From the Department of Molecular Medicine and Surgery
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ADDISON´S DISEASE
EPIDEMIOLOGICAL AND CLINICAL STUDIES

Sigridur Björnsdottir

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To Palli, Björn Andri & Helga Sif

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av
Sigridur Björnsdottir

Huvudhandledare:
Medicine doktor Sophie Bensing
Karolinska Institutet
Institutionen för molekylär medicin och kirurgi

Bihandledare:
Professor Jonas F. Ludvigsson
Karolinska Institutet
Institutionen för medicinsk epidemiologi och biostatistik

Docent Ylva Trolle Lagerros
Karolinska Institutet
Institutionen för medicin

Fakultetsopponent:
Professor Gudmundur Johannsson
Göteborgs Universitet
Sahlgrenska Akademin
Institutionen för medicin

Betygsnämnd:
Professor Angelica Lindén Hirschberg
Karolinska Institutet
Institutionen för kvinnors och barns hälsa

Docent Nathalie Ylitalo Helm
Göteborgs Universitet
Sahlgrenska Akademin
Institutionen för onkologi

Docent Marja Thorén
Karolinska Institutet
Institutionen för molekylär medicin och kirurgi

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ABSTRACT

Addison's disease (AD) is a potentially life-threatening condition that often presents with vague and nonspecific symptoms. Patients with AD have increased mortality risk. Data on parity and pregnancy outcome in women with AD are limited. Furthermore, there are no data on fracture risk or drug prescription patterns in patients with AD. Continuous subcutaneous hydrocortisone infusion (CSHI) is a novel treatment modality, but it has not yet been established whether the circadian hormone profiles and insulin sensitivity differ in patients on CSHI compared with conventional oral hydrocortisone treatment (OHC). The four studies in this thesis aim to enhance knowledge and clinical management of patients with AD.

Parity and pregnancy outcome: In all, 1188 women with AD were retrospectively evaluated. Women with AD had a reduced overall parity compared with controls ($P < 0.001$). Adjusted odds ratios (ORs) (95% confidence interval, CI) for infants born to mothers with deliveries ≤ 3 years before diagnosis of AD were 2.40 (1.27-4.53) for preterm birth, 3.50 (1.83-6.67) for low birth weight and 1.74 (1.02-2.96) for caesarean section. In comparison with controls, women who gave birth after their AD diagnosis were at increased risk for both caesarean delivery (adjusted OR, 2.35, 95% CI; 1.68-3.27) and preterm delivery (adjusted OR, 2.61, 95% CI; 1.69-4.05). No differences were found in risks of congenital malformations or infant death.

Hip fracture risk: Totally, 3219 patients with AD were retrospectively evaluated. Patients with AD had a higher risk of hip fracture (hazard ratio, HR 1.8, 95% CI; 1.6-2.1; $p < 0.001$) than matched controls. The increased risk was independent of age at diagnosis, sex and calendar period. A positive association between hip fracture and undiagnosed AD was noted with the highest risk estimates during the last year before AD diagnosis (OR 2.8, 95% CI; 1.8-4.2).

Drug prescription patterns: We identified 1305 patients with both a diagnosis of AD and on combination treatment with hydrocortisone/cortisone acetate and fludrocortisone. The yearly prevalence of AD increased from 12.2 to 13.1 (*P* for trend = .062); incidence varied between 0.5 and 0.6 (*P* for trend = .131) per 100 000 person-years during the period 2005-2009. Patients with AD received more prescribed drugs than controls. Both before and after AD diagnosis, patients used more gastrointestinal medications, antianemic preparations, lipid-modifying agents, antibiotics for systemic use, hypnotics and sedatives and drugs for obstructive airway disease (p-values < 0.05). Notably, an increased prescription of several antihypertensive drugs and high-ceiling diuretics was observed after AD diagnosis.

Circadian hormone profiles and insulin sensitivity: CSHI provided a more physiological circadian cortisol curve, including a late night cortisol surge, than OHC treatment. CSHI yielded a normalization of adrenocorticotropic hormone (ACTH) levels and showed a more normal circadian variation than OHC. CSHI prevented a continuous decrease in glucose during the night. No difference in insulin sensitivity was observed between the two treatment arms. The expected growth hormone (GH) peak during nighttime was more pronounced for CSHI, which, together with the higher insulin-like growth factor type 1 (IGF-1) and IGF-binding protein-3 (IGFBP-3) levels, suggest a more anabolic status.

Conclusion: This thesis demonstrates that both undiagnosed and diagnosed AD entail increased risks of unfavorable pregnancy outcome, hip fractures and altered drug prescription patterns compared to controls. In addition, parity is reduced in patients diagnosed with AD. This raises concerns about the conventional replacement therapy. CSHI is a safe and reliable mode of glucocorticoid replacement and might become a treatment option in selected patients.

RELATED PUBLICATION

Øksnes M, **Björnsdottir S**, Isaksson M, Methlie P, Carlsen S, Nilsen RM, Broman JE, Triebner K, Kämpe O, Hulting AL, Bensing S, Husebye ES, Løvås K.
Continuous subcutaneous hydrocortisone infusion versus oral hydrocortisone replacement for treatment of Addison's disease: A randomized clinical trial.
J Clin Endocrinol Metab. 2014 Feb 11:jc20134253. [Epub ahead of print]

LIST OF PUBLICATIONS

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals (I-IV):

I. **Björnsdottir S**, Cnattingius S, Brandt L, Nordenström A, Ekbom A, Kämpe O, Bensing S.
Addison's disease in women is a risk factor for an adverse pregnancy outcome.
J Clin Endocrinol Metab. 2010; 95(12): 5249-57

II. **Björnsdottir S,** Sääf M, Bensing S, Kämpe O, Michaëlsson K, Ludvigsson JF.
Risk of hip fracture in Addison's disease: a population-based cohort study.
J Intern Med. 2011; 270(2):187-95

III. **Björnsdottir S**, Sundström A, Ludvigsson JF, Blomqvist P, Kämpe O, Bensing S.
Drug prescription patterns in patients with Addison's disease: A Swedish population-based cohort study.
J Clin Endocrinol Metab. 2013; 98(5): 2009-18

IV. **Björnsdottir S**, Øksnes M, Isaksson M, Methlie P, Nilsen RM, Kämpe O, Hulting A-L, Husebye ES, Løvås K, Nyström T, Bensing S.
Circadian hormone profiles and insulin sensitivity in patients with Addison's disease: A comparison of continuous subcutaneous hydrocortisone infusion with conventional glucocorticoid replacement therapy.
Submitted.

LIST OF ABBREVIATIONS

11ß-HSD	11 beta-hydroxysteroid dehydrogenase
ACTH	Adrenocorticotropic hormone
AD	Addison's disease
APS	Autoimmune polyglandular syndrome
ATC	Anatomical Therapeutic Chemical
BMD	Bone mineral density
BMI	Body mass index
CBG	Corticosteroid-binding globulin
CI	Confidence interval
CRH	Corticotrophin releasing hormone
CSHI	Continuous subcutaneous hydrocortisone infusion
DHEAS	Dehydroepiandrosterone sulfate
E2	Estradiol
E3	Estratriol
GH	Growth hormone
GR	Glucocorticoid receptor
h	Hours
HDL	High-density lipoprotein
HPA-axis	Hypothalamus-pituitary-adrenal-axis
HR	Hazard ratio
HRQoL	Health related quality of life
11β-HSD	11-beta-hydroxysteroid dehydrogenase
ICD	International classification of diseases
IGF-1	Insulin-like growth factor I
IGFBP-1	Insulin-like growth factor binding protein-1
KCNRG	Potassium channel regulatory protein
LDL	Low-density lipoprotein
LBW	Low birth weight
MR	Mineralocorticoid receptor
OER	Observed to expected ratio
21-OH	21-hydroxylase
OHC	Oral hydrocortisone
OP	Osteoporosis
OR	Odds ratio
PIN	Personal identity number
RRR	Relative risk ratio

SD	Standard deviation
SNPR	Swedish National Patient Register
SPDR	Swedish Prescribed Drug Register
TPO	Thyroid peroxidase
TSH	Thyroid stimulating hormone

CONTENTS

1 INTRODUCTION

1.1 HISTORICAL MILESTONES

Primary adrenal insufficiency or Addison´s disease (AD) is named after the physician Thomas Addison (1793-1860). He worked at St. Guy's Hospital in London from 1817-1860 and described clinical and autopsy findings in 11 cases of AD in his classic monograph published in 1855 (1). He carefully delineated the clinical features that occur in patients with adrenal disease as "The leading and characteristic features of the morbid state to which I would direct attention are; anemia, general languor and debility, remarkable feebleness of the heart's action and peculiar change in the color of the skin occurring in connection with a diseased condition of the suprarenal capsules". A year later the French Professor Brown-Séguard demonstrated that the adrenal glands were organs essential for life by performing adrenalectomies in dogs, cats and guinea pigs (2).

In 1896, William Osler first administered adrenal extract to a patient with AD that revealed the clinical symptoms of AD (3). Before the 1940s, AD was always fatal. Between 1937 and 1955, the adrenocorticoidsteroid hormones were isolated, their structures defined and synthesized by Kendall and Reichstein. They together with Hench, who discovered the effectiveness of cortisone in treating rheumatoid arthritis (4), were awarded the Nobel Prize for their work in 1950. The availability of cortisone and the isolation of aldosterone in 1955 (5) made a life-saving treatment possible in patients with AD.

1.2 FETAL AND ADULT ADRENAL GLANDS

The fetal adrenal gland is evident from 6 to 8 weeks gestation, rapidly increasing in size and by midgestation it is larger than its adjacent kidney (6). In fetal life and up to 12 months postpartum two distinct zones are evident, an inner prominent fetal zone and an outer definitive zone that differentiates into the adult adrenal gland. The fetal zone comprises 80–85% of the volume of the fetal adrenal gland. It actively produces fetal steroids during gestation, but involutes rapidly after birth. The definitive cortex persists and develops into the functional adrenal cortex, with distinct zona glomerulosa and fasciculata present at birth.

The zona reticularis develops during the first year of life (6). The fetal zone is deficient in 3β-hydroxysteroid dehydrogenase enzyme activity and therefore secretes mainly dehydroepiandrostendione (DHEA) that serves as a substrate for estrogen biosynthesis by the placenta. At about the 28th week of pregnancy, the fetal adrenal gland initiates de novo glucocorticoid synthesis (7). It secretes large quantities of steroid hormones near term.

The adrenal glands are composed of two functionally distinct endocrine units (the adrenal cortex and medulla) contained within a single capsule. Adrenal medulla and cortex have different embryonic origins. The medulla of the adrenal gland is made up of 10-20% of the volume of a normal gland and originates from the neural crest. The outer adrenal cortex comprises 80-90% of the volume of a normal gland. It is derived from mesothelium and is further divided into three distinct layers: zona reticularis (10%), zona fasciculata (75%), zona glomerulosa (15%), each with distinct hormonal functions.

1.3 ADRENAL STEROID BIOSYNTHESIS

Three main types of hormone are produced by the adrenal cortex: Mineralocorticoids (aldosterone) from zona glomerulosa, glucocorticoids (cortisol) from zona fasciculata and sex steroids (mainly androgens) from the innermost layer, zona reticularis. Figure 1 presents an overview of the adrenal steroidogenesis.

Cholesterol is the precursor for the synthesis of all steroid hormones. The cells of the adrenal cortex can either take up cholesterol from the circulation or synthesize cholesterol *de novo* from acetyl coenzyme A. Both low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol are important sources of substrate for human adrenal steroidogenesis (8, 9).

Zona glomerulosa differs from the other layers in that it does not express 17α – hydroxylase. Thus, it is not able to synthesize glucocorticoids and androgens (10). Zona fasciculata and reticularis lack aldosterone synthase and therefore are not able to convert corticosterone to aldosterone. Zona fasciculata is the main

site for the production of glucocorticoids and zona reticularis for adrenal androgens, but both zones express enzymes for synthesizing both hormones (11).

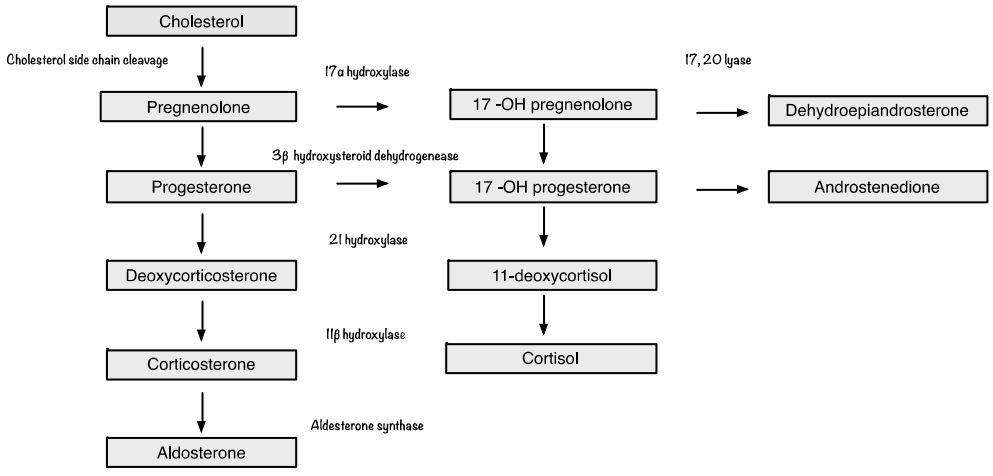

Figure 1. Overview of the adrenal steroidogenesis.

1.4 CORTISOL

Corticotropin-releasing hormone (CRH) secreted from the paraventricular nucleus in the hypothalamus, act on CRH receptors in the anterior pituitary and cause release of the adrenocorticotropic hormone (ACTH) into the bloodstream. ACTH acts on the melanocortin receptor type 2 in the adrenal cortex and stimulates production and release of cortisol. Cortisol has numerous actions, including feedback inhibition at the level of the paraventricular nucleus and the anterior pituitary, to control CRH or ACTH synthesis and release. In addition, the hypothalamus-pituitary-adrenal-axis (HPA-axis) receives important feedback from other areas of the brain (e.g., the hippocampus and amygdala) (Figure 2).

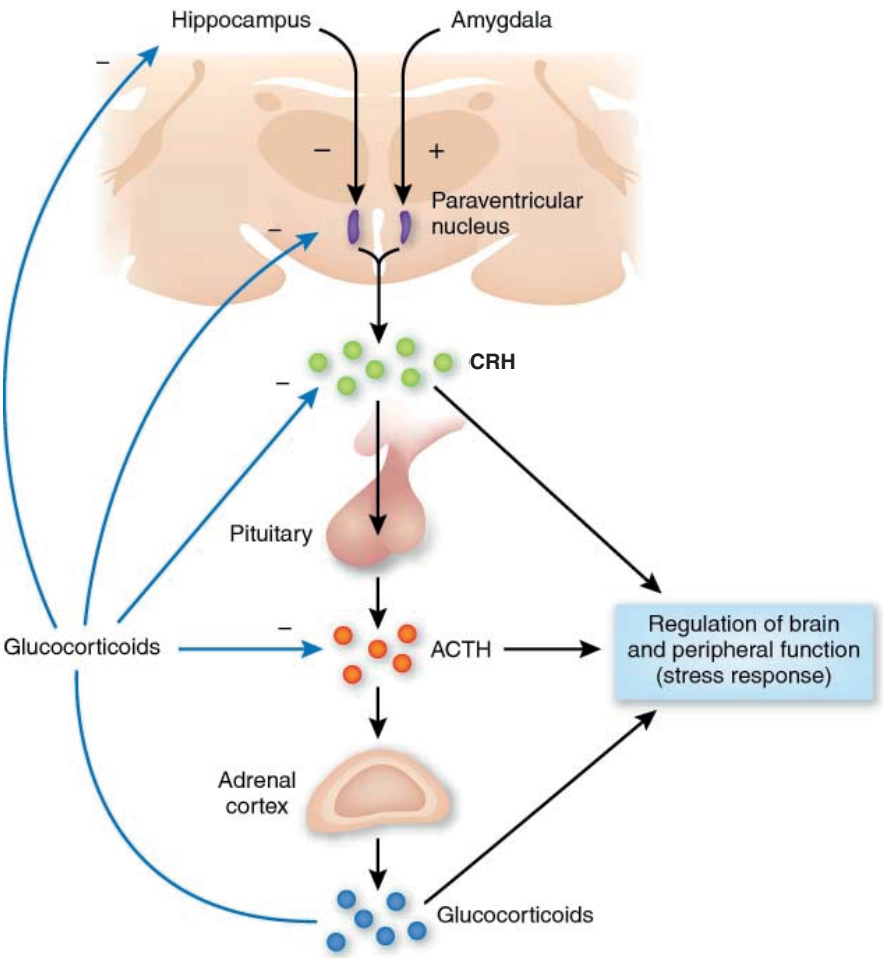

Figure 2. Hypothalamus-pituitary-adrenal-axis. Reprinted with permission from the author (Hyman SE, 2010 Nature Neuroscience 12; 241 - 243).

Cortisol is tightly bound to corticosteroid-binding globulin (CBG) (70%) and more loosely to albumin (20%). About 5% is "free" basally, although stress levels may exceed CBG capacity. CBG is saturated at 400-500 nmol/L; higher levels will increase the free fraction of cortisol (12). Only this free circulating fraction is available for transport into tissue for biological activity. At the cellular level, the effects of corticosteroids are largely a consequence of transcriptional

actions mediated via binding to two types of intracellular receptors: the high-affinity mineralocorticoid receptor (MR) and the lower affinity glucocorticoid receptor (GR) (13). GR is present in every cell type in the human body. Cortisol has an essential role in maintaining basal and stress-related homeostasis and has many effects on development and cell differentiation (14). It is also involved in growth, immune and inflammatory reactions as well as central nervous system and cardiovascular functions (15).

Cortisol secretion follows a circadian rhythm in healthy individuals. Information on light is received in the suprachiasmatic nucleus (SCN) that adapts the cortisol circadian rhythm to the day/night cycle. There is a nadir in cortisol secretion about midnight and a rise 2–3 h after sleep onset. It continues to rise into the early waking hours. The peak in cortisol occurs between 6 and 9 am; as the day progresses, a gradual decline in cortisol levels continues until nadir at midnight (16). New knowledge in the past years about the HPA axis has revealed an ultradian cortisol rhythm within the circadian rhythm (17). An ACTH pulse followed by a cortisol response is seen once every hour. The amplitude of the pulses varies in a circadian pattern, with the highest amplitude occurring before awakening (Figure 3).

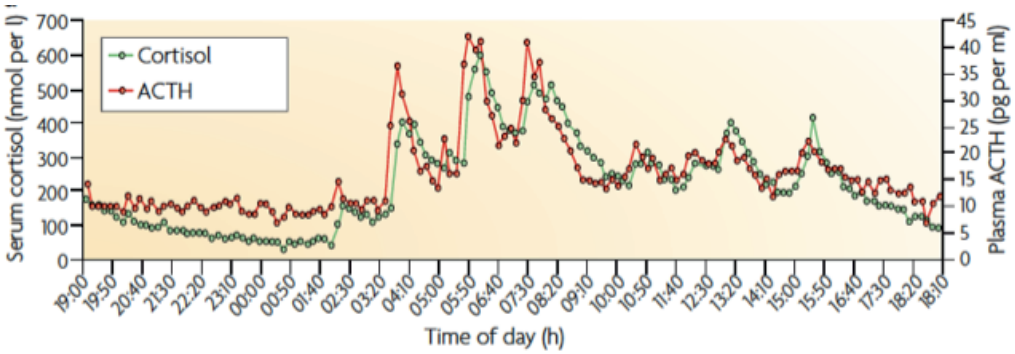

Figure 3. Circadian and ultradian rhythms for ACTH and cortisol in healthy humans. Reprinted with permission from author (Lightman SL, Conway-Campbell 2010 Nat Rev Neurosci 11:710-18)

1.5 ALDOSTERONE

Aldosterone synthesis is regulated by the renin-angiotensin system. Angiotensin II and a small rise in serum potassium concentration are the primary stimuli to aldosterone release. Aldosterone acts primarily in the distal nephron to increase the reabsorption of sodium and chloride and the excretion of potassium and hydrogen (18). Consequently, aldosterone regulates water and salt balance and blood pressure. The 11-beta-hydroxysteroid dehydrogenase type 2 (11β-HSD 2) converts cortisol to inactive cortisone. This enzyme protects the mineralocorticoid receptor in the kidney from the competitive action of the 1000-fold more abundant cortisol to bind to it. 11β-HSD 2 is inhibited by licorice, allowing cortisol to bypass the enzyme "barrier" and bind to and activate MR causing sodium retention, potassium loss and hypertension (19).

1.6 ADRENAL ANDROGENS

ACTH stimulates androgen secretion. Adrenal androgens represent an important component of circulating androgens in premenopausal females (20). This contribution is much smaller in males due to testicular production of androgens. In premenopausal women the major androgens in the serum are DHEA-S, DHEA, androstenedione, testosterone and dihydrotestosterone in descending order of serum concentrations. DHEA-S and DHEA may be considered pro-hormones, requiring conversion to testosterone or dihydrotestosterone in peripheral tissues to express their androgenic effects (20).

1.7 ADDISON'S DISEASE

1.7.1 Epidemiology

AD is a rare disease and its prevalence varies with geographical area. The lowest occurrence (five cases per million) has been reported in Japan (21) and the highest (144 cases per million) has been found in Norway (22). The highest incidence of AD reported is 4.4 cases per million/year (22). The most recent study on the prevalence of AD comes from Germany, where data were analyzed from a health insurance database (covering 10% of the German population) (23). The prevalence of AD was 87 per million in 2012 with an increased prevalence

during 2008-2012 in women, but not in men. The female: male ratio of AD is 3:2 (24, 25). AD can occur at any age but the peak age is around 40 years old, with women being older than men at diagnosis (26). AD is rare in children.

1.7.2 Causes

Autoimmunity is the predominant cause of primary AD in the Western world accounting for approximately 85% of all cases (27). The enzyme steroid 21-hydroxylase (21-OH) was identified in 1992 as the antigen for the auto-antibodies involved in AD (28). It is now known that 21-OH antibodies are markers for the ongoing autoimmune process but not a causative factor as the adrenal destruction results from T-cell-mediated autoimmunity (29). The prevalence of 21-OH antibodies in the general population is approximately 0.5%, but higher in patients with other autoimmune diseases and about 10% in first-degree relatives to patients with AD (30).

Other causes of primary AD include infectious diseases (e.g., tuberculosis, mycobacteria, meningococcus, HIV), adrenal infiltrative disorders (e.g., hemochromatosis, bilateral metastasis, lymphoma, amyloidosis), hemorrhage (e.g., anti-phospholipid syndrome, anticoagulation therapy), adrenal surgery (e.g., tumor surgery, Cushing's syndrome), genetic disorders (e.g., congenital adrenal hyperplasia, adrenal leukodystrophy) and medications (e.g.ketoconazole, mitotane, metyrapone) (31).

1.7.3 Symptoms and Signs

The autoimmune destruction of the adrenal glands is believed to develop gradually over time. Overt symptoms first appear when the adrenal residual function is very low (32).

Since the symptoms of AD are usually nonspecific, the diagnosis of AD is often delayed, a situation that may result in severe distress for the patient and the risk of suffering a life-threatening adrenal crisis. Recent data from Germany show that as many as 20% of patients with primary or secondary AD had symptoms for more than 5 years before diagnosis (42). They had consulted, on average, at

least three physicians before a correct diagnosis was made and frequently had incorrect diagnoses and treatments before the AD diagnosis. The most common complaints recorded at AD diagnosis are fatigue, loss of appetite, salt craving, vomiting and abdominal pain (22). Postural dizziness and pain in joints or muscles are also commonly reported. The most common signs are hyperpigmentation, orthostatic hypotension and weight loss. Symptoms diminish after replacement treatment is started, although 25% of patients still report fatigue and salt craving and 15% report postural dizziness while on replacement therapy (22).

1.7.4 Diagnosis

The diagnosis of AD should be considered in all patients presenting with unexplained collapse, hypotension, vomiting or diarrhea and hyperpigmentation (31). The combination of low early morning serum cortisol (usually < 165 nmol/L), but sometimes in the lower normal range and high plasma ACTH (> 22 pmol/L) is diagnostic for AD (33). Subnormal serum aldosterone concentrations (or within the lower normal range), with increased plasma renin activity are typical. Furthermore, the serum DHEA is low and often undetectable in women with AD (33). Hyponatraemia is present in 90% and hyperkalemia in 50% of newly diagnosed AD cases.

If an uncertainty exists about the AD diagnosis, a cosyntropin (synacthen or tetracosactide ACTH, 250 μg intramuscularly or intravenously) followed by measurement of serum cortisol after 30 and/or 60 min should be performed. In healthy individuals one of these values should exceed 500 or 550 nmol (31).

Once the AD diagnosis is confirmed, the next step is to identify the etiology. Since autoimmunity is the most common cause, the first test should be the measurement of 21-OH antibodies. Patients with 21-OH antibodies should be screened for concomitant autoimmune diseases, particularly autoimmune thyroid disease and diabetes mellitus. The rare autoimmune polyglandular syndrome type 1 (APS-1) should be considered in young patients and if other components of APS-1 are present (see below). If a patient with a diagnosis of AD is negative

for 21-OH antibodies, the next step is to perform a computer tomography scan of the adrenals to exclude tumors, hemorrhage or calcification typical of tuberculosis.

1.7.5 CONCOMITANT AUTOIMMUNE DISEASES

More than half of all patients with AD have concomitant autoimmune diseases (22, 34). Combinations of AD with different concomitant autoimmune diseases are classified into polyendocrine syndromes (APS).

APS-1, caused by mutations in the autoimmune regulator (AIRE) gene, is defined as the combination of two of the following three components: AD, hypoparathyroidism and chronic mucocutaneous candidiasis (35, 36) Other components of APS-1 can be dental enamel dysplasia, keratitis, autoimmune hepatitis, malabsorption and autoimmune premature ovarian failure. The prevalence of APS-1 is about 1:80 000 in most populations (37).

APS-2 comprises AD in combination with another organ-specific autoimmune disease. It most commonly involves primary hypothyroidism or autoimmune gastritis with vitamin B12 deficiency, type 1 diabetes and hyperthyroidism due to Graves' disease. Patients with isolated AD and patients with APS-2 share the same polygenic susceptibility with a complex pattern of inheritance (32).

Premature ovarian failure is the loss of ovarian function in women before 40 years of age. It is associated with sex steroid deficiency, amenorrhea, infertility and elevated serum gonadotropins. Autoimmune premature ovarian failure is found in 5% of all women with spontaneous premature ovarian failure (38). It may occur in the context of APS-1 or APS-2 or it may occur in isolation (39). Premature ovarian failure occurs in approximately 10 % of women with AD (40). In patients with APS-1 the frequency is about 50-60% (41).

1.7.6 TREATMENT

Patients with AD need lifelong steroid replacement, including both glucocorticoid and mineralocorticoid treatment (33).

1.7.6.1 Glucocorticoid treatment

Cortisol replacement in patients with AD most commonly consists of hydrocortisone or cortisone acetate. Oral hydrocortisone has high bioavailability (95%), but a short half-life (60-120 min) (42). Cortisone acetate requires conversion to hydrocortisone via the hepatic enzyme 11β-HSD type 1 and therefore has a lower cortisol peak, slower onset of action and slower decline to trough than hydrocortisone (43). Cortisol production rate in healthy individuals is estimated to be 5.4–6.1 mg/m^2/day (44). Long-acting glucocorticoid replacement, such as Prednisolone (half-life of 12–36h) and Dexamethasone (half-life of 36–72h), is rarely used in AD patients (43). Since prednisolone and dexamethasone have long duration of action, there is higher risk of long-term adverse effects (e.g., osteoporosis) (45).

It is recommended that the total hydrocortisone dose should be divided into two or three doses to mimic the circadian cortisol rhythm. The time of the last dose should be 6 h before bedtime to avoid sleep disturbances (31). A recent study demonstrated that once daily dual-release hydrocortisone, resulted in a reduced 24h cortisol exposure, reduced body weight, blood pressure and HbA1c and improved health-related quality of life (HRQoL) (46). This treatment successfully restored daytime cortisol levels to normal, but the late night increase in cortisol was not re-established. However, another modified-release hydrocortisone tablet restored the nighttime cortisol surge (47).

Although optimizing the glucocorticoid treatment is essential, no ideal monitoring parameter is presently available. ACTH cannot be used because it is invariably high during nighttime and early morning, rapidly declining after ingestion of the morning glucocorticoid dose (48). The use of the total cortisol value ignores any difference in glucocorticoid sensitivity, which may exist between individuals and does not reflect the cellular effects of glucocorticoids.

Therefore, glucocorticoid treatment efficacy is mainly assessed by clinical judgment, evaluating signs and symptoms indicating over-replacement (i.e. weight gain and skin alterations) or under-replacement (i.e. fatigue, nausea and myalgia).

The challenge of managing patients with AD is to determine the optimal daily maintenance glucocorticoid dose. It should be as low as possible to avoid morbidity of overtreatment. Further, the ideal treatment should mimic the normal glucocorticoid physiological rhythm as much as possible to avoid a high (unphysiological) exposure pattern during the afternoon and evening. Finally, it is important to inform patients with AD about the need for extra cortisol during illness and stress to prevent adrenal crisis.

1.7.6.2 Mineralocorticoid treatment

Mineralocorticoids (9α-fludrocortisone) are important for maintaining blood pressure and water and electrolyte homeostasis. Patients with AD usually require 50–200 μg depending on individual fluid and electrolyte intake/losses and type of glucocorticoid replacement used.

Mineralocorticoid replacement should be monitored clinically by asking about deficiency symptoms such as salt craving, lightheadedness and orthostatic blood pressure. The presence of hypertension, edema, hypokalemia and low plasma renin suggests excessive mineralocorticoid replacement (49). Measuring sodium, potassium and renin in which the goal for renin is in the upper normal range can be used to adjust the dosage. Salt intake should be liberal, especially when exercising, and the mineralocorticoid dose may have to be increased on warm days during the summer when salt loss in perspiration increases.

1.7.6.3 Adrenal androgen treatment

Studies of DHEA replacement in women with AD have generated variable and inconsistent results. Some studies have reported improvement in mood and sexual function (50, 51), whereas others have not (22, 52, 53) A meta-analysis of 10 randomized, placebo-controlled trials comparing DHEA with placebo

reported a small improvement in HRQoL and depression, but no significant effect on anxiety or sexual well-being was seen (54). Long-term effects of DHEA or testosterone replacement therapy in patients with AD are not known. A common clinical approach is to offer female patients with AD a 3-6-month trial of DHEA replacement if they have lack of libido, impaired mood or sense of well-being despite optimized glucocorticoid and mineralocorticoid replacement.

1.7.7 MORTALITY

For several years, the life expectancy of patients with AD on conventional glucocorticoid replacement therapy and adequate follow-up was considered normal (102). Recently, Swedish studies have shown that patients with AD have a more than twofold increased standardized mortality ratio (SMR), mainly due to cardiovascular, malignancy and infectious diseases (55, 56). Patients with APS-1 have higher mortality rates when compared with patients with APS-2. Another recent study from Norway found that males younger than 40 years of age suffered excess mortality due to adrenal crisis, infection and sudden death (57).

1.8 PREGNANCY

1.8.1 HPA axis during normal pregnancy

The HPA axis plays an important physiological role in normal pregnancy. During normal pregnancy, a gradual increase in circulating CRH, ACTH, total cortisol and 24-h urinary free cortisol occurs (58, 59). In the third trimester, free plasma cortisol levels also increase, suggesting greater tissue exposure to glucocorticoids during pregnancy (60).

The placenta functions partly as a hypothalamic-pituitary-end organ system, with stimulatory and inhibitory feedback mechanisms to regulate dynamic factors affecting fetal growth and development under a variety of conditions. The primary stimulus for the increase in activity of the fetal and maternal HPA axis appears to be placental CRH (60).

Plasma CRH levels rise exponentially by thousand-fold as pregnancy progress. At the 35 th week of pregnancy there is a sharp increase to a peak at the time of delivery (59). CRH normalizes to nonpregnant values within 24 h of delivery. The placenta is the main source of elevated circulating CRH during pregnancy. Cortisol stimulates placental CRH synthesis in a positive feedback loop.

Plasma ACTH levels rise during pregnancy, reaching maximal levels at delivery. The elevated ACTH levels with high plasma cortisol observed in late pregnancy do not suggest normal feedback control. The mechanisms underlying the increase in ACTH are not clear, but may include placental synthesis and release of biologically active ACTH, pituitary desensitization to cortisol feedback or enhanced pituitary responses to CRH and vasopressin (59).

Estrogen production from the placenta stimulates hepatic corticosteroid-binding globulin (CBG) production. A gradual increase in total plasma cortisol is found as early as the 11th week of pregnancy, with a peak during third trimester to levels that are threefold higher than those in nonpregnant women (58). Total plasma cortisol remains elevated until at least 2 weeks after delivery (59). During pregnancy, the circadian rhythm of cortisol is preserved, although it may be partly blunted (59).

The causes for the threefold rise in free cortisol during the third trimester are not known. They are thought to be due to alterations in the set point of the HPA axis or to the antiglucocorticoid effect of the increasing progesterone levels (60).

The maternal adrenal gland does not change morphologically during pregnancy. Levels of renin and angiotensin are known to rise during pregnancy, leading to elevated angiotensin II levels and markedly elevated levels of aldosterone. Plasma aldosterone concentrations are elevated 5- to 7-fold during the first trimester and continue to increase until the end of gestation when 10- to 20-fold elevations are reached (61).

Progesterone acts as a mineralocorticoid receptor antagonist and reduces sodium reabsorption and smooth muscle relaxation (62). Serum potassium levels remain

constant in pregnancy despite increased plasma aldosterone, which is most likely due to the antagonistic effects of progesterone.

Until approximately 33 weeks of gestation, more than 90% of fetal cortisol is derived from maternal sources (63). Cortisol levels in the maternal circulation are 5- to 10-fold higher than in the fetus, a gradient thought to be maintained by high placental 11β-HSD2 (19). Indeed, placental 11β-HSD2 inactivates the majority of maternal glucocorticoids passing to the fetus in humans. The fetus, unlike the adult, has little or no 11β-HSD1 in its tissues to regenerate cortisol from cortisone, at least until near term when 11β-HSD1 becomes expressed in organs, such as lung and liver (19).

1.8.2 Parity and pregnancy in women with AD

AD predominantly affects women in the reproductive age between the third and fourth decade of life (33). A recent study in Norway of 269 women with AD showed a significantly reduced fertility with the standardized incidence ratio (SIR) for childbirth (0.97 in women before AD diagnosis and 0.69 after diagnosis) (40). This ratio remained significantly low at 0.72 when women with premature ovarian failure were excluded.

The few published reports of pregnancy outcome in women with AD after the availability of cortisone in the 1950s demonstrated successful maternal outcome and no maternal death (64, 65). Intrauterine growth retardation and low birth weight (LBW) have been reported in infants born to mothers with untreated AD (66). Another study showed that careful treatment of AD with physiological glucocorticoid replacement in pregnancy could lead to successful pregnancy outcomes, including birth weights appropriate for gestational age (67). The few cases reporting intrauterine death were in women with undiagnosed AD or before the availability of glucocorticoids regimens (68, 69).

1.9 AD AND OSTEOPOROSIS

1.9.1 Glucocorticoid effects on bone cells

Glucocorticoids in a physiological amount are important for osteoblast differentiation to a mature phenotype (70-72). Glucocorticoids influence the expression of IGF-1, increasing bone formation and the synthesis of type I collagen and decreases bone collagen degradation (73).

Glucocorticoids in supraphysiological amounts have direct and indirect effects on the skeleton. The primary effects are on osteoblasts and osteocytes. Glucocorticoids impair the replication, differentiation and function of osteoblasts and induce the apoptosis of mature osteoblasts and osteocytes (74). These effects lead to a suppression of bone formation, a central feature in the pathogenesis of glucocorticoid-induced osteoporosis (OP) (75). Glucocorticoids favor osteoclastogenesis, and consequently, increase bone resorption. Glucocorticoids inhibit calcium absorption from the gastrointestinal tract by opposing vitamin D actions and decreasing the expression of specific calcium channels in the duodenum (76). Renal tubular calcium reabsorption is also inhibited by glucocorticoids (77).

1.9.2 Osteoporosis

Glucocorticoid replacement doses in AD are higher than what is normally produced by the adrenal glands (44), raising concerns about long-term adverse effects of glucocorticoid over-replacement on bone. Pharmacological doses of glucocorticoids and endogenous hypercortisolism (Cushing's disease) are known to increase fracture risk, affect peak bone mass and generate OP, especially in bones with high trabecular content (78). Premature ovarian failure and lack of adrenal androgens in AD patients may also contribute to OP (53, 79). In a randomized double-blind trial in patients with AD, 12-months treatment with DHEA reversed bone loss at the femoral neck, but not at other sites (53). Studies of AD and bone mineral density (BMD) are inconsistent or even contradictory (80-82). Most studies consist of small patient series and are insufficiently powered to determine BMD in a reliable manner. Some studies reported reduced

BMD in all patients with AD (83). However others have reported reduced BMD only in postmenopausal women (84, 85) or only in men (86, 87). In additional other studies found no significant differences in BMD in either men or women with AD (81, 82).

The largest cross-sectional study included 292 adult patients with AD from Norway, Britain and New Zealand. The study showed that patients with AD had a lower BMD at the femoral neck and lumbar spine compared with healthy subjects (88). However, the mean hydrocortisone equivalent dose for the Norwegian population was ≥ 30 mg/day. A study on BMD in 81 patients with AD found that patients treated with low-dose glucocorticoid replacement (hydrocortisone-equivalent doses, 21.9 ± 4.9 mg, mean ± SD) did not have any detrimental effect on BMD or biochemical markers of bone turnover (89). The authors concluded that the previously noted detrimental effects in this situation were primarily a consequence of supraphysiological glucocorticoid exposure.

1.10 AD AND CIRCADIAN HORMONE PROFILES

1.10.1 Cortisol and ACTH

The conventional glucocorticoid replacement therapy in AD does not restore a normal cortisol biorhythm. In fact, as judged by circulating cortisol levels, this treatment renders the patient over-treated immediately after oral administration and under-treated within a few hours (90). Additionally, during nighttime and early morning, the glucocorticoid levels are undetectable, which is in contrast with the rise seen in healthy individuals (91). Disruption of the cortisol rhythm has been associated with various metabolic disorders (92) as well as depression (93) and obstructive sleep apnea (94). A randomized, double-blind, crossover study tried to replicate the normal circadian rhythm in AD (95). They found that a four-dose regimen increased the availability of cortisol and enhanced the effect with a less elevated ACTH in the morning.

Merza et al. showed that it was possible to mimic the physiological circadian cortisol rhythm in AD by intravenous infusion of hydrocortisone (96). The authors found that the ACTH levels were normalized after 24 h of treatment.

Løvås and Husebye found that continuous subcutaneous hydrocortisone infusion (CSHI) was safe and enabled a fine-tuned control of glucocorticoid delivery that allowed restoration of the circadian biorhythm (97).

1.10.2 Glucocorticoids and circadian growth hormone secretion

Endogenous glucocorticoids play an important physiological role in the regulation of somatotroph function and modulate growth hormone (GH) synthesis including secretion at both the hypothalamic and pituitary level (98). In vivo studies support the hypothesis that glucocorticoids are both stimulators and inhibitors of GH secretion, with the final biological effect depending on hormonal concentrations and time of exposure (99). Chronic excess of glucocorticoids, either exogenous or endogenous, inhibits GH secretion (100).

The GH secretion is within the normal levels in patients with AD who receive chronic glucocorticoid replacement therapy (101). In patients with newly diagnosed AD and long-standing hypocortisolism, the GH response has been shown to be blunted but still within normal range (102). GH responsiveness to pharmacological stimuli starts to decrease several days (3–7 days) after withdrawal of replacement therapy (103), a finding suggesting that impairment of GH secretion occurs as a result of sustained low circulating concentrations of cortisol (104). Barkan et al. studied GH secretion and the circadian GH rhythmicity in AD (105). They found that cortisol deficiency leads to diminution of GH output with low GH pulse amplitude. However, circadian rhythmicity of GH secretion was glucocorticoid-independent.

Giustina et al. characterized the physiological relationship between GH and cortisol in patients with isolated ACTH deficiency (104). Using different doses of hydrocortisone, they found that serum cortisol above 700 nmol/l caused acute inhibition of GH secretion. Glucocorticoids influence on GH secretion in a biphasic way: a physiological glucocorticoid amount is needed for normal GH secretion and if patients with AD receive too much glucocorticoids, GH secretion might continue to be functionally suppressed by even a slight increase in levels of cortisol (101) (Figure 4).

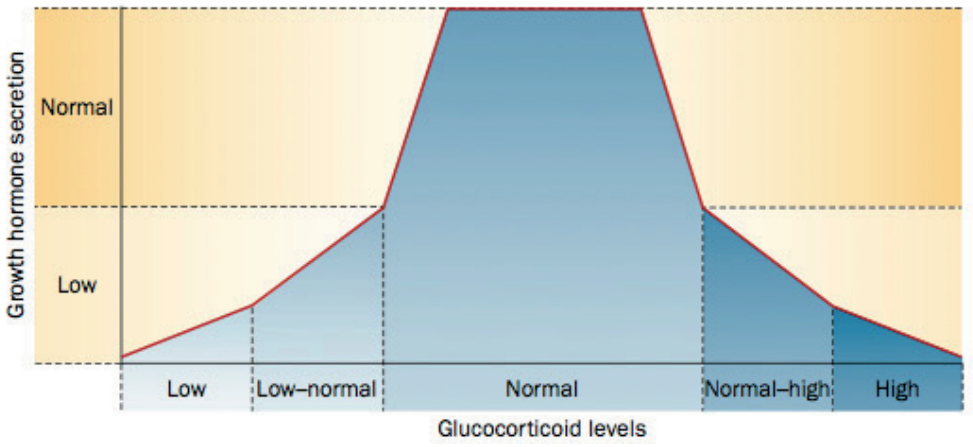

Figure 4. An integrated model of the effect of circulating glucocorticoid on GH secretion. Reprinted with permission from author (Giustina, A. 2013 Nat. Rev. Endocrinol. 9; 265–276)

1.11 AD AND METABOLISM

1.11.1 Lipid profiles in patients with AD

Glucocorticoids in high doses increase glycogen production and gluconeogenesis in the liver, inhibit peripheral glucose uptake and increase lipolysis (106). This can lead to increased insulin resistance, an increase in glucose levels and risk of type 2 diabetes.

A study in patients with secondary AD demonstrated that the cardiovascular risk profile was related to both the dose and type of glucocorticoid regimen used (107). Patients on hydrocortisone replacement ≥ 20 mg/d had increased total cholesterol, triglycerides, waist circumferences and glycosylated hemoglobin when compared with ACTH-sufficient subjects. An average dose of < 20 mg/day was not associated with a more adverse metabolic profile. Patients on hydrocortisone and prednisolone were more prone to develop an adverse metabolic profile than those using cortisone acetate (107).

An Italian study including 38 patients with AD found a higher prevalence of central adiposity, impaired glucose tolerance and dyslipidemia compared with matched controls (108). A study from South Africa found patients with AD to have significantly higher triglycerides, lower HDL, more small dense LDL particles and increased high sensitivity C - reactive protein (hs-CRP), a biochemical marker of inflammation, than controls (109). In another study, South African patients with AD were found to have worse lipid profiles and higher hs-CRP compared with matched Swedish patients, despite lower doses of hydrocortisone (110).

Overall, no significant effects on cardiovascular risk profile in patients with AD have been found in DHEA replacement trials. One study found DHEA replacement in women with AD to result in an unfavorable lipoprotein profile (111).

1.11.2 Glucose during the night in patients with AD

Patients with AD on conventional glucocorticoid therapy complain about fatigue, dizziness and concentration difficulties, especially in the early morning (22, 112). A recent study in patients with AD without concomitant type 1 diabetes showed that they can be susceptible to nocturnal hypoglycemia (113). Whether the low glucose level during the night could be related to morning fatigue seen in many patients with AD is not clear. In patients with AD and concomitant diabetes mellitus variation in cortisol levels can cause fluctuations in glucose levels that can influence insulin treatment. Such an event can occur when cortisol troughs increase insulin sensitivity and cortisol peaks decrease it (114, 115). In addition to the lack of cortisol, patients with AD have decreased adrenaline output from the adrenal gland, further impairing counter-regulatory responses to hypoglycemia (116). The incidence of sudden death is increased in patients with AD compared with the normal population, for which hypoglycemia could hypothetically be a contributing factor (57).

2 AIMS

The overall aim of this thesis was to study clinical, metabolic and epidemiological aspects of AD in order to increase knowledge of the disease and improve the clinical management of patients.

Specific aims were:

•To test the hypothesis that women with AD have reduced parity and worse pregnancy outcome compared with controls **(Paper I)**

•To test the hypothesis that patients with AD have increased hip fracture risk compared with controls **(Paper II)**

•To determine the prevalence and incidence of AD in Sweden and to describe the drug prescription patterns in patients with AD **(Paper III)**

•To test the hypothesis that hydrocortisone replacement via continuous subcutaneous infusion is a more physiological treatment than conventional treatment in terms of circadian hormone profiles and effect on insulin sensitivity **(Paper IV)**

3 SUBJECTS AND METHODS

3.1 STUDY SUBJECTS

We used inpatient and outpatient data from the Swedish National Patient Register (SNPR) to identify all women **(paper I)** and both men and women **(paper II and III)** with a main or secondary diagnosis of AD using the International Classification of Diseases (ICD): ICD-7: 274.40, ICD-8: 255.10, ICD-9: 255E, ICD-10: E27.1, E27.2. We focused our study on individuals suffering from AD of probable autoimmune origin. Table 1 shows exclusion diagnosis. In **paper IV** patients with evidence of autoimmune AD diagnosis identified from a patient registry (ROAS, Registry of Organ Specific Autoimmune Diseases) or from the hospital diagnosis registries were invited to participate.

Diagnosis	ICD10 1997-	ICD 9 1987-	ICD 8 1968-	ICD 7 1964-
Exclusion diagnosis				
Tuberculosis	A15-A19, P37.0, J65, B90	010-018, 137	010-019 Y34.09 Y34.19 Y34.29	001.99-019.20 Y03.00, 03.10, 03.20 Y53.00
Waterhouse-Friderichsens syndrome	A39.1	036D	036.11	057.11
HIV disease	B20-B24	079J, 279K	-	-
Neoplasm of the adrenal glands	C74, C79.7	194A, 198H	194.01	195.01
Mal. neoplasms of pituitary gland	C75.1	194D	194.31	195.31
Benign adrenal tumor	D35.0	227A	226.00-09	224.10-224.19
Benign neoplasms of pituitary gland	D35.2	227D	226.20	195.30, 224.40
Adrenal neoplasm of uncertain or unknown behaviour	D44.1	237C	239.10	195.00, 239.31
Neoplasm of uncertain or unknown behaviour of pituitary gland	D44.3	237A	239.90	239.34
Hyperfunction of pituitary gland	E22*	253*	253*	272*
Hypofunction and other disorders of pituitary gland	E23*	253*	253*	272*
Cushings syndrom	E24*	255A	258.00	277.10
Adrenogenital disorders	E25	255C	255.01, 255.02	-
Drug-induced adrenocortical insufficiency	E27.3	-	-	-
Other unspecified adrenocortical insufficiency	E27.4	255F	-	-
Disorder of adrenal glands in diseases classified elsewhere (Tuberculous (A18.7), meningococcal (A39.1))	E35.1	255F	-	-
Adrenoleukodystrophy	E71.3	330A	333.10	-
Postprocedural hypopituitarism	E89.3	253H	-	-
Postprocedural adrenocortical hypofunction	E89.6	255F	-	-
Neonatal adrenal hemorrhage	P54.4	772F	-	-
Congenital malfrm. of adrenal gland	Q 89.1	759B	758.10	-

Table 1. Exclusion diagnosis (ICD) in paper I-III.

Paper I: With inpatient and outpatient data from the SNPR, we identified 2349 women with a main or secondary diagnosis of AD during the period 1964-2006. To cover only the reproductive age we restricted our study to 1631 women aged 15–47 years for the period 1973 to 2006. Of those 1631 women, 443 had exclusion diagnosis. Thus, the analysis included 1188 women of reproductive age with AD of probable autoimmune cause. Using the Swedish Register of Population, we randomly selected 10 female controls, matched by birth year and county of residence, for each woman with AD.

Paper II: We identified 5405 patients with a diagnosis of AD in the SNPR for the period 1964-2006. Of these 5405 patients, 1398 had exclusion diagnosis. The remaining 4007 patients with AD were aged- and sex-matched with 40 040 controls. We then excluded 143 patients with AD and 724 controls with hip fracture before the beginning of the study. We restricted our sample to individuals aged ≥30 years at study entry because hip fracture before that age may be the result of misclassification or caused by factors other than osteoporosis. Hence, the main analysis of this study was based on 3219 patients with AD and 31 557 controls. In a subanalysis we examined the risk of hip fracture before AD diagnosis with hip fracture as exposure and AD as outcome.

Paper III: To increase the diagnostic accuracy of AD in our patient cohort we restricted all analyses to those patients who had both a diagnosis of AD of probable autoimmune origin in the SNPR and one or more prescriptions of both hydrocortisone/cortisone acetate and fludrocortisone in the Swedish Prescribed Drug Register (SPDR) during the period 2005-2009. The date of diagnosis was defined as the date of first registered AD diagnosis in the SNPR (1964-2009), or the date of the first prescription of hydrocortisone/cortisone acetate or fludrocortisone in the SPDR (2005-2009), whichever occurred first. Using the Swedish Register of Population, we randomly identified 10 controls individually matched by birth year, sex and county of residence for each patient with AD.

Paper IV: Patients were enrolled in a multicenter, randomized crossover study in Norway and Sweden. Inclusion criteria were verified AD of autoimmune origin and age between 18 and 70 years. Patients with concomitant

endocrine/autoimmune diseases had to be on stable treatment throughout the study. Exclusion criteria were concomitant diabetes mellitus, cardiovascular and malignant disease, pregnancy and pharmacological treatment with glucocorticoids or drugs (antiepileptics, rifampicin and St John's Wort) known to interfere with cortisol metabolism.

3.2 REGISTRIES

Sweden has excellent conditions for nationwide epidemiological studies. From 1947 and onwards, all Swedish residents alive have an unique, 10-digit personal identity number (PIN) (117). Through the PIN, register data can be linked to an individual.

3.2.1 The Swedish National Patient Register (Paper I, II and III)

The Swedish National Patient Register encompasses both inpatient and outpatient data. Inpatient data have been available since 1964 in parts of Sweden, becoming nationwide in 1987. In 2001, the National Board of Health and Welfare also began to collect data on outpatient diagnosis from hospitals. The register contains information on patient (sex, age, place of residence), dates of hospital admission and discharge, codes for all surgical procedures and discharge diagnosis (118). Through the register, we identified all patients with a main or secondary diagnosis of AD of probable autoimmune origin.

3.2.2 The Swedish Register of Population (Paper I, II and III)

The Swedish Register of Population contains data on all individuals living in Sweden since 1960. The register is based on reports from the Swedish tax authority. The register is kept by Statistics Sweden and includes data on name, PIN, current address, marital status, dates of death and migration. Through the register, we identified controls matched by gender, birth year and county of residence.

3.2.3 The Swedish Medical Birth Register (Paper I)

The Swedish Medical Birth Register includes prospectively collected and validated information from pregnancy, delivery and neonatal period on more than 99% of all births in Sweden since 1973 (119). Maternal characteristics are recorded in a standardized manner at the first visit to antenatal care, which occurs before the 15th week of gestation in more than 95% of pregnancies. Through the register, we collected data on parity, pregnancy, maternal and neonatal outcome in women with AD and controls.

3.2.4 The Swedish Prescribed Drug Register (Paper III)

The Swedish Prescribed Drug Register contains data since July 2005 on all drugs dispensed by prescription to the entire Swedish population (120). It contains information about age, sex, PIN, the prescriber's profession and practice, dispensed quantity, dosage, expenditure and reimbursement. The register does not include information about drugs sold over the counter or for use during hospital care, or clinical information on diagnosis/indication for treatment. All drugs are classified according to the Anatomical Therapeutic Chemical (ATC) classification system (121).

3.3 STUDY DESIGN

Paper I: This study was a retrospective matched population-based cohort study. Women with AD of reproductive age were matched with controls from the general population. Through the Swedish Medical Birth Register, we obtained information about pregnancy, antenatal period, delivery and neonatal period. When calculating parity, both singleton and multiple births were included. Because of higher pregnancy risk during multiple births, we included only singleton births when evaluating pregnancy outcome. Information about some variables such as body mass index and smoking were not available from the start of the register and therefore we restricted data to the period of full coverage.

Paper II: This study was a retrospective matched population-based cohort study. We linked data from the SNPR and the Swedish Register of Population to examine risk of hip fracture and in a subanalysis, risk of any fracture. The date of

diagnosis was defined as the date of first registered AD diagnosis in the SNPR (1964-2006). Study entry for controls began when their corresponding cases had their first registered AD diagnosis. Follow-up continued until death, hip fracture, or end of study period (December 31, 2006), whichever occurred first. In a subanalysis we examined the risk of hip fracture before AD diagnosis with hip fracture as exposure and AD as outcome. The end of the follow-up was the date of first recorded diagnosis of AD (the same date was used for the matched controls).

Paper III: In this retrospective matched population-based cohort study we restricted all analyses to those who had both AD diagnosis in the SNPR and one or more prescriptions of hydrocortisone/cortisone acetate and fludrocortisone in the SPDR. The date of diagnosis was defined as the date of first registered AD diagnosis in the SNPR (1964-2009), or the date of the first prescription of hydrocortisone/cortisone acetate or fludrocortisone in the SPDR (2005-2009), whichever first occurred. Study entry for the controls began when their matched cases were diagnosed with AD. The follow-up time for cases and controls ended December 31, 2009 or at death, whichever came first.

Paper IV: This study was an open, randomized, two-period, 12-weeks crossover multicenter trial in patients with AD from Norway and Sweden. The main analysis is presented in a related publication (122). The primary endpoint of the whole study was the ACTH level, which served as a marker for overall glucocorticoid effects and regulation. Secondary endpoints were safety and effects on other metabolic parameters, HRQoL and sleep. In paper IV we present data from a subgroup analysis on circadian hormone profiles and insulin sensitivity performed after 8 weeks of subcutaneous hydrocortisone infusion (CSHI) and conventional trice daily hydrocortisone treatment (OHC). The 10 first Norwegian patients with AD in the study were admitted to a clinical research facility for analysis of the circadian hormonal rhythm. The effects on cortisol, ACTH levels, carbohydrate metabolism and nighttime effects on growth hormone (GH), insulin-like growth factor I (IGF-1), IGF-binding protein-3 (IGFBP-3) and triglycerides were analyzed. Peripheral insulin sensitivity was

analyzed in 15 Swedish patients with AD using the euglycemic-hyperinsulinemic clamp technique.

3.4 METHODS

3.4.1 Intervention (Paper IV)

OHC treatment consisted of weight-adjusted thrice-daily (first dose at 07:00-08:00 h, second dose at 11:00-12:00 h and third dose at 16:00-17:00 h) administration of oral hydrocortisone 5 mg tablets as suggested by Mah et al. (90) (Table 2). The oral doses were titrated according to a serum cortisol nomogram 4 h after the morning dose at day 3-5 (90). For CSHI treatment, the patients received hydrocortisone (Solu-Cortef Act-o-Vial©, Pfizer Inc., NY, USA) administered by an insulin pump (Dana Diabecare©, SOOIL Development Company Ltd. Co, Seoul, South Korea), with doses adjusted to body surface area. The infusion gear was applied to the abdominal wall in a similar way as with continuous subcutaneous insulin infusion. The patients were instructed to clean the injection site with alcohol before needle insertion and replace the hydrocortisone solution and the infusion gear every third day.

Initial CSHI doses were 10.5 mg/m^2/d with the following infusion rate distribution: hours 08:00-14:00, 0.5 mg/ m^2/h; 14:00-20:00, 0.2 mg/ m^2/h; 20:00-02:00, 0.05 mg/ m^2/h; and 02:00-08:00, 1.0 mg/ m^2/h. The CSHI doses were adjusted according to salivary cortisol levels (h 06:00-08:00 and 23:00-24:00) and morning serum cortisol after 3-5 days.

The goal for the morning salivary cortisol was in the middle to upper reference range with an evening salivary cortisol in the lower range and furthermore a normal morning serum cortisol. During the washout phase, that lasted at least eight weeks, the patients were switched to their standard treatment. For both CSHI and OHC treatments, a smaller dose adjustment was allowed based on best clinical judgment during the dose titration, whereas after randomization all patients were treated with individually adjusted fixed daily doses for both treatments.

Weight (kg)	Total dose/day (mg)	First morning 07-08h (mg)	Second midday 11-12h (mg)	Third afternoon 16-17h (mg)
50-54	10.0	5.0	2.5	2.5
55-74	15.0	7.5	5.0	2.5
75-84	17.5	10.0	5.0	2.5
85-94	20.0	10.0	7.5	2.5
95-114	22.5	12.5	7.5	2.5
115-120	25.0	15.0	7.5	2.5

Table 2. OHC treatment consisted of weight-adjusted thrice-daily hydrocortisone tablets suggested by Mah et al (90).

3.4.2 Insulin sensitivity (Paper IV)

The insulin sensitivity group was admitted after 8 weeks of each treatment for euglycemic-hyperinsulinemic clamp according to De Fronzo et al. in a fasting state during the morning hours (123). Intravenous catheters were inserted into the right arm for substrate (insulin/glucose) infusion. A superficial dorsal hand vein was cannulated in retrograde fashion with a 21-gauge butterfly needle and the catheter was kept patent by a slow saline infusion. The hand was kept warm by a heated air box at 55°C (Department of Physiology and Pharmacology, University of Nottingham, Nottingham UK) for intermittent sampling of arterialized venous blood.

Insulin (Actrapid NovoNordisk A/S, Copenhagen, Denmark) was infused with a 10 min priming infusion followed by a constant infusion of 40 mU/ m^2/min for 110 min. Furthermore 20% dextrose (Fresenius Kabi, Stockholm, Sweden) was infused and the rate was adjusted to achieve a blood glucose level of 5.0 mmol/L based on arterialized samples drawn every 5 min from an ipsilateral dorsal hand vein (YSI 2300 STAT Plus™, Yellow Springs Instruments, OH, USA). When a steady state is attained the exogenous glucose infusion rate equals the amount of glucose disposed in body tissue.

Whole-body insulin sensitivity (M-value) was calculated from the amount of glucose infused during the last 30 min of the clamp divided by body weight (kg) and period (min) and expressed as mg/kg/min. The amount of infused glucose equals the whole-body glucose disposal when the endogenous glucose production is suppressed. In the vast majority of patients the standard version of the euglycemic clamp (40 mU/m2/min) will turn off the endogenous glucose output and give an estimate of peripheral insulin sensitivity (123). Serum cortisol and cortisone were measured every 30 min during the euglycemic-hyperinsulinemic clamp.

The glucose clamp-derived index of insulin sensitivity (SI) ((10^{-4} dl/kg*min)/(μ U/ml)) was calculated from the glucose infusion rate GIR, corrected for body weight, during the final 30 min as follows: SI = (GIRSS/GSS x delta ISS). GIRSS is the steady-state of glucose infusion rate (mg/min), GSS is the steady-state blood glucose concentration (mg/dl) and delta ISS is the difference between the basal and steady-state plasma insulin concentrations (μU/ml). This calculation is assumed to correct for differences in prevailing glucose and insulin concentration. All laboratory analyses were performed at Haukeland University Hospital, Bergen, Norway.

3.4.3 Circadian hormone profiles (Paper IV)

From an indwelling catheter in a forearm vein, serum and plasma samples were collected hourly and ACTH every third hour. All samples were stored at -80° C until the end of trial pending analysis. Cortisol and cortisone were analyzed by liquid chromatography mass spectrometry (124) and plasma ACTH by chemiluminescent immunometric assay (Immulite 2000, Siemens AG, Munich, Germany). S-glucose was assayed by UV-photometry (Roche Modular) and serum insulin, GH, IGF-1 and IGF-BP3 by Immulite 2000 (Siemens Healthcare, USA). Serum triglycerides were analyzed using standard measures. All analyses were performed at Haukeland University Hospital, Bergen, Norway.

3.4.4 Statistical analysis (Paper I-IV)

Results are presented as mean (± SD) if normally distributed data (otherwise as median and range, min-max).

Paper I: We used conditional regression analysis to calculate parity, presented as odds ratios (ORs) with 95% confidence intervals (CIs). Logistic regression analyses were used to estimate ORs and 95% CIs for all singleton pregnancy outcomes, adjusting for maternal age at delivery, calendar year at delivery and parity. To account for the dependence between births to the same mother we used a generalized linear model with births to the same mother as repeated measurements. The influence of type 1 diabetes and autoimmune thyroid disease on pregnancy outcomes was assessed by stratified analysis.

Paper II: We used Cox regression to estimate the risk of subsequent hip fracture in adults with AD. We analyzed each stratum (consisting of one patient with AD and his or her 10 matched controls) separately and then calculated a summary estimate. Risk estimates are given as hazard ratios (HRs). Additionally, we examined the risk of hip fracture according to time since AD diagnosis (intervals: 0-1 years, 1-5 years and >5 years). To examine whether there was an association between AD and fractures before AD diagnosis we used conditional logistic regression. In addition to ORs for fractures in AD, we estimated the risk of subsequent AD according to time since hip fracture.

Paper III: We calculated the observed/expected ratio (OER) by dividing the observed number of patients with AD who had been dispensed the drug or drug group with the expected number. Because we screened all dispensed medications, a large number of comparisons were performed. To attenuate the possible problem of multiple comparisons we calculated both 95% and 99% Poisson CIs around the OERs. The exact method was applied when the observed number was 20 or less; for more than 20 observations, a normal distribution approximation for the CI was used (125). When applicable, the two-sample test of proportions was used to assess differences in proportions while mean values were compared using student's t-test. The threshold for statistical significance was set to $p < 0.05$ (two-tailed). Stratifying drug prescription patterns by sex, we

used the relative risk ratio (RRR) to compare OER between men and women (126). In a subanalysis of the prescription pattern after diagnosis we excluded patients and controls with concomitant diabetes treatment when analyzing the prescription of drugs for the cardiovascular system.

Paper IV: To estimate time-specific differences in values between treatment groups we fitted mixed effects models for repeated measures using a PROC MIXED procedure. All models defined treatment, time and treatment-by-time interaction as fixed effects, whereas the patient within sequence was specified as a random effect to account for the intraindividual correlation between observations. The analyses were further adjusted for treatment period and sequence together with their time interactions. Additionally, to account for the heterogeneity in variance between treatment groups at the various time points separate variances were estimated together with the Satterthwaite approximation for correction of degrees of freedom. Finally, time-specific differences between treatment groups were estimated as the difference between least square means obtained from the PROC MIXED procedure.

Statistical analyses were performed using Statistical Analysis System (SAS) version 9.2 (SAS Institute, INC., Cary, NC, USA) (**Paper I**), SPSS 16.0 for Windows (SPSS Inc., Chicago, Il, USA, 2007) (**Paper II**), Stata 11(Collage Station, TX, USA) (**Paper III**), Excel, 2010 (Redmond, WA, USA) (**Paper III**) and PROC MIXED in SAS version 9.2 (SAS Institute, INC., Cary, NC, USA), (**Paper IV**).

3.4.5 Ethical approval (Paper I-IV)

The studies were approved by the Research Ethics Committee of Uppsala University, Uppsala, Sweden (Dnr 2006:026) (**Papers I, II and III**) and the Stockholm Ethical Review Board (Dnr 2010/1184-31/2) (**Paper IV**).

4 RESULTS

4.1 PARITY AND PREGNANCY OUTCOME IN WOMEN WITH AD (PAPER I)

Patient characteristics

During the observational period of 1188 women with AD, 386 gave birth to 665 infants (singleton and multiple births) before their first recorded diagnosis and 131 women gave birth to 199 singleton infants and five pairs of twins after the AD diagnosis.

Parity

There was a significantly reduced parity in women after AD diagnosis, compared with controls (OR, 0.55, 95 % CI; 0.44-0.69). No difference was found before diagnosis. Stratified analysis showed that the reduced parity was independent of calendar year of diagnosis, age at diagnosis or whether the women already had a child or not.

Overall pregnancy outcome

The majority of women independent if they were diagnosed with AD or not, had normal pregnancy outcomes and thus the overall risks must be considered low. There were no differences between groups for infant sex, Apgar score at 5 min or congenital malformations. No neonatal deaths were recorded in infants of patients or controls, nor any maternal deaths during labor or during the first year postpartum.

There was no difference in adjusted OR for edema, proteinuria and hypertension (including pre-eclampsia and eclampsia), placenta praevia, abruptio placentae or antepartum hemorrhage for mothers with deliveries 2 years before AD, similarly no differences could be found after AD diagnosis compared with controls.

In an attempt to shed light on whether AD was associated with increased risks of spontaneous or induced preterm birth we studied whether preterm infants born to mothers with undiagnosed or diagnosed AD were delivered by caesarean section more often than preterm infants to controls. As many as 70% (7/10) of preterm infants born to undiagnosed mothers and 56% (14/25) of preterm infants born to diagnosed mothers were delivered by a caesarean section compared with 33.8% (179/530) of the preterm infants born to controls. However, due to the small numbers, statistical interpretation of these results was not possible.

Pregnancy outcome before AD diagnosis

Adjusted ORs for infants born to mothers with deliveries ≤ 3 years before the diagnosis of AD were 2.40 (95% CI; 1.27-4.53) for preterm birth (< 37 weeks) and 3.50 (95% CI; 1.83-6.67) for low birth weight (< 2500 grams). The risks increased the closer the delivery was to the date of the AD diagnosis.

Pregnancy outcome after AD diagnosis

Compared with controls, women who gave birth after their AD diagnosis were at increased risk of both caesarean (adjusted OR, 2.35, 95% CI; 1.68-3.27) and preterm delivery (adjusted OR, 2.61, 95% CI; 1.69-4.05). Stratifying by isolated AD and concomitant type 1 diabetes and/or autoimmune thyroid disease in the mother did not essentially influence these odds ratios.

4.2 HIP FRACTURE RISK IN PATIENTS WITH AD (PAPER II)

Patient characteristics

The median age at study entry was 61 years (range 30-97) and the female to male ratio was 3:2. Hip fractures occurred from 32-98 years of age in the patients with AD and 40-101 years in the controls. The median duration between

registered diagnosis of AD and hip fracture was 5 years, whereas the median duration from study entry to fracture was 10 years in the controls.

Hip fracture risk before diagnosis

A positive association was found between hip fracture and undiagnosed AD (OR, 2.4, 95% CI; 2.1-3.0), with the highest risk estimates in the last year before AD diagnosis (OR, 2.8, 95% CI; 1.8-4.2).

Hip fracture risk after diagnosis

We observed 221 hip fractures (6.9%) in patients with AD and 846 (2.7%) in the control group. Patients with AD had almost twofold higher risk of hip fracture (HR, 1.8, 95% CI; 1.6-2.1) compared to controls. The relative risk was highest in the first year of follow-up (HR, 4.1, 95% CI; 2.9-5.8), but remained significantly higher more than 5 years after diagnosis (HR, 1.3, 95% CI; 1.1-1.6). This risk increase was independent of sex, age at diagnosis or calendar period of diagnosis. Risk estimates did not change with adjustment for type 1 diabetes, autoimmune thyroid disease, celiac disease or rheumatoid arthritis.

Stratifying patients by sex, we found that age at first registered diagnosis of AD affected the risk of hip fracture. Women ≤ 50 years at the time of registered AD diagnosis had a higher relative risk of hip fracture (HR, 2.7, 95% CI; 1.6-4.5) than those with a registered AD diagnosis > 50 years of age (HR, 1.7, 95% CI; 1.4-2.0) (P for interaction = 0.017). Age did not appear to modify the effect on hip fracture risk after AD diagnosis in men.

4.3 PREVALENCE, INCIDENCE AND DRUG PRESCRIPTION PATTERN IN PATIENTS WITH AD (PAPER III)

Patient characteristics

1305 patients with AD had combination treatment with hydrocortisone/cortisone acetate and fludrocortisone according to the SPDR. The mean age at the time of diagnosis was 39.1 years (SD 16.8): 42.2 years (SD 16.9) for women and 35.4 years (SD 16) for men. The mean age for the study population in 2005 when the SPDR started was 50.7 years (SD 18.5): 54% were female and 46% male.

In the analysis of the drug-prescribing pattern before the diagnosis of AD 176 patients diagnosed with AD at or later than July 1, 2006 were included, together with 1752 matched controls. This procedure allowed for at least 1 year of available data in the SPDR before diagnosis.

Prevalence and incidence of AD

In this material and with our strict inclusion criteria the yearly prevalence of AD changed from 12.2 to 13.1 (P for trend = .062); the incidence varied between 0.5 and 0.6 (P for trend = .131) per 100 000 person-years during the period 2005-2009.

Overall drug prescription pattern in patients with AD

Apart from hydrocortisone/cortisone acetate and fludrocortisone, 1260 AD patients (96.6%) and 10 491 controls (87.5%) had received ≥ 1 medication (difference 9.1%, 95% CI; 7.9-10.2%; P < .0001).

Drugs for peptic ulcer and gastroesophageal reflux disease, propulsives, laxatives, insulin and analogs, antianemic preparations, lipid-modifying agents, thyroid preparations, macrolides, hypnotics and sedatives and drugs for obstructive airway disease were prescribed more often (with statistically significant observed to expected ratios) to patients with AD both ≥ 1 year before

diagnosis and after diagnosis (on ATC third level, pharmacological subgroup, four positions).

Medications indicating APS-2 manifestations were prescribed to 59.3% of patients with AD; 46.6% had medication for autoimmune thyroid disease (controls, 5.9%), 17.7% for B12 deficiency, (controls, 5.5%) and 14.2% for insulin-treated diabetes mellitus (controls, 2.8%). Antithyroid drugs were prescribed to 1.1 % of the patients (controls, 0.2 %) before AD diagnosis and to 1.7 % (controls, 0.2 %) after diagnosis.

Hormonal contraceptives for systemic use were prescribed significantly less often to women with AD both before and after diagnosis.

Drug prescription pattern ≥ 1 year before AD diagnosis

Antihistamines and corticosteroids for systemic use (except hydrocortisone and cortisone acetate) were significantly more often prescribed before AD diagnosis but not after AD diagnosis.

Drug prescription pattern after AD diagnosis

The highest OER was found for anabolic steroids and androgens. This group consisted of prasterone (dehydroepiandrosterone) and nandrolone. Prasterone was prescribed to 41 patients (3.1%, 38 women and 3 men) and nandrolone to 1 patient (0.1%, 1 woman). Testosterone was prescribed to 16 patients (1.2%, 4 women and 12 men). Overall, 6% of the women with AD had prescription for either dehydroepiandrosterone or testosterone treatment. Of men with AD, 2% had testosterone treatment compared with 0.3% of the controls.

Unexpectedly, even when excluding patients and controls with diabetes treatment, more patients than controls were prescribed cardiovascular medicines (OER 1.14, 95% CI; 1.03-1.26). Stratifying these patients by age revealed that individuals < 40 years of age used more antihypertensive drugs and diuretics than controls (OER 1.90, 95%; CI 1.20-2.75). Patients 40 to 49 years old were prescribed lipid-lowering agents more frequently than controls (OER 1.97, 95%

CI; 1.15-3.01). For older age groups (50-59 and ≥ 60 years), the OERs for both antihypertensive and lipid-lowering drugs were very close to 1 and not statistically significant. There was no significant difference between patients with AD (4.8%) and controls (4.0%) regarding prescription of oral antidiabetic agents.

Patients with AD were prescribed significantly fewer diuretics and potassium-sparing agents in combination after diagnosis than controls.

4.4 CIRCADIAN HORMONE PROFILES AND INSULIN SENSITIVITY IN PATIENTS WITH AD (PAPER IV)

Patient characteristics

The Swedish insulin clamp cohort was significantly older and required lower hydrocortisone doses during CSHI treatment than the Norwegian circadian group undergoing the 24-h sampling for hormone profiles. In the circadian group four patients had isolated AD, three autoimmune hypothyroidism, one autoimmune premature ovarian insufficiency, one mild polycythemia vera and one was treated for hypercholesterolemia. The median hydrocortisone dose was 0.29 mg/kg/d (range, 0.20-0.50) and 0.23 (range, 0.25-0.50) mg/kg/d for CSHI and OHC treatments, respectively. In the insulin clamp group eight patients had isolated AD, seven were treated for autoimmune hypothyroidism and two for hypercholesterolemia. The median hydrocortisone dose was 0.27 mg/kg/d (range, 0.24-0.35) for CSHI and 0.22 (range, 0.21-0.32) mg/kg/d for OHC treatment.

Cortisol-ACTH profile

CSHI yielded a smooth circadian cortisol curve, with mean serum cortisol within the reference range for healthy individuals at all time points, including the physiological late night cortisol surge. As expected, OHC led to low nighttime cortisol levels and high post-dose peaks followed by troughs. The median serum

cortisol AUC was 5970 nmol/L/24 h (range 3910-7925) and 4911 nmol/L/24 h (range 2867-7831) for CSHI and OHC, respectively ($p = 0.028$). The CSHI median cortisone AUC was 867 nmol/L/24 h (range 718-1182) versus 499 nmol/L/24 h (range 416-1062) with OHC ($p = 0.005$). ACTH levels were clearly elevated with OHC, contrasting with the near normal circadian levels found with CSHI.

Glucose-insulin-triglyceride profile

The mean serum glucose levels were within the established normal reference range for non-diabetics for both treatment groups. Patients with AD on OHC had gradually decreasing glucose levels during the night, with the lowest levels at 0800 h. During CSHI treatment, the lowest glucose levels were observed at 0200 h. A further decrease in glucose levels was avoided during CSHI, resulting in a significantly improved mean serum glucose level during early morning when compared with OHC, with significantly between-treatment difference at 0700 h ($p< 0.001$) and 0800h ($p=0.011$). Conversely, daytime mean glucose levels tended to be higher (although not statistically significant) for OHC than for CSHI. Differences in serum insulin levels were generally small, with a non-significant trend towards higher insulin levels in the oral treatment group in the afternoon. Twelve-hour nighttime measurements of triglycerides were in the normal reference range and did not differ between the two therapies.

GH/IGF-I/IGFBP-3 profile

No difference in the GH/IGF-1/IGFBP-3 circadian rhythmicity was found between the hydrocortisone replacement modes. The expected nocturnal augmentation of GH release was more pronounced between 0100 and 0300 h in the CSHI group, but no statistically significant differences were detected. For IGF-1, there was a trend towards higher levels at each hour in the CSHI group, with significant differences at 0100 and 0800 h. Similarly, the IGFBP-3 was significantly higher with CSHI at 0800, 0900 and 1200 h, which correlated with the IGF-1 peak in the CSHI group at 0800 h.

Euglycemic-hyperinsulinemic clamp

No difference was noted in insulin sensitivity (M-value) between treatment groups (7.49 (SD=2.67) versus 8.13 (SD=3.64), p = 0.59) for the CSHI and OHC treatment, respectively. There was no difference in cortisol and cortisone at 0 and 30 min during the clamp. However, both cortisol and cortisone were significantly higher at 60, 90 and 120 min in the CSHI-treated group. Correcting for differences in prevailing glucose and insulin concentrations at steady state clamp, there was no difference in the glucose clamp-derived index of insulin sensitivity, SI = (15.9 (SD=5.7) versus 18.4 (SD=7.8) ((10^{-4} dl/kg*min)/(μU/ml), P=0.12) between the CSHI and OHC treatment groups.

5 DISCUSSION

5.1 METHODOLOGICAL CONSIDERATIONS

In our epidemiological studies we estimated exposure and outcome and measured whether there was an association and its strength. We sought to minimize errors in our measurements in order to be able to make correct estimations. Errors can be either random or systematic.

Validity

Validity is one of the most important issues in epidemiological studies. Validity of an assessment is the degree to which it measures what it is supposed to measure (127). It can be divided into two types***: internal and external***. Internal validity estimates the degree to which conclusions about associations can be made (e.g., cause and effect) based on the research setting and design. External validity is about whether findings can be generalized to other populations and is dependent on whether the study sample is representative of the general population.

Bias

Bias is a systematic error that alters estimations. To ensure high validity it is important to avoid bias. Bias can be divided into ***selection bias, information bias*** and ***confounding.***

Selection bias comes from the procedures used to select subjects and from factors influencing study participation. Such bias can arise when the association between exposure and disease differs for those who participate and those who do not participate in a study. Thereby, the study population is not representative of the theoretical cohort of all eligible individuals. Although selection bias can be prevented by study design, it can never be adjusted through statistical techniques. In **paper IV** there is a risk of selection bias. Potentially, only patients with a positive attitude to pump treatment accepted to participate in the study and

adhered to the protocol. The risk of selection bias affects both internal and external validity and therefore the participants in this study most likely do not represent the whole AD population. The strengths of **paper I-III** lie in the large sample sizes and comprehensive capture of those with AD, which enables representative groups that unlikely suffer from selection bias.

Information bias arises when collecting information on exposure or outcome. It cannot be corrected statistically. For example, if the variable is measured on a categorical scale and the error leads to a person being placed in an incorrect category, this is called misclassification (127). ***Misclassification*** can be ***differential*** or ***nondifferential***. Differential misclassification occurs when either the exposure is misclassified differentially according to a person's disease status, or the disease is misclassified differentially according a person's exposure. Differential misclassification can either exaggerate or underestimate an effect. In nondifferential misclassification either exposure or disease is misclassified, but the misclassification does not depend on a person's status for the other variable. This predominantly leads to bias towards the null, i.e. to an underestimation of the association between the exposure and the outcome. Misclassification can occur because of imprecise measurement of exposure, imprecise self-reports, missed/mistaken or wrong diagnoses/outcome.

Paper I and II may suffer from misclassification errors. For example, the AD diagnosis may have been wrongly reported to SNPR. This type of misclassification is most likely non-differential and therefore bias is probably towards null, which could underestimate the outcome measure. In **paper II** adjustment for rheumatoid arthritis was done to examine whether the relation between hip fracture risk and AD could be due to an inaccurate AD diagnosis in the patient register. Thereby, in cases when the AD was in fact iatrogenic we could identify it. However, this adjustment did not affect the risk estimates for hip fracture, indicating that the results were not affected by a falsely positive AD diagnosis. In **paper III** we used both the SNPR and SPDR to increase the accuracy of the AD diagnosis. Presumably, the risk of misclassification is thus smaller and hence there is an increased internal and external validity in this study

compared with **paper I and II**. Because the SPDR was not established until 2005, we did not have the possibility to use the same strict criteria in **paper I and II**. In **paper III** there is instead a potential risk that we excluded a number of "true" AD patients because we used such strict inclusion criteria. Some AD patients are treated with other glucocorticoids and some are not on fludrocortisone substitution. We believe that if we had included those patients we would have risked including patients with a false AD diagnosis in the SNPR. To prevent misclassification we chose to be restrictive in our inclusion criteria. At the same time, the condition that the AD patients should be alive after 1 July 2005 may have selected a subpopulation of AD patients with slightly less severe disease than the average patient. Hence, the risk estimates presented in our paper might underestimate the true risks.

Sensitivity and ***specificity*** describe the characteristics of a test in correctly classifying those who have or do not have a disease. Predictive value is a measure of the usefulness of a test in classifying people with disease. Predictive value is contingent on the prevalence of disease in the population being tested. ***Positive predictive value*** (PPV) is the probability that a person was truly exposed given that the person reported exposure, whereas ***negative predictive value*** (NPV) is the probability that the person was truly unexposed given the person reported no exposure. Evaluating the misclassification rates of the reported AD diagnosis compared with the "true" status obtained from 21-OH autoantibody analysis was done in a recent Swedish study (127). Using the same exclusion criteria as in **paper I and II,** the PPV was 79% and thus 79% of patients registered with an AD diagnosis in the SNPR were 21-OH antibody positive. The NPV was also 79%. Thus, 79% of patients reported not having AD in the SNPR were 21-OH antibody negative. Most likely, the PPV in the SNPR is higher as the 21-OH antibody titer may disappear after long duration of AD disease. NPV could also be higher than reported in that some of the patients with AD never required hospital care and were therefore only registered in the outpatient register.

Confounding refers to inseparability, i.e. when the effect of the exposure is mixed together with the effect of another variable. A confounder is a risk factor for the disease in the study and is associated with the exposure; it cannot be in the causal pathway between the exposure and outcome. As with other forms of bias, we can prevent confounding in the study design. Yet, unlike other types of bias we can control for confounding in the analysis of data: We can ***randomize, restrict*** or ***match*** the study participants and in the analysis ***stratify*** and conduct ***regression analysis***. In **paper I-III** we used matching and restriction in the study design and stratification and regression in the analysis of data to control for confounding. ***Residual confounding*** is the distortion that remains after controlling for confounding in the design, analysis of a study, or both. There are three causes of residual confounding: a confounding factor is not known, is not measured with sufficient precision or is not measured at all.

Matching makes groups directly comparable for potential confounders. In most studies four to five controls per case is considered enough and little statistical power is gained by further increasing this ratio. However, there are settings in which a higher control-to-case ratio may be desirable (e.g., when the cost of including additional controls is negligible and when there is concern for lack of a sufficient number of subjects in stratified analyses (127). Due to the latter reason, we chose 10 controls per AD case in **paper I-III.**

In **paper I** data were lacking on the prevalence of gestational diabetes in both the patient and control groups. Furthermore, we did not have any information about the quality of the endocrine management, either before, or during pregnancy in the patient group. Because maternal stress and socioeconomic status have been linked to preterm birth (128), there may be some residual confounding factors related to unmeasured social factors. A potential weakness of **paper II** was the lack of data on medication, especially because several studies have shown that different glucocorticoid replacement regimens have a different impact on bone turnover and BMD (88, 89). Nevertheless, it is notable that the risk of hip fractures in patients with AD was independent of the year of diagnosis, despite the likely temporal trend towards reducing doses of glucocorticoid replacement

therapy. Information on potential mediating factors and covariates (such as weight change, smoking, BMI, vitamin D status, thyroid status, sex hormone levels and the use of biphosphonates or hormone-replacement therapy) were unavailable for adjustment. Socioeconomic factors, such as educational level, income and occupation, have previously been shown to affect hip fracture risk (129). In **paper III** we were not able to adjust for dosage of hydrocortisone/cortisone acetate because of incomplete information.

Study design

In **paper I-III** the ideal study design would be a prospective follow-up study, but with such a rare disease as AD, it would take a long time and lead to a high cost. Therefore, the retrospective cohort study is the best option despite its limitations, including incomplete and missing data and problems with bias and confounding. Another option for the future would be to repeat the studies in **paper I-II** with an increased accuracy of the AD diagnosis using both the SNPR and SPDR.

In **paper IV** a double-blind study design would have been preferable. Initially, we aimed for this, given that an open label design is a limitation to any clinical trial. However, for numerous practical and economical reasons (unable to find a manufacturer able or willing to produce placebo for CSHI), we had to abandon this plan. To date no ideal double-blind placebo-controlled trial has been undertaken to show the benefit of insulin pump treatment either on metabolic parameters or on HRQoL in patients with type1 diabetes. Most likely, the metabolic parameters are not severely affected by placebo effects. A successful randomization of the study participants adjusts for all known and unknown confounders. The washout period of 2 months cannot completely exclude carry-over effects between the treatments, but this might be attenuated by randomization of the treatment sequence. The analyses in **paper IV** were further adjusted for treatment period and sequence together with their time interactions.

Randomized clinical trials, as in **paper IV**, can be analyzed by ***intention-to-treat*** or ***as-treated analysis***. In the latter analysis data from only subjects who complete the study and adhere to protocol requirements are analyzed. In intention to treat analysis data from all subjects, regardless of whether they completed the study and adhered to protocol requirements are analyzed. Intention to treat analysis avoids the effects of crossover and dropout that may violate the random assignment to the treatment groups in a study. It also estimates the treatment effect in real-world clinical practice, where patients do not always adhere to treatment regimens. On the other hand, intention to treat analysis can understate the treatment effect because a high rate of discontinuation may dilute the true treatment effect, which would decrease the power of the comparison. Data in **paper IV** were analyzed by as-treated analysis. After randomization, only a few patients (5/37) were withdrawn or dropped out of the study. However, a significant proportion of the patients (18/55) were excluded during the run-in phase. This loss was expected, CSHI is a demanding treatment and similar loss is seen with insulin pump treatment (130).

Precision

The error that remains after ***systematic error*** is eliminated is called ***random error*** and could be due to unexplained random variation in the data. This kind of error leads to lack of precision. P-value and CI obtain information about both precision and the size of our estimate. The width of the CI decreases as confidence increases, the sample size increases and the variability decreases. Thus, precision can be increased by large sample size such as in **paper I-III**. Precision can also be improved by efficient study design and methods resulting in more and better information per participant. The CI chosen was 95% in **paper I, II and IV** and 99% in **paper III** to attenuate the possible problem of multiple comparisons. Thus, we are 95 (and 99%) confident that the limits of interval cover the true value.

Power

Power is the probability to reject the false null hypothesis. The variation of the outcome parameters in AD and the expected effects of CHSI in **paper IV** were not known before this study, rendering power calculation difficult. While planning the whole study, sample size estimations were based on HRQoL data from cross-sectional studies. The effects observed in the pilot trial, indicating that a sample size of 40 would detect an improvement of 10 points on the SF-36 vitality scale with a statistical power exceeding 80%. However, we realized that HRQoL could not be chosen as the primary outcome measure because we planned to run an open-labeled study. We did not have pre-trial data to indicate the effects on ACTH, glucocorticoid metabolism or insulin sensitivity, but reasoned that these effects would be more pronounced and would be seen earlier than the effects on HRQoL. With the limitations in number of available patients and resources, we aimed for 40 patients, but eventually stopped inclusion after 37 patients. The limited number of participants in **paper IV** led to low statistical power and thereby risk of type II error.

6 INTERPRETATIONS

6.1 PARITY AND PREGNANCY OUTCOME IN WOMEN WITH AD (PAPER I)

Parity

The reason for the reduced parity in our study could be multifactorial. Having a chronic disease such as AD could affect women's desire to become pregnant. They could fear complications for themselves and their child during pregnancy. Patients with AD often suffer from concomitant autoimmune diseases. Overt autoimmune hypothyroidism and TPO autoantibody positivity are associated with a more than twofold increased risk of miscarriage (131). A study from Norway including 269 women with AD showed a reduced parity (40) even after excluding women with premature ovarian failure. It has been speculated that women with AD have low libido and impaired sexual function because of a lack of androgens. However, a survey from Norway found no reduced sexual activity in women with AD (40). Androgens are known to play a crucial role in normal folliculogenesis and a lack in androgens could consequently affect fertility in women with AD in a negative way (132).

Pregnancy outcome: low birth weight and preterm delivery

Relative adrenal insufficiency in undiagnosed women with AD with limited adrenal reserve may affect fetal growth, which could lead to a stressful environment for the fetus and increased fetal cortisol production. Fetal cortisol could stimulate CRH expression in the placenta inducing preterm labor.

The reason for low birth weight and preterm delivery after AD diagnosis could also be the opposite since excess glucocorticoids treatment can inhibit fetal growth and induce stress that could lead to preterm labor. Mediators or factors released in connection with the autoimmune immunoregulatory imbalance and cell destruction may negatively affect placental function and fetal growth (133, 134). Some of the women with undiagnosed or diagnosed AD could have had

TPO antibodies or undiagnosed autoimmune hypothyroid disease contributing to the higher preterm delivery.

Pregnancy outcome: caesarean delivery

Unfortunately, the Swedish Medical Birth Register does not separate elective and emergency caesarean sections nor does it specify the indications for a caesarean. Women with AD did not have increased rates of maternal complications (e.g., hypertensive diseases or antepartum bleedings), their infants did not have lower Apgar scores and no deaths among mothers or infants were recorded in relation to the delivery. Hence, maternal or neonatal complications evidently were not the underlying factors for performing the caesareans. The increased risk of caesarean deliveries is likely related to the strategy of the caring physicians who want the mother with AD to give birth in a well-controlled environment to avoid the risk of adrenal crisis.

6.2 HIP FRACTURE RISK IN PATIENTS WITH AD (PAPER II)

Increased hip fracture risk before and after AD diagnosis

Multiple disease-related factors before and at diagnosis, including decreased weight and weight-bearing physical activity, are known to negatively influence bone metabolic activity. A general decrease in well-being before and at the time of diagnosis may contribute to the increased fracture risk. A Norwegian survey showed that 15% of patients with AD on treatment had self-reported symptoms of postural dizziness and 12% had low blood pressure (135). Decreased postural stability could increase the risk of falls and fractures.

Another cause of the increased fracture risk both before and immediately after diagnosis could be the autoimmunity/inflammation per se. An optimal amount of cortisol is required for the differentiation of osteoblasts (70). Decreased cortisol production from the adrenal gland 1 year before AD diagnosis might oppose osteoblast differentiation, inducing a weaker bone and increased risk for fracture. A supraphysiological glucocorticoid substitution directly after AD diagnosis

could induce apoptosis in osteoblasts and osteocytes. The combination of a low cortisol concentration before diagnosis and a high dose of corticosteroid treatment in the first year after diagnosis could lead to a pronounced increase in the bone turnover rate, with a higher hip fracture risk as a consequence. Lack of adrenal androgens could also confer additional risks of OP and fractures.

Increased hip fracture in women with AD ≤ 50 years of age

The reason that women diagnosed with AD ≤ 50 years of age had higher risk for hip fracture than women diagnosed > 50 years could be multifactorial. This finding might imply that adrenal androgen deficiency in women with AD might be more important than previously thought. No study to date has evaluated fracture outcome in adrenal androgen-treated women with AD. Another reason could be premature ovarian failure associated with AD in approximately 10 % of women (135). Thus, by 50 years of age, women with AD are more likely to be postmenopausal than population controls, a fact that further could negatively affect their bone density.

6.3 PREVALENCE, INCIDENCE AND DRUG PRESCRIPTION PATTERN IN PATIENTS WITH AD (PAPER III)

Prevalence and incidence of AD

We found that the prevalence and incidence in patients with AD were almost identical to the high numbers reported from Norway (135). The numbers are higher than previously reported, indicating an increasing prevalence of AD (26, 136). This could be because of better diagnostics and treatment or a true change in incidence of AD.

Overall drug prescription pattern in patients with AD

Fifty-nine percent of patients with AD had medications indicating concomitant common APS-2 manifestations, which is in accordance with previous studies

(135, 137). The high frequency of thyroid disease emphasizes the need for repeated monitoring of the thyroid function in patients with AD.

A notable observation was that many patients seek medical attention for gastrointestinal discomfort both before and after diagnosis of AD. After diagnosis, patients with AD report symptoms of nausea, vomiting, abdominal pain and diarrhea according to recent studies from Norway and Germany (135, 138). A complete blood count is routine to exclude anemia as a cause of fatigue/lack of energy could explain the higher use of drugs for B12 deficiency, folic acid and iron preparations observed in our study. Another explanation is pernicious anemia, a condition known to be more common in patients with AD.

Both before and after diagnosis, patients with AD used more antibiotics commonly used for upper respiratory tract infections. One may expect that patients are more vulnerable to infections before the diagnosis of AD. After diagnosis it is likely that physicians initiate treatment for infection to avoid a potential adrenal crisis. Another explanation could be concomitant type 1 diabetes mellitus that is associated with more infections through defects in cellular immunity (139).

Previous studies have suggested that patients with AD have difficulties coping with emotional stress and increased anxiety (24, 140). An overall higher prescription of hypnotics and sedatives in patients with AD confirms this belief. In contrast, there was no increased prescription of antidepressants.

Drug prescription pattern before AD diagnosis

The reason for more prescriptions of corticosteroids other than hydrocortisone/cortisone acetate for systemic use before diagnosis is unclear. We studied the ICD codes in the SNPR in patients on synthetic corticosteroids ≥ 1 year before diagnosis and found diagnoses that included rheumatologic, pulmonary and inflammatory bowel diseases, which could represent misinterpretations of the usually vague symptoms of AD.

Another reason could be a bias, i.e. AD was suspected and initially treated with synthetic glucocorticoids before the date we defined as the date of diagnosis.

Drug prescription pattern after AD diagnosis

The prevalence of androgen prescription in women with AD was similar to the Norwegian data, where 5.3% of women with AD had dehydroepiandrosterone treatment (135). The higher androgen use in men with AD could possibly be explained by the higher prevalence of type 1 diabetes since these patients show a tendency to hypogonadism with lower free testosterone (141).

Patients with AD were prescribed more antihypertensive drugs than controls. Importantly, this increased use was evident in younger patients and persisted after exclusion of patients with concomitant diabetes treatment. In addition, lipid-lowering agents were prescribed more often to patients with AD both before and after diagnosis. Conventional glucocorticoid replacement doses could be too high (142) or delivered in a non-physiological pattern (143). This may result in excess glucocorticoid exposure, which is known to induce hypertension, obesity with abdominal fat distribution and altered glucose metabolism, all well-known independent risk factors for cardiovascular disease. Untreated androgen deficiency could be a contributing factor to high blood lipids after diagnosis. Concomitant autoimmune hypothyroidism is associated with high blood lipids and increased risk of atherosclerotic diseases (144). Thus the thyroid status inadequately monitored and treated may contribute to higher cardiovascular morbidity and explain the increased use of lipid-lowering drugs both before and after AD diagnosis.

6.4 CIRCADIAN HORMONE PROFILES AND INSULIN SENSITIVITY IN PATIENTS WITH AD (PAPER IV)

Cortisol-ACTH profile

The CSHI treatment successfully mimicked the circadian cortisol secretion, avoiding the peaks and troughs seen with conventional OHC treatment. The normalization of circadian cortisol levels was sufficient to return plasma ACTH to normal. The clinical significance of restoring the normal nighttime cortisol surge in patients with AD is not evident, but it might be important in achieving metabolic homeostasis.

The AUC for serum cortisol and cortisone was significantly higher with CSHI than with OHC, probably relating to the differences in cortisol and cortisone levels during the night. The two hydrocortisone administration modes (oral and subcutaneous) might result in differences in cortisol metabolism and elimination resulting in higher cortisol and cortisone AUC levels with CSHI.

Glucose profile

Studies in healthy persons have found endogenous circadian rhythms in glucose and insulin, with peaks around the usual time of awakening (103, 104). This glucose peak was achieved with CSHI treatment; however, with OHC treatment, there was a continuous fall in blood glucose during the night, reaching the lowest point at 0800 h. In fact, CSHI significantly improved glucose levels in the early morning compared with OHC, most likely as a direct effect of the higher cortisol levels at these time points.

Some patients with AD on conventional OHC therapy complain about fatigue, dizziness and concentration difficulties (135), especially in the early morning. These symptoms might represent neuroglycopenia. Since CSHI prevented low morning glucose levels it might become a therapeutic option to diminish the risk of nighttime hypoglycemia, particularly in AD patients with insulin-treated diabetes mellitus (115).

Patients with AD on OHC had a trend towards elevated late afternoon and evening cortisol levels compared with AD patients on CSHI. Plat et al. showed that elevation of plasma cortisol in the evening when the HPA-axis is normally quiescent had more deleterious metabolic effects than in increased morning cortisol (145).

Euglycemic-hyperinsulinemic clamp

Insulin resistance is important to evaluate because of its association with increased mortality. A cut-off value of < 6 mg/kg/min for the M-value is usually defined as insulin resistance (146). Both CSHI and OHC treatment groups had normal insulin sensitivity. Suliman et al. reported no impact of glucocorticoid dose or regimen on insulin sensitivity in patients with AD (147), findings consistent with ours.

GH/IGF-I/IGFBP-3 profile

A serum cortisol level > 700 nmol/l results in acute inhibition of GH secretion (101). One patient in our study reached this level with CSHI at 0800 h, whereas with OHC treatment, 6 of 10 patients had a peak cortisol response > 700 nmol/L after the morning dose and 4 of 10 patients after the midday dose. The important nocturnal GH peak was preserved in both treatment groups but was more pronounced in the CSHI group. In addition, the trend to higher IGF-1 and IGF-BP3 levels during morning hours suggests that CSHI might provide a more anabolic and physiological nighttime state.

7 CLINICAL IMPLICATIONS

The overall aim of this thesis was to study different clinical outcomes in patients with AD to enhance our knowledge of the disease and improve the quality of clinical management.

We have shown a reduced parity in patients with AD. The majority of women with AD had normal pregnancy outcomes, but still an increased risk of delivering children preterm and with low birth weight. This finding offers important clinical information. Women with AD should be informed about the reduced parity and carefully monitored before and through pregnancy.

The increased hip fracture risk suggests the need to adjust glucocorticoid therapy to the lowest possible dose. Risk assessment for OP and future fracture should be done in patients with AD. Patients should be educated about preventive measures such as the importance of physical activity, appropriate nutrition and cessation of smoking.

Our study on the prescription patterns in patients with AD raises serious concerns about unexpected comorbidity in the AD patient cohort. Gastrointestinal symptoms and anemia, especially in conjunction with autoimmune disorders, should alert the physician about the possibility of AD.

CSHI is a safe and reliable mode of glucocorticoid replacement and might become a treatment option in selected patients. Improved pump technology may attract larger groups of patients in the future, and avoidance of the oral route could be useful in patients with malabsorption or with frequent adrenal crisis due to gastrointestinal infections.

8 FUTURE PERPERSPECTIVES

A prospective multicenter study of pregnant women with AD, including clinical information about treatment, doses, concomitant diseases and antibody status could further enhance our knowledge about pregnancy outcome in these women. Studies of fertility preferences, social situation, nutritional status, menstrual pattern and antibody status would help to increase our knowledge on parity. It would also be worthwhile to study fertility in men with AD.

We found patients with AD to have a higher risk of hip fractures than controls. Well-designed prospective multicenter trials could elucidate the underlying mechanisms. Such information would add important knowledge about the role of glucocorticoid doses and regimens. Evaluating muscle strength, balance and risk factors for falling would also be important.

The use of more antihypertensive and lipid-lowering drugs in young patients with AD indicates a higher cardiovascular morbidity than previously known and needs to be further elucidated. A prospective study evaluating the cardiovascular risk profile in young patients with AD compared with matched controls would be beneficial in this regard.

Future CSHI trials should aim at replicating the full ultradian rhythm, which could possibly further improve outcome for patients with AD. Patients with type 1 diabetes on insulin pump treatment know quickly from capillary blood glucose if there is a problem with the pump. Development of an easy monitoring facility such as a bench top salivary or capillary blood cortisol assay is necessary if CSHI is to become a future treatment alternative.

9 CONCLUSIONS

•Women with an AD diagnosis have reduced parity as compared with controls. The majority of pregnancies were uncomplicated. There were no differences in risks of congenital malformations, infant or maternal death. Infants born to mothers before AD diagnosis had increased risk of preterm birth, low birth weight and low birth length. Infants born to mothers after AD diagnosis had increased risk of preterm birth and cesarean section.

•Patients with AD had an increased risk of hip fracture both before and after diagnosis. The highest risk was seen the year before and the year after AD diagnosis. Women diagnosed with AD before menopause (≤ 50 years of age) had a higher relative risk of hip fracture than women diagnosed after menopause.

•Patients with AD were prescribed more drugs both before and after diagnosis than controls. The drug prescription patterns were partly expected, but they also raise concerns about unexpected comorbidity in the AD patient cohort. The incidence and prevalence of AD in Sweden is comparable with the high figures reported in Norway.

•CSHI replacement reestablished a circadian cortisol rhythm with positive effects on circadian hormone profiles and nighttime glucose levels without compromising insulin sensitivity.

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