

# 1. Introduction

Adrenarche and puberty are two important events in human growth and biological maturation. Puberty is the transitional period between the juvenile state and adulthood during which the adolescent growth spurt occurs, secondary sexual characteristics appear, fertility is achieved, and profound psychological changes take place (1). Adrenarche is the process of increase of adrenal androgen (AA) production beginning several years before the onset of puberty (2, 3). Children with premature adrenarche or precocious puberty are at a risk for the development of hormone related cancers (4-6) or chronic disease (7) later in life.

The influence of childhood nutritional status, especially with regard to body fat mass (8, 9) and animal protein intake (10), on pubertal timing has been intensively discussed, however the evidence for the role of body composition and dietary intakes in the modulation of AA production or adrenarche is limited. Therefore, **the first aim** of this thesis was to examine whether body composition and certain dietary intakes may influence AA production in healthy children.

The link between nutritional status and the physiological variations in timing of puberty can be significant but it is not particularly strong, suggesting that this relationship may be mediated by one or more growth-, energy balance-, or sexual maturation-related hormones, such as insulin, IGF (Insulin-like growth factor)-1, leptin, glucocorticoids (GCs), adrenal or gonadal sex steroid hormones. The prospective Dortmund Nutritional and Anthropometric Longitudinally Designed (DONALD) Study, which observes diet, growth, and metabolism in healthy children, provides a good data basis for the investigation of the potential roles of several of these candidate hormones for the pubertal development. The DONALD Study is entirely observational and non-invasive until the participants are 18 years old. Therefore in this thesis the assessments of hormone status were based on 24-h urine data, which are regularly collected in yearly intervals by the DONALD participants. Since to date, the measurement of peptide hormones in urine samples has not yet been satisfactorily established, this thesis concentrated exclusively on steroid hormones.

Excess adrenal steroid hormone [i.e. AA (11, 12) and GC (13-16)] exposures can influence somatic growth and sexual maturation - two important physiological signs of pubertal development. The evidence for a role of steroid hormone variations in the physiological range in the pubertal timing is limited. Accordingly, **the second and the third aim** was to examine the association of prepubertal AA secretion and GC status (as a marker of stress activity) respectively with early and late pubertal markers, independent of

nutritional status. Few evidence of growth promoting properties of low circulating concentration of estrogens (Es) during childhood also exists; however, further elucidation of the role of Es in the pubertal timing has been hampered, so far by the lack of sensitive measurement method. **The last aim** was to investigate prepubertal urinary E excretion level [using a recently developed isotopic dilution/gas chromatography-mass spectrometry (ID/GC-MS) method] and its association with pubertal markers.

## Outline

The thesis begins with a Theoretical Background (**Chapter 2**). Firstly, the biosynthesis, metabolism, assessment and biological role of major steroid hormones during growth are introduced. Secondly, the current knowledge about the potential influences of nutritional status, hypothalamic and peripheral signals for the pubertal development, especially regarding the role of steroid hormones, is summarized. Based on this overview, several research questions are formulated (**Chapter 3**).

A General Methodology Section (**Chapter 4**) describes the DONALD Study as well as methodological considerations relevant to all, or the majority, of the analyses. This Chapter also includes a study as preliminary methodological work to examine the urine volume or water load dependency of AA and GC metabolites in healthy children (Chapter 4.8). The background for this is that observational and experimental studies have suggested that urine volume should be considered as a confounder when using urinary free cortisol (UFF) and cortisone (UFE) to assess GC status. However, to date the potential influence of water load on urinary excretion rates of other steroid hormone metabolites has never been investigated.

The major research questions will be addressed in a series of analyses of DONALD sub-samples which are referred to as Studies I-IV. They are presented in **Chapter 5** and comprise study-specific Introduction, Methods, Results, and Discussion Sections. A General Discussion (**Chapter 6**) brings the study results into a broader context and provides also overall conclusions and ideas for future research.

## 2. Theoretical Background

### 2.1 Adrenocortical hormones

There are three major groups of hormones produced by the adrenal cortex: mineralocorticoids, GCs, and sex steroids. GCs, primarily produced by the zona fasciculata (ZF), have an important role in metabolism of carbohydrate, lipid, and protein and multiple effects on immune, circulatory, skeletal, renal, and central nervous system. Mineralocorticoids, normally solely produced by the zona glomerulosa (ZG) are essential for the maintenance of blood volume and sodium balance (17). The major sex steroids, produced primarily in the zona reticularis (ZR), are AAs, which contribute to appearance of auxiliary and pubic hair and a transient physical maturation of children (18).

#### 2.1.1 Biosynthesis

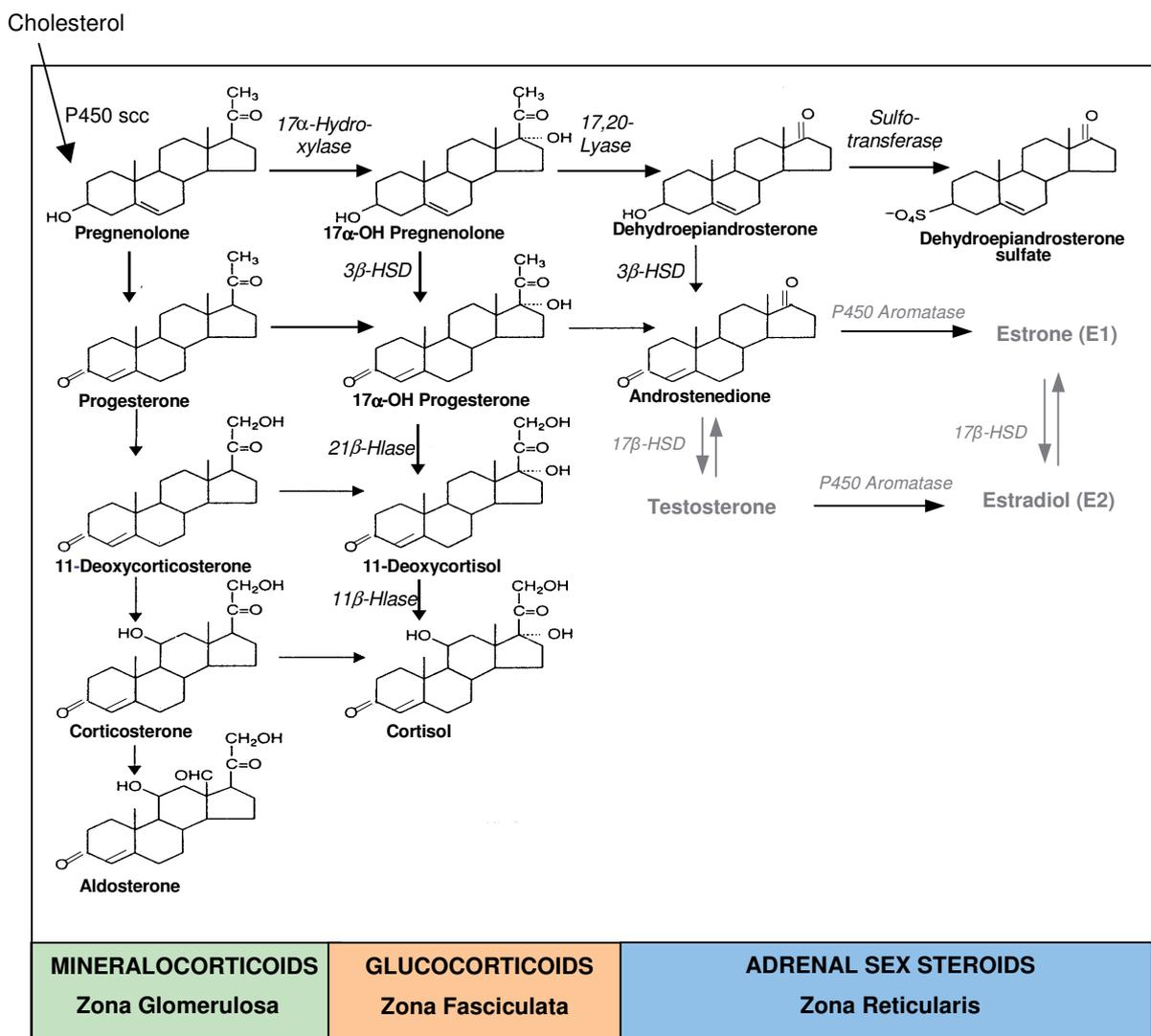
The precursor of steroid hormones is cholesterol, which has a 17-carbon steroid nucleus. The cells of the steroidogenic tissues can de novo synthesize cholesterol from acetate, but most of its supply comes from cholesterol derived from the plasma low density lipoproteins, which are influenced by dietary fats including dietary cholesterol. Cholesterol is converted to steroid hormones by steroidogenic enzymes in the mitochondria and smooth endoplasmic reticulum. Most steroidogenic enzymes are members of the cytochrome P450 group of oxidases, which are formally referred to with a common nomenclature consisting of "CYP" followed by a unique designator (19, 20). The hydroxysteroid dehydrogenases (HSDs) are another group of enzymes involved in the steroidogenic reactions. The zone specific steroid biosynthesis pathways of the adrenal gland are shown in the **Figure 1**.

#### ***Adrenal androgens***

Steroidogenic cells deliver large amounts of cholesterol to the cholesterol side-chain cleavage enzyme, termed P450<sub>scc</sub>, which catalyzes the first and rate-limiting step in steroidogenesis, converting cholesterol to pregnenolone. Once pregnenolone is produced from cholesterol, it may undergo 17 $\alpha$ -hydroxylation by P450<sub>c17</sub> to yield 17 $\alpha$ -hydroxypregnenolone, or it may be converted by 3 $\beta$ -hydroxysteroid dehydrogenase (3 $\beta$ HSD) to progesterone - the first biologically important steroid. 17 $\alpha$ -hydroxypregnenolone can be converted to 17 $\alpha$ -hydroxyprogesterone by 3 $\beta$ HSD or to a C19 steroid, dehydroepiandrosterone (DHEA) by 17,20-lyase, the second isoform of P450<sub>c17</sub>. DHEA can also be converted by DHEA-sulphotransferase to DHEA-sulfate (DHEA-S) or by 3 $\beta$ HSD to androstenedione (21).

### Glucocorticoids and Mineralocorticoids

Because of the existence of 21-hydroxylase (CYP21) in the ZG and ZF, progesterone or 17 $\alpha$ -hydroxyprogesterone can be hydroxylated at the 21-position, producing 11-deoxycorticosterone and 11-deoxycortisol, respectively. In the next step, the two isoforms of 11 $\beta$ -hydroxylase (CYP11B1 and B2), catalyze the conversion of 11-deoxycorticosterone and 11-deoxycortisol to the GCs, corticosterone and cortisol respectively. The biosynthetic pathway of the major mineralocorticoids, aldosterone initially parallels that of cortisol, except that 17-hydroxylation does not occur because of the absence of P450c17 in the ZG. On the other hand, only the ZG has the ability to catalyze the 18-oxidation of corticosterone to aldosterone, the major Mineralocorticoid (21)



**Figure 1.** Major steroid biosynthesis pathways

Mineralocorticoids, glucocorticoids, adrenal steroids are produced by the zona glomerulosa, fasciculata, and reticularis respectively. The major enzymes, which catalyze the steroidogenic reactions, are side-chain cleavage enzyme desmolase (P450scc), CYP17 (17 $\alpha$ -Hydroxylase and 17, 20-lyase), 3 $\beta$ -hydroxysteroid dehydrogenase (3 $\beta$ HSD), 21-Hydroxylase (21 $\beta$ -Hlase), and 11 $\beta$ -hydroxylase (11 $\beta$ -Hlase).

### 2.1.2 Development during growth

During childhood, the adrenal cortex changes in size, cell distribution, and function. This is impressively reflected by the progressively increase of AA production occurring several years before the onset of puberty (2, 3). As opposed to AAs, the production of mineralocorticoids and GCs does not follow such a clear age-associated pattern.

The onset of AA production from the adrenal ZR leads to the phenomenon of adrenarche, which occurs only in higher primates (22). Most researchers believe that adrenarche begins in mid-childhood at about 6-8 years (17). In contrast, two longitudinal studies – one performed in healthy children collecting 24-h urine samples at yearly intervals (3) and another in girls (with idiopathic central precocious puberty during long-term pituitary-gonadal suppression) providing blood samples at 3- to 6-month intervals (2) – suggest that adrenarche is not the result of sudden, rapid increase of AA production, but a gradual maturational process that begins in early childhood around 3 years. The AA secretion continues to increase during and after puberty and reaches maximum values in young adulthood. Thereafter follows a slow gradual decrease in AA levels in the elderly (adrenopause).

### 2.1.3 Abnormal adrenal androgen secretion

Premature adrenarche (PA) is defined as the appearance of pubic and/or axillary hair in the absence of breast or testicular development before the age of 8 yrs in girls and 9 yrs in boys. This condition associated with increased AA production occurs more commonly in girls than in boys with an unexplained sex ratio of nearly 10:1 (23). Hyperinsulinemia, insulin resistance, and unfavorable lipid profiles have been demonstrated in girls with PA (24, 25). Girls with PA are also at an increased risk for the development of polycystic ovarian syndrome (PCOS) (26). PCOS is characterized by hyperandrogenism and chronic anovulation with complications including hyperinsulinemia, insulin resistance, early onset of type 2 diabetes mellitus, dyslipidemia, cardiovascular disease and, infertility (27).

Congenital adrenal hyperplasia (CAH) is a group of inherited diseases caused by defective activity in enzymes that contribute to the synthesis of cortisol from cholesterol in adrenal cortex. 21-hydroxylase deficiency is the most common form of CAH. Patients with 21-hydroxylase deficiency can not adequately synthesize cortisol. To compensate this deficit of cortisol, more adrenocorticotropic hormone (ACTH) is secreted, resulting in the subsequent overproduction of AAs. The features of such patients include inappropriately rapid linear growth in childhood, early epiphyseal fusion of the long bones, short final stature, development of sexual hair, apocrine body odor, and penile or clitoral enlargement, and, infertility (28).