK, El-Garf W, El-Houssieny L. Steroid 5alpha reductase mRNA type 1 is differentially regulated by androgens and glucocorticoids in the rat liver. Endocr J. 2004;51 (1):37–46.
19. Hayes FJ, Crowley Jr WF. Gonadotropin pulsations across development. Horm Res. 1998;49 (3–4):163–8.
20. Matsumoto AM, Gross KM, Bremner WJ. The physiological significance of pulsatile LHRH secretion in man: gonadotrophin responses to physiological doses of pulsatile versus continuous LHRH administration. Int J Androl. 1991;14(1):23–32.
21. Delemarre-van de Waal HA. Regulation of puberty. Best Pract Res Clin Endocrinol Metab. 2002;16(1):1–12.
22. Ascoli M, Fanelli F, Segaloff DL. The lutropin/choriogonadotropin receptor, a 2002 perspective. Endocr Rev. 2002;23(2):141–74.

23. Dufau ML. Endocrine regulation and communicating functions of the Leydig cell. Annu Rev Physiol. 1988;50:483–508.
24. Baker J, Hardy MP, Zhou J, et al. Effects of an Igf1 gene null mutation on mouse reproduction. Mol Endocrinol. 1996;10(7):903–18.
25. Hu GX, Lin H, Chen GR, et al. Deletion of the Igf1 gene: suppressive effects on adult Leydig cell development. J Androl. 2010;31(4):379–87.
26. Lui WY, Lee WM, Cheng CY. TGF-betas: their role in testicular function and Sertoli cell tight junction dynamics. Int J Androl. 2003;26(3):147–60.
27. Svechnikov K, Landreh L, Weisser J, et al. Origin, development and regulation of human Leydig cells. Horm Res Paediatr. 2010;73(2):93–101.
28. Wikstrom AM, Bay K, Hero M, et al. Serum insulin-like factor 3 levels during puberty in healthy boys and boys with Klinefelter syndrome. J Clin Endocrinol Metab. 2006;91(11): 4705–8.
29. Bay K, Hartung S, Ivell R, et al. Insulin-like factor 3 serum levels in 135 normal men and 85 men with testicular disorders: relationship to the luteinizing hormone-testosterone axis. J Clin Endocrinol Metab. 2005;90(6):3410–8.
30. Foresta C, Bettella A, Vinanzi C, et al. A novel circulating hormone of testis origin in humans. J Clin Endocrinol Metab. 2004;89(12):5952–8.
31. Ivell R, Wade JD, Anand-Ivell R. INSL3 as a biomarker of Leydig cell functionality. Biol Reprod. 2013;88(6):147.
32. Zhu CC, Zhang H, Zhang JS, et al. Inhibition of ghrelin signaling improves the reproductive phenotype of male ob/ob mouse. Fertil Steril. 2013;99(3):918–26.
33. Sirotkin AV, Chrenkova M, Nitrayova S, et al. Effects of chronic food restriction and treatments with leptin or ghrelin on different reproductive parameters of male rats. Peptides. 2008;29(8):1362–8.
34. Sun Y, Asnicar M, Saha PK, et al. Ablation of ghrelin improves the diabetic but not obese phenotype of ob/ob mice. Cell Metab. 2006;3(5):379–86.
35. Fernandez-Fernandez R, Tena-Sempere M, Navarro VM, et al. Effects of ghrelin upon gonadotropin-releasing hormone and gonadotropin secretion in adult female rats: in vivo and in vitro studies. Neuroendocrinology. 2005;82(5–6):245–55.
36. Tena-Sempere M. Ghrelin as a pleotrophic modulator of gonadal function and reproduction. Nat Clin Pract Endocrinol Metab. 2008;4(12):666–74.
37. Ishikawa T, Fujioka H, Ishimura T, et al. Ghrelin expression in human testis and serum testosterone level. J Androl. 2007;28(2):320–4.
38. Pitteloud N, Dwyer AA, DeCruz S, et al. Inhibition of luteinizing hormone secretion by testosterone in men requires aromatization for its pituitary but not its hypothalamic effects: evidence from the tandem study of normal and gonadotropin-releasing hormone-deficient men. J Clin Endocrinol Metab. 2008;93(3):784–91.
39. Popkin BM, Adair LS, Ng SW. Global nutrition transition and the pandemic of obesity in developing countries. Nutr Rev. 2012;70(1):3–21.
40. Montague CT, O'Rahilly S. The perils of portliness: causes and consequences of visceral adiposity. Diabetes. 2000;49(6):883–8.
41. Zitzmann M, Nieschlag E. Effects of androgen replacement on metabolism and physical performances in male hypogonadism. J Endocrinol Invest. 2003;26(9):886–92.
42. De Maddalena C, Vodo S, Petroni A, et al. Impact of testosterone on body fat composition. J Cell Physiol. 2012;227(12):3744–8.
43. Walker BR. Steroid metabolism in metabolic syndrome X. Best Pract Res Clin Endocrinol Metab. 2001;15(1):111–22.
44. Al-Harithy RN. Relationship of leptin concentration to gender, body mass index and age in Saudi adults. Saudi Med J. 2004;25(8):1086–90.
45. Luukkaa V, Pesonen U, Huhtaniemi I, et al. Inverse correlation between serum testosterone and leptin in men. J Clin Endocrinol Metab. 1998;83(9):3243–6.

46. Araujo AB, Esche GR, Kupelian V, et al. Prevalence of symptomatic androgen deficiency in men. J Clin Endocrinol Metab. 2007;92(11):4241–7.
47. Laaksonen DE, Niskanen L, Punnonen K, et al. Testosterone and sex hormone-binding globulin predict the metabolic syndrome and diabetes in middle-aged men. Diabetes Care. 2004;27(5):1036–41.
48. Araujo AB, O'Donnell AB, Brambilla DJ, et al. Prevalence and incidence of androgen deficiency in middle-aged and older men: estimates from the Massachusetts Male Aging Study. J Clin Endocrinol Metab. 2004;89(12):5920–6.
49. Harkonen K, Huhtaniemi I, Makinen J, et al. The polymorphic androgen receptor gene CAG repeat, pituitary-testicular function and andropausal symptoms in ageing men. Int J Androl. 2003;26(3):187–94.
50. Chamberlain NL, Driver ED, Miesfeld RL. The length and location of CAG trinucleotide repeats in the androgen receptor N-terminal domain affect transactivation function. Nucleic Acids Res. 1994;22(15):3181–6.
51. Beilin J, Ball EM, Favaloro JM, et al. Effect of the androgen receptor CAG repeat polymorphism on transcriptional activity: specificity in prostate and non-prostate cell lines. J Mol Endocrinol. 2000;25(1):85–96.
52. Liu CC, Lee YC, Wang CJ, et al. The impact of androgen receptor CAG repeat polymorphism on andropausal symptoms in different serum testosterone levels. J Sex Med. 2012;9(9): 2429–37.