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CONGENITAL ADRENAL HYPERPLASIA IN ADULTS

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To my family

ABSTRACT

Congenital adrenal hyperplasia (CAH) is an autosomal recessive disorder affecting adrenal steroid synthesis. More than 95% of CAH cases are caused by reduced 21-hydroxylase function leading to variable extent of cortisol and aldosterone deficiency in addition to androgen excess. The foundation of CAH treatment is the use of glucocorticoids. However, overtreatment leads to Cushing's syndrome and undertreatment to hyperandrogenism and Addisonian crisis.

The aims of this thesis has been to evaluate the impact of CAH and its treatment on some factors that could lead to a reduced quality of life and increased morbidity or mortality during adult life.

In total 93 patients (32 males) with CAH and 93 (32 males) age- and sex-matched controls were studied. Subgroups of different ages (<30 years or older), phenotypes and the three most common genotype groups (null, I2 splice and I172N) were studied. Focus was on cardiovascular and metabolic risk, bone health in females and fertility in males.

Cardiovascular and metabolic risk: Younger female and male patients and controls had similar waist/hip ratio, lean and fat mass and insulin values. Older females had higher waist/hip ratio, lean mass and insulin values than controls. Fat mass was similar to controls but higher than in younger patients. Lipid profiles were slightly more favourable in older patients than in controls. Gestational diabetes was more common in patients. Few older female patients had hypertension, cardiovascular disease or diabetes. Despite moderate glucocorticoid doses, most patients had suppressed androgens. Serum liver enzymes were elevated in patients compared to controls. In patients, liver enzymes were correlated with waist circumference and with total body and trunk fat. Liver enzymes were increased even in non-obese patients mainly attributed to the patients ≥30 years who also demonstrated elevated insulin levels and HOMA-indices. In older males, waist/hip ratio, fat mass, and gamma-glutamyl transpeptidase were higher and heart rate faster than in controls. Insulin levels were increased during oral glucose tolerance test in all and older patients. Homocysteine was lower in all and in younger male patients which may be cardioprotective. Adverse cardiovascular profiles were mainly found in the mild genotype I172N. This group had normal urinary epinephrine concentrations whereas the more severe genotypes null and I2 splice had low levels. Few old male patients had cardiovascular disease and no patient had diabetes.

Bone health in females: Patients had lower bone mineral density (BMD) than controls at all measured sites. In patients ≥30 years old 73% were osteopenic or osteoporotic vs 21% in controls. BMD was similar in the two classic forms and had no obvious relationship to genotypes. More fractures were reported in patients than controls.

Fertility in males: Compared to national data the fertility was impaired in CAH males. The lifetime number of partners was smaller in all patients, in older patients and in the null group. Testicular tumours (TARTs) were found in 86% and 47% had pathological semen. Those with pathological semen had increased total and truncal fat mass, fat/lean mass ratio and heart rate. FSH was elevated and correlated negatively with sperm count and concentration.

Conclusions: Adult CAH females and males have a number of issues due to the disease and to corticoid supplementation. However, the findings in this thesis are more positive than many of the previous reports on CAH. Many parameters studied in our CAH individuals <30 years were not different from age- and sex-matched controls. This is likely to reflect improvements in management.

LIST OF PUBLICATIONS

I. **Falhammar H**, Filipsson H, Holmdahl G, Janson PO, Nordenskjöld A, Hagenfeldt K, Thorén M.

Metabolic profile and body composition in adult women with congenital adrenal hyperplasia due to 21-hydroxylase deficiency.

J Clin Endocrinol Metab 2007 92:110-116

II. **Falhammar H**, Filipsson H, Holmdahl G, Janson PO, Nordenskjöld A, Hagenfeldt K, Thorén M.

Increased liver enzymes in adult women with congenital adrenal hyperplasia due to 21-hydroxylase deficiency.

Endocr J 2009 56:601-608

III. **Falhammar H**, Filipsson Nyström H, Wedell A, Thorén M.

Cardiovascular risk, metabolic profile, and body composition in adult males with congenital adrenal hyperplasia 21-hydroxylase deficiency.

Submitted.

IV. **Falhammar H**, Filipsson H, Holmdahl G, Janson PO, Nordenskjöld A, Hagenfeldt K, Thorén M.

Fractures and bone mineral density in adult women with 21-hydroxylase deficiency.

J Clin Endocrinol Metab 2007 92:4643-4649

V. **Falhammar H**, Filipsson Nyström H, Ekström U, Granberg S, Wedell A, Thorén M.

Sexuality, fertility, and testicular adrenal rest tumors in adult males with congenital adrenal hyperplasia.

Manuscript.

RELATED PUBLICATIONS

1. **Falhammar H**, Thorén M 2005 An 88-year-old woman diagnosed with adrenal tumor and congenital adrenal hyperplasia: connection or coincidence? J Endocrinol Invest 28:449-453

2. Nordenskjöld A, Holmdahl G, Frisén L, **Falhammar H**, Filipsson H, Thorén M, Janson PO, Hagenfeldt K 2008 Type of mutation and surgical procedure affect long-term quality of life for women with congenital adrenal hyperplasia. J Clin Endocrinol Metab 93:380-386

3. **Falhammar H**, Thorén M, Hagenfeldt K 2008 A 31-year-old woman with infertility and polycystic ovaries diagnosed with non-classic congenital adrenal hyperplasia due to a novel CYP21 mutation. J Endocrinol Invest 31:176-180

4. Hagenfeldt K, Janson PO, Holmdahl G, **Falhammar H**, Filipsson H, Frisén L, Thorén M, Nordenskjöld A 2008 Fertility and pregnancy outcome in women with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Hum Reprod 23:1607-1613

5. **Falhammar H** 2008 Acne and non-classic congenital adrenal hyperplasia. N Z Med J 121(1275):94-95

6. Nygren U, Södersten M, **Falhammar H**, Thorén M, Hagenfeldt K, Nordenskjöld A 2009 Voice characteristics in women with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Clin Endocrinol (Oxf) 70:18-25

7. Frisén L, Nordenström A, **Falhammar H**, Filipsson H, Holmdahl G, Janson PO, Thorén M, Hagenfeldt K, Möller A, Nordenskjöld A 2009 Gender role behavior, sexuality, and psychosocial adaptation in women with congenital adrenal hyperplasia due to CYP21A2 deficiency. J Clin Endocrinol Metab 94:3432-3439

8. **Falhammar H** 2010 Non-classic congenital adrenal hyperplasia due to 21-hydoxylase deficiency as a cause of infertility and miscarriages. N Z Med J 123(1312):77-80

9. Nordenström A, Frisén L, **Falhammar H**, Filipsson H, Holmdahl G, Janson PO, Thorén M, Hagenfeldt K, Nordenskjöld A 2010 Sexual function and surgical outcome in women with congenital adrenal hyperplasia due to CYP21A2 deficiency: clinical perspective and the patients' perception. J Clin Endocrinol Metab 95:3633-3640

10. **Falhammar H**, Nordenström A, Thorén M 2010 Anthropometry in Congenital Adrenal Hyperplasia. In Handbook of Anthropometry: Physical Measures of Human Form in Health and Disease. Springer, USA. In Press.

CONTENTS

LIST OF ABBREVIATIONS

ACTH	Adrenocorticotropic hormone
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BMD	Bone mineral density
DHEAS	Dehydroepiandrostendione sulfate
DOC	Deoxycorticosterone
DXA	Dual energy X-ray absorptiometry
CAH	Congenital adrenal hyperplasia
CTX	β-C telopeptide of type I collagen
HDL	High-density lipoprotein
IGFBP	IGF-binding protein
ICSI	Intracytoplasmic sperm injection
GGT	Gamma-glutamyl transpeptidase
LDL	Low-density lipoprotein
NAFLD	Nonalcoholic fatty liver disease
NC	Non-classic
PCOS	Polycystic ovary syndrome
17OHP	17-hydroxyprogesterone
SV	Simple virilizing
SW	Salt-wasting
TART	Testicular adrenal rest tumor
WHO	World Health Organization

1. INTRODUCTION AND BACKGROUND

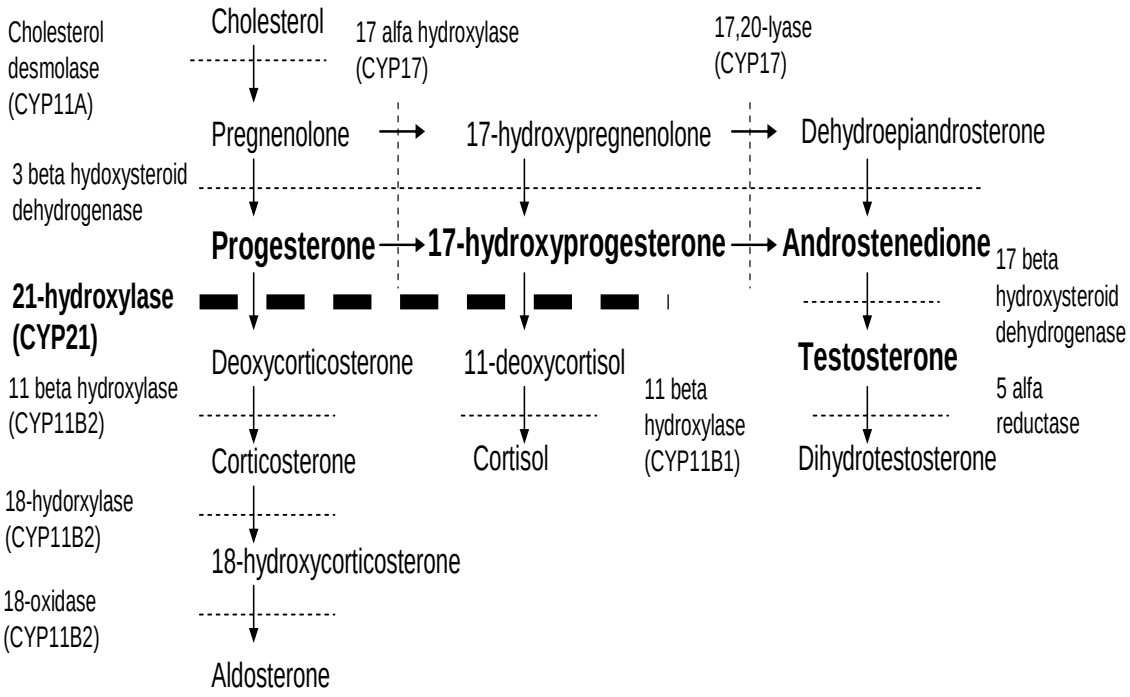
In 1865 the Neapolitan professor of anatomy Luigi De Crecchio described an astonishing case (De Crecchio 1865). He reported on the autopsy of a certain Joseph Marzo who had a 6 cm long penis with first grade hypospadias and no testes but with normal vagina, uterus, tubes, and ovaries. Moreover, the adrenal glands were clearly enlarged. Joseph had been considered a female at birth but had at 4 years of age been declared male. Prof De Crecchio conducted extensive interviews with people close to Joseph and all described him as a typical male socially and sexually. Joseph died in his 40s after one of many episodes of vomiting and diarrhea. This is the first reported case of congenital adrenal hyperplasia (CAH) and the description is still relevant today.

CAH is an autosomal recessive disorder affecting adrenal steroid synthesis. More than 95% of CAH cases are caused by reduced 21-hydroxylase function leading to variable degree of cortisol and aldosterone deficiency together with adrenal androgen excess (Merke and Bornstein 2005). The impaired cortisol secretion causes ACTH levels to rise and stimulate adrenocortical hormone secretion, resulting in hyperplasia of the adrenal cortex, accumulation of the precursors immediately proximal to the 21-hydroxylase enzyme in the pathway of cortisol and aldosterone synthesis, and the precursors are then directed into the androgen pathway (Fig. 1). Moreover, cortisol secretion from the adrenals is necessary for adrenomedullary organogenesis and epinephrine production which is therefore impaired in classic CAH (Merke and Bornstein 2005).

1. 1 Clinical presentation of 21-hydroxylase deficiency

Three distinct phenotypes are recognized in CAH due to 21-hydroxylase deficiency: salt-wasting (SW), simple virilizing (SV), and non-classic (NC) CAH. SW and SV are usually called classic CAH with newborn females demonstrating virilized external genitalia. This is in contrast to affected boys who have no overt signs of CAH except variable and subtle hyperpigmentation and penile enlargement. Males and females with SW phenotype have severe aldosterone deficiency resulting in salt loss which may be life-thretening in the neonatal period if not recognized and adequately treated.

Fig 1. Synthesis of steroid hormones in the adrenal cortex. The pathways of cortisol, aldosterone, and androgen synthesis and the different enzymes involved are shown. 21-hydroxylase is the enzyme most frequently deficient in congenital adrenal hyperplasia and is highlighted together with steroids elevated in this condition. Genes coding for the various enzymes are shown within brackets. From Falhammar et al 2010 Anthropometry in Congenital Adrenal Hyperplasia. In Handbook of Anthropometry: Physical Measures of Human Form in Health and Disease. Springer, USA. In Press.



Males with SV phenotype usually present with early virilization at age 2-4 years (Merke and Bornstein 2005), but cases with much later presentation, even during adult life, have been described.

The virilization of external genitalia in females demonstrates a wide range from very mild to severe with complete masculine appearance. In case of minimal clitoromegaly and fusion between the vagina and urthra near the perineum, surgery may be unnecessary. In the past the management of genital surgery has varied between clinics both with respect to extent of surgery and at what age it should be performed (6 months and 9 years) (Nordenskjöld et al 2008). It is nowadays recommended that the first surgery is done between 2 and 6 months and only in specialized centers to improve the surgical results and quality of life (Clayton et al 2002, Nordenskjöld et al 2008). If further surgery is needed it should, if possible, wait until the girl has reached adolescence and can make her own decision (Clayton et al 2002).

Severe neonatal virilization in girls can lead to erroneous sex assignment (White and Speiser 2000). These females are more likely to be brought up as males in cultures that value boys more than girls and/or in developing countries where the diagnosis often is delayed (Abdullah et al 1991, Kademir and Yordam 1997). However, even in the developed world, the female internal genitalia may escape discovery until late in life (Ravichandran et al 1996).

In NC CAH females, external genitalia are normal at birth, but signs and symptoms of androgen excess often develop in both males and females during the peripubertal period or in adulthood (New 2006). In NC females over 10 years of age the presenting symptoms reported were hirsutism (59%), oligomenorrhea (54%), acne (33%), infertility (13%), clitoromegaly (10%), alopecia (8%), primary amenorrhea (4%) and premature pubarche (4%) (Moran et al 2000). NC can also be found in the work-up of miscarriages, even late ones (Bidet et al 2010, Falhammar 2010).The symptoms and signs may be indistinguishable from those in the polycystic ovary syndrome (PCOS) (Falhammar et al 2008a), and exclusion of CAH is a prerequisite for the diagnosis of PCOS (Azziz et al 2009). Little has been published about males with NC CAH, and they are usually found during family screening (New 2006). Overrepresentation in males with severe acne may occur (Placzek et al 2005, Sharquie et al 2009). It has been claimed that all individuals diagnosed with NC based on genetic testing will develop signs of hyperandrogenism over time, including those who were initially asymptomatic (Levine et al 1980, New 2006). However, even in females symptoms and signs are sometimes very discrete and NC CAH may not be diagnosed until old age by coincidence (Falhammar and Thorén 2005).

1. 2 Neuropsychology

Androgens have been proposed to exert an organizational effect on higher brain function during fetal development and an activating effect during puberty (Goy and McEwen 1980). In females with CAH, having elevated prenatal and early postnatal adrenal androgens, the organizational effects of androgens on the developing brain have been studied. Since the 1960s numerous studies have demonstrated the masculinized behavior in girls with CAH e.g. regarding childhood play behavior, aggressive behavior, and spatial perception (Ehrhardt et al 1968, Berenbaum and Resnick 1997, Nordenström et al 2002, Hines et al 2003). Moreover, increased frequency of left-handiness in females with CAH has been proposed (Nass et al 1987), but has not been confirmed by others (Helleday et al 1994).

Prenatal androgens are believed to have a dose-dependent rather than a threshold effect. We found convincingly in our cohort of adult CAH women a high frequency of gender-atypical behavior regarding choise of occupations, spare time interests, and sports (Frisén et al 2009). The behavior was correlated with the severity of the *CYP21A2* mutations, and was most marked in genotypes with unmeasurable enzyme activity. Sexual orientation was also affected. Overall, bi- and homosexuality was present in 19% of the patients, being 50% in the most severe genotype group and declining with milder mutations.

1. 3 Neonatal screening

Early recognition and treatment of CAH due to 21-hydroxylase deficiency can prevent serious morbidity and mortality. The disease is appropriate for neonatal screening due to its relative commonness and can quite easily be diagnosed by 17-hydroxyprogesterone (17OHP) determination from a dried blood spot sample on filter paper. Currently, 49 states in the US and as a minimum 16 other countries have newborn screening for CAH, and 13 additional countries have pilot or local screening programs (White 2009). In Sweden newborn screening started in 1986 and clear benefits have been demonstrated as lowered age of definite diagnosis in boys from 21 days to 9 days, no more death reported due to neonatal salt-wasting, and virtually eradication of salt-losing crisis. Roughly half of the diagnosed CAH babies did benefit from the screening and the cost was reasonable (USD 26 700 per case CAH found) (Thilén et al 1998). No screening program will however detect all NC 21-hydroxylase deficiencies without an unacceptably high number of false positive cases. Thus, NC

CAH is still usually diagnosed by clinical signs and symptoms in older children and adults.

1. 4 Prevalence of 21-hydroxylase deficiency

Data from 13 neonatal screening programs (USA, France, Italy, New Zealand, Japan, UK, Brazil, Switzerland, Sweden, Germany, Portugal, Canada, and Spain) with more than 6.5 million newborns included demonstrated that 21-hydroxylase deficiency is quite common with a frequency of the classic form of one in 15 000 livebirths (Merke and Bornstein 2005). In Sweden the frequency was higher with one in 9 800 affected (Thilén et al 1998). Thus, the carrier incidence is roughly one in 50 individuals. However, the incidence of classic 21-hydroxylase deficiency varies widely depending on ethnicity and geographical location being highest in two very isolated areas with relatively small populations: the Ypic Eskimos in Alaska (one in 282), and on the island of La Réunion in the Indian Ocean (one in 2 141), but even a big cosmopolitan city as Rome in Italy has a high frequency (one in 5 580 Caucasians) (Pang et al 1988). In contrast, classic 21-hydroxylase deficiency is rare in Afro-Americans (one in 42 309) (Therrell et al 1998).

NC CAH is much more prevalent than classic CAH. In New York City with a very heterogenous population one in 111 was affected. Divided into different ethnicities the prevalence of the disease was in: Ashkenazi Jews 3.7%, Hispanics 1.9%, former Yugoslavs 1.6%, Italians 0.3%, and the remaining Caucasian population 0.1% (Speiser et al 1985). The high prevalence in the Caucasian population makes NC CAH due to 21-hydroxylase deficiency the most frequent autosomal recessive disorder in man (New 2006).

1. 5 Diagnosis

The biochemical hallmark of 21-hydroxylase deficiency is the elevation of 17OHP, the main substrate for 21-hydroxylase (Fig.1). In neonates a concentration of 17OHP > 240 nmol/L in a random blood sample is diagnostic of classic 21-hydroxylase deficiency (Merke and Bornstein 2005). The reference level is < 3 nmol/L at 3 days in a full-term infant. Premature, sick or stressed newborn babies have higher levels of 17OHP than healthy, term babies leading to many false-positive tests (White 2009). These newborns may need serial measurements of 17OHP to confirm or rule out classic CAH (Clayton

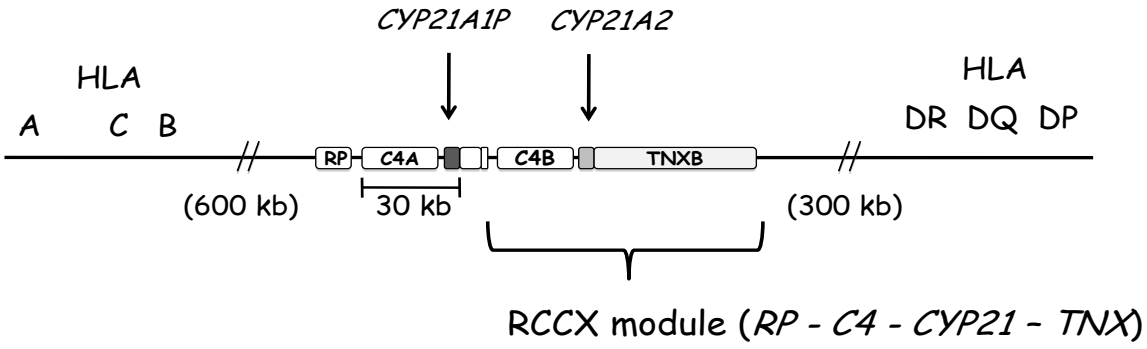
et al 2002). Typically, newborns with most severe genotypes have higher 17OHP concentrations than the other phenotypes (Nordenström et al 1999).
When the diagnosis is suspected later in childhood and onwards most experts advocate an early morning 17OHP as screening. A value < 2.5 nmol/L in children and < 6.0 nmol/L in adults will normally exclude CAH (Merke and Bornstein 2005). It has been estimated that 11% of NC CAH will be missed by this approach, at least in adults (Bachega et al 2000). Thus, if the clinical suspicion remains in spite of a normal basal 17OHP, the gold standard for diagnosis is the ACTH-stimulation test (250 mg of cosyntropin iv), with measurement of 17OHP at 60 min. NC CAH has traditionally been diagnosed by basal 17OHP > 15 nmol/L and/or ACTH-stimulated 17OHP > 30 nmol/L in males and in females during follicular phase (Bachega et al 2000). However, it was found in 58 patients with NC CAH that the ACTH-stimulated 17OHP levels ranged from 51 to 363 nmol/L (Bachega et al 2000). Consequently, it has been suggested to increase the ACTH stimulated cut-off value to > 45 nmol/L, and to allow testing at any time of the day and at any day during the menstrual cycle (Merke and Bornstein 2005). In patients with classic CAH, basal and ACTH-stimulated 17OHP will exceed 300 nmol/L.Twenty-four hour urinary pregnanetriol, the urinary metabolite of 17OHP, can be used to diagnose 21-hydroxylase deficiency (White and Speiser 2000), but normal values can be found in NC CAH. Two other variants of CAH with a different clinical presentation, 3β-hydroxysteroid dehydrogenase type 2 deficiency in women and 11β-hydroxylase deficiency in both sexes have rarely been misdiagnosed as 21-hydroxylase deficiency but will be identified by the serum steroid pattern following ACTH stimulation and/or the urinary steroid profile (White and Speiser 2000). The diagnosis is preferably confirmed by gene mutation analysis, which is particularly important in cases with symptoms and borderline elevation of 17OHP.

1. 6 Molecular genetics of *CYP21A2*

The structural gene encoding human 21-hydroxylase is a microsomal cytochrome P450 named *CYP21A2* (*CYP21*, or *CYP21B*). *CYP21A2* and the homologous inactive pseudogene, *CYP21A1P* (*CYP21P*, or *CYP21A*) are located in a complicated structure within the HLA major histocompatibility complex on chromosome 6p21.3 approximately 30 kb apart (Fig. 2). The genes are close to and alternating with the genes encoding the fourth component of serum complement, namely factor C4B and C4A (Carrol et al 1985, White et al 1985). *CYP21A2* and *CYP21A1P* each contain 10 exons with 98% homology of nucleotide sequences in the exons and roughly 96% in

the introns (Higashi et al 1986, White et al 1986). In spite of their almost identical nucleotide sequences, only *CYP21A2* is functional while *CYP21A1P* is a pseudogene with a number of harmful sequences. The similarities and proximity between the two genes predispose exchange of material. Approximately 95% of all mutations causing 21-hydroxylase deficiency are deletions/large gene conversions of the entire *CYP21A2* and/or a few point mutations that have been transferred from the inactive *CYP21A1P* to the active *CYP21A2* with the remaining 5% of the mutations occurring spontaneously without the involvement of the pseudogene (Wedell et al 1994, White and Speiser 2000).

Fig 2. The *CYP21A2* gene encoding steroid 21-hydroxylase is located in the HLA class III region in the major histocompatibility (MHC) locus on the short arm of chromosome 6 (band 6p21.3) together with a highly homologous pseudogene, *CYP21A1P*. Both genes are arranged in tandem repeat with the *C4A* and *C4B* genes encoding the fourth component of complement. The *C4/CYP21* unit is flanked by a telomeric *RP* gene and a centromeric *TNX* gene, forming what is referred to as RCCX modules (*RP-C4-CYP21-TNX*). Most haplotypes have a bimodular form composed of two sets of four genes arranged in tandem. Illustration kindly supplied by Prof Anna Wedell.



The frequency and spectrum of the most common mutations are similar between populations, even though some minor differences may exist between different ethnic

populations. In a Swedish cohort *CYP21A2* was entirely missing on 29.8% of the chromosomes, and the following point mutations were the most prevalent: I2 splice (27.7%), I172N (20.8%), V281L (5.4%), and R357W (3.8%) (Wedell et al 1994).

1. 7 Correlations between genotype and phenotype

Unlike most genetic diseases, a good genotype-phenotype correlation has been found in 21-hydroxylase deficiency (Wedell et al 1994, Jääskeläinen et al 1997), although some exceptions may occur. The advantage of performing mutation analysis is that the clinical presentation can be predicted and serious consequences prevented. Moreover, mutation analysis confirms the diagnosis.

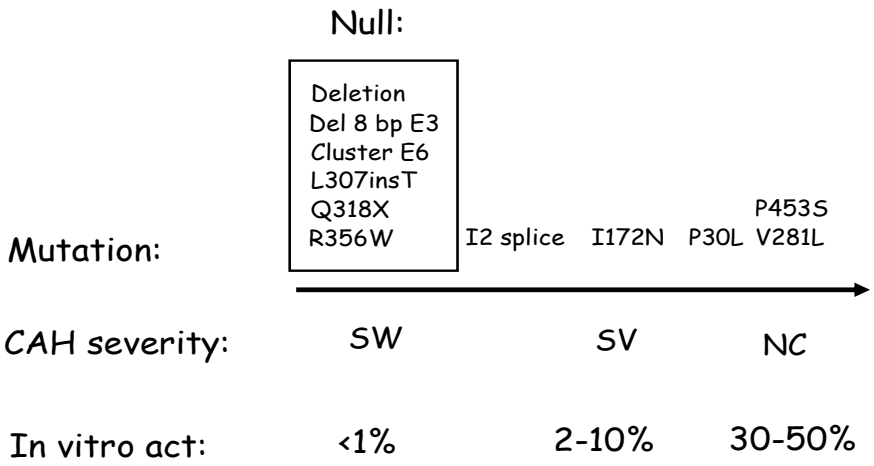
The milder mutation of the two affected alleles determines the phenotype. Although three different phenotypes exist, four distinct genotype groups can be identified with the mildest allele representing the group: null, I2 splice, I172N and NC genotype. Null refers to mutations completely abolishing enzyme activity and is associated with the SW phenotype. I2 splice retains a very low but measurable level of activity and is usually associated with SW but in a few cases SV. I172N is less severe and most often found in SV, but is rarely associated with SW. The final group includes mutations such as V281L and P30L with enzyme activities of between 30 and 50% and is associated with NC (Fig. 3) (Wedell et al 1994, White and Speiser 2000).

1. 8 Uncommon variants

As mentioned above, 21-hydroxylase deficiency constitutes about 95% of all CAH. Deficiencies of other adrenocortical enzymes shown in Fig. 1 can also be involved causing other phenotypes. All variants are, however, associated with cortisol insufficiency.

The most common CAH variant after 21-hydroxylase deficiency has an incidence of about one in 100 000 in the general population (White and Speiser 2000). This variant, 11β-hydroxylase deficiency, exhibits elevated levels of the steroid precursors deoxycorticosterone (DOC) and 11-deoxycortisol (Fig.1).

Fig. 3 The common, pseudogene-derived mutations in *CYP21A2*, their corresponding clinical disease presentations, and their residual activities assayed after expression of recombinant enzyme *in vitro*. Illustration kindly supplied by Prof Anna Wedell.



DOC is a strong mineralocorticoid, and affected patients show decreased serum potassium and hypertension which is also found in 17α-hydroxylase deficiency due to accumulation of DOC and another mineralocorticoid precursor, corticosterone. These two precursors have a weak glucocorticoid activity preventing salt-losing crisis, being rare in both 11β-hydroxylase and 17α-hydroxylase deficiency.

Genital ambiguity is not only a neonatal presentation of 46,XX individuals affected by 21-hydroxylase deficiency or 11β-hydroxylase deficiency, but is also found in 46,XY individuals affected by 17α-hydroxylase deficiency. Two CAH variants can display genital ambiguity in both sexes, 3β-hydroxysteroid dehydrogenase type 2 deficiency and P450 oxidoreductase deficiency. The genes for these four CAH variants are well studied and mutation analyses are available (Krone and Arlt 2009).

1. 9 Treatment

The foundation of CAH treatment is the use of glucocorticoids that will substitute the cortisol insufficiency and decrease ACTH production and secretion leading to lower adrenal androgens. Glucocorticoids first became available in the beginning of the

1950s. No currently living SW CAH individuals were born before the introduction since they did not survive the neonatal period. The launch of glucocorticoids led to survival and control of symptoms. Later, fear of long-term side effects of supraphysiological glucocorticoid supplementation has emerged. Nowadays, the practice is to give the lowest possible dose that suppresses excessive adrenal androgen and steroid precursor secretion and replaces cortisol deficiency. This is almost an impossible task as glucocorticoid doses needed to normalize androgens are often supraphysiological and there is no consensus regarding which laboratory and clinical parameters should be used to monitor therapy in adults.

Hydrocortisone (10–15 mg/m^2/day divided in three doses) has been recommended as the glucocorticoid of choice during childhood as it is considered to affect growth less than other preparations (Clayton et al 2002). Cortisone acetate has to be converted to cortisol for biological activity and this conversion can be impaired due to low 11β-hydroxysteroid dehydrogenase activity and is therefore a less favourable alternative (Nordenström et al 1999). Intermediary-acting glucocorticoids, such as prednisolone (5.0–7.5 mg per day divided in two doses) and long-acting glucocorticoids, such as dexamethasone (0.25–0.50 mg at bedtime or divided in two doses), may be an option at or near the completion of linear growth (Merke and Bornstein 2005). In adults the glucocorticoid of choice is often prednisolone (Ogilvie et al 2006, Nermoen et al 2010, Arlt et al 2010). In contrast to hydrocortisone, prednisolone and dexamethasone have minimal mineralocorticoid effect in the doses given.

Mineralocorticoid replacement is achieved with fludrocortisone and is usually mandatory in SW, but is also recommended in SV as it allows management with lower doses of glucocorticoids (Clayton et al 2002, Merke and Bornstein 2005). All individuals with 21-hydroxylase deficiency have some degree of aldosterone insufficiency, even NC patients (Fiet et al 1989), and mineralocorticoids have sometimes been used in NC (Falhammar et al 2008a, Williams et al 2010, Arlt et al 2010). The dose should be adjusted to maintain plasma renin in the range from mid-normal to slightly elevated. A typical daily dose of fludrocortisone ranges from 0.1 mg to 0.2 mg with higher doses in infancy and lower in adulthood. Very low doses are often used in older adults (0.05-0.025 mg) due to risk of side-effects (hypertension and oedema). Of note is that the dose is independent of body size. Salt tablets are usually used in infancy in addition to fludrocortisone (White and Speiser 2000).

1. 10 Outcome

Overall, data from children with CAH are quite abundant while data concerning adults are scanty. Most studies of adults with CAH have exclusively recruited patients up to 30 years of age and the majority of studies in adults have no or only few males included. Thus, information on the situation for adults with 21-hydroxylase deficiency aged ≥ 30 years is limited and studies are needed.

1. 10. 1 Metabolic and cardiovascular risk profiles

The long-term use of slightly supraphysiological glucocorticoid doses is potentially harmful and may bring an increased risk of obesitas, type 2 diabetes, dyslipidemia, hypertension and cardiovascular morbidity and mortality to patients with CAH. Previous reports on cardiovascular and metabolic risk profiles in adults with CAH have been conflicting, few parameters have been studied and mainly young adults below the age of 30 years have been included. In spite of an equal prevalence in males and females, predominantly females have been studied (Speiser et al 1992, Paula et al 1994, Cameron et al 1995, Hagenfeldt et al 2000, Stikkelbroeck et al 2003, Bayraktar et al 2004, Christiansen et al 2004, Saygili et al 2005, Hoepffner et al 2006, King et al 2006, Bachelot et al 2007, Sartorato et al 2007, Kroese et al 2009, Arlt et al 2010). These issues have recently been reviewed (Kim and Merke 2009, Mooij et al 2009).

1. 10. 1. 1 Body composition

BMI. Obesity is an important risk factor for type 2 diabetes and cardiovascular morbidity and mortality. Most studies have found increased BMI in adults and children with CAH (Helleday et al 1993, Cornean et al 1998, Hagenfeldt et al 2000, Paganini et al 2000, Stikkelbroeck et al 2003, King et al 2006, Völkl et al 2006a, Bachelot et al 2007, Völkl et al 2009, Arlt et al 2010), but not all (Cameron et al 1995, Guissinye et al 1997, Williams et al 2010). To avoid adult obesity, the management from infancy and onwards is extremely important. Over-treatment during infancy increased the risk of obesity in childhood despite adequate treatment for several years thereafter (Knorr et al 1988). Prepubertal children were shown to have increased BMI also when growth was unaffected. Normally, BMI increases rapidly to a peak in infancy and then reverses before increasing again later in childhood. An early age for this rebound is considered a reliable indicator of future adult obesity. Children with CAH had an earlier adiposity rebound by three years compared with the normal population. BMI SDS was greater

than one in all but one patient (of 22) in spite of unchanged height SDS (Cornean et al 1998). Parental obesity is another predisposing factor to childhood obesity. A 4.86 increased relative risk of obesity, defined as BMI > 2 SDS, has been reported in CAH children and adolescents with obese parents (Völkl et al 2006a).

Body circumferences. However, BMI is not an ideal estimate of body fat. For instance, body builders have elevated BMI but a very small proportion of body fat. Visceral obesity is an established risk factor for cardiovascular disease and type 2 diabetes. Waist circumference and the waist to hip ratio are established anthropometric measurements used for estimating visceral obesity and truncal fat. These measurements are better predictors of adverse outcomes than BMI (Ness-Abramof et al 2008). In spite of this, measurements of body circumferences have not been reported widely in CAH. In a small study of children affected with CAH aged 1.6 – 10.5 years, waist, hip, upper arm, and femur circumferences were increased compared to controls (Isguven et al 2008). The waist to hip ratio was, however, increased in girls while it was decreased in boys. A recent larger study of adults with CAH found increased waist circumference in females but not in males compared to national data (Arlt et al 2010).

DXA assessment of body composition. As mentioned above, BMI can be unreliable as an estimate of body fat in persons with CAH. Especially undiagnosed or poorly controlled females will have high androgen levels, leading to muscular hypertrophy (Fig. 4). High physical activity can also contribute (Frisén et al 2009). On the other hand, in physically very inactive patients or in those on severe overtreatment with glucocorticoids (exogenous Cushing´s syndrome), lean mass may be low and BMI may underestimate fat mass. Hence, a more reliable method of estimating body fat and lean mass is needed in addition to anthropometric measurements in individuals with CAH. Dual energy X-ray absorptiometry (DXA) provides a fairly accurate, reliable, and simple way of measuring total and regional fat and lean mass. Four studies in children and young adults with CAH each including 13-30 subjects (Cameron et al 1995, Hagenfeldt 2000, Stikkelbroeck et al 2003, Christiansen et al 2004) have all demonstrated increase in fat mass. Interestingly, two of the studies only found increased fat mass in male but not in female CAH patients (Cameron et al 1995, Christiansen et al 2004).

Fig. 4 Figure illustrating a short woman with classic CAH, poor compliance to glucocorticoid medication, and signs of muscular hypertrophy. Her serum testosterone was markedly elevated (16 nmol/L; reference interval in adult females 0.3 - 3.0 nmol/L, in adult males 10 - 30 nmol/L). From Falhammar et al 2010 Anthropometry in Congenital Adrenal Hyperplasia. In Handbook of Anthropometry: Physical Measures of Human Form in Health and Disease. Springer, USA. In Press.

1. 10. 1. 2 Liver enzymes

Increased frequency of elevated aminotransferase activity and signs of nonalcoholic fatty liver disease (NAFLD) have been reported in PCOS (Schwimmer et al 2005, Setji et al 2006). NAFLD is associated with increased risk of overall death compared to the general population (standard mortality ratio 1.34), with diabetes and cirrhosis being risk factors for death. It has been shown that subjects with NAFLD and elevated liver enzymes are at increased risk of developing metabolic complications, being three times more likely to develop type 2 diabetes and 50 % more likely to develop the metabolic

syndrome compared with the general population (Adams et al 2009). Thus, NAFLD and elevated liver enzymes are markers for increased future metabolic risk and could further predict the metabolic risk of CAH individuals. The frequency of NAFLD and/or liver enzymes has not been reported in CAH.

1. 10. 1. 3 Glucose, insulin, and lipids

No study has demonstrated increased frequency of diabetes in CAH but all have shown increased insulin resistance (Paula et al 1994, Speiser et al 1992, Charmandari et al 2002, Saygili et al 2005, Sartorato et al 2007, Völkl et al 2009, Williams et al 2010) except one (Bayraktar et al 2004). The latter study differed however, as only NC CAH patients were included, the diagnosis was not confirmed genetically and none of these patients was on glucocorticoid medication. Moreover, a recent study showed improved insulin sensitivity with pioglitazone treatment (Kroese et al 2009). Interestingly, in a recent study, only NC children and not classic CAH children were insulin resistant presumably due to adverse metabolic effects of prolonged postnatal androgen excess in NC (Williams et al 2010). Very few studies have included individuals over 30 years of age. Another way of predicting future type 2 diabetes is to study gestational diabetes. The frequency of this condition has not yet been reported in CAH. Studies of serum lipid profile in CAH have, in contrast to insulin resistance, shown normal values (Bayraktar et al 2004, Sartorato et al 2007, Bachelot et al 2007).

1. 10. 1. 4 Blood pressure

Studies evaluating blood pressure in patients with CAH have found divergent results (Sartorato et al 2007, Roch et al 2003, Völkl et al 2006b, Williams et al 2010, Arlt et al 2010). Single measurements in 29 adults with a mean age of 28 were normal and there were no differences compared with controls (Sartorato et al 2007), while in a large cohort of 199 adult 21-hydroxylase deficient patients (median age 34 years) females with classic phenotype demonstrated increased diastolic pressure compared to national data (Arlt et al 2010). On the other hand in children and adolescents, a single measurement of blood pressure was similar to controls in classic CAH while increased in NC CAH compared to controls (Williams et al 2010), and 24h ambulatory blood pressure measurements were elevated in classic CAH (Roch et al 2003, Völkl 2006b).

1. 10. 1. 5 Intima-media thickness

Intima-media thickness, a predictor of clinical arteriosclerosis and associated with cardiovascular risk, has been demonstrated to be elevated in adult individuals with CAH (Sartorato et al 2007). However, no individual had clinical cardiovascular disease, probably due to the small number (n = 19) and the fact that the subjects were too young to have manifest disease (mean age 28 ± 3.5 years).

1. 10. 2 Bone health

1. 10. 2. 1 Final height

Both over- and undertreatment with glucocorticoids during childhood and adolescence can compromise height development. Several studies of both males and females with CAH have verified a decreased final height. A meta-analysis of 18 studies published between 1977 and 2001 found that height was -1.37 SD under population mean which is equivalent to -10 cm (Eugster et al 2001). Later studies have confirmed this (Balsamo et al 2003, Arlt et al 2010), but also shown the importance of mineralocorticoid supplementation to optimize final height (Balsamo et al 2003). Early diagnosis and treatment improved height development in many (Bergstrand 1966, Eugster et al 2001, Balsamo et al 2003), but not in all studies (Urban et al 1978, Kirkland et al 1978, DiMartino-Nardi et al 1986). Good compliance to treatment is also important for a favourable outcome (Eugster et al 2001).

1. 10. 2. 2 BMD

It is well established that endogenous Cushing´s syndrome and pharmacological glucocorticoid therapy can generate osteoporosis via multiple mechanisms resulting in increased bone resorption followed by suppression of bone formation. Decreased intestinal calcium absorption and increased renal calcium excretion may lead to secondary hyperparathyroidism. Insulin-like growth factors, their binding proteins and the secretion of gonadal steroids may also be affected (Shaker and Lukert 2005). Data are conflicting concerning the effect of long-term glucocorticoid replacement therapy on bone mass and bone metabolism in patients with CAH. In adults bone mineral density (BMD) has been reported to be normal (Guo et al 1996, Mora et al 1996, Stikkelbroeck et al 2003, Christiansen et al 2004), or decreased (Jääskeläinen and Voutilainen 1996, Hagenfeldt et al 2000, Sciannamblo et al 2006, King et al 2006,

Bachelot et al 2007, Arlt et al 2010). In children and young adults BMD was found to be increased (Arisaka et al 2001), normal (Girgis et al 1997, Fleischman et al 2007) or decreased (Zimmermann et al 2009). Moreover, some studies of children found decreased BMD in males (Cameron et al 1995), at puberty (Paganini et al 2000) or in long-term treated girls (de Almeida Freire et al 2003). The discrepancies may reflect differences in age of patients, type and severity of enzyme deficiencies and various therapeutic regimes. If there is an increased prevalence of fractures, the ultimate outcome of decreased BMD, has not been documented probably due to the small number of CAH individuals studied, their low mean age and that fractures not have been recorded.

1. 10. 3 Fertility

1. 10. 3. 1 Female fertility

The frequency of pregnancies among women with CAH has constantly been reported as low compared with age-matched controls (Mulaikal et al 1987, Jääskeläinen et al 2000a, Krone et al 2001, Lo and Grumbach 2001, Gastaud et al 2007). In our cohort of 62 women with 21-hydroxylase deficiency only 16 had ever been pregnant compared to 41 of 62 age-matched controls. Moreover, the number of children was 25 in the women with CAH compared to 54 in controls. The fertility rate was clearly related to the severity of the *CYP21A2* mutation with no term pregnancy in the null group, 13% in the I2 splice group, 33% in the I172N group and 50% in the group of mutations consistent with NC CAH (Hagenfeldt et al 2008). The suggested reasons for low fertility in CAH women have been: delayed psychosexual development, low sexual activity, adrenal overproduction of androgens and steroid precursors, PCO, neuro-endocrine factors, and genital surgery (Jääskeläinen et al 2000a, Otten et al 2005). However, in our patients presented in details above, the main reason for lower fertility was that few lived in heterosexual relationships and few had ever tried to become pregnant. All CAH women who tried to become pregnant had succeeded, sometimes after some medical help, except for a few of the older patients (Hagenfeldt et al 2008).

1. 10. 3. 2 Male fertility

Increased adrenal androgens due to undertreatment with glucocorticoids leading to gonadotropin suppression can impair male CAH fertility (Claahsen-van der Grinten et

al 2009) and similar signs and symptoms may also be found in overtreatment (Reisch et al 2009). However, testicular adrenal rest tumours (TARTs) are considered the most important reason for reduced fertility.

Aberrant adrenal cells descend in the embryological period together with the testes in the majority of males. It is believed that these cells disappear if not stimulated. If adrenal cells are present and stimulated TARTs may arise. TARTs have receptors for both ACTH and angiotensin II, and high levels due to undertreatment with corticosteroids in CAH can enhance their growth (Claahsen-van der Griten 2007b). They are typically located in the rete testis and are associated with risk of obstruction of the seminal ducts, with subsequent permanent testicular damage. Few TARTs can be found by clinical examination as normally only TARTs > 2cm are detectable by palpation due to their location, buried within the testis (Claahsen-van der Grinten et al 2009). TARTs have been found in CAH children with a frequency of about 20% and down to 6 years of age (Claahsen-van der Griten et al 2007a, Martinez-Aguayo et al 2007). However, an old autopsy study found TARTs in 3 of 7 CAH boys less than 8 weeks (Shanklin et al 1963). TARTs have been diagnosed by palpation, ultrasound or MRI. The highest frequency of TARTs reported has been 94% (Stikkelbroeck et al 2001), while others report a prevalence of 0 - 69%, which probably reflects differences in mode of detection and age of patients (Urban 1978 et al, Avila et al 1996, Jääskeläinen et al 2000b, Cabrera et al 2001, Reisch et al 2009, Mouritsen et al 2010, Arlt et al 2010). TARTs are considered the most common reason for male CAH fertility problems which has been reported to be anything from normal (Urban et al 1978) to severely impaired (Jääskeläinen et al 2000b). The divergent results may be due to different modes of evaluation.

To suppress ACTH secretion by intensifying corticosteroid treatment is not always successful in reducing the TART size. Even in well-controlled CAH males with normal or suppressed plasma ACTH levels, TARTs have been found (Walker et al 1997, Stikkelbroeck et al 2004). In fact, parameters of hormonal control were positively correlated with adrenal volume but not TART volume (Reisch et al 2010). Thus, other unknown factors must also contribute to tumour growth.

When no decrease of the TARTs can be achieved with increased doses of corticoids, or if there is persistent azoospermia despite tumour reduction, testis-sparing surgery could be considered. Two small case series on steroid unresponsive TARTs have reported good results (Walker et al 1997, Tiryaki et al 2005). However, in a study of eight infertile males with 21-hydroxylase deficiency and TARTs, gonadal dysfunction and

oligo-azoospermia did not improve by surgery suggesting permanent damage of the surrounding testicular tissue (Claahsen-van der Grinten et al 2007c). The authors' conclusion was that surgery is only indicated for relief of pain and discomfort caused by TART. If surgery was still considered for longstanding TARTs, it should only be performed when testicular biopsies have demonstrated viable testicular tissue preoperatively.

However, TARTs may be present in CAH males who have children and a better indicator of fertility is semen analysis. Semen quality has recently been reported to be very poor in CAH males with all samples being pathologcal (Reisch et al 2009). But only one viable sperm is necessary to father a child and 22% had fathered children. In a Finnish study the child rate in CAH males was evaluated and compared with that of the whole Finnish male population with equal age distribution (Jääskeläinen et al 2000b). The CAH males had a child rate of 0.07 compared to 0.34 in the population. However, gonadotropin and inhibin B levels were similar to age-matched controls suggesting normal fertility. Thus, sexual and psychosocial factors may, like in CAH women discussed earlier, be a factor of importance for impaired fertility in CAH males. This has not been studied previously.

2. AIMS OF THE STUDY

The overall goal of this thesis has been to evaluate the impact of CAH and its treatment on some factors that could lead to reduced quality of life and increased morbidity or mortality during adult life.

The specific aims were to study in adult patients with CAH:

- Body composition in females and males (**Paper I and III**)
- Cardiovascular and metabolic risk factors in males and females (**Paper I, II and III**)
- Bone health in CAH including final height in males and females (**Paper I and III**) and bone mineral density and fracture prevalence in females (**Paper IV**)
- Fertility and fecundity in CAH males (**Paper V**)

3. MATERIALS AND METHODS

3. 1 Subjects

In total 93 patients with CAH and 93 age- and sex-matched controls were studied. The females were recruited by an appeal to all Swedish Departments of Gynaecology, Endocrinology, and Internal Medicine; advertisement in the Journal of the Swedish Medical Association; and information to the national CAH patient organization, whereas the male patients were recruited mainly from the two participating centers.

In all patients, diagnoses were verified by review of original pediatric and adult records including genital examinations, laboratory reports of adrenal steroids, and mutation analyses. All patients had 21-hydroxylase deficiency apart from one male who had 3-beta-HSD deficiency. Seventy-two (22 males) of the patients were recruited and examined at the Karolinska University Hospital and 21 (10 males) at the Sahlgrenska University Hospital.

Characteristics of the recruited females and males with CAH are given in Table 1. Two patients in **Paper V** deserve a more detailed presentation. In a 61-year-old patient with male phenotype (SV, genotype group I2 splice), a female karotype was diagnosed at age 7, the internal genitalia were extirpated, and glucocorticoid and testosterone treatments were initiated. He was working full-time in an academic profession, was happily married and had two adopted children. He had always had a male gender identity and had no objection what so ever to having been raised as a man. One 21-year-old male had been diagnosed with 3-beta-HSD deficiency (SW, genotype: C75R) as newborn, had ambiguous external genitalia at birth and surgery for hypospadias at 6 month of age; at inclusion he presented male external genitalia with micropenis. He was working full-time in a low qualified work, lived alone and had no children.

To find suitable controls to all recruited females and males, the National Population Registry was used. An invitation letter was sent to the person with the same sex in the National Population Registry following each patient. If these persons declined participation or did not answer despite reminding letters, the next person was invited and so on. Infants delivered at the same maternity ward at the same date were usually next to each other in the National Population Registry and were, or at least had been, living in the same area. The only exclusion criterion was severe mental or psychiatric disturbance with inability to consent to the study.

Table 1. Characteristics of the recruited adult females and males with CAH.

			Number, n (%)	Age, yrs Median (range)
Both sexes		All	93 (100)	32 (18-67)
Females		All	61 (100)	30 (18-63)
		< 30 years	27 (44.3)	24 (18-29)
		≥ 30 years	34 (55.7)	35 (30-63)
	Phenotypes	SW	27 (44.3)	30 (18-47)
		SV	28 (45.9)	32.5 (18-63)
		NC	6 (9.8)	29.5 (23-63)
	Genotype groups	Null	13 (21.3)	30 (18-34)
		I2 splice	15 (24.6)	30 (20-47)
		I172N	25 (41.0)	33 (18-63)
		NC	6 (9.8)	29.5 (23-63)
Males		All	32 (100)	34.5 (19-67)
		< 30 years	10 (31.3)	22.8 (19-29)
		≥ 30 years	22 (68.7)	38.6 (30-67)
	Phenotypes	SW	18 (56.2)	32.6 (19-52)
		SV	12 (37.5)	38.1 (21-67)
		NC	2 (6.3)	38.5 (31-45)
	Genotype groups	Null	7 (21.9)	34.6 (21-52)
		I2 splice	12 (37.5)	33 (19-61)
		I172N	9 (28.1)	34.4 (21-67)
		NC	3 (9.4)	36.6 (31-45)

3. 2 Study protocol

3. 2. 1 Female patients and controls (Paper I, II and IV)

A medical history was obtained from all female patients and controls. In addition, all participants answered questionnaires on social situation and previous and present health. Previous fractures were asked for. A general physical examination was performed including anthropometry (height, weight, waist and hip circumference). Blood pressure, supine and standing, was registered and signs of hypo/hypercortisolism and hyperandrogenism were recorded.

Blood samples were collected in the morning after an overnight fast for measurements of serum lipids [total cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol], liver enzymes [serum alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST)

and gamma-glutamyl transpeptidase (GGT)], electrolytes, plasma glucose, hormones [insulin, IGF-I, IGF-binding protein (IGFBP)-1, testosterone, DHEAS, androstenedione in all subjects and 17OHP, plasma ACTH, renin, PTH in patients], and β-C telopeptide of type I collagen (CTX). Twenty-four hour urinary pregnanetriol was measured only in the patients and samples were collected just prior to or after the examination. Total and regional fat and lean mass, and whole body, lumbar spine and femoral neck BMD were measured with DXA.

Results concerning anthropometry, metabolic and cardiovascular risk markers and body composition are presented in **Paper I**, liver function tests and their association to body composition and other parameters in **Paper II**, and fracture prevalence, bone mineral density and markers for bone metabolism in **Paper IV**.

3. 2. 2 Male patients and controls (Paper III and V)

The investigation of the male patients and controls was carried out in the same manner as in the females but some additional items were included. The questionnaires contained in addition to health and social factors items related to sexuality, fertility, and fecundity. Fecundity problems were defined as trying to father > 1 year. Estimations of testicular volume using orchidometer as well as assessment of testicular consistency and tumours were included in the physical examination. In addition, testicular ultrasound and analysis of a semen sample were included in the protocol. A 24h ambulatory blood pressure and heart rate monitoring started at the end of the main examination day. After collection of fasting blood samples an oral glucose (75 g) tolerance test (OGTT) followed. A morning urinary spot sample was collected for albumin determination. Catecholamines were analyzed in 24h urine samples. In patients, 24h urinary pregnanetriol and a diurnal 17OHP curve using dried blood spots were analyzed. Results associated with cardiovascular and metabolic risk factors are presented in **Paper III** and results concerning sexual function, fertility, fecundity, testicular function and imaging in **Paper V**.

3. 3 Methods

3. 3. 1 Body composition and BMD

Total and regional body fat, and lean mass, whole body, lumbar spine (L2–L4), and femoral neck BMD, were estimated by DXA in 57 CAH women and 60 control subjects using a Lunar Model DPX-L or Prodigy equipment (Lunar Radiation, Madison, WI) using a standard procedure as previously described (Mazess et al 1990). The two instruments were calibrated to each other. The body composition parameters lean mass and fat mass were divided by height^2 (kilograms per square meter) when analyzed to adjust for the difference in height between patients and controls. BMD values expressed as g/cm^2 were compared in patients and controls. BMD was also expressed as SD scores (SDS) from the mean of an age and sex-matched reference group (Z-score) provided by the manufacturers and SDS from the mean of young adults (T-score). In three CAH women, BMD was assessed by Hologic QDR 4500 (Hologic Inc., Waltham, MA) instead, and only T- and Z-scores were used. The World Health Organization (WHO) definitions of osteopenia and osteoporosis were applied (i.e. T-score between -1 and -2.5 SD at any measured site was defined as osteopenia, and values below -2.5 SD were defined as osteoporosis) (WHO 1994). However, the WHO criteria were set up to be applied to postmenopausal women, and not to premenopausal women with presumably low peak bone mass. Despite this, these criteria were preferred to identify different degrees of low bone mass.

The volume adjusted bone mineral apparent density (BMAD g/cm^3) was calculated of the vertebrae and femoral neck in order to explore the impact on the results of the differences in skeletal dimensions between the women, who were on average 6.8 cm shorter, and the age-matched controls, using the formulas previously proposed (Carter et al 1992): BMAD = bone mineral content (BMC)/bone area$^{1.5}$ for lumbar spine, and BMC/bone area^2 for femoral neck. To whole body measurements another formula was used: BMAD = BMC/ (bone area^2/ height) (Katzman et al 1991). Comparisons of Z-scores between patients and controls in the height range of 160 – 169 cm were applied as another method of controlling the influence of the height difference.

In males 32 CAH individuals and 31 controls were evaluated by the same principles as above all by Lunar Model Prodigy equipment but mainly body composition is presented in the thesis.

3. 3. 2 Glucocorticoid supplementation

The doses of glucocorticoids were converted to hydrocortisone equivalents using: antiinflammatory equivalents (30 mg hydrocortisone = 37.5 mg cortisone acetate = 7.5 mg prednisolone = 0.75 mg dexamethasone) (Liddle 1961); and growth-retarding equivalents (30 mg hydrocortisone = 37.5 mg cortisone acetate = 6 mg prednisolone = 0.375 mg dexamethasone) (Miller 1991). Thereafter, body surface area (BSA) was calculated as the square root of (height [cm] x weight [kg])/3600 (m^2) and was used to specify hydrocortisone equivalents in mg/m^2 (Mosteller 1987).

3. 3. 3 Blood pressure and heart rate

In males ambulatory 24h blood pressure and heart rate were determined with Meditech ABPM-05 (Meditech Ltd, Budapest, Hungary). An active (06:00 – 23:00) and a passive (23:00 – 06:00) part of the day were analyzed separately.

3. 3. 4 Testicular examination

The testicular ultrasounds were completed by one physician using a Voluson Expert 730 machine equipped with a 12 – 16 MHz real-time four-dimensional linear transducer (GE Healthcare, Kretz, Austria). Total testicular, TART and functional (total testicular-TART) volumes were estimated using the formula for a prolate ellipsoid (length x width x height x 0.523) (Fleischen et al 1990). The total number of TARTs in both testicles was summarized and noted.

3. 3. 5 Semen analysis

Seminal fluid was collected by the CAH males after 1 - 4 days of ejaculatory abstinence. The analysis included estimation of semen volume, sperm concentration, total sperm count, motile and immotile spermatozoa and morphology. Semen was evaluated according to the WHO standard (WHO 1999). The samples were divided into two groups: normal or pathological. A doubling of the WHO cut-off for normal sperm concentration to > 40 million/mL has been proposed and was also used as a stricter criterion for normality (Bonde et al 1998).

3. 3. 6 Hormones in serum and plasma

Serum DHEAS, serum PTH and plasma ACTH were measured on an Advantage automatic immune analyzer and plasma renin by immunoradiometric assay (both

from Nichols Institute Diagnostics, San Clemente, CA). The reference limits of PTH were 12 – 55 ng/L, ACTH 2.0 – 10 pmol/L and renin in standing position 4 – 46 ng/L. Serum insulin and testosterone were measured by fluoroimmunoassay (AutoDelfia; Wallac Inc, Turku, Finland). The reference value for fasting insulin was < 20 mU/L. To calculate the insulin resistance with a single fasting glucose and insulin value the HOMA-index [insulin/(22.5e-In glu)] was used (Matthew et al 1985). A HOMA-index ≥ 2.77 has been suggested to indicate insulin resistance (Bonora et al 1998). Bioactive testosterone was calculated with consideration of total testosterone, SHBG and albumin (Vermeulen et al 1999). To analyze serum IGF-I (Bang et al 1991), IGFBP-1 (Povoa et al 1982), 17OHP (CIS BioInternational, Gif-sur-Yvette, France), and androstenedione (DiaSorin S.p.A., Saluggia, Italy) RIA methods were used. A calculation of the regression line of the IGF-I concentrations in 448 healthy subjects, aged 20 – 96 years, was used to express IGF-I as SDS (Hilding et al 1999). The reference limits for serum 17OHP were 0.6 – 2.5 nmol/L (follicular phase), 2.2 – 6.5 nmol/L (midcycle phase), 2.5 – 10 nmol/L (luteal phase), and 0.5 – 2.0 nmol/L (menopause). Sexual hormone binding globulin (SHBG), dried blood spot 17OHP [measured at 08:00 (reference < 6 nmol/L), 14:00, 19:00, 01:00, and 06:00], FSH, LH, estradiol, total and free PSA were measured by fluoroimmunoassay (AutoDelfia, PerkinElmer, Waltham, MA). FSH and LH values between 1.0 - 10 U/L were considered normal. Prolactin was measured by immunoassay (Beckman Coulter Inc, Fullerton, CA); values between 3-13 µg/L were considered normal.

3. 3. 7 Hormones in urine

Urinary pregnanetriol was determined by gas chromatography and gas chromatography-mass spectrometry (Axelsson et al 1981). The reference limits were < 6 µmol/24 h (follicular phase and in males) and < 8 µmol/24 h (luteal phase). High performance liquid chromatography was used for determination of 24h urinary epinephrine and norepinephrine (reference limits < 80 and < 400 nmol/24h, respectively).

3. 3. 8 Routine clinical chemistry and bone markers

Serum cholesterol, triglycerides, HDL, ALP, ALT, AST, GGT, Lipoprotein-(a) [Lp(a)], and plasma homocysteine and glucose were measured on SYNCHRON LX Systems (Beckman Coulter Inc., Fullerton, CA). LDL concentration was calculated

(Friedewald et al 1972). The reference limits for females given by the manufacturer were for ALP: < 3.8 μkat/L; ALT and AST: < 0.60 μkat/L (the proposed definition of ALT > 0.317 μkat/L [19 U/L] as pathological in women was also applied [Prati et al 2002]); and GGT: < 0.80 μkat/L; and males ALP: < 1.9 μkat/L; ALT: < 1.20 μkat/L and GGT < 2.0 μkat/L. Serum CTX was measured on a Roche Elecsys 1010/2010 immunoassay analyzer (Roche Diagnostics Ltd., Basel, Switzerland) with the reference limits of < 550 ng/L in premenopausal women and of < 1000 ng/L in postmenopausal women. Sodium, potassium, creatinine and urinary albumin were measured using routine assays. High performance liquid chromatography was used to measure hemoglobin A1c (HbA1c) by the MonoS method (reference limits 3.6 – 5.3%).

3. 3. 9 Statistics

Data were analyzed using SigmaStat for Windows (Jandel Scientific, Erkarath, Germany). Results are presented as the mean ± SEM (**Paper I, II and IV**) or ± SD (**Paper III and V**) if not otherwise stated. Comparisons between two groups were made using the unpaired *t* test when values were normally distributed. Otherwise, the Mann-Whitney rank-sum test was used and, in these cases, the median and range are reported. When continuous variables were compared in three groups (**Paper III and V**), one-way ANOVA was used for normal distributions, otherwise the Kruskal-Wallis test was performed, both followed by *post hoc* Bonferroni *t* or Mann-Whitney rank-sum test with Dunn's method. Chi-square was used in frequency table calculations or, when the expected frequency was small (< 5), Fisher's exact test. All proportions were calculated discounting missing values. In **Paper I, III and IV** correlations between continuous variables were assessed using linear and multiple regression analysis. In these cases, IGFBP-1, insulin, ACTH, testosterone (not in males), androstenedione, DHEAS, 17OHP, and pregnanetriol concentrations were log transformed before analysis to obtain a more closely approximated Gaussian distribution. Spearman's correlation coefficient was used for correlation analyses in the remaining papers. Statistical significance was set at $P < 0.05$ and tendency at 0.05–0.10.

4. RESULTS AND DISCUSSION

4. 1 Age at diagnosis and glucocorticoid therapy

All included patients were born before the introduction of neonatal 17OHP screening. Thus, they were diagnosed on clinical suspicion. All individuals with the SW phenotype were diagnosed during the first weeks of life with salt-losing crisis (females and males) and the girls also presented ambiguous external genitalia. The females with the SV phenotype were diagnosed at 0 – 15 years of age with virilized external genitalia while the males were diagnosed at 3 – 28 years of age, both groups with mild or absent salt loss. NC females were diagnosed between 6 and 32 years of age and the males at 11 and 30 years of age both with nonambiguous external genitalia and no history of salt wasting (**Paper I - V**). The most commonly used glucocorticoid was prednisolone followed by hydrocortisone in both females and males. The glucocorticoid doses were similar in younger and older CAH females when comparing hydrocortisone equivalents (antiinflammatory equivalents: 18.1 ± 1.1 vs. 15.9 ± 0.9 mg/m^2; P = NS), and when comparing patients in the null, I2 splice, and I172N groups (17.8 ± 1.5 vs. 18.0 ± 1.2 vs. 15.9 ± 1.0 mg/m^2; P = NS) (**Paper I**). In males, hydrocortisone equivalents were similar in younger and older CAH males (16.3 ± 3.6 vs. 17.7 ± 5.6 mg/m^2) or between the genotype groups null, I2 splice and I172N (19.3 ± 3.5 vs. 16.2 ± 4.2 vs. 16.7 ± 7.2 mg/m^2) (partly presented in **Paper III**). When comparing CAH females with males the differences in hydrocortisone equivalents were non-significant.

4. 2 Cardiovascular morbidity

As expected, no cardiovascular morbidity was found in females and males with CAH below 30 years of age but a few older individuals were affected (**Paper I and III**). In females the diagnoses were: myocardial infarction (n = 1), angina pectoris (n = 1), hypertension (n = 3), and hyperlipidemia (n = 2). There was no history of cardiovascular disease, use of statins or antihypertensives in the female controls. In males, cardiovascular disease was revealed in two patients and two controls. Genotype and phenotype of the 7 individuals with cardiovascular morbidity were five with I172N and all SV, one with V281L and NC, and one I2 splice with SW. Thus, the incidence of manifest cardiovascular disease, including treated hypertension and dyslipidemia, was

low (CAH adults: 7.7% vs controls: 2.1%), as expected, since only 7% of the females (**Paper II**) and 13% of males (**Paper III**) were > 50 years old and the frequency of cardiovascular disease in female and males < 50 years old is very low (Booth et al 2006). Consequently, larger studies with more individuals > 50 years are needed to clarify if CAH females and males have increased cardiovascular morbidity and mortality. In the meantime risk factor analysis is the only option available.

4. 3 Body composition

4. 3. 1 BMI and body circumferences

In younger females and males BMI, waist circumference, waist to hip ratio, body fat, and lean body mass were similar to age-matched controls (**Paper I and III**). In contrast, CAH women and men older than 30 years had higher BMI and waist to hip ratios than both age-matched controls and younger CAH women.

4. 3. 2 Body composition studied by DXA

DXA measurements verified that total and regional fat and muscle mass were similar to controls in the younger CAH patients, whereas in the older group only males had increased total and truncal fat mass (**Paper I and III**). As previously mentioned, increased fat mass has been reported in young adults. Even increased fat mass only in male and not in female CAH patients has been observed (Cameron et al 1995, Christiansen et al 2004). The finding of elevated BMI but normal fat mass in older CAH females can be explained by a higher lean body mass than both controls and younger CAH females, while lean mass in older CAH males was similar to controls and to their younger counterparts. Interestingly, in contrast, lean mass was significantly higher in older than in younger control males. A strong correlation was found between lean mass and fat mass in females with CAH (Fig. 5) (**Paper I**). This was not found in males (CAH males: $r = 0.311$, $P = 0.083$; male controls: $r = 0.178$, $P = 0.346$). The reasons for the difference in body composition we found between older females and males with CAH are unclear and remain speculative. Overexposure to androgens in the CAH females in the past may have had a stimulatory effect on muscle mass but at the time of the present study, most females had suppressed androgens (Fig. 6).
Older CAH males had lower testosterone compared to male controls in spite of similar muscle mass. Another reason for the increased lean mass in CAH females could be

their gender-atypical behavior, with physically demanding professions and spare time interests (Frisén et al 2009). However, if there were differences in the amount of physical activity between younger and older CAH women or between male and female patients has not been studied in detail.

Fig. 5 Correlation between total lean mass/height^2 and total fat mass/height^2 in adult women with CAH. Filled triangles CAH women ≥ 30 years old or; unfilled triangles, CAH women < 30 years; filled circles, controls ≥ 30 years old; and unfilled circles, controls < 30 years. From Falhammar et al 2007 Metabolic profile and body composition in adult women with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. J Clin Endocrinol Metab 92:110-116. Copyright 2007, The Endocrine Society.

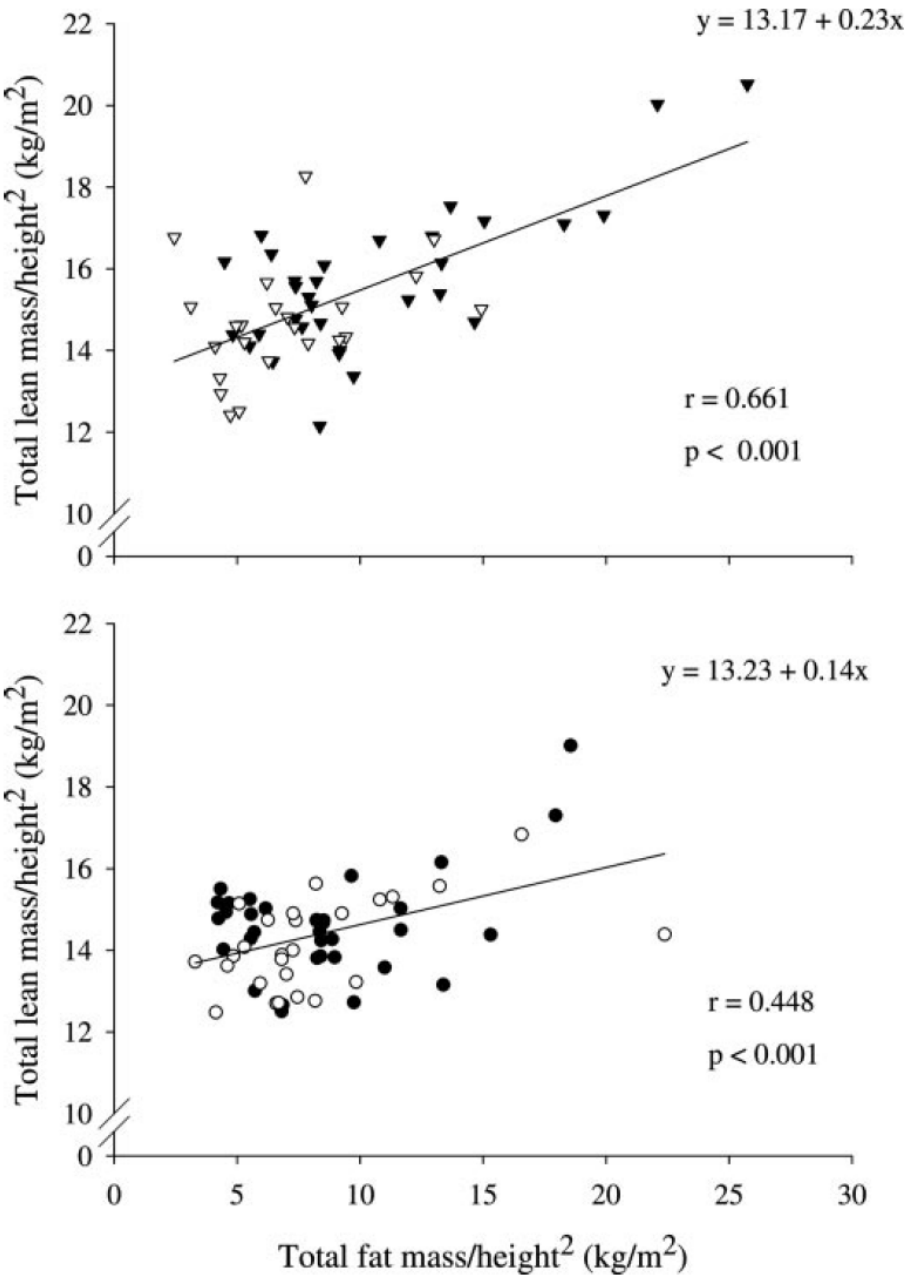
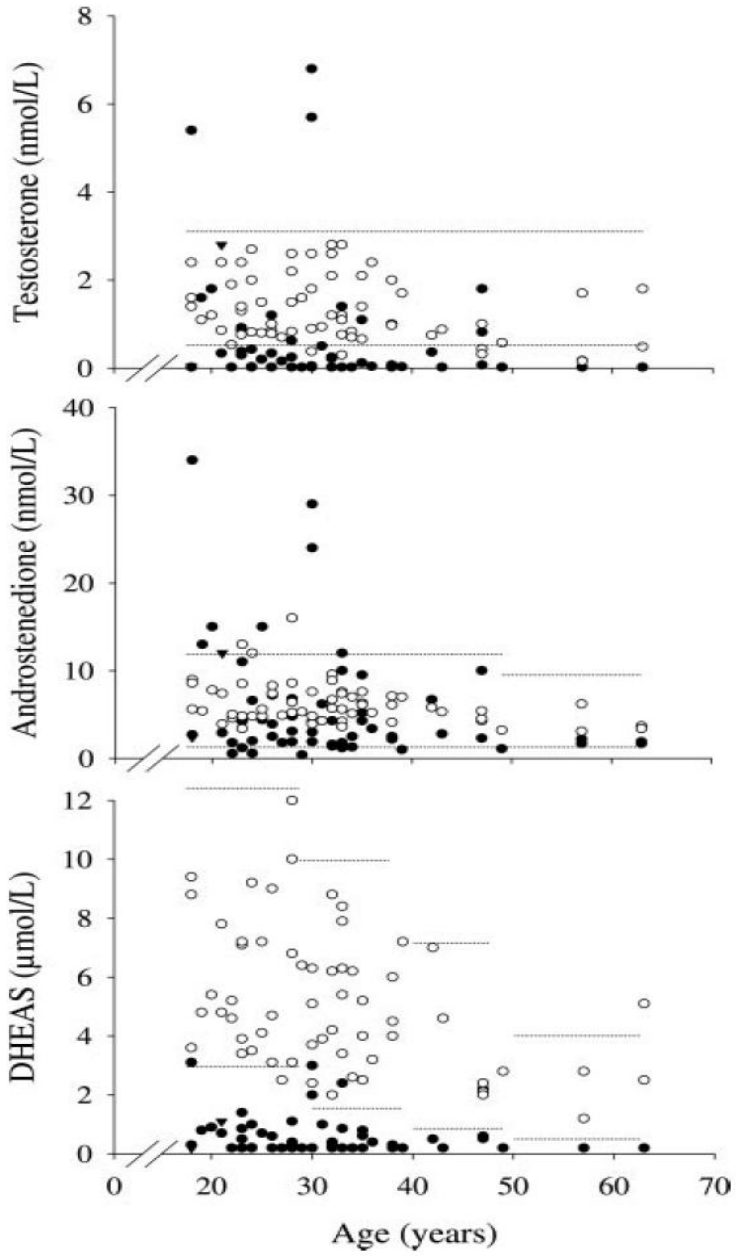


Fig 6. Serum androgens in adult women with CAH and controls. Filled circles, CAH women, those on antiandrogen excluded; filled triangles, CAH women on antiandrogen; and clear circles, controls. P < 0.001 between CAH women and controls in all comparisons. Reference limits for the different androgens and different age intervals are indicated with dotted lines. From Falhammar et al 2007 Metabolic profile and body composition in adult women with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. J Clin Endocrinol Metab 92:110-116. Copyright 2007, The Endocrine Society.



Younger CAH individuals and age- and sex-matched controls had similar body composition and this may reflect an improvement of therapy over time. Encouragement of physical activities, healthy food, and more optimal glucocorticoid therapy, as part of today's management of CAH patients, may be the explanation of lower fat mass and BMI in our younger patients.

4. 4 Liver enzymes

Elevation of liver enzymes can be a sign of NAFLD, which is associated with an increased risk of death (Adams et al 2007). Moreover, GGT has been demonstrated to be independently associated with cardiovascular mortality in a dose-response relationship even within the reference range (Ruttmann et al 2005). Women with CAH were found to have higher liver function tests (ALT, AST, GGT, ALP) than age- and sex-matched controls (**Paper II**). Compared with controls, patients with SW demonstrated elevation of all four liver enzymes, women with SV had only increased GGT and no differences were found in the six NC patients. In males we found elevated GGT in all males which was attributed to increase in older males and in the I172N genotype (**Paper III**). The increases were modest both in females and males, but 54% of women with CAH compared to 33% of controls displayed higher ALT using the lower cut off limit for women suggested (Prati et al 2002) (**Paper II**). We found positive correlations between liver enzymes and waist circumference (**Paper II**), and with trunk and total body fat measured by DXA (**Paper II and III**).

One probable reason for higher liver enzymes in our patients compared to controls is increased liver fat storage supported by the positive associations to estimates of truncal fat. Positive correlations with BMI, waist to hip ratio and hepatic steatosis have been demonstrated previously (Lonardo and Trande 2000). Interestingly, when comparing non-obese CAH women and controls (i.e. BMI < 30 kg/m^2 and waist circumference ≤ 88 cm) patients still had elevated liver enzymes. The finding of higher liver function tests in non-obese CAH women than in non-obese controls suggests that not only central obesity but also glucocorticoids *per se* influence liver enzymes in women with CAH. Hepatic steatosis has been demonstrated both in endogenous Cushing's syndrome (Rockall et al 2003) and in long-term pharmacological glucocorticoid therapy (Itoh et al 1977). In CAH the glucocorticoid doses needed to suppress elevated androgens are often slightly supraphysiological. Our female cohort had both markedly suppressed adrenal androgens (Fig. 6) and low bone mineral density, suggesting past and present overtreatment (**Paper I and IV**). We propose that the life-long glucocorticoid treatment is the most probable reason for higher liver enzymes in patients compared with controls. The cumulative life-time doses, not calculated in the present studies, have most likely been higher in older patients.

4. 3 Glucose, insulin, and lipids

No increase of diabetes could be demonstrated. Only one CAH female ≥ 30 years old had elevated fasting plasma glucose and another had diet-controlled diabetes mellitus. The male cohort underwent a glucose tolerance test which was normal in all (**Paper I and III**).

Compared to controls, women with CAH had lower fasting glucose levels in particular the younger patients. Insulin levels were similar to controls in younger patients and somewhat elevated in the older ones (**Paper I**). HOMA–index above the suggested level of insulin resistance (≥ 2.77) was found in 21% in the whole cohort of female patients and in 6% of controls (P = NS) with a trend to higher HOMA-indices in older patients (**Paper II**). In males glucose metabolism was similar in younger patients and controls, but older patients tended to have higher HbA1c compared to younger ones (**Paper III**). During OGTT, the area under the curve (AUC) for S-insulin was increased in all patients compared to controls. The 2h insulin level was higher in older patients than in their controls.

S-IGFBP-1 was similar in all female groups (**Paper I**). As insulin is considered the principal regulator of hepatic production of IGFBP-1, hyperinsulinemia will lead to decreased levels of IGFBP-1 (Söderberg et al 2001). Hence, the expected negative relationship between IGFBP-1 and insulin was found both in patients and controls. Normal IGFBP-1 concentrations indicate that there was no major aberration in insulin sensitivity at the group level.

Most reports to date have demonstrated increased insulin resistance in CAH (Paula et al 1994, Speiser et al 1992, Charmandari et al 2002, Saygili et al 2005, Sartorato et al 2007, Völkl et al 2009, Williams et al 2010). Our findings are contradictory concerning our younger patients, where we were unable to demonstrate increased insulin resistance. The included CAH individuals in previous studies have been of a similar age or younger. The only study reporting normal insulin sensitivity is carried out in untreated NC CAH women (Bayraktar et al 2004). However, our older CAH patients did show indices of increased insulin resistance so the frequency of diabetes may be increased when this population gets older. The very modest increase in insulin concentrations despite higher BMI in the older female patients might be explained by a larger lean body mass. Some of the previous reports in children with classic CAH and adult women with NC CAH have shown higher insulin levels as well as higher testosterone concentrations than controls (Charmandari et al 2002, Saygili et al 2005). Elevated androgens in women may lead to insulin resistance (Livingstone and

Collison 2002). If the suppressed androgens in our women with CAH may have modified insulin sensitivity remains tentative.

The HOMA-index has been the most frequently used method for evaluating insulin resistance in CAH. The test is based on fasting glucose and insulin. The low fasting glucose found in our patients (**Paper I, II and III**), which was probably due to cortisol insufficiency before the morning glucocorticoid medication may lead to underestimation of insulin resistance.

Gestational diabetes is a strong predictor for future type 2 diabetes (Cheung and Helmink 2006) and the frequency was increased in CAH women (**Paper I**). Three of 14 patients (21%), all in the older group, compared with none of 31 controls who had accomplished a full-term pregnancy, had a history of gestational diabetes. If this preliminary observation is verified in larger studies, the frequency is even higher than in the Australian Indigenous populations which have been considered to have one of the highest risks of diabetes in pregnancy (Davis et al 2009, Falhammar et al 2010). The doses of prednisolone (5 – 7.5 mg daily) during pregnancy in the CAH women when becoming diabetic did not differ from the other CAH females.

We found, similar to previous studies (Bayraktar et al 2004, Bachelot et al 2007, Sartorato et al 2007), normal lipid values in younger CAH patients. Our older females had even more favorable lipid values than both younger CAH females and controls (**Paper I**), while older males had more unfavorable lipid values compared to younger ones (**Paper III**), possibly reflecting differences in lifestyle between older females and males with CAH.

4. 4 Other cardiovascular risk markers

Lp(a) has in many studies been associated with coronary heart disease and stroke (Emerging Risk Factors Collaboration 2009) and has not previously been measured in CAH. Lp(a) was found to be similar in male CAH patients and controls (**Paper III**).

Several clinical and epidemiologic studies have recognized elevated blood homocysteine as a strong independent risk factor for cardiovascular disease in the general population (McCully 2007). Homocysteine has previously been analyzed in one study of NC CAH and found to be similar to that in controls (Bayraktar et al 2004). In contrast, homocysteine was decreased in younger male patients compared to controls and to older male patients which may give cardiovascular protection.

Albuminuria has been shown to be a marker of vacular dysfunctionan and even increasing urinary albumin in the reference range has been associated with increasing cardiovascular disease (Danziger 2008). Urinary albumin in CAH individuals has not been reported previously and tended to be increased in our cohort of older CAH males compared to controls.

4. 5 Adrenomedullary function, blood pressure, and heart rate

4. 5. 1 Adrenomedullary function

Adrenomedullary function was studied for the first time in a mature CAH cohort (**Paper III**). In our male patients, significantly reduced urinary epinephrine was convincingly demonstrated in the two more severe genotype groups (null and I2 splice), but not in the milder I172N group (Fig. 7). There was no difference between older and younger patients.

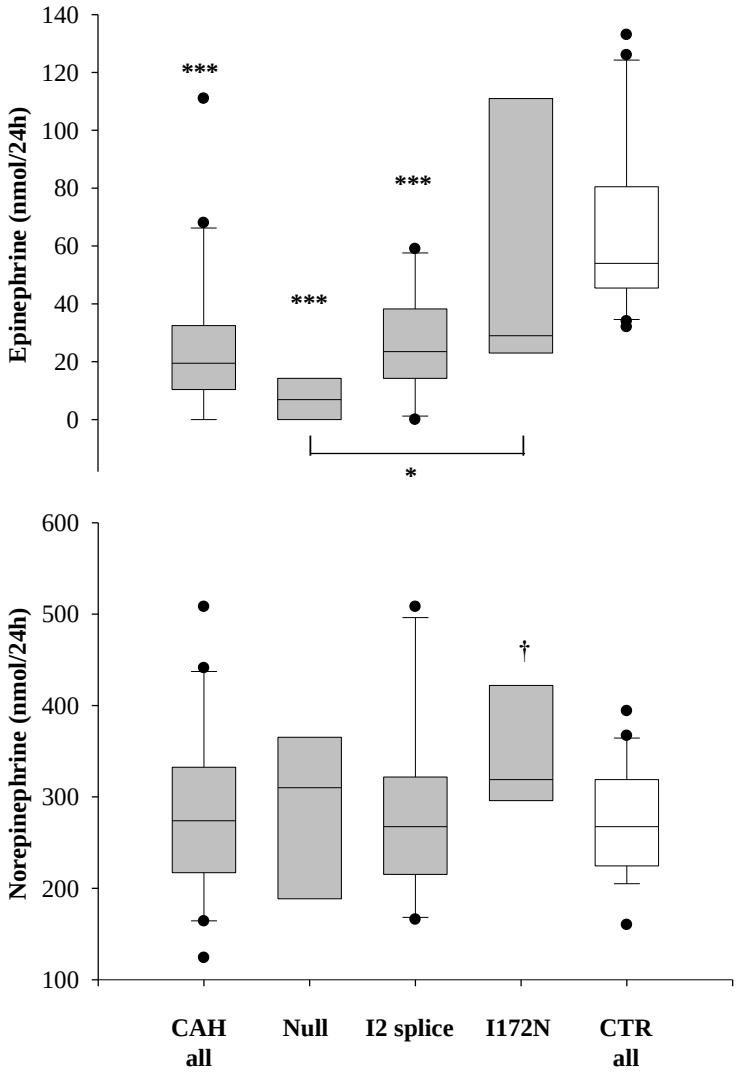
Plasma epinephrine has been shown to be impaired in children, adolescents and young adults with classic CAH (Merke and Bornstein 2005, Weise et al 2004, Riepe et al 2006, Green-Golan et al 2007). Adequate cortisol secretion from the adrenal cortex is necessary for normal adrenomedullary organogenesis and epinephrine production (Merke and Bornstein 2005). These adrenomedullary defects are certainly a result of insufficient prenatal cortisol production and were not ameliorated by postnatal glucocorticoid supplementation. Epinephrine insufficiency can aggravate Addisonian crisis in individuals with CAH and lead to hypoglycemia (Merke and Bornstein 2005). This, together with more severe mineralocorticoid deficiency, is probably the reason for more severe Addisonian crisis in SW, especially in the null genotype.

4. 5. 2 Blood pressure

We found no difference in supine blood pressure in adult females with CAH compared to controls (**Paper I**), which agrees with one of the two other reports on blood pressure in adult CAH (Sartorato et al 2007). There was a small, but significant, elevation of upright diastolic blood pressure in older CAH females not seen in younger CAH females (**Paper I**). If this has any clinical significance is uncertain. The other study with blood pressure reported in adult CAH patients reported also increased diastolic blood pressure, however, only sitting diastolic blood pressure in classic CAH females and not in NC females or classic CAH males (Arlt et al 2010). In males 24h ambulatory

blood pressure measurement was performed and no significant difference was seen between patients and control, irrespective of age group (**Paper III**).

Fig. 7 Urinary catecholamines in adult males with congenital adrenal hyperplasia and in the three most common *CYP21A2* (Null, I2 splice, and I172N) and age- and sex-matched controls. Box plot demonstrates the 10th, 25th, 50th, 75th and 90th percentiles. All P-values compared to controls if not indicated otherwise. P-value: *<0.05, **<0.01, ***<0.001, †= 0.057.



The two other reports on 24h ambulatory blood pressure measurements, both in children and adolescents, included almost exclusively SW individuals with only a small proportion of SV patients. They demonstrated, in contrast to our patients, elevated day and night time systolic pressures in CAH individuals (Roch et al 2003, Völkl et al

2006b). The median BMI in both reports was significantly elevated and the elevated systolic levels correlated with the degree of overweight and obesity explaining the difference to our leaner patients.

4. 5. 3 Heart rate

An unexpected finding when analyzing the 24h ambulatory monitoring in CAH males and controls was increased heart rate in the CAH cohort as well as in the I2 splice and I172N groups compared to controls (Fig. 8) (**Paper III**). This increase was attributed to a higher frequency in the older patients. Our younger patients had normal heart rates in accord with previous studies of young patients (Weise et al 2004, Riepe et al 2006, Green-Golan et al 2007). In the older groups, the mean difference between patients and controls during night hours was 27 beats/min while it was lower during day time, 13 beats/min. Increased heart rate is a known risk factor for cardiovascular and noncardiovascular death, especially in men, with some studies finding heart rate to be independent of other cardiovascular risk factors (Kannel et al 1987, Shaper et al 1993, Mensink and Hoffmeister 1997). Even a small increment in heart rate in the normal range can increase the cardiovascular risk. The heart rate in our CAH males was correlated with other cardiovascular risk factors. Decreased testosterone levels and the extent of glycemic control explained around 50% of the elevated heart rate. The adrenomedullary hypofunction in severe CAH is not likely to be involved as this would lead to a decreased heart rate. Cardiovascular effects of catecholamines are dependent on cortisol concentrations. This is probably not important because the mean glucocorticoid dose was similar in all groups.

4. 6 Cardiovascular risk and age

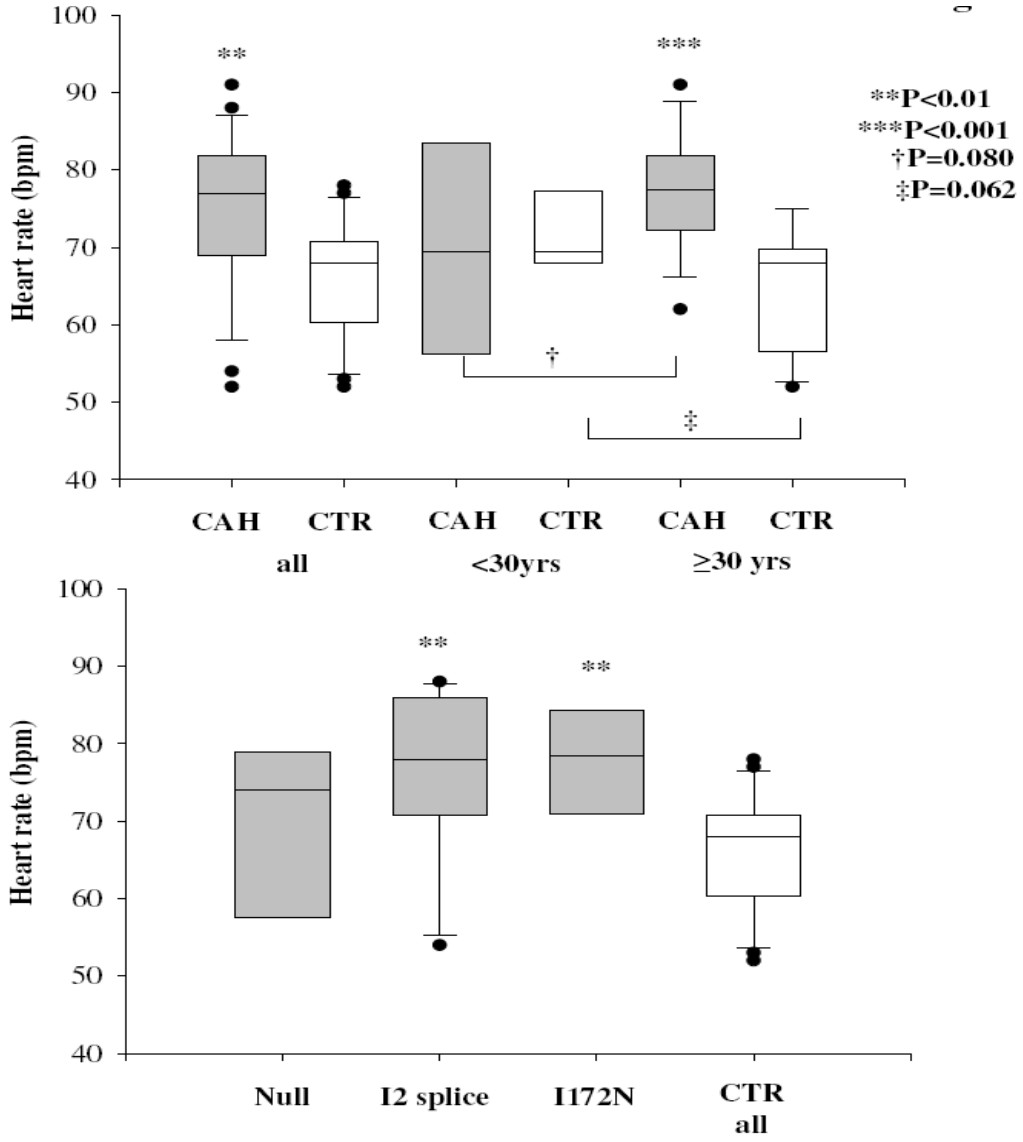
As presented above most metabolic and cardiovascular risk factors were elevated in older CAH individuals, especially in males, while they were normal in younger. Why the risk appears to be increased compared to age-matched controls is speculative but a higher lifetime exposure to exogenous glucocorticoids may be one explanation. Moreover, the majority of the older patients were treated in general pediatric care during childhood, but the younger ones have been treated within or with back-up from pediatric endocrinology units, most likely with a more optimal corticosteroid therapy and access to life-style interventions.

4. 7 Cardiovascular risk and genotype

For the first time, the three most common *CYP21A2* genotype groups and their association to cardiovascular and metabolic risk profiles were explored.

In males, heart rate was elevated in the I172N and I2 splice groups (Fig. 8).

Fig. 8 Heart rate measured with 24h ambulatory monitor in adult males with congenital adrenal hyperplasia, divided into younger than 30 years or older (upper panel), and into the three most common *CYP21A2* genotype groups, (Null, I2 splice, and I172N) (lower panel) and age- and sex-matched controls. Box plot demonstrates the 10th, 25th, 50th, 75th and 90th percentiles. All P values compared to controls if not indicated otherwise.



Moreover, there was an increased waist to hip ratio but also increased lean mass together with tendencies of increased BMI and fat mass in the I172N group, not seen in the null or I2 splice group. GGT was elevated in the I172N group, but not in the null or

I2 splice groups. The I172N genotype group had higher truncal fat mass than the other genotypes probably explaining their higher GGT (**Paper III**).

The null genotype group had lower fasting insulin values than controls which tended to be lower than in the other groups. The I172N group had higher insulin AUC than controls and the other genotypes. Lp(a) was more unfavorable in the null group compared to the other genotypes. In the I172N group there was a tendency to a decreased homocysteine compared to controls. Urinary albumin tended to be increased in the I2 splice group compared to controls. A higher average 24h systolic and night diastolic blood pressure was found in the I172N group while the null and I2 splice groups had similar blood pressure as controls (**Paper III**).

Thus, in males, as seen in table 2, a statistically significant increase in 5/10 risk parameters was found in the genotype group I172N, vs 1/10 and 0/10 in the I2 splice and null individuals. If this is attributed to a high dose of glucocorticoids and mineralocorticoids in I172N males (the doses were similar to those in null and I2 splice) in relation to the milder *CYP21A2* mutation or to a higher fat mass or if it is genotype specific is presently unclear. Moreover, none of the I172N males had been screened with 17OHP at birth and it can be speculated if a late diagnosis with prolonged postnatal androgen excess could lead to adverse metabolic effects. Some support of this can be found in a recent study where NC CAH boys and girls had more parameters of insulin resistance and higher systolic blood pressure compared to controls, which were not seen in classic CAH boys and girls (Williams et al 2010). These NC children were diagnosed on average more than 5 years later than the classic CAH children. One could also hypothesize that the demonstrated adrenomedullary hypofunction in our null and I2 splice groups could be a protective factor.

Preliminary results in the female CAH cohort indicate a different pattern where all genotype groups have similar fat mass as controls and a higher lean mass in the null and NC group. In females liver enzymes were increased in all the three most common genotype groups compared to controls (**Paper II**). The differences between sexes may be attributed to earlier diagnosis in females and that they are more affected by adrenal androgens neuropsychologically and physically. Certainly there is a need for long-term follow-up and further studies of insulin sensitivity, cardiovascular and metabolic risk in adult CAH.

Table 2. Genotype and cardiovascular risk factors in males with CAH

	Null	I2 splice	I172N
Waist/hip ratio	↔	↔	↑
Fat mass	↔	↔	(↑)
Blood pressure	↔	↔	↑
Heart rate	↔	↑	↑
Insulin	↔	↔	↑
Lipids	↔	↔	↔
GGT	↔	↔	↑
Lp(a)	(↑)	↔	↔
Homocysteine	↔	↔	(↓)
U-albumin	↔	(↑)	↔

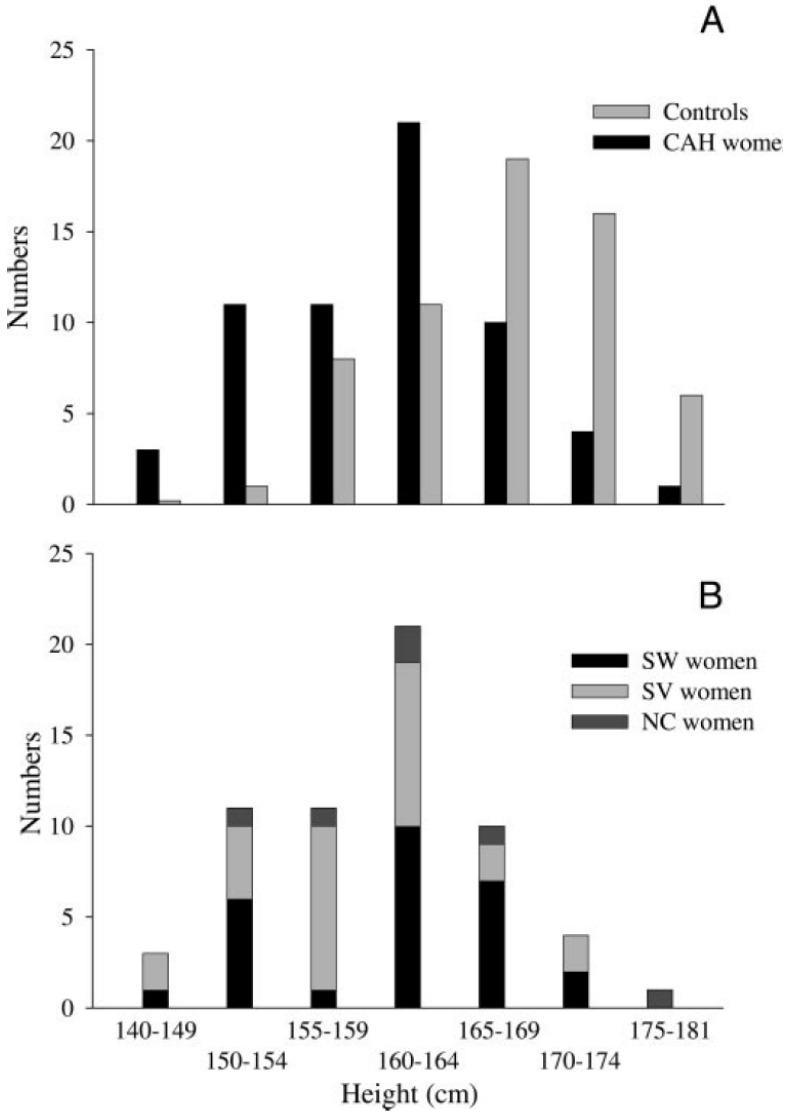
Cardiovascular risk compared to controls: ↔ similar; ↑ increased; ↓ decreased. ( ) tendency.

4. 8 Bone health

4. 8. 1 Final height

In accordance with previous reports (Eugster et al 2001, Arlt et al 2010), we demonstrated that individuals with CAH were significantly shorter than controls (Fig. 9) (**Paper I and III**). Males were more affected than females. Mean height SDS was -1.85 ± 1.3 in males and -1.3 ± 1.1 in females (P = 0.035) when compared to a Swedish reference population (Wikland et al 2002). As expected, patients with classic CAH who were diagnosed within the first year of life were significantly taller (P < 0.001) than those who were diagnosed later (Fig. 10). The mean difference in the females was 1.1 SD (P < 0.001) but in the males only 0.4 SD (NS) in spite of the fact that diagnosis after the first year was present in 40 % of males vs 28 % of women and mean glucocorticoid doses were similar. This suggests that other circumstances affecting height development may differ between males and females, for instance compliance to medication. In classic CAH we also found a trend to a better height achievement in younger than in older patients, mean SDS being -1.2 ± 1.1 vs -1.7 ± 1.2 (P = 0.075), whereas there was no difference in the control group. This might reflect earlier diagnosis and improved management.

Fig. 9 A, Body height (centimeters) in 61 adult women with CAH and age-matched controls. B, Body height divided into SW, SV, and NC in patients. From Falhammar et al 2007 Metabolic profile and body composition in adult women with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. J Clin Endocrinol Metab 92:110-116. Copyright 2007, The Endocrine Society.



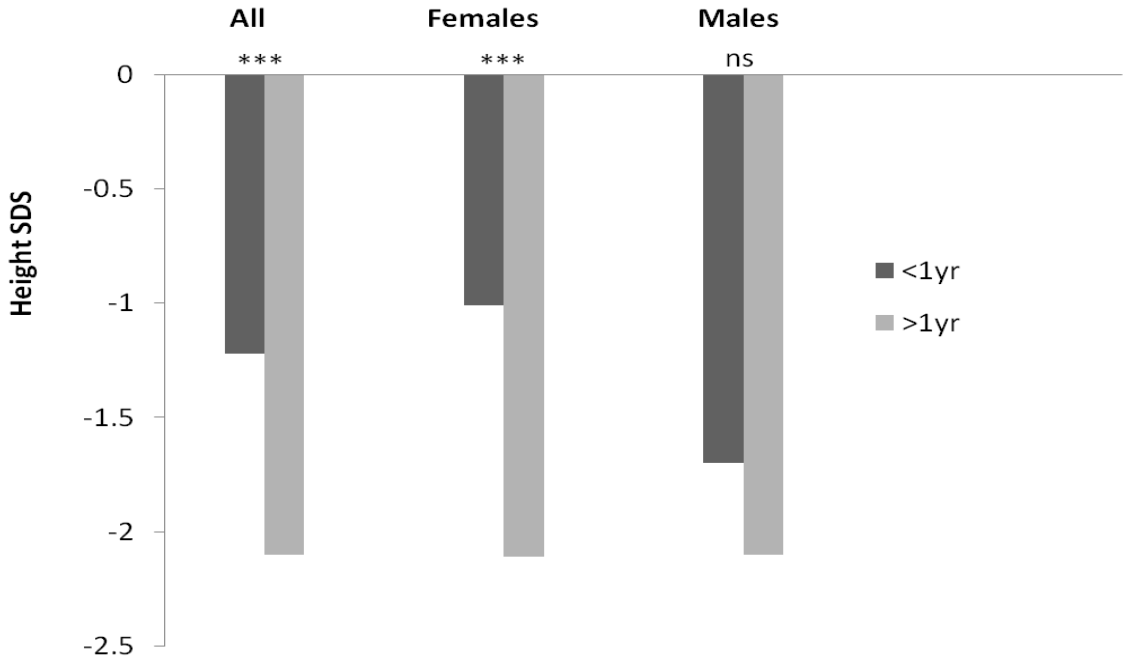
4. 8. 2 BMD and fractures in females

Both younger and older women with CAH exhibited lower BMD at all measured sites compared with controls (Fig. 11) **(Paper IV).** These differences were also found in subgroup analysis of patients with the three common classic genotypes (Fig.12) or with the SW and SV phenotypes. In the milder NC form, BMD did not differ between patients and controls.

A total of 40 patients (62%) fulfilled the WHO criteria for osteopenia/osteoporosis compared with 10 controls (16%). The frequency of osteoporosis/osteopenia was also

significantly higher compared with controls in SW and SV patients and in the null and I172N genotype groups.

Fig. 10 Mean final height SDS in relation to Swedish reference data in patients with classic CAH diagnosed before and after one year of age. The entire cohort (n = 86), females (n = 56), and males (n = 30) are shown. ***P < 0.001, ns = not significant.

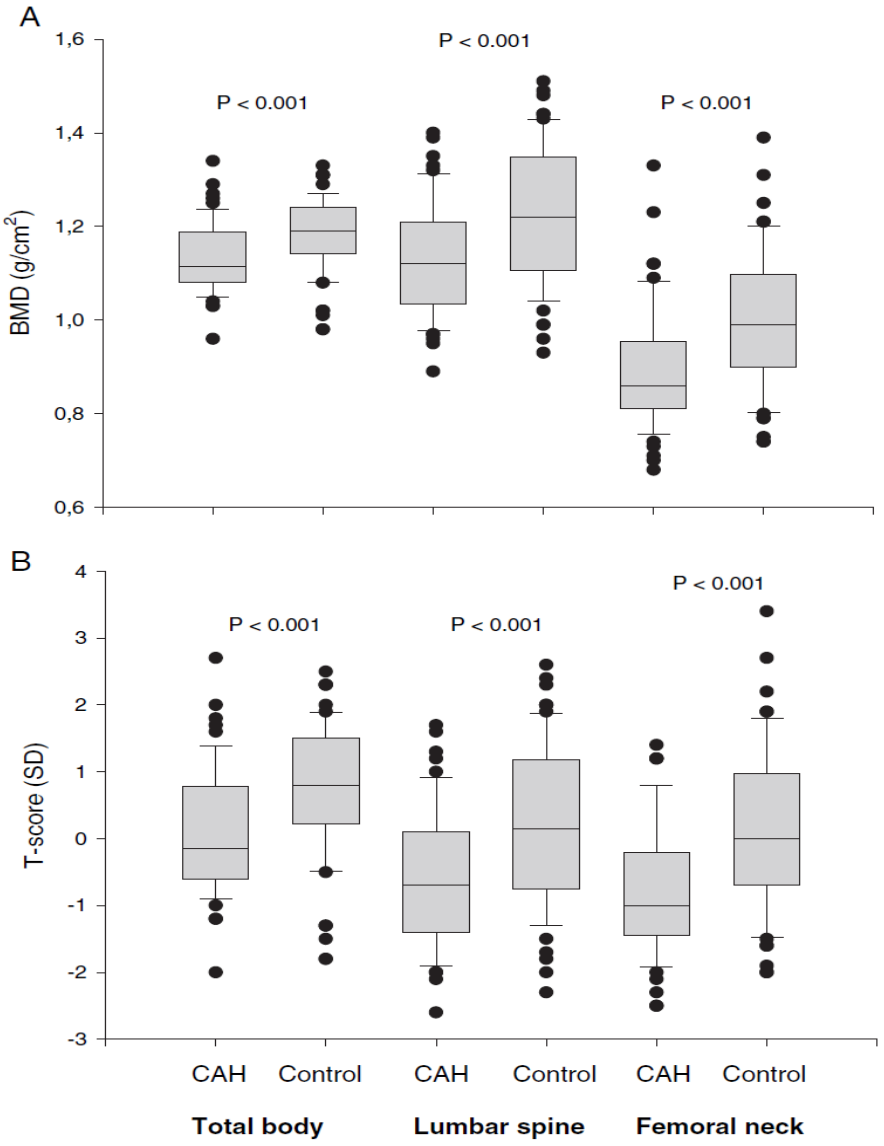


BMD differences between patients and controls were not attributed to the patients' shorter height. When calculating volume-adjusted BMAD or comparing patients and controls within the same height range 160 – 169 cm differences persisted.

Our finding of low BMD in females with CAH is in accordance with the majority of studies in adults (Jääskeläinen and Voutilainen 1996, Hagenfeldt et al 2000, Sciannamblo et al 2006, King et al 2006, Bachelot et al 2007, Arlt et al 2010), but not all (Guo et al 1996, Mora et al 1996, Stikkelbroeck et al 2003, Christiansen et al 2004). However, the latter four studies only included between 6 and 19 women each with 21-hydroxylase deficiency and very few patients were above 30 years of age, probably explaining the difference.

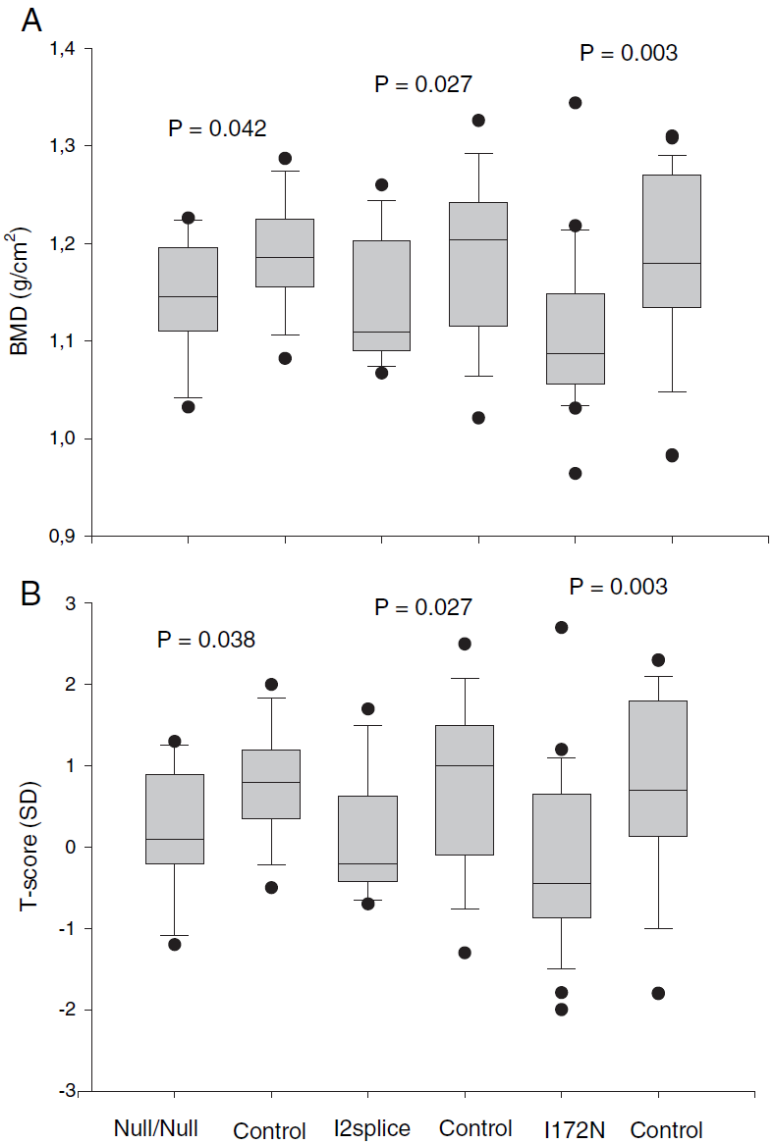
The ultimate outcome of decreased BMD, fractures, has not previously been reported. We found significantly more fractures in patients than in controls (31 fractures in 18 CAH women vs. two fractures in two controls).

Fig. 11 BMD in 61 adult females with CAH due to 21-hydroxylase deficiency, and age- and sex-matched controls expressed as g/cm^2 (A) and as T-score (SD) (B). Box plot demonstrates the 10th, 25th, 50th, 75th, and 90th percentiles. From Falhammar H et al 2007 Fractures and bone mineral density in adult women with 21-hydroxylase deficiency. J Clin Endocrinol Metab 92:4643-4649. Copyright 2007, The Endocrine Society.



The difference between patients and controls in osteoporotic fractures (i.e. vertebrae, wrist, and hip) almost reached significance. Subgroups with significant differences in fracture prevalence compared with controls were: older women, the SW and SV groups and the genotypes null and Il72N. However, the trauma that led to fractures was not ascertained, thus we cannot entirely exclude that the differences in lifestyle between women with CAH and controls partly influenced the results.

Fig. 12 BMD in adult females with CAH due to 21-hydroxylase deficiency divided into three genotypes, with the mildest of the two mutations as representative of the genotype (Null/Null, n = 13; I2 splice, n = 15; and I172N, n = 25), and age- and sex-matched controls expressed as g/cm^2 (A) and as T score (SD) (B). Box plot demonstrates the 10th, 25th, 50th, 75th, and 90th percentiles. From Falhammar H et al 2007 Fractures and bone mineral density in adult women with 21-hydroxylase deficiency. J Clin Endocrinol Metab 92:4643-4649. Copyright 2007, The Endocrine Society.



The bone resorption marker CTX was significantly lower in the older patients than in controls. This was unexpected, as it was previously observed both elevated CTX and bone-specific ALP concentrations when investigating young CAH individuals, some still growing (Sciannamblo et al 2006). The effect of elevated glucocorticoid doses on the skeleton comprises however an initial period of bone resorption when bone density decreases rapidly followed by a slower progressive phase when bone mineral declines because of impaired bone formation (Canalis et al 2004). Possibly our female patients

treated for many years had predominantly low bone formation. Because we only measured total ALP, we cannot decide whether the slight increase in older CAH women was attributed to bone or liver-derived ALP.
Preliminary, in CAH males we have found similar results of decreased BMD and increased frequency of osteoporosis/osteopenia. When comparing the CAH males and women, preliminary data show no difference in T- and Z-score in lumbar spine or femoral neck. Hence, in the management of CAH it is important to monitor BMD and optimize the glucocorticoid doses. Other measures are calcium and vitamin D supplementation, life-style intervention and when needed conventional pharmacotherapy to prevent future osteoporosis related fractures.

4. 9 Fecundity, fertility, and sexuality in males

Compared to controls, more fecundity problems were found in CAH males (**Paper V**). Ten percent (3/31) had tried to father but never succeeded compared to 3% among controls (1/32). One of these patients had, however, a child a year after being included in the study. The I172N genotype group reported more fecundity problems compared to controls and no fecundity problems were reported in the null group. Fertility was decreased when compared to national Swedish data (mean 0.9 children per CAH male vs 1.8 in national data [SCB 2005]) and was found in all three classic genotypes. Our results fall within the wide range of previous studies reporting from normal (Urban et al 1978) to severely impaired fertility (Jääskeläinen et al 2000b). In the latter study however, when examining a subset of the males their inhibin B values were normal indicating normal fertility. Thus, the low fertility may partly be due to psychosexual factors.
In our CAH females, one reason for a low fertility rate was increased non-heterosexuality being 50% in the null genotype (Frisén et al 2009). In contrast, no homo- or bisexuality was found in our male patients (**Paper V**). This is in agreement with the only other report on sexual orientation that included 9 CAH males (Hines et al 2004). Erroneous information from the medical profession may to some extent have influenced fertility. The CAH males aged > 50 years reported that they had been informed in their youth that all CAH patients were infertile. Some of them were now fathers but one man with a normal semen sample had never tried to have children due to this information! Our patients also had less sexual experience with different partners, especially those older than 30 years and the null genotype.The increased frequency of marriage with age seen in controls was not seen in patients. Most likely

however, the main reason for decreased fecundity and fertility was the high frequency of TARTs being found in 86% of examined patients (74% were bilateral) which is similar to the high frequency reported by Stikkelbroeck et al (2001). The varying frequency in previous reports may partly be explained by differences in ages of included patients and the mode of detection, with our patients being the older and our ultrasound equipment the most advanced. A typical image demonstrating a TART from one of the patients is shown in Fig. 13.

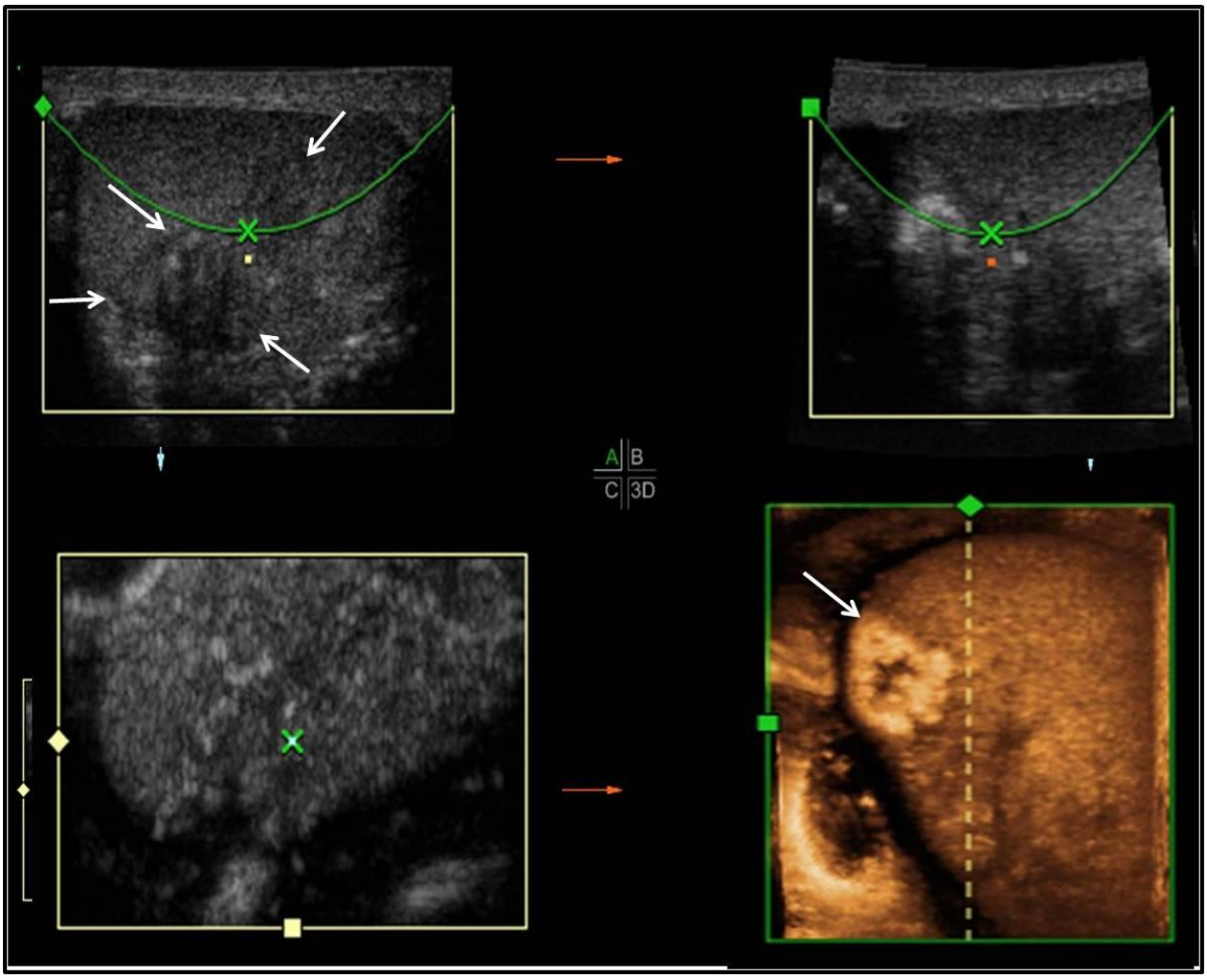
A recent study has reported higher frequency of TARTs in the null compared to I2 splice genotype group, and no case of TART was found in the I172N group or in the NC patients (Mouritsen et al 2010). In contrast, we found TARTs in high frequency in all genotype groups including I172N. The higher mean age in our I172N patients (41 vs 24 years) probably explains the difference. We even found small TARTs in two of three NC genotype patients with normal ACTH, previously only reported with clearly elevated ACTH or AII (Claahsen-van der Griten et al 2009).

Consistent with previous reports, only 10% of our TARTs were detected by manual palpation (Claahsen-van der Grinten et al 2009). Due to their location within the testis usually TARTs < 2 cm will escape detection. The three patients with palpable TARTs were all poorly controlled. In the whole patient cohort, no correlation between TARTs and ACTH or renin was found, but those with very poor control had smaller functional testicular volumes and a tendency to larger TARTs compared to rest of the CAH males.

TART volume correlated positively with LH and negatively with sperm motility (just above the level of significance, $P = 0.058$) probably reflecting Leydig cell destruction. Total functional testicular volume, on the other hand was positively correlated with sperm concentration, sperm count and volume (tendencies), and testosterone/estradiol ratio. These associations are in favour of a negative impact by TARTs on testicular function described below.

If the WHO criterion (WHO 1999) for sperm concentration was used, 7% (1/15) of samples were pathological. With the stricter criteria for normality 33% were abnormal (5/15) and applying all WHO criteria 47% (7/15) were pathological. A selection bias may however have influenced our results. Although not statistically significant, those who produced a sample reported more fecundity problems and had fewer biological children than those who refrained from semen analyses (**Paper V**).

Fig. 13 Steps in the 3D inversion rendering process of right testicle of a 30-year-old male with congenital adrenal hyperplasia (genotype null/null) with two children. Acquisition in the three orthogonal planes and the rendered volume in the bottom right box. By eliminating the surrounding nonpertinent structures with the electronic scalpel the structures of interest were left as the final inverted and rendered volume. In this case it could be seen that the tumor was calcified and had a cystic structure (right bottom corner the tumor marked with an arrow). In the left upper box the tumor is marked with arrows (the size of the tumor was measured to be: 2.6 x 1.1 x 0.9 cm corresponding to a volume of 1.4 cm^3).

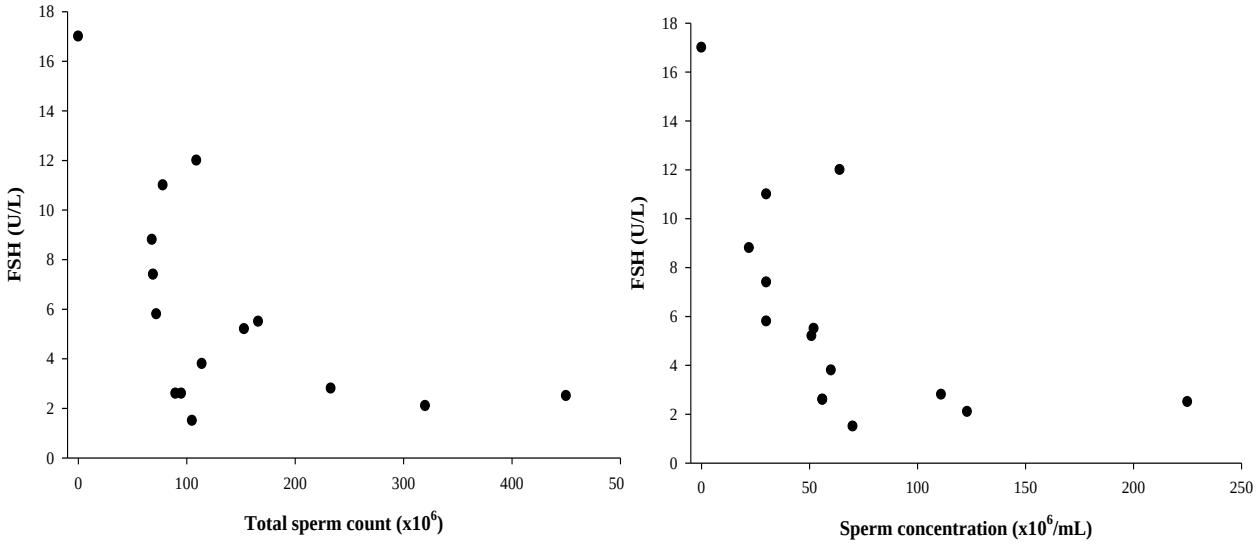


Producing a semen sample may be uncomfortable and a high motivation is needed for participation. Still, our results are more favourable than those in a recent report were semen quality was found to be very poor in CAH males with 58% being pathological if the stricter criteria of sperm concentration were used or 100% if all WHO criteria were considered (Reisch et al 2009).

All the poorly controlled patients with an ultrasound or a semen sample analysed were pathological (6/6 and 1/1 respectively). Only one of the poorly controlled had

reported fecundity problems and 63% (5/8) had biological children. None of the NC patients had a semen sample analysed, however, one had fathered three children. FSH levels were significantly increased compared to controls in the entire CAH group and in subgroups and was negatively correlated with total sperm count and sperm concentration (Fig. 11).

Fig. 11 Correlation between FSH and total sperm count (left panel, Spearman's correlation = -0.620, P = 0.012) or sperm concentration (right panel, Spearman's correlation = -0.756, P < 0.001) in CAH.



These findings strongly indicate Sertoli cell dysfunction in CAH males. On the other hand, LH was no different from controls. None showed suppression of gonadotropin levels below the normal range. Decreased values of testosterone/estradiol ratio were found in patients due to decreased concentrations in older patients and could possibly affect fecundity and fertility. However, the lower ratio in patients with pathological semen was not significantly different compared to patients with normal semen.
The CAH males with abnormal semen demonstrated higher truncal and total fat mass, total fat/lean mass ratio, and heart rate, thus showing increased metabolic and cardiovascular risk, in addition to fecundity issues. These signs are compatible with overtreatment with glucocorticoids, even though all groups had similar 17OHP levels and corticosteroid doses at the time of the study. Moreover, the I172N genotype group had a tendency to poorer sperm parameters compared to the I2 splice patients, possibly reflecting their negative cardio-metabolic profile (**Paper IV**), since increased metabolic risk has been associated with decreased fertility and impaired sperm quality (Kasturi et al 2008). One of the I172N males had for example severe teratozoospermi

with impaired motility and had previously been subjected to intracytoplasmic sperm injection (ICSI) resulting in one child.
The patient with 3-beta-HSD deficiency had hardly any normal testicular tissue left as both testes were full of TARTs. His semen sample contained no sperms but a biopsy was able to retrieve approximately 100 severely abnormal sperm which were saved for future ICSI. Thus he was infertile. Fertility in 3-beta-HSD deficiency has not previously been studied (Pang 2001).
Some of our patients with TARTs had experienced increased doses of glucocorticoids, surgery, biopsy and ICSI which may be one of the explanations why most of our males with CAH due to 21-hydroxylase deficiency who wished to father children appeared to be able to eventually.

4. 10 Limitations

The major limitations of the present study are, despite being larger and including older CAH individuals than most others, its size and involvement of few individuals >50 years old. Negative findings must be interpreted with caution as the studies have limited power and the mean age of women was only 30 and of men only 35 years. Larger studies including more individuals above 50 years of age are needed to finally determine the long-term consequences of CAH, and to evaluate if the genotype influences the outcome.

5. CONCLUSIONS

The present thesis aimed to evaluate some long-term consequences of CAH and its treatment. Little has previously been reported, especially concerning individuals with CAH above the age of 30 years. Our main focus was on cardiovascular and metabolic risk profiles in females and males, bone health in females, and sexuality and fertility in males.

There was no clear evidence of unfavorable cardiovascular risk factors. Few patients had hypertension, manifest cardiovascular disease, or diabetes. Older patients, however, had higher BMI and waist to hip ratio than controls. Older women with CAH also had higher lean mass than controls. Adults with CAH had raised liver enzymes compared to controls. Only the group ≥ 30 years of age in both females and males, the null, I2 splice and I172N genotypes in women and the I172N group in males, demonstrated elevation of liver enzymes compared to controls. Women and men with CAH may have increased frequency of NAFLD. The occurrence of gestational diabetes was significantly elevated, indicating an increased risk for type 2 diabetes in the future.

We demonstrated that epinephrine concentrations were very low in the null and I2 splice groups while in the slightly less severe genotype, I172N, the concentrations were normal. Increased heart rate was found in older CAH males, the I2 splice and the I172N group. Moreover, the I172N group also demonstrated increased blood pressure together with indices of increased body fat and insulin levels indicating a higher cardiovascular risk not seen in the males with the null genotype.

Both women and men with CAH were shorter than age- and sex-matched controls. The frequency of fractures was significantly elevated in women with CAH 30 years or older. Moreover, the frequency of osteopenia and osteoporosis in the women was increased. The reason was probably due to overtreatment with glucocorticoids, indicated by low androgens.

Impaired fertility and fecundity were found in adult men with CAH. The most obvious cause is the presence of TARTs, but other causes probably contribute. In contrast to women with CAH, low fecundity does not seem to be mainly due to sexual orientation or psychosocial factors. Decreased semen quality was prevalent and patients with pathological semen also had more cardio-metabolic risk factors. The male with 3-beta-HSD deficiency was infertile. In spite of these findings, most males

with CAH due to 21-hydroxylase deficiency seemed to be able to father children eventually if they wished.

Even though increased risks and worse outcomes were found in females and males with CAH the findings in this thesis were more positive than many of the previous reports on CAH. Many parameters studied in our CAH individuals < 30 years were in fact no different from age- and sex-matched controls. A larger study recently presented from United Kingdom indicated worse outcomes than our younger patients (Arlt et al 2010). One reason for a more favourable outcome for younger than older CAH patients in our cohort is most likely a better follow-up and management, especially during recent years. This emphasizes the importance of regular follow-ups of CAH women and men with lifestyle interventions and dose adjustments of corticoids, monitoring of anthropometric measurements, BMD, fractures, testicular ultrasound, and biochemical parameters together with a thorough clinical examination, as this seems to directely influence outcome. All patients in the present cohort were born before the introduction of neonatal screening. However, all CAH indivdiuals born in Sweden entering adulthood nowadays have been screened so outcomes will most likely improve futher.

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7. REFERENCES

Abdullah MA, Katugampola M, al-Habib S, al-Jurayyan N, al-Samarrai A, Al-Nuaim A, Patel PJ, Niazi M 1991 Ambiguous genitalia: medical, socio-cultural and religious factors affecting management in Saudi Arabia. Ann Trop Paediatr 11:343–348

Adams LA, Waters OR, Knuiman MW, Elliott RR, Olynyk JK 2009 NAFLD as a risk factor for the development of diabetes and the metabolic syndrome: an eleven-year follow-up study. Am J Gastroenterol 104:861-867

de Almeida Freire PO, de Lemos-Marini SH, Maciel-Guerra AT, Morcillo AM, Matias Baptista MT, de Mello MP, Guerra G Jr 2003 Classical congenital adrenal hyperplasia due to 21-hydroxylase deficiency: a cross-sectional study of factors involved in bone mineral density. J Bone Miner Metab 21:396-401

Arisaka O, Hoshi M, Kanazawa S, Numata M, Nakajima D, Kanno S, Negishi M, Nishikura K, Nitta A, Imataka M, Kuribayashi T, Kano K 2001 Preliminary report: effect of adrenal androgen and estrogen on bone maturation and bone mineral density. Metabolism 50:377-379

Arlt W, Willis DS, Wild SH, Krone N, Doherty EJ, Hahner S, Han TS, Carroll PV, Conway GS, Rees DA, Stimson RH, Walker BR, Connell JM, Ross RJ; the United Kingdom Congenital Adrenal Hyperplasia Adult Study Executive (CaHASE) 2010 Health Status of Adults with Congenital Adrenal Hyperplasia: A Cohort Study of 203 Patients. J Clin Endocrinol Metab Aug 18 [Epub ahead of print]

Avila NA, Premkumar A, Shawker TH, Jones JV, Laue L, Cutler GB Jr 1996 Testicular adrenal rest tissue in congenital adrenal hyperplasia: findings at Gray-scale and color Doppler US. Radiology 198:99-104

Avila NA, Premkumar A, Merke DP 1999 Testicular adrenal rest tissue in congenital adrenal hyperplasia: comparison of MR imaging and sonographic findings. AJR Am J Roentgenol 172:1003-1006

Axelson M, Sahlberg BL, Sjövall J 1981 Analysis of profiles of conjugated steroids in urine by ion-exchange separation and gas chromatography-mass spectrometry. J Chromatogr 224:355–370

Azziz R, Carmina E, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Futterweit W, Janssen OE, Legro RS, Norman RJ, Taylor AE, Witchel SF; Task Force on the Phenotype of the Polycystic Ovary Syndrome of The Androgen Excess and PCOS Society 2009 The Androgen Excess and PCOS Society criteria for the polycystic ovary syndrome: the complete task force report. Fertil Steril 91:456-488

Bachega TA, Billerbeck AE, Marcondes JA, Madureira G, Arnhold IJ, Mendonca BB 2000 Influence of different genotypes on 17-hydroxyprogesterone levels in patients with nonclassical congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Clin Endocrinol (Oxf) 52:601–607

Bachelot A, Plu-Bureau G, Thibaud E, Laborde K, Pinto G, Samara D, Nihoul-Fékété C, Kuttenn F, Polak M, Touraine P 2007 Long-term outcome of patients with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Horm Res 67:268-276

Bang P, Eriksson U, Sara V, Wivall IL, Hall K1991 Comparison of acid ethanol extraction and acid gel filtration prior to IGF-I and IGF-II radioimmunoassays: improvement of determination in acid ethanol extracts by the use of truncated IGF-1 as radioligand. Acta Endocrinol (Copenh) 124:620–629

Bayraktar F, Dereli D, Ozgen AG, Yilmaz C 2004 Plasma homocysteine levels in polycystic ovary syndrome and congenital adrenal hyperplasia. Endocr J 51:601-608

Berenbaum SA, Resnick SM 1997 Early androgen effects on aggression in children and adults with congenital adrenal hyperplasia. Psychoneuroendocrinology 22:505–515

Bergstrand CG 1966 Growth in congenital adrenal hyperplasia. Acta Paediatr Scand 55:463-472

Bidet M, Bellanné-Chantelot C, Galand-Portier MB, Golmard JL, Tardy V, Morel Y, Clauin S, Coussieu C, Boudou P, Mowzowicz I, Bachelot A, Touraine P, Kuttenn F 2010 Fertility in women with nonclassical congenital adrenal hyperplasia due to 21-hydroxylase deficiency. J Clin Endocrinol Metab 95:1182-1190

Bonde JP, Ernst E, Jensen TK, Hjollund NH, Kolstad H, Henriksen TB, Scheike T, Giwercman A, Olsen J, Skakkebaek NE 1998 Relation between semen quality and fertility: a population-based study of 430 first-pregnancy planners. Lancet 352:1172-1177

Bonora E, Kiechl S, Willeit J, Oberhollenzer F, Egger G, Targher G, Alberiche M, Bonadonna RC, Muggeo M 1998 Prevalence of insulin resistance in metabolic disorders: the Bruneck Study. Diabetes 47:1643-1649

Booth GL, Kapral MK, Fung K, Tu JV 2006 Relation between age and cardiovascular disease in men and women with diabetes compared with non-diabetic people: a population-based retrospective cohort study. Lancet 368:29-36

Cabrera MS, Vogiatzi MG, New MI 2001 Long term outcome in adult males with classic congenital adrenal hyperplasia. J Clin Endocrinol Metab 86:3070-3078

Cameron FJ, Kaymakci B, Byrt EA, Ebeling PR, Warne GL, Wark JD 1995 Bone mineral density and body composition in congenital adrenal hyperplasia. J Clin Endocrinol Metab 80:2238-2243

Canalis E, Bilezikian JP, Angeli A, Guistina A 2004 Perspectives on glucocorticoid-induced osteoporosis. Bone 34:593–598

Carroll MC, Campbell RD, Porter RR 1985 Mapping of steroid 21-hydroxylase genes adjacent to complement component C4 genes in HLA, the major histocompatibility complex in man. Proc Natl Acad Sci USA 82:521–525

Carter DR, Bouxsein ML, Marcus R 1992 New approaches for interpreting projected bone densitometry area. J Bone Miner Res 7:137–145

Charmandari E, Weise M, Bornstein SR, Eisenhofer G, Keil MF, Chrousos GP, Merke DP 2002 Children with classic congenital adrenal hyperplasia have elevated serum leptin concentrations and insulin resistance: potential clinical implications. J Clin Endocrinol Metab 87:2114-2120

Cheung NW, Helmink D 2006 Gestational diabetes: the significance of persistent fasting hyperglycemia for subsequent development of diabetes mellitus. J Diabetes Compl 20:21–25

Christiansen P, Molgaard C, Muller J 2004 Normal bone mineral content in young adults with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Horm Res 61:133-136

Claahsen-van der Grinten HL, Sweep FC, Blickman JG, Hermus AR, Otten BJ 2007a Prevalence of testicular adrenal rest tumours in male children with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Eur J Endocrinol 157:339-344

Claahsen-van der Grinten HL, Otten BJ, Sweep FC, Span PN, Ross HA, Meuleman EJ, Hermus AR 2007b Testicular tumors in patients with congenital adrenal hyperplasia due to 21-hydroxylase deficiency show functional features of adrenocortical tissue. J Clin Endocrinol Metab 92:3674-3680

Claahsen-van der Grinten HL, Otten BJ, Takahashi S, Meuleman EJ, Hulsbergen-van de Kaa C, Sweep FC, Hermus AR 2007 Testicular adrenal rest tumors in adult males with congenital adrenal hyperplasia: evaluation of pituitary-gonadal function before and after successful testis-sparing surgery in eight patients. J Clin Endocrinol Metab 92:612-615

Claahsen-van der Grinten HL, Otten BJ, Stikkelbroeck MM, Sweep FC, Hermus AR 2009 Testicular adrenal rest tumours in congenital adrenal hyperplasia. Best Pract Res Clin Endocrinol Metab 23:209-220

Clayton PE, Miller WL, Oberfield SE, Ritzen EM, Sippell WG, Speiser PW 2002 Consensus statement on 21-hydroxylase deficiency from the European Society for Paediatric Endocrinology and the Lawson Wilkins Pediatric Endocrine Society. Horm Res 58:188–195

Cornean RE, Hindmarsh PC, Brook CG 1998 Obesity in 21-hydroxylase deficient patients. Arch Dis Child 78:261-263

Danziger J 2008 Importance of low-grade albuminuria. Mayo Clin Proc 83:806-812

Davis B, Bond D, Howat P, Sinha AK, Falhammar H 2009 Maternal and neonatal outcomes following diabetes in pregnancy in Far North Queensland, Australia. Aust N Z J Obstet Gynaecol 49:393-399

De Crecchio L 1865 Sopra un caso di apparenzi virili in una donna. Morgagni 7:154–188

DiMartino-Nardi J, Stoner E, O'Connell A, New MI 1986 The effect of treatment of final height in classical congenital adrenal hyperplasia (CAH). Acta Endocrinol Suppl (Copenh) 279:305-314

Ehrhardt AA, Epstein R, Money J 1968 Fetal androgens and female gender identity in the early-treated adrenogenital syndrome. Johns Hopkins Med J 122:160–167

Emerging Risk Factors Collaboration, Erqou S, Kaptoge S, Perry PL, Di Angelantonio E, Thompson A, White IR, Marcovina SM, Collins R, Thompson SG, Danesh J 2009 Lipoprotein(a) concentration and the risk of coronary heart disease, stroke, and nonvascular mortality. JAMA 302:412-423

Eugster EA, Dimeglio LA, Wright JC, Freidenberg GR, Seshadri R, Pescovitz OH 2001 Height outcome in congenital adrenal hyperplasia caused by 21-hydroxylase deficiency: a meta-analysis. J Pediatr 138:26-32

Falhammar H, Thorén M 2005 An 88-year-old woman diagnosed with adrenal tumor and congenital adrenal hyperplasia: connection or coincidence? J Endocrinol Invest 28:449-453

Falhammar H, Thorén M, Hagenfeldt K 2008a A 31-year-old woman with infertility and polycystic ovaries diagnosed with non-classic congenital adrenal hyperplasia due to a novel CYP21 mutation. J Endocrinol Invest 31:176-180

Falhammar H 2010 Non-classic congenital adrenal hyperplasia due to 21-hydoxylase deficiency as a cause of infertility and miscarriages. N Z Med J 123(1312):77-80

Falhammar H, Davis B, Bond D, Sinha AK 2010 Maternal and neonatal outcomes in the Torres Strait Islands with a sixfold increase in type 2 diabetes in pregnancy over six years. Aust N Z J Obstet Gynaecol 50:120-126

Fiet J, Gueux B, Raux-DeMay MC, Kuttenn F, Vexiau P, Brerault JL, Couillin P, Galons H, Villette JM, Julien R, et al 1989 Increased plasma 21-deoxycorticosterone (21-DB) levels in late-onset adrenal 21-hydroxylase deficiency suggest a mild defect of the mineralocorticoid pathway. J Clin Endocrinol Metab 68:542-547

Fleischer AC, McKee MS, Gordon AN, Page DL, Kepple DM, Worrell JA, Jones HW 3rd, Burnett LS, James AE Jr 1990 Transvaginal sonography of postmenopausal ovaries with pathologic correlation. J Ultrasound Med 9:637-644

Fleischman A, Ringelheim J, Feldman HA, Gordon CM 2007 Bone mineral status in children with congenital adrenal hyperplasia. J Pediatr Endocrinol Metab 20:227-235

Friedewald WT, Levy RL, Fredrickson DS 1972 Estimation of concentration of low density lipoprotein cholesterol in plasma without the use of the preparative ultracentrifuge. Clin Chem 18:499–502

Frisén L, Nordenström A, Falhammar H, Filipsson H, Holmdahl G, Janson PO, Thorén M, Hagenfeldt K, Möller A, Nordenskjöld A 2009 Gender role behavior, sexuality, and psychosocial adaptation in women with congenital adrenal hyperplasia due to CYP21A2 deficiency. J Clin Endocrinol Metab 94:3432-3439

Gastaud F, Bouvattier C, Duranteau L, Brauner R, Thibaud E, Kutten F, Bougnères P 2007 Impaired sexual and reproductive outcomes in women with classical forms of congenital adrenal hyperplasia. J Clin Endocrinol Metab 92:1391-1396

Girgis R, Winter JS 1997 The effects of glucocorticoid replacement therapy on growth, bone mineral density, and bone turnover markers in children with congenital adrenal hyperplasia. J Clin Endocrinol Metab 82:3926-3929

Goy RW, McEwen BS 1980 Sexual differentiation of the brain. Cambridge, MA: MIT Press

Green-Golan L, Yates C, Drinkard B, VanRyzin C, Eisenhofer G, Weise M, Merke DP 2007 Patients with classic congenital adrenal hyperplasia have decreased epinephrine reserve and defective glycemic control during prolonged moderate-intensity exercise. J Clin Endocrinol Metab 92:3019-3024

Guo CY, Weetman AP, Eastell R 1996 Bone turnover and bone mineral density in patients with congenital adrenal hyperplasia. Clin Endocrinol (Oxf) 45:535-541

Gussinyé M, Carrascosa A, Potau N, Enrubia M, Vicens-Calvet E, Ibáñez L, Yeste D 1997 Bone mineral density in prepubertal and in adolescent and young adult patients with the salt-wasting form of congenital adrenal hyperplasia. Pediatrics 100:671-674

Hagenfeldt K, Ritzen EM, Ringertz H, Helleday J, Carlstrom K 2000 Bone mass and body composition of adult women with congenital virilizing 21-hydroxylase deficiency after glucocortioid treatment since infancy. Eur J Endocrinol 143:667-671

Hagenfeldt K, Janson PO, Holmdahl G, Falhammar H, Filipsson H, Frisén L, Thorén M, Nordenskjöld A 2008 Fertility and pregnancy outcome in women with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Hum Reprod 23:1607-1613

Helleday J, Siwers B, Ritzén EM, Carlström K 1993 Subnormal androgen and elevated progesterone levels in women treated for congenital virilizing 21-hydroxylase deficiency. J Clin Endocrinol Metab 76:933-936

Helleday J, Siwers B, Ritzen EM, Hugdahl K 1994 Normal lateralization for handedness and ear advantage in a verbal dichotic listening task in women with congenital adrenal hyperplasia (CAH). Neuropsychologia 32:875–880

Higashi Y, Yoshioka H, Yamane M, Gotoh O, Fujii-Kuriyama Y 1986 Complete nucleotide sequence of two steroid 21-hydroxylase genes tandemly arranged in human chromosome: a pseudogene and a genuine gene. Proc Natl Acad Sci USA 83:2841–2845

Hilding A, Hall K, Wivall-Helleryd IL, Sääf M, Melin AL, Thorén M 1999 Serum levels of insulin-like growth factor 1 in 152 patients with growth hormone deficiency, aged 19–82 years, in relation to those in healthy subjects. J Clin Endocrinol Metab 84:2013–2019

Hines M, Fane BA, Pasterski VL, Mathews GA, Conway GS, Brook C 2003 Spatial abilities following prenatal androgen abnormality: targeting and mental rotations performance in individuals with congenital adrenal hyperplasia. Psychoneuroendocrinology 28:1010–1026

Hines M, Brook C, Conway GS 2004 Androgen and psychosexual development: core gender identity, sexual orientation and recalled childhood gender role behavior in women and men with congenital adrenal hyperplasia (CAH). J Sex Res 41:75-81

Hoepffner W, Herrmann A, Willgerodt H, Keller E 2006 Blood pressure in patients with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. J Pediatr Endocrinol Metab 19:705-711

Isguven P, Arslanoglu I, Mesutoglu N, Yildiz M, Erguven M 2008 Bioelectrical impedance analysis of body fatness in childhood congenital adrenal hyperplasia and its metabolic correlates. Eur J Pediatr 167:1263-1268

Itoh S, Igarashi M, Tsukada Y, Ichinoe A 1977 Nonalcoholic fatty liver with alcoholic hyalin after long term glucocorticoid therapy. Acta Hepatogastroenterol 24:415-418

Jääskeläinen J, Voutilainen R 1996 Bone mineral density in relation to glucocorticoid substitution therapy in adult patients with 21-hydroxylase deficiency. Clin Endocrinol (Oxf) 45:707-713

Jääskeläinen J, Levo A, Voutilainen R, Partanen J 1997 Population-wide evaluation of disease manifestation in relation to molecular genotype in steroid 21-hydroxylase (CYP21) deficiency: good correlation in a well defined population. J Clin Endocrinol Metab 82:3293-3297

Jääskeläinen J, Hippeläinen M, Kiekara O, Voutilainen R 2000a Child rate, pregnancy outcome and ovarian function in females with classical 21-hydroxylase deficiency. Acta Obstet Gynecol Scand 79:687-692

Jääskeläinen J, Kiekara O, Hippeläinen M, Voutilainen R 2000b Pituitary gonadal axis and child rate in males with classical 21-hydroxylase deficiency. J Endocrinol Invest 23:23-27

Kandemir N, Yordam N 1997 Congenital adrenal hyperplasia in Turkey: a review of 273 patients. Acta Paediatr 86:22–25

Kannel WB, Kannel C, Paffenbarger RS 1987 Heart rate and cardiovascular mortality: the Framingham study. Am Heart J 113:1489-1494

Kasturi SS, Tannir J, Brannigan RE 2008 The metabolic syndrome and male infertility. J Androl 29:251-259

Katzman DK, Bachrach LK, Carter DK, Marcus R 1991 Clinical and anthropometric correlates of bone mineral acquisition in healthy adolescent girls. J Clin Endocrinol Metab 73:1332–1339

Kim MS, Merke DP 2009 Cardiovascular disease risk in adult women with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Semin Reprod Med 27:316-321

King JA, Wisniewski AB, Bankowski BJ, Carson KA, Zacur HA, Migeon CJ 2006 Long-term corticosteroid replacement and bone mineral density in adult women with classical congenital adrenal hyperplasia. J Clin Endocrinol Metab 91:865-869

Kirkland RT, Keenan BS, Holcombe JH, Kirkland JL, Clayton GW 1978 The effect of therapy on mature height in congenital adrenal hyperplasia. J Clin Endocrinol Metab 47:1320-1324

Knorr D, Hinrichsen de Lienau SG 1988 Persistent obesity and short final height after corticoid overtreatment for congenital adrenal hyperplasia (CAH) in infancy. Acta Paediatr Jpn 30 Suppl:89-92

Kroese JM, Mooij CF, van der Graaf M, Hermus AR, Tack CJ 2009 Pioglitazone improves insulin resistance and decreases blood pressure in adult patients with congenital adrenal hyperplasia. Eur J Endocrinol 161:887-894

Krone N, Wachter I, Stefanidou M, Roscher AA, Schwarz HP 2001 Mothers with congenital adrenal hyperplasia and their children: outcome of pregnancy, birth and childhood. Clin Endocrinol (Oxf) 55:523-529

Krone N, Arlt W 2009 Genetics of congenital adrenal hyperplasia. Best Pract Res Clin Endocrinol Metab 23:181-192

Levine LS, Dupont B, Lorenzen F, Pang S, Pollack M, Oberfield S, Kohn B, Lerner A, Cacciari E, Mantero F, Cassio A, Scaroni C, Chiumello G, Rondanini GF, Gargantini L, Giovannelli G, Virdis R, Bartolotta E, Migliori C, Pintor C, Tato L, Barboni F, New MI 1980 Cryptic 21-hydroxylase deficiency in families of patients with classical congenital adrenal hyperplasia. J Clin Endocrinol Metab 51:1316-1324

Livingstone C, Collison M 2002 Sex steroids and insulin resistance. Clin Sci 102:151–166

Liddle GW 1961 Clinical pharmacology of anti-inflammatory steroids. Clin Pharmacol Ther 2:615–635

Lo JC, Grumbach MM 2001 Pregnancy outcomes in women with congenital virilizing adrenal hyperplasia. Endocrinol Metab Clin North Am 30:207-229

Lonardo A, Trande P 2000 Are there any sex differences in fatty liver? A study of glucose metabolism and body fat distribution. J Gastroenterol Hepatol 15: 775-782

Martinez-Aguayo A, Rocha A, Rojas N, García C, Parra R, Lagos M, Valdivia L, Poggi H, Cattani A; Chilean Collaborative Testicular Adrenal Rest Tumor Study Group 2007 Testicular adrenal rest tumors and Leydig and Sertoli cell function in boys with classical congenital adrenal hyperplasia. J Clin Endocrinol Metab 92:4583-4589

Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC 1985 Homeostatis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28:412-419

Mazess RB, Barden HS, Bisek JP, Hansson J 1990 Dual energy x-ray absorptiometry for total body and regional bone mineral and soft tissue composition. Am J Clin Nutr 51:156–163

McCully KS 2007 Homocysteine, vitamins, and vascular disease prevention. Am J Clin Nutr 86:1563S-1568S

Mensink GB, Hoffmeister H 1997 The relationship between resting heart rate and all-cause, cardiovascular and cancer mortality. Eur Heart J 18:1404-1410

Merke DP, Bornstein SR 2005 Congenital adrenal hyperplasia. Lancet 365:2125-2136

Miller WL 1991 The adrenal cortex. In: Rudolph AM, Hoffman JIE, eds. Rudolph's pediatrics. 19th ed. Norwalk, CT: Appleton & Lange; 1584–1613

Mooij CF, Kroese JM, Claahsen-van der Grinten HL, Tack CJ, Hermus AR 2009 Unfavorable trends in cardiovascular and metabolic risk in pediatric and adult patients with congenital adrenal hyperplasia? Clin Endocrinol Aug 29. [Epub ahead of print]

Mora S, Saggion F, Russo G, Weber G, Bellini A, Prinster C, Chiumello G 1996 Bone density in young patients with congenital adrenal hyperplasia. Bone 18:337-340

Moran C, Azziz R, Carmina E, Dewailly D, Fruzzetti F, Ibañez L, Knochenhauer ES, Marcondes JA, Mendonca BB, Pignatelli D, Pugeat M, Rohmer V, Speiser PW, Witchel SF 2000 21-Hydroxylase-deficient nonclassic adrenal hyperplasia is a progressive disorder: a multicenter study. Am J Obstet Gynecol 183:1468-1474

Mosteller RD 1987 Simplified calculation of body-surface area. N Engl J Med 317:1098

Mouritsen A, Jørgensen N, Main KM, Schwartz M, Juul A 2010 Testicular adrenal rest tumours in boys, adolescents and adult men with congenital adrenal hyperplasia may be associated with the CYP21A2 mutation. Int J Androl 33:521-527

Mulaikal RM, Migeon CJ, Rock JA 1987 Fertility rates in female patients with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. N Engl J Med 316:178-182

Nass R, Baker S, Speiser P, Virdis R, Balsamo A, Cacciari E, Loche A, Dumic M, New M 1987 Hormones and handedness: left-hand bias in female congenital adrenal hyperplasia patients. Neurology 37:711–715

Nermoen I, Husebye E, Svartberg J, Lovas K 2010 Subjective health status in men and women with congenital adrenal hyperplasia: A population-based survey in Norway. Eur J Endocrinol In Press

Ness-Abramof R, Apovian CM 2008 Waist circumference measurement in clinical practice. Nutr Clin Pract 23:397-404

New MI 2006 Extensive clinical experience: nonclassical 21-hydroxylase deficiency. J Clin Endocrinol Metab 91:4205-4214

Nordenskjöld A, Holmdahl G, Frisén L, Falhammar H, Filipsson H, Thorén M, Janson PO, Hagenfeldt K 2008 Type of mutation and surgical procedure affect long-term quality of life for women with congenital adrenal hyperplasia. J Clin Endocrinol Metab 93:380-386

Nordenström A, Marcus C, Axelson M, Wedell A, Ritzén EM 1999 Failure of cortisone acetate treatment in congenital adrenal hyperplasia because of defective 11beta-hydroxysteroid dehydrogenase reductase activity. J Clin Endocrinol Metab 84:1210-1213

Nordenström A, Thilén A, Hagenfeldt L, Larsson A, Wedell A 1999 Genotyping is a valuable diagnostic complement to neonatal screening for congenital adrenal hyperplasia due to steroid 21-hydroxylase deficiency. J Clin Endocrinol Metab 84:1505-1509

Nordenström A, Servin A, Bohlin G, Larsson A, Wedell A 2002 Sextyped toy play behavior correlates with the degree of prenatal androgen exposure assessed by CYP21 genotype in girls with congenital adrenal hyperplasia. J Clin Endocrinol Metab 87:5119–5124

Ogilvie CM, Crouch NS, Rumsby G, Creighton SM, Liao LM, Conway GS 2006 Congenital adrenal hyperplasia in adults: a review of medical, surgical and psychological issues. Clin Endocrinol 64:2-11

Otten BJ, Stikkelbroeck MM, Claahsen-van der Grinten HL, Hermus AR 2005 Puberty and fertility in congenital adrenal hyperplasia. Endocr Dev 8:54-66.

Paganini C, Radetti G, Livieri C, Braga V, Migliavacca D, Adami S 2000 Height, bone mineral density and bone markers in congenital adrenal hyperplasia. Horm Res 54:164-168

Pang SY, Wallace MA, Hofman L, Thuline HC, Dorche C, Lyon IC, Dobbins RH, Kling S, Fujieda K, Suwa S 1988 Worldwide experience in newborn screening for classical congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Pediatrics 81:866-874

Pang S 2001 Congenital adrenal hyperplasia owing to 3 beta-hydroxysteroid dehydrogenase deficiency. Endocrinol Metab Clin North Am 30:81-99

Paula FJ, Gouveia LM, Paccola GM, Piccinato CE, Moreira AC, Foss MC 1994 Androgen-related effects on peripheral glucose metabolism in women with congenital adrenal hyperplasia. Horm Metab Res 26:552-556

Placzek M, Arnold B, Schmidt H, Gaube S, Keller E, Plewig G, Degitz K 2005 Elevated 17-hydroxyprogesterone serum values in male patients with acne. J Am Acad Dermatol 53:955-958

Povoa G, Roovete A, Hall K 1984 Crossreaction of somatomedin-binding protein a radioimmunoassay developed for somatomedin binding protein isolated from human amniotic fluid. Acta Endocrinol (Copenh) 107:563–570

Prati D, Taioli E, Zanella A, Torre ED , Butelli S, Del Vecchio E, Vianello L, Zanuso F, Mozzi F, Milani S, Conte D, Colombo M, Sirchia G 2002 Updated definitions of healthy ranges for serum alanine aminotransferase levels. Ann Intern Med 137:1–9

Ravichandran R, Lafferty F, McGinniss MJ, Taylor HC 1996 Congenital adrenal hyperplasia presenting as massive adrenal incidentalomas in the sixth decade of life: report of two patients with 21-hydroxylase deficiency. J Clin Endocrinol Metab 81:1776–1779

Reisch N, Flade L, Scherr M, Rottenkolber M, Pedrosa Gil F, Bidlingmaier M, Wolff H, Schwarz HP, Quinkler M, Beuschlein F, Reincke M 2009 High prevalence of reduced fecundity in men with congenital adrenal hyperplasia. J Clin Endocrinol Metab 94:1665-1670

Reisch N, Scherr M, Flade L, Bidlingmaier M, Schwarz HP, Müller-Lisse U, Reincke M, Quinkler M, Beuschlein F 2010 Total adrenal volume but not testicular adrenal rest tumor volume is associated with hormonal control in patients with 21-hydroxylase deficiency. J Clin Endocrinol Metab 95:2065-2072

Riepe FG, Krone N, Krüger SN, Sweep FC, Lenders JW, Dötsch J, Mönig H, Sippell WG, Partsch CJ 2006 Absence of exercise-induced leptin suppression associated with insufficient epinephrine reserve in patients with classic congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Exp Clin Endocrinol Diabetes 114:105-110

Roche EF, Charmandari E, Dattani MT, Hindmarsh PC 2003 Blood pressure in children and adolescents with congenital adrenal hyperplasia (21-hydroxylase deficiency): a preliminary report. Clin Endocrinol (Oxf) 58:589-596

Rockall AG, Sohaib SA, Evans D, Kaltsas G, Isidori AM, Monson JP, Besser GM, Grossman AB, Reznek RH 2003 Hepatic steatosis in Cushing's syndrome: a radiological assessment using computed tomography. Eur J Endocrinol 149: 543-548

Ruttmann E, Brant LJ, Concin H, Diem G, Rapp K, Ulmer H; Vorarlberg Health Monitoring and Promotion Program Study Group 2005 Gamma-glutamyltransferase as a risk factor for cardiovascular disease mortality: an epidemiological investigation in a cohort of 163,944 Austrian adults. Circulation 112:2130-2107

Sartorato P, Zulian E, Benedini S, Mariniello B, Schiavi F, Bilora F, Pozzan G, Greggio N, Pagnan A, Mantero F, Scaroni C 2007 Cardiovascular risk factors and ultrasound evaluation of intima-media thickness at common carotids, carotid bulbs, and femoral and abdominal aorta arteries in patients with classic congenital adrenal hyperplasia due to 21-hydroxylase deficiency. J Clin Endocrinol Metab 92:1015-1018

Saygili F, Oge A, Yilmaz C 2005 Hyperinsulinemia and insulin insensitivity in women with nonclassical congenital adrenal hyperplasia due to 21-hydroxylase deficiency: the relationship between serum leptin levels and chronic hyperinsulinemia. Horm Res 63:270-274

SCB 2005 www.scb.se/statistik/_publikationer/BE9999_2005A01_BR_BE96ST0605.pdf (accessed July 2010)

Schwimmer JB, Khorram O, Chiu V & Schwimmer WB 2005 Abnormal aminotransferase activity in women with polycystic ovary syndrome. Fertil Steril 83:494–497

Sciannamblo M, Russo G, Cuccato D, Chiumello G, Mora S 2006 Reduced bone mineral density and increased bone metabolism rate in young adult patients with 21-hydroxylase deficiency. J Clin Endocrinol Metab 91:4453-4458

Setji TL, Holland ND, Sanders LL, Pereira KC, Diehl AM, Brown AJ 2006 Nonalcoholic steatohepatitis and nonalcoholic fatty liver disease in young women with polycystic ovary syndrome. J Clin Endocrinol Metab 91:1741-1747

Shaker JL, Lukert BP 2005 Osteoporosis associated with excess glucocorticoids. Endocrinol Metab Clin North Am 34:341-356

Shanklin DR, Richardson AP Jr, Rothstein G 1963 Testicular hilar nodules in adrenogenital syndrome. The nature of the nodules. Am J Dis Child 106:243-250

Shaper AG, Wannamethee G, Macfarlane PW, Walker M 1993 Heart rate, ischaemic heart disease, and sudden cardiac death in middle-aged British men. Br Heart J 70:49-55

Sharquie KE, Noaimi AA, Saleh BO, Anbar ZN 2009 The frequency of 21-alpha hydroxylase enzyme deficiency and related sex hormones in Iraqi healthy male subjects versus patients with acne vulgaris. Saudi Med J 30:1547-1550

Speiser PW, Dupont B, Rubinstein P, Piazza A, Kastelan A, New MI 1985 High frequency of nonclassical steroid 21-hydroxylase deficiency. Am J Hum Genet 37:650-667

Speiser PW, Serrat J, New MI, Gertner JM 1992 Insulin insensitivity in adrenal hyperplasia due to nonclassical steroid 21-hydroxylase deficiency. J Clin Endocrinol Metab 75:1421-1424

Stikkelbroeck NM, Otten BJ, Pasic A, Jager GJ, Sweep CG, Noordam K, Hermus AR 2001 High prevalence of testicular adrenal rest tumors, impaired spermatogenesis, and Leydig cell failure in adolescent and adult males with congenital adrenal hyperplasia. J Clin Endocrinol Metab 86:5721-5728

Stikkelbroeck NM, Oyen WJ, van der Wilt GJ, Hermus AR, Otten BJ 2003 Normal bone mineral density and lean body mass, but increased fat mass, in young adult patients with congenital adrenal hyperplasia. J Clin Endocrinol Metab 88:1036-1042

Stikkelbroeck NM, Hermus AR, Suliman HM, Jager GJ, Otten BJ 2004 Asymptomatic testicular adrenal rest tumours in adolescent and adult males with congenital adrenal hyperplasia: basal and follow-up investigation after 2.6 years. J Pediatr Endocrinol Metab 17:645-653

Söderberg S, Ahrén B, Eliasson M, Dinesen B, Brismar K, Olsson T 2001 Circulating IGF binding protein-1 is inversely associated with leptin in nonobese men and obese postmenopausal women. Eur J Endocrinol 144:283–290

Tiryaki T, Aycan Z, Hücümenoğlu S, Atayurt H 2005 Testis sparing surgery for steroid unresponsive testicular tumors of the congenital adrenal hyperplasia. Pediatr Surg Int 21:853-855

Therrell BL Jr, Berenbaum SA, Manter-Kapanke V, Simmank J, Korman K, Prentice L, Gonzalez J, Gunn S 1998 Results of screening 1.9 million Texas newborns for 21-hydroxylase-deficient congenital adrenal hyperplasia. Pediatrics 101:583-590

Thilén A, Nordenström A, Hagenfeldt L, von Döbeln U, Guthenberg C, Larsson A 1998 Benefits of neonatal screening for congenital adrenal hyperplasia (21-hydroxylase deficiency) in Sweden. Pediatrics 101:E11

Urban MD, Lee PA, Migeon CJ 1978 Adult height and fertility in men with congenital virilizing adrenal hyperplasia. N Engl J Med 299:1392-1396

Vermeulen A, Verdonck L, Kaufman JM 1999 A critical evaluation of simple methods for the estimation of free testosterone in serum. J Clin Endocrinol Metab 84:3666-3672

Völkl TM, Simm D, Beier C, Dörr HG 2006a Obesity among children and adolescents with classic congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Pediatrics 117:e98-105

Völkl TM, Simm D, Dötsch J, Rascher W, Dörr HG 2006b Altered 24-hour blood pressure profiles in children and adolescents with classical congenital adrenal hyperplasia due to 21-hydroxylase deficiency. J Clin Endocrinol Metab 91:4888-4895

Völkl TM, Simm D, Körner A, Rascher W, Kiess W, Kratzsch J, Dörr HG 2009 Does an altered leptin axis play a role in obesity among children and adolescents with classical congenital adrenal hyperplasia due to 21-hydroxylase deficiency? Eur J Endocrinol 160:239-247

Walker BR, Skoog SJ, Winslow BH, Canning DA, Tank ES 1997 Testis sparing surgery for steroid unresponsive testicular tumors of the adrenogenital syndrome. J Urol 157:1460-1463

Wedell A, Thilén A, Ritzén EM, Stengler B, Luthman H 1994 Mutational spectrum of the steroid 21-hydroxylase gene in Sweden: implications for genetic diagnosis and association with disease manifestation. J Clin Endocrinol Metab. 78:1145-1152

Weise M, Mehlinger SL, Drinkard B, Rawson E, Charmandari E, Hiroi M, Eisenhofer G, Yanovski JA, Chrousos GP, Merke DP 2004 Patients with classic congenital adrenal hyperplasia have decreased epinephrine reserve and defective glucose elevation in response to high-intensity exercise. J Clin Endocrinol Metab 89:591-597

White PC, Grossberger D, Onufer BJ, Chaplin DD, New MI, Dupont B, Strominger JL 1985 Two genes encoding steroid 21-hydroxylase are located near the genes encoding the fourth component of complement in man. Proc Natl Acad Sci USA 82:1089–1093

White PC, New MI, Dupont B 1986 Structure of human steroid 21-hydroxylase genes. Proc Natl Acad Sci USA 83:5111–5115

White PC, Speiser PW 2000 Congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Endocr Rev. 21:245-291

White PC 2009 Neonatal screening for congenital adrenal hyperplasia. Nat Rev Endocrinol 5:490-498

WHO 1994 Assessment of osteoporotic fracture risk and its role in screening for postmenopausal osteoporosis. WHO Technical Report Series 843. Geneva: World Health Organization

WHO 1999 WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction. 4th ed. Cambridge, United Kingdom: Cambridge University Press.

Wikland KA, Luo ZC, Niklasson A, Karlberg J 2002 Swedish population-based longitudinal reference values from birth to 18 years of age for height, weight and head circumference. Acta Paediatr 91:739-754

Williams RM, Deeb A, Ong KK, Bich W, Murgatroyd PR, Hughes IA, Acerini CL 2010 Insulin sensitivity and body composition in children with classical and nonclassical congenital adrenal hyperplasia. Clin Endocrinol (Oxf) 72:155-160

Zimmermann A, Sido PG, Schulze E, Al Khzouz C, Lazea C, Coldea C, Weber MM 2009 Bone mineral density and bone turnover in Romanian children and young adults with classical 21-hydroxylase deficiency are influenced by glucocorticoid replacement therapy. Clin Endocrinol (Oxf) 71:477-484