Anabolic-androgenic steroids (AAS):

Anabolic-androgenic steroids are synthetically derived compounds based upon the structure of male sex hormone testosterone (Thiblin and Petersson, 2005). To strengthen the anabolic properties of testosterone, more than 100 synthetic steroid derivatives have been described for human purposes. The anabolic effect promotes protein synthesis, muscle growth and erythropoiesis. In clinical practice, substances with anabolic effect are needed to overcome various catabolic states. However, none of these compounds are devoid of androgenicity. Androgenic and anabolic properties of anabolic steroids cannot be totally separated. Therefore, it is more appropriate to use the term anabolic androgenic steroids (Yesalis, 1993; Shahidi, 2001).

Testosterone and its derivatives are well absorbed from the gastrointestinal tract but are rapidly metabolized during hepatic first-pass metabolism without reaching systemic circulation. Testosterone is inactivated primarily by the cytochrome P450 hepatic isoenzyme (Fotherby and James, 1972). To increase systemic availability, AAS are modified as injectable 17β-esters or orally administered 17α-alkylated steroids. Orally administered testosterone undecanoate also avoids hepatic metabolism because it is absorbed from the alimentary canal through the lymphatic system. After absorption from the gastrointestinal tract during hepatic first-pass metabolism, testosterone and AAS undergo biotransformation and are partly excreted via bile to the faeces. Testosterone in systemic circulation is also prone to metabolism in the liver, and once excreted, the steroid can be reabsorbed from the gastrointestinal tract. In peripheral tissues, testosterone is susceptible to glucuronization to androsterone and etiocholanolone, two major metabolites of testosterone, which are excreted to urine (Fotherby and James, 1972; Gagliardi, 1991). In vivo, different AAS are also potential
targets for aromatization and reduction. Since the AAS molecule is susceptible to such enzymatic conversion, it possesses various biological properties (Yesalis, 1993).

Fig. II.1: Chemical Structures of some of the common Anabolic Steroids (Fragkaki et al., 2009).
Chemical structure of AAS:
The chemically synthesized AAS are structurally related to the native testosterone molecule. They were designed to enhance the protein anabolic effect of testosterone while reducing the unwanted androgenic effect, and to improve the pharmacological properties of the molecule. Accordingly, AAS can roughly be divided into three groups:

i. Analogs produced via esterification of the 17β-hydroxyl group.

ii. Analogs that have been alkylated at the 17α-position.

iii. Analogs where A, B or C rings are modified.

Esterification of the 17β-hydroxyl group with carboxylic acids enables intramuscular administration of the drugs. Alkylation at the 17α position protects steroid against first pass metabolism and enables its oral use. Most of the 17-alkylated AAS are methyl derivatives but ethylated and ethinylated derivatives also exist. The great majority of AAS has structural modification in either A, B or C rings (Basaria et al., 2001). These steroids are often available for oral use. The most common modifications are introduction of a double bond between C-1 and C-2 and reduction of the double bond.

Mechanism of action:
The effects of AAS on genes and consecutive gene expression are poorly understood. Recently, human myostatin has been cloned and is considered to be a negative regulator of muscle growth. Basaria et al. (2001) speculated that AAS might act by influencing myostatin concentration. Further, all tissues are susceptible to androgen action. No tissues are devoid of androgen receptors, and all androgen receptors distributed throughout the body possess the same binding affinity for a particular steroid.
Receptor-binding studies have not demonstrated marked differences between AAS in receptor-binding affinity. Young adolescents are more susceptible to androgen action of AAS because they possess a higher number of cytosol androgen receptors. Even with the biologically active unbound fraction of testosterone in circulation, androgen receptor sites are already saturated in striated muscles (Sheridan, 1983; Huhtaniemi et al., 1992).

Supraphysiological doses of AAS induce gain in muscle size and strength, even without concomitant exercise. At a supraphysiological dosage, AAS interacts with various receptors, including progesterone, estrogen, and mineralo- and glucocorticoid receptors (Alen et al., 1984; Bhasin et al., 1996; Giorgi et al., 1999).

Supraphysiological doses of AAS have been speculated to mediate their anabolic action through interaction with glucocorticoid receptors by preventing glucocorticoid’s catabolic effect (Hickson et al., 1990; Rogol and Yesalis, 1992; Haupt, 1993). Testosterone has in fact been shown to have a high affinity for glucocorticoid receptors and in vivo it acts as an antagonist to endogenous circulating glucocorticoids (Danhaive and Rousseau, 1986, 1988). AAS lower the levels of certain hormone-binding proteins in circulation. Thyroxine, cortisol, sex hormone, growth hormone and D-vitamin-binding globulin concentrations in circulation are decreased after AAS administration (Barbosa et al., 1971; Small et al., 1984; Ruokonen et al., 1985; Alen et al., 1987; Karila et al. 1998). Alterations in carrier protein concentration levels may increase biologically active steroid concentrations. It is hypothesized that AAS-mediated anabolism could be partly due to increased concentrations of circulating biologically active human growth hormone (GH) and insulin-like growth factor-I (IGF-I), particularly when supraphysiological doses are used (Hobbs et al., 1993; Karila et al., 1998). Alen et al. (1987) found 5 to 60 times higher serum GH concentrations in
subjects on AAS administration, even without concomitant use of exogenous GH. Local stimulation of IGF-I may be required in the process of anabolic action (Fryburg, 1994). Androgens are known to be needed in local production of IGF-I within the skeletal muscle (Mauras et al., 1998). These could partly explain the mechanism of action of supraphysiological doses of AAS. On the other hand, these findings might also provide an explanation for the unpredictable adverse effects of AAS (Bahrke et al., 1996; Parssinen and Seppala, 2002).

Anabolic androgenic steroids have established their usefulness in treating various types of anaemia, osteoporosis, androgen replacement therapy, muscle-wasting conditions, cachexia caused by various cancers, and HIV infection. Long-standing hypogonadism in adult males is associated with reduced bone remodelling and decreased bone formation. In treating muscle-wasting disorders with AAS, none of AAS preparations has proved to be superior to another (Francis et al., 1986; Strawford et al., 1999; Shahidi, 2001; Parssinen and Seppala, 2002). Recently, AAS have been studied for male andropause replacement therapy, but more studies are required before AAS can be used broadly for improving the quality of life of ageing males (Swerdloff et al., 1992).

Anabolic androgenic steroid-induced adverse effects:
AAS have been extensively studied and it is found that within clinical dosages, they are well tolerated. Many of the AAS-induced adverse effects are reversible. Most adverse effects are gender-dependent, females, for instance, experiencing virilizing effects (Shahidi, 2001). In the literature, there are numerous case reports of myocardial infarction (McNutt et al., 1988; Ferenchick and Adelman, 1992; Huie, 1994), coronary atherosclerosis (Mewis et al., 1996), sudden death (Fineschi et al., 2001), congestive heart disease, serious arrhythmia (Appleby et al., 1994; Nieminen et al., 1996), atrial
fibrillation (Sullivan et al., 1999), intraventricular thrombosis, pulmonary embolus (Gaede and Montine, 1992) and arterial and venous thrombosis (Ferenchick, 1991) associated with AAS abuse. AAS abuse has also been associated with hepatic dysfunction and various neoplasias. Alen (1985) reported that the use of AAS significantly increased serum concentrations of hepatic amino transferases, although measurements remained within normal limits. He concluded that sustained high-dose use of AAS produces mild impairment in liver function. AAS do not, however, cause irreversible damage to liver function (Zimmerman and Lewis, 1987). Previous reports stating that AAS administration causes hepatic dysfunction are mainly based on elevated serum amino transferase concentrations (Dickerman et al., 1999). Cholestatic jaundice is related to use of 17α-alkylated AAS but not to structurally different steroids. Peliosis hepatitis (dilated hepatic venous sinuses), by contrast, is not related to C17-alkylation, but manifesting with testosterone administration. Evidences are available for AAS promoting tumour formation in mice by enhancing the effects of carcinogens (Lesna and Taylor, 1986), without AAS being mutagenic in the Ames test (Ingerowski et al., 1981).

AAS have proven to be aetiological factors for some cancers. According to Chen et al. (1997), AAS are included as a risk factor for hepatocellular carcinoma together with viral hepatitis, alcohol consumption and some genetic factors. Benign hepatic neoplasia, diffuse hyperplasia, nodular regenerative hyperplasia and focal nodular hyperplasia have also been attributed to the use of 17α-alkylated AAS (Ishak and Zimmerman, 1987). Histologically, a rare androgen-specific form of a hepatic tumour can be distinguished in man that appears to act more like a benign hepatocellular adenoma (Anthony, 1975; Craig et al., 1989). Interestingly, these androgen-related tumours have
a tendency to regress after androgen medication has ceased (Cocks, 1981; Drew, 1984; McCoughan et al., 1985). Hepatocellular carcinoma is connected to long-term treatment with AAS (Ishak and Zimmerman, 1987). However, the malignant nature of AAS-induced hepatocellular carcinoma is questionable since regression occurs in the majority of cases after withdrawal of AAS administration. In addition, there is contradictory evidence about the role of androgens in prostate cancer (Signorello et al., 1997). AAS have also been associated with development of soft tissue sarcomas (Zahm and Fraumeni, 1997). While clear convincing evidence of the mutagenicity of AAS is still lacking, they do at least possess tumour growth-promoting activity.

Depending on the administration route, infections at the injection site of bacterial or fungal aetiology have been reported. There is also an increased risk of hepatitis and AIDS as a result of shared needles and syringes (Rich et al., 1999). AAS abuse is also reported to increase the prevalence of acne formation. AAS abuse is associated with various psychiatric and behavioural effects. One-fourth of AAS abusers report major mood syndromes, such as mania, hypomania or major depression (Pope and Katz, 1994). Many of the studies in this area clearly demonstrate that increased aggression and irritability are associated with AAS abuse (Bahrke et al., 1990). Moreover, evidence exists that severity of psychiatric adverse effects is dose-related (Porcerelli and Sandler, 1998). However, contradictory reports suggest that at least some psychiatric symptoms are associated with life-style and exercise regimen (Bahrke and Yesalis, 1994). Various psychiatric symptoms are related to withdrawal of AAS, and these increase AAS dependence (Brower et al., 1991).

AAS are derivatives of testosterone and, via negative feedback to the hypothalamus, they induce hypogonadotrophic hypogonadism associated with decreased serum
testosterone concentrations (unless exogenous testosterone used), testicular atrophy, impaired steroidogenesis and spermatogenesis (Kilshaw et al., 1975, Schurmeyer et al., 1984, Jarow and Lipshultz, 1990). There is a marked depression of serum testosterone and sex hormone-binding globulin (SHBG), especially when C17α-alkylated steroids are used, as well as of gonadotrophins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (Sader et al., 2001). LH and FSH control steroidogenesis and spermatogenesis, and their secretion is regulated by gonadal steroids and inhibin through negative feedback (de Kretser et al., 1998, 2000). LH and FSH secretion are equally suppressed after 4-6 months of testosterone administration at a physiological to moderately supraphysiological dosage (25-300 mg/week) (Matsumoto, 1990). Normal spermatogenesis requires a 50-fold higher androgen concentration in the testis than in the peripheral serum (Adamopoulos et al., 1984; Turner et al., 1984). AAS also induce changes in other hormone levels and endocrinological systems, probably mediated by multiple receptor interactions, but these effects seem to be reversible (O’Connor et al., 1990; Brower, 1993).

Effects on male fertility:

Androgens have been used to treat male subfertility, but inadequate evidence exists to evaluate the usefulness of androgens for this purpose (Vandekerckhove et al., 2000). A few studies have also been published on AAS abuse-induced impaired fertility during a steroid cycle and following the cessation of abuse. In these reports, lowered fertility has always been reversible (Yesalis, 1993). AAS abuse induces oligozoospermia and sometimes azoospermia (Schurmeyer et al., 1984). Torres-Calleja et al. (2001) showed that abuse of AAS not only reduces the concentration of sperm, but in some subjects also impairs the percentage of morphologically normal semen. Lower semen density is
supposed to occur because of AAS-induced hypogonadotrophic hypogonadism. No reports of the direct effects of AAS on testicular semen production are available.

In healthy male subjects, human chorionic gonadotrophin (HCG) used alone at a dosage of 5000 IU three times a week can maintain normal spermatogenesis (Matsumoto et al., 1983). The role of FSH in spermatogenesis is controversial, but at least it has a qualitative role in human spermatogenesis (Tapanainen et al., 1997). However, long-term HCG treatment has been shown to suppress spermatogenesis in an experimental model (Cusan et al., 1982), and HCG has a direct effect on spermatogenesis which leads to poorer sperm quality (Dunkel et al., 1997). Large variability exists in interindividual fertility, and thus the androgen dose required to suppress spermatogenesis also varies substantially between individuals (Schurmeyer et al., 1984; Knuth and Nieschlag, 1987). Despite using a moderately large testosterone dosage (300mg/week), azoospermia is not reliably achieved in normal men, further, this dosage failed to stimulate spermatogenesis (Matsumoto, 1990).

**Chemistry of nandrolone decanoate:**

Nandrolone (19-nortestosterone) was synthesized in 1950 (Birch, 1950) and 1953 (Wilds and Nelson, 1953) (Fig. II.2). Its metabolism is similar to that of testosterone (Engel et al., 1958) and its main metabolites are found to be 3α-hydroxy-5α-estrane-17-one (norandrosterone) and 3α-hydroxy-5β-estrane-17-one (noretiocholanolone), the structures of which were elucidated by Kupfer et al. (1960). Besides these metabolites, a 3β-hydroxy isomer, 3β-hydroxy-5α-estrane-17-one, is also excreted into urine as a 3β-sulfate in an amount similar to that of the 3α-hydroxy metabolites (Fig. I.2).
Despite its similarity to testosterone, nandrolone is found to be more anabolic. The substitution of a hydrogen atom in the C19 methyl group of testosterone creates a new asymmetric centre at C10, which is responsible for the drugs’ favourable anabolic to androgenic ratio.

**Oxidative stress:**

Free radicals are chemical species possessing an unpaired electron that can be considered as fragments of molecules and which are generally very reactive. They are produced continuously in cells either as accidental by-products of metabolism or deliberately during phagocytosis. The most important reactants in free radical biochemistry in aerobic cells are oxygen and its radical derivatives (superoxide and hydroxyl radical), hydrogen peroxide and transition metals. Cells have developed a comprehensive array of antioxidant defenses to prevent free radical formation or limit their damaging effects. Reactive free radicals formed within cells can oxidize biomolecules and lead to cell death and tissue injury (Cheeseman and Slater, 1993). Reactive free radicals or reactive oxygen species generation often exceeds the cell’s antioxidant capacity, resulting in a condition termed oxidative stress.

Over the past few decades, free radicals that are highly reactive and destructive molecules and their simultaneous effect called oxidative stress have come to be
appreciated increasingly for their importance in human health and disease. Many common and life threatening human diseases, including cancer, atherosclerosis and ageing have free-radical reactions as underlying mechanisms of injury. Over this period of time, the conceptual understanding of the interaction of such reactive oxygen species (ROS) with living organisms has undergone a remarkable evolution (Halliwell and Cross, 1991).

In the modern terminology, a free radical is defined as any atom, group of atoms or molecules having at least one unpaired electron in its outermost orbital which alters the chemical reactivity of the atom or molecule, usually making it more reactive than the non-radical. Free radicals have cationic, anionic or neutral characteristics and are extremely reactive. Gamma radiation, UV rays, environmental pollutants and certain pharmaceutical drugs are among the many exogenous initiators of free radical reaction. However, the most important source of these radical species in vivo are univalent biochemical redox reactions involving oxygen (Halliwell and Gutteridge, 1984).

Oxygen is required to transform various substrates for the release of energy to oxidize endogenous compounds and to detoxify xenobiotics. During this process, oxygen acts as a terminal 4-electron acceptor and is eventually converted to more stable chemical state water. Some biological reduction of oxygen occurs by the monovalent pathway and necessarily produces first superoxide radical (O$_2^\cdot$) and hydrogen peroxide (H$_2$O$_2$) and then, if these are not efficiently scavenged, hydroxyl radical (OH) and singlet oxygen are formed as well. Living cells cannot tolerate the high quantity of these highly reactive products.

The hydroxyl radical, in particular, is incredibly reactive and is the third intermediate in the monovalent pathway of oxygen reduction. Its production can be avoided by
efficiently removing the first two namely $\text{O}_2^-$ and H$_2$O$_2$. Molecular oxygen or dioxygen is a stable triplet diradical in the ground state with a kinetic preference for undergoing radical reaction such as initial univalent reduction in enzymatic reaction where H$_2$O$_2$ is formed. Free radicals damage the cell parts (cell membrane, nuclear membrane, DNA, membrane lipids and proteins) by:-

- Breaking off membrane proteins and thus destroying the cell’s identity.
- Hardening the cell by fusing together membrane lipid and proteins.
- Puncturing the cell membrane viruses and bacteria can enter.
- Disrupting the nuclear membrane and thus opening up the nucleus and exposing the genetic material.
- Mutating and destroying genetic material.
- Damaging cells of the immune system and thus threatening its integrity. Among the most important of these are the actions of free radicals on the fatty acid side chains of lipids in the various cellular membrane especially mitochondrial membrane (which are directly exposed to the superoxide anions produced during cellular respiration) (Witting, 1965).

Reactive oxygen species (ROS), whether produced endogenously as a consequence of normal cell function or derived from external sources, pose a constant threat to cells living in an aerobic environment as they can result in severe damage to DNA, protein and lipids (Martindale and Holbrook, 2002). The reactions of activated oxygen with organic substrates are complex even in vitro with homogeneous solutions, but in biological systems, there are even more complications due to the surface properties of membranes, electrical charges, binding properties of macromolecules, and compartmentalization of enzymes, substrates and catalysts. Thus, various sites even
within a single cell differ in the nature and extent of reactions with oxygen. The nature of the oxidative injury that causes cell death is not always obvious. The mechanisms by which oxygen radicals damage membrane lipids are well accepted and consequently oxidative damage is often exclusively associated with peroxidation reactions in membrane lipids as well as oxidative degeneration of proteins and nucleic acids. Thus, Reactive oxygen species (ROS) cause lipid peroxidation and oxidation of some specific proteins, thereby affecting many intra and intercellular systems (Mates and Sanchez-Jimenez, 2000). Oxidatives stress can be measured by monitoring the change in blood and tissue malondialdehyde and carbonyl content (Durdi et al., 2005).

**Lipid peroxidation:**

Lipid peroxidation can be defined as the oxidative deterioration of lipids containing any number of carbon-carbon double bond (Kale and Sitaswad, 1990). Among the different components associated with membrane lipids, the unsaturated fatty acid essential for formation of either phospholipids or sphingolipids is the most susceptible target for oxidative stress induced peroxidation (Witting, 1965). Lipid peroxidation proceeds by an auto catalytic chain reaction since peroxide products of the reaction also acts as initiator. Lipid peroxidation is a radical initiated chain reaction that is self-propagating in cellular membranes. Autooxidation can be triggered by diverse agents such as –

a) Redox metal ion as iron or copper in the presence of reductants such as thiols or ascorbates,

b) Ionizing or ultraviolet radiation, and

c) Photoactivated dyes and pigments.

Non-radical reactions are also known to occur involving singlet molecular oxygen as the primary oxidant (Halliwell and Gutteridge, 1984). Lipid peroxidation may also be
driven enzymatically, important example being (i) xanthine oxidase induced reaction (Kellog and Fridovich, 1975; Lynch and Fridovich, 1978) (ii) the NADPH-cytochrome p450 reductase-dependant reaction in microsomes and (iii) cyclooxygenase dependent peroxidation of arachidonate to prostaglandin and thromboxane precursors.

Lipid peroxidation involves three discrete phases of chain reactions that include initiation, propagation and termination. In the course of LPO initiation, activated oxygen species are produced as a result of consequent one-electron reduction. These species are the oxygen radicals, superoxide anion-radical and the hydroxyl radical. These oxyradicals are formed with the participation of transient metal ions, which often takes place outside the hydrophobic zone of the membrane on its surface or in aqueous phase. The superoxide anion-radicals have a relatively low reactivity, as a result of which their interaction with the membrane phospholipids is not very important for the initiation of LPO reaction (Witting 1965; Halliwell and Gutteridge, 1984, 1985). Conversely, the interaction of highly reactive OH radicals with membrane lipids results in the formation of intermediate free radical products of a lipid nature: Alkyl (L), alkoxy (LO⁻) and peroxyalkyl (LO₂⁻) radicals. This stage of the LPO process takes place in the hydrophobic zone of the membrane. The maintenance and development of the LPO process as well as the involvement in it of newer lipid substrates are guaranteed by the constant “regeneration” of LO⁻ and LO₂⁻ radicals and by their interaction with membrane phospholipids. An important source of such lipid radicals are the primary molecular LPO products, hydroperoxides, which are decomposed into alkoxy or peroxyalkyl radicals in the presence of transition metals (Halliwell and Gutteridge, 1984).
The lipid bilayer membrane is composed of a mixture of phospholipids and glycolipids that have fatty acid chains attached to carbon 1 and 2 of the glycerol backbone by an ester linkage. The peroxidation reactions differ among these fatty acids depending on the number and position of the double bonds on the acyl chain. The initiation reaction between an unsaturated fatty acid (e.g. linoleate) and the hydroxyl radical involves the abstraction of an H-atom from the methylvinyl group on the fatty acid (reaction-I) at carbon-11 (in case of linoleate). The remaining carbon centred radical forms a resonance structure sharing this unpaired electron among carbons 9 to 13. In the propagation reactions, this resonance structure reacts with triplet oxygen, which is a biradical having two unpaired electrons and therefore reacts readily with other radicals. This reaction forms a peroxy radical. In the case of linoleate addition occurs at either carbon 9 or 13. The peroxy radical then abstracts an H-atom from a second fatty acid forming a lipid hydroperoxide and leaving another carbon centred free radical (reaction-3) that can participate in second H abstraction (reaction-2). Therefore, once one hydroxyl radical initiates the peroxidation reaction by abstracting a single H-atom, it creates a carbon radical product that is capable of reacting with ground state oxygen in a chain reaction. The basis for the hydroxyl radical’s extreme reactivity in lipid systems is that at very low concentrations it initiates a chain reaction involving triplet oxygen, the most abundant form of oxygen in the cell.

\[
\cdot\text{OH} + \text{RH} \rightarrow \cdot\text{R} + \text{H}_2\text{O} \quad \text{............................ (1)}
\]
\[
\cdot\text{R} + \text{O}_2 \rightarrow \text{ROO}\cdot \quad \text{................................. (2)}
\]
\[
\text{ROO}\cdot + \text{RH} \rightarrow \text{R}\cdot + \text{HOOH} \quad \text{......................... (3)}
\]
\[
\text{ROOH} + \text{Fe}^{2+} \rightarrow \cdot\text{OH} + \text{RO}\cdot + \text{Fe}^{3+} \quad \text{...................... (4)}
\]
\[
\cdot\text{R} + \text{R}\cdot \rightarrow \text{R-R} \quad \text{............................... (5)}
\]
\[ \cdot R + ROO\cdot \rightarrow ROOR \] (6)

\[ ROO\cdot + ROO\cdot \rightarrow ROOR + O_2 \] (7)

The lipid hydroperoxide (ROOH) is unstable in the presence of Fe or other metal catalysts because ROOH participates in a Fenton reaction leading to the formation of reactive alkoxy radicals. Among the degradation products of ROOH are aldehydes, such as malondialdehyde and hydrocarbon, such as ethane and ethylene, which are commonly measured end products of lipid peroxidation. The peroxidation reaction in membrane lipids are terminated when the carbon or peroxyl radicals cross link to form conjugated products that are not radicals (equation 5 to 7).

Typically high molecular weight, cross linked fatty acids and phospholipids accumulate in peroxidised membrane lipid samples. Singlet oxygen can react readily with unsaturated fatty acids producing a complex mixture of hydroperoxides. Again the chemistry of these reactions is based on foods. Oxidation of unsaturated fatty acid by singlet oxygen produces distinctly different products than the hydroxyl radical (Bradley and Min, 1992). Once formed the lipid hydroperoxides decompose into a variety of products, some of which can produce oxygen free radicals in the presence of metal catalysts.

The rate constant of hydroxide forming reaction sharply increases when the number of double bonds in the oxidized molecule is increased (Witting, 1965). This is the reason for the preferable oxidation of unsaturated lipids in the course of the LPO process in biomembranes. As a result of these reactions, a considerable amount of membrane polyenoic phospholipids can be involved in the LPO process.

There are two alternative ways for the oxidation of polyenoic lipids of biomembranes by means of free radical reaction. Polyenoic fatty acyls can be subjected to free-radical
oxidation both after preliminary hydrolysis of phospholipids by type A2 phospholipases as well as in esterified form, directly in the molecules of the membrane phospholipids. In the first case the reaction products catalyzed by cyclooxygenases or lipoxygenases are two groups of physiologically active compounds.

(i) Prostaglandins, thromboxanes and prostacyclins.

(ii) Leukotrienes, lypoxins and lypoxenes (Vliegenthart and Veldink, 1982). The oxidation of fatty acyls in the phospholipids can also be catalyzed by lipoxygenases (e.g., lipoxygenase from reticulocytes, micro vessels) with the formation of stereo specific hydroperoxidation of the fatty acid residues of phospholipids, taking place at the expense of the interaction with the oxygen radicals, results as a rule, in the formation not of one or several stereoscopic products, but of a wide range of different compounds.

There is evidence that the LPO products are capable of forming single ionic channels in the lipid bilayer (Lebedev et al., 1982). ROS mediated oxidative damage is a common pathway for a number of diseases. Almost any disease is likely to be accompanied by increased formation of reactive oxygen species. The list of diseases in which their formation has been implicated is long and is growing longer. For carcinogenesis, atherosclerosis (Esterbauer et al., 1989; Halliwell and Cross, 1991), rheumatoid arthritis, some forms of adult respiratory distress syndrome (ARDS), reoxygenation injury (McCord, 1985), and traumatic or ischemic damage to the central nervous system, there is reasonable evidence to suggest that oxidative damage by free radical reactions make a significant detrimental contribution to the pathologic and degradative process.
**Protein Peroxidation:**

Oxidative modified forms of proteins have been reported to accumulate during aging, oxidative stress and in some pathological conditions which focused attention on physiological and non-physiological mechanisms for the generation of reactive oxygen species and on the modification of biological molecules by various kinds of ROS. Basic principles that govern the oxidation of proteins by ROS were established in the pioneering studies of Swallow (1960), Garrison (1987), and Schuessler and Schilling (1984). Oxidative attack on proteins results in site-specific amino acid modifications, fragmentation of the peptide chain, aggregation of cross-linked reaction products, altered electrical charge and increased susceptibility to proteolysis. The amino acids in a peptide differ in their susceptibility to attack, and the various forms of activated oxygen differ in their potential reactivity. Primary, secondary, and tertiary protein structures alter the relative susceptibility of certain acids.

Oxidative attack of the polypeptide backbone is initiated by the •OH dependent abstraction of the α-hydrogen atom of an amino acid residue to form a carbon-centered radical. The •OH needed for this reaction may be obtained by radiolysis of water or by metal catalyzed cleavage of H₂O₂. The carbon centered radical thus formed reacts rapidly with O₂ to form an alkyl-peroxyl radical intermediate which can give rise to the alkyl peroxide followed by formation of an alkoxy radical and finally to a hydroxyl protein derivative. Many of these steps in this pathway mediated by interactions with H₂O₂ can also be catalyzed by Fe²⁺. The alkyl, alkylperoxyl and alkoxy radical intermediates in this pathway may undergo side reactions with other amino acid residues in the same or a different protein molecule to generate a new carbon centered radical capable of undergoing reactions with oxygen free radical (Berlett and Stadtman,
In the absence of oxygen, the carbon-centered radical may react with another carbon-centered radical to form a protein-protein cross-linked derivative.

The generation of alkoxyl radicals sets the stage for cleavage of the peptide bond either by the diamide or α-amidation pathway. The resulting peptide fragment of cleavage pathway derived from the N-terminal portion of the protein possesses a diamide structure at the C-terminal end, whereas the peptide derived from the C-terminal portion of the protein possesses an isocyanate structure at the N-terminal end. On cleavage by the α-amidation pathway, the peptide fragment obtained from the N-terminal portion of the protein possesses an amide group at the C-terminal end, whereas the N-terminal amino acid residue of the fragment derived from the C-terminal portion of the protein exists as an N-α-Ketoacyl-derivative. The peptide fragments obtained on acid hydrolysis by the diamide pathway yield CO₂, NH₃ and a free carboxylic acid whereas hydrolysis of the fragment obtained by the α-amidation pathway yields NH₃ and a free α-ketocarboxylic acid.

Sulphur containing amino acids, and thiol groups specifically, are very susceptible sites. Activated oxygen can abstract an H-atom from cysteine residues to form a thiyl radical that will cross-link to a second thiyl radical to form disulphide bridges. Aromatic amino acid residues are among the preferred targets for ROS attack. Methionine and cysteine residues of proteins are particularly vulnerable to oxidation by peroxynitrite (PN) and tyrosine and tryptophan residues are selective targets for PN-dependent nitration.

Direct oxidation of lysine, arginine, proline and threonine residues also yield carboxyl derivatives. Carbonyl groups may be introduced into proteins by reactions with aldehydes (4-hydroxy-2-nonenal (HNE), malondialdehyde) produced during lipid peroxidation or with reactive carbonyl derivatives (ketoamines, ketoaldehydes,
deoxyosones) generated as a consequence of the reaction of reducing sugars or their oxidation products with lysine residues of proteins (Berlett and Stadtman, 1997).

The intracellular level of oxidized protein reflects ‘the balance between the rate of protein oxidation and the rate of oxidized protein degradation’. Different physiological and environmental processes, administered drugs, pollutants lead to the formation of ROS. Collectively, these processes can promote the generation of a battery of ROS including a number of free radicals (\( 'OH, 'O_2^-, ROO', RO', NO', RS', ROS', RSOO' and RSSR'^\cdot \)), various non-radical oxygen derivatives (\( H_2O_2, ROOH, O_2, O_3, HOCl, ONOO^-, O=NOCO_2^-, O_2NOCO_2^-, N_2O_2, NO_2^+ \)), and highly reactive lipid or carbohydrate derived carboxy compounds, as 4-hydroxy-2-nonenal (HNE), malondialdehyde (MDA) ketoamines, ketoaldehyde, and deoxyosones capable of promoting the modification of proteins (Berlett and Stadtman, 1997).

The oxidative modification of proteins by reactive species, especially reactive oxygen species, is implicated in the etiology or progression of panoply of disorders and diseases. These reactive species are formed through a large number of physiological and non-physiological reactions. An increase in the rate of their production or decrease in their rate of scavenging increases the oxidative modifications of cellular molecules, including proteins (Stadtman and Levine, 2000).

Exposure of animals cell cultures to various conditions of oxidative stress such as hyperoxia, forced exercise, ischemia, reperfusion, rapid correction of hyponatremia, paraquat toxicity, magnesium deficiency, ozone, neutrophil activation, cigarette smoking, \( \alpha \)-radiation, chronic alcohol treatment, mixed function oxidation systems etc. leads to an increase in the level of oxidized protein (Berlett and Stadtman, 1997).
Accumulation of oxidized protein (protein carbonyls) is associated with a number of diseases, including amyotrophic lateral sclerosis, Alzheimer’s disease, respiratory distress syndrome, muscular dystrophy, cataractogenesis, rheumatoid arthritis, progeria, and Werner’s syndrome. Although the level of carbonyl has not been directly determined, there is reason to believe that oxidative modification of proteins is also implicated in atherosclerosis, diabetes, Parkinson’s disease, essential hypertension, cystic fibrosis, and ulcerative colitis (Berlett and Stadtman, 1997).

**Gonadotropins:**

The gonadotropins viz., FSH (follicle-stimulating hormone) and LH (luteinizing hormone) are synthesized and secreted by gonadotroph cells of the anterior pituitary gland. Both the hormones are non-covalently linked dimeric glycoproteins consisting of a common α-subunit (chorionic gonadotropin alpha [CGA]) and unique β-subunits (FSH-β and LH-β). The latter confer biologic specificity to the two ligands. FSH and LH play necessary and complementary roles in the control of mammalian reproduction. In female mammals, FSH stimulates ovarian follicle growth and maturation, as well as estradiol (E2) synthesis by granulosa cells, whereas LH stimulates androgen production by theca cells and ovulation of the dominant follicles. The role of FSH in female reproduction is clearly demonstrated both clinically and in animal models. Women with loss-of function mutations in the FSHB or FSH receptor (FSHR) genes present clinically with primary or secondary amenorrhea and associated arrest in follicle development at the preantral stage (Themmen and Huhtaniemi, 2000; Huhtaniemi and Themmen, 2005; Themmen, 2005). Similar phenotypes are observed in Fshb- and Fshr deficient (knockout) mice (Kumar et al., 1997; Dierich et al., 1998; Danilovich et al., 1998).
In male mammals, LH stimulates androgen production by interstitial Leydig cells. Although FSH targets Sertoli cells in the testes to regulate spermatogenesis, its absolute necessity for reproduction has been a matter of some debate. Men homozygous for one particular inactivating mutation in the FSHR gene have variable extents of oligozoospermia (Tapanainen et al., 1997). Similarly, male Fshr-deficient mice have reduced testis size and sperm counts but are fertile (Kumar et al., 1997). Reports indicate that FSH plays a role in maintaining quantitatively normal spermatogenesis, but may not be fundamentally required for fertility in males. However, men with inactivating mutations in the FSHB subunit gene, who are unable to produce the dimeric ligand, are azoospermic (Lindstedt et al., 1998; Phillip et al., 1998; Berger et al., 2005). Other cases of idiopathic isolated FSH deficiency have also been reported and are similarly associated with azoospermia or oligoteratozoospermia and infertility (Mantovani et al., 2003; Giltay et al., 2004).

FSH plays a crucial role in spermatogenesis. In the fetal and neonatal development stages, FSH activates the proliferation of the Sertoli cells and successively, during the pubertal phase, it influences the mitotic activity of the spermatogonia and encourages cellular differentiation, until arrival at the round spermatid stage. Because of its physiological role in spermatogenesis, various attempts have been made to treat idiopathic oligozoospermic men with FSH. However, the results obtained so far are still controversial (Foresta et al., 2007). The causes of Azoospermia such as failure of spermatogenesis and obstruction of the ductal system particularly the vas deferens have been investigated (Ojengbede et al., 1992). Emokpac et al. (2006) reported that hormonal abnormalities and underlying testicular pathology are common in infertile Azoospermia males. 40% of azoospermia males are reported to have defect in testes
despite increased level of FSH and LH. Marked elevation of FSH in the Azoospermia patients strongly suggest testicular failure and a poor prognosis. Spermatogenic arrest, testicular atrophy and hypospermatogenesis are the most common testicular abnormalities. Testicular failure is also a responsible factor for decrease in sperm count. Defect in testes leads to poor quality sperm cells (Winters, 1990; Khan et al., 2004). Oligospermia is another important clinical condition. There may be many responsible factors for oligospermia, including endocrine inter-relationship, testicular function, genetic factors or conditions of the vas and genital tract, the seminal vesicles, pH of the vaginal fluid and the motility and general health of the spermatozoa in the seminal fluid and their viability in the female genital tract (Madan and Madan 1985).

Testosterone:

Testosterone is a steroid hormone produced by various tissues in the human body although it is mainly the product of endocrine glands, i.e. testes, ovaries and adrenal glands. Testosterone synthesis is under hypothalamic-pituitary-gonadal-axis control. Testicular steroid production is controlled by gonadotrophins viz., luteinizing hormone (LH) and follicle-stimulating hormone (FSH). In men, the majority of testosterone is of gonadal origin, and healthy adult males produce between 2.5 and 11 mg of testosterone daily. Male circulating testosterone levels are 10-fold higher than female levels. In women, the ovaries and adrenals contribute equally to testosterone production, each supplying about 25% of the total circulating level. The remaining 50% is derived from peripheral conversion of androstenedione in the liver, skin, brain and adipose tissue (Rosenfield, 1972; Longcope, 1986; Gagliardi, 1991). In men about 44% of the secreted testosterone is bound to the sex hormone binding globulin (SHBG), and around 2% occurs in free form. The remaining 54% is loosely bound to albumin, from which it can
dissociate within the capillary beds (Pardridge, 1986). Free and bound testosterone exists in equilibrium. Through aromatization and reduction, the molecule is converted to estrogen and more androgenic 5α-dihydrotestosterone respectively (Huhtaniemi et al., 1992).

The testosterone molecule is a four-ring structure of cyclic cholesterol rings. Both reproductive and non-reproductive tissues possess possible targets for testosterone. Testosterone has two different kinds of biological effects: 1) The androgen effect is responsible for the development of the male reproductive organs and secondary sexual characteristics, and 2) the anabolic effect stimulates increased nitrogen fixation and protein synthesis (Huhtaniemi et al., 1992). To increase the therapeutic value of the molecule, scientists have been keen on developing molecules with emphasized anabolic and prolonged biological activity. Further, to avoid first-pass metabolism to increase systemic availability, the parent molecule needed modification, which led to the invention of numerous anabolic steroids (Shahidi, 2001).

![Structure of testosterone](image)

**Fig. II.3: Structure of testosterone.**

**Structure and biotransformation of testosterone:**

The main natural androgen viz., testosterone, undergoes a series of biotransformation by oxido-reductive reactions at C3, C4, C5 and C17. The first step in the metabolism of
testosterone is reduction of C4,5 double bond, resulting in formation of 5α and 5β derivatives in which the hydrogen at C5 are respectively above and below the planar molecule, making them different in spatial configuration. Among the metabolites, 17β-hydroxy-5α derivatives such as 5α-dihydrotestosterone (DHT) are androgen whereas 5β steroids are not. 5α- DHT is produced by 5α-reductase in target organs such as brain and reproductive tract and 5β steroids are formed in liver by 5β reductase (Celotti et al., 1992). Reduction of the C4,5 double bond is irreversible. The testosterone molecule and some of its synthetic derivatives (e.g., C4,5 testosterone esters) with unsaturated C4,5 can be converted to estradiol by aromatase in tissues such as adipocytes and the brain, whereas DHT and other 5α synthetic derivatives cannot be converted. After reduction of the C4,5 double bond, the 3-keto group of the 5α isomer is rapidly reduced by either 3α-hydroxysteroid dehydrogenase or 3β-hydroxysteroid dehydrogenase (Shahidi, 2001).

After oral administration or intramuscular injection, testosterone is metabolized mainly to 3α-hydroxyl isomers, with formation of only small amounts of the 3β–hydroxyl-5α metabolite. Another important metabolic pathway of testosterone is oxidation of the 17β-hydroxyl group in the D-ring. The step is mediated by 17β-hydroxyl dehydrogenase with formation of 17–keto. However, the 17 – keto group can be converted back to the hydroxyl group by the same enzyme. 17 – keto derivatives such as androsterone and etiocholanolone are the main urinary metabolites of testosterone. Thus, on oral or parenteral administration, the 17 β-hydroxy groups are rapidly oxidized to biologically inactive polar metabolites.

To overcome the rapid biotransformation of testosterone and to synthesize longer acting and orally active compounds with lower androgenicity and higher anabolic activity,
more than 100 synthetic steroids have been developed. Among the many modifications that have been made to the testosterone molecule, the 17α-alkylation and 17 β-esterification have been most effective in achieving the active compounds.

Fig. II.4: Part of the biochemical cascade of metabolism of testosterone (Gao et al., 2005). (HSD- hydroxysteroid dehydrogenase)

When the testosterone molecule is modified by 17α-alkylation, a methyl group (CH₃) is commonly introduced at position C17α. Methyltestosterone, thus synthesized, was among the first 17α alkylated androgens. Others in this group include oxymetholone, oxandrolone and stanozolol. In other 17α alkylated androgens such as norethandrolone, ethylestrenol and norbolethone, an ethyl group (C2H₅) is introduced at position C17α. 17α-alkylation markedly retards hepatic inactivation of testosterone and thus allows these products to become orally active. With the exception of methenolone, which is alkylated in the C1 position, all orally active androgens are 17α-alkylated.

Esterification of the 17 hydroxy groups with long-chain hydrocarbon molecules delays biodegradation of testosterone to ketosteroids. The products in this group are active only on parenteral administration. The type of acid used to acidify the 17 β-hydroxy group
determines the duration of anabolic action. Short chain esters (C$_2$-C$_3$) give rise to short acting steroids, whereas long-chain esters (C$_7$-C$_{10}$) are long acting compounds. These derivatives are also highly androgenic and can be aromatized by virtue of their unsaturated C4,5 double bond. Substitution of hydrogen for the methyl group of C19 results in the formation of 19-nor testosterone (nandrolone). This hydrogen substitution has the same β configuration as the methyl group of testosterone. Esterification of the 17-hydroxyl group of nandrolone with phenylpropionic acid (nandrolone phenylpropionate) or cylopentylpropionate (nandrolone cypionate) yields products that are more stable and more anabolic. Esterification of the 17-hydroxyl group of nandrolone with decanoic acid, a long chain fatty, yields nandrolone decanoate which is released into the circulation slowly on deep intramuscular injection which exerts its optimal anabolic activity over 6 to 7 days. Despite unsaturated with C4, 5 double bond, 19-nortestosterone derivatives have significantly less androgenic activity compared with testosterone ester. The most commonly used testosterone derivatives include nandrolone decanoate, oxandrolone, oxymetholone and stanozolol. However, nandrolone decanoate is reported to be the most abused drug in the present scenario (Shahidi, 2001).

**Estrogen:**

From a historical perspective, the role of estrogen in human male has been investigated when Zondek (1934) reported estrogen production in males through the discovery of the intratesticular conversion of androgen into estrogen in male stallions (Rochira et al., 2005). Several years later, MacDonald et al. (1979) demonstrated estrogen production in human males. Afterwards, numerous studies followed year after year in order to expand the comprehension of the role of estrogen in men (Simpson et al., 2005; Zirilli
et al., 2008). Several studies provide evidence that estrogen exert a wide range of biological effects in men along with women (Sharpe, 1998; Deroo and Korach, 2006).

**Estrogen Biosynthesis:**

The principal source of systemic estrogen in the pre-menopausal non-pregnant woman is the ovary whereas other sites of estrogen biosynthesis are also present throughout the body which become the major sources of estrogen beyond menopause which include the mesenchymal cells of the adipose tissue and skin (Simpson et al., 1997), osteoblasts (Bruch et al., 1992) and perhaps osteoclasts (Jakob et al., 1995) in bone, possibly vascular endothelial (Bayard et al., 1995) and aortic smooth muscle cells (Murakami et al., 1998) as well as a number of sites in the brain viz., medial preoptic/anterior hypothalamus, the medial basal hypothalamus and the amygdala (Naftolin et al., 1975). These extragonadal sites are dependent on circulating precursor C₁₉ steroids for estrogen biosynthesis. These tissues have the capacity to convert C₁₉ steroids to C₁₈ steroids, unlike ovaries they lack the ability to synthesize C₁₉ precursors (Labrie et al., 1997). The total volume of estrogen synthesized by these extragonadal sites may be small, but the local tissue concentrations achieved are probably quite high which exert significant biological influence locally. After menopause, the mesenchymal cells of the adipose tissue become the chief source of estrogen (Simpson et al., 1997).

In males, the Leydig cells (Tsai-Morris et al., 1985) and other cells of the testes, including germ cells in various stages of differentiation, produce estrogen which has an important role in spermatogenesis. It has been estimated that testes account for 15% of circulating estrogen (Hemsell et al., 1974) and therefore, in the male, local production of estrogen, both intratesticular and extragonadal is of physiological significance throughout adult life. In the case of males, estrogen is derived from circulating
androgen. The key step in estrogen biosynthesis is the aromatization of the C\textsubscript{19} androgens, viz., testosterone and androstenedione, to form estradiol and estrone, respectively. The step is under the control of aromatase enzyme (Fig II.5).

The aromatase enzyme is a P\textsubscript{450} mono-oxygenase enzyme complex present in the smooth endoplasmic reticulum, which acts through three consecutive hydroxylation reactions, whose final effect is the aromatization of the A ring of androgens (Simpson et al., 1994; Gruber et al., 2002) (Fig II.2). This enzymatic complex is composed of an ubiquitous and non-specific NADPH-cytochrome P\textsubscript{450} reductase, together with the regulated form of cytochrome P\textsubscript{450} aromatase (Carreau et al., 2012). The conversion of androgen to estrogen takes place mainly in the testes, adipose tissue and muscle tissue (Fig II.5).

Circulating estrogens are mainly reversibly bound to sex hormone binding globulin (SHBG), a β-globulin, and, to a lesser degree, to albumin. Estrogen action is mediated by the interaction with specific nuclear estrogen receptors (ERs), which are ligand-inducible transcription factors regulating the expression of target genes after hormone binding (Deroo and Korach, 2006). Two subtypes of ERs have been reported: estrogen receptor α (ERα) and the more recently discovered estrogen receptor β (ERβ). Aromatase enzyme and ERs are widely expressed in the male reproductive tract both in animals and humans, suggesting that estrogen biosynthesis occurs at this site and that both locally produced and circulating estrogens may interact with ERs in an intracrine / paracrine or endocrine fashion (Gruber et al., 2002). The concept of a key role of estrogen in the male reproductive tract is strongly supported by the ability of the male reproductive structures to produce and respond to estrogen (O’Donnell et al., 2001).
Estradiol is the most potent estrogen produced in the body. The total estradiol production rate in human male has been reported to be 35-45 µg (0.130-0.165 µmol) per day, of which approximately 15-20% is directly produced by testes (Baird et al., 1969; MacDonald et al., 1979). Roughly 60% of circulating estradiol is derived from peripheral aromatization of circulating testosterone and 20% is the product of peripheral conversion of estrone (Baird et al., 1969).
**Estrogen and Fertility:**

The demonstration of wide expression of aromatase enzyme, ERα and ERβ throughout both the male reproductive system and the human sperm, underlines the role of estrogen in male reproductive function (Rochira et al., 2005; Rochira and Carani, 2009; Carreau et al., 2012). Reports indicate that estrogen modulates sperm maturation, since spermatozoa also express ERα and ERβ, and they are responsive to estrogen throughout their run from the testes to the urethra (Luconi et al., 1999; Carreau et al., 2012). The exposure to the excess of environmental estrogen has been proposed as a possible cause of impaired fertility. Thus, administration of aromatase inhibitors to infertile men with impaired testosterone-to-estradiol ratio results in an improvement of their fertility rate (Raman and Schlegel, 2002; Saylam et al., 2011). Over-expression of aromatase causes an increased conversion of androgens to estrogen with a consequent excess of estrogen. Excess estrogen in boys causes gynaecomastia, a premature growth spurt, early fusion of epiphyses, and decreased adult height (Hemsell et al., 1977). In adult men elevated serum estradiol levels are associated with an abnormal sex hormonal pattern such as a mild hypogonadotropic hypogonadism probably due to a direct estrogen negative feedback effect on pituitary gonadotropins (Stratakis et al., 1998; Shozu et al., 2003).

On the basis of the role of estrogen on gonadotropic feedback inhibition, some clinical insights on the management of male infertility have been made (Raven et al., 2006; Rochira et al., 2006; Pitteloud et al., 2008). However, it is yet to be established if estrogen in men could be a good target for improving male fertility, since results available in the area of aromatase inhibitors used for the treatment of male infertility are conflicting (Raman and Schlegel, 2002; Rochira and Carani, 2009; Saylam et al., 2011; Carreau et al., 2012; Rochira et al., 2013).
Prolactin (PRL):

Among the hormones of the anterior pituitary, prolactin (PRL) with more than 185 functions documented among various vertebrate species is by far the most versatile (Nicoll, 1974). Prolactin (PRL), also known as luteotropic hormone or luteotropin, was first discovered in 1928, based upon its ability to cause lactation in pseudopregnant rabbits (Stricker and Gmeter, 1928). The suckling induced release of PRL, a universal response in mammals, has emerged as a classic experimental model for the study of neuroendocrine interactions (Leong et al., 1983). In the late 1980’s, the cloning and characterization of Pit-1 as a tissue specific transactivator of PRL gene transcription enhanced the understanding of pituitary cell development and PRL gene regulation (Rosenfeld, 1991).

Biosynthesis of Prolactin:

The capacity of the pituitary to synthesize hormones predates histological differentiation of the pituitary cells into lactocrophs (PRL secreting cells) and somatotrophs (growth hormone secreting cells). In all the species studied, growth hormone (GH) secretion predates PRL secretion. Data have accumulated supporting the concept that GH and PRL secreting cells present in the adult animal may be inter convertible. This has been suggested by the observation of marked increase in dual secreting mammosomatotrophs in male rat pituitaries exposed to estrogen associated with a commensurate decrease in GH-secreting cells (Kendell and Hymer, 1987).

The morphology of the lactotrophs has been best described in the rat where PRL containing cells are sparsely distributed in the lateroventrical portion of the anterior lobe and are present as band adjacent to the intermediate lobes (Nakane, 1970; Tougard and
Tixier-Vidal, 1994). Pituitary preprolactin like all secreted proteins is synthesized and its signal peptide is removed on the membrane-bound ribosomes of the rough endoplasmic reticulum. From there processed PRL is transported to the golgi, where it is glycosylated to a variable extent and packaged into secretory granules. PRL was originally thought to be secreted by two pathways, the regulated secretory granule pathway and a constitutive pathway, characterized by lack of secretory granule formation and a rapid transport to the cell surface. The constitutive pathway was considered to be the bulk flow default pathway, whereas the secretory granule pathway was considered to be the pathway responding to secretagogues.

The first observation that PRL is produced in the brain was by Fuxe et al. (1977), who found PRL immunoreactivity in hypothalamic axon terminals. PRL immunoreactivity was subsequently found in the telencephalon in the cerebral cortex, hippocampus, amygdala, septum, caudate putamen, brain stem, cerebellum, spinal cord, choroid plexi and the circumventricular organs (Thompson, 1982; Emanuele et al., 1987; Devito, 1988; Seroogy et al., 1988; Siaud et al., 1989).

PRL immunoreactivity is found within numerous hypothalamic areas in a variety of mammals (Thompson, 1982; Alonso et al., 1988; Harlan et al., 1989; Siaud et al., 1989; Nishizuka et al., 1990; Griffond et al., 1993; 1994). Within the rat hypothalamus, PRL immunoreactivity is detectable in the dorsomedial, ventromedial, supraoptic and paraventricular nuclei (Hansen et al., 1982; Griffond et al., 1993). Several approaches have been taken to prove that PRL found in the hypothalamus is synthesized locally, independent of PRL synthesis in the pituitary gland. Indeed, hypophysectomy has no effect on the amount of immunoreactive PRL in the male hypothalamus and only
diminishes but does not abolish the quantity of immunoreactive PRL in the female rat hypothalamus (Devito, 1988).

It has now been established that the primary structure of PRL of the hypothalamic and pituitary origin is identical (Emanuele et al., 1992). Although the role of PRL of hypothalamic origin is not apparent, it has been speculated that PRL of central origin may exert its effect as neurotransmitter, neuromodulator or a central cytokine regulating vascular growth and glial functions. It is difficult to differentiate between the effects of PRL of pituitary versus hypothalamic origin in the CNS. One cause of these difficulties is that pituitary PRL from the circulation bypasses the blood brain barrier and enters the CNS through the choroid plexi of the brain ventricles (Freeman et al., 2000). Aside from passage from the blood to the cerebrospinal fluid by way of the choroid plexus, pituitary PRL may also reach the brain by retrograde blood flow from anterior pituitary to the hypothalamus (Mezey and Palkovitis, 1982). Therefore, the actions of PRL in the CNS can be due to the hormone of the pituitary or hypothalamic origin.

In addition to its major site of synthesis in the pituitary, human PRL is also synthesized in the decidua basalis of the pregnant uterus (Clements et al., 1983). PRL is present in amniotic fluid at levels 100-fold higher than in maternal or fetal blood. PRL produced by the decidual cell appears to be 50% glycosylated, whereas that produced by the pituitary is only 10% glycosylated. The PRL found in the amniotic fluid is heavily glycosylated (Lee and Markoff, 1986).

PRL has been detected in epithelial cells of the lactating mammary gland as well as in the milk itself (Nolin and Witorsch, 1976; Grosvenor and Keenan, 1992).
Regulation of Secretion:

Like all pituitary hormones, PRL is secreted episodically, with a distinctive 24-hour pattern. In normal human subjects, there are about 14 pulses of PRL secretion in 24 hours, approximately one every 95 minutes (Van Cauter et al., 1981). Superimposed upon this pattern is a bimodal 24-hour pattern of secretion, with a major nocturnal peak beginning after sleep onset and peaking in mid sleep. Minimal levels (the smallest spikes) occur around noon followed by a lesser peak of secretion in the evening (Sassin et al., 1973; Van Cauter et al., 1981). The enhancement of secretion during the night is due to increase in the amplitude of each pulse, unaccompanied by an increase in pulse frequency (Veldhuis and Johnson, 1988). PRL secretion remains pulsatile in patients with prolactinomas, whereas circadian variation is abolished. Furthermore, rat anterior pituitaries transplanted under the pituitary capsule of hypophysectomized rats release PRL in pulses of 8 to 10 minute intervals. Because hypothalamic connections have been severed, this short periodicity appears to be intrinsic to the lactotroph (Shin and Reifel, 1981). The data from both human and rat studies support the concept that these short pulses are not controlled by the hypothalamus but arise within the gland (Samuels et al., 1991).

Serum PRL levels are generally higher in women than in men. This reflects the effects of estrogen. The day time peak of PRL secretion is more pronounced during the luteal phase of the menstrual cycle (Tennekoon and Lenton, 1985). During pregnancy, maternal serum PRL levels begin to rise during the first trimester and increase steadily throughout pregnancy, resulting in about 10-fold increase by term, presumably due to high levels of estrogen during pregnancy (Rigg et al., 1977). After delivery, serum PRL
levels rise markedly, peaking about two hours postpartum and then falling again six hours later (Rigg and Yen, 1977).

Prolactin is released in high quantity in response to stress that is physical and psychological in nature. Human PRL secretion has been shown to increase after many types of stress, including general anesthesia, surgery, exercise and insulin induced hypoglycemia. In each case the stress causes a significantly greater PRL increase in women than men. Following general surgery, levels as high as five-fold over basal have been reported (Noel et al., 1972). It has been postulated that the stress induced increase in PRL is partially mediated by the opiate peptides, particularly β-endorphin (Pontiroli et al., 1982). Neuronal histamine and arginine vasopressin have been implicated as mediators in other studies, whereas melanocyte-stimulating hormone (MSH) has been implicated as an inhibitor of stress induced increase in PRL (Khorram et al., 1984; Kjaer et al., 1991).

Estrogen regulates the secretion of PRL in many different species. Surveys of human pituitaries at autopsy have demonstrated that estrogen increases the number of PRL-secreting cells (Asa et al., 1982). Physiological doses of insulin stimulate PRL expression in GH3 cells; PRL mRNA levels increase 3 to 10-fold and secretion is accelerated (Stanley, 1988).

Several examples of neuroendocrine control of PRL secretion are sleep related, stress and suckling induced surges of PRL. The suckling stimulus represents a neuroendocrine reflex and is a popular experimental model: the magnitude of PRL response is closely coupled to the intensity of the stimulus (Neill, 1988).

Hyperprolactinemia is the most common hypothalamic pituitary disorder encountered in clinical endocrinology. The causes of pathological hyperprolactinemia are diverse, and
treatment depends upon identification of precise cause. In humans, pathological hyperprolactinemia is defined as a consistently elevated serum PRL level (greater than 20 mg/ml) in the absence of pregnancy or postpartum lactation (Vance and Thorner, 1995).

**Prolactin and male infertility:**

Two-thirds of patients with oligozoospermia, asthenozoospermia and azoospermia are reported to have normal PRL levels. Infertility in men due to moderate hyperprolactinemia could be associated with these sperm disturbances (Merino *et al*., 1997). Male hyperprolactinemia was detected in 4% of infertile men (Segal *et al*., 1979). Hyperprolactinemia causes infertility in around 11% of oligospermic males (Masud *et al*., 2007). Hyperprolactinemia inhibits the secretion of the gonadotropin releasing hormone, which causes decreased pulsatile release of FSH, LH and testosterone, which in turn causes spermatogenic arrest, impaired sperm motility and altered sperm quality which eventually leads to secondary hypogonadism and infertility. Hyperprolactinemia directly influences spermatogenesis and steroidogenesis by acting on prolactin receptors present in Sertoli cells and Leydig cells in testes, and produces primary hypogonadism and infertility (Masud *et al*., 2007; Singh *et al*., 2011). Reports indicate that oligospermic or azoospermic patients with normal serum levels of gonadotropins show relatively higher serum levels of prolactin. Studies confirm a definite role of hyperprolactinemia in male infertility which is one of the reversible causes of male infertility (Fernandez *et al*., 1990, Buvat, 2003; De Rosa *et al*., 2003; Masud *et al*., 2007; Singh *et al*., 2011).