# Chapter 5
# How is the synthesis of testosterone regulated?

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Testosterone, the major male sexual hormone, is secreted primarily by Leydig cells of the testis. The amount of testosterone formed by the Leydig cells reflects specific needs of the body for cell growth, organ formation, masculinization, and maintenance of androgen-dependent functions. During development, the differentiation of Leydig cell precursors leads to the establishment of specific Leydig cell populations that are responsible for the formation of the amounts of testosterone needed at various ages. In the fetus, Leydig cells are considered the main source of testosterone essential for sexual differentiation and the prenatal masculinization of the male urogenital system. However, additional sources have emerged from studies in mouse, where fetal Sertoli cells were shown to generate testosterone from Leydig cell-produced androstenedione. Moreover, the existence of a "human backdoor pathway of androgen synthesis" was recently described, in which androsterone produced from placental progesterone may be as critical for human fetal masculine-ization as testosterone produced by fetal Leydig cells and dihydrotestosterone. After birth, the fetal Leydig cell population disappears and the adult Leydig cell population, evolving in an environment free of maternal factors, develops from a small pool of undifferentiated, self-renewing stem Leydig cells via a sequence of stages that include Leydig cell progenitors, immature Leydig cells and adult Leydig cells. These changes reflect the need for testosterone for development of male characteristics, puberty, and androgen-dependent functions. Changes in cell structure and gene expression are responsible for the morphological and functional differences among these various cell types; the basic components of the steroidogenic machinery are present but not used to the same extents. Thus, testosterone production changes during development are due to alterations in the cellular environment and are designed for the optimal production of testosterone for specific biological needs.

How is the synthesis of testosterone regulated?

The conversion of cholesterol to testosterone is a tightly regulated process influenced by the pituitary gonadotrophin luteinizing hormone (LH). For Leydig cells to respond to LH and to function optimally, it is critical that the integrity of proteins involved in steroidogenesis, from the LH receptor to cholesterol transporting

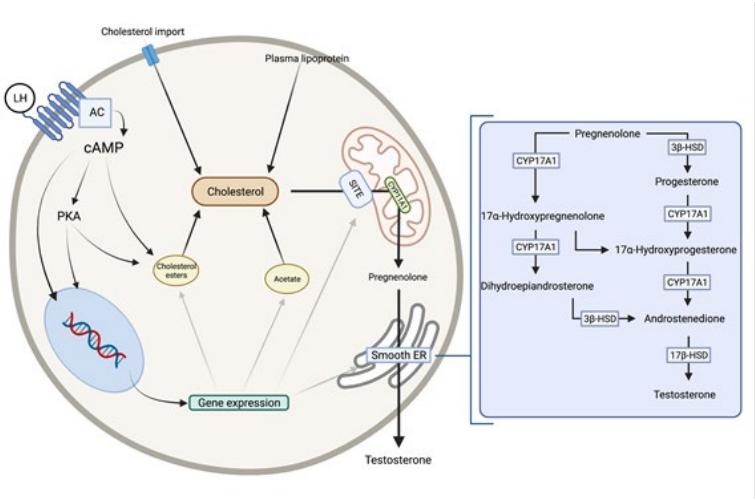


**Figure 1**. Schematic representation of the steps involved in testosterone formation. Luteinizing hormone (LH) binds to a G-protein coupled receptor leading to activation of adenylate cyclase (AC) that produces cAMP, the major second messenger of LH action in Leydig cells. cAMP subsequently activates the cAMP-dependent protein kinase (PKA), an event that triggers a series of reactions including the de-esterification of cholesterol from lipid droplets and activation of proteins, PKA substrates, involved in cholesterol transport into mitochondria. cAMP, as well as PKA act also in the nucleus activating steroidogenic protein and enzyme gene expression. Free cholesterol is transported and imported into mitochondria via SITE formed to amplify the effect of LH and cAMP. This complex included proteins such as the steroidogenesis acute regulatory protein (STAR), translocator protein (18-kDa; TSPO), and voltage-dependent anion channel (VDAC) 1. Imported cholesterol is metabolized to pregnenolone by the cytochrome P450 side chain cleavage (CYP11A1). Pregnenolone is subsequently metabolized in the smooth endoplasmic reticulum by a series of enzymes 3β-hydroxysteroid dehydrogenase (3β-HSD), CYP17A1 and 17β-hydroxysteroid dehydrogenase (17β-HSD) to form testosterone.

proteins and steroidogenic metabolizing enzymes, are maintained. There are several key points that are critical for the establishment and optimal function of the steroidogenic machinery responsible for testosterone synthesis in the Leydig cell (Fig. 1).

(i) Integrity of the plasma membrane LH receptor signal transduction cascade responsible for sensing and responding to the blood-borne LH. The LH receptor is a G protein-coupled receptor whose activation by LH upregulates the production of cyclic adenosine 3',5'-monophosphate (cAMP). This step precedes and initiates cholesterol mobilization and activation of transcription factors that upregulate steroidogenic genes.

(ii) Availability of sufficient amounts of the substrate cholesterol coming from the blood or synthesized de novo. Leydig cells can synthesize cholesterol de novo from acetate or source it from plasma lipoprotein, cholesterol esters, and the plasma membrane for testosterone biosynthesis. Leydig cells can also use receptor-mediated endocytic uptake to acquire lipoprotein-derived cholesterol (LDL, HDL). The de-esterification of stored cholesterol provides an ample pool of substrate for steroidogenesis.

(iii) Integrity of the mechanism responsible for transporting cholesterol from intracellular stores into mitochondria. An initial protein scaffold known as the transduceosome, comprising cytoplasmic and outer mitochondrial membrane (OMM) proteins, receives cholesterol via vesicular or non-vesicular pathways and responds to LH/cAMP. Hormone-induced proteins will join this scaffold to accelerate cholesterol import. OMM proteins further interact with proteins spanning the OMM and inner mitochondrial membrane (IMM), such as VDAC 1, mediating cholesterol loading onto the first enzyme of the steroidogenic cascade, the cytochrome P450 side chain cleavage (CYP11A1). The larger steroidogenic complex that encompasses cytoplasmic, OMM and IMM proteins is defined as the Steroidogenic InteracTomE (SITE). Some of the key proteins of the SITE include STAR, TSPO, VDAC, and 14-3-3 adaptor proteins,

(iv) Availability of appropriate levels and combinations of the nuclear transcription factors controlling the expression of proteins involved in cholesterol transport and in testosterone biosynthesis. The differential expression of steroidogenic enzymes is regulated by numerous transcription factors. These precisely regulate steroid output and Leydig cell function, preventing

either testosterone insufficiency or over production.

(v) Maintenance of appropriate organelle structures required for optimal testosterone formation. Steroidogenic enzymes reside in the mitochondria and smooth endoplasmic reticulum. The organelles' integrity and proper function are essential for normal steroid formation.

(vi) Appropriate spatial and temporal expression of steroidogenic enzymes. Cytochrome P450 monooxygenases and de-hydrogenases are responsible for metabolizing cholesterol to various intermediates leading to testosterone formation. The availability of the co-factors is also necessary for steroidogenic enzyme action.

The concepts of transduceosome and SITE have been instrumental in understanding the regulation of Leydig cell testosterone production, as well as that of steroidogenic adrenal cortical cells, which share the main steps of cholesterol transport and steroidogenic cascade. The identification of the components of the SITE complex, uncovering the spatial organization and interactions of the cytoplasmic and OMM elements, and the relationships between OMM and IMM proteins in response to hormone, have led to a better understanding of this dynamic and plastic network of proteins converging for optimal steroid hormone biosynthesis. This process can be altered by several factors. In aging, various components of the steroidogenic machinery fail to function at an optimal level, leading to a decline in androgen formation. In some cases, this can lead to significantly reduced testosterone, a condition known as hypogonadism. Indeed, the integrity of the transduceosome seems to be compromised in hypogonadism leading to Leydig cell dysfunction. The accumulation of fat mass, declining energy, alterations in mood, and decreased bone mineral density are common symptoms of low testosterone levels. Administering exogenous testosterone, testosterone replacement therapy, is commonly used to ameliorate many hypogonadism symptoms. However, monitoring and maintaining optimal testosterone levels is challenging and adverse effects have been observed, including polycythemia, peripheral edema, as well as cardiac and hepatic dysfunction. Stimulating Leydig cells to increase testosterone production is an active area of research with several identified drug targets and novel chemical entities under investigation. Stem cell-based therapy to re-establish androgen producing Leydig cells in the body has also been an active area of research for the treatment of hypogonadism.

How is the synthesis of testosterone regulated?

Inborn errors in steroid biosynthesis in the testis and the adrenal cortex are linked to mutations that can be lethal or lead to disease states such as pseudohermaphroditism, hypogonadism, and infertility. These mutations can impact numerous stages in the steroidogenic pathway, such as cholesterol transport, and steroid metabolism.

(i) Mutations in the LH receptor cause either overactivation or inactivation and disrupt the development of secondary sex characteristics. Activating mutations stimulate Leydig cells during fetal and prepubertal stages, causing autonomous testosterone production and early onset of puberty. Antiandrogen and aromatase inhibitors are effective at restoring normal prepubertal development. Mutations causing inactivation of the LH receptor result in resistance to LH stimulation and Leydig cell hypoplasia (LCH). LCH patients display varying symptoms from hypogonadism to pseudohermaphroditism.

(ii) Congenital adrenal hyperplasia (CAH) is a rare heritable disorder caused by mutations in enzymes of the steroidogenic pathway, most commonly 21-hydroxylase, and impacts nearly 1 in 5000-18000 children worldwide. CAH is characterized by reduced cortisol and aldosterone, and increased progesterone, 17-OH-prog, and sex steroids resulting in early virilization of the male.

(iii) Rare cases of 17α-hydroxylase (CYP17A1), 3β-hydroxysteroid dehydrogenase and 20,22-desmolase (part of CYP11A1) have been linked to altered androgen formation and ambiguous genitalia in boys.

(iv) Mutations in the steroidogenic acute regulatory protein (STAR), an essential protein in cholesterol transport, cause mineralocorticoid deficiency and a lipoid congenital adrenal hyperplasia phenotype among patients STAR mutations also cause CAH conditions that result in the buildup of lipid droplets in Leydig cells.

(v) TSPO mutations limit the translocation of cholesterol into the mitochondria and cause esterified cholesterol accumulations and disruptions to steroid formation

(vi) Mutations in steroidogenic factor 1, which drives the expression of many steroidogenic genes, may also result in testicular failure leading to disorders of sex development.

Testosterone production is also influenced by external factors such as drugs and environmental compounds. Numerous pharmaceuticals, agricultural and industrial chemicals act as endocrine disrupting compounds (EDCs) that can affect male reproductive functions and health, transcriptional regulation, or androgen receptor binding EDCs exposure can occur via ingestion, inhalation, or skin absorption. Bisphenols, perfluoroalkyls, phthalates, flame retardants, fungicides, herbicides and parabens, as well as dietary natural compounds have been reported to exert EDC properties on components of the steroidogenic cascade, such as LH signal transduction, cholesterol transport, and steroidogenic enzymes. The risk impact of EDCs exposure on steroidogenesis is not fully understood, given their ability to disrupt various signaling mechanisms within Leydig cells and other testicular cell types involved in male reproductive functions. Studies evaluating the impact of individual EDC's exposure on steroidogenesis may not accurately represent the risk of EDC mixtures and their metabolites, found at detectable levels in blood, such as phthalate and pesticide mixtures reported to induce reproductive defects in a cumulative manner despite individual doses being below the no-observed-adverse-effect levels.

Although the pathway of testosterone formation and its regulation by LH, as well as its susceptibility to drugs and the environment are now well established, there are many steps yet to be defined in the physiology and pathology of this complex process. In particular, the possible existence of adaptative, alternate or redundant mechanisms, especially during the developmental periods in response to environmental stressors and in aging, leave the field open for further investigations.

## Suggested reading

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