From the Department of Clinical Science, Intervention and Technology
Division of Speech and Language Pathology
Karolinska Institutet, Stockholm, Sweden

Effects of increased levels of androgens on voice and vocal folds in women with congenital adrenal hyperplasia and female-to-male transsexual persons

Ulrika Nygren

Karolinska Institutet

Stockholm 2014

Published by Karolinska Institutet.

ISBN 978-91-7549-725-9

Printed by
REPROPRINT AB
Stockholm 2014
www.reproprint.se
Gårdsvägen 4, 169 70 Solna

Karolinska Institutet

The Department of Clinical Science, Intervention and Technology
Division of Speech and Language Pathology

Effects of increased levels of androgens on voice and vocal folds in women with congenital adrenal hyperplasia and female-to-male transsexual persons

THESIS FOR DOCTORAL DEGREE (Ph.D.)
which, by due permission of Karolinska Institutet, will be publicly defended in lecture hall R 64, Rehabgatan 4, floor 6, Karolinska University Hospital, Huddinge, for the degree of Doctor of Medicine

Friday, December 12, 2014 at 9 am

By
Ulrika Nygren
Speech and Language Pathologist

Principal Supervisor:
Associate professor Maria Södersten
Karolinska Institutet
Department of Clinical Science, Intervention and Technology
Division of Speech and Language Pathology

Co-supervisors:
Professor Agneta Nordenskjöld
Karolinska Institutet
Department of Women's and Children's Health
Center of Molecular Medicine

Associate professor Stefan Arver
Karolinska Institutet
Department of Medicine/Huddinge

Opponent:
Associate professor Jennifer Oates
La Trobe University, Melbourne, Australia
Department of Human Communication Sciences and Faculty of Health Sciences

Examination Board:
Associate professor Elisabeth Lindström
Åbo Akademi
Department of Psychology and Logopedics
Division of Logopedics

Professor Jan Gustafsson
Uppsala University
Department of Women's and Children's Health

Associate professor Riitta Möller
Karolinska Institutet
Department of Medical Epidemiology and Biostatistics

Stockholm 2014

ABSTRACT

Voice virilization in women may occur due to increased levels of androgens. Women with congenital adrenal hyperplasia (CAH) are at risk for voice virilization due to an enzyme deficiency that causes increased production of androgens and lack of cortisol. Female-to-male transsexual persons, trans men, are treated with testosterone, with virilization of the voice as a desired outcome. The overall aim of the project was to provide new knowledge on how female voice and vocal folds are affected by endogenous and exogenous androgen exposure, and the consequences virilization of the voice may have in a patient's life.

Study I: Thirty-eight women with CAH and 24 age-matched controls participated. Their voices were recorded and acoustically and perceptually analyzed. They answered questions about subjective voice problems. Endocrine data were obtained from medical journals. The results showed that women with CAH spoke with significantly lower mean fundamental frequency (F0), had darker voice quality, and rated higher on the statement "my voice is a problem in my daily life" than the controls. Voice virilization was associated with late diagnosis or problems with glucocorticoid medication, but not with severity of mutation. Proper treatment with glucocorticoids is important to avoid long periods of increased androgen levels to prevent irreversible voice virilization.

Study II: Forty-two women with CAH and 43 age-matched controls filled out the Voice Handicap Index (VHI) and answered questions about voice function related to virilization. Endocrine data were obtained from medical journals. Women with CAH scored significantly higher than the controls on VHI when the results were divided into groups by voice handicap: none/mild, moderate, and severe. A virilized voice in women with CAH correlated with less voice satisfaction. Seven percent of the women with CAH had voice problems related to voice virilization. Voice virilization was associated with long periods of under-treatment with glucocorticoids and higher bone mineral density, confirming results and conclusions from study I. It is recommended that women with CAH who experience voice problems are referred for voice assessment.

Study III: Four women with CAH and virilized voices, and 5 female and 4 male controls participated. A procedure for magnetic resonance imaging of the vocal folds was developed. The results showed that the cross-sectional area of the thyroarytenoid (TA) muscle was larger in women with virilized voices than in female controls, and smaller than in males. The larger TA area correlated with lower F0 values obtained from acoustic analysis of habitual speech range profiles. Thus, the anatomical explanation for voice virilization may be a larger cross-sectional area of the TA muscle, suggesting androgen receptors in the vocal folds. These findings need to be confirmed in a larger study.

Study IV: Fifty trans men participated in a longitudinal study. Voice assessments, performed before testosterone treatment started and regularly up to 24 months, included audio-recordings of speech and voice range profiles and self-ratings of voice function. A significant lowering of mean F0 was found after 3 months, after 6 months, and up to 12 months, when group data were congruent with reference data for males. No correlations were found between levels of testosterone, EVF, Hb, SHBG or LH, and F0 values. Lower F0 values correlated with greater satisfaction with the voice. A quarter of the participants had received voice therapy for problems associated with virilization, such as vocal fatigue or unstable voice. Voice assessment during testosterone treatment is important to detect the potentially large subgroup of trans men that needs voice therapy.

SAMMANFATTNING

Röstvirilisering hos kvinnor kan förekomma på grund av ökade halter av androgener. Kvinnor med kongenital binjurehyperplasi (congenital adrenal hyperplasia, CAH) riskerar att få en viriliserad röst på grund av en enzymdefekt som medför ökad produktion av androgener och kortisolbrist. Transsexuella kvinnor-till-män, transmän, behandlas med testosteron med röstvirilisering som önskat resultat. Det övergripande syftet med projektet var att tillhandahålla ny kunskap om hur kvinnors röster och stämband påverkas av endogen och exogen exponering för androgener och vilka konsekvenser röstvirilisering kan ha i patienters liv.

Studie I: Trettioåtta kvinnor med CAH och 24 åldersmatchade kontrollpersoner deltog. Deras röster spelades in och analyserades akustiskt och perceptuellt. De besvarade frågor om subjektiva röstproblem. Endokrinologiska data inhämtades från journaler. Resultaten visade att kvinnor med CAH talade med signifikant lägre grundtonsfrekvens (MF0), hade mörkare röstkvalitet och skattade högre värden på påståendet ”min röst utgör ett problem i mitt dagliga liv” jämfört med kontrollpersoner. Röstviriliseringen var associerad med sen diagnosticering eller problem med kortisonmedicinering, och inte med svårighetsgraden av CAH. En korrekt medicinering med kortison är avgörande för att undvika långa perioder av ökade halter av androgener med risk för irreversibel röstvirilisering.

Studie II: Fyrtiotvå kvinnor med CAH och 43 åldersmatchade kontrollpersoner besvarade Rösthandikappindex (RHI) och frågor gällande röstfunktionen relaterade till röstvirilisering. Endokrinologiska data inhämtades från journaler. Kvinnor med CAH skattade signifikant högre värden på RHI när resultaten delades in i grupper: ingen/mild, måttlig och hög grad av rösthandikapp. En viriliserad röst hos kvinnor med CAH korrelerade med att de var mindre nöjda med sina röster. Sju procent av kvinnorna med CAH hade röstproblem relaterade till röstvirilisering. Röstvirilisering var associerad med långa perioder av underbehandling med kortison och högre benmineraldensitet, vilket bekräftade resultat och slutsatser från delstudie I. Kvinnor med CAH som har röstproblem bör remitteras för röstbedömning.

Studie III: Fyra kvinnor med CAH och med viriliserade röster, 5 kvinnliga och 4 manliga kontrollpersoner deltog. En procedur för inspelning och analys med magnetisk resonanstomografi utvecklades för stämbandsmätningar. Resultaten visade att tvärsnittsarean av thyro-arytenoidmuskeln (TA) var större hos kvinnor med CAH jämfört med de kvinnliga kontrollpersonerna, och mindre jämfört med de manliga. En större TA-area korrelerade med lägre F0-värden från akustisk analys av talfonetogram. Den anatomiska förklaringen till en viriliserad röst kan därför vara en större tvärsnittsarea av TA-muskeln, vilket tyder på att androgenreceptorer finns i stämbanden. Resultaten behöver bekräftas av större studier.

Studie IV: Femtio transmän deltog i en longitudinell studie. Röstbedömningar, innan testosteronbehandling inleddes och regelbundet upp till 24 månader, omfattade röstinspelningar av tal- och maxfonetogram samt egenskattningar av röstfunktionen. En signifikant sänkning av MF0 skedde efter 3 månader, efter 6 månader och efter 12 månader, då gruppdata var i nivå med referensvärden för manliga talare. Ingen korrelation kunde påvisas mellan nivåerna av testosteron, EVF, Hb, SHBG eller LH och F0-värden. Lägre F0-värden korrelerade med att deltagaren var mer nöjd med rösten. En fjärdedel av deltagarna hade fått röstbehandling för röstproblem relaterade till viriliseringen, såsom röstträtthet och instabilitet. Därför är röstbedömning under testosteronbehandling viktig för att upptäcka den relativt stora grupp transmän som behöver röstbehandling.

LIST OF SCIENTIFIC PAPERS

I. **Nygren U**, Södersten M, Falhammar H, Thorén M, Hagenfeldt K, Nordenskjöld A. Voice characteristics in women with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Clinical Endocrinology (Oxf)*, 2009,70(1), 18-25.

II. **Nygren U**, Filipsson Nyström H, Falhammar H, Hagenfeldt K, Nordenskjöld A, Södersten M. Voice problems due to virilization in adult women with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Clinical Endocrinology (Oxf)*, 2013,79(6), 859-66.

III. **Nygren U**, Isberg B, Arver S, Hertegård S, Södersten M, Nordenskjöld A. Magnetic resonance imaging of the vocal folds in women with congenital adrenal hyperplasia and virilized voices. Submitted manuscript.

IV. **Nygren U**, Nordenskjöld A, Arver S, Södersten M. Effects on voice fundamental frequency in trans men during testosterone treatment - a longitudinal study. Manuscript.

CONTENTS

LIST OF ABBREVIATIONS

ACTH	Adrenocorticotropic hormone
CAH	Congenital adrenal hyperplasia
EVF	Erythrocyte volume fraction
F0	Fundamental frequency
Hb	Hemoglobin
Leq	Equivalent continuous sound level
LH	Luteinizing hormone
MF0	Mean fundamental frequency
MRI	Magnetic resonance imaging
NC CAH	Non classic CAH
SHBG	Sex hormone binding globulin
SPL	Sound pressure level
SRP	Speech range profile
ST	Semitones
SW CAH	Salt wasting CAH
SV CAH	Simple virilizing CAH
Sw-VHI	Swedish validated version of the Voice Handicap Index
T	Testosterone
TA	Thyroarytenoid muscle
TS	Transsexualism
VRP	Voice range profile
VT	Vocal tract
VTL	Vocal tract length

1 INTRODUCTION

The voice is necessary in verbal communication and a crucial part of an individual's identity. The voice contains acoustic cues, which give a listener information about the speaker's gender and age, and can sound more or less feminine or masculine. The voice and the vocal folds can be affected by many medical conditions resulting in a changed voice pitch or quality, which can severely affect a person's quality of life. One such condition is abnormal increase of male hormones, androgens, in women. Females can respond to increased androgen stimulation with changes in the larynx and a virilized voice. However, knowledge is insufficient about voice virilization and its consequences, which have previously been described only scarcely. Two patient groups are highly relevant to include in this thesis representing two different principles of androgen stimulation. Women with congenital adrenal hyperplasia (CAH) are born with an enzyme deficiency that causes lack of cortisol and often aldosterone and increased production of endogenous androgens and thus risk undesirable voice virilization. Female-to-male transsexual persons receive testosterone treatment with virilization of the voice as one of the desired outcomes.

1.1 VIRILIZATION OF VOICE AND VOCAL FOLDS IN WOMEN

Symptoms of voice virilization in women have been described as a lowered fundamental frequency (F0), loss of high frequencies, vocal instability such as involuntary shift between the modal and falsetto registers, hoarse or rough voice quality, creakiness, changes in timbre and difficulties to project the voice. These characteristics are based on results from case studies, small group studies, clinical experience and one prospective study (Baker, 1999; Berendes, 1962; Boothroyd & Lepre, 1990; Damsté, 1964, 1967; Eliakim, et al., 2011; Gerritsma, Brocaar, Hakkesteegt, & Birkenhager, 1994; Juniarto, et al., 2013; Martin, 1988; Nordenskjöld & Fex, 1984; Pattie, Murdoch, Theodoros, & Forbes, 1998; Shepperd, 1966; Spooner, 1977; Strauss, Liggett, & Lanese, 1985; Wollina, et al., 2007). A virilized voice may also be emotionally difficult as described by Tsuji et al. (2003), Baker (1999) and Van Gelder (1974).

Effects of virilization on the vocal folds and the larynx have been described not to be visible during a laryngoscopic examination (Damsté, 1964, 1967). Three studies focused on investigating virilized vocal folds and larynxes in vitro. Bauer (1968) compared one woman's excised virilized larynx with larynxes from vocally healthy men and women. The virilized vocal fold had larger dimensions of the thyroarytenoid (TA) muscle and smaller dimensions of connective tissue. In another study, female mice with virilized larynxes were examined. Hypertrophy and hyperplasia of the inner part of the TA muscle, as well as slight edema in the mucous membrane and the connective tissue were seen (Talaat, et al., 1987). A more recent study examined the vocal folds in virilized rats and it was found that the muscle fibers

were hypertrophic (Amer, Asker, & Mazroa, 2011). Thus, it is important to further investigate effects of virilization on the vocal folds in larger groups and in vivo.

1.2 VOICE PRODUCTION

Voice production requires three parts, presented below:

- The respiratory system regulates the air pressure from the lungs during exhalation and the subglottal pressure generates the *driving force* for vocal fold vibrations.
- The vocal folds generate the sound of the voice when they are set into vibration, thus being the voice *source*.
- The vocal tract (VT) is the cavity in the area immediately above the vocal folds up to the lips, including the pharynx, oral and nasal cavities, and acts as a *filter*. Changes in the vocal tract by moving the jaw, tongue, lips, velum, and the larynx position affect the articulation and resonance of the voice, see Figure 1a and b.

The vocal folds contain the *cover*, i.e. epithelium and the superficial layer of the lamina propria and the *body*, i.e. the intermediate and deep layers of the lamina propria and the TA muscle, also called the vocalis muscle (Hirano, 1974). The subglottal pressure and the Bernoulli effect generate the forces that produce the vocal fold vibrations (Van Den Berg & Zantema, 1956). The fundamental frequency (F0), the number of vocal fold vibrations per second (Hz), is closely related to the perceived voice pitch. Longer and thicker vocal folds, as in males, result in slower vibrations and a lower F0, as compared with the faster vibrations caused by the shorter and thinner vocal folds in females (Hirano, 1974; Titze, 1989). However, there is a complicated association between vocal fold mass and F0 according to Titze (2011), who claimed that an increase only in the vertical thickness of the vocal folds does not lower F0, whereas an increase of the vocal fold length or of the lateral depth of the vocal folds will lower F0. F0 is also regulated through stretching of the vocal folds, controlled mainly by activity in the cricothyroid muscle, and shortening of the vocal folds, i.e. changing the tonus in the TA muscle (Titze, Luschei, & Hirano, 1989). A balance between the cricothyroid muscle and the TA muscle activity is needed for pitch regulation and vocal stability. The sound pressure level (SPL) of the voice is closely related to perceived loudness and is regulated mainly by subglottal pressure. A higher subglottal pressure typically corresponds to higher SPL (Holmberg, Hillman, & Perkell, 1988). The voice quality is associated with both the resonances in the vocal tract and the glottal waveform (Gauffin & Sundberg, 1989).

There are anatomical differences between females and males, such as females having a shorter vocal tract, shorter and thinner vocal folds, and a less prominent Adam's apple (Fitch & Giedd, 1999). See Figure 1a and b.

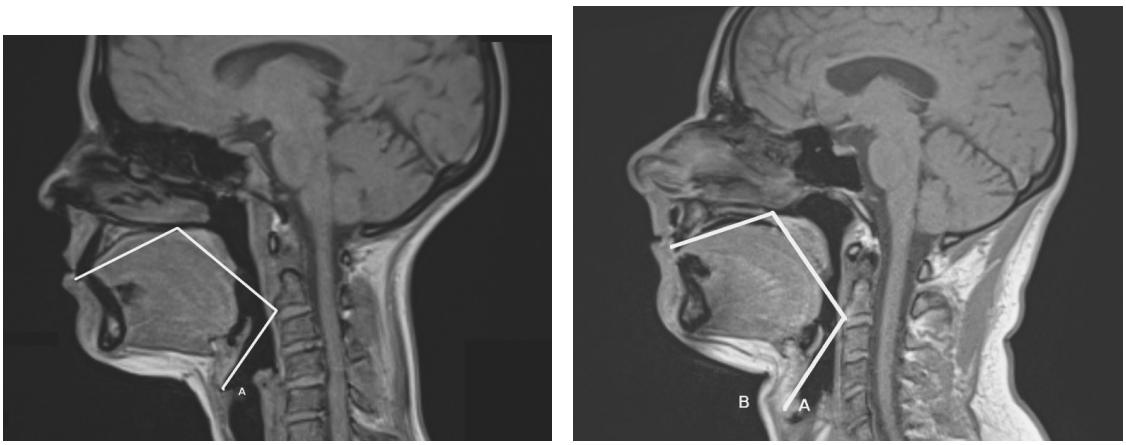

Figure 1a and b. Images acquired in sagittal plane using MRI of a female (left) and a male (right) control in study III. The vocal folds (A), the Adam's apple (B) and the vocal tract (straight lines), are pointed out. The VTL was 158 mm for the female and 181 mm for the male.

There are differences between female and male voices as regards the acoustic parameters F0, SPL, the glottal waveform and the acoustic spectrum. Females typically speak with higher F0 and lower SPL, are perceived as having more breathy voice quality and a "brighter" speech due to the approximately 20 percent higher formant frequencies (Coleman, 1983; Hallin, Fröst, Holmberg, & Södersten, 2012; Holmberg, et al., 1988; Klatt & Klatt, 1990; Pegoraro Krook, 1988; Sanchez, Oates, Dacakis, & Holmberg, 2014; Södersten, Hertegård, & Hammarberg, 1995; Södersten & Lindestad, 1990; Södersten, Lindestad, & Hammarberg, 1991; Södersten, Ternström, & Bohman, 2005). The main acoustic variables of interest in the present thesis are F0 and SPL related to voice virilization in both women with CAH and female-to-male transsexual persons. Reference values for mean F0 (MF0) in adult vocally healthy Swedish females are 188 Hz and for males 116 Hz (Pegoraro Krook, 1988).

1.3 TESTOSTERONE PRODUCTION AND ADMINISTRATION

Testosterone and its derivative dihydrotestosterone are the principle androgenic virilizing hormones in men. These androgens are responsible for sex differentiation during the early fetal period, virilization at puberty, and maintenance of androgen-dependent functions in adult life. The potential to respond to androgen signaling remains intact throughout life in certain tissues, i.e. genital structures, larynx, skin, muscle, bone, the central nervous system, and adipose tissue. Thus, women may respond to increased androgen stimulation with changes in the larynx and vocal folds. Independent of age, normal serum levels of testosterone in adult women are 0.5-3.0 nmol/L and 10-30 nmol/L in adult men (Nieschlag, Behre, & Nieschlag, 2012).

1.3.1 Steroid hormone synthesis

The adrenal glands produce glucocorticoids (cortisol), mineralocorticoids (aldosterone) and androgens. Androgens are also derived from the ovaries, which produce 50 percent of testosterone in females, and the testis, which produce 90 percent of circulating testosterone in adult males. The steroid hormone synthesis starts with conversion of cholesterol to pregnenolone regulated by adrenocorticotropic hormone (ACTH)-controlled expression of the rate-limiting enzyme. ACTH is produced in the anterior pituitary and pregnenolone is a precursor for the various steroids produced by the adrenal glands. The feedback system regulating ACTH is mainly based on circulating levels of cortisol. Impaired production of cortisol leads to raised levels of ACTH and excessive secretion of adrenal androgens. To synthesize aldosterone the enzyme 21-hydroxylase is needed for conversion of pregnenolone, and 17-hydroxyprogesterone is needed for conversion of pregnenolone to produce cortisol (White & Speiser, 2000).

1.3.2 Testosterone treatment in female-to-male transsexual persons

Testosterone treatment in female-to-male transsexual persons aims to induce and maintain virilization and testosterone levels within the normal male range. Monitoring of testosterone treatment in trans men includes assessment of testosterone levels, suppression of luteinizing hormone (LH), increase in erythropoesis and also sex hormone binding globulin (SHBG), a binding protein that mitigate the levels of free testosterone. Side effects of testosterone therapy may include acne and excessive stimulation of erythropoesis, while severe adverse reactions are rare if testosterone levels are maintained in the normal male range (Hembree, et al., 2009).

1.4 CONGENITAL ADRENAL HYPERPLASIA IN WOMEN

1.4.1 Clinical presentation and incidence

Women with congenital adrenal hyperplasia (CAH), an inherited autosomal recessive disorder, are born with an enzyme deficiency in the adrenal glands and a defective or absent production of cortisol and often also of aldosterone. The lack of these hormones causes a feedback on the pituitary by increased ACTH stimulation, which leads to high androgen secretion from the adrenal glands (Merke & Bornstein, 2005; White & Speiser, 2000). For a description of the steroid hormone synthesis in CAH, see Figure 2. Prenatal androgen excess results in virilization of the external genitals in females, which can make gender decision difficult at birth. Treatment with glucocorticoids is vital and substitutes the cortisol insufficiency to prevent adrenal crisis, as well as decreasing ACTH production, which suppresses the increased production of adrenal androgens. Neonatal screening for CAH started 1986 in Sweden and the level of 17-hydroxyprogesterone is used as a disease marker; raised levels indicate CAH (Gidlöf, Wedell, Guthenberg, von Döbeln, & Nordenström, 2014). In the study by Gidlöf and colleagues (2014), the efficiency of the neonatal screening

for CAH in Sweden was investigated and it was concluded that all cases with the most severe form of CAH were detected, but not all cases with milder forms of CAH. Based on data from the neonatal screening program in Sweden, the incidence was found to be more than one in 9 000 live births between 1990 and 2000 (Gidlöf, et al., 2013) or one in 9 800 (Thilén, et al., 1998), corresponding to a total of 5 girls and 5 boys receiving this diagnosis each year. CAH is characterized by impaired activity of one of the five enzymes in the adrenal gland. CAH due to 21-hydroxylase deficiency accounts for more than 90-95 percent of all patients with CAH (Merke & Bornstein, 2005).

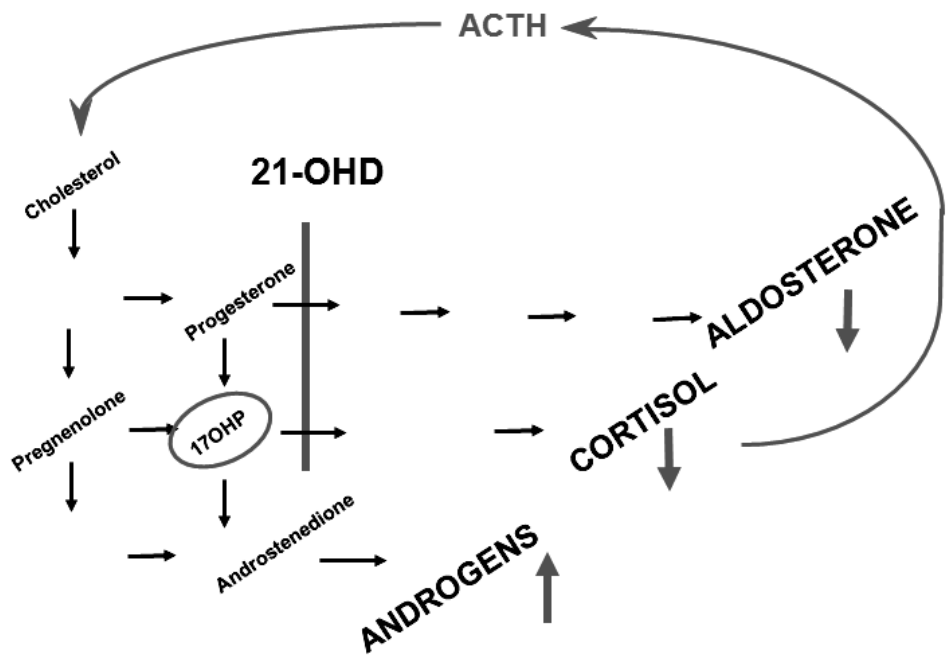

Figure 2. **S**teroid hormone synthesis defect in CAH. The production of cortisol, aldosterone and androgens in the adrenal cortex and the enzymes involved in CAH are shown as well as the feedback system regulating adrenocorticotropic hormone (ACTH). A lack of the enzyme 21-hydroxylase (21-OHD) results in insufficient cortisol production, an accumulation of 17-hydroxyprogesterone (17-OHP) and excessive androgen secretion. (Modified from an illustration by Anna Wedell, with permission).

Clinically, CAH due to 21-hydroxylase deficiency is divided in three sub-groups: salt-wasting (SW), simple virilizing (SV) and non-classic (NC) (Wedell, Thilén, Ritzen, Stengler, & Luthman, 1994). SW and SV are called classic CAH, and affected girls are often born with virilized external genitals. In SW, which is the most severe form, both cortisol and aldosterone deficiency occurs. SV, a milder phenotype, shows mainly cortisol deficiency and NC shows symptoms due to virilization later in life, i.e. early onset of puberty, hirsutism and infertility (Pinto, et al., 2003). A good correlation between phenotype and genotype has been

found (Wedell, et al., 1994). There are four genotype groups that are the most common, identified by to the mildest representing allele: null, I2splice, I172N and V281L, see Figure 3. Null is a mutation where no activity of the enzyme (21-hydroxylase) occurs and is associated with SW. I2splice mutation with very low activity of the enzyme is usually associated with SW, but sometimes with SV. Further, I172N is associated with SV and V281L with NC.

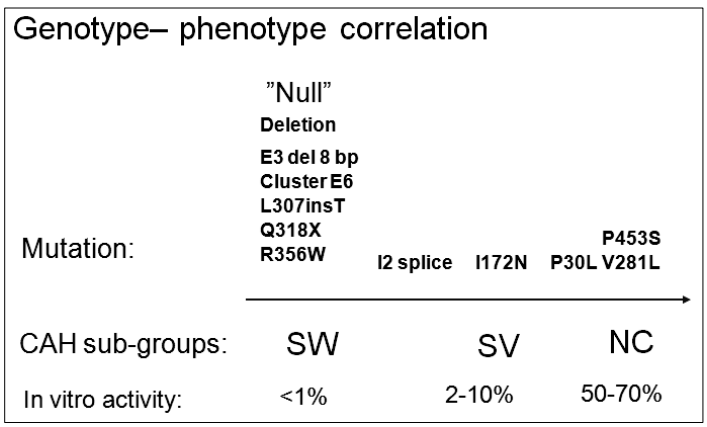

Figure 3. The 10 genotypes that encompass 95 percent of all mutations in the population and their corresponding phenotypes (clinical sub-groups of CAH) and diminished enzyme activity compared to the normal enzyme. (Modified from an illustration by Anna Wedell, with permission).

1.4.2 Voice characteristics

Women with CAH may develop voice virilization, as described in three case reports (Fürst-Recktenwald, Dörr, & Rosanowski, 2000; Heinemann, 1974; Tsuji, et al., 2003), see Table 1. Heinemann (1974) reported perceptual analysis of pitch and voice quality in 10 girls and 3 boys with CAH, whose voices were found to have dark voice "timbre" (quality), low pitch and sometimes hoarse voice quality. In one case report of a 17-year-old girl with CAH, data on "middle F0" of 104 Hz was presented (Fürst-Recktenwald, et al., 2000). In another case report, a 16-year-old girl had a virilized voice with an MF0 of 160 Hz and presence of prominent Adam's apple, causing discomfort to the patient (Tsuji, et al., 2003). Cricothyroid approximation, Isshiki's type IV was performed to increase the pitch (Isshiki, Morita, Okamura, & Hiramoto, 1974). After the surgery and six sessions of voice therapy, with the aim of expanding F0 range and reducing strain voice quality, MF0 was raised to 220 Hz.

Table 1. Previous studies and voice findings in girls with CAH.

Study	n	Voice	Voice assessment
Heinemann, 1974	10	Lowering of voice pitch Dark voice quality	Perceptual
Fürst-Recktenwald et al., 2000	1	Deep voice "Middle F0" = 104	Not described
Tsuji et al., 2003	1	Lowering of F0, MF0 = 160 Small speaking frequency range	Acoustic analyses

1.5 FEMALE-TO-MALE TRANSSEXUAL PERSONS

1.5.1 Clinical presentation and incidence

Transsexualism (TS) is a medical diagnosis with the code F64.0 in the International Classification of Diseases (ICD-10) and the criteria "a desire to live and be accepted as a member of the opposite sex, usually accompanied by a sense of discomfort with, or inappropriateness of, one's anatomic sex, and a wish to have surgery and hormonal treatment to make one's body as congruent as possible with one's preferred sex" (World Health Organization, 2009). The diagnosis according to DSM-5 is Gender Dysphoria (American Psychiatric Association, 2013). In Sweden change of legal status and surgical sex-reassignment is formally applied for at the National Board of Health and Welfare and regulated by Swedish law since 1972. The applicable law was amended in 2013, when the requirements of being sterilized and unmarried were removed. Incidence of applications for legal and surgical sex-reassignment in Sweden have increased significantly between 1972 and 2010: from 0.16 to 0.42/10 000 per year for trans men and from 0.23 to 0.73/100 000 per year for trans women, with the sex ratio (trans men:trans women) being 1:1.66 for the time period (Dhejne, Öberg, Arver, & Landen, 2014). Between 2010 and 2012, in average 27 trans men and 31 trans women per year formally applied for change of legal status and surgical sex reassignment in Sweden. After the change of the law 2013 the number has increased considerably.

Individuals who have been assigned female sex at birth and experience gender incongruence, transsexual female-to-males, will henceforth be referred to as trans men in the thesis. Trans men who receive the TS diagnosis experience gender incongruence, i.e. the gender identity contradicts the sex assigned at birth. Treatment include cross sex hormones, surgery to alter the chest, change of legal status, and sometimes surgical sex-reassignment. The treatment program is described in "Standards of care for the health of transsexual, transgender, and gender-nonconforming people" (Coleman, et al., 2012). Trans men receive testosterone treatment and today the administration contains mainly of intramuscular depot injections or transdermal testosterone gels. The reversible changes that occur require life-long treatment to maintain the effects and sex hormone levels within the normal range for males. The reversible changes are increased muscle mass, decreased fat mass, cessation of menses, and secondary

hair growth. The irreversible changes are increased clitoromegaly and deepening of the voice (Hembree, et al., 2009; Nieschlag, et al., 2012).

1.5.2 Terminology

There is a large variety in the literature as regards to the terminology for transsexualism: Gender dysphoria, Gender Identity Disorder, Gender incongruence and Transgender. Regarding individuals who are assigned female sex at birth and change their legal gender, there are also different terms used, such as *female-to-male*, *FtM*, and *transmasculine* (Hansbury, 2005). Trans men is a term used more lately (Wierckx, et al., 2014). The term transmasculine is used as an umbrella term for individuals who describe themselves as FtM transsexuals, trans men, or having a gender that do not fit into the societies binary understanding of gender (Azul, 2014). In this thesis, the term "trans men" has been chosen to describe individuals assigned female sex at birth and diagnosed with Transsexualism. This decision was based on the latest recommendation from the international organization World Professional Association for Transgender Health, WPATH (personal communication with Cecilia Dhejne).

1.5.3 Voice characteristics

There is a lack of information in the research literature concerning voice characteristics in trans men during testosterone treatment (Adler, Constansis, & Van Borsel, 2012). Previous studies on voice changes in trans men are presented in Table 2. Van Borsel and colleagues (2000) presented longitudinal data from two trans men during treatment. These trans men received intramuscular injections of testosterone undecanoate for 12 and 13 months. The largest change in F0 was noticed after four months of treatment and MF0 decreased to 155 Hz and 132 Hz. The frequency range was also measured and found to be reduced. The highest F0 values were lowered, but the lowest F0 values were not lowered enough to maintain the range before treatment. Another case report presented longitudinal data of a trans man before and during 16 months of testosterone treatment with intramuscular injections, showing a marked decrease in F0 between the third and the fourth month (Damrose, 2009). MF0 of sustained /a: / for 5 seconds was analyzed and had decreased from 228.47 Hz before treatment to 116.52 Hz. A recent controlled cross-sectional study by Cosyns and colleagues (2014) investigated voice characteristics and hormonal levels in 38 trans men after testosterone treatment (9 months to 22 years). Age-matched male controls were included. The results showed that the median F0 and F0 variation during speaking did not differ significantly compared with controls. However, in 10 percent of the participants, the median F0 were insufficient, including participants whose F0 values were within the gender-ambiguous range in which the speaker's gender is unidentifiable, and participants who had undergone voice therapy or pitch lowering surgery, i.e. Type 3 thyroplasty (Isshiki, Taira, & Tanabe, 1983). Higher levels of hematocrit and longer cysteine-adenine-guanine (CAG) repeats in the androgen receptor (*AR*) gene were reported to be associated with the lowest F0 values, which would suggest a dose-response relationship between androgen exposure and lowering of the voice (Cosyns, et al., 2014). Further, Scheidt et al. (2004)

investigated voice characteristics in 14 trans men who had been treated with intramuscular injections from 2.5 months up to 9 years. They were divided in two groups, one for those who had been treated for less than one year, including participants before start of treatment (n=6) and another group for those who had been treated for more than one year (n=8). The MF0 values for the group treated > 12 months varied between 103 Hz to 140 Hz. A Swedish follow-up study by Söderpalm and colleagues (2004) reported on 22 trans women and 3 trans men. The initial MF0 values for the trans men were between 140 Hz and 150 Hz. One of the participants underwent 6 months of voice therapy and the MF0 declined from 148 Hz to 133 Hz during treatment. After 22 months, MF0 was 113 Hz and problems with vocal fatigue were no longer present.

Table 2. Previous studies on voice changes in trans men during testosterone treatment.

Study	n	Testosterone duration	Voice	Voice assessment
Van Borsel et al., 2000	2	12 and 13 months	MF0 = 155 and 123 Hz Loss of high frequencies	Acoustic analyses
Söderpalm et al., 2004	3	No information	MF0 = 140 to 150 Hz Vocal instability	Acoustic analyses
Scheidt et al., 2004	8	> 12 months	MF0 = 103-140 Hz F0 lowering	Acoustic analyses
Damrose, 2009	1	16 months	MF0 = 113 Hz	Acoustic analyses
Cosyns et al., 2014	38 + 38 contr	9 months - 22 years	Median F0 = 109 Hz F0 lowering	Acoustic analyses

1.6 OTHER CONDITIONS CAUSED BY EXOGENOUS ADMINISTRATION OR ENDOGENOUS PRODUCTION OF ANDROGENS

Previous studies on voice virilization due to exogenous administration or endogenous production of androgens are summarized in Table 3.

Conditions for which women are treated with medications including androgens are for example, menopausal problems (such as osteoporosis), endometriosis, gynecological carcinoma, fibrocystic breast disease, sexual dysfunction, and Turner's syndrome (Andersson-Wallgren & Albertsson-Wikland, 1994; Baker, 1999; Gerritsma, et al., 1994; Shepperd, 1966), see Table 3 for voice results. The use of anabolic steroids is also an instance where women receive exogenous androgens (Strauss, et al., 1985; Wollina, et al., 2007). Girls with Turner's syndrome treated with hormones were followed in a longitudinal study and a decrease of speaking F0 was reported (Andersson-Wallgren & Albertsson-Wikland, 1994). Final F0 values after four years of hormone treatment were within the normal range for adult women in comparison to reference data, so there was no large change of F0 for this patient

group based on acoustic analyses. However, during the first year of treatment, voice breaks, rough voice quality, instability in terms of register, and difficulties singing high notes were noticed.

Studies on patients' subjective experience of voice symptoms related to voice virilization are scarce. Boothroyd & Lepre (1990) reported of a woman who was perceived as male on the phone and therefore was unable to receive employment. Further, in a case study of five women with virilized voices, it was mentioned in the discussion that singers complained more about smaller singing ranges and the inability to hit certain notes accurately (Shepperd, 1966). Gerritsma et al. (1994) studied the effects on voice in 22 women with postmenopausal osteoporosis after 12 months medication with 50 mg nandrolone decanote and compared these subjects with 17 control subjects without medication. After one year of medication, the patients reported a significantly higher number of unspecified voice complaints as well as of changed timbre, vocal instability, voice pitch lowering and loss of high frequencies as compared with their controls. In addition to these self-reported results, the patients' F0 values in speech were significantly lower. Perceptual analysis of their voice quality showed significantly higher degrees of creakiness and instability as compared with the control group (Gerritsma, et al., 1994). In a retrospective study, Baker (1999) described four female singers who developed voice changes after treatment with medicine having androgenic effects. Results from perceptual and acoustic analyses indicated that all four women spoke with low-pitched voices. Reduced vocal power and endurance were also reported. All subjects had lost about one octave from the highest tone and gained one octave on the lowest tone as compared with analyses prior to medication with virilizing agents. The subjects experienced the virilizing effect on their voices as traumatic since it affected both their careers and social lives negatively (Baker, 1999).

Eliakim et al. (2011) reported of endogenous production of androgens because of a virilizing adrenal tumor. Virilization of the voice and masculine appearance with hirsutism and acne in a 14 female athlete was described. Another case study of a patient with an ovarian Leydig cell tumor reported voice deepening and a prominent Adam's apple (Juniarto, et al., 2013). Polycystic ovary syndrome (PCOS) affects 5 to 15 percent of women in reproductive age and is also characterized by hyperandrogenism (Gugatschka, et al., 2012). The voice change in women with PCOS has subsequently been investigated and compared with controls. In two studies, a lower mean F0 was detectable but no significant differences were found (Gugatschka, et al., 2012; Hannoun, et al., 2011). In the study by Hannoun and colleagues (2011), interviews with the women showed a significant difference concerning deepening of the voice between women with PCOS (35.3 percent of the 17 women with PCOS) and controls.

Table 3. Studies on voice virilization in women due to exogenous administration or endogenous production.

Study	Type of study	n	Voice	Voice assessment
Exogenous administration of androgens				
Damsté, 1964	Case reports	6	Low F0 Instability No pitch control	Not described
Shepperd, 1966	Case reports	5	Deep voice Low pitch for high and low notes Hoarseness	Perceptual
Damsté, 1967	Case reports	6	Hoarseness Loss of singing voice	Not described
Strauss et al., 1985	Case reports of anabolic steroids	10	Lower voice pitch Undesirable change	Interview
Boothroyd et al., 1990	Case report	1	Deep voice 18 month post treatment	Perceptual
Gerritsma et al., 1994	Prospective Controlled	22 + 17 controls	Voice lowering Hoarseness Changed timbre Loss of high F0 Instability	Voice history Acoustic analyses
Andersson-Wallgren et al., 1994	Case reports	4	Decreased F0	Acoustic analyses
Baker, 1999	Case reports	4	Low habitual voice pitch Loss of control of singing voice Less in vocal power MF0=104, 113, 99, 104 Hz	Acoustic and perceptual Analyses
Wollina et al., 2007	Case reports of anabolic steroids	2	Low F0, MF0=170 Rough and instable	Not described
Endogenous androgen production				
Eliakim et al., 2011	Case report Virilizing adrenal tumor	1	Voice deepening	Interview
Hannoun et al., 2011	Controlled Women with PCOS	17 + 21 controls	Deepening of voice pitch Detectable lower MF0	Interview Acoustic analyses
Gugatschka et al., 2012	Controlled of Women with PCSO	24 + 10 controls	Trend toward lower MF0	Acoustic analyses
Juniarto et al., 2013	Case report Ovarian Leydig cell tumor	1	Deepening of the voice	Interview

2 AIMS OF THE PROJECT

The overall aim of the project was to provide new knowledge on a) how female voice and vocal folds are affected by endogenous and exogenous androgen exposure, and b) the consequences voice virilization may have on patients' daily lives, with the goal to improve medical treatment and care for women with CAH and female-to-male transsexual persons, trans men.

2.1 SPECIFIC AIMS

The specific aims were to:

- describe voice characteristics such as fundamental frequency (F0) and voice quality in adult women with CAH as compared with control subjects. (Study I)

- examine if women with CAH have subjective voice problems. The following questions were adressed: Do women with CAH experience voice problems in daily life? Are the voice problems caused by voice virilization? (Study II)

- investigate wether a virilized voice and subjective voice problems in women with CAH correlate with severity of the disease (genotype or phenotype) or androgen load, i.e. late diagnosis after first symptoms or poorly controlled medication, such as under-treatment with glucocorticoids. (Studies I and II)

- study if the area of the thyroarytenoid muscle, the length of the vocal folds and vocal tract length were different in women with virilized voices as compared with female and male controls and to correlate any such findings with F0 and voice sound pressure level (SPL). (Study III)

- examine longitudinal changes of the voice during testosterone treatment in trans men. The following questions were adressed: To what extent and for how long a period of time does voice F0 decreases? Is voice SPL affected by testosterone treatment? Are trans men satisfied with the voice changes? Is voice and communication therapy needed? Is there a relationship between voice F0 and voice satisfaction, and between F0 and androgen levels? (Study IV)

3 MATERIALS AND METHODS

3.1 PARTICIPANTS

All participants in studies I, II and III were recruited from a Swedish project studying medical and psychosocial health in women with CAH (Falhammar, et al., 2007a, 2007b; Frisen, et al., 2009; Hagenfeldt, et al., 2008; Nordenskjöld, et al., 2008), including 61 women with CAH and 61 age-matched female control subjects.

The participants in study IV were recruited from a total of 104 trans men who, during the years 2006-2014, were referred to the Department of Speech and Language Pathology, Karolinska University Hospital, after the diagnosis TS was confirmed by the psychiatric gender team at the Department of Psychiatry, Karolinska University Hospital.

3.1.1 Women with CAH and controls: Study I

Thirty-eight women with CAH, ranging in age between 18 and 63 years and 24 age-matched female controls (19 to 63 years) participated. All participants were examined at the Department of Speech and Language Pathology, Karolinska University Hospital in connection with the data collection for the larger follow-up study at the hospital. The women with CAH comprised 15 patients with the clinical sub-group (phenotype) SV, 10 with NC and 17 with SW including two different types of mutation (genotype): ten null and seven I2splice. All women with CAH were under good control regarding treatment with glucocorticoids at the time for data collection, except for two who had testosterone levels of 5.7 and 6.8 nmol/L respectively.

3.1.2 Women with CAH and controls: Study II

All 61 women with CAH and 61 age-matched controls participating in the national follow-up project were asked to participate in study II. Forty-two women with CAH and 43 controls (range 25 to 71 years) accepted (response rates 69 percent and 70 percent respectively). Of these participants, 25 women with CAH and 18 controls were also participants in study I. The number of women with CAH in each of the clinical sub-groups of CAH were 20 with SW, 18 with SV and 4 with NC, also representing four genotype groups: null (n = 10), I2splice (n = 12), I172N (n = 16) and V281L (n = 4). All women with CAH were under good control regarding treatment with glucocorticoids, except for the two presented in study I.

3.1.3 Women with CAH and controls: Study III

The participants were four women with CAH (range 26 to 40 years) among those with the most virilized voices from study I (3 SW and 1 NC) and four age- and height-matched controls, also recruited from study I. Four vocally healthy age-matched males and one female control were recruited among colleagues. All women with CAH were under good control regarding glucocorticoid treatment.

3.1.4 Female-to-male transsexual persons: Study IV

Fifty trans men (range 18 to 64 years) fulfilled the inclusion criteria, i.e. voice assessment had been carried out before start of testosterone treatment, and at least once more during treatment. The clinical routine for voice assessment was before start of testosterone treatment and after 3, 6, 12, 18, and 24 months. Since all trans men did not come to all visits or at the exact same time points, the recordings were divided into six groups with a time range in each group. A total of 211 recordings were included. Twenty-nine of the participants received intramuscular injections. Four received transdermal testosterone gel. Eight started with transdermal administration, which was then substituted with intramuscular injections. Nine participants were 18 years of age and had recently initiated testosterone treatment with gradual increment of intramuscular injections.

3.2 VOICE RECORDINGS

Digital audio recordings were made in a sound-treated booth at the Department of Speech and Language Pathology, Karolinska University Hospital following clinical routines. The voices were recorded using the computer programs Soundswell and Phog (Electronix Hitech, Täby, Sweden). An electret microphone (Sennheiser MKE-2, Sennheiser, Wennebostel, Germany) was mounted on a headset at a distance of 15 cm from the participant's mouth. The SPL values were corrected for a mouth-to-microphone distance of 30 cm. The recordings made in Phog provided phonetograms: two-dimensional graphs of the voice showing the range of F0 and SPL (Sulter, Wit, Schutte, & Miller, 1994). Details about the speech and voice tasks, analyses and measurements are presented in Table 4. For more specific information on the analyses, see sections 3.2.1, 3.2.2 and 3.2.3.

3.2.1 Recordings and analysis of habitual voice

The habitual speaking voice was assessed from recordings of reading a Swedish standard text aloud (40 seconds duration) and narration of a series of six pictures. These voice samples were recorded to provide a speech range profile (SRP) using Phog.

For acoustic analyses of the SRP, the analysis tools in Phog and Swell were used for the following variables: mean fundamental frequency (MF0), F0-mode, lowest frequency (Min F0), highest frequency (Max F0), in Hz, lowest SPL (Min SPL), highest SPL (Max SPL), equivalent continuous sound level (Leq) in dB, and total area in semitones times decibel (ST*dB).

Table 4. Speech and voice tasks, types of analyses, and measures in study I, III and IV.

Speech and voice tasks	Analysis	Measure	Study
SRP: reading	Acoustic	MF0	I
		Min F0	I
		Max F0	I
	Perceptual	Dark voice quality	I
SRP: reading and narrating	Acoustic	MF0	III, IV
		F0-mode	III, IV
		Min F0	III,
		Max F0	III,
		Min SPL	III
		Max SPL	III
		Leq	III, IV
		Area	III, IV
VRP: sustained vowels /a: /	Acoustic	Min F0	III, IV
		Max F0	III, IV
		Min SPL	III
		Max SPL	III, IV
		Area	III, IV

Note: SRP – speech range profile, VRP – voice range profile, MF0 – mean fundamental frequency, Min F0 – minimum F0, Max F0 – maximum F0, SPL – sound pressure level, Leq – equivalent continuous sound level.

3.2.2 Recordings and analysis of the physiological voice range

Recordings of the physiological voice range were assessed to provide a voice range profile (VRP), following the guidelines presented by Hallin et al. (2012). The participants performed a VRP standing facing the computer screen changing F0 and SPL on sustained phonations and glissandi using the vowel /a: /. The participants were prompted by the instructor. The task was to use the maximal variation of F0 and SPL without using strained voice or feeling any vocal pain and took approximately 20-30 minutes to complete.

For acoustic analyses of VRP the analysis tools in Phog were used for the following variables: lowest frequency (Min F0), highest frequency (Max F0), in Hz, lowest SPL (Min SPL), highest SPL (Max SPL) in dB and total area in semitones times decibel (ST*dB).

3.2.3 Perceptual analyses

Perceptual analyses were performed of 69 voice recordings (38 women with CAH, 24 controls and seven duplications) in study I. The duplicated recordings were added to the material for calculation of inter- and intra-judge reliability. Three trained listeners (speech and language pathologists) with several years of experience as voice clinicians performed the analyses. The Stockholm Voice Evaluation Approach, SVEA (Hammarberg, 2000) was used as a basis for the perceptual evaluation. Two of the authors (UN and MS) listened through the

material prior to the perceptual analyses, in order to choose appropriate parameters for the perceptual analyses as recommended by Hammarberg (2000). The following variables were selected for the perceptual analyses: Pitch, dark voice quality (timbre), vocal fry and instability. Dark voice timbre was chosen since the term was described in a previous study (Heinemann, 1974) and because the first author's clinical observations when meeting women with CAH was that a dark voice was a characteristic feature and different from low pitch. Ratings were made on 100 mm visual analogue scale (VAS) where 0 mm meant "not at all" and 100 mm meant "high degree of". Pitch was rated on a 200 mm VAS, where -100 corresponded to "low pitch", 0 to normal pitch and +100 to "high pitch". The recordings, including the duplicates, were listened to in randomized order. Before the listening test started, a training session was conducted for "calibration" with the three judges and the first author. They listened in consensus to three voices (not included in the material) and discussed the perceptual terms so that they agreed on the meaning, especially the difference between dark voice timbre and low pitch. Since *dark voice* is not a parameter used routinely in perceptual analyses or in the clinic, this was an important procedure. Only the results from the ratings of dark voice quality are presented in study I. Information about the ratings of the other variables are given in an unpublished thesis report (Nygren, 2008). The patients spoke with significantly lower pitch, but the ratings of vocal fry and instability were very low for both patients and controls, thus, statistical analyses could not be performed for these parameters.

3.3 SELF RATINGS

In study I, a questionnaire was used concerning smoking habits, profession and ratings of voice function based on four statements: "my voice is hoarse", "my voice is dark", "my voice is a problem in my daily life" and "I get tired in my throat when speaking" rated on 100 mm VAS (0 mm = "not at all" and 100 mm = "high degree of").

Voice Handicap Index (VHI) is a questionnaire for self-assessment of voice problems including 30 statements on voice-related aspects in daily life (Jacobson, et al., 1997). The statements can be grouped into three subscales: a physical, a functional and an emotional scale. Each subscale includes scores from 0 to 40, which makes a total score of 120, where a higher score indicates larger voice handicap. A validated translation of VHI in Swedish (Sw-VHI) was used in study II (Ohlsson & Dotevall, 2009). A total score > 20 indicates that an individual has a voice disorder according to Olsson et al. (2009). In study II, the total scores were divided into groups as described in Lundström et al. (2009): a score between 0 and 30 reflected no/mild voice handicap, between 31 and 60, moderate voice handicap and between 61 and 120, severe voice handicap. Supplementary questions with a focus on voice function related to voice virilization were added to Sw-VHI. Some questions were chosen from a rating form used for trans women, developed at La Trobe University, Australia (Dacakis, 2000). The questions covered issues such as: amount of voice use, satisfaction with voice

pitch, if the voice was dark, if the voice was perceived as male on the phone, and if the speaker got negative reactions to their voice.

In study IV, four statements about voice function and voice problems were rated The statements were: “I am perceived as male when speaking on the phone”; “I am satisfied with my voice”; “I am worried that my voice will reveal my native sex”; and “I get tired in my throat/voice or hoarse when speaking”. The rating scale changed slightly during the years of data collection. First, a 7-point interval scale was used where 1 indicated “never” and 7 “always”. In the end of data collection, a 5-point scale was used where 0 indicated “never”, 1 “almost never”, 2 “sometimes”, 3 “almost always” and 4 “always”. The statement “I am satisfied with my voice” which was used on the 7-point scale was formulated on the 5 point-scale to “I am not satisfied with my voice“. In order to compare the results the answers were converted. Ratings from the 5-point scale were also converted to fit the 7-point scale: 0 was converted to 1 on the 7-point scale, 1 to 2.5, 2 to 4, 3 to 5.5 and 4 to 7.

3.4 MAGNETIC RESONANCE IMAGING

In study III magnetic resonance imaging (MRI) was performed using a 1.4 T MR scanner (Avanto, Siemens AG, Erlangen, Germany). Measurements of laryngeal anatomical details have been performed in previous studies (Kazemirad, Bakhshaee, Mongeau, & Kost, 2014; Sakai, Gamsu, Dillon, Lynch, & Gilbert, 1990) as well as measurements of VTL (Fitch & Giedd, 1999). Vocal fold length or the cross-sectional area of the TA muscle have not previously been measured in women with virilized voices. The method and procedure for using MRI measurements of the vocal folds TA muscle was developed in a series of pilot sessions.

The participants were asked to breathe quietly and avoid swallowing during imaging acquisition (nearly 2 minutes) in order to reduce artefacts due to movement of the larynx and the vocal folds. Images in transverse oblique orientation were used for measurements of the vocal fold length (membranous part): i.e. from the vocal process of the arytenoid cartilage to the anterior commissure. Measurements of the cross-sectional area of the TA muscle were made from oblique coronal images, perpendicular to the left vocal fold. A method described by Roers et al. (2009) was used for measurements of the VTL using images in sagittal orientation.

A routine PACS work station (Sectra-Imtec AB, Linköping, Sweden) was used for the measurements, which were performed by two observers. One of them was blinded to the material. The measurements were performed twice, with an interval of six or twelve months. Intra- and inter-observer reliabilities are presented in the result section 4.3.

3.5 INDIRECT LARYNGOSCOPY AND ANALYSES

In study III the participants were assessed with indirect laryngoscopy with videostroboscopy of the vocal folds according to clinical routines at the Department of Otorhinolaryngology, Karolinska University Hospital. For analyses, a protocol was developed for ratings of vocal folds movement, vocal fold closure pattern, presence of edema using a rating scale: no, little, moderate and heavy edema (while the participants phonated during inhalation), and a final judgment concerning if the vocal fold status was normal or not normal. Two phoniatricians performed the analyses blindly and independently. Analysis of the ratings showed total agreement between the two judges.

3.6 ENDOCRINE AND MEDICAL DATA FROM MEDICAL JOURNALS

Data on which of the three clinical sub-groups of CAH (phenotype) each woman had was collected from medical journals (studies I, II and III), as well as data on type of mutation (genotype) (study II). In studies I, II and III, information on medical history was collected, i.e. age at diagnosis, period of time from first symptom to diagnosis and the period of time during which the women with CAH had been under-treated with glucocorticoids. Late diagnosis after first symptoms was defined as > 2 years of symptoms before treatment was initiated and under-treatment with glucocorticoids as > 12 months with high circulating androgen levels. Data on height, testosterone levels, body mass index, waist-hip ratio were also collected, as well as data on total bone mineral density (BMD), fat and lean mass measured with dual energy X-ray absorptiometry (DXA).

In study IV, information on start of testosterone treatment, type of treatment regimen, doses and frequencies were collected. Androgen levels and related assessments from blood samples were collected, i.e. S-testosterone (T), S-luteinizing hormone (LH), S-sex hormone binding globulin (SHBG), B-hemoglobin (Hb) and B-hematocrit (EVF).

3.7 STATISTICAL ANALYSES

All statistical tests used in the four studies are presented in Table 5. Descriptive statistics presented as mean, median, standard deviation, range from minimum to maximum values, and interquartile range (P25-P75), were calculated in all four studies. The Shapiro-Wilk test and Levene's test were used to check for the assumptions to use analyses of variance (ANOVA) and t-test. A significant effect of the within-group factor in a linear mixed model was further investigated through pair-wise comparisons between time points and the p-values were then corrected using the Bonferroni procedure. Power analysis was made for calculation of suitable sample size in study II.A p-value < 0.05 was considered to be statistically significant.

Table 5. Presentation of statistical tests in the four studies.

Statistical test	Study I	Study II	Study III	Study IV
Unpaired t-test	x	x		
Mann-Whitney U-test	x	x		
Paired t-test	x			x
Sign test	x	x		
Two group chi-squared test		x		
Chi-squared test		x		
Fischer's exact test	x	x		
One-way ANOVA	x			
Kruskal-Wallis ANOVA by Ranks	x			
Linear mixed model				x
Two-way repeated measures ANOVA	x		x	
Scheffe's post hoc test			x	
Spearman's rank-order correlation	x	x		x
Pearson's correlation coefficients			x	
Intra class correlation ICC	x		x	

3.8 ETHICAL CONSIDERATIONS

The Regional Ethics board at Karolinska Institutet, Stockholm approved the studies in the doctoral project: Dnr 02713 (study I), Dnr 2010/1833-32 (study II), Dnr 2009/1338-32 (study III), and Dnr 2010/1829-31/3 (study IV).

4 RESULTS

4.1 STUDY 1

Voice recordings were performed, on which acoustic analyses of F0 and perceptual analyses of dark voice quality, were carried out in adult women with CAH and age-matched controls. The participants also rated voice symptoms. Endocrine data were obtained from medical journals. The results showed that women with CAH had significantly lower MF0, lower Min F0 and lower Max F0 in habitual voice as compared with age-matched control subjects. The result from calculations of the intrajudge reliabilities using intraclass correlation (ICC) regarding dark voice quality indicated very good reliability (varying between r = 0.82 to r = 0.98) and the inter-judge reliability indicated good reliability (r = 0.71), according to guidelines by Landis & Kock (1977). Women with CAH were perceptually rated to have significantly darker voice quality than control subjects. In self-ratings, the women with CAH had significantly higher values for the statement “my voice is a problem in my daily life” as compared with controls. The 38 women with CAH were divided into two groups, one group with “dark voices” and one group with “normal voices”. The “dark voice“ group consisted of thirteen women with CAH who had lower MF0 values compared with reference values for vocally healthy women (Pegoraro Krook, 1988) and were perceptually rated to have dark voice quality. The women in the “dark voice” group had significantly higher BMI and lean body mass compared with their age-matched controls, which indicated long periods of high levels of androgens. After review of the medical history and since voice virilization was not correlated with severity of the disease, it was concluded that voice virilization in women with CAH was associated with a late diagnosis after the first symptom or with noncompliance/under- treatment for a longer period of time with glucocorticoids postnatally.

4.2 STUDY 2

The Sw-VHI and questions on voice function related to voice virilization were answered by the participants and information on endocrine data from medical journals in women with CAH were compared with corresponding data for age-matched controls. Women with CAH scored significantly higher on the Sw-VHI when the ratings were subdivided into the groups: no/mild, moderate and severe voice handicap. In comparison with age-matched control subjects, a larger proportion of patients rated moderate voice handicap, and severe voice handicap was only found among the women with CAH. They also rated significantly higher values regarding the statements “my voice is dark” and “I am perceived as male on the phone”. Voice problems that were related to voice virilization were found in 7 percent of the women with CAH. There were significant negative correlations between the ratings for “dark voice” and “satisfaction with voice” in women with CAH. A significant association was found between high ratings of dark voice and patients who had been under-treated with glucocorticoids for long periods and had thus had high level of androgens.

4.3 STUDY 3

MRI of the vocal folds, voice recordings and acoustic analyses were assessed in women with CAH and virilized voices, age- and length-matched female controls and age-matched male controls. Measurements of vocal fold length, maximum mid-membranous cross-sectional area of the TA muscle, and VTL were performed by two observers using MR images. Intra- and interobserver reliability was calculated using ICC, and interpretation of the results was made according to guidelines by Landis & Kock (1977). A very good intra-observer reliability was found for both observers regarding all measurements (varying between r = 0.846 and r = 0.997) and also a very good interobserver reliability (varying between r = 0.839 and r = 0.949). Women with CAH had a significantly larger cross-sectional TA muscle area as compared with female controls and smaller compared with male controls. We also found a correlation between larger TA area and lower F0 values extracted from SRPs and VRPs. All F0 values of the patients extracted from the SRPs were significantly lower than for female controls and higher than for male controls. Analyses of VRP revealed that women with CAH had significantly lower Max F0 and smaller area as compared with female controls, and significantly higher Min F0 and smaller area as compared with male controls. SPL values did not differ, nor were there any differences regarding vocal fold length or VTL from the MRI analyses. The laryngoscopic analyses showed normal vocal fold status in all participants, except for two women with CAH where a small degree of edematous mucosa bilaterally was revealed.

4.4 STUDY 4

Voice recordings of habitual voice and physiological voice range, self-ratings of voice function and voice problems were assessed, and endocrine data from medical journals were collected in trans men before and during testosterone treatment. The F0 in trans men decreased significantly during the first 3 months of testosterone treatment regarding MF0 and F0-mode of the habitual voice, and continued to decrease significantly between 3 and 6 months and also between 6 and 12 months. MF0 was 192 Hz before treatment, 155 Hz after 3 months and decreased to 125 Hz after 12 months. After 12 months of treatment there was no further significant lowering of frequency values. The participants' MF0 before start of testosterone treatment did not affect the final values of MF0. SPL values did not change significantly during testosterone treatment. Regarding SRP area and VRP areas, no significant differences were demonstrated. A significant increase was found for testosterone, EVF and Hb levels and a significant decrease in SHBG and LH levels. No or weak correlations were found between these levels and F0 values. Self-ratings regarding voice satisfaction significantly increased up to 6 months of treatment and lower F0 values correlated with greater satisfaction with the voice. Voice and communication therapy was needed for 24 percent of the trans men, due to problems with vocal fatigue, vocal instability, strained voice quality, insufficient lowering of pitch, problems to project the voice, and problems with the voice sounding too young.

5 DISCUSSION

In this project, the voice and the vocal folds have been investigated in women with CAH, who are at risk of undesired voice virilization, and in trans men, who are treated with testosterone with virilization of voice as a desired outcome.

The main findings were that: a) women with CAH had lower voice F0 compared with female controls on a group level, b) voice virilization was due to long periods of high levels of androgens, c) women with CAH may develop subjective voice problems, d) a larger area of the TA muscle may be the anatomical explanation for a virilized voice, e) the voice F0 in trans men decreased during the first three months of testosterone treatment and continued to decrease up to 12 months, and f) a quarter of the trans men needed voice and communication therapy.

5.1 METHODOLOGICAL ISSUES

5.1.1 Participants

Study I, with a total of 38 women with CAH, study II, with 42, are the hitherto the largest group studies, and the only ones with age-matched controls, focusing on virilized voices in this small patient group. It was possible to perform statistical calculation for matched pairs concerning some of the variables in study I, but not all, since age-matched controls could not be recruited for every patient due to logistical issues. In study III, the intention was to recruit the seven women with the most virilized voices from study I. Three of them declined to participate due to lack of time or because they had already been part of other research projects. Despite the fact that there was a small sample size in this study, the results showed significant differences between cross-sectional area for women with virilized voices compared with both female and male controls, with no result overlap. A larger sample would have been desirable in order to strengthen the results.

Study IV is the first longitudinal study of a larger group of trans men during testosterone treatment. The goal was to assess the voice regularly before start of testosterone treatment and after 3, 6, 12, 18 and 24 months of treatment. Because of the great variety of numbers of voice assessments and also variety regarding the time-point for each visit a linear mixed model was the most appropriate statistical method for analyzing the voice change from 3 to 24 months.

5.1.2 Acoustic analyses

When recording VRP, it is important to ensure that a maximum voice range profile has been reached. Therefore, a VRP usually requires a great amount of prompting from the instructor and multiple attempts to reach Max and Min F0 and Max and Min SPL. Repeated trials are

especially important regarding phonation with soft voice to reach the lowest contour in the VRP. Thus, a recording of a VRP takes time; our experience is 20 to 30 minutes, in agreement with Hallin et al. (2012). Results from a VRP which has not been properly done are not reliable. Therefore, it is important to follow guidelines such as those described in Hallin et al. (2012) and Sanchez et al. (2014). Besides the VRP measurements, a recording of a VRP also gives information about voice function such as register shifts and instability, which are often seen in women with CAH with virilized voices and trans men during testosterone treatment. Some trans men can be reluctant to produce high pitch phonation, which may have influenced the results of Max F0 and thus of the VRP area. This is important to consider when recording trans men, so that they feel comfortable with the recording. Sometimes it may not be appropriate to force them to perform a VRP.

5.1.3 Perceptual and subjective ratings of dark voice

It should be noted that the term "dark voice" was used and defined differently in studies I and II. In the perceptual analysis in study I the term dark voice was used to describe the voice quality specific for a virilized voice. The listeners in study I rated both dark voice quality and the pitch. This was done in order to try to perceptually discriminate low voice pitch from dark voice quality. The women with CAH and controls in studies I and II performed subjective ratings of the statement "my voice is dark", but probably did not differentiate between low pitch and dark voice quality. Furthermore, in study I the women with CAH were divided into two groups: those with "dark voices" and those with "normal voices". The dark voice group was defined as women with CAH who had low MF0 compared with reference data for vocally healthy women (Pegoraro Krook, 1988) in combination with dark voice quality as perceptually rated. The group of women with CAH in study II was also divided into two groups: dark voice and normal voice. This division was based on their subjective ratings of dark voice on visual analogue scales. The ratings were analyzed using cluster analyses, verified through generalized cluster analysis.

5.1.4 MRI

Measurement of the vocal folds using MRI is difficult since the vocal folds slightly move during breathing with the risk of motion artefacts which may destroy the image. It was a challenge to train the participants to keep the vocal folds as still as possible while breathing and especially to avoid swallowing for 2 minutes, since the mean unstimulated saliva flow rate is 0.55 ml/minute (Lagerlöf & Dawes, 1985). Cotton swabs were used to absorb saliva. We performed several pilot MR recordings to evaluate which sequence to use to obtain the images with minimal artefacts, sufficient signal to noise ratio, optimal soft tissue visualization and reasonable imaging time. The final procedure for imaging of the cross-sectional vocal fold images had a fairly long imaging time of nearly 2 minutes. Due to the long imaging time, one data point for each subject had to be excluded because of motion artefacts making it unreliable for analysis. The measurements of vocal fold length, VTL and cross-sectional area of the TA muscle from the images were performed by two observers, who performed measurements of the complete material twice. The measurements showed very good intra-

and inter-observer reliability. One of the observers made the measurements blinded; this was not possible to arrange for the other observer (first author, who conducted the study). The possibility that this observer could be biased was considered. Since the analyses of the inter-observer reliability showed very high ICC-values, the risk for bias was considered minimal. Thus, the results were presented as means of the four measurements.

5.2 F0 IN WOMEN WITH CAH AND TRANS MEN

Our results from studies I and III, that women with CAH had lower F0 compared with female controls, support earlier findings from case studies (Fürst-Recktenwald, et al., 2000; Heinemann, 1974; Tsuji, et al., 2003). Low F0 values in women with virilized voices have also been reported in case studies of women who were treated with medication containing androgens (Baker, 1999; Boothroyd & Lepre, 1990; Damsté, 1964, 1967; Shepperd, 1966) and in trans men during testosterone treatment (Cosyns, et al., 2014; Damrose, 2009; Scheidt, et al., 2004; Söderpalm, et al., 2004; Van Borsel, et al., 2000). F0 and SPL were investigated in study III from SRPs of habitual voice and VRPs. Results showed that women with CAH and virilized voices had lower F0 values compared with female controls and higher compared with male controls. Smaller VRP areas were found in the women with CAH and the shapes of the areas demonstrated register breaks between modal and falsetto registers and also loss of high frequencies. VRPs have not been performed previously in women with virilized voices, but symptoms such as instability and loss of frequencies have been reported previously (Baker, 1999; Damsté, 1967; Gerritsma, et al., 1994).

Mean F0 decreased significantly in the trans men group during the first 3 months of androgen treatment according to the results in study IV. This is in accordance with previous longitudinal case studies of 3 trans men, which reported that the largest change in F0 was between the third and the fourth month of androgen treatment (Damrose, 2009; Van Borsel, et al., 2000). Our results showed that MF0 was in congruence with reference data for vocally healthy Swedish males after 12 months of androgen treatment (Hallin, et al., 2012). The results were also in accordance with those from a controlled cross-sectional study by Cosyns and colleagues (2014) of 38 trans men who had been treated with testosterone for a long time. This study did not find any significant differences between trans men and male controls regarding median F0 and F0 variation. In contrast to the findings reported in Cosyns et al. (2014), we could not find any correlation between F0 and hematocrit (a surrogate marker for androgen exposure). This lack of correlation may be due to a difference in dose-response relation between testosterone levels and erythropoiesis, and between testosterone and changes in F0. It is known that erythropoiesis responds to increasing levels of androgens beyond the normal upper reference levels for testosterone, while a series of other responses to androgens become saturated at a certain threshold level beyond which there is no further increase. It seems likely that the changes of the voice and F0 are regulated in the same way.

5.3 VOICE QUALITY IN WOMEN WITH CAH

As found in study I, the women with CAH with virilized voices sounded darker in terms of voice quality. Since VTL was measured in study III and we found no differences between women with CAH and female controls, it is likely that the darker voice quality was not associated with lower formant frequencies, but rather with the voice source. It may be hypothesized that vibrations produced with virilized vocal folds, with a larger cross-sectional area of the TA muscle, are characterized by a longer closed phase and a shorter closing time, generating stronger harmonics in the higher partials of the spectrum as compared with non-virilized vocal folds at the same pitch. Perceptual ratings of dark voice quality were assessed in study I. It was found to be possible to discriminate dark voice quality from ratings of voice pitch. Therefore, we suggest that the term dark voice is included in perceptual analyses of virilized voices. It is also important to investigate voice source parameters from flow glottograms and acoustic spectra, to define the term dark voice and to fully describe the characteristics of virilized voices.

5.4 ANATOMICAL EXPLANATIONS FOR VOICE VIRILIZATION

To our knowledge, measurements of the vocal folds in women with virilized voices have not been performed with MRI previously. In our study based on four women with CAH with virilized voices the main finding was that they had a larger cross-sectional TA area than female controls and smaller than males. A larger cross-sectional area correlated with lower F0. No differences were found regarding the vocal fold length or VTL between the women and the female controls. VTL had a tendency to be shorter in women with CAH, which is consistent with the fact that these women have a shorter body length (Falhammar, et al., 2007b), which in turn is associated with shorter vocal tract (Fitch & Giedd, 1999). Our results from study III indicate that the underlying anatomical explanation for a virilized voice is the larger cross-sectional area of the TA muscle. This finding supports Bauer's (1968), who investigated an excised larynx from a woman with a virilized voice and compared that with the larynxes of six males and six females. Our findings also support findings from two studies investigating changes of the larynx in female mice (Talaat, et al., 1987) and rats (Amer, et al., 2011) after androgen treatment. An overall thickening of the vocal folds, vertically and horizontally, probably affects the closed phase during the vocal fold vibrations, which becomes longer compared with the closed phase in thin vocal folds. In thicker vocal folds, the adduction starts with the lower part of the vocal folds and continues with the upper part, causing the closed phase to become longer than in thin vocal folds. Titze (2011) described the complicated association between vocal fold mass and F0 and claimed that an increase only of the vertical thickness of the vocal folds does not lower F0, whereas an increase of the lateral depth of the vocal folds will lower F0. In study III, it seems likely that the larger cross-sectional TA area was due to both an increase of vertical thickness and an increase of the lateral depth, since a larger TA area correlated with lower F0. In our measurements of the TA

area, depth and vertical thickness were not analyzed separately, which would be interesting to do in future studies.

5.5 VOICE PROBLEMS AND VOICE TREATMENT IN WOMEN WITH CAH

Studies I and II investigated subjectively rated voice problems in women with CAH. Tsuji et al. (2003) described that voice virilization in a young girl with CAH was emotionally difficult. Our results showed that voice problems due to voice virilization may occur in some, but not all, women with CAH. A virilized voice correlated with less voice satisfaction and seven percent had voice problems related to voice virilization. Some patients did not find a virilized voice, or to be perceived as male on the phone, to be a problem. Women with CAH seldom seek help for voice problems, which was not the case for women who developed virilized voices due to exogenous androgen administration (Baker, 1999; Damsté, 1964, 1967; Shepperd, 1966). The reasons why women with CAH do not seek help are not investigated, but one reason may be that a virilized voice is not considered to be a problem compared with the severe consequences CAH may have in daily life (Falhammar, et al., 2007a, 2007b; Frisen, et al., 2009; Hagenfeldt, et al., 2008; Nordenskjöld, et al., 2008). Another explanation could be that women with CAH have a much higher tendency to choose a male-dominant occupation and thus a virilized voice may be of somewhat smaller concern (Frisen, et al., 2009). One could assume that another reason is lack of knowledge about voice virilization among medical staff and patients, meaning that the question of voice problems may not be discussed.

Based on the results from studies I and II it was suggested that the women who have voice problems should be referred for voice assessment and eventual voice therapy. Therapy programs need to be developed for women with virilized voices including exercises to changes the voice source characteristics. However, vocal exercises and the therapy programs for trans women may also be relevant with the goals to raise F0 in habitual speech and decrease the degree of dark voice quality (Carew, Dacakis, & Oates, 2007; Dacakis, 2000). Although it is not likely that the formant frequencies are lower in women with CAH, based on our VTL results, it may be useful to increase the vowel formant frequencies by changing the dimensions in the vocal tract to make the voice sound brighter. Carew and colleagues (2007) described techniques for trans women with the goal to increase the vowel formant frequencies by spreading the lips (first formant frequency) and fronting the tongue (second formant frequency) during speech. An important success factor is that the patient must be highly motivated to practice both during therapy sessions and also at home for carry-over. Another option for women who have problems with virilized voices and low F0 could be pitch-raising surgery as sometimes performed for trans women (Kanagalingam, et al., 2005; Isshiki, et al., 1974). Such surgery was performed in one girl with CAH who had a virilized voice (Tsuji, et al., 2003), and has also been described to be a method for other women with virilized voices (Tanabe, Haji, Honjo, & Isshiki, 1985). It is important to be aware of the potential side effects after surgery that have been reported in studies of trans women, such as

weak voice, reduced frequency range, and vocal fatigue and that voice therapy should be given after surgery (Hertegård, et al., 2013; Mastronikolis et al., 2013; Wagner et al., 2003). There is a need of long- term follow-up studies after pitch raising surgery.

The questionnaire used in study II comprised questions with a focus on voice function related to voice virilization. The questions, relevant to use among women with CAH, were chosen from a questionnaire developed for trans women (Dacakis, 2000). This questionnaire could be used for women who develop a virilized voice due to either endogenous production or exogenous administration of androgens. A questionnaire of this kind needs to be validated for future use in Speech and Language clinics, as this is important for evaluation of voice therapy results.

5.6 VOICE PROBLEMS AND VOICE TREATMENT FOR TRANS MEN

For most trans men, it is important that the voice is congruent with their identity and with the physical changes of the body during testosterone treatment. A problem for some trans men can be that they are perceived by others to sound younger than their age. This could depend on vocal instability and register shifts during running speech, as can be heard in the voices of boys during puberty. The reason could also be associated with their short VTL, with the effect that the vowel formant frequencies are higher than in native male speakers.

Most trans men do not need voice therapy, since the testosterone has a good effect causing a voice virilization. However, a quarter of the trans men in study IV did require voice therapy. Based on this result, it is suggested that trans men be referred for voice assessment during testosterone treatment, in order to detect any voice problems, which is supported by findings from previous studies (Scheidt, et al., 2004; Söderpalm, et al., 2004; Van Borsel, et al., 2000). Van Borsel et al. (2000) conducted a sample survey including 16 trans men. Eight of them had hoped for a faster and/or more distinct voice change, two liked to have a “heavier” voice, and one had problems due to strained voice quality. Another study by Scheidt et al. (2004) examined subjective voice function among other things, in 14 trans men. The results showed that most of them found it important to be recognized as male by their voice and 64 percent desired support regarding their voice problems such as reduced vocal power. Taken together, results from those earlier studies and from study IV, referral for voice therapy should be offered to this patient group.

Therapy programs need to be developed for trans men including exercises to decrease vocal instability, vocal fatigue, and strained voice quality. Exercises to lower the larynx in order to decrease the formant frequencies may also be relevant. Interesting experiments were made by Coleman (1971, 1976) to investigate how listeners perceived gender in relation to F0 and formant frequencies. The tests were conducted with the use of an electrolarynx. In the first study a panel listened to recordings when female and male speakers used an electrolarynx with F0 of 85 Hz (Coleman, 1971). The panel identified the speaker’s gender correctly in 88 percent of cases and was more confident rating gender for males with lower formant

frequencies than for females. It was concluded that resonance is an important cue for identification of a speaker's gender. In the next listening test, male and female speakers used an electrolarynx, now with F0 at both 120 Hz and 240 Hz (Coleman, 1976). The results showed that the combination of male F0 and female vocal tract resonances was identified as male. The combination of male vocal tract resonances and female F0 values was a weak indicator to identify gender as female. Based on these results it was concluded that formant frequencies, not only F0, are important for the perception of an individual's gender. This means that there is a greater chance that trans men, who have female vocal tract resonances and male F0 values, are identified by others as male speakers, than that trans women, with male vocal tract resonances and high female F0 values, are identified as female speakers.

There is a need for a questionnaire developed for trans men, capturing voice function and voice problems relevant for this patient group, as well as questions about voice and identity (Azul, 2014; Hansbury, 2005). Questions used for trans women are not necessarily relevant for trans men. It seems important to develop and validate a questionnaire for trans men in the same way as the Transsexual Voice Questionnaire TVQ^{MtF} for trans women (Dacakis, Davies, Oates, Douglas, & Johnston, 2013). Such a questionnaire can be of value for evaluation of voice therapy outcomes.

6 CONCLUSIONS AND CLINICAL IMPLICATIONS

Women with CAH spoke with significantly lower MF0 and had darker voice quality than vocally healthy controls. They were at risk for developing a virilized voice due to late diagnosis or suboptimal treatment with glucocorticoids and not due to severity of the disease. It is important to inform both medical staff and patients that voice virilization may occur in women with CAH. Physicians caring for this patient group need to acknowledge the risk for altered voice characteristics and avoid long periods of high androgen influence, to prevent irreversible voice virilization. (I)

Seven percent of the women with CAH had subjective voice problems associated with voice virilization and a virilized voice correlated with less voice satisfaction. The virilized voice was associated with long periods of under-treatment with glucocorticoids and higher bone mineral density, confirming the results and conclusions from study I. Based on the results, it is recommended that patients who experience voice problems are referred for voice assessment and eventually voice treatment. (II)

A larger cross-sectional area of the thyroarythenoid muscle in the vocal folds was found in women with CAH and virilized voices as compared with vocally healthy female controls, while the area was smaller than in male controls. This result may be the anatomical explanation for a virilized voice in women, suggesting presence of androgen receptors in the vocal folds. Those findings need to be confirmed in large studies. A possible method using MRI for measurements of the vocal folds was presented. (III)

In **trans men**, a significant lowering of MF0 was found after 3 months of testosterone treatment, after 6 months, and after 12 months, when group data was congruent with MF0 reference data for Swedish adult males. Lower MF0 correlated with greater voice satisfaction. A quarter of the trans men had received voice therapy for problems associated with testosterone treatment such as vocal instability, vocal fatigue, and insufficient lowering of voice pitch. It is important to offer trans men voice assessment to detect the potentially large subgroup who need voice therapy during testosterone treatment. (IV)

7 FUTURE STUDIES

The result of study III, that women with CAH had a significant larger cross-sectional TA muscle area as compared with female controls and smaller compared with male controls, needs to be verified in larger groups of women with virilized voices. MRI studies of the vocal fold muscle TA, also need to be conducted using faster gradient echo, in order to shorten the imaging time. Furthermore, MRI studies of the laryngeal structures would be of interest to perform in trans men before and after 12 months of testosterone treatment, when the male voice F0 has been established.

More detailed analyses of voice quality parameters would be of interest to perform both in women with virilized voices and in trans men such as voice source parameters from flow glottograms and acoustic spectra to fully describe the characteristics of virilized voices.

Listening tests should be performed on order to investigate how trans men are perceived by others regarding gender and age, as well as for more detailed perceptual analyses of voice quality in virilized voices.

Validated questionnaires are needed for evaluating subjective voice problems in trans men and in women with virilized voices. It is also important to further investigate how trans men perceive their own voice in relation to their identity.

Voice therapy programs for trans men and women with virilized voices need to be developed. Studies evaluating voice therapy intervention for those patient groups are highly necessary.

8 ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to all the individuals who participated in the studies of this doctoral project – without you this thesis would not exist.

I gratefully wish to acknowledge the financial support making it possible for me to complete this research project, visit researchers abroad and participate in national and international conferences, from: the Swedish Research Council, the Aina Börjeson Foundation for Speech Language Pathology Research and Treatment, Röstfonden, the Stockholm City Council, the Department of Clinical Science, Intervention and Technology at Karolinska Institutet, Capio Travel Grant, HRH Crown Princess Lovisa Foundation, Foundation Frimurare Barnhuset Stockholm, Gothenburgs Medical Society, the Samariten Foundation, Karolinska Institutet, and funding provided by Centre for Andrology and Sexual medicine (CASM).

Throughout the years of my doctoral project there are many persons who have helped and supported me in many ways. In particular I want to thank:

Maria Södersten, my main supervisor. You were the first SLP I met, my supervisor as a SLP student and you have guided me in my professional life and through my doctoral studies. Thank you for your never-failing support and professional supervision in my progress, for always knowing what to say to make me believe in my ability, for always being there, for your inspiring way of sharing your knowledge, and for our constructive discussions in Nacka, Budapest, Prague, Bangkok, and Midsummer's Eve in Oslo to mention a few. I have had so much fun together with you during many late evenings working with the project, always filled with many laughs.

Agneta Nordenskjöld, my co-supervisor, for introducing me to the project on women with CAH, your fantastic support and inspiring way to include me and making me dare to present my research at large conferences and meet people with different professions, and for being so enthusiastic.

Stefan Arver, my co-supervisor, for introducing me to the exciting field of endocrinology, for great support and teaching, constructive discussions, for being so encouraging during the project, and for always taking time when I have desperately needed your help.

I will miss our supervising meetings!

Kerstin Hagenfeldt, my co-author, for acting as an extra supervisor during the work with the studies on women with CAH. Thanks also for believing in me to become a doctoral student.

Ewa Vanhoenacker Söderpalm, my external mentor, for reflective talks and discussions about the process and transgender issues, at many different restaurants in Stockholm museums.

Bengt Isberg, my co-author, for helping me understand a little about MRI and for invaluable collaboration which was time consuming when developing the procedure and collecting data for study III.

Stellan Hertegård, my co-author, for constructive collaboration during the process of developing the procedure for MRI analyses, for performing the measurements and for valuable comments on the manuscript.

My co-authors *Henrik Falhammar* for sharing your knowledge in statistical analyses and valuable support and comments during the manuscript writing, and *Marja Thorén* and *Helena Filipsson Nyström,* for valuable help with the studies on women with CAH.

My recent employer and former nearest voice colleague *Elisabet Lundström,* who inspired and pushed me to start with further academic studies and attend the course "vetenskaplig metodik och *magisteruppsats i logopedi*", and my former employers *Christina Blom* and *Jonas Karling. Jonas*: you always believed in me and you made it possible for me to start collecting data for study I. Thank you all for your support during my doctoral studies.

Anette Lohmander, head of the Division of Speech and Language Pathology at Karolinska Institutet, for your support and valuable feedback, and for your way of making individuals around you feel that their presence and contributions at meetings, seminars, and retreats are important.

Britta Hammarberg, for support, sharing of knowledge and for helping me understand articles written in the German language.

Eva B Holmberg, for sharing the interest in transgender and voice, which has led to fruitful discussions during the process, and thanks also for revising my English including constructive comments on the manuscripts.

Elisabeth Berg, for help and advice regarding statistical analyses.

Elisabet Borg, for valuable discussions on rating scales and questionnaires.

The staff at Medicinsk Röntgen at Läkarhuset Odenplan, for being so obliging and helpful, especially *Mansour Haghgou* for making the MRI examination and for good collaboration.

Gunnar Björck and *Per-Åke Lindestad* for strong expertise in laryngoscopic investigations and analyses. Thank you *Gunnar* also for recommending me to perform the voice recordings of the women with CAH and for enjoyable collaboration at our "Fallkonferenser" through the years.

Eva Borell and *Anna Starbäck* for your "good ears" in perceptual analyses.

John Van Borsel for sharing knowledge and expertise during a study visit.

Cecilia Dhejne for valuable comments on manuscripts and fruitful discussions about terminology and for being a very pleasant companion at conferences and panel discussions.

The members of the Gender team at Karolinska University Hospital for valuable and instructive meetings.

The Division of Speech and Language Pathology at Karolinska Institutet. Special thanks to *Christina Hedestedt* for all kinds of out-of-sight help concerning all types of administration and for always being there, *Hans Larsson* for support regarding analyses of images and technical issues, and for being such a nice travel companion at conferences, especially your calm manner during air turbulence sitting in an airplane, *Svante Granqvist*, my new room mate, for support in acoustical issues, *Per Östberg* for advice regarding statistical analyses and *Annika Sääf-Rothoff* for your support.

The Department of Clinical Science, Intervention and Technology at Karolinska Institutet. Special thanks to *Agneta Wittlock* and *Annika Aspnor* for help with the administration and the computer.

The staff at Karolinska Institutet Library. Special thanks to *Klas Moberg* for invaluable support regarding EndNote.

Janet and *Linnea Holmén* for revision of my English.

Robert Liljebäck, and *Lucinda Lindvall* for help with voice recordings.

My dearest doctoral fellows, especially *Kerstin Johansson* for never-failing support with "stort och smått" i.e. valuable comments on manuscripts and how to create tables in Microsoft Office Word, and last but not least for always being there, and *Päivikki Aarne* for our talks and sharing thoughts during the process of becoming a "researcher" and both of you for our invaluable "Hammam" visits. Furthermore, *Kicki Klintö* for constructive help and support with writing of the "Kappa" at the end. Thanks also to the rest of the members of the doctoral research program HÖST at KI, we had fruitful research discussions and a delightful time.

All former and present colleagues – SLPs and administrative staff – at the Speech and Language Pathology Department at the Karolinska University Hospital. I am especially grateful to my nearest and dearest colleagues for unfailing support, interesting discussions and for having such fun over the years, to mention a few: *Naima el Ghalbzouri, Victoria Kelly, Karin Wier, Anna Lundblad, Liisi Raud Westberg, Jill Nyberg, Maria Lundberg, Karin Nyberg, Catarina Ingebro* and especially *Therese Engström* for coming up with the idea of using cotton swabs.

Thank you all!

There are several friends and relatives outside my research and clinical work who deserve acknowledgement for moral support, helpfulness and for putting up with me during these past years.

My friends from growing up in Bromma, some of you since we were six years old, class-mates from “Adolf Fredriks musikklasser”, and friends who are in Södermalm in winter time and on Blidö in summer time. Thank you for always being there.

My close family: my mother, father and sisters with their families, for being encouraging, for being such wonderful and loving persons, and for always showing such interest in everything I do. Thank you for our invaluable time on “Blidö, ön i solen”. I love to be there with you!

And last, but not least, because this thesis would never have been written without these three persons:

Harald, my beloved husband, my best friend and my most important coach. Thank you for always telling me that everything is gonna be alright, for cooking the best meals, always looking at the bright side of life, and for being so fun to be with.

My darling daughter and son. *Kajsa*, for wanting to talk with me about everything (I think), for asking me how my day was, telling me I am good at what I am doing and letting me practice choir singing with you. *Karl*, for being so understanding and cool, for playing the guitar and singing to me, our talks over a cup of tea, and for your inspiring laughs.

You three make my life worth living!

9 REFERENCES

Adler, R. K., Constansis, A. N., & Van Borsel, J. (2012). Female-to-male transgender/transsexual consideration. In R. K. Adler, S. Hirsch & M. Mordaunt (Eds.), *Voice and communication therapy for the transgender/transsexual client : a comprehensive clinical guide*

(pp. 153-185). San Diego, CA: Plural Pub.

Amer, H. E., Asker, S. A., & Mazroa, S. A. (2011). Structural changes and immunohistochemical localisation of epidermal growth factor receptor in the true vocal fold of female albino rats administered anabolic, androgenic steroids, and effects of anti-androgen therapy. *J Laryngol Otol, 125*(8), 829-836.

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders [Elektronisk resurs] : DSM-5*. Arlington, VA: American Psychiatric Association.

Andersson-Wallgren, G., & Albertsson-Wikland, K. (1994). Change in speaking fundamental frequency in hormone-treated patients with Turner's syndrome--a longitudinal study of four cases. *Acta Paediatr, 83*(4), 452-455.

Azul, D. (2014). Transmasculine people's vocal situations: a critical review of gender-related discourses and empirical data. *Int J Lang Commun Disord*.

Baker, J. (1999). A report on alterations to the speaking and singing voices of four women following hormonal therapy with virilizing agents. *J Voice, 13*(4), 496-507.

Bauer, H. (1968). Relation to phoniatrics to endocrinology. *Folia Phoniatr (Basel), 20*(6), 387-393.

Berendes, J. (1962). Changes in the female voice caused by virilizing and anabolic hormones. *Folia Phoniatr (Basel), 14*, 265-271.

Boothroyd, C. V., & Lepre, F. (1990). Permanent voice change resulting from Danazol therapy. *Aust N Z J Obstet Gynaecol, 30*(3), 275-276.

Carew, L., Dacakis, G., & Oates, J. (2007). The effectiveness of oral resonance therapy on the perception of femininity of voice in male-to-female transsexuals. *J Voice, 21*(5), 591-603.

Coleman, E., Bockting, W., Botzer, M., Cohen-Kettenis, P., DeCuypere, G., Feldman, J., et al. (2012). Standards of care for the health of transsexual, transgender, and gender-nonconforming people, version 7. *Int J Transgenderism, 13*, 165-232.

Coleman, R. O. (1971). Male and female voice quality and its relationship to vowel formant frequencies. *J Speech Hear Res, 14*(3), 565-577.

Coleman, R. O. (1976). A comparison of the contributions of two voice quality characteristics to the perception of maleness and femaleness in the voice. *J Speech Hear Res, 19*(1), 168-180.

Coleman, R. O. (1983). Acoustic correlates of speaker sex identification: implications for the transsexual voice. *J Sex Res, 19*(3), 293-295.

Cosyns, M., Van Borsel, J., Wierckx, K., Dedecker, D., Van de Peer, F., Daelman, T., et al. (2014). Voice in female-to-male transsexual persons after long-term androgen therapy. *Laryngoscope, 124*(6), 1409-1414.

Dacakis, G. (2000). Long-term maintenance of fundamental frequency increases in male-to-female transsexuals. *J Voice, 14*(4), 549-556.

Dacakis, G., Davies, S., Oates, J. M., Douglas, J. M., & Johnston, J. R. (2013). Development and preliminary evaluation of the transsexual voice questionnaire for male-to-female transsexuals. *J Voice, 27*(3), 312-320.

Damrose, E. J. (2009). Quantifying the impact of androgen therapy on the female larynx. *Auris Nasus Larynx, 36*(1), 110-112.

Damsté, P. H. (1964). Virilization of the voice due to anabolic steroids. *Folia Phoniatr (Basel), 16*, 10-18.

Damsté, P. H. (1967). Voice change in adult women caused by virilizing agents. *J Speech Hear Disord, 32*(2), 126-132.

Dhejne, C., Öberg, K., Arver, S., & Landen, M. (2014). An Analysis of All Applications for Sex Reassignment Surgery in Sweden, 1960-2010: Prevalence, Incidence, and Regrets. *Arch Sex Behav*.

Eliakim, A., Cale-Benzoor, M., Klinger-Cantor, B., Freud, E., Nemet, D., Feigin, E., et al. (2011). A case study of virilizing adrenal tumor in an adolescent female elite tennis player--insight into the use of anabolic steroids in young athletes. *J Strength Cond Res, 25*(1), 46-50.

Falhammar, H., Filipsson, H., Holmdahl, G., Janson, P. O., Nordenskjöld, A., Hagenfeldt, K., et al. (2007a). Fractures and bone mineral density in adult women with 21-hydroxylase deficiency. *J Clin Endocrinol Metab, 92*(12), 4643-4649.

Falhammar, H., Filipsson, H., Holmdahl, G., Janson, P. O., Nordenskjöld, A., Hagenfeldt, K., et al. (2007b). Metabolic profile and body composition in adult women with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *J Clin Endocrinol Metab, 92*(1), 110-116.

Fitch, W. T., & Giedd, J. (1999). Morphology and development of the human vocal tract: a study using magnetic resonance imaging. *J Acoust Soc Am, 106*(3 Pt 1), 1511-1522.

Frisen, L., Nordenström, A., Falhammar, H., Filipsson, H., Holmdahl, G., Janson, P. O., et al. (2009). Gender role behavior, sexuality, and psychosocial adaptation in women with congenital adrenal hyperplasia due to CYP21A2 deficiency. *J Clin Endocrinol Metab, 94*(9), 3432-3439.

Fürst-Recktenwald, S., Dörr, H. G., & Rosanowski, F. (2000). Androglottia in a young female adolescent with congenital adrenal hyperplasia and 21-hydroxylase deficiency. *J Pediatr Endocrinol Metab, 13*(7), 959-962.

Gauffin, J., & Sundberg, J. (1989). Spectral correlates of glottal voice source waveform characteristics. *J Speech Hear Res, 32*(3), 556-565.

Gerritsma, E. J., Brocaar, M. P., Hakkesteegt, M. M., & Birkenhager, J. C. (1994). Virilization of the voice in post-menopausal women due to the anabolic steroid nandrolone decanoate (Decadurabolin). The effects of medication for one year. *Clin Otolaryngol Allied Sci, 19*(1), 79-84.

Gidlöf, S., Falhammar, H., Thilén, A., von Döbeln, U., Ritzén, M., Wedell, A., et al. (2013). One hundred years of congenital adrenal hyperplasia in Sweden: a retrospective, population-based cohort study. *Lancet Diabetes Endocrinol, 1*(1), 35-42.

Gidlöf, S., Wedell, A., Guthenberg, C., von Döbeln, U., & Nordenström, A. (2014). Nationwide neonatal screening for congenital adrenal hyperplasia in sweden: a 26-year longitudinal prospective population-based study. *JAMA Pediatr, 168*(6), 567-574.

Gugatschka, M., Lichtenwagner, S., Schwetz, V., Lerchbaum, E., Graupp, M., Gerstenberger, C., et al. (2012). Subjective and Objective Vocal Parameters in Women With Polycystic Ovary Syndrome. *J Voice*.

Hagenfeldt, K., Janson, P. O., Holmdahl, G., Falhammar, H., Filipsson, H., Frisén, L., et al. (2008). Fertility and pregnancy outcome in women with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Hum Reprod, 23*(7), 1607-1613.

Hallin, A. E., Fröst, K., Holmberg, E. B., & Södersten, M. (2012). Voice and speech range profiles and Voice Handicap Index for males - methodological issues and data. *Logoped Phoniatr Vocol, 37*(2), 47-61.

Hammarberg, B. (2000). Voice research and clinical needs. *Folia Phoniatr Logop, 52*(1-3), 93-102.

Hannoun, A., Zreik, T., Husseini, S. T., Mahfoud, L., Sibai, A., & Hamdan, A. L. (2011). Vocal changes in patients with polycystic ovary syndrome. *J Voice, 25*(4), 501-504.

Hansbury, G. (2005). The middle men: an introduction to the transmasculine ideintities. *Studies in Gender and Sexuality., 6*(3), 241-264.

Heinemann, M. (1974). Laryngeal and voice findings in congenital adrenogenital syndrome with adrenocortical hyperplasia. *Folia Phoniatr (Basel), 26*(6), 450-460.

Hembree, W. C., Cohen-Kettenis, P., Delemarre-van de Waal, H. A., Gooren, L. J., Meyer, W. J., 3rd, Spack, N. P., et al. (2009). Endocrine treatment of transsexual persons: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab, 94*(9), 3132-3154.

Hertegård, S., Kelly, V., Södersten M. (2013). Evidence for the effectiveness of pitch raising surgery and potential side-effects. Invited paper for presentation at the special session "Voice and speech in male-to-female transgender clients: Current knowledge on effectiveness of voice therapy and surgery, and outcome measures", at *the 10th Pan European Voice Conference (PEVOC)*, August 21-24, Prague.

Hirano, M. (1974). Morphological structure of the vocal cord as a vibrator and its variations. *Folia Phoniatr (Basel), 26*(2), 89-94.

Holmberg, E. B., Hillman, R. E., & Perkell, J. S. (1988). Glottal airflow and transglottal air pressure measurements for male and female speakers in soft, normal, and loud voice. *J Acoust Soc Am, 84*(2), 511-529.

Isshiki, N., Morita, H., Okamura, H., & Hiramoto, M. (1974). Thyroplasty as a new phonosurgical technique. *Acta Otolaryngol, 78*(5-6), 451-457.

Isshiki, N., Taira, T., & Tanabe, M. (1983). Surgical alteration of the vocal pitch. *J Otolaryngol, 12*(5), 335-340.

Jacobson, B. H., Johnson, A., Grywalski, C., Silbergleit, A., Jacobson, G., Benninger, M. S., et al. (1997). The voice handicap index (VHI): development and validation. *American Journal of Speech-Language Pathology, 6*, 66-70.

Juniarto, A. Z., Setiawati, B. A., Ediati, A., van der Zwan, Y. G., Looijenga, L. H., de Jong, F. H., et al. (2013). Virilization due to androgen hypersecretion in a patient with ovarian leydig cell tumor: diagnostic and psychosocial implications. *Acta Med Indones, 45*(2), 130-135.

Kanagalingam, J., Georgalas, C., Wood, G. R., Ahluwalia, S., Sandhu, G., & Cheesman, A. D. (2005). Cricothyroid approximation and subluxation in 21 male-to-female transsexuals. *Laryngoscope, 115*(4), 611-618.

Kazemirad, S., Bakhshaee, H., Mongeau, L., & Kost, K. (2014). Non-invasive in vivo measurement of the shear modulus of human vocal fold tissue. *J Biomech, 47*(5), 1173-1179.

Klatt, D. H., & Klatt, L. C. (1990). Analysis, synthesis, and perception of voice quality variations among female and male talkers. *J Acoust Soc Am, 87*(2), 820-857.

Lagerlöf, F., & Dawes, C. (1985). The effect of swallowing frequency on oral sugar clearance and pH changes by Streptococcus mitior in vivo after sucrose ingestion. *J Dent Res, 64*(10), 1229-1232.

Landis, J. R., & Koch, G. G. (1977). The measurement of observer agreement for categorical data. *Biometrics, 33*(1), 159-174.

Lundström, E., Hammarberg, B., & Munck-Wikland, E. (2009). Voice handicap and health-related quality of life in laryngectomees: assessments with the use of VHI and EORTC questionnaires. *Folia Phoniatr Logop, 61*(2), 83-92.

Mastronikolis, N., Remacle, M., Biagini, M., Kiagiadaki, D., Lawson, G. (2013). Wendler glottoplasty: an effective pitch raising surgery in male-to-female trassexuals. *J Voice 27*(4), 516-522.

Martin, G. F. (1988). Drugs and Vocal Function. *J Voice, 2*(4), 338-344.

Merke, D. P., & Bornstein, S. R. (2005). Congenital adrenal hyperplasia. *Lancet, 365*(9477), 2125-2136.

Nieschlag, E., Behre, H. M., & Nieschlag, S. (2012). *Testosterone [Elektronisk resurs] : Action, Deficiency, Substitution*. Cambridge: Cambridge University Press.

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

Nordenskjöld, F., & Fex, S. (1984). Vocal effects of danazol therapy. A preliminary report. *Acta Obstet Gynecol Scand Suppl, 123*, 131-132.

Nygren, U. (2008). Röstkarakteristika relaterade till förhöjda halter av androgener hos kvinnor med kongenital binjurehyperplasi (CAH). *Thesis master-one-year. Karolinska Institutet.*

Ohlsson, A. C., & Dotevall, H. (2009). Voice handicap index in Swedish. *Logoped Phoniatr Vocol, 34*(2), 60-66.

Pattie, M. A., Murdoch, B. E., Theodoros, D., & Forbes, K. (1998). Voice changes in women treated for endometriosis and related conditions: the need for comprehensive vocal assessment. *J Voice, 12*(3), 366-371.

Pegoraro Krook, M. I. (1988). Speaking fundamental frequency characteristics of normal Swedish subjects obtained by glottal frequency analysis. *Folia Phoniatr (Basel), 40*(2), 82-90.

Pinto, G., Tardy, V., Trivin, C., Thalassinos, C., Lortat-Jacob, S., Nihoul-Fekete, C., et al. (2003). Follow-up of 68 children with congenital adrenal hyperplasia due to 21-hydroxylase deficiency: relevance of genotype for management. *J Clin Endocrinol Metab, 88*(6), 2624-2633.

Roers, F., Murbe, D., & Sundberg, J. (2009). Voice classification and vocal tract of singers: a study of x-ray images and morphology. *J Acoust Soc Am, 125*(1), 503-512.

Sakai, F., Gamsu, G., Dillon, W. P., Lynch, D. A., & Gilbert, T. J. (1990). MR imaging of the larynx at 1.5 T. *J Comput Assist Tomogr, 14*(1), 60-71.

Sanchez, K., Oates, J., Dacakis, G., & Holmberg, E. B. (2014). Speech and voice range profiles of adults with untrained normal voices: methodological implications. *Logoped Phoniatr Vocol, 39*(2), 62-71.

Scheidt, D., Kob, M., Willmes, K., & Neuschaefer-Rube, C. (2004). Do we need voice therapy for female-to-male transgenders? *B. E. Murdoch, J. Goozee, B. - M. Whelan, K. Docking (Eds.), 2004 IALP-Congress-Proceedings. Brisbane: Speech Pathlogy Australia.*

Shepperd, H. W. (1966). Androgenic hoarseness. *J Laryngol Otol, 80*(4), 403-405.

Spooner, J. B. (1977). Classification of side-effects to danazol therapy. *J Int Med Res, 5 Suppl 3*, 15-17.

Strauss, R. H., Liggett, M. T., & Lanese, R. R. (1985). Anabolic steroid use and perceived effects in ten weight-trained women athletes. *JAMA, 253*(19), 2871-2873.

Sulter, A. M., Wit, H. P., Schutte, H. K., & Miller, D. G. (1994). A structured approach to voice range profile (phonetogram) analysis. *J Speech Hear Res, 37*(5), 1076-1085.

Söderpalm, E., Larsson, A., & Almquist, S. A. (2004). Evaluation of a consecutive group of transsexual individuals referred for vocal intervention in the west of Sweden. *Logoped Phoniatr Vocol, 29*(1), 18-30.

Södersten, M., Hertegård, S., & Hammarberg, B. (1995). Glottal closure, transglottal airflow, and voice quality in healthy middle-aged women. *J Voice, 9*(2), 182-197.

Södersten, M., & Lindestad, P. A. (1990). Glottal closure and perceived breathiness during phonation in normally speaking subjects. *J Speech Hear Res, 33*(3), 601-611.

Södersten, M., Lindestad, P. A., & Hammarberg, B. (1991). Vocal fold closure , perceived breathiness, and acoustic characteristics in normal adult speakers. In J. Gauffin & B. Hammarberg (Eds.), *Vocal fold physiology: Acoustic, perceptual, and physiological aspects of voice mechanisms.* (pp. 217-224): San Diego: Singular Publishing Group.

Södersten, M., Ternström, S., & Bohman, M. (2005). Loud speech in realistic environmental noise: phonetogram data, perceptual voice quality, subjective ratings, and gender differences in healthy speakers. *J Voice, 19*(1), 29-46.

Talaat, M., Talaat, A. M., Kelada, I., Angelo, A., Elwany, S., & Thabet, H. (1987). Histologic and histochemical study of effects of anabolic steroids on the female larynx. *Ann Otol Rhinol Laryngol, 96*(4), 468-471.

Tanabe, M., Haji, T., Honjo, I., & Isshiki, N. (1985). Surgical treatment for androphonia. An experimental study. *Folia Phoniatr (Basel), 37*(1), 15-21.

Thilén, A., Nordenström, A., Hagenfeldt, L., von Dobeln, U., Guthenberg, C., & Larsson, A. (1998). Benefits of neonatal screening for congenital adrenal hyperplasia (21-hydroxylase deficiency) in Sweden. *Pediatrics, 101*(4), E11.

Titze, I. R. (1989). Physiologic and acoustic differences between male and female voices. *J Acoust Soc Am, 85*(4), 1699-1707.

Titze, I. R. (2011). Vocal fold mass is not a useful quantity for describing F0 in vocalization. *J Speech Lang Hear Res, 54*(2), 520-522.

Titze, I. R., Luschei, E. S., & Hirano, M. (1989). Role of the thyroarytenoid muscle in regulation of fundamental frequency. *Journal of Voice, 3*(3), 213-224.

Tsuji, D. H., Ubirajara Sennes, L., Bohadana, S. C., & Rebelo Pinho, S. M. (2003). Management for voice fundamental frequency in the congenital adrenal hyperplasia through Isshiki´s Type IV thyroplasty. *International Archives of Otorhinolaryngology 7*(3), 244.

Wagner, I., Fugain, C.,Monneron-Girard, L., Cordier, B., Chabolle, F. (2003). Pitch-raising surgery in fourteen male-to-female transsexuals. *The Laryngoscope,113*, 1157-1165.

Van Borsel, J., De Cuypere, G., Rubens, R., & Destaerke, B. (2000). Voice problems in female-to-male transsexuals. *Int J Lang Commun Disord, 35*(3), 427-442.

Van Den Berg, J., & Zantema, J. T. (1956). The resistance and the Bernoulli effect of the glottis. *Acta Physiol Pharmacol Neerl, 5*(2), 239-240.

van Gelder, L. (1974). Psychosomatic aspects of endocrine disorders of the voice. *J Commun Disord, 7*(3), 257-262.

Wedell, A., Thilén, A., Ritzen, E. M., 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*(5), 1145-1152.

White, P. C., & Speiser, P. W. (2000). Congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Endocr Rev, 21*(3), 245-291.

Wierckx, K., Van Caenegem, E., Schreiner, T., Haraldsen, I., Fisher, A., Toye, K., et al. (2014). Cross-sex hormone therapy in trans persons is safe and effective at short-time follow-up: results from the European network for the investigation of gender incongruence. *J Sex Med, 11*(8), 1999-2011.

Wollina, U., Pabst, F., Schonlebe, J., Abdel-Naser, M. B., Konrad, H., Gruner, M., et al. (2007). Side-effects of topical androgenic and anabolic substances and steroids. A short review. *Acta Dermatovenerol Alp Panonica Adriat, 16*(3), 117-122.

World Health Organization. (2009). *International statistical classification of diseases and related health problems : ICD-10*. Geneva: World Health Organization.