Mortality and Morbidity in Patients with Addison's Disease

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Gothenburg 2015

This thesis is dedicated to my mother,
Guðrún Hjörleifsdóttir
GUGGÚ
õAum Sri Sai Ramö

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ABSTRACT

Addison's disease (AD) or primary adrenal insufficiency is a rare disease with an estimated prevalence of 100-140 per million inhabitants and deadly unless treated with glucocorticoids (GCs). Very limited information is available on the morbidity and mortality in this patient group. A few old studies report near normal mortality and several studies indicate impaired bone health and reduced health-related quality of life (HR-QoL). It has been suggested that GC treatment, with both too high GC doses and a replacement regime which cannot replicate the physiological cortisol rhythm, may partly explain the impaired outcome in AD patients.

This thesis is based on studies with the main objective of studying mortality and morbidity in patients with AD receiving long-term GC replacement therapy. In a large nation-wide register-based study patients with AD had a more than two-fold higher mortality rate than the general population which was mainly explained by excess mortality from cardiovascular diseases, cancer and infectious diseases. The mortality was further increased among patients with AD who also had diabetes mellitus (DM).
In a case-control study, comparing AD patients to healthy controls matched for age, gender, BMI and smoking habits; cardiometabolic risk factors, visceral abdominal adipose tissue (VAT), bone health and HR-QoL were studied. The patients did not have increased VAT measured using computerized tomography, but a greater proportion of patients had the metabolic syndrome (MetS) and type 2 diabetes mellitus (T2DM). The patients had reduced bone mineral density (BMD) and an increased frequency of osteoporosis and osteopenia and patients using higher GC doses for replacement had increased risk of osteoporosis and osteopenia. Finally, using four different validated questionnaires we could demonstrate that the patients experienced more fatigue and had impaired HR-QoL.

In conclusion, patients with AD in Sweden have increased mortality, which is mainly explained by cardiovascular diseases. Despite compatible VAT between the AD patients and controls, the patients have an increased prevalence of MetS and T2DM, both of which are known to be related to increased cardiovascular risk. Patients with AD also have impaired bone health and reduced HR-QoL. The thesis strongly suggests that there is a need for improvement in the overall management of patients with AD.

Key words: Addison's disease, mortality, glucocorticoid(s), glucocorticoid replacement therapy, cardiovascular diseases, bone mineral density, osteoporosis, quality of life

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by the Roman numerals.

Paper I.   Bergthorsdottir R , Leonsson-Zachrisson M, Oden A, Johannsson G.
                **Premature mortality in patients with Addison's disease: a population-based
                study.** *J Clin Endocrinol Metab 2006; 91(12):4849-53*

Paper II.   Bergthorsdottir R, Ragnarsson O, Skrtic S, Ross IL, Leonsson-Zachrisson M,
                Johannsson G.
                **Visceral fat mass and cardiovascular risk factors in patients with Addison's
                disease: a case-control study.** *Manuscript.*

Paper III.   Bergthorsdottir R, Chantzichristos D, Skrtic S, Ragnarsson O,
                Johannsson G.
                **Patients with Addison's disease have decreased bone mineral density and
                increased prevalence of osteoporosis: a case-control study.** *Manuscript.*

Paper IV.   Bergthorsdottir R, Pappakokkinou E, Skrtic S, Ragnarsson O, Johannsson G.
                **Health-related quality-of-life (HR-QoL) is compromised in patients with
                Addison's disease: a case-control study.** *Manuscript.*

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ABBREVIATIONS

11 -HSD	11 -Hydroxysteroid dehydrogenase
ACTH	Adrenocorticotropic hormone
AD	Addisons's disease
ADDIQoL	Addison's disease-specific quality-of-life questionnaire
BMC	Bone mineral content
BMD	Bone mineral density
BMI	Body mass index
CBG	Corticosteroid-binding globulin
CEM	Centre for Endocrinology and Metabolism
CI	Confidence interval
CRH	Corticotropin-releasing hormone
CS	Cushing's syndrome
CSHI	Continuous subcutaneous hydrocortisone infusion
CT	Computed tomography
CV	Coefficient of variation
DEXA	Dual-energy X-ray absorptiometry
DHEA	Dehydroepiandrosterone
DM	Diabetes mellitus
DR	Dual release
FIS	Fatigue Impact Scale
FPG	Fasting plasma glucose
GC	Glucocorticoid
GR	Glucocorticoid receptor
GRE	Glucocorticoid response element
HbA1c	Glycated hemoglobin
HC	Hydrocortisone
HDL-C	High-density lipoprotein cholesterol
HOMA1-IR	Insulin resistance according to the homeostatic model assessment
HPA	Hypothalamic-pituitary-adrenal
hs-CRP	High-sensitivity C-reactive protein
HR-QoL	Health-related quality of life
HU	Hounsfield units
ICD	International Classification of Diseases
IQR	Interquartile range
IR	Insulin resistance
LDL-C	Low-density lipoprotein cholesterol
MetS	Metabolic syndrome
MSH	Melanocyte-stimulating hormone
NHP	Nottingham Health Profile
OGTT	Oral glucose tolerance test
P1NP	Procollagen type 1 aminoterminal propeptide
PAI-1	Protein tissue-type plasminogen activator-1
PGWB	Psychological General Well-Being Index
PTH	Parathyroid hormone
RCT	Randomized controlled trial
RR	Risk ratio
SAT	Subcutaneous adipose tissue
s-Ca	Serum calcium

SD	Standard deviation
SF-36	Short Form-36
SNBW	Swedish National Board of Health and Welfare
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
T4	Free thyroxin
TC	Total cholesterol
TG	Triglycerides
TSH	Thyroid-stimulating hormone
UFC	Urinary free cortisol
VAT	Visceral adipose tissue
WHO	World Health Organisation

1   INTRODUCTION

1.1   History of Addison's disease

*"The leading and characteristic features of the morbid state to which I would
direct attention are, anaemia, general languor and debility, remarkable feebleness of the
heart's action, irritability of the stomach, and a peculiar change of the colour in the skin
occurring in connexion with a diseased condition of the 'suprarenal capsules'..."*

In a monograph dated 1855, Thomas Addison, the British physician, reported six patients with symptoms of adrenal insufficiency and concomitant pathological changes in their suprarenal glands. He described a progressive disease with increased weakness, decreased appetite, body-wasting, and a weak pulse, with the condition ultimately being fatal. In 1856, the year after Addison's discovery, Trousseau named the disease "La maladie d'Addison" (1). The disease later became known as Addisons's disease (AD) and the symptoms described preceding death are typical in patients with Addisonian or adrenal crisis. Unfortunately, there was no treatment available for patients with AD, who remained untreated with a high mortality rate for about 100 years. According to a publication addressing survival in patients with adrenal insufficiency before cortisol became available in the 1950s (2), approximately nine out of ten patients died within 1 year following symptom onset and no patients survived more than 5 years.

The first positive attempt to treat adrenal crisis in humans was with an animal cortical extract in the 1930s (3). Salt was added thereafter to become part of maintenance therapy, with additional clinical and electrolyte (sodium and potassium) improvements (4). The search for more effective treatment for this chronic disease continued and, in 1937, desoxycorticosterone, a mineralocorticoid, was introduced, which influences sodium retention and potassium excretion (5). However, a dramatic improvement in survival did not eventuate until the adrenal corticoid steroid, cortisone [also called compound E (Kendall's compound)], was introduced: cortisone was first given to a patient with AD in 1948 (6).

"The response was prompt and striking." (6)

During this time, it had become clear that the adrenal glands were essential to life and that there was something, later known to be steroids, coming from the adrenal glands, which needed to be replaced in patients with AD. They had an effect on mineral metabolism (sodium, potassium, and water): however, it was apparent that replacement therapy with salt-retaining steroids was insufficient to stop patients from dying. The steroids also influenced carbohydrate metabolism. There was some mechanism which makes steroids vital in stressful situations (7).

A few years after the implementation of cortisone treatment for replacement therapy, the improvement in life expectancy was described by the words:

"í with care the expectation of life should not be shortened í " (2)

Shortly after the introduction of cortisone (compound E), cortisol (compound F) was produced and the pharmaceutical name became hydrocortisone (HC) (7). Nevertheless, old survival data for patients with AD still indicated an increased mortality, which mainly

occurred in undiagnosed patients, patients with psychiatric diseases, and patients living in poverty (8) . This confirmed the importance of the glucocorticoids (GCs) for survival.

1.2   Glucocorticoids

GCs (cortisol, cortisone) are steroid hormones produced from cholesterol in the adrenal cortex and their secretion is controlled through a negative feedback mechanism by the hypothalamic-pituitary-adrenal (HPA) axis. Cortisol exerts a negative feedback on the corticotropin-releasing hormone (CRH) and adrenocorticotropic hormone (ACTH) release from the hypothalamus and pituitary gland, respectively. However, low serum cortisol concentrations stimulate release of CRH and ACTH, resulting in increased cortisol secretion from the adrenals. In healthy subjects, the plasma concentration profile of cortisol demonstrates a strong diurnal variation with the highest concentrations early in the morning and lowest in the late afternoon and around midnight. During stressful conditions, cortisol production increases in order to meet physiological demands (9).

Cortisol is the biologically active form of the most important GC in humans. Only a small proportion of cortisol circulates in a free, active form in plasma. Cortisol is mainly transported bound to transcortin, corticosteroid-binding globulin (CBG). The peripheral metabolism of cortisol and, thus, its tissue availability is regulated at a tissue-specific level by two isoforms of the enzyme 11 -hydroxysteroid dehydrogenase (11 -HSD) 1 and 2. 11 -HSD-1 regulates the conversion of the inactive metabolite, cortisone, to the active hormone, cortisol, in the liver, adipose tissue, and the central nervous system. 11 -HSD-2 (expressed particularly in the kidney, colon, and salivary glands) inactivates cortisol to cortisone and prevents cortisol binding to the mineralocorticoid receptor. This inactivation of cortisol is of most importance in the kidneys where it prevents abnormal mineralocorticoid activation with resulting hypertension and hypokalemia (10).

GCs exert their genomic effects via the glucocorticoid receptor (GR), an intracellular nuclear receptor. The building of the GC-GR complex enables interaction with the glucocorticoid response elements (GREs) in DNA with subsequent activation or repression of transcription and is characterized by slow onset and response. Repression of transcription mediates many anti-inflammatory GC-mediated effects while transactivation is thought to be responsible for many of the metabolic effects (11). Data on non-genomic effects acting faster on a membrane and at an enzymatic level is appearing but these mechanisms are still poorly understood (12).

1.3   Addison's disease

1.3.1   Epidemiology

In AD (primary adrenal insufficiency), the adrenal cortex is affected with impaired production and secretion of GCs (cortisol being the most important), adrenal androgens, and mineralocorticoids (aldosterone) (13). Adrenal insufficiency has multiple etiologies but autoimmune adrenalitis is the most common cause of AD in Europe today where the disease has a reported prevalence of approximately 90-140 per million, with the highest prevalence reported from Scandinavia (14-17). Most patients are diagnosed in the fourth decade of life and women are twice as likely to be affected as men (13,18).

1.3.2 Diagnosis

The clinical state results in fatigue, nausea, loss of appetite, salt craving, weight loss, and muscle, joint, and abdominal pain. Skin hyperpigmentation and postural hypotension may occur as well as biochemical disturbances (18). Hyponatremia, hyperkalemia, hypoglycemia, hypercalcemia, increased thyroid-stimulating hormone (TSH), and anemia have all been described, and women may experience reduced libido (13). If unrecognized, patients develop acute adrenal crisis with abdominal pain, vomiting, fever, hypotensive shock, tachycardia, unconsciousness, and death (13). Diagnosis is made by measuring early morning serum cortisol and ACTH. If serum cortisol is inappropriately low according to the local laboratory references and accompanied by increased ACTH, a confirmatory test, the short cosyntropin (Synacthen) test, is often performed. The functional capacity of the adrenal cortex can be measured by injecting synthetic ACTH (Synacthen), which stimulates the adrenal cortex to produce cortisol. A cortisol response less than 500 nmol/L is diagnostic of AD (19). ACTH and melanocyte-stimulating hormone (MSH) share the same precursor hormone, pro-opiomelanocortin, and in cortisol deficiency with increased ACTH, ACTH and MSH stimulate the melanocyte receptors in the skin, explaining the hyperpigmentation in many patients with AD (13).

About 85% of patients with newly diagnosed adrenal insufficiency in Scandinavia have autoimmune disease with positive autoantibodies against the adrenal cortex, mostly 21-hydroxylase (18,20). If autoantibodies are negative, other etiologies such as infection (tuberculosis, HIV), adrenal hemorrhage, or genetic disorder should be suspected and further investigations are needed. As in other autoimmune diseases, AD is often accompanied by concurrent autoimmune disease. In a Norwegian study on patients with AD, autoimmune polyendocrine syndrome 2 with associated thyroid disease was reported in 47% of the patients and 12% had concomitant type 1 diabetes mellitus (T1DM) (18).

1.3.3 Treatment

The conventional therapy for AD, is replacement therapy with an oral GC and a mineralocorticoid. The aim of the GC therapy is to mimic the physiological circadian rhythm of cortisol, to respond to the increased cortisol requirement during various stimuli and to obtain normal metabolism with improved long-term outcome (13). Patients are educated to increase their GC dose in the case of concomitant illness or stressful situations and to seek care for rescue therapy with intravenous GC and saline infusion if they become acutely ill, are unable to take or retain their GC tablets, or are about to be exposed to major physical stress (surgery, birth, etc.). Patients are requested to carry a medical emergency card for patients with adrenal insufficiency that can guide healthcare personnel in their treatment of acute illness (21). Studies on the importance of adrenal androgen replacement in females with adrenal insufficiency have been inconsistent and routine supplementation therapy is not advised given the absence of efficiency and safety data (22).

1.4 Effects of glucocorticoids

The GC receptor is present on almost all cells in the human body. GCs therefore have diverse effects in the body including regulation of intermediary metabolism and the immune response. The immune-modulating effects of GC will not be discussed in this thesis but intermediary metabolism in the physiological state and during cortisol excess will be given consideration.

1.4.1   Body composition and cardiometabolic risk factors

Energy homeostasis in mammals is maintained with GCs, particularly carbohydrate metabolism with the availability of energy (glucose) through gluconeogenesis, lipolysis, and proteolysis. GCs promote increased hepatic glucose production (gluconeogenesis) and decreased insulin-mediated uptake of glucose in skeletal muscles, thereby increasing the availability of glucose in the circulation (insulin antagonist effect). However, GC excess by these mechanisms can result in impaired glucose tolerance, hyperglycemia, or type 2 diabetes mellitus (T2DM) (23).

Furthermore GCs help the body to mobilize energy substrates from triacylglycerides in fat depots through lipolysis during stress. These catabolic effects mediated by GC-GR are well known but the mechanisms favoring lipid accumulation, such as the development of abdominal obesity, are less understood (24).

GR expression shows regional differences in the human body with increased expression in the abdominal region that may explain the central obesity with visceral fat accumulation seen in GC excess. In addition, tissue-specific intracellular activation of GC by 11 -HSD-1 has been associated with increased visceral fat accumulation as the expression of this enzyme seems to be increased in visceral fat depots as compared to subcutaneous fat. In addition, the GC-mediated increase in lipoprotein lipase activity and/or the GC-mediated increase in appetite and increased food intake could also be explanatory or complementary factors. Secondary to the excessive visceral fat, high amounts of free fatty acids can be released into the circulation, causing lipid dysregulation (23-25).

To sum up, the long-term excessive and unphysiological levels of cortisol result in metabolic disorders with fat redistribution, central obesity, hyperglycemia, and dyslipidemia. Whether the metabolic consequences in the form of hyperglycemia and dyslipidemia are a direct effect of GC or are only secondary to the long-term effects on body fat distribution has not yet been elucidated.

Physiological levels of GC do not appear to influence muscle metabolism. However, high GC levels cause proteolysis with skeletal muscle wasting (23).

Hypertension and hypokalemia may occur secondary to activation of the mineralocorticoid receptor by high cortisol levels (10).

All the negative metabolic consequences described here can be seen in patients with endogenous cortisol excess, Cushing's syndrome (CS) (26).

1.4.2   Bone

There is a constant modeling and remodeling in healthy bones. GCs influence bone homeostasis through direct effects on bone-reforming and bone-resorbing cells, osteoblasts and osteoclasts, respectively (27). Decreased osteoblast production and their shorter life span may explain reduced formation of bone, and increased osteocyte apoptosis might deteriorate the bone microarchitecture, making the bone more fragile. In addition, there is reduced osteoclast production, which is accompanied by a longer half-life for osteoclasts: the importance of this finding has not yet been elucidated. Overall, however, GCs in excess disturb the balance of bone formation in a biphasic way with excessive bone resorption

accompanied by impaired bone formation (28). They also have a negative influence on calcium and vitamin D metabolism with decreased calcium absorption from the intestine and a secondary rise in parathyroid hormone (PTH), stimulating osteoclast-induced bone resorption. In addition, the gonadal axis is suppressed with reduced production of sex hormones, estrogen and testosterone (29). This results in a GC-induced loss of bone with secondary osteoporosis as seen in patients with CS who have increased risk of osteoporotic fractures with vertebral fractures being the most common (30).

1.4.3   Brain

GCs are important for adequate memory processing and the hormonal actions in the brain are mediated through two types of receptors, the mineralocorticoid receptors mainly located in the hippocampus and the glucocorticoid receptors in the hippocampus and the frontal lobes. The hippocampus is part of the limbic system in the brain located deep in the temporal lobe and responsible for declarative and spatial memory and the frontal lobes at the front of the brain are responsible for working memory and emotions (31). Patients with CS show memory dysfunction and reduced hippocampal volume in patients with CS has been associated with increased serum cortisol concentrations (32).

1.4.4   Morbidity and mortality

Published data on morbidity and mortality in patients with AD who receive long-term replacement therapy with glucocorticoids is sparse. The mortality data is briefly reviewed in the historical part of this thesis. Data on morbidity in patients with AD receiving long-term replacement therapy was limited at the initiation of this thesis, but indicated reduced bone mineral density (33-37) as well as impaired health-related quality of life (HR-QoL) (38).

2 AIMS

The main objective of this thesis was to study mortality and morbidity in patients with AD receiving GC replacement therapy.

Specific aims were:

- To study the mortality rate in patients with AD.

- To study body composition by measuring visceral adipose tissue (VAT) and to determine the prevalence of metabolic syndrome (MetS) in patients with AD.

- To study bone mineral density and the prevalence of osteoporosis and osteopenia in patients with AD.

- To study self-reported HR-QoL and general well-being in patients with AD.

3 SUBJECTS AND METHODS

3.1. Paper I

3.1.1 Study design, subjects, and methods

This was a retrospective, observational, population-based study aimed at investigating the risk ratio (RR) for mortality in patients with AD in Sweden.

Patients with a diagnosis of AD and/or adrenal crisis from 1987 to 2001 were identified from the Swedish National Hospital Register by using the system for International Classification of Diseases (ICD). We searched for codes 255 E (AD, Addisonian crisis, ICD-9), E 27.1 (primary adrenocortical insufficiency), and E 27.2 (Addisonian crisis, ICD-10). Each patient was followed from the first registered hospitalization where the codes 255 E, 27.1, or 27.2 appeared until end of follow-up or death. The unique, personal identification code ensures that each individual is only counted once. Patients were also coded for age, sex, and death date in order to be able to define the cause of death that was obtained from the National Cause of Death Register. Concomitant diabetes mellitus (DM) at the time of identification was registered as a potential confounder related to mortality. Patients diagnosed with CS [255A (ICD-9), E 24 (ICD-10)] or pituitary disease [253 (ICD-9), E 23 (ICD-10)] during the study period were excluded.

The quotient between the number of observed deaths and expected deaths was used to calculate the RR for mortality after hospitalization for primary adrenocortical failure or Addisonian crisis. The RR was then compared with that of the general Swedish population considering age and calendar date, with separate analysis for men and women. The background population was the whole population of Sweden during the period 1987-2001 for which there is a hazard function of death depending on age, sex, and calendar date.

The Swedish National Board of Health and Welfare (SNBW) has well established registers based on the favorable epidemiological conditions in Sweden. We applied similar techniques to data collection and processing as previously described in Swedish surveys (39,40). The intention was to include all patients with AD in Sweden from 1987-2001 (a 15-year period).

The SNBW does internal validation on their databases (registers) by comparing listed diagnosis with information in medical records. Ninety-nine percent of all hospital admissions are registered in The National Hospital Register and the rate of miscoding reported during quality control checks was less than 8.3% (41). More than 99% of deaths are reported in the Cause of Death Register and the frequency of miscoding is 1.2ó6.3% (42).

A local hospital register was used to validate the selection criteria (ICD-codes) used in the national study. Patients with primary adrenocortical insufficiency, Addisonian crisis, drug-induced adrenocortical insufficiency, and other and unspecified adrenocortical insufficiency (ICD 10 codes E 27.1ó4) who received inpatient or outpatient medical care between 1999 and 2003 were identified and their medical records reviewed.

We chose a broader search strategy in the local register to estimate the proportion of patients with AD who were not identified in the national study as well as the proportion incorrectly classified with Addisonian crisis or primary adrenocortical insufficiency.

With the broader search criteria, 122 patients were identified, of whom 105 were included in the analysis. The remaining 17 patients were excluded due to secondary and tertiary adrenal insufficiency (n=2 and n=3, respectively), Waterhouse-Friedrichsen syndrome (n=1), and adrenalectomy due to CS or malignant disease (n=4 and n=2, respectively). In addition, two patients were misclassified and three had insufficient medical records. As a result, seven (6%) or six (5%) of the subjects according to ICD 9 and ICD 10 coding, respectively, could have been incorrectly included in our national cohort.

3.2   Papers II-IV

3.2.1   Study design and subjects

This was a cross-sectional, single-center, case-control study performed at the Centre for Endocrinology and Metabolism (CEM), Sahlgrenska University Hospital, Gothenburg, Sweden. Subject enrolment was between 2005 and 2009. All participants were studied during a single visit.

Information regarding medical history, current medical conditions, and concomitant medications were obtained; physical examination performed; and blood samples collected. Body composition [bone mineral density (BMD) and VAT] was estimated with dual-energy x-ray absorptiometry (DEXA) and computed tomography (CT). Four validated questionnaires were used to measure HR-QoL and general well-being.

Patients from western Sweden with a diagnosis of primary adrenal failure at the age of 18 years or older were invited to participate. In Gothenburg, patients were identified using the Hospital Diagnosis Register. Additionally, endocrinologists in four local regional hospitals asked suitable patients at their clinics to participate. All eligible patients were invited to participate at their regular clinical visit or by an invitation letter. Patients with adrenal insufficiency secondary to a pituitary disease, those who were receiving or had recently been treated with GC, and those with any severe diseases that would interfere with successful participation were excluded.

Healthy controls were recruited from the Swedish Population Registry. Randomly selected individuals matched for age and gender from the Gothenburg region were asked to participate in the study by an invitation letter. Responding subjects were included who additionally matched the patients according to smoking habits and body mass index (BMI).

Ethical approval for the study was obtained from the Ethics committee at the University of Gothenburg. Informed consent was obtained from all participants and the study was conducted in accordance with the Declaration of Helsinki.

3.2.2   METHODS

Blood pressure and anthropometrical measurements

Blood pressure was measured indirectly with a mercury sphygmomanometer using a standard cuff with the subjects in a seated position after resting for 5 minutes. The mean of three

measurements at 1-minute intervals was used. Height was measured to the nearest 1 cm and weight to the nearest 0.1 kg with the subjects barefoot and wearing light indoor clothing. Waist circumference was measured with a soft tape at the umbilical level.

Definition of metabolic syndrome, osteopenia, and osteoporosis

MetS was defined according to International Diabetes Federation (IDF) criteria published in a consensus statement in 2006 (43). Osteopenia was defined as a T-score between ó1.0 and ó2.5 and osteoporosis as T-score Öó2.5 according to the World Health Organisation (WHO) 1994 diagnostic criteria (44).

Blood and urine sampling

Fasting blood samples were collected between 8-10 AM and included: hematology, chemistry, creatinine, liver enzymes, glucose, insulin, glycated hemoglobin (HbA1c), lipids, high-sensitivity C-reactive protein (hs-CRP), protein tissue-type plasminogen activator-1 (PAI-1), adiponectin, serum cortisol, TSH, free thyroxin (T4), parathyroid hormone (PTH), serum calcium (s-Ca), and procollagen type 1 aminoterminal propeptide (P1NP). Patients with AD were instructed to take their usual morning HC dose at home with a glass of water prior to the blood sampling at the clinic. Urine was collected over 24 hours and used to perform analyses of urinary free cortisol (UFC). The completeness of the urine collection was estimated from creatinine measurements.

Insulin resistance (IR) was calculated according to the homeostatic model assessment (HOMA1-IR) from fasting plasma insulin and glucose concentrations (45). Oral glucose tolerance test (OGTT) with a 75-g glucose loading was performed in the morning after an overnight fast. The subjects were asked to refrain from tobacco use and alcohol intake for at least 12 hours prior to the visit according to the OGTT protocol. Fasting blood samples were collected prior to and 30, 60, 90, and 120 minutes after the oral glucose load for glucose and insulin determination. Patients with T1DM (n=5) did not perform the test. Determination of serum total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were run in one batch for all subjects at the end of study using enzymatic methods with within-assay coefficients of variation (CV) of 3-5%. Serum cortisol was measured using competitive an electro-chemiluminescence immunoassay (Cortisol Elecsys, Roche Diagnostics Scandinavia AB, Bromma, Sweden) and UFC using a radioimmunoassay (SpectRia Cortisol 125I, Orion Diagnostica, Espoo, Finland).

Body composition

DEXA (GE Lunar Prodigy, Scanex, Helsingborg, Sweden) was used to evaluate total bone mineral content (BMC, kg) and BMD (g/cm^2) at the lumbar spine (L1-4) and hips. After transforming the BMD to T- and Z-scores, the combined NHANES/Lunar (v112) reference population (white race) for the region was used. The T-score reflects the value compared to that of healthy control subjects (at their peak BMD) whereas the Z-score is compared to that of age- and sex-matched subjects (46) . The coefficients of variation for BMC (whole body), BMD for lumbar spine (L1-4), and BMD for right and left hips were 1.4%, 1.4%, and 1%, respectively. With respect to the equipment used for measurement, quality controls were

performed according to the manufacturer's protocol and, in addition, phantom measurements were performed every week.

DEXA is the standard method to evaluate BMD (47,48) and it can also be used to evaluate body composition (49). It is a non-invasive, easily applied, well-validated, cost-effective method where radiation exposure is low. It represents a three-compartment model where attenuation of radiation (x-rays) at two energy levels is used to differentiate between two components of the attenuating tissue, fat and fat-free mass. The fat-free mass can be divided into bone mineral and lean tissue mass (49).

When measuring areal BMD and not volumetric BMD, there is a risk of BMD overestimation in individuals with large bones and in those with osteoarthritis or fractures, contributing to a falsely high bone density. Falsely low BMD may, on the other hand, be the result of osteomalacia. In addition, DEXA cannot be used to differentiate between cortical and trabecular bone (47) and bones containing a higher percentage of trabecular bone seem to be more vulnerable for GC-induced osteoporosis (29).

CT [General Electric High Speed Advantage CT system (HAS), version RP2, GE Medical Systems, Milwaukee, WI] was used to estimate regional body fat, lean body mass, and abdominal subcutaneous and visceral fat mass. CT scanning was conducted with subjects in a supine position. The voltage was 120 kV and slice thickness 5-mm. A dose reduction protocol (50) was used and the total absorbed dose was calculated to be less than 0.8 mSv. The 4th lumbar vertebra level (L4) was used to determine VAT and subcutaneous adipose tissue (SAT), and the mid-thigh region was used to determine subcutaneous and the intermuscular adipose tissue as well as the cross-sectional area for the thigh, with a CV of <1% for both the fat and muscle area. In brief, the x-rays produced during CT imaging penetrate the study object, are assembled by a detector, and the pixels are transformed into attenuation values expressed as Hounsfield units (HU). HU for air is set to 1000 and water is 0 HU. This generates a tomographic image for which tissue area (cm^2, single image) with different attenuation can be measured. We used a previously described multicompartment body composition technique and pixels with Hounsfield values from -190 up to -30 were defined as adipose tissue (51). Hepatic fat content was determined by the attenuation of the liver compared to the spleen in HU units.

CT has been applied for several years to estimate body composition (51-53). The advantage of CT compared to DEXA is that adipose tissue distribution can be studied with separation of subcutaneous and visceral components (54,55). Visceral adipose tissue is of interest as it is strongly related to the risk of cardiovascular disease (56). The main disadvantage by using CT is the exposure to ionizing radiation; however, by using a method with reduced radiation dose (50), the risks are markedly reduced for the patients. Also, CT is costly and time consuming.

Assessment of self-reported HR-QoL and general well-being

Three questionnaires, the Nottingham Health Profile (NHP) (57), the Psychological General Well-Being (PGWB) index (58), and the Short Form-36 (SF-36) (59,60) were used for measurements of HR-QoL and self-reported health. The Fatigue Impact Scale (FIS) (61,62) was used to measure fatigue. NHP and SF-36 are well-known generic questionnaires and PGWB is a dimension-specific questionnaire. These are all well-known questionnaires, which have been translated and validated in Swedish. After instructions from experienced study staff, the participants answered the questionnaires between 8-10 AM on the day of the study visit.

An AD-specific quality-of-life questionnaire (ADDIQoL) with good correlation to vitality and general health in SF-36 and to the PGWB index has been developed (63). Consistent with previous findings, patients with AD in Norway have worse outcome measured by the ADDIQoL than the general background population (64). It would have been interesting to use this disease-specific questionnaire, ADDIQoL, but this instrument was not available when this study was designed.

NHP

The NHP has two parts. In the first part, information on sleep, physical abilities, energy level, pain, emotional reactions, and social isolation is obtained. Part two includes seven complementary questions on areas of daily life that impaired health may affect. The answers to part one are assigned appropriate weightings and the sum of the weighted values in each subarea adds up to 100, with higher scores implicating more severe problems. The Swedish version of NHP as well as the original questionnaire have established validity and reliability (65).

PGWB index

The PGWB index evaluates self-perceived well-being and psychological health with the advantage of measuring mental health. The index includes six domains: anxiety (5 items), depressed mood (3 items), positive well-being (4 items), self-control (3 items), general health (3 items), and vitality (4 items). Each item has scores on a 1-6 scale (total score range 22-132) and the results are expressed as a summary of scores, with higher score representing better well-being. It is well validated and reliable, and has been adapted in Swedish (58).

SF-36

SF-36 is a widely used questionnaire that has been evaluated across numerous patient populations. It consists of a 36-item scale including eight health domains: 1) limitations in physical activities (10 items); 2) limitations in social activities (2 items); 3) limitations in physical role activities (4 items); 4) bodily pain, 5) general mental health (5 items); 6) emotional limitations in usual role activities (3 items); 7) vitality (4 items); and 8) general health perceptions (5 items). The scoring system is from 0 to 100, with higher values reporting better health. SF-36 has been translated into Swedish and has good validity and reliability (60,66).

FIS

FIS includes 40 questions evaluating perceived limitations in cognitive (10 questions), physical (10 questions), and psychosocial (20 questions) function caused by fatigue. Each question has scores from 0-4, with higher scores indicating a more severe degree of fatigue. FIS has good validity and reliability (60) and has acceptable cross-cultural stability when used in a Swedish population (61,62).

3.3 STATISTICAL ANANLYSIS

3.3.1 Paper I

Statistical analysis can be divided into two major categories. The risk of hospital admission in Sweden resulting from AD as the main diagnosis and the risk of death after the diagnosis of AD. The chi-square test was used to assess if there was a difference between regions in the detection of AD as the main diagnosis at hospitalization. A Poisson model (39,67) was used to estimate the risk of AD with respect to latitude. Furthermore, it was used to study whether there was clustering with respect to calendar date, communes, and/or calendar date and year of birth. Such types of clustering might emerge if AD was to some degree dependent on an infection, with varying numbers of infections over the years or during early childhood. The expected number of deaths were calculated for men and women separately, taking age and calendar date into account. Patients were compared to the age- and sex-matched Swedish background population. Comparisons between observed and expected numbers of deaths were performed using Poisson distributions, which were also applied to the calculation of the 95% confidence intervals (CI) of RRs. Poisson regression was also used to estimate the hazard function of death as a continuous function of time from the initial diagnosis of AD and depending on the presence of DM at the time of diagnosis. This was done to investigate the impact of DM on outcome. We have used the term "hazard ratio" for the quotient between two hazard functions and RR for the quotient between observed and expected number of deaths. The two terms essentially reflect the same function. All tests were two-tailed.

3.3.2 Papers II-IV

SPSS (IBM® SPSS® 22.0, Somers, NY) was used for statistical analyses. Data were presented as mean values ± standard deviation (SD) or median with the interquartile range (IQR). Group differences were compared with independent samples t-test for normally distributed data and Mann-Whitney U-test for non-normally distributed data. For differences in proportions, Pearson chi-square or Fisher's exact tests were used. All statistical tests were two-tailed, and P-values <0.05 were considered to be statistically significant. To study the influence of cortisol exposure (Urine-Cortisol/creatinine estimate) and HC dose on VAT and MetS, respectively, we used multiple logistic regression analysis after adjustment for age, gender, and weight. To take advantage of the matched controls, a paired test design was used when comparing HR-QoL and FIS questionnaires. The matched study design allows for both independent t-test and paired samples test. The advantages of the independent t-test is that the risk of type 1 error with falsely positive results is minimized, which is reasonable when studying VAT, a previously unknown outcome. To increase the probability of detecting significant differences in HR-QoL, we choose a paired design to increase the power.

4 RESULTS

4.1 Paper I

A total of 1675 patients with AD were identified in the National Hospital Register between 1987ó2001 (Table 1). Mean age (±SD) at initial identification was 52.8±22.0 years. Mean follow-up was 6.5 years: 3.4 years for the deceased and 7.9 years for those who survived during the observation period. The geographical distribution of AD within Sweden did not differ significantly considering the number of cases reported between regions or with respect to latitude (*i.e.* a north-to-south gradient). No clustering was found with respect to calendar date and communes and/or calendar date and year of birth.

Table 1. The number (N) of men and women with Addisonøs disease obtained from The National Hospital and Cause of Death Registers at the SNBW during the period 1987ó2001

	All (N) subjects	Subjects (N) with DM	Percentage with DM	N of deaths
Men	*680*	*86*	*12.6*	*208*
Women	*995*	*113*	*11.4*	*299*
Total	*1675*	*199*	*12*	*507*

The number and percentage of patients with concomitant diabetes mellitus (DM) at the time of detection is also shown. *Abbreviations:* N: number, SNBW: Swedish National Board of Health and Welfare, DM: diabetes mellitus.

4.1.1 Mortality

During the time period 1987ó2001, 507 deaths were observed compared to 199 expected (Table 1). The RR for death was 2.19 (95 % CI 1.91-2.51) for men and 2.86 (95% CI 2.54-3.20) for women (Fig 1). Deaths occurring from cardiovascular (n=239) and malignant (n=73) diseases were most prevalent followed by endocrine (n=64), respiratory (n=45), and infectious (n=12) diseases. The RR for death from cardiovascular and malignant diseases was increased in both men and women (Fig 2). The most common cardiovascular cause of death was ischemic heart disease (n=133). We found no clustering of cancer type or cancer from a specific organ system. There was increased risk of death from infectious disease in both men and women. Thirty-five patients died from infection, the majority (66%) from pneumonia, and one death related to tuberculosis was listed. Of the 36 patients reported to have died from adrenal insufficiency, five died within 2 days and five within 3 weeks of hospitalization. The ICD 9 classification does not distinguish between primary adrenal insufficiency and adrenal crisis but no patient was reported to have died from adrenal crisis according to ICD 10. The increased risk of death among patients with AD was most pronounced close to the initial detection in the register.
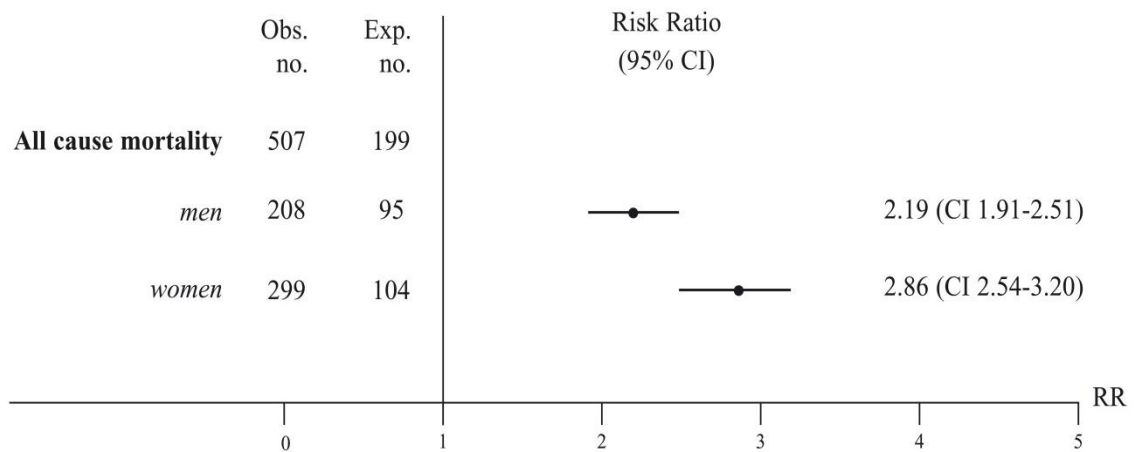
	Obs. no.	Exp. no.	Risk Ratio (95% CI)	
All cause mortality	507	199		
men	208	95		2.19 (CI 1.91-2.51)
women	299	104		2.86 (CI 2.54-3.20)

Figure 1. The Risk Ratio and 95% CI for all-cause mortality in patients with Addison's disease in Sweden from 1987–2001. This was calculated for men and women separately, taking age and calendar time into account. Obs. no., observed number; Exp. no., expected number; CI, confidence interval.
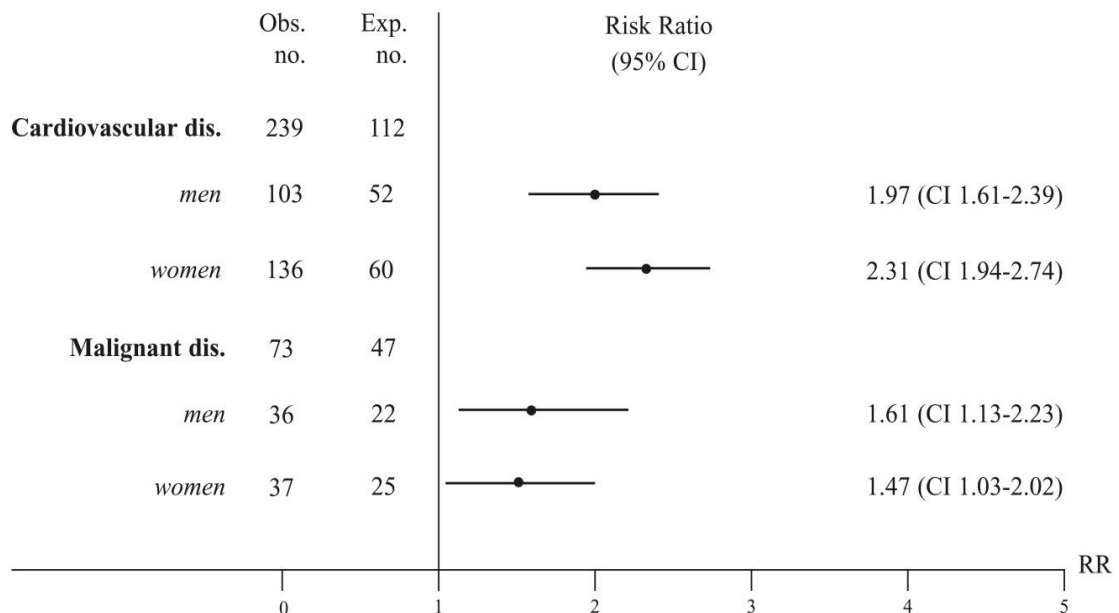
	Obs. no.	Exp. no.	Risk Ratio (95% CI)	
Cardiovascular dis.	239	112		
men	103	52		1.97 (CI 1.61-2.39)
women	136	60		2.31 (CI 1.94-2.74)
Malignant dis.	73	47		
men	36	22		1.61 (CI 1.13-2.23)
women	37	25		1.47 (CI 1.03-2.02)

Figure 2. The Risk Ratio and 95% CI for cardiovascular mortality and mortality from neoplastic disorders in patients with Addison's disease in Sweden from 1987–2001. This was calculated for men and women separately, taking age and calendar time into account. Obs. no., Observed number; Exp. no., expected number; CI, confidence interval.

20

4.1.2   Impact of concurrent DM

Concomitant diagnosis of DM at the time of the initial identification in the register was 12.6% for the men and 11.4% for the women. When men and women with AD and DM were compared to those patients with AD but without DM, the RR for death was 1.82 (95% CI 1.29-2.06) and 1.52 (95% CI 1.11-2.07) for men and women, respectively. Thus, DM had a significant impact on total mortality in both genders. However, the impact of DM on the excess mortality in the whole group of patients with AD was limited as the RR of death for patients with AD without DM was 2.04 (95% CI 1.74-2.37) and 2.68 (95% CI 2.36-3.04) for men and women, respectively, when compared to the background population. Accordingly, a 7% lower risk of death was observed for men and women without co-existing DM.

4.2   Papers II-IV

4.2.1   Subject characteristics

The clinical characteristics of the patients and controls are shown in Table 2. Mean (±SD) age was 53±14 years and the mean duration of AD was 17±12 year. Mean BMI was 25±4 kg/m^2. The cohorts did not differ with respect to smoking habits. Median daily HC and fludrocortisone doses were 30 mg (range 10-50 mg) and 0.1 mg (range 0-0.2 mg), respectively. Most patients (71%) received HC twice daily. Forty-five percent of the patients and 3% of controls had treated hypothyroidism (*P*<0.001). Antihypertensive medications were more common in the patients compared to controls (21% *vs* 5%, respectively; *P*=0.004). Thirty-three (65%) women with AD and 33 control women were postmenopausal (×52 years of age). Two (4%) women with AD reported premature menopause. Six (12%) women with AD and three (6%) controls were on oral estrogen therapy (*P*=0.5) and seven (14%) women with AD had dehydroepiandrosterone (DHEA) substitution. Four (5%) AD women, no men with AD, and no controls received bisphosphonate treatment (*P*=0.12). Serum cortisol, 24-hour UFC, and urinary cortisol/creatinine estimates were higher in the patients than in controls (*P*<0.001 for all variables).

4.2.2   VAT and the prevalence of MetS

Median (IQR) VAT did not differ between the patients and controls [76 (93) *vs* 71 (92) cm^2, respectively; *P*=0.7] (Fig 3). Neither did abdominal SAT, thigh intermuscular AT, thigh SAT, nor thigh muscle area (Table 3). No correlation was found between VAT and dose of HC expressed as mg per day. Liver attenuation was increased in the patients compared to controls [59 (4) *vs* 57 (7) HU, respectively; *P*=0.03].

The criteria for MetS were fulfilled in 25 patients (33%) and 12 controls (16%) (*P*=0.02). No association was found between the dose of HC or UFC and the presence of MetS after adjustment for age, weight, and gender in logistic regression analysis.

Table 2. Clinical characteristics of patients with Addisonøs disease and their matched controls (mean ± SD, (%) or median (IQR))

Characteristics	Patients	Controls	P
N	76	76	
Age years	53.2 ± 13.9	53.7 ± 14.0	0.8
Female *N* (%)	51 (67%)	51 (67%)	1.0
BMI kg/m^2	25.3 ± 4.0	24.8 ± 3.8	0.4
Waist circumference cm	89.7 ± 11.5	87.8 ± 9.8	0.3
Diabetics *N* (%)	12 (16%)	1 (1%)	**0.001**
Type 1	5	0	0.06
Type 2	7	1	0.06
Hypothyroidism *N* (%)	34 (45%)	2 (3%)	**<0.001**
TSH mIU/L	2.2 (1.8)	1.9 (1.0)	0.2
Antihypertensive treatment *N* (%)	16 (21%)	4 (5%)	**0.004**
Systolic BP (mmHg)	127 ± 18	124 ± 18	0.4
Diastolic BP (mmHg)	75 ± 9	73 ± 10	0.3
Lipid lowering therapy *N* (%)	11 (15%)	1 (1%)	**0.003**
Bisphosphanates *N* (%)	4 (5%)	0 (0%)	0.12
DHEA treatment *N* (%)	7 (9.2%)	0 (0%)	**0.014**
Smokers *N* (%)	3 (4%)	4 (5%)	1.0
Metabolic syndrome *N* (%)	25 (33%)	12 (16%)	**0.020**
Serum Cortisol nmol/L	770 (450)	415 (180)	**<0.001**
24h-UFC nmol	359 (408)	175 (104)	**<0.001**
U-Cortisol/creatinine estimates	27.6 (28.4)	14.3 (5.8)	**<0.001**

Abbreviations; SD: standard deviation, IQR: interquartile range, N: number, BMI: body mass index, TSH: thyroid stimulating hormone, HRT: Hormone Replacement Therapy, DHEA: Dehydroepiandrosterone, 24h-UFC: 24 hour urinary free cortisol, U: urinary
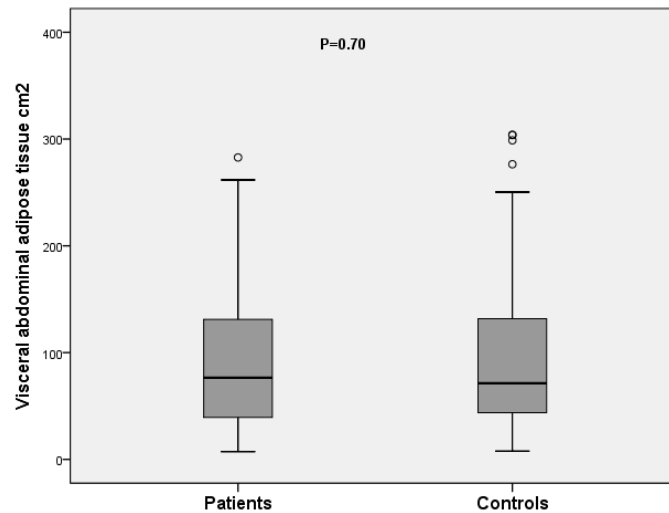
Figure 3. Box plot showing visceral abdominal adipose tissue in 76 patients with Addison's disease and their BMI, age and gender matched controls.

Table 3. Body composition of the patients with Addison's disease and their matched controls (median (IQR))

	Patients	Controls	P
N	76	76	
L4-subcutaneous adipose tissue (cm^2)	226 (164)	225 (124)	0.5
L4-visceral abdominal adipose tissue (cm^2)	76 (93)	71 (92)	0.7
Thigh-subcutaneous adipose tissue (cm^2)	75 (59)	74 (59)	0.3
Thigh-intermuscular adipose tissue (cm^2)	1.6 (1.6)	1.4 (1.9)	0.3
Thigh-muscle (cm^2)	109 (44)	110 (42)	0.9
L4-Circumference (cm)	90 (14)	90 (14)	0.7
Liver attenuation (HU)	59 (4)	57 (7)	**0.03**

Abbreviations; IQR: interquartile range, N: number, HU: Hounsfield units

4.2.3 Glucose metabolism

Twelve patients (16%) had DM [5 T1DM and 7 T2DM] and one control (1%) had T2DM (*P*=0.001) and the patients had higher HbA1c (*P*=0.007) (Table 4, Fig 4). Fifteen patients had HbA1c greater than 5%, of whom four had T1DM and five had T2DM, compared to one control subject (*P*<0.01). Median (IQR) HbA1c remained higher in patients compared to controls [4.5 (0.7) *vs* 4.4 (0.5); *P*<0.05] after excluding patients with T1DM.

Table 4. Glucose variables, lipids and inflammatory/ fibrinolytic markers in patients with Addisonøs disease and their matched controls (median (IQR))

N	76 patients	76 controls	P
Fasting plasma-glucose mmol/L	4.6 (0.7)	4.8 (0.8)	0.2
HbA1c %	4.5(0.8)	4.4 (0.5)	**0.007**
HbA1c mmol/mol*	36	35	
TG mmol/L	1.1 (0.8)	0.9 (0.5)	**0.03**
TC mmol/L	5.1 (1.2)	5.10 (1.4)	0.6
HDL-C mmol/L	1.0 (0.5)	1.3 (0.7)	**<0.0005**
LDL-C mmol/L	2.9 (1.3)	3.3 (1.2)	**0.03**
Hs-CRP mg/L	1.1 (2.0)	0.9 (1.8)	0.7
Adiponectin µg/ml	11.4 (8.6)	10.6 (6.6)	0.1
PAI 1 µg/L	8.6 (15.5)	7.7 (13.7)	0.6

Abbreviations:N: number, IQR: interquartile range, glycosylated haemoglobin: HbA1c, TG: triglyceride, TC: total cholesterol, HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol, hs-CRP: high sensitive C-reactive protein, PAI 1: tissue-type plasminogen activator 1.

*HbA1c % was measured by Mono-S (Sweden) and by calculating to International Federation of Clinical Chemistry (IFCC) Standardization of HbA1c according to IFCC = (10.11*Mono-S) - 8.94, HbA1c 4.5 % and 4.4% are equivalent to 36 and 35 mmol/mol, respectively (68).
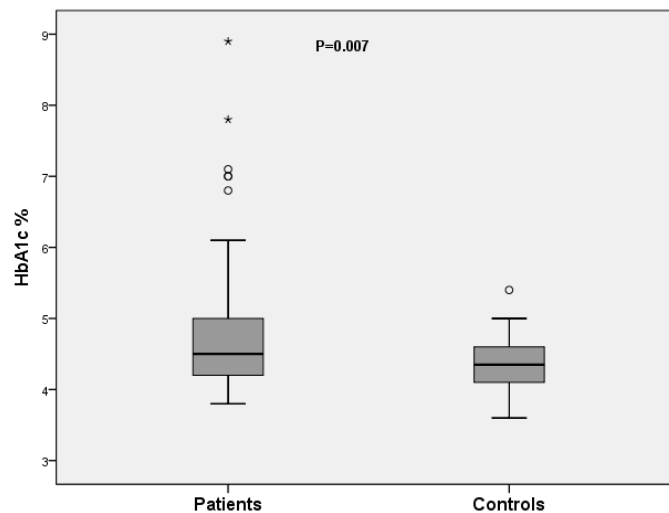
Figure 4. Box plot showing HbA1c in 76 patients with Addisonøs disease and their BMI, age and gender matched controls

4.2.4   Lipids, and inflammatory and fibrinolytic markers

More patients than controls were using lipid-lowering treatment (15% *vs* 1%; *P*=0.003). Serum TG concentrations were higher (*P*=0.03), but HDL-C and LDL-C were lower (*P*<0.0005 and *P*=0.03, respectively) in the patients than in controls (Table 4, Fig 5). Eighteen patients compared to six controls had a TG level ×1.7 mmol/L (*P*=0.007). TG remained higher [1.1 (0.8) *vs* 0.9 (0.5) mmol/L; *P*=0.051] and the HDL-C [1.0 (0.5) *vs* 1.3 (0.7) mmol/L; *P*<0.0005] remained lower in the patients after excluding those receiving lipid-lowering therapy (11 patients and 1 control). Elevated TG and reduced HDL-C persisted when all patients with DM were excluded from the analysis. hs-CRP, adiponectin, and PAI-1 did not differ between the groups (Table 4).
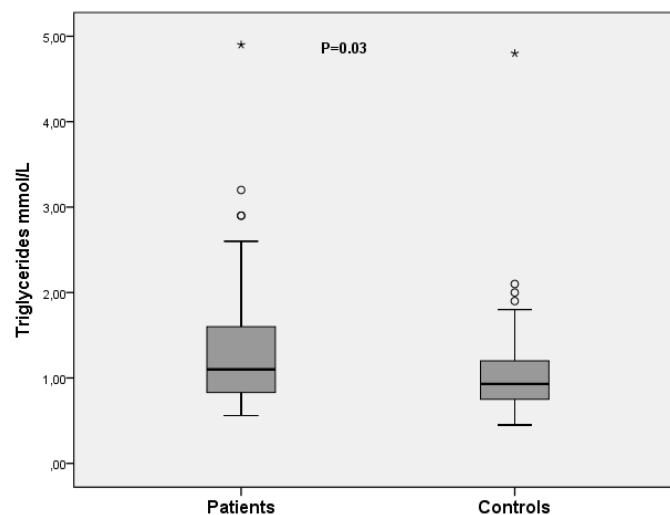
Figure 5. Box plot showing triglycerides in 76 patients with Addison's disease and their BMI, age and gender matched controls.

4.2.5   Bone mineral density and the prevalence of osteoporosis

Mean (±SD) whole body BMC was lower in the patients (2.65±0.60) than in controls (2.83±0.51; *P*<0.05; Fig 6). Mean total BMD (1.16±0.13 *vs* 1.21±0.09; *P*<0.05) and BMD in lumbar spine (1.11±0.22 *vs* 1.18±0.15; *P*<0.05) were also reduced in patients compared to controls, respectively. The T- and Z-scores in lumbar spine and the Z-score in right hip were reduced in patients. Figure 7 illustrates the BMD and Z-scores in lumbar spine.
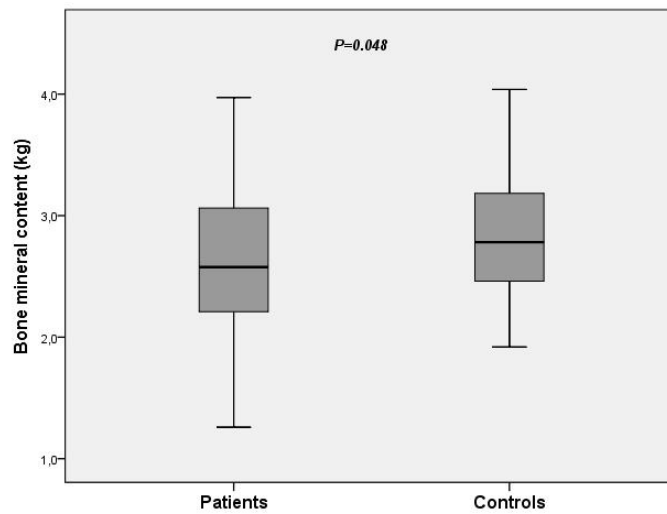
Figure 6. Boxplot showing total bone mineral content (BMC, kg) in 76 patients with Addison's disease and their age, sex, BMI and smoking habits matched controls.
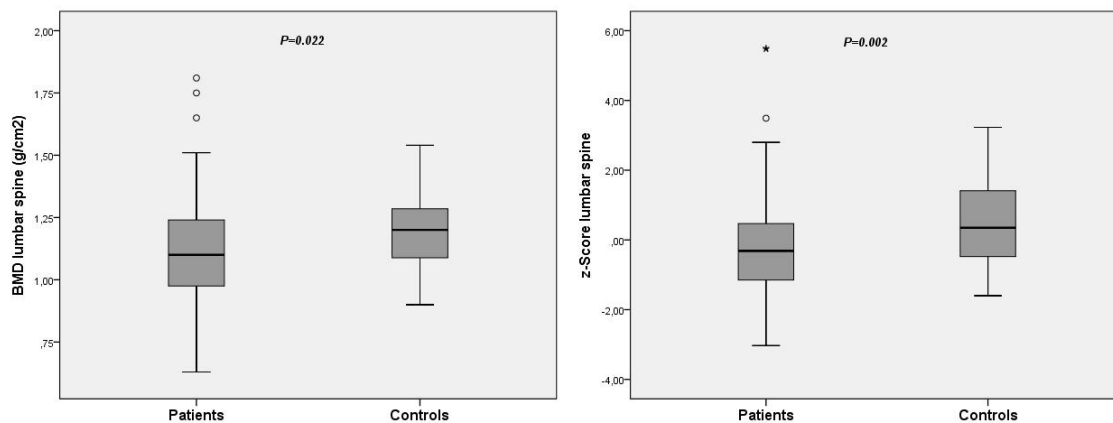

Figure 7. Boxplots showing bone mineral density (BMD, g/cm2) and z-Score in lumbar spine in 76 patients with Addison's disease and their age, sex, BMI and smoking habits matched controls.

4.2.6   Osteoporosis and osteopenia

Nine patients (12%) and no controls had osteoporosis ($P<0.01$) and 37 (49%) patients and 27 (36%) controls had osteopenia ($P=0.1$). When the patients with osteopenia and/or osteoporosis were grouped together, they had received higher HC doses more frequently than patients with normal T-scores (Fig 8). By analyzing genders separately, women on HC ×30 mg/day were more likely to have osteopenia/osteoporosis ($P<0.05$) whereas men did not ($P=0.5$).
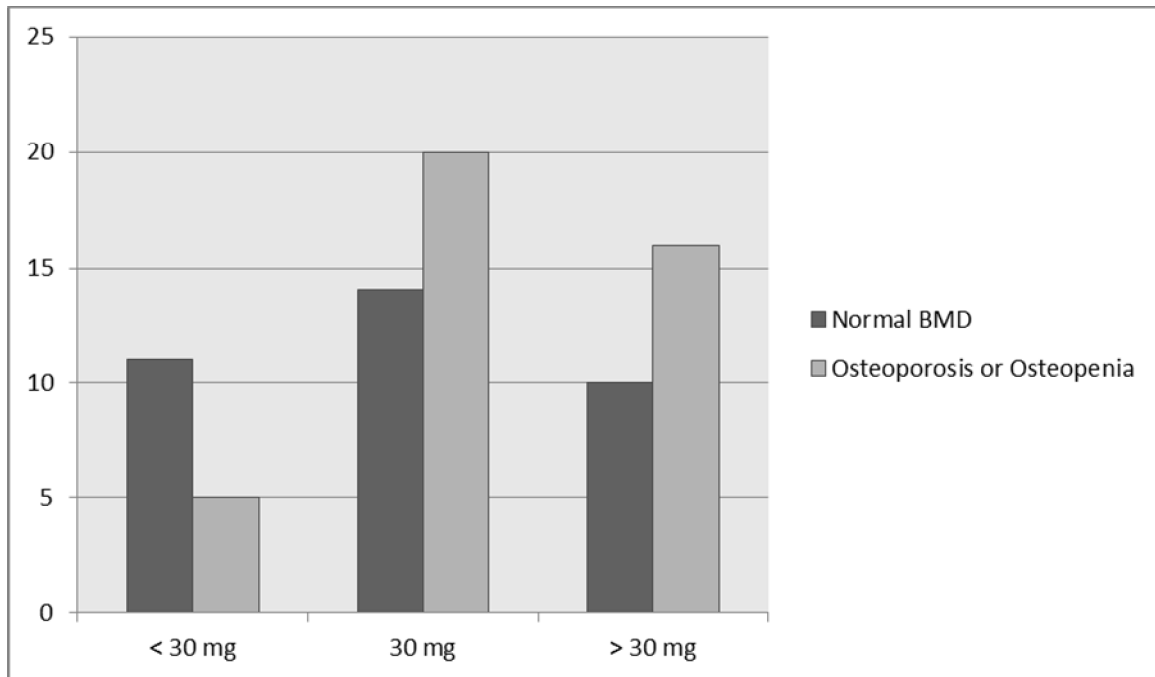
Figure 8. Bar Graphs demonstrating the prevalence (expressed as number of patients) of osteoporosis and/or osteopenia in relation to the daily hydrocortisone dose (in mg) in 76 patients with Addisonøs disease.

4.2.7   Self-reported HR-QoL and general well-being

NHP

Patients reported worse HR-QoL [mean (±SD) higher total score (8.28±11.59 *vs* 4.25±7.52; *P*<0.01] and lower energy level [16.2 ± 29.6 *vs* 4.15 ± 13.32; *P*<0.01] compared to the controls.

SF-36

Mean (±SD) for general health (64.1±24.3 *vs* 83.2±15.3; *P*<0.0001) and vitality scores (64.7±24.2 *vs* 73.1±19.8; *P*<0.05) were lower in the patients than in controls, indicating worse subjective health. In addition, the patients had worse scale scores in the: "Role Physical" and "Role Emotional".

PGWB

Mean (±SD) vitality scores were worse in patients compared to controls [17.0±4.1 *vs* 19.0±3.0; *P*<0.01]. Measures for anxiety and depressed mood did not differ significantly between the groups.

FIS

Mean (±SD) total FIS scores (26.1±25.8 *vs* 14.8±16.6; *P*<0.05) as well as all composites of the cognitive- (7.17±7.23 *vs* 4.31±5.26; *P*<0.05), physical- (7.52±8.76 *vs* 3.35±4.91; *P*<0.01),

and psychosocial-functioning (11.5±12.1 *vs* 7.17±7.88; *P*<0.05) scales were higher (worse outcome) in patients compared to controls.

4.3   Papers I-IV: Gender considerations

VAT did not differ between men with AD and their matched controls [median (IQR), 103 (78) *vs* 109 (122); *P*=0.66] or between the women with AD and their controls [64 (81) *vs* 67 (83); *P*=0.86]. More men (8 *vs* 3; *P*=0.088) and women (17 *vs* 9; *P*=0.077) with AD had MetS than their controls. Based on a polar chart plot, women with AD are more likely to have higher fasting plasma glucose (FPG) and receive more lipid lowering medication, whereas men with AD have a higher proportion with hypertriglyceridemia (Fig 9).
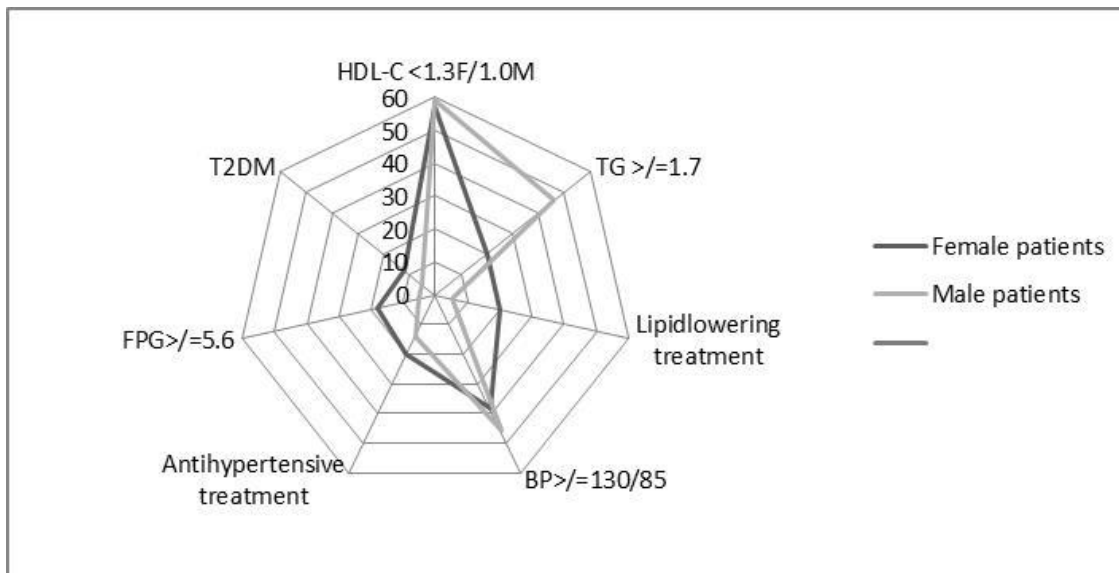
Figure 9. Polar chart illustrating the frequency in percent of the composite of the MetS (IDF 2006 definition) in female and male patients with AD, after excluding T1DM from the analysis. All variables are reported dichotomously and the scale moves from 0 to 60% from centre to outer line, with line divisions for every 10%.

5 DISCUSSION

5.1 Paper I ó Mortality

In the retrospective, observational, population-based study (the national study) we found that patients with AD in Sweden have a two-fold higher mortality rate than the background population. The increased mortality was mainly caused by cardiovascular, malignant, and infectious diseases and was highest close to the time of first detection of AD. This increased risk for death remained during the study period but not to the same extent as during the first few years after diagnosis. Our results have been confirmed in a later Swedish study (69). However, a study on Norwegian patients with AD showed an increased risk of death, mainly from adrenal crisis, infectious diseases, and from sudden death, with the highest risk observed in young male patients (70). Of note is that the patients in the local validation study received replacement therapy with a median daily cortisone acetate dose of 50 mg (equivalent to 40 mg HC), usually as a twice-daily regimen, which is consistent with an overly high GC dose for GC replacement therapy (71,72).

DM is an important confounder in this study. The risk of death from cardiovascular disease is increased in patients with DM (73). Moreover, the prevalence of DM is increased in the patients with AD. The frequency of DM among the patients with AD in the national cohort was 12.0%, four-times higher than the expected prevalence of DM in Sweden (73,74). We were unable to differentiate between T1DM and T2DM but later findings in a large epidemiological drug prescription study on Swedish patients has found medication for insulin-treated DM in 14% of the cohort (75) and a 12% rate for T1DM has been reported in a Norwegian cohort of patients with AD (18). The risk of death was significantly higher in the AD patients with DM as compared to those who had AD without DM but the impact on overall mortality in the total study population was minor. We therefore conclude that having AD with or without DM increases the risk of death in the cohort.

We observed a five-times increased mortality rate from infectious diseases, which is an uncommon cause of death in the age-adjusted background population. Increased risk of death from infectious diseases amongst patients with AD has been reported in a later Swedish study (69) and in a Norwegian cohort of AD patients (70). More antibiotics are prescribed to patients with AD compared to the background population (75). AD patients are also more often hospitalized for infectious diseases (76) and may therefore be more prone to infections or more often treated for infectious diseases.

Moreover, a recently published prospective German study on patients with primary and secondary adrenal insufficiency (77) reported an incidence of eight adrenal crises per 100 patient-years, with gastrointestinal infections and fever as common precipitating factors, and 6% of the patients died in relation to an adrenal crisis. No patient was reported to have died from adrenal crisis in our study but 36 patients (7% of the deceased) were reported as having died from adrenal insufficiency. Thus, these data on death from both infections and adrenal insufficiency are probably both related to adrenal crisis and, therefore, avoidable. This should raise the awareness concerning continuous education of patients and healthcare personnel about this risk.

The epidemiological study is based on well-validated Swedish registers and, even if the local validation study confirms what is reported with respect to quality control of the registers,

there are some limitations. Diagnostic coding errors are unavoidable, so a small proportion of the patients may have been incorrectly coded. Unusual causes of AD other than autoimmune, are probably identified as autoimmune AD, but the contribution to overall mortality is probably small and only one subject had concomitant tuberculosis. Selection of the subjects at hospitalization may occur when the patient is first diagnosed with AD or when the patient with AD is admitted because of an intercurrent illness. That could explain the higher average age in the national cohort as compared to the mean age at diagnosis in the local study and compared to that reported by others (16). Post-mortem detection of subjects has been described (8,70) and it is likely that some patients had died before diagnosis and they are not therefore listed in the register. Thus, the study may underestimate the risk of death.

The strength of this study is its size with 1675 patients with AD identified during a 15-year period, which is in accordance with an estimated prevalence of 140 per million and an incidence of six per million per year (14-17), and included longitudinal follow-up from initial detection to either death or the end of the study period. Since 80% of the patients in the validation study were diagnosed during in-patient care and, with the expected eight adrenal crisis with hospital admission per 100 patient-years, it is likely that the study is exhaustive. The homogenous distribution of AD within Sweden with no regional variation in the detection of the disease diminishes the risk of bias when choosing one local hospital for the validation study.

5.2 Paper II ó Cardiometabolic risk factors

In the cross-sectional, case-control study, we investigated whether the patients with AD receiving long-term standard HC replacement therapy have increased VAT and prevalence of MetS compared to an age-, gender-, and BMI-matched population. If so, traditional cardiovascular risk factors might explain the increased risk of death from cardiovascular diseases.

VAT was, however, not increased in the patients compared to the controls but a larger proportion of the patients had MetS and more frequent prescription of antihypertensive and lipid-lowering medication, supporting the assumption that patients with AD have an increased cardiovascular risk. The increased prescription of antihypertensive and lipid-lowering medications in our study has also been described by others (75). The patients with AD did not have excess abdominal fat, which is characteristic in patients with CS who have both increased exposure and loss of cortisol diurnal rhythm, never reaching nadir (26). As previously mentioned, a higher GR density in VAT and/or increased 11 -HSD-1 activity with increased cortisol exposure has been mentioned as possible explanations to why cortisol excess is followed by visceral fat accumulation in patients with CS (23-25). It is important to keep in mind that the GC replacement therapy received by patients with AD does not restore the cortisol diurnal rhythm and, even if we try to mimic the day-time cortisol profile, the night-time hours with secretion late at night and cortisol peak in the morning are lost, so the patients are in the "nadir state" longer than physiologically expected. Thus, in contrast to patients with CS, patients with AD do not have increased cortisol values overnight and are probably not exposed to continuously high cortisol levels during the daytime. This difference might explain the absence of increased VAT in the patients with AD despite receiving a high total daily HC dose and, consequently, VAT may not be a sensitive marker of cortisol exposure in patients with AD.

We observed increased serum TG concentrations in the patients with AD, which is associated with an increased risk for cardiovascular disease (78). In the absence of increased VAT, the higher TG may be explained by increased hepatic TG output induced by an acute effect of the supra-physiological cortisol level hours after the patient's intake of their morning HC doses. A short-term prednisone treatment study reporting increased TG in healthy men supports this (79). Another explanation could be decreased lipoprotein lipase activity during the night with lipolysis and increased free-fatty acid efflux to the portal circulation secondary to the low night-time cortisol exposure, the prolonged "nadir state" (80). Increased lipolysis and TG mobilization could also be secondary to catecholamine release during the night and early morning (25). The above mentioned mechanisms would be consistent with the finding that TG was increased and VAT was not. The reduced liver attenuation suggesting reduced hepatic fat content in the patients may further support this mechanism of action. However, increasing the over-night and early morning exposure of cortisol using infusion pump administration of HC did not change TG concentration or intermediate metabolites, arguing against low night-time cortisol exposure as an explanation for increased serum TG concentrations in the morning (81,82).

DM was more common in our patients, as expected. However, surprisingly, it was not only due to autoimmune T1DM, a common co-morbidity in patients with AD and previously reported by others (16,18,75), but also due to T2DM. The adverse effects of excess cortisol on glucose metabolism are well known, with induction of insulin resistance and increased plasma glucose levels (83,84). Therefore, cortisol withdrawal for 24 hours in patients with AD decreases endogenous glucose production and increases insulin sensitivity (85). After excluding patients with T1DM from our cohort, the patients had reduced FPG compared to controls. Thus, the low night-time cortisol exposure may partly explain the reduced FPG concentration in the morning, as this can be raised with increased night-time cortisol exposure (81). So a possible explanation is that the morning HC dose has not yet reached its full effect on glucose metabolism in the morning, when blood was collected.

The higher HbA1c in the patients could, on the other hand, reflect higher glucose concentrations or impaired glucose tolerance because of reduced insulin sensitivity later during the day and evening. Supporting this hypothesis is a study demonstrating that patients with both AD and T1DM require lower total daily insulin doses despite requiring both higher pre-meal insulin doses and higher insulin doses in the latter part of the day as compared to patients who only have T1DM (86). The increased HbA1c and TG, and reduced HDL-C in the patients were not explained by the presence of DM since these changes remained when all patients with DM were excluded from the analysis.

The prevalence of MetS was increased in the patients with AD, a syndrome associated with an increased risk of T2DM and cardiovascular disease (87). Taken together, the adverse cardiometabolic profile we observed in the patients compared to controls may partly explain the increased cardiovascular mortality that we and others have observed in patients with AD (69,88). This increased risk may be due to GC replacement therapy.

The adverse cardiovascular profile in patients with AD may also be explained by their hypothyroidism (89) or their unreplaced DHEA. However, the TSH concentration did not differ between the groups and, in a randomized controlled trial evaluating DHEA substitution in patients with AD, no effects on fat mass or blood lipids were shown (90). The mineralocorticoid dose may be inappropriately high, explaining more frequent use of antihypertensive medication (91,92), but this could also be due to the careful and regular

medical surveillance of the patients. The expected increase in hs-CRP, and inflammatory and coagulation markers seen in other populations with MetS was not seen in the patients in this study, which could be explained by the absence of increased VAT and by the relatively high doses of HC exerting anti-inflammatory effects (93).

5.3   Paper III – Bone metabolism

Bone health was impaired in the AD patients with decreased whole body BMC and BMD compared to that in matched controls. Total body and lumbar spine BMD as well as the T- and Z-scores for the lumbar spine and the Z-score for right hip were also reduced in the patients. Patients using higher doses of HC are more likely to have osteoporosis and osteopenia. The adverse outcome in the lumbar spine is consistent with trabecular bone being more vulnerable to GC-induced osteporosis (29). Of note is that all patients who were smokers in the study, even if few, had osteopenia and/or osteoporosis, suggesting the importance of additional risk factors for osteoporosis in susceptible patients.

Our findings are in agreement with some previously published studies in patients with AD (33-37,94), but in contrast to others (95-97). BMD is dependent on many factors such as age, gender, genetics, smoking, and physical activity (44) and, in our study, we matched the controls for age, gender, BMI, and smoking habits. Therefore, it is possible that we are observing a GC-induced reduction in BMD secondary to the relatively high and unphysiological GC replacement regimen in susceptible patients, a concern that has been raised by others (72,98-100). Some studies have reported decreased BMD (101,102) and increased fracture risk (103) with the use of inhaled GCs. Thus, mildly increased GC exposure may have a negative impact on BMD and fracture risk, and it is therefore possible that patients with AD with moderately increased GC exposure and reduced BMD are at increased risk for fractures.

GCs can induce osteoporosis indirectly through changes in calcium and vitamin D deficiency but the patients had higher s-Ca concentrations and similar PTH levels compared to controls, so decreased calcium absorption from the intestine and a secondary rise in PTH (29) is an unlikely explanation for the adverse skeletal outcome in the group. Neither the s-Ca nor the PTH pattern support vitamin D deficiency as a likely explanation for the reduced BMD in the patients. Concomitant endocrine diseases (gonadal, thyroid, or DM), autoimmune disorders (celiac disease), or the loss of adrenal androgens, which often occur in patients with AD, may also have adverse skeletal effects. A randomized, controlled trial evaluating DHEA substitution in patients with AD showed an increase in BMD of the femoral neck (90) and a cross-sectional study found lower bone resorption markers and a higher lumbar spine Z-score in DHEA-substituted women with AD (97). Thus, adrenal androgen deficiency could contribute to the reduced BMD seen in patients with AD, in particular in women. Furthermore, GC-induced myopathy with muscle weakness and reduced physical activity could also be a contributing factor to the reduced BMD (104).

Whether the decreased BMC and BMD, and the increased prevalence of osteoporosis in patients with AD will increase their risk for fractures remains to be demonstrated. An epidemiological study has shown an increased risk for hip fracture in patients with AD adjacent to the time of diagnosis (105). The risk of vertebral fractures in patients with AD is not known as many vertebral fractures do not come to clinical attention (106). Increased fracture risk in patients with pharmacological GC-induced osteoporosis is thought to be partly independent of BMD, but may be more related to changes in bone quality with increased

skeletal fragility (107), so we may underestimate the fracture risk in patients with AD when measuring BMD with DEXA.

5.4 Paper IV – HR-QoL and general well being

In keeping with previous findings, the case-control study confirms impaired HR-QoL and an increased level of fatigue in the patients with AD. In brief, the patients had reduced general health, vitality, and energy levels as well as an increased level of fatigue. Our findings are consistent with other European studies (38,64,108,109). The careful matching of the patients and controls for age, gender, BMI, and smoking habits, as well as adequate thyroxin substitution according to clinical and biochemical assessments, is a strength in our study. Furthermore, we used well-validated and established generic HR-QoL questionnaires.

The impaired HR-QoL may be confounded by co-morbidities such as DM and hypothyroidism since DM has been associated with impaired HR-QoL in many studies, and all the functional domains (physical, psychological, and social functioning) may be compromised in patients with DM (110). Hypothyroidism has also been linked to impaired HR-QoL (111) and reduced well-being (112), both in untreated and treated patients. Very few women in our study group received DHEA replacement, which may also influence outcome in terms of HR-QoL. However, data regarding the beneficial effects of DHEA is scarce and conflicting (90,113,114). One study reported that patients with autoimmune polyendocrine syndromes had worse outcome and that adrenal androgen depletion was associated with reduced physical function in women with AD (38), whereas another study showed that the impaired health status was irrespective of concomitant autoimmune disease and whether the patients received DHEA or not (108).

It is difficult to prove the actual reason for the compromised HR-QoL in the patients as we cannot differentiate between the burden of the disease itself and the impact of its treatment. For example, concerns among patients with AD about the necessity of HC and its adverse effects have been associated with a more negative illness perception (115), which may also contribute to their reduced HR-QoL. Considering the increased fatigue one randomized double blind, cross-over (with HC and placebo) study reports rapid eye movement (REM) sleep disturbances in patients with AD, with less REM sleep in HC deprived patients (116). Another study did not identify any specific sleep disturbance pattern for AD patients and no more daytime sleepiness than normal but some of the patients reported problems with awakening early in the morning (117).

The main study limitations are incomplete data on socioeconomic status and educational level in the patients and controls. The disease-specific questionnaire, ADDIQoL, could not be used as it was not available when the study started.

On the whole, successful matching in the case-control study for age, gender, BMI, and smoking habits is a strength, particularly as the primary end-points were VAT, BMD and osteoporosis. The matching is also important with respect to secondary end-points as conventional cardiovascular risk factors. Furthermore, we expect the patient population to be representative of patients with AD in Sweden. On the other hand, we acknowledge that our control group, although randomly selected from the population, may be healthier than the general population by selection. Another important limitation is the fact that lifestyle, dietary habits, alcohol consumption, exercise, and socio-economic status were not controlled for in the matching of the patients and controls.

5.5 Gender considerations

In the epidemiological study, we observed higher mortality rate in women with AD than in men (88) and the case-control study suggests that the cardiovascular risk burden is higher in women than in men with AD. The reason for this remains unexplained but higher total cortisol exposure in women than in men using the same daily HC dose may be one explanation (118). Healthy premenopausal women have been shown to have lower total daily cortisol amount than men probably because of a lower peak cortisol in the morning (119). Therefore it is possible that women with AD are more susceptible to the sub-optimal HC replacement therapy with overly high cortisol levels in the morning and later in the day inducing more adverse effects in them than in men.

5.6 Glucocorticoid replacement therapy

The daily cortisol production rate in healthy individuals corresponds to approximately 15-25 mg of oral HC per day (120,121), clearly indicating that the patients in the national and case-control studies are receiving inappropriately high GC doses. The majority of the patients are on a twice-daily regimen and, taken together, this results in an unphysiological cortisol circadian rhythm. Our hypothesis is, therefore, that the increased mortality, cardiometabolic risk factors and the impaired bone health and HR-QoL may be associated with the current practice of GC replacement therapy.

Physicians started to consider the pharmacokinetics of GC replacement therapy in patients with adrenal insufficiency in the 1980s and found that a more physiological cortisol profile, improved well-being and increased bone formation markers could be achieved with lower doses and by changing from a twice-daily to a three-times daily dosing regimen (98-100). However, HC, the most widely used GC for replacement in Europe (122), has its restrictions as it is an immediate-release preparation with a short half-life (~1.8 hour), which results in an immediate high cortisol peak after oral intake followed by a low trough between doses. All attempts to mimic the physiological rhythm and achieve an individual and physiological cortisol exposure have also been limited by the fact that there are no well-established methods to monitor treatment. ACTH cannot be used because of high inter-individual variability and sensitivity to inhibition (123,124). Serum cortisol day curves and 24-hour UFC have been advocated but their clinical value is limited because of high inter-individual pharmacokinetic variability, variability in the saturation of CBG, and the transient increase in UFC during serum cortisol peaks (72,99,125,126). Salivary cortisol measurements are also of limited value and they have poor correlation to serum cortisol (127). Body weight is an important predictor of HC clearance so a weight-related dosing regime is therefore associated with less over-exposure of cortisol, although this dosing strategy does not account for differing GC sensitivity between patients (128).

The first, short-term studies investigating clinical response in relation to a GC replacement regimen suggested reduction of the daily dose and, preferably, a three-times daily regimen in order to more closely mimic the circadian cortisol rhythm (98-100). A three-times daily dosing regimen may not be an option for many patients, as shown in a large multinational postal survey (122). Considering the possible adverse effects of unphysiological GC replacement therapy, studies have been performed with the objective of ameliorating the metabolic effects and HR-QoL in patients with adrenal insufficiency by improving old and

applying new therapeutic approaches.

A dual release (DR) tablet formulation of HC, with the advantage of once-daily dosing and simulating the cortisol circadian rhythm, has been developed (129). In a randomized, controlled trial (RCT) with a crossover design comparing once-daily DR-HC to three-times-daily immediate-release HC at the same total daily dose of HC, a significant reduction in body weight, blood pressure, and HbA1c was achieved, suggesting that the profile of GC exposure is of importance (130). A method for continuous subcutaneous HC infusion (CSHI) has also been developed for replacement therapy (131). CSHI as compared to a three-times-daily oral HC regimen has been associated with improvements in the serum cortisol profile in a crossover RCT, and in addition it results in higher night-time glucose levels. In another attempt to improve outcome, a modified-release HC formulation with delayed release for twice-daily administration has been developed (132) but no outcome data except for pharmacokinetic profiling is available at present.

A prospective study has reported a more physiological cortisol profile and an increase in serum osteocalcin in response to a reduction in the GC dose in patients with AD (72). An early study from 1988 suggested that, by administering the same daily GC dose using a three-times-daily instead of twice-daily schedule, well-being could be improved, in particular in the afternoon (98). A cross-sectional study has shown that impaired QoL was unrelated to the type of GC replacement being used (HC, cortisone acetate, or prednisolone) (133) and another study showed that reduced HR-QoL was associated with GC doses above 30 mg/day but that a three-times-daily regimen was not superior to a twice-daily regimen (134).

Using the DR-HC formulation and CSHI was also accompanied by improvement in HR-QoL (130) (135) but neither study was blinded. In contrast, no improvements in subjective health status were obtained in a double-blind study comparing CSHI with conventional HC replacement when HR-QoL was evaluated using both AddiQoL and SF-36(136).

All these attempts to reproduce a physiological circadian rhythm suggest that further improvement in the outcome of patients with AD can be achieved. These studies also support our hypothesis that many of the adverse effects observed in patients with AD are related to GC replacement therapy and not entirely to the underlying disease or its co-morbidities.

6   CONCLUSIONS

- The mortality rate in patients with AD is more than two-fold higher than in the background population. The excess mortality was mainly explained by death from cardiovascular diseases and cancer but there was increased mortality from infectious diseases as well. Concomitant DM increased the risk of death further but explained the increased mortality rate in the whole group only to a minor extent.

- Patients with AD receiving standard HC replacement therapy do not have increased VAT compared to BMI-matched controls. A higher proportion of the patients had: MetS and T2DM; more frequent use of antihypertensive and lipid-lowering medications; and higher TG and HbA1c, and lower HDL-C.

- Patients with AD have reduced BMD and increased frequency of osteoporosis and osteopenia. The risk for osteoporosis/osteopenia was particularly increased in the patients with higher daily doses of HC and in women.

- Patients with AD have impaired HR-QoL and an increased level of fatigue.

All this may be explained by the relatively high and unphysiological HC replacement dose regimens that patients with AD receive in Sweden. Replacement therapy could probably be optimized using a dosing method that provides a more physiological cortisol exposure profile to achieve improvement of morbidity and mortality in patients with AD. Greater understanding of the individual response to GCs is warranted and biological markers of GC activity are needed.

7   FUTURE PERSPECTIVES

It would be interesting to investigate whether the mortality rate in patients with AD has changed over the years during which HC doses have probably been reduced in Sweden. Furthermore, a new national emergency card was developed for patients in 2010 (21) and, hopefully, this has raised awareness about the importance of rescue therapy during concomitant illness. It is also important to bear in mind that a good patient-doctor/nurse relationship, encouraging patients to effective self-management, may convey confidence to patients and minimize the risk for adrenal crisis.

We observed an increased cardiovascular burden in the patients in the case-control study that may be related to GC replacement therapy. It is maybe not realistic to perform longitudinal, randomized, controlled studies with different preventive measures and/or treatment regimens and to use cardiovascular death as the primary outcome variable, although the underlying mechanisms related to increased cardiovascular death should be investigated. It is possible to study whether AD patients have a greater degree of atherosclerosis than matched controls. Regarding traditional modifiable risk factors for cardiovascular disease, it is maybe wise to confide in both FPG and HbA1c, since FPG is influenced by low night-time cortisol and an impaired glucose profile the rest of the day and its probable clinical importance may have been overlooked.

Considering impaired bone health in AD patients, it is important to determine whether it confers a greater risk of vertebral fractures. Moreover, the microarchitecture of bone should be studied to see whether there are changes that may be weakening bone and making it more vulnerable to fractures. A case-control study, as previously described, with quantitative CT (137) of the spine and bone marker measurements of the best defined bone markers, would be a good start. Of course, we should screen AD patients with the currently recommended tools and be generous in measuring BMD with DEXA to be able to offer appropriate prevention and/or treatment.

The mechanisms behind the impaired HR-QoL in patients with AD are sparsely studied and more physiological GC replacement therapy in AD patients should be studied in randomized, controlled studies with respect to impact on HR-QoL and fatique.

SAMMANFATTNING PÅ SVENSKA

Addisons sjukdom (AS) eller primär binjurebarkssvikt är en livshotande sjukdom om den inte behandlas med glukokortikoider (GK). Det är en ovanlig sjukdom med en förekomst på 100-140 personer per miljon invånare. Både sjukligheten (morbiditeten) och dödligheten (mortaliteten) hos denna patientgrupp har hittills varit sparsamt studerade. I några äldre studier rapporteras huvudsakligen normal dödlighet och några studier tyder på försämrad benhälsa och livskvalitet hos patienter med AS. Ersättningsbehandling med GK med för höga doser och en dosregim som inte efterliknar den fysiologiska dygnsprofilen av kortisol, är troligen bidragande orsaker till den försämrade hälsan hos patienter med AS.

Avhandlingen bygger på studier vars huvudsyfte var att studera dödlighet och sjuklighet hos patienter med AS som får livslång ersättningsbehandling med GK.
I en stor nationell registerstudie hade patienter med AS mer än dubbelt så hög dödlighet som bakgrundspopulationen vilket förklarades främst av död i hjärt- och kärlsjukdomar, cancer och infektioner. Dödligheten var ännu högre hos patienter som hade AS och åtföljande diabetes mellitus (DM).

I en fall-kontrollstudie där patienter med AS jämfördes med friska kontroller matchade för ålder, kön och kroppsmasseindex (body mass index=BMI) studerades sedan riskfaktorer för hjärt- och kärlsjukdomar, bukfett, benhälsa och livskvalitet.
Patienterna hade inte ökat bukfett mätt med skiktröntgen (CT) men en större andel av de hade metabolt syndrom (MetS) och typ 2DM (T2DM). Patienterna hade nedsatt bentäthet och ökad förekomst av benskörhet (osteoporos). Påfallande var att patienter som stod på en högre GK dos hade ökad risk för benskörhet och låg bentäthet. Till sist såg vi med fyra validerade frågeformulär att patienterna upplevde sig trötta och hade nedsatt livskvalitet.

Sammanfattningsvis visar denna avhandling att patienter med AS i Sverige har en ökad dödlighet som förklaras främst av död i hjärt- och kärlsjukdomar. De har också ökad risk för död i cancer och infektioner. Därtill kommer att trots det att bukfettvolymen inte skiljer sig mellan patienter och kontroller så har patienterna ökad förekomst av MetS och T2DM. Både diabetes och MetS är viktiga riskfaktorer för hjärt- och kärlsjukdom. Patienterna hade också försämrad benhälsa och nedsatt livskvalitet. Avhandlingen talar starkt för att det finns behov av förbättringar vad gäller behandling och omhändertagande av patienter med AS.

ACKNOWLEDGEMENTS

Matias Arkeklint for excellent computer support.

All our *patients* and *controls* who took the time to participate in the study.

My grandmother *Ragnhildur Bergþórsdóttir* the most amazing woman I have ever met and my father, *Bergþór Atlason* who has been there for me through thick and thin.

My dear husband *Sigurbergur*, and our children *Hekla Mekkín*, *Hlynur Snær* and *Sindri Freyr*. I∅l never walk alone! I love you so much...

REFERENCES

1. Bishop PM. The history of the discovery of Addison's disease. Proceedings of the Royal Society of Medicine 1950; 43:35-42
2. Dunlop D. Eighty-Six Cases of Addison's Disease. Br Med J 1963; 5362:887-891
3. Rowntree LG, Greene CH, Swingle WW, Pfiffner JJ. The Treatment of Patients with Addison's Disease with the "Cortical Hormone" of Swingle and Pfiffner. Science 1930; 72:482-483
4. Loeb RF. Chemical Changes in the Blood in Addison's Disease. Science 1932; 76:420-421
5. Loeb RF. The Adrenal Cortex and Electrolyte Behavior: Harvey Lecture, December 18, 1941. Bulletin of the New York Academy of Medicine 1942; 18:263-288
6. Kendall EC, Knowlton AI. The centenary of Addison's disease; discussion. Bulletin of the New York Academy of Medicine 1956; 32:837-843
7. Sorkin SZ. The centenary of Addison's disease. Bulletin of the New York Academy of Medicine 1956; 32:819-836
8. Mason AS. Epidemiological and clinical picture of Addison's disease. Lancet 1968; 2(7571):744-747
9. Arlt W, Stewart PM. Adrenal corticosteroid biosynthesis, metabolism, and action. Endocrinology and metabolism clinics of North America 2005; 34:293-313, viii
10. Tomlinson JW, Walker EA, Bujalska IJ, Draper N, Lavery GG, Cooper MS, Hewison M, Stewart PM. 11beta-hydroxysteroid dehydrogenase type 1: a tissue-specific regulator of glucocorticoid response. Endocrine reviews 2004; 25:831-866
11. DeRijk RH, Schaaf M, de Kloet ER. Glucocorticoid receptor variants: clinical implications. The Journal of steroid biochemistry and molecular biology 2002; 81:103-122
12. Lowenberg M, Stahn C, Hommes DW, Buttgereit F. Novel insights into mechanisms of glucocorticoid action and the development of new glucocorticoid receptor ligands. Steroids 2008; 73:1025-1029
13. Arlt W, Allolio B. Adrenal insufficiency. Lancet 2003; 361:1881-1893
14. Kong MF, Jeffcoate W. Eighty-six cases of Addison's disease. Clin Endocrinol (Oxf) 1994; 41:757-761
15. Laureti S, Vecchi L, Santeusanio F, Falorni A. Is the prevalence of Addison's disease underestimated? J Clin Endocrinol Metab 1999; 84:1762
16. Lovas K, Husebye ES. High prevalence and increasing incidence of Addison's disease in western Norway. Clin Endocrinol (Oxf) 2002; 56:787-791
17. Willis AC, Vince FP. The prevalence of Addison's disease in Coventry, UK. Postgrad Med J 1997; 73:286-288
18. Erichsen MM, Lovas K, Skinningsrud B, Wolff AB, Undlien DE, Svartberg J, Fougner KJ, Berg TJ, Bollerslev J, Mella B, Carlson JA, Erlich H, Husebye ES. Clinical, immunological, and genetic features of autoimmune primary adrenal insufficiency: observations from a Norwegian registry. J Clin Endocrinol Metab 2009; 94:4882-4890
19. Husebye ES, Allolio B, Arlt W, Badenhoop K, Bensing S, Betterle C, Falorni A, Gan EH, Hulting AL, Kasperlik-Zaluska A, Kampe O, Lovas K, Meyer G, Pearce SH. Consensus statement on the diagnosis, treatment and follow-up of patients with primary adrenal insufficiency. Journal of internal medicine 2014; 275:104-115
20. Winqvist O, Karlsson FA, Kampe O. 21-Hydroxylase, a major autoantigen in idiopathic Addison's disease. Lancet 1992; 339:1559-1562

21.   Dahlqvist P, Bensing S, Ekwall O, Wahlberg J, Bergthorsdottir R, Hulting AL. [A national medical emergency card for adrenal insufficiency. A new warning card for better management and patient safety]. Lakartidningen 2011; 108:2226-2227

22.   Alkatib AA, Cosma M, Elamin MB, Erickson D, Swiglo BA, Erwin PJ, Montori VM. A systematic review and meta-analysis of randomized placebo-controlled trials of DHEA treatment effects on quality of life in women with adrenal insufficiency. J Clin Endocrinol Metab 2009; 94:3676-3681

23.   Dallman MF, Strack AM, Akana SF, Bradbury MJ, Hanson ES, Scribner KA, Smith M. Feast and famine: critical role of glucocorticoids with insulin in daily energy flow. Frontiers in neuroendocrinology 1993; 14:303-347

24.   Peckett AJ, Wright DC, Riddell MC. The effects of glucocorticoids on adipose tissue lipid metabolism. Metabolism 2011; 60:1500-1510

25.   Bjorntorp P. Abdominal obesity and the metabolic syndrome. Ann Med 1992; 24:465-468

26.   Newell-Price J, Bertagna X, Grossman AB, Nieman LK. Cushing's syndrome. Lancet 2006; 367:1605-1617

27.   Manolagas SC. Birth and death of bone cells: basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis. Endocrine reviews 2000; 21:115-137

28.   Weinstein RS. Clinical practice. Glucocorticoid-induced bone disease. N Engl J Med 2011; 365:62-70

29.   Lafage-Proust MH, Boudignon B, Thomas T. Glucocorticoid-induced osteoporosis: pathophysiological data and recent treatments. Joint, bone, spine : revue du rhumatisme 2003; 70:109-118

30.   Mancini T, Doga M, Mazziotti G, Giustina A. Cushing's syndrome and bone. Pituitary 2004; 7:249-252

31.   de Kloet ER, Oitzl MS, Joels M. Stress and cognition: are corticosteroids good or bad guys? Trends in neurosciences 1999; 22:422-426

32.   Starkman MN, Gebarski SS, Berent S, Schteingart DE. Hippocampal formation volume, memory dysfunction, and cortisol levels in patients with Cushing's syndrome. Biological psychiatry 1992; 32:756-765

33.   Devogelaer JP, Crabbe J, Nagant de Deuxchaisnes C. Bone mineral density in Addison's disease. British medical journal 1987; 295:214

34.   Florkowski CM, Holmes SJ, Elliot JR, Donald RA, Espiner EA. Bone mineral density is reduced in female but not male subjects with Addison's disease. The New Zealand medical journal 1994; 107:52-53

35.   Heureux F, Maiter D, Boutsen Y, Devogelaer JP, Jamart J, Donckier J. [Evaluation of corticosteroid replacement therapy and its effect on bones in Addison's disease]. Annales d'endocrinologie 2000; 61:179-183

36.   Valero MA, Leon M, Ruiz Valdepenas MP, Larrodera L, Lopez MB, Papapietro K, Jara A, Hawkins F. Bone density and turnover in Addison's disease: effect of glucocorticoid treatment. Bone and mineral 1994; 26:9-17

37.   Zelissen P, Croughs R, van Rijk P, Raymakers J. Effect of glucocorticoid replacement therapy on bone mineral density in patients with Addison disease. Annals of internal medicine 1994; 120:207-210

38.   Lovas K, Loge JH, Husebye ES. Subjective health status in Norwegian patients with Addison's disease. Clin Endocrinol (Oxf) 2002; 56:581-588

39.   Kanis JA, Oden A, Johnell O, De Laet C, Jonsson B, Oglesby AK. The components of excess mortality after hip fracture. Bone 2003; 32:468-473

40. Svensson J, Bengtsson BA, Rosen T, Oden A, Johannsson G. Malignant disease and cardiovascular morbidity in hypopituitary adults with or without growth hormone replacement therapy. J Clin Endocrinol Metab 2004; 89:3306-3312

41. SNBW. The National Swedish Board of Health and Welfare Patient registret 1964á2003.

42. SNBW. The National Swedish Board of Health and Welfare Causes of Death 2002.

43. Alberti KG, Zimmet P, Shaw J. Metabolic syndrome--a new world-wide definition. A Consensus Statement from the International Diabetes Federation. Diabetic medicine : a journal of the British Diabetic Association 2006; 23:469-480

44. Kanis JA. Assessment of fracture risk and its application to screening for postmenopausal osteoporosis: synopsis of a WHO report. WHO Study Group. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA 1994; 4:368-381

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

46. Kanis JA, Burlet N, Cooper C, Delmas PD, Reginster JY, Borgstrom F, Rizzoli R, European Society for C, Economic Aspects of O, Osteoarthritis. European guidance for the diagnosis and management of osteoporosis in postmenopausal women. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA 2008; 19:399-428

47. Kanis JA. Diagnosis of osteoporosis and assessment of fracture risk. Lancet 2002; 359:1929-1936

48. Kanis JA, Delmas P, Burckhardt P, Cooper C, Torgerson D. Guidelines for diagnosis and management of osteoporosis. The European Foundation for Osteoporosis and Bone Disease. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA 1997; 7:390-406

49. Plank LD. Dual-energy X-ray absorptiometry and body composition. Current opinion in clinical nutrition and metabolic care 2005; 8:305-309

50. Starck G, Lonn L, Cederblad A, Forssell-Aronsson E, Sjostrom L, Alpsten M. A method to obtain the same levels of CT image noise for patients of various sizes, to minimize radiation dose. The British journal of radiology 2002; 75:140-150

51. Chowdhury B, Sjostrom L, Alpsten M, Kostanty J, Kvist H, Lofgren R. A multicompartment body composition technique based on computerized tomography. International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity 1994; 18:219-234

52. Gradmark AM, Rydh A, Renstrom F, De Lucia-Rolfe E, Sleigh A, Nordstrom P, Brage S, Franks PW. Computed tomography-based validation of abdominal adiposity measurements from ultrasonography, dual-energy X-ray absorptiometry and anthropometry. The British journal of nutrition 2010; 104:582-588

53. Kvist H, Chowdhury B, Grangard U, Tylen U, Sjostrom L. Total and visceral adipose-tissue volumes derived from measurements with computed tomography in adult men and women: predictive equations. The American journal of clinical nutrition 1988; 48:1351-1361

54. Cornier MA, Despres JP, Davis N, Grossniklaus DA, Klein S, Lamarche B, Lopez-Jimenez F, Rao G, St-Onge MP, Towfighi A, Poirier P, American Heart Association

Obesity Committee of the Council on N, Physical A, Metabolism, Council on A, Thrombosis, Vascular B, Council on Cardiovascular Disease in the Y, Council on Cardiovascular R, Intervention, Council on Cardiovascular Nursing CoE, Prevention, Council on the Kidney in Cardiovascular D, Stroke C. Assessing adiposity: a scientific statement from the American Heart Association. Circulation 2011; 124:1996-2019

55.   Shuster A, Patlas M, Pinthus JH, Mourtzakis M. The clinical importance of visceral adiposity: a critical review of methods for visceral adipose tissue analysis. The British journal of radiology 2012; 85:1-10

56.   Fox CS, Massaro JM, Hoffmann U, Pou KM, Maurovich-Horvat P, Liu CY, Vasan RS, Murabito JM, Meigs JB, Cupples LA, D'Agostino RB, Sr., O'Donnell CJ. Abdominal visceral and subcutaneous adipose tissue compartments: association with metabolic risk factors in the Framingham Heart Study. Circulation 2007; 116:39-48

57.   Hunt SM, McEwen J, McKenna SP. Measuring health status: a new tool for clinicians and epidemiologists. The Journal of the Royal College of General Practitioners 1985; 35:185-188

58.   Wiklund I, Karlberg J. Evaluation of quality of life in clinical trials. Selecting quality-of-life measures. Controlled clinical trials 1991; 12:204S-216S

59.   Persson LO, Karlsson J, Bengtsson C, Steen B, Sullivan M. The Swedish SF-36 Health Survey II. Evaluation of clinical validity: results from population studies of elderly and women in Gothenborg. Journal of clinical epidemiology 1998; 51:1095-1103

60.   Ware JE, Jr., Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. Medical care 1992; 30:473-483

61.   Fisk JD, Ritvo PG, Ross L, Haase DA, Marrie TJ, Schlech WF. Measuring the functional impact of fatigue: initial validation of the fatigue impact scale. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 1994; 18 Suppl 1:S79-83

62.   Flensner G, Ek AC, Soderhamn O. Reliability and validity of the Swedish version of the Fatigue Impact Scale (FIS). Scandinavian journal of occupational therapy 2005; 12:170-180

63.   Lovas K, Curran S, Oksnes M, Husebye ES, Huppert FA, Chatterjee VK. Development of a disease-specific quality of life questionnaire in Addison's disease. J Clin Endocrinol Metab 2010; 95:545-551

64.   Oksnes M, Bensing S, Hulting AL, Kampe O, Hackemann A, Meyer G, Badenhoop K, Betterle C, Parolo A, Giordano R, Falorni A, Papierska L, Jeske W, Kasperlik-Zaluska AA, Chatterjee VK, Husebye ES, Lovas K. Quality of life in European patients with Addison's disease: validity of the disease-specific questionnaire AddiQoL. J Clin Endocrinol Metab 2012; 97:568-576

65.   Wiklund I, Romanus B, Hunt SM. Self-assessed disability in patients with arthrosis of the hip joint. Reliability of the Swedish version of the Nottingham Health Profile. International disability studies 1988; 10:159-163

66.   Sullivan M, Karlsson J, Ware JE, Jr. The Swedish SF-36 Health Survey--I. Evaluation of data quality, scaling assumptions, reliability and construct validity across general populations in Sweden. Social science & medicine 1995; 41:1349-1358

67.   Breslow NE, Day NE. Statistical methods in cancer research. Volume II--The design and analysis of cohort studies. IARC Sci Publ 1987:1-406

68.   Hoelzel W, Weykamp C, Jeppsson JO, Miedema K, Barr JR, Goodall I, Hoshino T, John WG, Kobold U, Little R, Mosca A, Mauri P, Paroni R, Susanto F, Takei I, Thienpont L, Umemoto M, Wiedmeyer HM, Standardization IWGoHc. IFCC reference system for measurement of hemoglobin A1c in human blood and the

national standardization schemes in the United States, Japan, and Sweden: a method-comparison study. Clinical chemistry 2004; 50:166-174

69. Bensing S, Brandt L, Tabaroj F, Sjoberg O, Nilsson B, Ekbom A, Blomqvist P, Kampe O. Increased death risk and altered cancer incidence pattern in patients with isolated or combined autoimmune primary adrenocortical insufficiency. Clin Endocrinol (Oxf) 2008; 69:697-704

70. Erichsen MM, Lovas K, Fougner KJ, Svartberg J, Hauge ER, Bollerslev J, Berg JP, Mella B, Husebye ES. Normal overall mortality rate in Addison's disease, but young patients are at risk of premature death. Eur J Endocrinol 2009; 160:233-237

71. Crown A, Lightman S. Why is the management of glucocorticoid deficiency still controversial: a review of the literature. Clin Endocrinol (Oxf) 2005; 63:483-492

72. Peacey SR, Guo CY, Robinson AM, Price A, Giles MA, Eastell R, Weetman AP. Glucocorticoid replacement therapy: are patients over treated and does it matter? Clin Endocrinol (Oxf) 1997; 46:255-261

73. Berger B, Stenstrom G, Sundkvist G. Incidence, prevalence, and mortality of diabetes in a large population. A report from the Skaraborg Diabetes Registry. Diabetes care 1999; 22:773-778

74. Andersson DK, Svardsudd K, Tibblin G. Prevalence and incidence of diabetes in a Swedish community 1972-1987. Diabetic medicine : a journal of the British Diabetic Association 1991; 8:428-434

75. Bjornsdottir S, Sundstrom A, Ludvigsson JF, Blomqvist P, Kampe O, Bensing S. Drug prescription patterns in patients with Addison's disease: a Swedish population-based cohort study. J Clin Endocrinol Metab 2013; 98:2009-2018

76. Smans LC, Souverein PC, Leufkens HG, Hoepelman AI, Zelissen PM. Increased use of antimicrobial agents and hospital admission for infections in patients with primary adrenal insufficiency: a cohort study. Eur J Endocrinol 2013; 168:609-614

77. Hahner S, Spinnler C, Fassnacht M, Burger-Stritt S, Lang K, Milovanovic D, Beuschlein F, Willenberg HS, Quinkler M, Allolio B. High incidence of adrenal crisis in educated patients with chronic adrenal insufficiency - a prospective study. J Clin Endocrinol Metab 2014:jc20143191

78. Abdel-Maksoud MF, Hokanson JE. The complex role of triglycerides in cardiovascular disease. Seminars in vascular medicine 2002; 2:325-333

79. Ettinger WH, Jr., Hazzard WR. Prednisone increases very low density lipoprotein and high density lipoprotein in healthy men. Metabolism 1988; 37:1055-1058

80. Bjorntorp P. Hormonal control of regional fat distribution. Human reproduction 1997; 12 Suppl 1:21-25

81. Bjornsdottir S, Oksnes M, Isaksson M, Methlie P, Nilsen RM, Hustad S, Kampe O, Hulting AL, Husebye ES, Lovas K, Nystrom 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. Clin Endocrinol (Oxf) 2014;

82. McConnell EM, Bell PM, Ennis C, Hadden DR, McCance DR, Sheridan B, Atkinson AB. Effects of low-dose oral hydrocortisone replacement versus short-term reproduction of physiological serum cortisol concentrations on insulin action in adult-onset hypopituitarism. Clin Endocrinol (Oxf) 2002; 56:195-201

83. Arnaldi G, Angeli A, Atkinson AB, Bertagna X, Cavagnini F, Chrousos GP, Fava GA, Findling JW, Gaillard RC, Grossman AB, Kola B, Lacroix A, Mancini T, Mantero F, Newell-Price J, Nieman LK, Sonino N, Vance ML, Giustina A, Boscaro M. Diagnosis and complications of Cushing's syndrome: a consensus statement. J Clin Endocrinol Metab 2003; 88:5593-5602

84. van Raalte DH, Ouwens DM, Diamant M. Novel insights into glucocorticoid-mediated diabetogenic effects: towards expansion of therapeutic options? European journal of clinical investigation 2009; 39:81-93

85. Christiansen JJ, Djurhuus CB, Gravholt CH, Iversen P, Christiansen JS, Schmitz O, Weeke J, Jorgensen JO, Moller N. Effects of cortisol on carbohydrate, lipid, and protein metabolism: studies of acute cortisol withdrawal in adrenocortical failure. J Clin Endocrinol Metab 2007; 92:3553-3559

86. Elbelt U, Hahner S, Allolio B. Altered insulin requirement in patients with type 1 diabetes and primary adrenal insufficiency receiving standard glucocorticoid replacement therapy. Eur J Endocrinol 2009; 160:919-924

87. Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. Lancet 2005; 365:1415-1428

88. Bergthorsdottir R, Leonsson-Zachrisson M, Oden A, Johannsson G. Premature mortality in patients with Addison's disease: a population-based study. J Clin Endocrinol Metab 2006; 91:4849-4853

89. Becker C. Hypothyroidism and atherosclerotic heart disease: pathogenesis, medical management, and the role of coronary artery bypass surgery. Endocrine reviews 1985; 6:432-440

90. Gurnell EM, Hunt PJ, Curran SE, Conway CL, Pullenayegum EM, Huppert FA, Compston JE, Herbert J, Chatterjee VK. Long-term DHEA replacement in primary adrenal insufficiency: a randomized, controlled trial. J Clin Endocrinol Metab 2008; 93:400-409

91. Oelkers W, Diederich S, Bahr V. Diagnosis and therapy surveillance in Addison's disease: rapid adrenocorticotropin (ACTH) test and measurement of plasma ACTH, renin activity, and aldosterone. J Clin Endocrinol Metab 1992; 75:259-264

92. Oelkers W, L'Age M. Control of mineralocorticoid substitution in Addison's disease by plasma renin measurement. Klinische Wochenschrift 1976; 54:607-612

93. Brotman DJ, Girod JP, Garcia MJ, Patel JV, Gupta M, Posch A, Saunders S, Lip GY, Worley S, Reddy S. Effects of short-term glucocorticoids on cardiovascular biomarkers. J Clin Endocrinol Metab 2005; 90:3202-3208

94. Lovas K, Gjesdal CG, Christensen M, Wolff AB, Almas B, Svartberg J, Fougner KJ, Syversen U, Bollerslev J, Falch JA, Hunt PJ, Chatterjee VK, Husebye ES. Glucocorticoid replacement therapy and pharmacogenetics in Addison's disease: effects on bone. Eur J Endocrinol 2009; 160:993-1002

95. Braatvedt GD, Joyce M, Evans M, Clearwater J, Reid IR. Bone mineral density in patients with treated Addison's disease. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA 1999; 10:435-440

96. Jodar E, Valdepenas MP, Martinez G, Jara A, Hawkins F. Long-term follow-up of bone mineral density in Addison's disease. Clin Endocrinol (Oxf) 2003; 58:617-620

97. Koetz KR, Ventz M, Diederich S, Quinkler M. Bone mineral density is not significantly reduced in adult patients on low-dose glucocorticoid replacement therapy. J Clin Endocrinol Metab 2012; 97:85-92

98. Groves RW, Toms GC, Houghton BJ, Monson JP. Corticosteroid replacement therapy: twice or thrice daily? J R Soc Med 1988; 81:514-516

99. Howlett TA. An assessment of optimal hydrocortisone replacement therapy. Clin Endocrinol (Oxf) 1997; 46:263-268

100. Oelkers. Recent advances in diagnosis and therapy of Addison´s disease. In: Bhatt HR, James VHT, Besser GM, et.al., eds. Advances in Thomas Addison´s disease. Journal of Endocrinology 1994; 1:69-80

101.	Israel E, Banerjee TR, Fitzmaurice GM, Kotlov TV, LaHive K, LeBoff MS. Effects of inhaled glucocorticoids on bone density in premenopausal women. N Engl J Med 2001; 345:941-947

102.	Richy F, Bousquet J, Ehrlich GE, Meunier PJ, Israel E, Morii H, Devogelaer JP, Peel N, Haim M, Bruyere O, Reginster JY. Inhaled corticosteroids effects on bone in asthmatic and COPD patients: a quantitative systematic review. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA 2003; 14:179-190

103.	van Staa TP, Leufkens HG, Cooper C. Use of inhaled corticosteroids and risk of fractures. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research 2001; 16:581-588

104.	Woolf AD. An update on glucocorticoid-induced osteoporosis. Current opinion in rheumatology 2007; 19:370-375

105.	Bjornsdottir S, Saaf M, Bensing S, Kampe O, Michaelsson K, Ludvigsson JF. Risk of hip fracture in Addison's disease: a population-based cohort study. Journal of internal medicine 2011; 270:187-195

106.	Wasnich RD. Vertebral fracture epidemiology. Bone 1996; 18:179S-183S

107.	van Staa TP, Leufkens HG, Cooper C. The epidemiology of corticosteroid-induced osteoporosis: a meta-analysis. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA 2002; 13:777-787

108.	Hahner S, Loeffler M, Fassnacht M, Weismann D, Koschker AC, Quinkler M, Decker O, Arlt W, Allolio B. Impaired subjective health status in 256 patients with adrenal insufficiency on standard therapy based on cross-sectional analysis. J Clin Endocrinol Metab 2007; 92:3912-3922

109.	Tiemensma J, Andela CD, Kaptein AA, Romijn JA, van der Mast RC, Biermasz NR, Pereira AM. Psychological morbidity and impaired quality of life in patients with stable treatment for primary adrenal insufficiency: cross-sectional study and review of the literature. Eur J Endocrinol 2014; 171:171-182

110.	Rubin RR, Peyrot M. Quality of life and diabetes. Diabetes/metabolism research and reviews 1999; 15:205-218

111.	Watt T, Groenvold M, Rasmussen AK, Bonnema SJ, Hegedus L, Bjorner JB, Feldt-Rasmussen U. Quality of life in patients with benign thyroid disorders. A review. Eur J Endocrinol 2006; 154:501-510

112.	Wekking EM, Appelhof BC, Fliers E, Schene AH, Huyser J, Tijssen JG, Wiersinga WM. Cognitive functioning and well-being in euthyroid patients on thyroxine replacement therapy for primary hypothyroidism. Eur J Endocrinol 2005; 153:747-753

113.	Arlt W, Callies F, van Vlijmen JC, Koehler I, Reincke M, Bidlingmaier M, Huebler D, Oettel M, Ernst M, Schulte HM, Allolio B. Dehydroepiandrosterone replacement in women with adrenal insufficiency. N Engl J Med 1999; 341:1013-1020

114.	Lovas K, Gebre-Medhin G, Trovik TS, Fougner KJ, Uhlving S, Nedrebo BG, Myking OL, Kampe O, Husebye ES. Replacement of dehydroepiandrosterone in adrenal failure: no benefit for subjective health status and sexuality in a 9-month, randomized, parallel group clinical trial. J Clin Endocrinol Metab 2003; 88:1112-1118

115.	Tiemensma J, Andela CD, Pereira AM, Romijn JA, Biermasz NR, Kaptein AA. Patients with adrenal insufficiency hate their medication: concerns and stronger beliefs about the necessity of hydrocortisone intake are associated with more negative illness perceptions. J Clin Endocrinol Metab 2014; 99:3668-3676

116. Garcia-Borreguero D, Wehr TA, Larrosa O, Granizo JJ, Hardwick D, Chrousos GP, Friedman TC. Glucocorticoid replacement is permissive for rapid eye movement sleep and sleep consolidation in patients with adrenal insufficiency. J Clin Endocrinol Metab 2000; 85:4201-4206

117. Lovas K, Husebye ES, Holsten F, Bjorvatn B. Sleep disturbances in patients with Addison's disease. Eur J Endocrinol 2003; 148:449-456

118. Ragnarsson O, Nystrom HF, Johannsson G. Glucocorticoid replacement therapy is independently associated with reduced bone mineral density in women with hypopituitarism. Clin Endocrinol (Oxf) 2012; 76:246-252

119. Van Cauter E, Leproult R, Kupfer DJ. Effects of gender and age on the levels and circadian rhythmicity of plasma cortisol. J Clin Endocrinol Metab 1996; 81:2468-2473

120. Esteban NV, Loughlin T, Yergey AL, Zawadzki JK, Booth JD, Winterer JC, Loriaux DL. Daily cortisol production rate in man determined by stable isotope dilution/mass spectrometry. J Clin Endocrinol Metab 1991; 72:39-45

121. Kraan GP, Dullaart RP, Pratt JJ, Wolthers BG, Drayer NM, De Bruin R. The daily cortisol production reinvestigated in healthy men. The serum and urinary cortisol production rates are not significantly different. J Clin Endocrinol Metab 1998; 83:1247-1252

122. Forss M, Batcheller G, Skrtic S, Johannsson G. Current practice of glucocorticoid replacement therapy and patient-perceived health outcomes in adrenal insufficiency - a worldwide patient survey. BMC endocrine disorders 2012; 12:8

123. Feek CM, Ratcliffe JG, Seth J, Gray CE, Toft AD, Irvine WJ. Patterns of plasma cortisol and ACTH concentrations in patients with Addison's disease treated with conventional corticosteroid replacement. Clin Endocrinol (Oxf) 1981; 14:451-458

124. Scott RS, Donald RA, Espiner EA. Plasma ACTH and cortisol profiles in Addisonian patients receiving conventional substitution therapy. Clin Endocrinol (Oxf) 1978; 9:571-576

125. Arlt W, Rosenthal C, Hahner S, Allolio B. Quality of glucocorticoid replacement in adrenal insufficiency: clinical assessment vs. timed serum cortisol measurements. Clin Endocrinol (Oxf) 2006; 64:384-389

126. Monson JP. The assessment of glucocorticoid replacement therapy. Clin Endocrinol (Oxf) 1997; 46:269-270

127. Thomson AH, Devers MC, Wallace AM, Grant D, Campbell K, Freel M, Connell JM. Variability in hydrocortisone plasma and saliva pharmacokinetics following intravenous and oral administration to patients with adrenal insufficiency. Clin Endocrinol (Oxf) 2007; 66:789-796

128. Mah PM, Jenkins RC, Rostami-Hodjegan A, Newell-Price J, Doane A, Ibbotson V, Tucker GT, Ross RJ. Weight-related dosing, timing and monitoring hydrocortisone replacement therapy in patients with adrenal insufficiency. Clin Endocrinol (Oxf) 2004; 61:367-375

129. Johannsson G, Bergthorsdottir R, Nilsson AG, Lennernas H, Hedner T, Skrtic S. Improving glucocorticoid replacement therapy using a novel modified-release hydrocortisone tablet: a pharmacokinetic study. Eur J Endocrinol 2009; 161:119-130

130. Johannsson G, Nilsson AG, Bergthorsdottir R, Burman P, Dahlqvist P, Ekman B, Engstrom BE, Olsson T, Ragnarsson O, Ryberg M, Wahlberg J, Biller BM, Monson JP, Stewart PM, Lennernas H, Skrtic S. Improved cortisol exposure-time profile and outcome in patients with adrenal insufficiency: a prospective randomized trial of a novel hydrocortisone dual-release formulation. J Clin Endocrinol Metab 2012; 97:473-481

131. Lovas K, Husebye ES. Continuous subcutaneous hydrocortisone infusion in Addison's disease. Eur J Endocrinol 2007; 157:109-112

132. Whitaker MJ, Debono M, Huatan H, Merke DP, Arlt W, Ross RJ. An oral multiparticulate, modified-release, hydrocortisone replacement therapy that provides physiological cortisol exposure. Clin Endocrinol (Oxf) 2014; 80:554-561

133. Bleicken B, Hahner S, Loeffler M, Ventz M, Allolio B, Quinkler M. Impaired subjective health status in chronic adrenal insufficiency: impact of different glucocorticoid replacement regimens. Eur J Endocrinol 2008; 159:811-817

134. Bleicken B, Hahner S, Loeffler M, Ventz M, Decker O, Allolio B, Quinkler M. Influence of hydrocortisone dosage scheme on health-related quality of life in patients with adrenal insufficiency. Clin Endocrinol (Oxf) 2010; 72:297-304

135. Oksnes M, Bjornsdottir S, Isaksson M, Methlie P, Carlsen S, Nilsen RM, Broman JE, Triebner K, Kampe O, Hulting AL, Bensing S, Husebye ES, Lovas K. Continuous subcutaneous hydrocortisone infusion versus oral hydrocortisone replacement for treatment of addison's disease: a randomized clinical trial. J Clin Endocrinol Metab 2014; 99:1665-1674

136. Gagliardi L, Nenke MA, Thynne TR, von der Borch J, Rankin WA, Henley DE, Sorbello J, Inder WJ, Torpy DJ. Continuous subcutaneous hydrocortisone infusion therapy in Addison's disease: a randomized, placebo-controlled clinical trial. J Clin Endocrinol Metab 2014; 99:4149-4157

137. Adams JE. Quantitative computed tomography. European journal of radiology 2009; 71:415-424