Glucocorticoid Treatment and Quality of Life in Addison's disease

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Learn from yesterday, live for today, hope for tomorrow. The important thing is not to stop questioning.

Albert Einstein

Scientific environment

This work was carried out at **Department of Clinical Science**, Section for Endocrinology, Faculty of Medicine and Dentistry, University of Bergen; in cooperation with **Department of Medicine**, section for Endocrinology, Haukeland University hospital, Bergen, Norway. The endocrinology research group is led by Professor Eystein Husebye.

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Abbreviations

AC:	Addisonian crisis
ACTH:	Adrenocorticotropic hormone
AD:	Addison's disease
AIRE:	Autoimmune regulator
allo-THF:	allo- tetrahydrocortisol
APS:	Autoimmune Polyendocrine Syndrome
AUC:	Area under the curve
BMD:	Bone mineral density
BMI:	Body mass index
BSA:	Body surface area
CA:	Cortisone acetat
CAH:	Congenital adrenal hyperplasia
CBG:	Cortisol binding globulin
CRH:	Corticotropin-releasing hormone
CSHI:	Continous subcutaneous hydrocortisone infusion
CTX-1:	C-terminal crosslinked C-telopeptide of type 1 collagen
CVD:	Cardiovascular disease
CYP3A4 :	6β-hydroxylase
DHEA(S):	Dehydroepiandrosterone (sulphate)

DIF: Differential item functioning

DOCA: Desoxycorticosterone-acetat

EEG: Electroencephalogram

EFA: Exploratory factor analysis

FT4: Free T4, Free thyroxine

GC(s): Glucocorticoid(s)

GR(s): Glucocorticoid receptor(s)

GH: Growth hormone

GRE: Glucocorticoid responsive elements

HbA1c: Hemoglobin A1c

HDL: High-density lipoprotein

HIV: Human immunodeficiency virus

HOMA: Homeostatic model assessment

HPA axis: Hypothalamus-Pituitary-Adrenal axis

HRQoL: Health Related Quality of Life

IFN-ω: Interferon omega

LC-MS/MS: Liquid chromatography and sequential mass spectrometry

LDL: Low-density lipoprotein

M-value: Whole-body insulin sensitivity

MR: Mineralocorticoid receptor

MC1-5R: Melanocortin receptor 1-5

MCID: Minimal clinically important difference

MSH: Melanocyte stimulating hormone

NEFA: Non-esterified fatty acids

OHC: Oral hydrocortisone

PAI: Primary adrenal insufficiency

PER1: Period 1 gene

PGWB: Psychological general well-being index

PNMT: Phenylethanolamine-N-methyltransferase

P1NP: Procollagen type 1 N-terminal peptide

POMC: Pro-opiomelanocortine

PRA: Plasma renin activity

PSI: Person separation index

PSQI: Pittsburgh sleep quality index

PTH: Parathyroid hormone

PVN: Paraventricular nucleus

QoL: Quality of Life

REM: Rapid eye movement

ROAS: Registry of Organ specific Autoimmune Diseases

SAI: Secondary adrenal insufficiency

SCC: Side-chain cleavage enzyme

SCN : Suprachiasmatic nucleus

SF-36: Short Form-36

SHBG: Sex hormone binding globulin

SMR: Standard mortality rate

SWS: Slow wave sleep

THE: Tetrahydrocortisone

THF: Tetrahydrocortisol

TSH: Thyrotropin

VAS: Visual-analog scale

VLCFA: Very long chain fatty acids

WHO: Word Health Organisation

WHR: Waist hip ratio

β-LPH: Beta-lipotropin

11β-HSD: 11 beta-hydroxysteroid dehydrogenase

17OHP: 17-hydroxyprogesterone

21-OH: 21-hydroxylase

21OHAb: 21-hydroxylase antibodies

Abstract

Addison's disease (AD) is rare and result in lack of the adrenal hormones cortisol, aldosterone and adrenal androgens. Despite conventional oral replacement therapy, mortality is increased and Health-Related Quality-of-life (HRQoL) is reduced. Currently, the non-physiological circadian cortisol profile is suspected to be a major cause, although evidence has been lacking. Here, we aimed to develop a better tool for evaluating HRQoL in AD, and to investigate whether a more physiological circadian cortisol profile would result in benefit for AD patients.

An AD-specific HRQoL questionnaire (AddiQoL) was developed through a multistep approach. After testing the original AddiQoL in 86 patients in UK, the AddiQoL was translated to five European languages and tested in further 615 AD patients in respective countries. Applying Rasch analysis, a valid and reliable 30 item AddiQoL was produced.

In a randomized controlled multicenter trial with cross-over design, we compared the effects of three months treatment with continuous subcutaneous hydrocortisone infusion (CSHI) to the effects of three months treatment with conventional oral hydrocortisone (OHC) in 33 AD patients. The primary endpoint was the effect on ACTH levels. Secondary endpoints were effects on metabolism, HRQoL and sleep. CSHI produced a more physiological circadian cortisol biorhythm than conventional therapy and induced normalization of morning ACTH and cortisol levels, restoration of nighttime cortisol levels and changes in glucocorticoid metabolism resembling healthy individuals. The late night decrease in glucose seen with OHC was counteracted, without decreasing overall insulin sensitivity. CSHI did not significantly affect sleep but might have positive HRQoL effects.

The AddiQoL development provided a valid and reliable new tool for HRQoL evaluation in AD. Mimicking the physiological cortisol rhythm with CSHI proved safe and provides a means for further improving replacement therapy in AD.

List of publications

Paper 1: *Development of a Disease specific Quality of Life Questionnaire in Addison's Disease;* J.Clin.Endocrinol.Metab. 2010, 95:545-551
Løvås K, Curran S, Øksnes M, Husebye ES, Huppert FA, Chatterjee VK

Paper 2: *Quality of life in European patients with Addison's disease; Validity of the disease-specific questionnaire AddiQoL;* J Clin Endocrinol Metab. 2012, 97(2):568-576
Marianne Øksnes, Sophie Bensing, Anna Lena Hulting, Olle Kämpe, Annika Hackemann, Gesine Meyer, Klaus Badenhoop, Corrado Betterle, Anna Parolo, Roberta Giordano, Alberto Falorni, Lucyna Papierska, Wojciech Jeske, Anna A. Kasperlik-Zaluska, V. Krishna K. Chatterjee, Eystein S. Husebye, Kristian Løvås

Paper 3: *Continuous subcutaneous hydrocortisone infusion versus oral hydrocortisone replacement for treatment of Addison's disease: A randomized clinical trial;* J Clin Endocrinol Metab. 2014, in press
Marianne Øksnes, Sigridur Björnsdottir, Magnus Isaksson, Paal Methlie, Siri Carlsen, Roy M. Nilsen, Jan-Erik Broman, Kai Triebner, Olle Kämpe, Anna-Lena Hulting, Sophie Bensing, Eystein S. Husebye, Kristian Løvås

Paper 4: *Circadian hormone profiles and insulin sensitivity in patients with Addison's disease: A comparison of continuous subcutaneous hydrocortisone infusion with conventional glucocorticoid replacement therapy;* Submitted manuscript
Sigridur Björnsdottir*, Marianne Øksnes*, Magnus Isaksson, Paal Methlie, Roy M. Nilsen, Olle Kämpe, Anna-Lena Hulting, Eystein S. Husebye, Kristian Løvås, Thomas Nyström, Sophie Bensing

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2. Introduction

Normal physiology

The adrenals

The adrenals are endocrine organs located above each kidney, consisting of a cortex and a medulla. The adrenal medulla consists of chromaffin cells, specialized for the production of the catecholamines adrenaline and noradrenaline in response to stimulation from the sympathetic nerve system. Although the medulla is of different embryonic origin and regulated independently of the adrenal cortex, medullary adrenaline production is dependent on cortisol production from the adrenal cortex. The production of adrenalin from its precursor noradrenalin is rate-limited by the enzyme phenylethanolamine-N-methyltransferase (PNMT), which is induced by high local concentrations of cortisol in the medulla; glucocorticoids (GCs) are also necessary for maintenance of chromaffin cells (1).

The adrenal cortex's characteristic architecture reflects the different hormones produced, each hormone being derived from cholesterol (Figure 1). The outer layer, zona glomerulosa, is the source of mineralocorticoids; the most important is aldosterone. Aldosterone is crucial for regulating water- and salt homeostasis, hereby regulating blood pressure. The effect of aldosterone is mediated via the mineralocorticoid receptor (MR); located in the kidneys, salivary glands and colon. Activation of the MR in the kidneys will increase urinary reabsorption of sodium and water, and increase excretion of potassium. Aldosterone production is regulated by the renin-angiotensin system, which is responsive to changes in blood pressure and potassium balance. Although aldosterone production is regulated independently of cortisol production, the MR is not aldosterone specific, and cortisol excess will result in stimulation of the MR producing an aldosterone-like effect (2).

GCs are produced in the middle cortex layer, zona fasciculata, where the principal hormone is cortisol. GCs have diverse and wide-spread effects, which is not surprising given the fact that glucocorticoid receptors (GRs) are present in virtually every cell type in the human body. Briefly, cortisol is important for modulation of the immune system, for neuron function as well as for maintaining energy metabolism and bone metabolism (further outlined below).

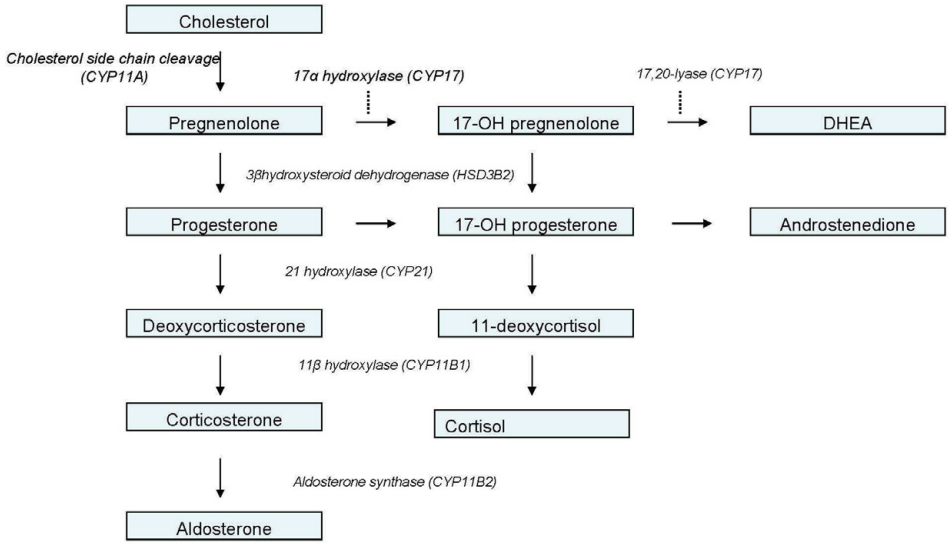

Figure 1. Adrenal steroidogenesis.
Modified from Williams Textbook of endocrinology (10.edition, page 495)

The adrenal androgens dehydroepiandrosterone (DHEA) and androstenedione are produced from zona reticularis, the innermost level of the adrenal cortex. DHEA is further metabolized to dehydroepiandrosterone sulphate (DHEAS) in other tissues. Androgen production is stimulated by ACTH. Their physiological role in humans is still not clear as they have very little intrinsic androgen effect, and are thought to function as circulating pro-hormones that can be converted to active androgens in androgen sensitive tissues (3). The adrenals contribute 30-50% of androgens in men, but are responsible for 50-100% of androgens in pre- and postmenopausal women (4, 5).

The Hypothalamus-Pituitary-Adrenal (HPA) axis

Adrenal production of cortisol is under tight control of the physiological HPA axis feed-forward/feed-back system (Figure 2, (6)). A state of cortisol deficiency will stimulate the paraventricular nucleus (PVN) to secrete corticotropin releasing hormone (CRH), which again will stimulate the anterior pituitary to secrete ACTH. ACTH promotes cortisol production from the adrenals. Conversely, a state of cortisol sufficiency or excess will suppress CRH and ACTH, leading to lack of adrenal stimulation and reduction of cortisol production. There is a delay in the positive feed-forward cortisol response to ACTH because cortisol has to be synthesized de novo in the adrenal cortical cell before secretion. Cortisol exerts a negative feed-back effect by binding to low-affinity GRs and high affinity MRs in the brain. It has been suggested that the effect of MR dominates in the early nighttime when cortisol levels are low (to maintain low basal HPA activity), whereas the GR inhibitory effect on the HPA axis dominates in the morning when cortisol levels peak (to constrain HPA activity), although this view has recently been disputed (7, 8).

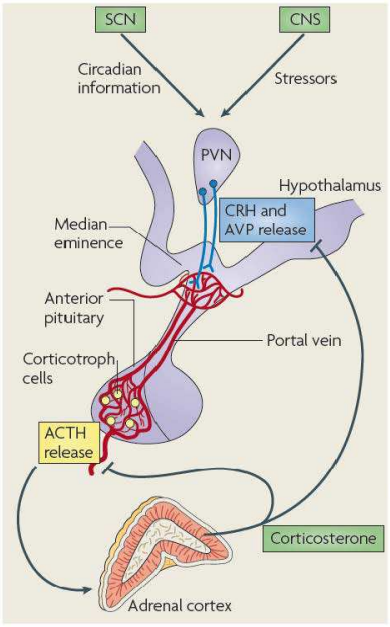

Figure 2. The feed-forward / feed-back system of the HPA-axis.
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ACTH is derived from the Pro-opiomelanocortine (POMC) peptide produced in the anterior pituitary, and is enzymatically split into γ-melanocyte-stimulating hormone (γ-MSH), ACTH and β-lipoprotein (β-LPH) (Figure 3). ACTH and β-LPH is further split to produce two other melanocortins; α-MSH and β-MSH. All of these melanocortins share a common core sequence of four amino acids, which produces overlap in receptor binding. In conditions characterized with high ACTH levels, β-LPH is also elevated (9). γ-MSH, but not α-MSH, is increased by stressors, and decreased with negative GC feed-back (10, 11).

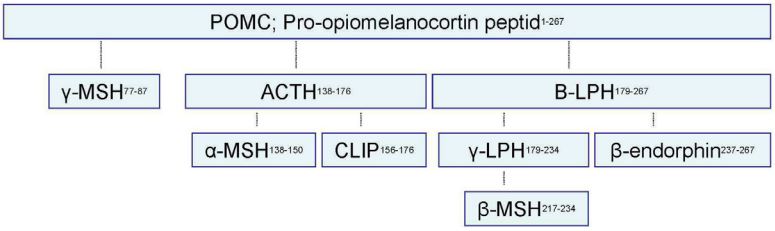


Figure 3. Cleavage of the common precursor Pro-opiomelanocortin to ACTH and the melanocortins α-MSH, β-MSH and γ-MSH.
Modified from Williams textbook of endocrinology (10.edition, page 245) and Catania A, Gatti S, Colombo G, Lipton JM 2004 Pharmacological reviews 56:1-29

The recent decades have produced new insight in the melanocortin system, particularly after identification of the five melanocortin receptors (MC1R-MC5R). Activation of these receptors has diverse effects, such as immune modulation, lipolysis and effects on feeding behaviour and metabolism (12). The MC2R is ACTH specific and predominantly found in the adrenals, where stimulation will increase cortisol production, and in adipocytes, where in vitro studies have shown that activation induces lipolysis (13). The best described function of the MC1R is induction of melanogenesis, but the receptor is also identified in non-pigmented cells

(14). MC3R and MC4R are both involved in energy homeostasis and feeding (15, 16), and mediates the central effects of melanocortins, including neuroprotection (17). MC3R has additional effects on control of inflammation (18). MC5R is widely distributed, and has been shown to have immune modulating effect on B- and T-lymphocytes and mast cells, and effects on exocrine secretions (19). Hence, immune modulating effects of melanocortins can be mediated by several of the receptors, although the mechanism is not fully understood. However, studies have shown that α-MSH can inhibit antigen stimulated T cell proliferation and inhibit production of proinflammatory cytokines through inhibition of a nuclear transcription factor (17).

Glucocorticoid rhythm - circadian and ultradian

The word circadian derives from the Latin *circa* (around) and *dies* (day), and physiological oscillations with 24 h periodicity are referred to as circadian rhythms. Oscillation of shorter duration, i.e. a few hours, is referred to as ultradian. Circadian rhythms control a wide range of physiological events, such as sleep, body temperature, feeding patter and even metabolism (20).

In healthy subjects, cortisol levels fluctuate in a circadian manner, with nadir around midnight, followed by gradually increasing levels during the last part of the night culminating in the highest peak near awakening (21).The circadian rhythm of cortisol production depends on activity in the suprachiasmatic nucleus (SCN), via the PVN in the hypothalamus (22). The SCN also receives input regarding light, and can adapt the circadian cortisol rhythm to the day/night cycle. CRH is derived mainly from the PVN, and act on CRH receptors in the pituitary to promote the production and secretion of ACTH. This effect can be potentiated by other neuropeptides, especially vasopressin. CRH is secreted in a circadian and pulsatile fashion, with the highest levels occurring during the early morning and the lowest levels during the first part of the night.

In recent years new technology has made it possible to examine the rhythm/pattern of the HPA axis more closely, revealing an underlying ultradian rhythm, with cortisol pulses approximately once per hour, preceded by pulses of ACTH (Figure 4). Every secretory pulse of ACTH is followed by a delayed response of cortisol (6). The amplitude of the pulses varies with the circadian pattern, with the highest amplitude before awakening. This cortisol pulse rhythm is synchronized between the blood, the subcutaneous tissue and the brain (23). Pulsed stimulation of the GR has also been shown to result in pulsed transcription of GC-responsive genes, and pulsatile signalling produces a different transcript when compared to constant signalling (24). This dynamic transcriptional system provides a sensitive mechanism for the maintenance of homeostasis by enabling cells to rapidly detect and respond to changes in circulating cortisol (6).

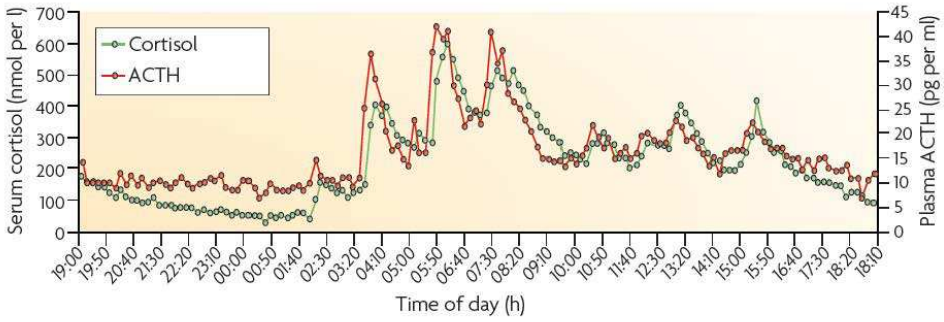


Figure 4. Circadian and ultradian rhythms for ACTH and cortisol in healthy humans. Reprinted with permission from author (Lightman SL, Conway-Campbell BL 2010 Nat Rev Neurosci 11:710-718)

Destruction of the SCN will abolish the circadian rhythm, while the ultradian rhythm is maintained (25). This has challenged the earlier view that also the ultradian rhythm is the results of a hypothalamic pulse generator. Recent studies have suggested that these oscillations result from a pituitary-adrenal system producing a complex feed-

forward/feed-back system (26). It is hypothesized that the delayed feed-forward response in cortisol in combination with rapid GC feed-back inhibition at the level of the pituitary is the primary factor behind the ultradian rhythm.

Disruption of the cortisol rhythm has been associated with various disorders, for example depression (27), obstructive sleep apnoea (28) and sleep disorders (29). Interestingly, Plat and co-workers were able to show that elevation of plasma cortisol in the evening when the HPA axis is normally quiescent has more deleterious metabolic effects than a similar elevation in the morning when the HPA axis is maximally activated (30), implicating that maintenance of the circadian cortisol rhythm is of importance also in carbohydrate metabolism. Studies have also shown that GCs regulate the expression of the clock gene *PER1,* suggesting a role for GCs in modulating the biological circadian clock (31-33).

Glucocorticoid receptors

The peripheral effects of GCs are primarily mediated through the intracellular GR. The concentration of GRs in a given tissue can modify the effect magnitude of receptor binding (34). The effect of ligand binding differs from one cell type to another, implicating that several mechanisms are involved in signal transduction. The GR belongs to the nuclear receptor family, and upon cortisol binding, the GR-ligand complex undergoes conformational changes that result in translocation to the nucleus. Through interactions with GC responsive elements in the genome the complex functions as a transcription factor for target genes; however, multiple possible genomic binding sites exist (35). Access to the genome, and hence transcription, is also dependent on the presence of protein co-activators or repressors which may facilitate or hinder the GR's access to RNA II polymerase by remodelling chromatin (34). In addition to the direct effect on DNA transcription, the GR also interacts with other non-receptor transcription factors and co-regulators through protein-protein interactions, which can either induce or repress transcription, producing another mechanism for cell-specific effects of GCs (36, 37). Finally, GCs

also have rapid (anti-inflammatory) non-genomic effects, although the physiology and function of these are still not fully understood (38, 39).

The GR has a lower affinity for cortisol than the MR. In the brain, notably the hippocampus and the pituitary, GCs can also exert their effects through the MR. The MR has a high affinity for cortisol and has a high degree of occupancy during basal conditions, as the MR's aldosterone specificity seen in the periphery is lacking due to the lack of 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2). Hence, the MR can maintain receptor activation throughout 1h intervals between secretory cortisol bursts lasting 20 minutes, whereas activation of the GR follows the ultradian rhythm more closely (40). Tissue differences in distribution of receptor type may therefore allow similar pulses to convey different information to different tissues (8).

Glucocorticoid effects

The widespread distribution of the GR in combination with the distribution of GC to virtually every tissue by the circulation enable GCs to modulate the expression of about ten percent of our genes, although few if any genes are exclusively controlled by GCs (34, 41). This is mirrored in the complex picture of GC function in the human body. Historically, the physiological effect of cortisol is sometimes confused with the effects of supra-physiological pharmacological GC doses, as there are few studies on GC effects during physiological sub-saturating conditions (42). Unfortunately, the role of GCs in maintaining homeostasis in a non-stressful situation is less clear (43).

It was Philip Hench who incidentally discovered the anti-inflammatory effects of cortisone, an observation which granted him the Nobel Prize in medicine and physiology in 1950 (44). The GCs are now known as key players in the regulation of inflammation and immunity, with well-known and important immunosuppressive effects of pharmacological doses (45). Cytokines and other pro-inflammatory mediators can activate the HPA axis. The resulting increased cortisol levels will exert a negative feed-back by inhibiting the inflammatory process, hence constraining the

immune response from going overboard (38, 43, 45). Hypercortisolism in the absence of inflammation, i.e. Cushing syndrome or high-dose GC treatment, can cause immunosuppression and increase the risk of infection (45).

High doses of GCs also have important effects on carbohydrate and lipid metabolism by increasing glycogen production and gluconeogenesis in the liver (46), inhibiting peripheral glucose uptake and increasing lipolysis. The net effect is an increase in insulin resistance and increased levels of glucose, that is, acting as an insulin antagonist, producing a diabetogenic effect with concomitant catabolism of lipids and proteins (47). This effect is important when the body requires rapidly available energy substrates to cope with a major stressor. Chronic hypercortisolism causes hyperglycaemia and may lead to type 2 diabetes mellitus, whereas chronic hypocortisolism (i.e. adrenal insufficiency) predisposes for hypoglycaemia. However, the influence of GCs on glucose metabolism in normal subjects in a non-stressful situation is not fully understood, and some authors have suggested that the name *gluco*corticoid is misleading (38, 48). Thus, although cortisol may act synergistically with other factors to influence carbohydrate metabolism, being especially important in a stressful situation, the role of GCs in carbohydrate metabolism in the normal situation is less clear. Also, GCs act in concert with other hormones such as insulin, glucagon, growth hormone (GH), among others, which also have profound effect on carbohydrate metabolism.

Multiple other effects of GCs are described; most of them are associated with supra-physiological doses (47): In the skin, connective tissue and in muscle, GCs cause atrophy. In bone, osteoblasts are inhibited, decreasing bone formation. Also, calcium absorption from the intestines is reduced, resulting in elevated parathyroid hormone (PTH) levels and further deteriorating bone metabolism. GCs can increase blood pressure by several mechanisms. Synthesis of angiotensinogen is increased, the sensitivity to catecholamines and angiotensin II is increased in vascular smooth muscle and the nitric oxide mediated dilatation of endothelia is reduced. While these

effects are important in the event of a major stressor, chronic high endogenous or exogenous delivery of GC will cause hypertension. GCs also have cerebral dose-dependent effects. In addition to effects on sleep (29), GCs are also linked to feeding behaviour, cognitive function and psychiatric disturbances, depression being the most common diagnosis (49).

In the later chapters of this thesis I will focus on the effects of GC in the context of replacement therapy rather than on effects of GCs in general.

Glucocorticoid metabolism

The concentration of free cortisol accounting for biological activity accounts for less than 5% of the total cortisol concentration (50). The half-life of circulating free cortisol is short, approximately 70-120 min. More than 90% of circulating cortisol is bound, predominantly to cortisol-binding-globulin (CBG, 70%), and to a lesser degree to albumin (20%) (51). CBG is saturated at 400-500 nmol/L, higher levels will increase the biological active free fraction rapidly (52). CBG is synthesized in the liver – production is enhanced by estrogens – and levels might also be elevated in patients with chronic active hepatitis and acute lymphatic or myeloid leukemia. CBG levels are reduced by GCs, and in patients with hyperthyroidism, cirrhosis and nephrotic syndrome (53). In AD patients, both the total and the free cortisol levels are reduced.

A crucial step in the metabolism of cortisol is the interconversion of cortisol to cortisone, governed by the intracellular 11β-hydroxysteroid dehydrogenase (11β-HSD) enzymes, and local tissue concentrations of cortisol are modulated by tissue-specific 11β-HSD. Hereby 11β-HSD can modulate the magnitude of the cortisol response on the tissue level. This enzyme has 2 different isoforms: 11β-HSD type 1 (11β-HSD1) and 11β-HSD2.

11β-HSD1 is widely expressed in the human body and functions predominantly as a reductase, regenerating cortisol from the biologically inactive counterpart cortisone to maintain adequate activation of the GR in local tissue. 11β-HSD1 activity is especially abundant in the liver (54), although a significant contribution from adipose tissue and skeletal muscle has been demonstrated (55, 56). This implies that 11β-HSD1 might modulate circulating cortisol levels (57). In healthy humans, the proportion of the total cortisol pool derived from 11β-HSD1 mediated regeneration of cortisol from cortisone has been quantified to approximately 11 nmol/min when the adrenal cortisol production rate was 38 nmol/min (58). This suggests that the inactive metabolite cortisone function as a systemic GC reservoir, which can be locally activated when needed, in the presence of 11β-HSD1 (59). Others have suggested that although the primary function of 11β-HSD1 functions is to regenerate cortisol from cortisone, it may also function as a dehydrogenase, that is, promote conversion of cortisol to cortisone. This dual function facilitates recycling between cortisol and cortisone in local tissues (41, 56). 11β-HSD1 activity can be stimulated by thyroid hormones and inhibited by growth hormone (60).

Circulating cortisone levels in healthy people are primarily dependant on renal 11β-HSD2 activity, approximating one fifth of circulating cortisol levels (61). ACTH stimulation has been shown to decrease cortisone levels in addition to increasing cortisol levels, hence increasing the cortisol/cortisone ratio (59). This suggests that ACTH may modulate peripheral metabolism of cortisol by modulating 11β-HSD activity, which might be important under stressful conditions.

11β-HSD2 provides a barrier against GC excess in tissues with high aldosterone sensitivity, such as the kidneys and salivary glands were 11β-HSD2 inactivates cortisol to cortisone. In a state of GC excess, renal 11β-HSD2 activity can be exceeded, increasing loss of cortisol in the urine and increasing the urine cortisol/cortisone ratio (57). 11β-HSD2 genetic insufficiency or inhibition causes

apparent mineralocorticoid excess and hypertension due to inappropriate GC activation of the renal MR (62-64).

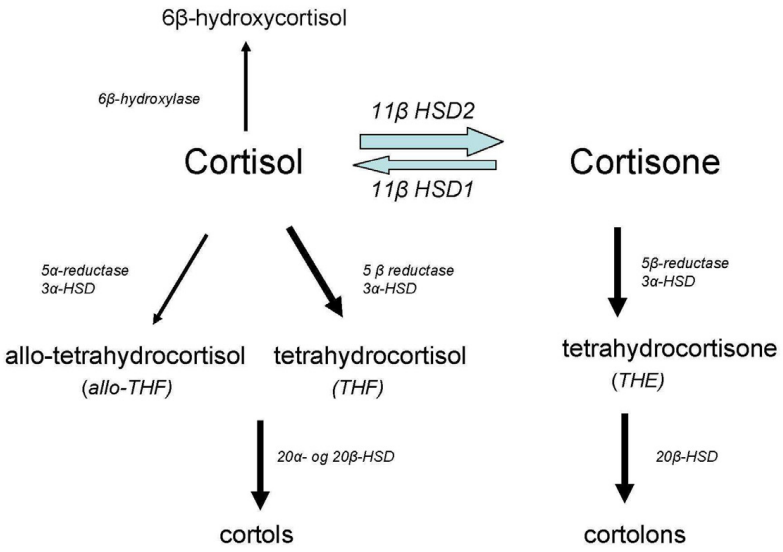


Figure 5. Overview of cortisol metabolism.
Modified from Williams textbook of endocrinology (10.edition, page 504)

Both cortisol and cortisone can be further metabolised by similar pathways, to dihydrocortisol and dihydrocortisone, respectively (Figure 5). These metabolites are further hydroxylated to form tetrahydrocortisol (THF) and tetrahydrocortisone (THE). The reduction to THF can be performed by 5α-reductase or 5β-reductase, giving rise to two different isoforms: THF and allo-THF. In normal subjects the 5β-reductase dominates (THF/allo-THF 2:1). Approximately 50% of secreted cortisol appears in the urine as THE, THF and allo-THF (47) . Cortisol can also be metabolised to 6β-hydroxycortisol by 6β-hydroxylase (CYP3A4). Normally this is a minor pathway; 6β-hydroxycortisol represents about 1% of the total metabolites in the urine. However, CYP3A4 can be induced by hypercortisolism and certain drugs/substances (anticonvulsant, rifampicin, pioglitazone, St. John's Wart, statins, gapefruit juice), hence increasing the proportion of cortisol metabolized through this pathway. Some drugs inhibit this enzyme, but usually the degree of inhibition is small (e.g. clarithromycine, danazol, fluconazol, levonorgestrel, fluoxetine, diliazem, cimetidine)

(60). There is a large inter-individual variance in urinary excretion of 6β-hydroxycortisol, but the ratio 6β-hydroxylase/cortisol can be used for estimating the CYP3A4 enzyme induction capacity of a drug when patients are their own controls (65).

Urine cortisol, cortisone and metabolites have been used to indirectly calculate the activity of cortisol metabolising enzymes. The urinary cortisone/cortisol ratio has been primarily used as an index for 11β-HSD2 activity, the ratio of allo-THF/cortisol for 5α-reductase, the ratio THF/cortisol and THE/cortisone for 5β-reductase and the (THF + allo-THF)/THE ratio as an overall measure of 11β-HSD activity (55, 66, 67). In healthy humans, urine free cortisone excretion is larger than free cortisol excretion, and the normal urinary free cortisol/cortisone ratio is around 0.54, whereas the normal urinary free (THF + allo-THF)/THE ratio is around 1.21 (67). In that study, urinary free cortisone excretion was found to be normal in patients with pituitary insufficiency on GC replacement therapy, whereas in patients with ACTH-dependent Cushing syndrome the urinary cortisone excretion and the cortisol/cortisone ratio were highly elevated.

Sleep and the HPA axis

Normally, sleep consists of cycles with light sleep (stage 1 and 2), deep sleep (3 and 4) and rapid eye movement (REM) sleep. More time is spent in deep sleep (slow wave sleep, SWS) during the first part of the night, and most REM sleep occurs during the last part of the night. REM sleep typically appears in 90 minute cycles. Sleep structure can also be describes as having only 3 stages, that is REM, non-REM and stage wake (29). Each sleep stage is characterized by specific electroencephalographic (EEG) sleep waves and frequency; decreased frequency is associated with deeper sleep, and increased frequency with wakefulness. The sleep stages and architecture can be determined by polysomnography; where EEG waveform, eye movement and electromyography determine each sleep stage.

The HPA axis is implicated in influencing sleep patterns (29, 68, 69). Physiologically, HPA axis activity is at its lowest the first part of the night when the deepest sleep (SWS) occur. The gradual increase in ACTH and cortisol during the last part of the night coincides with lighter sleep and increasing amount of time spent in REM sleep. It has been suggested that awakening results in a following cortisol peak, superimposed on the circadian cortisol fluctuation (70). Efforts to identify isolated effects of CRH, ACTH and cortisol on sleep have been made, but not all have been consistent (71, 72). Exogenous CRH has been shown to increase EEG frequency in healthy males, hereby decreasing SWS and REM sleep and increasing light sleep and awakenings, and this effect was more pronounced during the last part of the night (73). Both pulsatile and continuous nocturnal cortisol infusion has been documented to increase SWS and reduce REM in normal controls (71). Since cortisol and CRH have opposite effects on SWS, it has been suggested that the observed cortisol effects were due to negative feedback inhibition of CRH (74). A dose-dependent cortisol effect has been documented; while lower levels of cortisol is thought to be necessary for SWS, too high levels will promote fragmentation of sleep. It has been suggested that this is due to the type of receptor being activated, suggesting that while low doses will only activate the high affinity MR and increase SWS, higher doses will also activate the GR, increasing light sleep fragmentation and wakefulness (29). This is consistent with the decreased SWS found in patients with hypercortisolism (75).

Quality of life

Definition

Although the term "Quality of life" historically is relatively new, the concept has been known to man through centuries, through synonyms like well-being, happiness, having a good life, life satisfaction, etc. In the Nichomachean Ethics, Aristoteles (384-322 BC) wrote: *Both the multitude and persons of refinement…conceive "the good life" or "doing well" to be the same thing as "being happy". But what constitute happiness (*which includes well-being according to the translator Harris

Rackham) *is a matter of dispute*. Quality of life was then rarely mentioned before the twentieth century, although Thomas Jefferson has been cited for including the pursuit of happiness as an inalienable right in the United States Constitution (76).

In 1948 the WHO declared health as: *a state of complete physical, mental and social well-being, and not merely the absence of disease or infirmity* (77). Hence, health professionals cannot merely focus on saving patient lives (improving quantity of life); restoring the well-being of the patient should also gain importance. Quality of life usually refers to a person's general well-being including physical, mental and social aspects; but more philosophical use of the word is broader, and the term is used in a wide range of contexts. There are many other factors impacting how a person views his own well-being, including personality, environment, socioeconomic status, etc. Hence, the term quality-of-life is broad and ill-defined, and can be viewed in terms of an individual, group or large population of patients (78). In health sciences we are most interested in evaluating those aspects affected by disease or disease treatment. The term "Health Related Quality of life" (HRQoL) is used to emphasise the difference, which usually includes aspects concerning perception of general health (overall questions about how a person view his health), physical health (symptoms and disabilities), mental health (emotional and cognitive functioning), and sometimes also social well-being (the impact on their social life) (79).

HRQoL is influenced by how a person cope with the challenge of a disease, and a person's coping abilities are of course influenced by numerous factors, such as personality, earlier experiences or expectations, social support or influences, educational level and more. Therefore, it is not unexpected that two persons with the same objective disease burden will experience the HRQoL impact caused by the disease differently. Hence, HRQoL is sometimes referred to as "subjective health status". Furthermore, how a person copes with a challenge over time, for instance a chronic disease will also influence the HRQoL scores measured later in life; this phenomenon is called response shift. Response shift can be described as a change in

the meaning of the patient's self-evaluation (score), and is suggested to be due to recalibration, reprioritisation, and reconseptualisation (80). HRQoL changes can be underestimated if response shift is not taken into account (81), particularly when comparing patient scores with normative data.

Measuring HRQoL

So how is it possible to measure the concept of well-being, or HRQoL, in individuals or patient groups? The key feature of HRQoL measurement when compared to other clinical tests is the incorporation of patient values, judgement and preference, attempting to bridge the gap between the patient views and the views of researchers or clinicians; with the words of Galileo Galileo (1564-1642): *to measure what is measurable, and to make measurable what is not so*. Since the WHO declaration in 1948, HRQoL measurement has become a field of science on its own, generating a myriad of publications, and new questionnaires for measuring HRQoL are constantly being developed (82).

Generally, HRQoL is measured with self-administered or interviewer-administered questionnaires consisting of a group of questions, called items, for the patient to answer. The response scoring system can differ between the simplest types, such as a visual-analogue scale, to a Likert scale, where the patient chooses between different response options for each question. Typically, there are between two to six response options, ranging from the worst possible to the best possible option (78).

Questionnaires can be designed with the purpose of differentiating between patient groups at a point of time, i.e. *discriminative* questionnaires. Such questionnaires are typically apt for cross-sectional surveys. On the other hand there are *evaluative* questionnaires, designed to evaluate longitudinal changes in HRQoL within patients, more apt for detecting improvement or deterioration in HRQoL for instant during a clinical trial. There is also a distinction between *disease-specific* and *generic* questionnaires. Generic questionnaires can be used for any group of patients, and are

particularly apt for comparing HRQoL between patient groups, i.e. measuring the relative burden of a disease. Disease-specific, or disease-sensitive, questionnaires have the opportunity of covering areas known to be affected by a given disease, and are thought to be more able to detect HRQoL changes over time within the specific populations (76). With this in mind, each type of HRQoL instrument has its strengths and weaknesses, and there are criteria all questionnaires should fulfil to demonstrate their measurement ability (83).

The process of development and validation of measurement instruments, i.e. HRQoL-questionnaires, should secure that the questionnaire measures what it is intended to measure, i.e, its *validity* is good, and that the HRQoL score produced is precise, reproducible and free from error; i.e. its *reliability* is good. In addition, particularly evaluative questionnaires should demonstrate the ability to detect real changes in HRQoL score over a period of time, even if these changes are small; this ability is called *responsiveness*.

There are different ways to test for validity, reliability and responsiveness. Validity testing is the process of demonstrating that an instrument actually quantifies what it seeks to measure, and that it is useful for this purpose. In lay terms, this means to demonstrate that the items chosen are rational, cover all the aspects that we want to measure and that they are not redundant or biased. The questionnaire should also be easy to understand, not too long and available in the appropriate language for the patient. If a gold-standard instrument exists, validity can be tested by examining the correlation of scores between the gold standard and the new questionnaire; this is called *concurrent validity*. A more comprehensive way of testing validity is to examine the construct of the questionnaire, i.e. *construct validity*, which usually involves testing of correlation between the items. Traditionally this has been done by factor analysis, demonstrating that sub-groups of items in the questionnaire actually correlate to each other and that these groups of items actually represents the areas or domains intended to measure when constructing the questionnaire.

Reliability implies the degree to which an instrument is free from random error. The traditional reliability coefficient, Cronbach's α, indicates how well an individual item correlates with the other items in a questionnaire; this is also called *internal consistency*. A reliability coefficient above 0.85 is said to be necessary if the questionnaire is to be used at the individual level. Test-retest reliability or repeatability tests the correlation between scores from the same individual (or group) assessed on two separate occasions, given that their clinical condition is stable. If reliability is good, the variability of scores between patients (the signal) should be larger than the intra-individual variability (the noise).

Demonstrating responsiveness is commonly done by examining statistically different changes in mean score for patient groups over time, for instance by demonstrating a significant improvement of HRQoL scores after an intervention. Responsiveness is compromised by floor effects (when persons with the worst score can deteriorate further without this being detected by the questionnaire) and ceiling effects (when persons with the highest score can improve further without this being detected).

Questionnaires

The Short Form-36 (SF-36) is a generic multidimensional HRQoL questionnaire, measuring eight general health concepts: Physical functioning, role limitations due to physical health problems (role-physical), bodily pain, general health perceptions, vitality, social functioning, role limitations due to emotional problems (role-emotional), and mental health. The physical functioning, role-physical and bodily pain scales measures primarily physical health, whereas the mental health, social functioning and role-emotional scale primarily measure mental health. The vitality and general health scale are sensitive to both physical and mental health. The scale is extensively used in different medical fields, and has proved to be a valid and reliable tool for measuring HRQoL (84-86). The SF-36 has been used in previous studies of HRQoL in AD (87, 88).

The Psychological General Well-Being Index (PGWB) is a validated generic HRQoL questionnaire that has been translated into several languages, intended to measure the subjective feeling of psychological well-being (89-91). The 22 items group in six sub-dimensions, each measuring six concepts: anxiety, depression, positive well-being, self-control, general health and vitality. Each item is scored from zero to five on a six-point Likert scale, and the sum of all items is used to calculate a score for each dimension and a total score; a higher score indicates a higher HRQoL level.

In other endocrine disorders, both the SF-36 and the PGWB have been used to validate disease-specific questionnaires, i.e., for growth hormone deficiency (92, 93), acromegaly (94) and Cushing syndrome (95). Until now, no AD-specific HRQoL questionnaire has been developed. However, a valid and reliable AD-specific questionnaire is needed to better detect changes in HRQoL over time, both in clinical trials, and during the follow-up of individual AD patients.

Addison's disease

Definition

In primary adrenal deficiency (PAI), i.e., Addison's disease (AD), the adrenal cortex produces insufficient amounts of GCs, mineralocorticoids and adrenal androgens. Irrespective of cause, the lack of these hormones produces a characteristic clinical picture, first described by Thomas Addison in 1855. In his classic paper "On the constitutional and local effects of disease of the suprarenal capsule" he described 11 cases of adrenal failure, combining patient history, clinical features and necropsy findings to a recognisable clinical syndrome excellently illustrated by hand-made illustrations (96). In this publication he gives the first clinical description of PAI: *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 colour of the skin, occurring in connexion with a diseased condition of the "supra-renal capsules".*

During his lifespan, Thomas Addison was not acknowledged for his work, and it was eventually Trousseau who accredited him for his work and named the syndrome Addison's disease (97).

Epidemiology

AD is rare, with a prevalence of 110-140 per million and an incidence of 4.4 – 5.6 per million (98, 99). There is a female preponderance, and although AD can occur at any age, the peak age is around 40 years.

AD may appear isolated, however more than 50% of AD patients have other autoimmune diseases, that is, an autoimmune polyendocrine syndrome (APS) (100, 101). APS1 is an autoimmune recessive disorder, caused by mutations in the autoimmune regulator gene (AIRE); more than 70 mutations have been identified (102). AIRE is involved in the thymus' process of identifying and destructing immature autoreactive T lymphocytes; a defective AIRE gene results in loss of self-tolerance by allowing autoreactive T-lymphocytes to enter the circulation (102). APS1 patients are at risk of developing additional autoimmune diseases over time, but the three main manifestations are autoimmune hypoparathyroidism, AD and chronic mucocutaneous candidiasis (103). The prevalence of APS1 is around 1:80000 in most populations (104), except for higher prevalence in more homogeneous populations (105).

APS2 comprises AD in combination with another organ-specific autoimmune disease, most commonly autoimmune thyroiditis (~50%), but other autoimmune diseases such as primary gonadal insufficiency (~7%), type 1 diabetes mellitus (~10%), vitiligo, pernicious anaemia, alopecia, celiac disease and other are not unusual. The term APS2 is sometimes used for AD in combination with any organ-specific autoimmune disease (106). Of note, some authors includes only AD in combination with either hypothyroidism or type 1 diabetes mellitus in APS2, and uses the term APS3 for

thyroid autoimmunity in combination with another autoimmune disease and APS4 for two or more organ-specific autoimmune diseases to describe other combinations of autoimmune diseases (107), but these terms are not in common use.

Pathogenesis

AD can be caused by developmental defects, destruction of the adrenals by tumours, bleeding or infection, and impaired steroidogenesis. In Caucasians, the most common cause is autoimmune inflammation and destruction of the hormone-producing cells in the adrenal cortex (108). The autoimmune destruction of the adrenal cortex is believed to develop gradually over time, with overt symptoms first appearing when adrenal residual function is very low (106) (Figure 6).

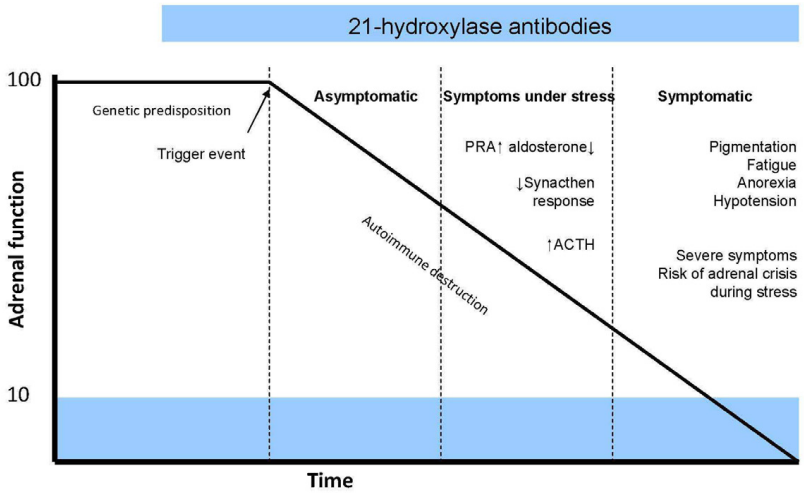


Figure 6. Natural history of autoimmune adrenal failure.
Modified from Eisenbarth GS, Gottlieb PA. N Engl J Med 2004;350:2068-2079 and Betterle C, Dal Pra C, Mantero F, Zanchetta R. Endocr Rev2002; 23:327-364

The high prevalence of concomitant autoimmune disease in AD is linked to a common genetic susceptibility for autoimmune diseases. A combination of multiple genes acting together with an environmental trigger is believed to elicit the autoimmune destruction of the adrenal cortex, although the precise pathogenesis is still uncertain (106). To date, environmental triggers have not yet been demonstrated.

In 1992, the enzyme steroid 21-hydroxylase (21OH) was identified as the antigen for the auto-antibodies involved in AD (109), but later it was demonstrated that the auto-antibodies do not inhibit the enzyme activity in vivo (110). 21OH antibodies (21OHAb) are now seen as a bystander for the ongoing autoimmune process, more than a causative factor, present in more than 95% of recently diagnosed AD patients (100). The frequency of positive 21OHAb in the general population is around 0.5%, but higher in patients with other autoimmune diseases and in first degree relatives to AD patients (111). Studies have shown that the predictive value of 21OHAb for future clinical autoimmune AD is 30-40% at 10 years (107, 111, 112). Determinants of the future risk for clinical AD are the response to ACTH stimulation test, 21OHAb titre, male gender and the number of associated autoimmune diseases (111).

Although T-lymphocytes reactive to adrenal cortex cells were discovered already in the 1960s (113), the exact mechanism for T-cell mediated adrenal destruction in AD is still not clear. In APS2 a breach of peripheral tolerance due to defective CD4+CD25+ regulator T-cells and reduced expression of caspase-3 have been demonstrated (114, 115).

Symptoms and Diagnosis

The difficulty of diagnosing AD lies in considering the possibility, as symptoms may be nonspecific and subtle in the early stages and progress insidious. A German survey found that less than 50 percent of women and less than 30 percent of men were diagnosed within six months with symptoms, and more than 67 percent of patients had consulted three doctors or more before they were diagnosed (116). The most common presenting symptoms are fatigue, loss of appetite with nausea and other gastrointestinal symptoms, salt craving and muscle and joint pain. Usually there are accompanying signs of pigmentation, weight loss and orthostatic hypotension (98). Once the suspicion of AD arises, measurement of basal cortisol and ACTH is often sufficient to establish the diagnosis. A morning cortisol below or in the lower

reference range in combination with an ACTH level above reference range confirms the diagnosis (117). Aldosterone is typically below, plasma renin activity (PRA) above, and DHEAS below their reference ranges.

When basal tests are equivocal, testing with cosyntropin (synthetic ACTH) can be indicated. Administration of Synacthen 250µg i.v. or s.c. should elevate cortisol above 500 nmol/L after 30 or 60 minutes (108). It has been argued that the conventional Synacthen dose is supra-physiological, risking to overlook early adrenal insufficiency, and that a low dose Synacthen at 1 µg i.v. or s.c would be more appropriate (118). However, others have pointed out that in PAI, as opposed to in secondary adrenal insufficiency (SAI), basal ACTH levels are already maximally elevated, and that the low dose test will not provide a higher diagnostic sensitivity (119).

Once primary adrenal insufficiency has been confirmed, aetiology should be determined. As the most common cause in adults is autoimmune adrenalitis, testing for 21OHAb is the test of first choice (120), reported to be present in 96 percent of the patients (98, 99). If positive, the patient should also be screened for concomitant autoimmune disease, particularly autoimmune thyroid disease and diabetes mellitus (APS2). In patients suspected to have mucocutaneous candidiasis or hypoparathyroidism, testing for APS1 with anti-Interferon-ω antibody is warranted (121).

In the rare case that 21OHAb is negative; a CT of the adrenals can identify any causal adrenal bleedings, tumours or infection (tuberculosis, HIV, fungal infections). In male patients with no other apparent cause, especially if concomitant neurological symptoms, testing for very long chain fatty acids (VLCFA) can identify patients with adrenoleukodystrophy (122). Testing for 17-hydroxy progesterone (17OHP) can identify patients with congenital adrenal hyperplasia (CAH); however these patients are generally diagnosed in childhood.

Replacement therapy

AD is a chronic disease, and patients depend on appropriate lifelong replacement therapy with GCs in combination with a mineralocorticoid. The lack of GCs, or cortisol, mandates appropriate replacement therapy. This subject will be covered comprehensively below.

The lack of aldosterone will lead to increased loss of sodium in the urine and increased potassium levels, which in turn will lower the blood pressure and induce hypovolemia. Untreated, this can lead to overt hypovolemic shock, or adrenal crisis. Aldosterone is difficult to synthesize; therefore, the synthetic mineralocorticoid Fludrocortisone is used for replacement. Once daily doses of 0.05-0.2 mg are recommended (108). One study found that 0.2 mg was the most appropriate dose, and concluded that the most common doses of 0.05-0.1 mg fludrocortisone might be too small for many AD patients (123). It is possible that a fludrocortisone dose increase could facilitate reduction of GC dose when needed, although the evidence for this is scarce. On the other hand, overdosage may lead to hypertension, peripheral oedema and possibly increase the risk of cardiovascular disease (CVD). Nevertheless, a dose increase and/or increased salt intake are warranted for patients during hot climate and during strong perspiration (124). Fludrocortisone do not replicate the physiological dip in aldosterone during the night; this has unknown significance (125). Higher fludrocortisone doses may be needed in patients treated with synthetic GCs, as these have a lower mineralocorticoid effect than hydrocortisone and cortisone.

There is an absolute lack of adrenal androgens in both AD men and women, but the decrease in total androgens is much larger in women. This is particularly true for women with ovarian failure (both premature failure and normal menopause).
There is still no consensus on the need for DHEA replacement therapy in AD. The first clinical trial reported improved well-being and improvement in mood and sexual function for women with primary or secondary adrenal failure with DHEA 50 mg when compared to placebo (126). Subsequent studies have reported partly conflicting

results and partly minor effects on HRQoL, possible beneficial effects on bone and no effect on sexual function (5, 127-129). However, most clinicians will consider prescribing androgens to individual AD females who are still symptomatic despite appropriate dosage of GCs and mineralocorticoids. It is generally recommended to start with low doses such as 25 mg once a day, doses can then be increased to 50 mg depending on clinical effect, the occurrence of side-effects (acne, sweating, hirsutism) and resulting androgen levels (testosterone, androstenedione, DHEAS) (130). If no positive subjective effects are noted within 6 months, discontinuation of DHEA treatment is recommended (5, 60). Transdermal patches supplying testosterone 150 μg/day have been tested only in women with SAI; only minor effects were noted on HRQoL, but bone density, mood and sexual function improved (131).

Acute adrenal crisis

Acute adrenal failure, or Addisonian crisis (AC), is a life-threatening complication of chronic AD, and can also be the presenting symptom leading to the diagnosis of AD. An untreated absolute or relative lack of cortisol and aldosterone will lead to hypotension and/or hypovolemic shock, often accompanied with abdominal pain, vomiting and fever (108).

Hahner et al. documented a frequency of AC at 6.6 events per 100 patient years, the main precipitating events being infectious disease (57%), major surgery (7.2%) and physical stress (7.7%). The risk was increased for patients with additional comorbidities (132). In a postal survey, White et al. found that 47% of the patients reported that they had been in need of hospital treatment since the time of diagnosis, 10 percent on more than four occasions (133).

In a retrospective study in AD, Erichsen and co-workers found that AD was given as the principal death diagnosis in 15%, and infectious disease in 10% of patients. In addition, sudden death accounted for 9.2% of the deaths, compared with 5.3% in the general population. It is likely that AC contributed to deaths in all the three diagnose

groups (134), as AC is often precipitated by infectious diseases and can result in sudden death if appropriate treatment is not provided.

In healthy subjects, endogenous production of cortisol is increased during stress, and patients with AD need to increase their GC doses accordingly to cover the increased demand in case of events such as infections, strenuous activity, major surgery and labour. Especially in conditions leading to compromised absorption of cortisol and fludrocortisone such as gastroenteritis, patients are at risk of rapidly developing AC unless appropriately treated with intravenous fluids and cortisol. Hence, both patients and health care professionals need to be properly educated about the required action in the event of AC symptoms.

Mortality

Despite conventional replacement therapy, the standard mortality rates (SMR) have been found to be more than doubled in AD, with higher mortality rates for patients with APS1 when compared to APS2 (135). The major causes of death are cardiovascular disease, malignancy and infectious diseases (136). Another study did not find an increased overall SMR, but mortality was increased in patients diagnosed before the age of 40, especially in males, and acute adrenal failure was found to be a major cause of death (134). This study did not find any increase in CVD in AD.

Risk of cardiovascular disease

Exogenous or endogen hypercortisolism is known to increase the risk of cardiovascular disease, and several studies have demonstrated a strong relationship between GC dose and CVD risk factors in adrenal insufficiency. In SAI, reduction of GC doses resulted in reduction of cholesterol, triglycerides and weight (137). Filipsson and co-workers demonstrated that the metabolic CVD risk profile in SAI is related to the daily dose of GC. Hydrocortisone equivalent doses of less than 20 mg/d did not adversely affect metabolic risk factors. Furthermore, patients treated with hydrocortisone and prednisolone had higher HbA1c and waist-hip-ratio respectively,

than patients treated with cortisone acetate (138). This is interesting, knowing that the replacement therapy in the mortality study showing increased risk of CVD in AD were predominately OHC (136), while in the study showing no CVD risk patients were mainly treated with cortisone acetate (134).

In AD, a higher prevalence of central adiposity, impaired glucose tolerance and dyslipidemia, all well-known independent risk factors for cardiovascular disease, has been demonstrated (139). The same study showed that patients with AD use more antihypertensive drugs and lipid-lowering agents than the background population, indicating an unfavourable metabolic state and possibly increased cardiovascular morbidity. Another study found increased prescription of lipid-lowering drugs and antihypertensive agents in AD when compared to the general population (140). Explanations might be supra-physiological GC replacement doses, and altered diurnal profile (141).

Carbohydrate metabolism

While too high GC doses can impair glucose tolerance in AD, the very low nighttime cortisol levels seen with conventional OHC may reduce late night glucose levels. Patients with SAI on OHC treatment have reduced glucose levels during the night, which could possibly impair sleep quality and reduce daytime energy (142). A recent study demonstrated that also AD patients without concomitant type 1 diabetes can be susceptible to low night time glucose levels (143). In AD patients with concomitant diabetes mellitus inherent variation in cortisol levels on OHC can also cause fluctuations in glucose levels that can be difficult to handle; as cortisol troughs will increase insulin sensitivity and cortisol peaks will decrease it (144, 145). In addition to the lack of cortisol, AD patients have a decreased adrenal adrenaline output, further impairing counter-regulatory responses to hypoglycaemia (1, 146). The incidence of sudden death is increased in AD patients when compared with the normal population (134), for which hypoglycemia could hypothetically be a contributing factor.

Patients with AD on conventional OHC therapy complain about fatigue, dizziness and concentration difficulties (147, 148), especially in the early morning. In one study high-calorie glucose-rich food alleviated these symptoms (149), but another showed no effect of glucose infusion on neurocognitive function (150). Whether these morning symptoms could relate to low glucose levels and neuroglycopenia is not clear.

During parts of the day, especially in the afternoon, AD patients have higher cortisol levels than normal (151, 152). Increased evening exposure to cortisol reduced glucose tolerance, insulin secretion and insulin sensitivity in healthy young adults (30). Hence, mimicking the circadian cortisol rhythm might be of importance both during the night and day, and a recent study found that reduced cortisol exposure during the afternoon reduced weight, blood pressure and HbA1c in AD patients (153).

Bone metabolism

GCs inhibit osteoblasts, stimulate osteoclasts and inhibit intestinal absorption of calcium, hereby reducing bone mineral density (BMD). Too high doses of GCs in AD can therefore lead to reduced BMD in AD, and studies have demonstrated an inverse correlation between GC dose and BMD (154-156) or bone formation markers (157). Type of GC also seems to matter, as synthetic long acting GCs seem to have a worse impact on bone than OHC (158, 159). Studies on BMD in AD have been inconsistent, but the largest study including 293 AD patients found lower BMD in patients than in controls (155). On the other hand, a recent study which included patients on lower GC doses, demonstrated BMD within reference range in patients with AD or CAH when compared to controls (159). A register-based study identified hip fractures in 6.9% of AD patients versus in 2.7% of controls (160). Surprisingly, the risk of fracture was highest around time of AD diagnosis (one year before and one year after), suggesting that both too low and too high GC levels could affect bone.

AD patients also lack adrenal androgens, which may contribute to loss of bone mass in females. In a randomized double-blind trial, Gurnell et al. found that six months treatment with DHEA reversed bone loss at the femoral neck, but not at other sites, in female AD patients (128). In addition, the prevalence of premature menopause is 7% in AD (98), which also could influence bone health in this patient group. Given the slightly conflicting results, BMD measurements are not always recommended for regular follow-up in AD (108), but might be indicated in patients with premature ovarian failure and in patients on high doses of GCs, especially when synthetic GCs are used.

Infections

AD patients lack the physiological increase in cortisol levels normally produced under stressful conditions, and during infections they depend on extra doses of GC. Mortality studies found that infections are a more common cause of death in AD than in reference populations (134, 136). A recent study showed high rate of prescription of systemic antibiotics in AD, both before and after the time of diagnosis (140). A new cohort study applying pharmaceutical records found that the incidence of infections was one and a half times higher in AD than in controls, with higher risk for fungal and viral infections than for bacterial infections (161). Here, the overall incidence of hospital admissions due to serious infections was five times higher, the incidence of pneumonia 10 times higher and of urinary infection four times higher in AD than in controls.

Even though studies on defects in immunity in AD are lacking, the increased risk of infection could be a result of the autoimmune disease per se, as defects in the cellular innate immunity have been found for other autoimmune diseases such as type 1 diabetes (162). One could speculate that the high ACTH levels in AD patients could convey anti-inflammatory effect through the melanocortin receptors, although evidence is lacking. However, given the important role of GCs in inflammation,

suboptimal GC replacement therapy could theoretically contribute to an increased risk of infection in AD.

Quality of life in AD

Patients with AD reproducibly report of reduced HRQoL when compared with healthy reference populations. Løvås and co-workers reported HRQoL scores in 79 Norwegian AD patients; lower scores were found in the domains vitality and general health for the SF-36 questionnaire, and for the Fatigue questionnaire (87). Lower scores for physical function were found only among women, and lower scores for mental health only in men. No significant differences in scores were found between patients with isolated AD and patients with APS. Working ability was lower in AD than in the reference population. These findings were confirmed in a nation-wide study from Norway involving 426 AD patients (98). Here, the vitality, general health, social functioning and role physical scores were low in AD. Thirty percent of the working age patients received full or part time disability benefit compared with 11% in the reference population, indicating that AD has a serious impact on the daily lives of the patients. In a cross-sectional study including 256 German patients, Hahner and co-authors found significantly reduced HRQoL levels in patients on conventional replacement therapy, irrespective of age, sex, concomitant disease and primary or secondary adrenal failure. The largest impairment was seen in the general health, physical function and vitality scores with the SF-36, and for exhaustion tendency and anxiety with the two other generic questionnaires applied. When comparing the results for the SF-36 general health and vitality scores from different AD studies in different countries, there is a remarkable consistency of score levels (87, 88, 98, 128), indicating that affection of these domains are indeed characteristic for AD. Both German and Norwegian studies showed that the SF-36 general health scores for AD patients were similar to the published scores for patients with type 1 diabetes (163) and patients with diabetic foot ulcers (164), but higher than in patients with rheumatoid arthritis (165).

In contrast to the normal mental health scores mentioned in studies applying the SF-36 questionnaire (87, 128), studies applying alternative questionnaires have shown psychological and mood impairments in AD (126, 128, 166). Also, a Danish registry study investigated the risk of affective disorders in AD by identifying AD patients among patients admitted for affective disorders. Compared to patients with osteoarthritis, AD patients had a significant two times greater rate of affective disorders, but the rate of depression was not significantly increased, and the overall incidence of affective disorders in AD was low (167). The highest rate was found the first year after diagnosis.

The reason for the reduced HRQoL in AD is not clear cut. Currently, unphysiological or insufficient GC replacement is believed to be the major explanation for the reduced HRQoL in AD (60, 88). Some authors have suggested that the lack of DHEA could be a causative factor (126), although the largest placebo-controlled randomized study detected only minor HRQoL benefits of DHEA in AD (128). Another theory is that the autoimmune state in itself will compromise HRQoL, given that HRQoL are affected in several autoimmune conditions (163, 165, 168). A recent study in patients on thyroid replacement therapy compared SF-36 scores between patient with autoimmune hypothyroidism and postoperative hypothyroidism, demonstrating lower scores in patients with anti-thyroid peroxidase antibodies (169), which supports this theory.

A survey showed that although achieving HRQoL improvement was seen as both a major goal and a major challenge in AD, HRQoL measurement in AD patients is not routinely assessed by clinicians (60). This could be because no AD-specific HRQoL questionnaire has been available, or of the difficulty of interpreting HRQoL scores in individual patients. An AD-specific questionnaire containing questions related to AD symptoms could be of assistance in identifying patients with poor functioning, who are in need of a more individualized treatment strategy.

Sleep

In AD patients on conventional replacement therapy, nocturnal levels of cortisol are unphysiologically low, especially during the last part of the night. In the same period, ACTH levels, and probably CRH levels, are higher than normal. How this affects sleep in AD is not clear, as results from previous studies are not entirely consistent (72, 170, 171). Because of the close links between the sleep cycle and the circadian activity of the HPA axis, restoring the physiological circadian cortisol rhythm in AD patients could possibly affect sleep.

Glucocorticoid treatment in AD

A historical perspective

When Thomas Addison in his classical publication "On the constitutional and Local Effects of Diseases of the Supra-Renal capsules" in 1855 first described the typical symptoms and findings of adrenal failure, no treatment was available and the disease was always lethal (96). At the end of the 18th century there was a growing interest for the function of what were then called "the ductless glands" (thyroid, adrenals, thymus, pituitary), although there were conflicting views of their function. Some believed that the glands had an excretory function and hence that removal of the gland would lead to accumulation of toxins causing disease; others believed that their function was primarily to secrete substances to the blood. The adrenals were believed to produce a medullary derived substance to the blood, essential for the muscular function especially in the heart and the great arteries (172). This secretory theory was based on the physiological effects demonstrated from experimental injections of adrenal extracts, and in 1896 William Osler published a report of six cases of Addison's disease of whom one patient radically improved after the use of adrenal extracts (173), describing the method for preparing the extract as well as the clinical result: *On May 16 the treatment with suprarenal extracts begun. Thirty-six pigs' suprarenals were obtained at the time of slaughtering, cut up finely, thoroughly powdered with pestle and mortar, and to this mass about six ounces of pure glycerine were added,*

and the whole allowed to macerate for thirty-six hours in a refrigerator. The mixture was then filtered several times through fine-meshed gauze. The filtrate consisted of a reddish-brown syrupy fluid of a rather disagreeable odor. After filtering there were thirty-eight drachms of the extract, so that one drachm corresponded to a capsule. The patient began with half a drachm three times a day.
...He left the hospital on September 10. The change in his condition had been very remarkable. When admitted he could scarcely walk to the bed, and was profoundly asthenic and emaciated. The general appearance had improved wonderfully; he was bright and active, and said he felt vigorous. His weight on discharge was one hundred and eighteen pounds, a gain of nineteen pounds. The pigmentation was unaltered.

Although this early report seemed very promising, the treatment results were not always that positive. A year later, in 1897, Francis Kinnicutt published the results of 48 AD cases treated with some form of adrenal extract, describing that six patients were cured, two improved, 18 unimproved and in two patients the symptoms were aggravated. This is maybe as expected as the content of cortisol required to ameliorate symptoms probably was highly variable and the mineralocorticoid effect small, and that a high content of adrenaline (slaughtered pigs) could give detrimental side-effects.

In 1925, Dr. Rowntree of Minnesota presented encouraging results obtained from “the Muirhead treatment” at the Annual Meeting of the Association of American Physicians (90). This consisted of adrenaline given hypodermically or rectally three times a day, and the whole adrenal gland given by mouth three times a day. The treatment was named after the first patient, Dr. A. L. Muirhead, a professor in pharmacology, who presented at the Mayo clinic in 1920, suffering from advanced AD. He improved to describe his experience in the Journal of American Medical Association under the title “An Autograph History of a Case of Addison’s disease” in 1924, but unfortunately he died 16 months later. Of the 12 patients on the Muirhead

regimen reported in the 1925 publication, five died without apparent benefits of the treatment, and seven were reported still alive.

In the late 1920's two groups of physiologists, Hartman and associates at the University of Buffalo and Swingle and Pfiffner at Princeton University, had developed adrenal extracts that prolonged survival in adrenalectomised animals and controlled symptoms in AD (174). They manufactured the extract on a large scale, which Dr Rowntree at the Mayo Clinic used in treatment trials of AD patients in 1931 (175). The extract preparation was an expensive and time-consuming process; 1000 kg of beef adrenals yielded about 1 kg of dry residue to be refined further to 25 g adrenal extract (176). Hence, the hunt for the active component called "cortin" from adrenal cortical extracts begun.

At the Mayo Clinic in Rochester US, Edward C. Kendall and associates succeeded in preparing what they first believed was a pure cortin extract in 1934. In Basel, Thaddeus Reichstein found 29 structurally similar steroids, of which six possessed "cortin-activity". Half of these were identified by Reichstein, and half by Kendall, who also identified an additional substance. Probably the most important substance named "Compound E" was later identified as cortisone at four different laboratories, including Reichstein's and Kendall's. For their achievements they, together with Philip Hench who discovered the effectiveness of cortisone in treating rheumatoid arthritis, were awarded the Nobel Prize in Medicine and Physiology already in 1950 (44, 176, 177).

The first semisynthetic steroid available for treatment in AD was desoxycorticosterone acetate (DOCA) synthesised by Reichstein from deoxycholic acid in 1937 (178, 179), administered once daily i.m. or s.c. DOCA was later shown to lack glucocorticoid and anti-inflammatory effects, and AD patients were therefore instructed to remain on a diet rich in carbohydrates to avoid hypoglycaemia. Although

mortality was reduced by DOCA, patients were still at risk for AD crisis during stress (180).

After cortisone became available in 1948, Reichsteins "Compound F" was in 1951 identified as hydrocortisone, and it became clear that hydrocortisone was the substance produced by the normal adrenal cortex (178, 181). The following decade it was discovered that modification of anti-inflammatory and mineralocorticoid effects could be achieved by alterations in the steroid skeleton, producing prednisone, prednisolone and fluorocortisone in 1954, triamcinolone in 1956, methyl-prednisolone in 1957 and dexamethasone in 1958.

Although low blood pressure, a reduced sodium concentration and a high potassium level were recognised features of AD, it was Robert F. Loeb that in 1932 demonstrated that excessive urinary salt loss was responsible (174). He examined the blood of three patients with AD, discovering a striking decrease in the sodium content in their blood. Further studies in adrenalectomised dogs and cats confirmed this finding (182), and later gave evidence for prolonged survival with sodium supplements. Although it was noted that adrenal extracts had remarkably low mineralocorticoid activity, evidence for adrenal aldosterone production was presented as late as 1948 (183, 184). After DOCA and oral cortisone became commercially available, combination treatment was common, as DOCA possessed mineralocorticoid effect. In 1955, 9-α-fluorocortisone was discovered to have a superior mineralocorticoid effect and favourable pharmacokinetics, and replacement with oral cortisone or hydrocortisone in combination with fludrocortisone became possible (185, 186) (Figure 7).

Present conventional glucocorticoid replacement therapy

Available oral replacement therapy ensured that AD was no longer a fatal disease, and made patients live near normal lives. In 1963 Sir Derrick Dunlop published a report

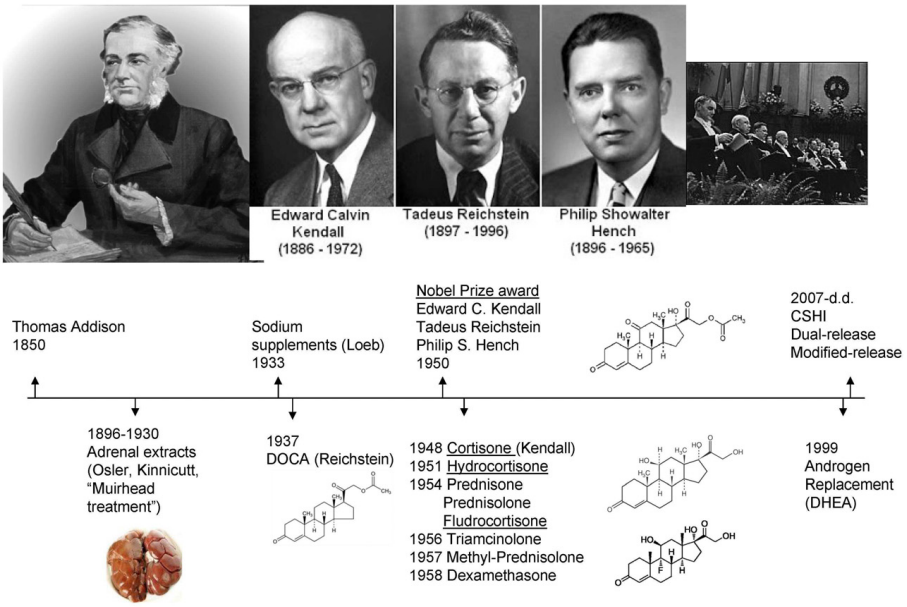


Figure 7. Glucocorticoid treatment history

of 86 AD patients he had cared for between 1928 and 1958, showing that while all patients died during the first 10 years, after cortisone became available only one of his patients had died (180). That paper also stated that conventional AD replacement by then was cortisone 25-37.5 mg/d in combination with fludrocortisone 0.1-0.2 mg/d.

Over the following 50 years the GC replacement in AD has been virtually unchanged. Being the normal GC produced by the adrenal, OHC is the most common treatment choice worldwide (187). Cortisone acetate (CA) is considered equally effective as OHC and is preferred in some countries, where OHC is not easily commercially available. CA needs to be converted to hydrocortisone by liver 11β-HSD to have biological effect. In some countries long-acting GCs such as prednisolone and dexamethasone are also used (188, 189), despite the resulting unphysiological pharmacodynamic effect (108). There are also concerns that the synthetic long-acting GCs results in continuous stimulation of the GR, probably increasing the risk of detrimental side-effects (24, 60, 190).

In the recent decade there has been a number of reports showing that patient on conventional replacement therapy have reduced HRQoL (87, 98, 128), increased risk of osteoporosis (155, 159, 160), increased mortality rate (134-136) and possibly increased risk of cardiovascular disease (191, 192). Newer estimates of the endogen cortisol production rate at only 5.4–6.1 mg/m^2/day have caused concern that AD patients are receiving too high doses (193, 194), and slightly lower daily doses than before of hydrocortisone 15-25 mg/d or cortisone acetate 20-30 mg/d are now recommended, allowing for less than 100% bioavailability and first-pass liver metabolism. These concerns have sparked interest in improving the therapeutic regimes.

Efforts have been made to optimise treatment regimens by various strategies. A cross-sectional study examined cortisol day curves in 32 AD patients (PAI and SAI), showing that 75% of patients required a dose reduction and 56% a change of drug or drug regimen to achieve acceptable cortisol levels during the day (157). The only randomised placebo-controlled trial available demonstrated that twice daily dosing was better than once daily (195). Howlett and Groves lead studies that examined cortisol day curves and suggested that thrice daily OHC normalised cortisol levels better than twice daily dosing (152, 196). Mah and colleagues demonstrated large inter-individual differences in pharmacokinetics after intake of 10 mg hydrocortisone in patients with adrenal insufficiency. By individually adjusting doses of thrice daily OHC according to weight or body-surface-area (BSA), the variability in peak cortisol and cortisol area under the curve (AUC) were significantly reduced (151). In a cross-sectional analysis, Bleicken and co-workers examined impact of number of OHC doses on HRQoL; only minor differences were found between twice and thrice daily dosage, favouring two daily doses (197). However, patients on doses more than 30 mg/d scored significantly worse than patients on lower daily doses. The same authors also found that type of GC replacement (OHC, CA, Prednisolone) did not impact HRQoL scores in this patient group (188). A recent double-blind study compared two with four doses, with the same daily dose. They found that four doses increased the

serum cortisol AUC, but could not detect any significant difference in HRQoL scores (198). Current recommendations state that OHC should be given in two or three daily doses, with one-half to two-thirds of the daily dose in the morning, and the subsequent dose approximately five hours later (60, 130, 190).

The available conventional OHC or CA cannot reproduce the physiological circadian cortisol variation (199). The ideal GC replacement therapy would mimic the endogenous cortisol rhythm, with a nadir at bedtime, gradually rising levels until the early morning peak before waking, followed by decreasing levels during the day (200). Instead, with OHC, cortisol levels are very low during the night and early morning, which may contribute to low nighttime glucose levels and symptoms like fatigue, mild nausea or headache experienced by AD patients especially in the morning (143, 147). During daytime the cortisol level fluctuates, with supra-physiological post-dose peaks followed by troughs before the next dose (151). This non-physiological cortisol profile is currently believed to be the major explanation for poor outcome in AD, but clinical trials confirming this hypothesis are lacking (60, 88).

Physical activity

The adrenocortical and the adrenomedullary stress responses are closely linked. Both responses are compromised in AD (201-203), and strenuous exercise can trigger an adrenal crisis (132). Some authors advice patients to use an additional dose of hydrocortisone 5-10 mg during strenuous or long-lasting exercise and long-lasting psychological stress (124, 204). However, exercise studies in CAH patients have demonstrated a normal exercise capacity when compared with controls, despite lower cortisol and adrenaline levels. An extra stress dose of hydrocortisone was not found beneficial (146, 205, 206). Likewise, DHEA replacement produced no benefit on physical capacity in AD patients (207). More research is needed to clarify why AD patients experience fatigue in relation to physical activity, and how this should be prevented and treated.

Prevention and treatment of adrenal crisis

Patients should be able to recognise stressful events that possibly result in AC, and be instructed to adjust their oral dose accordingly, to prevent progression to AC (124). They should be provided with a steroid card, stating their diagnosis and recommended treatment in the event of AC symptoms (208). Also, patients should be equipped with hydrocortisone for intramuscular or subcutaneous use in emergency to avoid delay of treatment (209). As a thumb rule, we advise patients to double or triple their daily oral GC doses during intercurrent disease, i.e., depending on the degree of body temperature. When modified-release hydrocortisone is used as replacement therapy, the additional dose should be given 6-8 h after the normal dose, due to the pharmacokinetic properties of the drug (60).

Patients presenting with severe AC symptoms, vomiting or diarrhoea need to be admitted for treatment with intravenous hydrocortisone in combination with saline infusion. An immediate dose of 100 mg hydrocortisone is recommended, followed by infusion of 100-200 mg over the next 24 hours (108).

In normal subjects, ACTH and cortisol increase significantly at start of surgery, but the largest increase is seen during reversal of anaesthesia. Earlier treatment recommendations were 100 mg hydrocortisone every 8 hour, starting at the induction of anaesthesia (210). These doses are supra-physiological (130, 211, 212) and hydrocortisone doses of i.v.100-150 mg/d are now recommended to prevent AC in the setting of major surgery, or during critical illness (108, 213).

Pregnancy

There is a physiological increase in serum cortisol levels during pregnancy due to the increase in CBG. The free cortisol level also increases during the last trimester. In the last trimester progesterone levels also increase, exerting an anti-mineralocorticoid effect. The foetus is relatively protected from maternal hypercortisolism due to

placental 11β-HSD2 production. It is recommended to use the most physiological replacement therapy possible, to minimize the risk for mother and foetus. There is no consensus of routinely increasing the GC doses in all patients, as some authors state that doses rarely need to be increased (214), while others encourage a dose increase of 50% in the last trimester to mimic the physiological increase in free cortisol (108). Mineralocorticoid replacement should be adjusted to blood pressure and potassium levels during pregnancy, as PRA is physiologically increased. Due to the anti-mineralcorticoid effect of progesterone, an increase of the Fludrocortisone dose may be necessary during late pregnancy (204). During labour, some authors recommend to double the oral dose, or give hydrocortisone 50-100 mg i.v. (108, 204). During Caesarean section, i.v. hydrocortisone should be given as with major surgery. The doses can be tapered to normal within two days if no complications occur. During breastfeeding, OHC can be continued as usual, as less than 0.5% of the absorbed dose will appear in breast milk (215).

Recent developments

Lately, two new modified-release hydrocortisone formulations have been developed, both aiming for a more physiological circadian pattern of cortisol. Recent studies demonstrated that 24 h exposure of cortisol was reduced by dual-release hydrocortisone, resulting in reduction of body weight, blood pressure and HbA1c (153, 216). Also improvement in HRQoL was noted. Although such treatment successfully restored daytime cortisol levels to normal with once daily dosage, the normal late night increase in cortisol is not re-established. Restoration of this nighttime cortisol surge was however obtained by another modified-release hydrocortisone preparation (200, 217, 218). This preparation was later shown to have reduced bioavailability and too early drug release, leading to further modifications. A recent study with healthy dexamethasone-suppressed volunteers showed that twice daily dosing of DIURF-006 (10 mg morning and 30 mg evening) resulted in near normal daily cortisol AUC and peak cortisol; maximal absorption was also delayed, mimicking the physiological morning peak (219). Potential effects on well-being will

have to be investigated in future studies. Also, modified-release prednisolone was tested in AD patients in an open label pilot trial (220). One daily dose at bedtime improved HRQoL and reduced fatigue when compared to one daily dose of prednisolone in the morning.

Merza and co-workers showed that mimicking the physiological circadian cortisol rhythm in AD is possible by intravenous infusion of hydrocortisone; in fact, ACTH levels were reduced towards normal levels already after 24 h treatment (221). Løvås and Husebye found that continuous subcutaneous hydrocortisone infusion (CSHI) enabled a fine-tuned control of GC delivery that allowed restoration of the circadian biorhythm in a few AD patients (222), suggesting that CSHI is a versatile method for further refinement of the GC replacement therapy. This method of GC delivery also provides us with a means to explore whether restoring the circadian cortisol rhythm can improve replacement therapy in AD.

Monitoring glucocorticoid replacement

Although the recent decades have produced increasing knowledge both in the field of AD and of replacement therapy, there is still no universal agreement about how to appropriately monitor GC replacement therapy. Some endocrinologists rely primarily or exclusively on clinical parameters and judgement to evaluate GC doses. One study proposed a clinical scoring system to identify over- and under-replacement (223), but although the scoring system correlated with serum cortisol measurements, there was no difference in score between the perceived overdosed and underdosed patients. There has also been concern that subtle over- and under-replacement might produce detrimental effects without obvious clues from the patient history and clinical examination. Monitoring HRQoL could be another possible method of detecting suboptimal replacement therapy, but until now an appropriate disease-specific questionnaire has been lacking.

Several laboratory tests have been proposed either as the main parameter for appropriate dosage, or as a supplement to clinical judgement. However, the major objection to this strategy is that levels of hormones in plasma or other samples do not necessarily reflect cortisol action on tissue or cellular level, and a relevant biomarker for optimal GC effect is lacking (224).

Given the pulsatile nature of ACTH secretion and the short half time of ACTH in plasma, ACTH levels will be strongly influenced of the cortisol exposure the last hours before measurement. Due to the low nighttime cortisol levels on conventional oral treatment, ACTH will remain elevated in the morning and early daytime in most patients, despite adequate doses of GCs. Daytime ACTH levels will also be influenced by the timing of the measurement in relation to the dosage time, and can be difficult to interpret. Some papers have advocated that normalisation of ACTH levels imply over dosage, whereas others suggest that measuring ACTH has some clinical benefit (225). However, random ACTH measurement is of little value in monitoring conventional GC replacement (108, 226).

Serum total cortisol has been the easiest and most available analysis for many years, although the measurement has obvious disadvantages. Interpretation requires knowledge about timing of doses and type of GC used, and preferably standardised absorption conditions. Random serum cortisol measurements have little clinical utility. Some have suggested measuring serum cortisol day curves despite that this is cumbersome to perform in all patients (152). Nevertheless, day curves could be useful in special situations, such as evaluating absorption issues or drug interactions. Mah and coworkers showed that serum cortisol four hours after the OHC morning dose explained 78% of the variability in cortisol AUC; therefore they suggested using weight-adjusted doses thrice daily, and produced a nomogram for optimisation of individual doses (151). This approach is easier and more effective than day curves, but use of the nomogram is limited to patients on thrice daily OHC. Also, this study was criticised for proposing too low doses for AD patients, because the study also

included patients with SAI, who might have residual adrenal cortisol production. A major limitation of serum total cortisol is that the correlation with the free and biologically active cortisol proportion depend on the CBG levels (227). This is particularly important under acute stress such as critical illness, because binding proteins will be down regulated resulting in a higher biologically active cortisol level undetectable when measuring total cortisol (59, 228). Measuring free cortisol is possible, but time consuming and cumbersome; therefore, this is rarely feasible in clinical practice. Several formulas for estimating the free proportion of cortisol have been published (51); these are less accurate as they will contain the variability in both the CBG and the cortisol analyses.

The urinary free cortisol represents approximately 1% of the total cortisol secretion rate. The 24 h urinary free cortisol level has been used to evaluate overall cortisol load per day (157), but cannot be used to evaluate modern tailored twice or thrice daily dosage. Also, after an OHC dose CBG is rapidly saturated and the excess free cortisol secreted in the urine, which can give spurious results (190), especially if spot urinary samples are used.

Salivary cortisol has the advantage of measuring the free cortisol, it is easy to use, and can be performed in an outpatient setting. There are caveats concerning pre-analytical errors, such as sample contamination from blood or OHC tablet residue; therefore patients should be instructed not to eat, drink, brush teeth or smoke the last hour before the sample is taken. The swab should be sufficiently soaked, either by chewing or keeping it in the mouth for 1-3 minutes. Elevated late night salivary cortisol is well validated as a marker for Cushing's syndrome (229), but results from monitoring GC treatment are more conflicting. One study showed poor correlation between serum and salivary cortisol profiles both after oral and intravenous administration, as well as large inter- and intra-individual variability (230), and suggested together with others (231, 232) that salivary cortisol should not be used for monitoring GC replacement. Of note, these studies correlated salivary and serum total cortisol, which will increase

the variability (50). Other authors have reported more promising results, and have suggested using salivary cortisol in clinical practice (233, 234). Of interest, in both studies claiming too high variability in salivary results patients were treated with OHC, and some of the samples were taken a short time (15 minutes) after OHC administration (230), which increases the risk of saliva contamination considerably. On the other hand, in the two studies reporting less variability, patients were treated with CA (233, 234), reducing the risk of contamination. A recent study showed very high salivary cortisol AUC levels in 31 AD patients receiving OHC treatment compared with matched controls (235), the largest variability in salivary cortisol levels were found in the first measurements after the oral morning dose. Taken together, OHC possibly gives more sample contamination than earlier perceived.

By using liquid chromatography with sequential tandem mass spectrometry (LC-MSMS) method for analysis of salivary cortisol, an accurate measurement of salivary cortisone can be obtained, and the up to 30% cross-reactivity for cortisone seen with most cortisol immunoassays can be eliminated (236). Conversely, LC-MSMS provides a much more reliable measurement of cortisone than immunoassays. Lately, salivary cortisone has been suggested as a more valid estimate for the unbound cortisol level in serum (50). Here, salivary cortisone had the highest correlation to measured serum free cortisol, and the authors proposed that salivary cortisone should be the preferred biomarker for serum free cortisol under basal and stimulated conditions, and also during OHC treatment.

Another interesting analysis under investigation is hair cortisol content, as a recent study showed that hair cortisol content correlated with GC dose in patients with adrenal insufficiency (237). Previous studies have shown that hair cortisol is elevated in patients with Cushing syndrome, myocardial infarction, alcoholism and with increased stress (238-242). Hair grows approximately 1 cm per month, and a hair sample of the proximal 1 cm is thought to reflect the cortisol exposure over the last four weeks. Although this analysis is useless for the detection of cortisol variation

during the day and for identifying unphysiological cortisol peaks and troughs, the idea of having a biochemical marker of dosage over time in AD, similar to the use of HbA1c for glycaemic control in diabetes, certainly is appealing.

Follow-up

As AD is a rare disease with particular issues relevant for follow-up, we recommend annual controls at an endocrinology hospital clinic. Annual check-ups should include monitoring of the replacement therapy, reviewing episodes of potential or actual adrenal crises, repeating patient education if necessary and regular screening for concomitant autoimmune disease.

As outlined above, the surveillance of GC replacement in clinical practice is currently based on clinical assessment (223). Too low GC doses increase the risk of adrenal crisis and reduced well-being, whereas too high doses increase the risk of complications such as osteoporosis, obesity and impaired glucose tolerance (108). Symptoms such as weight gain, abdominal obesity and peripheral oedema may indicate a too high daily dose, whereas symptoms like fatigue, myalgia or joint pain, nausea and anorexia may indicate under-dosage. Symptoms of both categories are unspecific, and hence an overall assessment of patient health is more important than isolated symptoms. Increased pigmentation due to high ACTH levels may suggest that doses should be increased; however, patients on conventional oral replacement therapy will invariably display elevated ACTH levels due to low cortisol levels during the night; hence some degree of pigmentation will usually persist. To evaluate the frequency of doses, asking the patients if they have low spots during the day is useful. If the patient is clock-watching for a particular dose this could indicate that the time interval between doses is too long and splitting the daily GC dose in more frequent doses should be considered. Morning fatigue or nausea indicates very low cortisol levels and possibly low glucose levels during the night. Some symptom relief could be gained by asking the patient to administer the morning dose 30-60 minutes before they get up, otherwise a small evening dose of prednisolone may be useful. In the

future, new treatment options which restore the nighttime cortisol levels can be useful for these patients. For patients on OHC who complains of “roller-coaster days”, switching to cortisone acetate could be a solution. Problems with sleep initiation can suggest that the last dose of the day is taken too late or is too high. Since reduced well-being or HRQoL is a characteristic feature of AD, an AD-specific HRQoL questionnaire could be of assistance in identifying problematic issues in individual AD patients.

PRA is useful for monitoring mineralocorticoid dosage, aiming for PRA levels at or above the upper reference range to avoid too high doses (117). PRA levels reflect the mineralocorticoid dose better than sodium and potassium levels, but can be falsely elevated in GC overdosage, as GCs will increase the renin precursor angiotensinogen (243). Measuring orthostatic blood pressure and asking for dizziness are useful, as a drop in blood pressure in the erect position indicate clinically relevant mineralocorticoid deficiency. The presence of peripheral oedema suggests too high doses, and salt cravings suggest that the fludrocortisone dose should be increased.

If DHEA treatment in females is tried, some authors suggest monitoring serum DHEAS, androstenedione, testosterone and SHBG, aiming at the middle normal range for healthy young people (108, 130). A clinical assessment of well-being, skin, pubic and axillary hair and possible androgen side-effects is of assistance (60).

There are no international consensus guidelines concerning screening for concomitant autoimmunity in AD. Regular screening for thyroid disease and diabetes mellitus is generally recommended (130); in our practice this translates to measuring thyrotropin (TSH), free thyroxin (FT4), HbA1c and fasting morning glucose annually. If menses are irregular, screening for premature ovarian failure with antibodies against side chain cleavage enzyme should be considered in women; and if well-being and fatigue is present despite optimal replacement therapy, screening for vitamin B12 deficiency should be undertaken. Also, screening for celiac disease every five years is done at

our centre. Routine BMD measurement is not recommended (108), but should be considered done at least once in patients with high GC doses or concurrent ovarian insufficiency.

3. AIMS OF THE STUDY

Currently, the non-physiological cortisol levels resulting from conventional replacement therapy is suspected to be a major cause for the increased morbidity and the reduced HRQoL described in AD, although evidence is insufficient. In order to improve treatment and care for AD patients our aim was to develop a better tool for monitoring HRQoL and to improve the replacement therapy. Ultimately, we aimed to test whether a more physiological GC replacement would result in benefits over the conventional oral replacement therapy.

Specifically, our aims were to:

- Generate an AD-specific quality-of-life questionnaire apt for detecting changes in HRQoL status over time in AD patients.
- Translate the original UK version to various European languages and to perform a large validation study.
- Examine the construct validity, concurrent validity, reliability and repeatability, in order to improve and possibly shorten the questionnaire.
- Test the final validated questionnaire in a large group of European AD patients.
- Investigate whether CSHI in AD patients could mimic the physiological circadian cortisol rhythm and normalise ACTH levels (primary endpoint)
- Investigate the effects of CSHI and OHC on GC metabolism and other metabolic parameters such as carbohydrate and bone metabolism; and effects on HRQoL and sleep (secondary endpoints). We also wanted to investigate whether CSHI was safe and well tolerated.

4. MATERIALS AND METHODS

The AddiQoL

Study design, Patients and Ethics

The AddiQoL was developed by a multi-step approach involving initial generation of multiple relevant items and subsequent item reduction by expert evaluation and pretesting of items. The resulting questionnaire was translated to several languages, and all language versions were tested for validity and reliability, see below.

For AddiQoL development, AD patients were recruited through the UK Addison's disease self-help group. For validation, patients with verified AD were recruited locally from patient registries, or consecutively from outpatient clinics, in several countries. The patients received an invitation letter containing study information and the AddiQoL; by returning the pre-coded questionnaires they were included in the study. There were no exclusion criteria.

The AddiQoL subject codes were used to retrieve patient characteristics such as age, sex and concurrent autoimmune diseases from registries, or via an additional pre-coded registration form. To examine concurrent validity, the patients in Norway and Sweden also received the SF-36 and the PGWB questionnaires. For analysis of longitudinal reliability a subgroup of at least 20 clinically stable patients from Norway, Sweden and Italy completed AddiQoL a second time, 2-6 weeks after the first questionnaire. A random sample of 2000 persons with patient-matched sex, age and geographical distribution was drawn from the Norwegian People Registry. They received a letter with invitation to participate as control subjects by returning the completed anonymous AddiQoL questionnaire, with registration of age and sex only. The study was approved by Regional Ethics committees in each country.

AddiQoL development

HRQoL issues in AD were identified by literature search and in-depth interviews with UK AD patients and their partners (78). The list of items thus generated was reviewed

for clarity and relevance by nine expert clinicians and five experienced patients, and the number of items was reduced. The resulting preliminary questionnaire was tested in anonymous AD patients. The distribution in score for each item was examined; items with a narrow or skewed distribution were eliminated. If two items correlated significantly, one of them was considered redundant and eliminated.

The resulting AddiQoL questionnaire containing 36 items (AddiQoL-36) was used for field-testing in 86 AD patients in the UK. For response categories, a six point Likert scale was chosen, ranging from 1 (none of the time) to 6 (all of the time) to indicate frequency, or 1 (strongly disagree) to 6 (strongly agree) to indicate severity. In items that were negative statements, the scoring was reversed; hence, a higher score indicate a higher HRQoL level. Furthermore, a preliminary analysis of validity and reliability was performed.

Translation

First, the AddiQoL-36 was translated into Norwegian, Swedish, German, Italian and Polish. The translations were performed locally in each country by a multi-step approach (78, 244). Preliminary translations were performed individually by 3-5 members of the study group, each with good knowledge of the English language. After discussing each version, a panel of experts agreed upon a final version to be evaluated further. Two independent professional translators then assessed the quality of the translations and evaluated the conceptual equivalence with the original, the clarity and use of a familiar language. The professional translators both made suggestions to improve overall quality; their suggestions were re-evaluated by the study groups in each country, who decided upon a final version. Ultimately, large cohorts of AD patients in each country completed the final translated AddiQoL versions.

Rasch analysis

The Rasch model rests on the idea that useful measurement involves examination of only one attribute at a time (unidimensionality). The model allows quantitative assessment (i.e. item scores can be added to a total score) from data that are ordinal, based on logistic transformation of the item responses (245). The objective is to test how well the observed data fit with the expectations of the mathematical measurement model (245, 246), which estimates how the patient group should have scored on each item if a logical hierarchical order is present for the items (91). If the observed patient scores do not fit with such a hierarchical pattern, validity is compromised. In lay terms, this translates to identifying at which HRQoL level each item provides the most information; and then organising the items in a hierarchy accordingly. If hierarchical ordering is present, the patients with the lowest HRQoL scores should be able to endorse the items covering the lowest HRQoL levels, but not the items covering a higher HRQoL level. Patients with a little higher HRQoL levels should be able to endorse both items covering the lowest HRQoL level, but also items covering a little higher HRQoL level, etc.

Overall fit statistics examines fit to the model for both the items and the patients, calculated as mean item location and mean person location, and also include item-person interaction statistics. Both person fit and item fit are transformed to approximate a z-score; this represents a standardized Normal Distribution. Therefore, if the items and persons fit the model perfectly, the mean Fit Residual is expected to be zero with standard deviations around 1. Overall fit statistics also includes χ^2 statistics for item-trait test-of-fit to the model. This tests whether the items work as expected at group level along the range of the scale. A non-significant χ^2 probability implies that the hierarchical ordering of items and persons do not vary across the range of the scale (247).

A practical consequence of the fit statistic is that problematic items can be identified and eliminated to improve the validity of the questionnaire. Any item with a *fit residual* greater than +/- 2.5 is a cause for concern; a high positive item fit residual

indicates that the item does not separate well between high and low person ability (or HRQoL), and a high negative item fit residual indicates redundancy or local dependency (see below) of the item. Other causes of misfit to the Rasch model are *disordered thresholds* and *differential item functioning (DIF)*, that is, item bias (246). A threshold is the point where the probability of endorsing two neighbouring response alternatives is equal; one threshold exists for each transition between one scoring alternative and the next. To obtain ordered thresholds, each item and response alternative is assessed, and collapsed or rescored when necessary. This can improve item fit and the validity of an item. DIF analysis explores item performance and questionnaire performance across different patient groups. DIF exists if one patient group scores significantly different on an item compared with another patient group with similar overall HRQoL level (246). For example, if an item works differently in men and women, this item would give a different contribution to the total score in men when compared with women. This would compromise validity of the questionnaire. However, if two items contain DIF for the same person factor, it is possible to group these to items together as a combined item. If DIF disappears in the combined item, the detected DIF do not compromise the validity of the total score of the questionnaire.

Each item's difficulty (item location) and each person's ability (person location) are organized in ordered hierarchies (245, 248). By plotting item location and person location on the same scale, the *targeting of the items to the sample* population can be explored. A perfect targeting is indicated if average person location is zero.

To produce a psychometrically meaningful total score, the scale has to be *unidimensional* (245). Fit to the model and an absence of a significant pattern among the Fit Residuals supports the scale being unidimensional, i.e., there is only one concept being measured. *Local dependency* exists when there is covariance between the response pattern of items, and this is considered a breach of the strict unidimensionality that the Rasch model requires. This can be corrected for by

grouping items with covariance together, i.e. treating the item group mathematically as a single combined item (249).

In Rasch analysis, the Person Separation index (PSI) is equivalent to the *reliability* coefficient (Crohnbach's α), and represents the power of the construct to discriminate between respondents (247). PSI is calculated as the ratio of true variance to observed variance and represents the proportion of variance that is not due to error (247, 248). A PSI of 0.85 is generally required if the scale is to be used on the individual level.

Examining validity and reliability

To examine the construct of the questionnaire, the responses from the patients were first analyzed by exploratory factor analysis (EFA), the purpose being to identify items that do not correlate with the rest of the items in the questionnaire. The construct validity was further explored applying Rasch analysis, to identify problematic items compromising validity and to investigate the psychometric measurement quality of the questionnaire. All analyses were performed for individual countries as well as for the pooled data.

The overall fit statics including individual person- and item fit and item-trait interaction was evaluated by χ^2 statistics incorporated in the RUMM2020 software. Misfitting items were identified and considered for elimination. Thresholds were examined and response categories collapsed and rescored if thresholds were disordered. To identify item bias we examined DIF with regard to patient sex, age, country and concurrent autoimmune disease. If DIF was present for a person factor, we considered either splitting of the item or grouping the item with another item with DIF in the opposite direction, otherwise the item was eliminated. Local dependency was identified by examination of correlation of the fit residuals. If local dependency was present, mathematical grouping of the affected items in to a "super-item" was considered. Unidimensionality was assessed by identifying items loading positively and negatively on the first principal component of the residuals. Person estimates

from these to item groups were compared by independent T-tests incorporated in RUMM2020. If less than five percent of the T-tests were found significant different, the questionnaire was considered unidimensional. Targeting of the items to the distribution in person scores was evaluated by comparing person location and item- or threshold location.

The reliability coefficient Cronbach's α and PSI were calculated for the pooled data, and PSI was also calculated for individual countries. Longitudinal reliability was examined by comparing scores on two occasions in the same clinically stable sample. A test-retest DIF analysis was performed for patient subgroups with stable clinical condition in Norway, Italy and Sweden, over two to six weeks' intervals. Concurrent validity was examined by comparing AddiQoL scores with SF-36 and PGWB scores in Norway and Sweden. Spearman's ρ with two-tailed significance was calculated for the correlation analysis. The Mann-Whitney U-test was used to compare Norwegian patients' AddiQoL scores with AddiQoL results from a random Norwegian population sample. However, for disease-specific questionnaires comparisons between patients and normative data from a reference population should be interpreted with caution, as items are optimized for specific patients groups. Comparison of HRQoL scores in different subgroups of patients was performed by multiple linear regression analysis with sex, age, country and comorbidity as independent variables.

The clinical trial

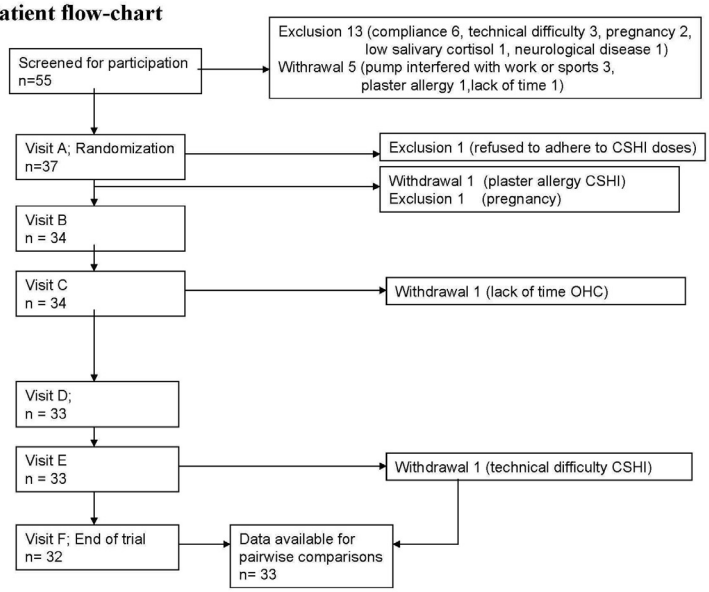
Study design, Patients and Ethics

The clinical trial was designed to prospectively compare the effects of mimicking the physiological circadian cortisol rhythm by CSHI to the effects of conventional OHC. As AD is rare, a multi-centre study was necessary to recruit enough participants. After discussing realistic recruitment possibilities and statistical considerations, a cross-over study design was selected. A treatment period length of three months with each treatment type was considered long enough to be able to detect relevant changes in

endpoints; and a between treatment wash-out period of minimum eight weeks enough to minimise the risk of carry-over effects.

The patient registry (ROAS, Registry of Organ specific Autoimmune Diseases) or the hospital diagnosis registries were used to identify eligible AD patients. Patients who fulfilled the inclusion and exclusion criteria and signed the consent form were invited to a screening visit, where they participated in a practical group course on pump treatment. The patients then underwent a period of dose adjustments (see below) of both treatments (OHC and CSHI). The investigators considered withdrawal for safety reasons if a patient had major difficulties managing the infusion pump. Thereafter, the patients were randomized to either treatment sequence with fixed individual doses of CSHI and OHC. A flow-chart describing patient screening, inclusion and withdrawal are presented in figure 8.

Figure 8. Patient flow-chart



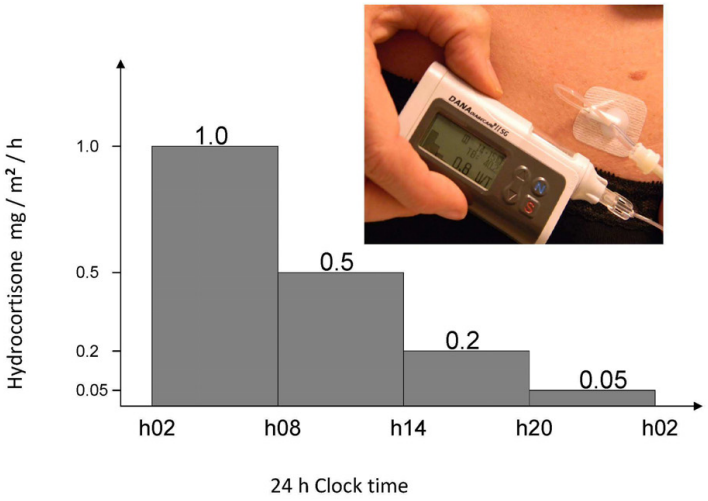
The study was approved by Regional Ethics committees and the National Medicines Agencies in both countries (EudraCT # 2009-010917-61). We conducted the study in accordance with the principles of Good Clinical Practice (CPMP/ICH/135/95) and the Declaration of Helsinki on ethical principles for medical research involving human subjects (1989 version).

Intervention

The CSHI dosing algorithm was derived from the pilot trial (222). A proposed CSHI dose and dose interval was created based on the estimated normal endogen cortisol production (193, 194), and tested in patients while repeated measurement of salivary cortisol was performed. The results suggested a starting dose of 10 mg/m^2/d divided in four dosing intervals: h 08:00-12:00, 0.5 mg/m^2/h; h 12:00-20:00, 0.2 mg/m^2/h; h 20:00-02:00, 0.05 mg/m^2/h; h 02:00-08:00, 1.0 mg/m^2/h.

For the current trial, we used the dosing recommendations and the four dosing intervals as suggested in the pilot trial. Some of those patients had complained of low energy levels at the end of their working day, suggesting that the dose was too low at that time point. Therefore the first dosing interval was prolonged two hours (h 08:00-14:00) in the current study, producing an increase in the suggested starting dose to 10.5 mg/m^2/h, see figure 9. Thus, the daily CSHI starting doses was adjusted to body size, and furthermore individualized during a period of dose adjustment prior to randomisation.

Figure 9. CSHI starting doses (10.5 mg / m^2 / 24 hours)



Since CSHI is technically more complicated than OHC, patient education had to be secured before randomisation. At the screening visit, the patients attended a practical group course on pump treatment, led by an experienced diabetes nurse, with long experience in teaching diabetic patients continuous subcutaneous insulin infusion. Here, the patients learned the proper procedure for self-injections, how to mix the Solu-Cortef act-o-vial and how to fill the pump and injection gear with hydrocortisone. They were also taught how to program the pump with the correct doses, and how to check for treatment malfunction such as battery failure and leakage, and how to check if there was enough hydrocortisone left in the pump. They were instructed to replace the injection gear and refill the pump every third day. Because of the immunosuppressant effects of hydrocortisone, we advised the patients to clean the injection site with alcohol prior to an injection. They were instructed to contact the investigator if signs of infection developed. CSHI doses were evaluated by measuring morning salivary and serum cortisol and late evening salivary cortisol, aiming for normal levels.

For the oral treatment, we chose thrice daily OHC, partly because this is the drug of choice in most countries, and partly because we wanted to achieve a comparator treatment as physiological as possible with conventional treatment. The OHC dose algorithm suggested by Mah et al. was chosen to secure that also OHC doses were not too high, that they were individually adjusted, and adjusted to body size (151).
For both treatments, we allowed smaller dose adjustments at the discretion of the investigators based on best clinical judgment during the dose-adjusting period.

Glucocorticoids, ACTH and CBG

Morning plasma ACTH, serum cortisol and CBG were measured at baseline, eight weeks and 12 weeks in each treatment arm.

Twenty-four hour urine cortisol, cortisone and their metabolites were measured once during each treatment type, and used to calculate indices of enzyme activity. Overall 11β-HSD activity was calculated as (THF + allo-THF)/THE, 11β-HSD2 activity as cortisone/cortisol, 5α-reductase activity as allo-THF/cortisol, 5β-reductase activity (reduction of cortisol) was calculated as THF/cortisol, 5β-reductase activity (reduction of cortisone) as THE/cortisone, and CYP3A4 activity as 6β-OH-cortisol/cortisol.

Circadian salivary cortisol and cortisone were sampled twice within each treatment period, at hours 08:00, 09:30, 11:00, 12:30, 14:00, 15:30, 17:00, 18:30, 19:00, 21:00, 24:00, 03:00 and 06:00. The patients were asked to take their prescribed OHC doses at hours 08:00, 12:30 and 17:00 on the days when the salivary samples were collected. The area under the curve (AUC) was calculated by the composite trapezoidal rule, and reported for 24 hours (AUC_{24h}) as well as for daytime (AUC_{08-24}) and nighttime (AUC_{24-08}) for each patient and for each treatment arm.

Metabolic parameters

At each visit, measurements of blood pressure, weight, waist and hip circumference were measured, and body mass index (BMI) and waist-hip ratio (WHR) calculated. Fasting blood samples were drawn at 08:00 at baseline and after 8 and 12 weeks on each treatment. The samples were stored at -80°C until the end of trial. Carbohydrate metabolism was assessed by measuring morning glucose, insulin and insulin C-peptide. The insulin resistance (homeostatic model assessment; HOMA) index was calculated as fasting plasma glucose (mmol/L)* fasting serum insulin (mIE/L)/22.5) (250). Samples suited for HbA1c analysis were available only for 14 Norwegian patients. Serum total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and triglycerides were measured. To investigate impact on bone, we measured the bone formation marker procollagen type 1 N-terminal peptide (P1NP) and the bone resorption marker C-terminal crosslinked C-telopeptide of type 1 collagen (CTX-1).

HRQoL

At each visit, the patients completed three HRQoL questionnaires: the SF-36, the PGWB and the AddiQoL (appendices 1, 2 and 3). The SF-36 and the PGWB scores were calculated and presented as recommended. The AddiQoL comprised 30 items with six response options, which were scored 1, 2, 2, 3, 3 and 4, according to the results of the AddiQoL validation study. The score of the negative statement items were reversed before calculating a total score; thus, a higher score indicates a higher HRQoL level. Also, the score of the eight-item AddiQoL short version was calculated in the same manner (appendix 4). The resulting AddiQoL score range from 30 (lowest possible HRQoL level) to 120 (highest possible HRQoL level), and the AddiQoL short version score range from 8 to 32.

Sleep

To measure subjective sleep quality during the last month the patients completed the Pittsburgh Sleep Quality Index (PSQI) at each visit (appendix 5). The PSQI consists of 16 items producing seven component scores as well as a total index ranging 0-21

(251). A higher score indicates more sleep problems and a score above 5 is indicative of poor sleep quality. For objective sleep registration, the study participants completed a 7-day actigraph registration a minimum of eight weeks after baseline during both CSHI and OHC treatment. Concomitantly a sleep diary (appendix 6) for the period was administered. The actigraph continuously records information about intensity and frequency of movement using an accelerometer. The following variables were chosen for analysis: time in bed, sleep time, wake time, sleep efficiency, sleep onset latency, sleep bouts, wake bouts and sleep fragmentation.

Circadian study

The 10 first Norwegian patients (the circadian group) were included in an extended protocol and admitted twice, i.e. during OHC and CSHI treatment at approximately 8-12 weeks, to a clinical research facility for 24 h blood sampling. The aim was to determine whether circadian cortisol rhythm was restored, and to analyze the circadian effects on ACTH levels. The AUC for serum cortisol was calculated. Also, the effects on metabolic fuel were assessed by 24 h glucose and insulin levels and triglyceride levels during the night. Nighttime levels of growth hormone, insulin-like growth factor 1 (IGF-1) and insulin-like growth factor binding protein 3 (IGFBP3) were also investigated. Serum and plasma samples were collected hourly from an indwelling catheter in a forearm vein; ACTH was sampled every three hours. During OHC the patients administered their medication at 08:00, 12:30 and 17:00 while admitted.

Euglycaemic clamp

All the fifteen Swedish patients (the insulin sensitivity group) were admitted twice, i.e. after eight weeks of each treatment, for euglycaemic-hyperinsulinaemic clamp according to De Fronzo et al. in a fasting state during the morning hours (252). Intravenous catheters were inserted into the right arm for substrate (insulin/glucose) infusion. During the 120 min of the test, insulin (Actrapid [40 mU * $m^{2(-1)}$ * min^{-1}], NovoNordisk A/S, Copenhagen, Denmark) was infused together with 20% dextrose

(Fresenius Kabi, Stockholm, Sweden). The rate of dextrose infusion was adjusted to achieve a blood glucose level of 5.0 mmol/L based on arterialized samples withdrawn every five minutes from an ipsilateral dorsal hand vein. Whole-body insulin sensitivity (M-value) was calculated from the amount of glucose infused during the last 30 min of the clamp divided by body weight (kg) and period (min) and expressed as mg/kg/min. Serum cortisol and cortisone were measured every 30 min during the euglucaemic-hyperinsulinemic clamp.

Statistics

Data were presented as percentages, medians or means together with a variability measure such as standard error or confidence interval. All *p*-values were two-sided and values less than 0.05 were considered statistically significant. The AUC_{24h} for salivary and serum profiles was calculated by the composite trapezoidal rule, and for salivary samples also reported for daytime (AUC_{08-24}) and nighttime (AUC_{24-08}) for each patient and for each treatment arm. Statistical analysis of paired data (24 h urine and AUC analyses in Paper 3 and euglycemic clamp in Paper 4) was done by Wilcoxon signed rank non-parametric test for related samples.

To estimate differences in metabolism, HRQoL and sleep between CSHI and OHC treatments a linear mixed effects model for repeated measures was used (253). Our model defined treatment, visit time, treatment sequence, treatment period and treatment-by-time interaction as fixed effects. To account for the intra-individual correlation between repeated measures a random effect with patients nested within sequence of treatment was specified. Because of skewed distributions, several variables from the SF-36 domain, glucose, HOMA-index and CTX-1 were transformed according to recommendations before model testing (254).

To investigate a treatment-by-time effect on metabolism, HRQoL and sleep we used the likelihood-ratio test by comparing the log-likelihood between models with and without the treatment-by-time term. To obtain p for trend in means within treatment

groups the effect of time was included as a linear term in the mixed effect model using the z-test. To obtain p for model-predicted mean differences between treatment groups for different visit time we performed a post-hoc test for pairwise comparisons (z-test).

5. RESULTS AND SUMMARY OF PAPERS

Paper 1

Paper 1 describes the development of AddiQol, and reports the initial results of its reliability and validity. A list of 70 items relevant for AD was generated; after revision the number was reduced to 60 items, which was pretested in 100 AD patients in the UK. The number of items was further reduced to 36 after scrutinising each item for distribution of scores, relevance, redundancy, and possible sensitivity. The resulting 36 item AddiQoL questionnaire was then tested in 86 AD patients in the UK.

The AddiQoL-36 displayed a high reliability with a Crohnbach's alpha of 0.95 and a Pearson Separation index (PSI) of 0.94. Rasch analysis suggested some misfitting items with item fit residuals above 2.5. Overall, there was a misfit to the Rasch model of the total 36 items (item-trait interaction χ^2 <0.0001), but exclusion of six items with extreme fit residuals improved fit to the Rasch model (item-trait interaction χ^2 >0.09) to an acceptable level. The difficulty of the items (item location) gave a satisfactory coverage of the distribution of patient scores (patient location) with no apparent floor or ceiling effect, indicating overall good targeting of the items to this patient group. Because of the relatively small sample size, it was difficult to draw any firm conclusions regarding misfitting items and revision of the questionnaire; therefore all 36 items were kept pending a larger validation study.

Paper 2

Paper 2 reports the results of a large European AddiQoL validation study. A total of 615 patients were recruited from registries or outpatient clinics in Norway (n=107), Sweden (n=101), Germany (n=200), Italy (n=157) and Poland (n=50), and completed the AddiQoL-36. The data was pooled with the original UK data (n=86). Reliability and validity were assessed for all countries pooled together, as well as for individual countries.

EFA showed that the items could be grouped in four domains; eight items covered energy level (sub-dimension Fatigue), eight items covered emotional or mental aspects (Emotions), 11 items common AD symptoms (Symptoms), and six items basic aspects like sleep, sexuality and impact of intercurrent disease (Miscellaneous). Three items did not fit into any domain, their misfit was confirmed with subsequent Rasch analysis, and they were therefore eliminated. The Rasch analysis revealed three additional problematic items, which were eliminated.

Disordered thresholds were present, indicating that the patients had problems differentiating between the six scoring categories (1, 2, 3, 4, 5, 6). Collapsing the middle adjacent response options by rescoring categories (1, 2, 2, 3, 3, 4) resulted in ordered thresholds and improved fit to the Rasch model.

There was no significant DIF between the genders, and no DIF when comparing the results from patients with isolated AD with patients with autoimmune polyendocrine syndromes. Significant DIF for age was present in the Emotions sub-dimension. Significant DIF for country was present in the Fatigue, the Symptom and the Miscellaneous sub-dimensions.

The 30 remaining items arranged in the four sub-dimensions fitted the Rasch model (item-trait interaction $\chi 2$ $p=0.56$) and proved unidimensional. Person-item targeting was good in all countries. Reliability was high as Cronbach's α was 0.93 and PSI 0.86. Test-retest reliability was excellent; no item bias for time was detected in clinically stable patients in Norway (n=37), Italy (n=25) and Sweden (n=29).

Correlation between the AddiQoL scores and concurrent scores for the PGWB and SF-36 subdimension scores Vitality, General Health and Physical Function were high in Norwegian and Swedish patients. The AddiQoL scores were significantly lower in AD patients ($p<0.001$) than in a random population sample (n=539) in Norway. Regression analysis revealed that women scored worse than men ($p<0.001$), and the

scores worsened with increasing age ($p<0.001$). No difference was found between patients with isolated AD and those with polyendocrine syndromes.

In conclusion, the AddiQoL was translated to five European languages, the number of items reduced to 30 and the item scoring range altered, resulting in a psychometrically valid and reliable AddiQoL questionnaire. This study design did not provide an opportunity to further test the responsiveness of the AddiQoL. Also, an AddiQoL short version (i.e. the Fatigue dimension) was produced; suitable for use when measuring fatigue is the main objective. In patients without fatigue, the short version could be less sensitive to change due to ceiling-effects, as the short version displayed less optimal item-person targeting when compared to the AddiQoL.

Paper 3

Paper 3 reports the results of the randomised controlled cross-over clinical trial "Glucocorticoid treatment in Addison's disease" as per study protocol (NCT 01063569). Thirty-seven patients were randomised, and 32 completed the trial. Results from both treatment arms were available for 33 patients (see Figure 8). The mean patient age was 48 (SD 12) years, the mean AD duration 12.4 (SD 10.1) years, and 25 patients (75.8%) were female. The OHC doses (mean 0.26 mg/kg/d; SD 0.08) were slightly lower than the CSHI doses (mean 0.31 mg/kg/d; SD 0.07); both were lower than the hydrocortisone equivalent pre-trial doses (mean 0.36 mg/kg/d, SD 0.13).

CSHI brought the morning ACTH levels back to normal, contrasting the very high levels seen with OHC. CSHI also normalized the morning cortisol levels, and produced 24 h salivary cortisol and cortisone day curves resembling the normal circadian variation including the late night cortisol surge. In contrast, OHC produced the typical daytime supra-physiological post-dose cortisol peaks followed by troughs before the next dose. Nighttime and early morning cortisol levels were very low. There was no between-treatment difference in salivary cortisol AUC_{24h}, but salivary

cortisone AUC_{24h} was higher with CSHI. Effects on other metabolic parameters were generally small, with the exception of significantly higher morning glucose on CSHI than on OHC. No between-treatment difference was found for insulin, insulin C-peptide or HOMA-index. There were no between-treatment difference in blood pressure, weight, WHR or BMI, and no difference in cholesterol, HDL, LDL or triglycerides. Effects on bone markers and between-treatment differences were small, and the CTX-1 and P1NP means were within the reference range on either treatment. However, both CTX-1 and P1NP declined significantly during CSHI treatment, whereas during OHC treatment P1NP increased without changes in CTX-1.

CSHI did not significantly affect sleep compared to OHC, but might have positive HRQoL effects. CSHI increased AddiQoL and AddiQoL short version scores, whereas no effect was found for OHC, and AddiQoL scores were significantly better for CSHI than for OHC. CSHI yielded significantly superior scores in the SF-36 physical function scale and the PGWB vitality score. The other subscales of both generic questionnaires detected no changes and no between-treatment differences, implying that the AddiQoL is better apt to detect changes in HRQoL in AD than the generic questionnaires.

Paper 4

Paper 4 reports the results from two sub-populations of the study in whom circadian hormone levels and metabolic effects were studied in more detail.

In the circadian group, the median hydrocortisone dose was 0.28 mg/kg/d (range 0.20-0.50) and 0.23 (0.25-0.50) mg/kg/d for CSHI and OHC treatments, respectively. CSHI yielded a smooth circadian cortisol curve, with mean serum cortisol within the reference range for healthy individuals, including the physiological low evening levels and a late night cortisol surge culminating in an early morning peak. In contrast, OHC brought about low nighttime cortisol levels and high post-dose peaks followed by troughs. The median serum cortisol AUC_{24h} was higher for CSHI than for

OHC (p=0.028). Furthermore, ACTH levels remained clearly elevated with OHC, contrasting the near normal circadian levels found with CSHI.

On OHC, the patients had gradually decreasing glucose levels during the late night, with the lowest levels at h 08:00. During CSHI treatment, the glucose nadir was observed at h 02:00. A further decrease in glucose levels was avoided, resulting in improved mean serum glucose level during early morning when compared with OHC. Differences in 24 h serum insulin levels were generally small. Twelve-hour nighttime measurements of triglycerides were in the normal reference range and did not differ between the two therapies.

During both treatments there was a GH peak during the first part of the night, which during CSHI tended to be more pronounced than during OHC. For IGF-1 there was a trend for higher levels at each hour in the CSHI group with a significant difference at h 01:00 and h 08:00. The IGFBP3 was significantly higher with CSHI at h 08:00, 09:00 and h 12:00, correlating with the IGF-1 peak in the CSHI group at h 08:00.

In the insulin sensitivity group the median hydrocortisone dose was 0.27 mg/kg/d (0.24-0.35) for CSHI and 0.23 (0.21-0.32) mg/kg/d for OHC treatment. No difference was noted in insulin sensitivity (M-value) between treatment groups (p=0.59) for the CSHI and OHC treatment. There was no difference in cortisol and cortisone at 0 and 30 min during the clamp, but both cortisol and cortisone were significantly higher at 60, 90 and 120 min in the CSHI-treated group.

6. GENERAL DISCUSSION

AddiQoL

AddiQoL development

The development of the AddiQoL provided a valid and reliable AD-specific questionnaire, which will hopefully become useful for detecting changes in HRQoL in patient groups or individual patients over time. The multi-step approach of item generation and reduction secured a satisfactory breadth of HRQoL range coverage for AD patients, which is essential for a disease-specific questionnaire. The resulting high reliability and validity secures that changes in AddiQoL scores obtained over time, or differences found between patients groups, can be trusted. Retrospectively, testing for threshold ordering revealed that the chosen 6-point Likert scale did not fit all items. If Rasch analysis had been applied earlier in the development process, this might have been avoided.

Methodological considerations

Rasch analysis is increasingly used in somatic medicine and endocrinology, and was previously applied in the validation of QoL-AGHDA (255), AcroQoL (256) and CushingQoL questionnaires (95). We chose Rasch analysis to explore the psychometric properties of AddiQoL, that is, to examine construct validity, because fit to the Rasch model confirms that basic properties of a measurement instrument are secured, such as hierarchical ordering and unidimensionality. These properties should be present to allow adding the score of individual items to a total score. The Rasch model also allows the researcher to examine the measurement properties for individual items, which we found extremely useful in the process of validating the AddiQoL. The opportunity to examine how well the item location target the patient location is valuable; here we could demonstrate how well the items as a group target the distribution of HRQoL scores in the patient group, which is an important issue for a disease-specific questionnaire.

High reliability and good discriminating properties usually indicate good evaluative properties (responsiveness) as well. However, the items with the best fit to the Rasch model might not necessarily be the items most sensitive to change (257). Clinical issues such as disease-specific symptoms, might not have the best fit to the stringent Rasch model, yet such items are important to include in evaluative disease-specific questionnaires as they are expected to be more responsive to change during disease intervention. Also, while high reliability indicates a high signal to noise ratio, a too high reliability might compromise responsiveness of the questionnaire (257). Reliability may also be inflated by the presence of redundant items or items with local dependency (245). Therefore, the validation process had to secure a balance between retaining highly relevant items without compromising the basic psychometric requirements of a measurement instrument.

The rescoring of response options in the AddiQoL reduced the score range of the questionnaire, which possibly reduces responsiveness of the AddiQoL. However, this was necessary as the disordered thresholds found initially indicated that basic hierarchical ordering was not present. In this situation a score change from response option two to response option three might be random and not represent real HRQoL change. Rescoring the response option secured that basic hierarchical ordering now is present, rendering the detection of a small change in HRQoL score more reliable. We detected DIF between countries, indicating that several items functioned somehow different in different countries, which is common for HRQoL questionnaires. Whether this is due to small translational differences, cultural differences or other issues such as differences in patient characteristics cannot be clarified. If the aim is to compare HRQoL scores between countries, the biased items will have to be statistically adjusted for each country before comparisons can be done (258). However, the country DIF may have a negligible clinical impact when evaluating the HRQoL scores from a multicentre clinical trial (259).

Future perspectives

A challenge to HRQoL measurement in clinical practice is the interpretability of the HRQoL score. Even if scores do significantly improve or deteriorate over time, how are this interpreted to give meaningful clinical information about the HRQoL change? The minimal clinically important difference (MCID) is defined as the smallest difference a patient will perceive, that would mandate a change in clinical management (260). Whenever the detected difference in mean scores is less than MCID, the difference is considered not clinically relevant. The MCID can be calculated by an anchor-based approach, i.e., by linking the difference in HRQoL scores found in a trial to a clinically relevant endpoint, to the results of a symptom score, or to patient opinion of whether the detected change is clinically relevant. This approach has been criticised for being imprecise, but has nonetheless been useful for several disease-specific questionnaires (261, 262). Alternatively, the MCID can be calculated by a distribution-based approach. Some authors suggest calculating MCID as half the standard deviation at baseline, although this is controversial (263, 264). Another possibility is to calculate the effect size, i.e. to compare the HRQoL change over time to the standard deviation at baseline (265). Presently, the most common distribution-based approach is to define MCID as one standard error of the mean (SEM) (266). Ideally, the MCID should be calculated by several approaches, as results may vary between methods (266, 267). The use of one cut-off value for the MCID has been criticised, as it is based on mean changes in one patient group, and also because the meaning of a HRQoL change depend on the starting point and on the direction of change (268).

To demonstrate responsiveness, it is necessary to identify AD patients who truly change, and compare their AddiQoL results to HRQoL scores with scores in patients who do not change. Compared to other endocrine diseases such as acromegaly or growth hormone deficiency, it is difficult to sample HRQoL in untreated AD patients as AD treatment has to be started without delay. Hence, to demonstrate responsiveness and define MCID we rely on demonstrating differences in HRQoL scores between different treatment types, and the detected difference in HRQoL

scores will therefore be smaller. In AD, there is no clinical endpoint or physical test available for anchor-based definition of MCID. We have correlated our results with the best "gold-standard" available, and shown that the AddiQoL detected improved HRQoL levels with CSHI, whereas with SF-36 and the PGWB differences were smaller and significant only on a few subscales. Future studies on responsiveness are needed for the AddiQoL, and should possibly include both estimation of the MCID and calculation of the responsiveness index (269).

Since new and more expensive AD treatment options are under development, it may become important to better define what constitutes a clinical relevant increase in AddiQoL score. From the view of a health economist, the cost-benefit balance of new treatment options should be considered, and a benefit in HRQoL documented by the AddiQoL could possibly be used for these kinds of calculations in the future.

Future application of the AddiQoL depends on the availability of translations to relevant language versions. We know from personal communications that Danish, Spanish and Japanese versions have been created, although no validation studies have been published yet. To encourage the use of AddiQoL, it is free to use, and new language versions are welcomed.

Pump treatment in AD

CSHI dosing and monitoring

Because the administration mode of hydrocortisone is different with CSHI and OHC, we could not assume equal daily doses in the two conditions. The dosing algorithms applied in our study were different for the two treatment types, and resulted in slightly higher daily doses of hydrocortisone with CSHI than with OHC, although both the OHC and the CSHI doses were reduced compared with pre-trial doses. However, the main difference between treatments was the dissimilar circadian distribution of the daily dose. The AUC_{24h} for salivary cortisol did not show significant between-treatment differences, but the AUC_{24h} for salivary cortisone were significantly higher

with CSHI than with OHC. Salivary cortisone may better reflect the free serum cortisol levels in serum (50), suggesting a higher cortisol bioavailability with CSHI than with OHC. The elevated 24 h urine cortisol and cortisone levels found with CSHI are in line with this (see below).

However, we did not find any proof of overdosage with CSHI; while there was a trend towards increasing BMI with CSHI, there was no significant between-treatment difference. Likewise, there was a significant trend towards higher morning HOMA-index with CSHI, but no between-treatment difference was found. In addition, as no between-treatment in insulin sensitivity was detected with euglucaemic clamp, the trend towards increased morning HOMA-index was probably due to the timing of the glucose and insulin samples in the morning when cortisol levels were much higher with CSHI than with OHC. The fact that HbA1c levels decreased with both treatments and that no changes were found in other parameters such as WHR, blood pressure or lipids was also reassuring.

In contrast to diabetic patients, AD patients are not as experienced in self-injections or the mixing of drugs for injection. Hence, proper education of both the patient and health care professionals are mandatory before starting CSHI treatment. One worry prior to starting this trial was the risk of local infection at the injection site. We experienced no adverse events due to local infections during the trial. Another concern was that AD patients have no means to monitor if the pump treatment is working as expected, whereas diabetic patients can rely on glucose levels to monitor if insulin is delivered as expected. With CSHI, we had to rely on indirect signs such as the decreasing amount of drug in the pump, no leakage, and most importantly the well-being of the patient. During the trial, the patients reported that they were reassured by hearing the pump deliver the drug every fourth minute. Experience from the dose adjustment period taught us that it was practical to use the pump program to check which dose had been delivered the previous day, to check if everything was working properly. Checking the refill dates and alarm history also proved helpful in a

few patients. Patients were instructed to use extra oral doses of their pre-trial drug if they felt unwell, and to contact the investigator as soon as possible.

Pump treatment also has other potential positive sides, which we did not explore in this study. Vomiting or diarrhoea is the most common precipitating cause of adrenal crisis in AD patients (132). With parenteral replacement like CSHI the risk of AC might be reduced in these situations. The pumps can also deliver extra hydrocortisone boluses if needed, and the size of the dose can be lower than feasible with the current oral replacement drugs. Increased dosage can also be delivered by temporarily increasing the basal rate of the infusion, for example by doubling the infusion rate during days with febrile illness, or increase the basal rate to 150% during strenuous exercise.

Salivary cortisol or cortisone is useful in monitoring CSHI treatment. However, in the trial we detected a few results with extremely high salivary cortisol levels (above 1000 nmol/L), most likely a result of pre-analytical error. The cortisol concentration in Solu-Cortef is substantially higher than in saliva, and even small amounts of Solu-Cortef contaminating the saliva swab will result in extremely high salivary cortisol results. With this exception, our results indicate that CSHI can be monitored by morning and late evening salivary cortisol samples, or day curves. We recommend aiming for morning salivary cortisol in the middle reference area, and evening salivary cortisol within the reference area (< 2.8 nmol/L) for optimal dosage. If the patient is not feeling well, measuring morning serum cortisol in the normal reference range can exclude under-dosage, and measuring a morning ACTH at the upper reference range can reassure that doses are not too high.

Effects on cortisol metabolism

The higher AUC_{24h} for salivary cortisone found with CSHI suggests that the free cortisol levels were higher than with OHC (50). In addition, the 24 h urine cortisol and cortisone were significantly higher with CSHI than OHC. Also, the circadian sub-

study revealed that after the dose increase at h 02:00, cortisol levels increased faster than expected from the pilot trial, suggesting that this dose interval could preferably be delayed. As a consequence, we suggest postponing this dose interval by one hour, i.e. to h 03:00-08:00, hence lowering the CSHI starting dose to 9.55 mg/m^2/d.

Furthermore, the indices for 11β-HSD2 activity, CYP3A4 activity, β-reductase activity and α-reductase activity were all lower with CSHI than with OHC. There was no between-treatment difference in overall 11β-HSD activity. When comparing our results with those from control persons in a recently published study we found that OHC increased all these enzyme indices, whereas CSHI restored them closer to normal. However, the high 24 h urine cortisone level seen with CSHI complicates the interpretation of the estimated index for 11β-HSD2 activity, as this enzyme has nonlinear kinetic properties (67, 270). We cannot exclude the possibility that CSHI constantly saturated the 11β-HSD2 enzyme, resulting in increased excretion of free cortisol in the urine.

Effects on carbohydrate and lipid metabolism

We have shown that with OHC there is a progressive unphysiological decrease in late night glucose levels, probably resulting from the lack of cortisol. Although we did not identify any hypoglycaemias, other studies have reported the occurrence of nighttime hypoglycaemia in AD (271). With CSHI, the lowest glucose levels were observed at h 03:00 as in healthy people, and a further decrease in glucose levels was avoided. Only small between-treatment differences were found in insulin levels. Theoretically, glucose levels seemed more physiological with CSHI than with OHC, but the clinical benefit of increasing glucose levels within the reference range in a non-diabetic AD patient with no hypoglycaemia is uncertain. Our results do however suggest that mimicking the late night cortisol surge with CSHI or modified release hydrocortisone can be beneficial in AD patients with concomitant diabetes mellitus. Conversely, during daytime the glucose and insulin levels tended to be higher with OHC than with CSHI, but these differences were not statistically significant. OHC doses were

significantly reduced from pre-trial doses, possibly contributing to the lack of between-treatment difference in daytime glucose and insulin levels. In the current study, reassuringly, we found no between-treatment difference in M-value with euglucaemic hyperinsulinemic clamp despite the between-treatment difference in hydrocortisone dose.

al-Shoumer and co-authors found that in addition to low glucose levels during the night, patients with hypopituitarism on conventional GC replacement also had low concentrations of other metabolic fuels such as non-esterified fatty acids (NEFA) and 3-hydroxybutyrate (272). In another study from the same group, fasting total cholesterol, LDL, triglycerides and apolipoprotein B were elevated in patients compared to controls, and the AUC_{24h} for cholesterol and triglycerides were significantly higher in patients than in controls (272). Furthermore, they later performed a small study in patients with hypopituitarism replaced with GCs; here, they showed that GH replacement increased nighttime NEFA levels, but had no effect on glucose levels (273). In line with their findings, in our study fasting levels of cholesterol, LDL, HDL and triglycerides did not differ between CSHI and OHC, and we found no difference in nighttime triglyceride levels, suggesting that mimicking the physiological nighttime cortisol surge does not influence the lipid levels. Possibly, the total GC dose has a higher impact on lipids than restoring the circadian cortisol rhythm, as a daily dose of more than 20 mg/d increases BMI, total cholesterol, LDL and triglycerides in patients with hypopituitarism (138). The daily hydrocortisone equivalent dose in our trial was relatively low for both CSHI and OHC.

Effects on bone markers

When evaluating effects on bone metabolism in our study, it is a challenge that patient number was low and the study relatively short. OHC produced an increase in P1NP probably related to the reduction of hydrocortisone dose, and no alteration in CTX-1. This suggests that a small reduction of the conventional daily GC replacement dose has beneficial effects on bone formation without altering bone resorption.

Surprisingly, CSHI resulted in a reduction of P1NP despite the dose reduction, while CTX-1 decreased; possibly indicating an overall reduced bone turnover with CSHI. Since only CTX-1 and P1NP were measured, we cannot relate our findings to other relevant measures such as levels of calcium, vitamin D, parathyroid hormone or osteocalcin. Importantly, the changes in CTX-1 and P1NP were small for both CSHI and OHC, and mean levels were within reference ranges for both treatments. Furthermore, as the intra-individual variability is high for both P1NP and CTX-1, the least significant change for both has been calculated to around 30%, suggesting that the changes in P1NP and CTX-1 observed in our trial may not be clinically relevant (274, 275). Also, bone resorption markers such as CTX-1 display a circadian rhythm with a peak during the night followed by decreasing levels towards a nadir in the middle of the day (276, 277). Likewise, a similar circadian rhythm with lower amplitude has been described for bone formation markers, especially for osteocalcin and P1CP (278). Some authors have suggested that the circadian cortisol rhythm is implicated in the circadian variation in bone metabolism (279), which could imply that the decrease in P1NP with CSHI in our study may result from restoring the circadian cortisol rhythm. In contrast, a Danish study examined circadian levels of bone markers during OHC replacement in seven patients with SAI and four with AD. Here, the daily OHC doses (20-30 mg) were divided into four equal doses administered at h 06:00, 12:00, 18:00 and 24:00; although this distorted the circadian cortisol rhythm, the circadian rhythm was maintained for bone resorption markers and the bone formation markers osteocalcin and PICP (280). This suggests that the cortisol rhythm is of little importance in the regulation of circadian bone metabolism. Alternatively, the decrease in P1NP with CSHI could imply that continuous delivery of hydrocortisone inhibits bone formation. Osteoblasts contain glucocorticoid receptors (279), which may respond differently to a continuous hydrocortisone delivery as opposed to the ultradian delivery in healthy people (24). The higher cortisol bioavailability seen with CSHI could possibly also contribute.

Although changes in bone markers were small and within the normal reference range,

our results suggest that future studies on circadian cortisol delivery should include circadian measurement of bone markers. CSHI provides an opportunity to study the relationship between the circadian rhythms of cortisol and bone markers.

HRQoL

This study did not have statistical power to reach firm conclusions on HRQoL effects, but the observed improvement of AddiQoL scores suggests that CSHI diminishes fatigue and improves HRQoL in AD. Importantly, a reduction of GCs from the pre-trial doses both in the CSHI and OHC arms did not adversely affect HRQoL. Thus, our results suggest that replicating the circadian cortisol pattern by CSHI might improve HRQoL. However, since HRQoL scores are subjective, results have to be interpreted with caution due to the un-blinded study design.

The HRQoL results were not as striking as in the pilot trial, probably because this trial recruited unselected patients whereas the patients in the pilot trial were selected because they had poor functioning. The SF-36 scores at the start of each treatment arm in our study were a little higher than previously published SF-36 scores in AD (87, 88, 128). Also, the majority of the participants were full-time workers (60.6%); only 12.1% did not work. Only 21% did not engage in physical activity, whereas 55% exercised more than 3h per week. Taken together, this indicates that the randomised patients were relatively healthy. Nevertheless, we found that the SF-36 Physical Function score was better with CSHI than with OHC. The PGWB scores at baseline were very similar to the Norwegian and Swedish results in the AddiQoL validation study (Paper 2). There was a significant trend towards improvement of the PGWB total score during CSHI treatment, but no significant between-treatment difference. The complexity of CSHI in combination with all the security precautions in the trial could possibly compromise HRQoL and render the patients more anxious and worried. Therefore, it was reassuring to find that anxiety levels did not increase, in fact, they tended to decrease on CSHI.

A better estimate of the responsiveness of a questionnaire can be obtained by examining the ratio of the mean change in HRQoL score after treatment to the variability in score at baseline (269). For the AddiQoL scores obtained in the clinical trial this ratio is promising, indicating that the sample size needed to detect statistical significant differences over time is lower with the AddiQoL, and the responsiveness better, than with the two generic questionnaires. As mentioned above, the clinical trial was not designed for testing AddiQoL responsiveness; hence, future studies are needed for clarification.

Theoretically, the between-treatment differences found in HRQoL levels could be due to the between-treatment differences in GC doses. However, there is no evidence indicating that higher glucocorticoid doses increase HRQoL in AD; to the contrary, studies have suggested that supra-physiological GC doses are associated with decreased HRQoL (88, 197). Furthermore, increasing the serum cortisol AUC by more frequent dosage of the same total daily dose did not improve HRQoL levels in another study (198).

Sleep

Associations between the circadian cortisol rhythm and sleep architecture are well known (29). Actigraphy is a good tool for evaluating circadian rhythm disorders (281), but cannot give information about sleep architecture, i.e., the length and distribution of sleep stages. Hence, information about whether restoring circadian rhythm improves sleep architecture in AD could be lost by our choice of method. However, our results suggest that overall, sleeping problems are a minor issue in AD, and we found no objective sleep benefit of replicating the circadian cortisol rhythm compared with conventional OHC. The shorter sleep time reported with CSHI could suggest that increasing late night cortisol results in more alertness in the morning, but given the subjective nature of these data in combination with the non-blinded study design, it seems prudent to rely more on the objective actigraphy recordings. Future

studies in circadian replacement therapy should include polysomnography, which is the gold standard of sleep evaluation.

Methodological considerations

Initially, we aimed for a double-blind study design. We were, however, unable to find a manufacturer willing to produce a placebo for CSHI, which may not be surprising since Solu-Cortef is an Act-O-Vial (powder and fluid to be mixed by the patient); hence the powder, the fluid and the mixed product would have to look identical to the active drug. In addition the product would have to be approved for subcutaneous use. Even though saline do not have an identical appearance to Solu-Cortef, we also considered using isotonic saline as placebo. Because mixed Solu-Cortef Act-o-Vial is approved for use within 3 days only, this implied shipping of study drugs to each patient's home every three days, which proved logistically too challenging. As longer shelf-life has been reported for hydrocortisone (282), we also tested the stability of pre-mixed Solu-Cortef at room temperature versus lower temperatures, to see if it could be possible to ship the study drug refrigerated and less frequently without compromising safety. Premixed Solu-Cortef samples were stored cold and in room temperature, and then subjected to measurement of Solu-Cortef concentration (LC-MSMS), the appearance of degrading products (LC-MSMS scan), pH measurement and a visual inspection for colour and precipitation. The preliminary results showed that both the concentration of hydrocortisone and pH were stable for seven days when refrigerated, but the concentration declined somewhat in room temperature. Visual inspections did not differ, and we did not identify any degrading products except hydrocortisone succinate and sodium cortisol. However, even weekly shipment of refrigerated solutions would have been hampered by logistics, because we knew that some of the participants would live far from the hospital, otherwise the number of participants would be dramatically reduced. We therefore finally decided to choose an un-blinded study design.

The open-label crossover design of the clinical trial implies some important limitations to the interpretation of the treatment effects. Most likely, the primary outcome ACTH and the metabolic parameters are not severely affected by placebo effects; however, caution is required when interpreting effects on subjective HRQoL and sleep scores.

Future perspectives

In conclusion, we demonstrate in a controlled fashion that CSHI is a safe and more physiological replacement therapy than OHC, and might become a treatment option for selected AD patients. CSHI can be an option for capable AD patients who are symptomatic despite up to date conventional treatment with OHC or oral cortisone in combination with optimal fludrocortisones dosage. We know from clinical practice and personal communication that long-term CSHI has already been applied in several selected AD patients world-wide. Also, a few cases have been reported in the literature of patients with congenital adrenal hyperplasia successfully treated with CSHI during adolescence (283, 284). That patient group is especially suited for more physiological GC replacement, as the lack of cortisol during the night with conventional treatment results also result in high androgen levels (217). Our results also suggest that with CSHI the nighttime decrease in blood glucose levels in patients with AD can be avoided, which might be advantageous for AD patients with concomitant diabetes mellitus. Because the oral route is bypassed, CSHI also has the potential to reduce the risk of acute adrenal crisis precipitated by vomiting and diarrhoea, and may become a treatment option for patients with malabsorption.

Patients who are functioning well with OHC may perceive CSHI as complicated and disturbing, whereas patients with poor functioning may experience significant gain and readily accept CSHI treatment. On the other hand, the pumps are getting smaller and easier to manage, and due to the development in our society in general patients are less afraid of, and more accustomed to, the use of technology in their daily lives. In our trial, most patients managed the pump treatment well, without major problems.

Five of the 18 Norwegian patients in this trial insisted on continuing CSHI treatment at the end of our trial; in Sweden no one continued, as this was not given as an option.

Another argument against pump treatment is that it is more expensive than conventional oral treatment; although not necessarily so when compared to modified-release hydrocortisone. Hopefully, with improvement of pump technology, the cost of such treatment can be reduced, making CSHI an affordable choice for a larger group in the future.

Furthermore, when now proved feasible and safe we believe that CSHI is a versatile method for further refinement and studies of the GC replacement therapy. While our results have shown that CSHI can restore the physiological circadian variation in cortisol levels, resulting in normalisation of ACTH levels and possibly HRQoL improvement. However, the ultradian rhythm cannot be replicated with the current dosing (6). The rapidly developing pump technology in combination with new knowledge on cortisol rhythms enables us to further refine the therapy. It may become possible to mimic the ultradian rhythm, by programming the pump to deliver pulses of hydrocortisone superimposed on a circadian delivery. Future CSHI trials should aim at replicating the full ultradian rhythm, which could possibly further improve outcome for AD patients.

7. LIMITATIONS

The AddiQoL

Although disease-specific questionnaires usually are more sensitive for detecting changes in HRQoL scores over time, testing for such responsiveness was not included in the AddiQoL validation study. Even though the AddiQoL detected significant HRQoL improvement with CSHI during the clinical trial, our results should preferably be confirmed by other interventional trials. However, formal testing of AddiQoL responsiveness is hampered by the lack of a clinical relevant correlate, as no such objective suitable test exists for AD.

Also, the clinical utility of the AddiQoL when following individual patients could possibly be improved. This implies relating individual AddiQoL scores to that of other groups of patients in order to pinpoint an individual score as good or bad, aiming to identify patients with poor functioning in need of intervention. The published scores from the validation study were scores calculated from the person location score, hence scored range from zero to 100, making comparisons more difficult. Publishing the percentiles of AddiQoL raw scores ranging from 30 to 120 from individual countries might aid interpretation of scores in individual patients.

The clinical trial

Rejecting the null hypothesis when it is in fact true is called a statistical type 1 error. Here, one null hypothesis is that HRQoL scores do not differ between CSHI and OHC. In that case, a type 1 error would translate to finding a significantly improved HRQoL on CSHI, when in fact the observed improvements in HRQoL score are a result of chance. When examining HRQoL, most studies use multiple questionnaires each containing multiple subscales. As multiple testing increases the risk of Type 1 error, the significance level should preferably be lowered to minimize the risk. Pretrial, the statistical power analyses where performed assuming significant difference equals $p < 0.05$. In retrospect, we acknowledge that a more conservative approach ($p<0.01$) might be warranted due to statistical testing of multiple variables,

to reduce the risk of type 1 error. If we this way retrospectively consider our results, the between-treatment differences in HRQoL scores is only borderline significant (AddiQoL p for interaction = 0.012). However, there is still a significant HRQoL improvement with CSHI, as evaluated by p for trend for the AddiQoL, the AddiQoL short version and the PGWB vitality score.

On the other hand, endorsing the null hypothesis when it is in fact false is called a type 2 error. In our study this translates to rejecting effects that were not statistically significant, because the sample size was too small, i.e., the statistical power too low. When studying a rare disease like AD, there is always a risk of making a type 2 error because the sample size is small. Of course, we would have preferred a higher sample number in this trial to reduce the risk of statistical errors.

Anyway, the open-labelled study design is most likely a more important limitation for the interpretation of the HRQoL and sleep outcomes than the p-values. Firm conclusions cannot be reached without blinding.

8. CONCLUSIONS

- We have successfully developed the disease-specific quality-of-life questionnaire AddiQoL to improve evaluation of HRQoL in AD patients both in clinical trials and in clinical practice.
- The original 36-item AddiQoL UK was translated to five other European languages and further validated resulting in a revised 30-item version, tested on 615 European AD patients; AddiQoL proved valid and reliable, with a good targeting to AD patients.
- AddiQoL demonstrated a superior ability to detected HRQoL improvement when compared with the generic questionnaires SF-36 and PGWB, but larger studies testing the AddiQoL's responsiveness are needed.
- CSHI reinstated the circadian cortisol rhythm, resulting in near normal ACTH levels in sharp contrast to the typical daytime cortisol peaks and troughs and elevated morning ACTH seen on OHC treatment.
- Whereas OHC yielded major alterations in the pattern of urinary GC metabolites and estimated metabolic enzyme activities, CSHI restored GC metabolism closer to normal; urinary excretion of cortisol and cortisone was high with CSHI, indicating a higher bioavailability for parenteral administration.
- HRQoL improved with CSHI, whereas no HRQoL benefit was seen with OHC, despite individually adjusted doses and a reduction of the daily hydrocortisone doses.
- CSHI did not alter sleep when compared to OHC.
- Restoration of the nighttime cortisol surge with CSHI prevented the gradual nighttime decline in glucose levels seen with OHC, without compromising insulin sensitivity.

9. ERRATUM

10. Literature

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