Mortality and Morbidity in Patients with Addison's Disease

Ragnhildur Bergthorsdottir

Institute of Medicine at Sahlgrenska Academy
University of Gothenburg
Sweden

UNIVERSITY OF GOTHENBURG

Gothenburg 2015
This thesis is dedicated to my mother,
Guðrún Hjörleifsdóttir
GUGGÚ
"Aum Sri Sai Ram"
MORTALITY AND MORBIDITY IN PATIENTS WITH ADDISON'S DISEASE

Ragnhildur Bergthorsdottir

Institute of Medicine at Sahlgrenska Academy,
University of Gothenburg, Sweden

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

E-publication: http://hdl.handle.net/2077/37995
LIST OF PAPERS

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


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.

Health-related quality-of-life (HR-QoL) is compromised in patients with Addison's disease: a case-control study. Manuscript.
# TABLE OF CONTENTS

## ABBREVIATIONS

<table>
<thead>
<tr>
<th>ABBREVIATIONS</th>
<th>5</th>
</tr>
</thead>
</table>

## INTRODUCTION

<table>
<thead>
<tr>
<th>1</th>
<th>INTRODUCTION</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>History of Addison's disease</td>
<td>7</td>
</tr>
<tr>
<td>1.2</td>
<td>Glucocorticoids</td>
<td>8</td>
</tr>
<tr>
<td>1.3</td>
<td>Addison's disease</td>
<td>8</td>
</tr>
<tr>
<td>1.3.1</td>
<td>Epidemiology</td>
<td>8</td>
</tr>
<tr>
<td>1.3.2</td>
<td>Diagnosis</td>
<td>9</td>
</tr>
<tr>
<td>1.3.3</td>
<td>Treatment</td>
<td>9</td>
</tr>
<tr>
<td>1.4</td>
<td>Effects of glucocorticoids</td>
<td>9</td>
</tr>
<tr>
<td>1.4.1</td>
<td>Body composition and cardiometabolic risk factors</td>
<td>10</td>
</tr>
<tr>
<td>1.4.2</td>
<td>Bone</td>
<td>10</td>
</tr>
<tr>
<td>1.4.3</td>
<td>Brain</td>
<td>11</td>
</tr>
<tr>
<td>1.4.4</td>
<td>Morbidity and mortality</td>
<td>11</td>
</tr>
</tbody>
</table>

## AIMS

| 2 | AIMS | 12 |

## SUBJECTS AND METHODS

<table>
<thead>
<tr>
<th>3</th>
<th>SUBJECTS AND METHODS</th>
<th>13</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>Paper I</td>
<td>13</td>
</tr>
<tr>
<td>3.1.1</td>
<td>Study design, subjects and method</td>
<td>13</td>
</tr>
<tr>
<td>3.2</td>
<td>Papers II-IV</td>
<td>14</td>
</tr>
<tr>
<td>3.2.1</td>
<td>Study design and subjects</td>
<td>14</td>
</tr>
<tr>
<td>3.2.2</td>
<td>Methods</td>
<td>14</td>
</tr>
<tr>
<td>3.3</td>
<td>Statistical analysis</td>
<td>18</td>
</tr>
<tr>
<td>3.3.1</td>
<td>Paper I</td>
<td>18</td>
</tr>
<tr>
<td>3.3.2</td>
<td>Papers II-IV</td>
<td>18</td>
</tr>
</tbody>
</table>

## RESULTS

<table>
<thead>
<tr>
<th>4</th>
<th>RESULTS</th>
<th>19</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1</td>
<td>Paper I</td>
<td>19</td>
</tr>
<tr>
<td>4.1.1</td>
<td>Mortality</td>
<td>19</td>
</tr>
<tr>
<td>4.1.2</td>
<td>Impact of concurrent DM</td>
<td>21</td>
</tr>
<tr>
<td>4.2</td>
<td>Papers II-IV</td>
<td>21</td>
</tr>
<tr>
<td>4.2.1</td>
<td>Subject characteristics</td>
<td>21</td>
</tr>
<tr>
<td>4.2.2</td>
<td>VAT and the prevalence of MetS</td>
<td>21</td>
</tr>
<tr>
<td>4.2.3</td>
<td>Glucose metabolism</td>
<td>23</td>
</tr>
<tr>
<td>4.2.4</td>
<td>Lipids, and inflammatory and fibrinolytic markers</td>
<td>25</td>
</tr>
<tr>
<td>4.2.5</td>
<td>Bone mineral density and the prevalence of osteoporosis</td>
<td>25</td>
</tr>
<tr>
<td>Section</td>
<td>Page</td>
<td></td>
</tr>
<tr>
<td>------------------------------------------------</td>
<td>------</td>
<td></td>
</tr>
<tr>
<td>4.2.6 Osteoporosis and osteopenia</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>4.2.7 Self-reported HR-QoL and general well-being</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>4.3 Paper I-IV Gender considerations</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>5 DISCUSSION</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>5.1 Paper I Mortality</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>5.2 Paper II Cardiometabolic risk factors</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>5.3 Paper III Bone metabolism</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>5.4 Paper IV HR-QoL and general well-being</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>5.5 Gender considerations</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>5.6 Glucocorticoid replacement therapy</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>6 CONCLUSION</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>7 FUTURE PERSPECTIVES</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>SAMMANFATTNING PÅ SVENSKA</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>REFERENCES</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>ORIGINAL PAPERS</td>
<td>50</td>
<td></td>
</tr>
</tbody>
</table>
ABBREVIATIONS

11β-HSD  11β-Hydroxysteroid dehydrogenase
ACTH  Adrenocorticotropin 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
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SF-36</td>
<td>Short Form-36</td>
</tr>
<tr>
<td>SNBW</td>
<td>Swedish National Board of Health and Welfare</td>
</tr>
<tr>
<td>T1DM</td>
<td>Type 1 diabetes mellitus</td>
</tr>
<tr>
<td>T2DM</td>
<td>Type 2 diabetes mellitus</td>
</tr>
<tr>
<td>T4</td>
<td>Free thyroxin</td>
</tr>
<tr>
<td>TC</td>
<td>Total cholesterol</td>
</tr>
<tr>
<td>TG</td>
<td>Triglycerides</td>
</tr>
<tr>
<td>TSH</td>
<td>Thyroid-stimulating hormone</td>
</tr>
<tr>
<td>UFC</td>
<td>Urinary free cortisol</td>
</tr>
<tr>
<td>VAT</td>
<td>Visceral adipose tissue</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organisation</td>
</tr>
</tbody>
</table>
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 Addison'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, cortisol [also called compound E (Kendall's compound)], was introduced: cortisol 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 cortisol treatment for replacement therapy, the improvement in life expectancy was described by the words:

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

Shortly after the introduction of cortisol (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 11β-hydroxysteroid dehydrogenase (11β-HSD) 1 and 2. 11β-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. 11β-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 11β-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 255E (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 255E, 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 1.2–6.3% (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 27.1–4) 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 $1.0$ and $2.5$ and osteoporosis as T-score $<2.5$ 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 imunoassay (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 (cm², 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 ANALYSIS

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 1987-2001 (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 Addison’s disease obtained from The National Hospital and Cause of Death Registers at the SNBW during the period 1987-2001

<table>
<thead>
<tr>
<th></th>
<th>All (N) subjects</th>
<th>Subjects (N) with DM</th>
<th>Percentage with DM</th>
<th>N of deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Men</strong></td>
<td>680</td>
<td>86</td>
<td>12.6</td>
<td>208</td>
</tr>
<tr>
<td><strong>Women</strong></td>
<td>995</td>
<td>113</td>
<td>11.4</td>
<td>299</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>1675</td>
<td>199</td>
<td>12</td>
<td>507</td>
</tr>
</tbody>
</table>

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 1987-2001, 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.
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.

<table>
<thead>
<tr>
<th>Obs. no.</th>
<th>Exp. no.</th>
<th>Risk Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>All cause mortality</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>507</td>
<td>199</td>
</tr>
<tr>
<td><strong>men</strong></td>
<td>208</td>
<td>95</td>
</tr>
<tr>
<td><strong>women</strong></td>
<td>299</td>
<td>104</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Obs. no.</th>
<th>Exp. no.</th>
<th>Risk Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cardiovascular dis.</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>239</td>
<td>112</td>
</tr>
<tr>
<td><strong>men</strong></td>
<td>103</td>
<td>52</td>
</tr>
<tr>
<td><strong>women</strong></td>
<td>136</td>
<td>60</td>
</tr>
<tr>
<td><strong>Malignant dis.</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>73</td>
<td>47</td>
</tr>
<tr>
<td><strong>men</strong></td>
<td>36</td>
<td>22</td>
</tr>
<tr>
<td><strong>women</strong></td>
<td>37</td>
<td>25</td>
</tr>
</tbody>
</table>
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 for 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/m². 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) cm², 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 Addison’s disease and their matched controls (mean ± SD, (%) or median (IQR))

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patients</th>
<th>Controls</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>76</td>
<td>76</td>
<td></td>
</tr>
<tr>
<td>Age years (years)</td>
<td>53.2 ± 13.9</td>
<td>53.7 ± 14.0</td>
<td>0.8</td>
</tr>
<tr>
<td>Female N (%)</td>
<td>51 (67%)</td>
<td>51 (67%)</td>
<td>1.0</td>
</tr>
<tr>
<td>BMI kg/m²</td>
<td>25.3 ± 4.0</td>
<td>24.8 ± 3.8</td>
<td>0.4</td>
</tr>
<tr>
<td>Waist circumference cm</td>
<td>89.7 ± 11.5</td>
<td>87.8 ± 9.8</td>
<td>0.3</td>
</tr>
<tr>
<td>Diabetics N (%)</td>
<td>12 (16%)</td>
<td>1 (1%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Type 1</td>
<td>5</td>
<td>0</td>
<td>0.06</td>
</tr>
<tr>
<td>Type 2</td>
<td>7</td>
<td>1</td>
<td>0.06</td>
</tr>
<tr>
<td>Hypothyroidism N (%)</td>
<td>34 (45%)</td>
<td>2 (3%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TSH mIU/L</td>
<td>2.2 (1.8)</td>
<td>1.9 (1.0)</td>
<td>0.2</td>
</tr>
<tr>
<td>Antihypertensive treatment N (%)</td>
<td>16 (21%)</td>
<td>4 (5%)</td>
<td>0.004</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>127 ± 18</td>
<td>124 ± 18</td>
<td>0.4</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>75 ± 9</td>
<td>73 ± 10</td>
<td>0.3</td>
</tr>
<tr>
<td>Lipid lowering therapy N (%)</td>
<td>11 (15%)</td>
<td>1 (1%)</td>
<td>0.003</td>
</tr>
<tr>
<td>Bisphosphates N (%)</td>
<td>4 (5%)</td>
<td>0 (0%)</td>
<td>0.12</td>
</tr>
<tr>
<td>DHEA treatment N (%)</td>
<td>7 (9.2%)</td>
<td>0 (0%)</td>
<td>0.014</td>
</tr>
<tr>
<td>Smokers N (%)</td>
<td>3 (4%)</td>
<td>4 (5%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Metabolic syndrome N (%)</td>
<td>25 (33%)</td>
<td>12 (16%)</td>
<td>0.020</td>
</tr>
<tr>
<td>Serum Cortisol nmol/L</td>
<td>770 (450)</td>
<td>415 (180)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>24h-UFC nmol</td>
<td>359 (408)</td>
<td>175 (104)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>U-Cortisol/creatinine estimates</td>
<td>27.6 (28.4)</td>
<td>14.3 (5.8)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

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))

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Controls</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>76</td>
<td>76</td>
<td></td>
</tr>
<tr>
<td>L4-subcutaneous adipose tissue (cm²)</td>
<td>226 (164)</td>
<td>225 (124)</td>
<td>0.5</td>
</tr>
<tr>
<td>L4-visceral abdominal adipose tissue (cm²)</td>
<td>76 (93)</td>
<td>71 (92)</td>
<td>0.7</td>
</tr>
<tr>
<td>Thigh-subcutaneous adipose tissue (cm²)</td>
<td>75 (59)</td>
<td>74 (59)</td>
<td>0.3</td>
</tr>
<tr>
<td>Thigh-intermuscular adipose tissue (cm²)</td>
<td>1.6 (1.6)</td>
<td>1.4 (1.9)</td>
<td>0.3</td>
</tr>
<tr>
<td>Thigh-muscle (cm²)</td>
<td>109 (44)</td>
<td>110 (42)</td>
<td>0.9</td>
</tr>
<tr>
<td>L4-Circumference (cm)</td>
<td>90 (14)</td>
<td>90 (14)</td>
<td>0.7</td>
</tr>
<tr>
<td>Liver attenuation (HU)</td>
<td>59 (4)</td>
<td>57 (7)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

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 Addison’s disease and their matched controls (median (IQR))

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

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 Addison’s 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 ≥80 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 Addison’s 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).

![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%.

**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: 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 cortisol 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 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 11β-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

31
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 osteoporosis (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 fatigue.
Addisons sjukdom (AS) eller primär binjurebarksvikt 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 huvudsytte 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 älder, kön och kroppssmassaindex (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 (ostoporos). 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.

**Key words:** Addisons sjukdom, mortalitet, glukokortikoider, glukokortikoid ersättningsbehandling, hjärt- och kärlsjukdom, bentäthet, benskörhet, livskvalitet.

ACKNOWLEDGEMENTS

I would like to thank all the wonderful people who contributed in some way to the work described in this thesis. Especially, I would like to thank:

**Gudmundur Johannsson**, my main supervisor, for giving me the opportunity to join his research group for many years ago and for never giving up on me, it has been such a pleasure and honour. I would like to thank him for sharing his visionary clinical and academic expertise with me, for his understanding and support and all interesting discussions we have had through the years.

My co-supervisors and co-authors, **Oskar Ragnarsson, Stanko Skrtic** and **Maria Leonsson-Zachrisson** for their valuable contribution, insightful comments and motivating discussion. In particular I would like to thank **Óskar** for being my friend and always available and for bringing me down-to-earth when I fly away in speculation and his wife **Ósk** for her tolerance and for being my good friend.

My co-authors, **Anders Odén**, **Ian Louis Ross**, **Dimitris Chantzichristos** and **Eleni Pappakokkinou** who all contributed in a joint effort to make this thesis come true.

The personnel at the Centre of Endocrinology and Metabolism (CEM): **Ingrid Hansson, Anna-Lena Jönsson, Lena Wirén, Annica Alklind, Jenny Tiberg, Kristina Cid Käll**, **Ann-Charlotte Olafsson (Lotta), Anna Olsson** and **Annika Reibring** for their tireless efforts in helping me in every way through the years. Hjartans þakkir 😊

The present and former heads of the Institute of Medicine at Sahlgrenska Academy, University of Gothenburg and the Department of Medicine at the Sahlgrenska University Hospital for encouraging scientific research and for providing the resources necessary to perform them.

All my colleagues at the Department of Endocrinology, Diabetology and Metabolism, Sahlgrenska University Hospital, for their continuous friendly support through the years.

To my present and previous room-mates and dear friends at Sahlgrensa, **Helena Filipsson, Penelope Trimpou, Eleni Pappakokkinou, Dimitris Chantzichristos, Daniel Olson** and **Camilla Glad** for their support and lots of fun and laughter.

**Håkan Widell, Erik Almqvist, Eva Ekerstad** and **Jonathan Mulenga** for their lively help in recruiting patients.

**Jan-Erik Angelhed** for all the technical information I received about the CT.

**Lars Ellegård** and **Vibeke Malmros** for the technical information on the DEXA system.
Matias Arkekling 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'll never walk alone! I love you so much...

The projects in this thesis received financial support from the Swedish federal government under the LUA/ALF agreement, The Health & Medical Care Committee of the Regional Executive Board, Region Västra Götaland and The Göteborg Medical Society.
REFERENCES

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
24. Peckett AJ, Wright DC, Riddell MC. The effects of glucocorticoids on adipose tissue lipid metabolism. Metabolism 2011; 60:1500-1510
31. de Kloet ER, Oitzl MS, Joels M. Stress and cognition: are corticosteroids good or bad guys? Trends in neurosciences 1999; 22:422-426
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
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


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


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


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
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
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


118. Ragnarsson O, Nyström 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


