Effect of once-daily, modified-release hydrocortisone versus standard glucocorticoid therapy on metabolism and innate immunity in patients with adrenal insufficiency (DREAM): a single-blind, randomised controlled trial

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Summary

Background Conventional treatment of patients with adrenal insufficiency involves administration of glucocorticoids multiple times a day and has been associated with weight gain and metabolic impairment. The optimal glucocorticoid replacement therapy for these patients is highly debated because of the scarcity of evidence from randomised trials. We aimed to establish whether the timing and pharmacokinetics of glucocorticoid replacement therapy affect the metabolism and immune system of patients with adrenal insufficiency.

Methods We did a single-blind randomised controlled trial at two reference university hospitals in Italy. Eligible patients (aged 18–80 years) with adrenal insufficiency were on conventional glucocorticoid therapy and had been stable for at least 3 months before enrolment. Patients were randomly assigned (1:1) with a computer-generated random sequence stratified by type of adrenal insufficiency and BMI to continue conventional glucocorticoid therapy (standard treatment group) or to switch to an equivalent dose of once-daily, modified-release oral hydrocortisone (switch treatment group). Outcome assessors were masked to treatment allocation. The primary outcome was bodyweight change from baseline to 24 weeks. Secondary outcomes included immune cell profiles, susceptibility to infections, and quality of life. Efficacy analyses included all patients who received at least one dose of the study drug. This trial is registered with ClinicalTrials.gov, NCT02277587.

Findings Between March 1, 2014, and June 30, 2016, 89 patients with adrenal insufficiency were randomly assigned to continue standard glucocorticoid therapy (n=43) or to switch to once-daily, modified-release hydrocortisone (n=46). At 24 weeks, bodyweight reduction was superior in patients in the once-daily hydrocortisone group compared with those in the standard treatment group (–2·1 kg [95% CI –4·0 to –0·3] *vs* 1·9 kg [–0·1 to 3·9]; treatment difference –4·0 kg, 95% CI –6·9 to –1·1; p=0·008). Additionally, patients in the once-daily hydrocortisone group had more normal immune cell profiles, reduced susceptibility to infections, and improved quality of life compared with the standard glucocorticoid therapy group. We observed no difference in frequency or severity of adverse events between the two intervention groups, although a lower cumulative number of recurrent upper respiratory tract infections was observed with once-daily hydrocortisone than with standard treatment (17 *vs* 38; p=0·016). Most adverse events were mild; three serious adverse events occurred in each group, of which one adverse advent (arthritis) in the switch treatment group could be considered drug related.

Interpretation Patients with adrenal insufficiency on conventional glucocorticoid replacement therapy multiple times a day exhibit a pro-inflammatory state and weakened immune defence. Restoration of a more physiological circadian glucocorticoid rhythm by switching to a once-daily, modified-release regimen reduces bodyweight, normalises the immune cell profile, reduces recurrent infections, and improves the quality of life of patients with adrenal insufficiency.

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Introduction

Adrenal insufficiency, caused by adrenal failure (primary) or hypothalamo-pituitary failure (secondary), is a public health problem with a prevalence of 250–450 per 1 million population.[1] Adrenal insufficiency is potentially life-threatening and requires lifelong glucocorticoid replacement therapy. The incidence of adrenal crisis is increasing worldwide, at an estimated 5–9% per year among patients with primary adrenal insufficiency and 3–6% per year among patients with secondary adrenal insufficiency.[2] Adrenal crisis has a 1% mortality rate.[2] Management of adrenal insufficiency involves balancing the need to increase the usual glucocorticoid dose during stress to prevent adrenal crisis with the need to reduce overexposure during uneventful daily life. Dose adjustment is the main strategy to reduce the complications of glucocorticoid replacement therapy, whereas altering the timing of glucocorticoid administration remains largely

Research in context

Evidence before the study

We searched PubMed, ScienceDirect, and the Cochrane Library for articles published between Jan 1, 1965, and Jan 30, 2014, using various combinations of the search terms "adrenal insufficiency", "Addison's disease", "therapy", "glucocorticoid", "treatment", "metabolism", "body weight", "glycaemia", "lipid", "immunity", "immune system", "immunology", "immune phenotype", "infection", "infectious diseases" and "quality of life". We found that, although the negative effect of overtreatment with glucocorticoids on bodyweight, glucose, and lipids has repeatedly been shown, few trials have investigated the effect of once-daily, modified-release hydrocortisone on metabolism as the primary outcome. Additionally, although the role of the immune system in the pathogenesis of Addison's disease has been extensively investigated, the effect of lifelong glucocorticoid replacement therapy on the immune function of patients with adrenal insufficiency has not been addressed.

In June, 2017, after completing the study, we did another search of the databases up to June 30, 2017. No randomised controlled trials analysing the effect of once-daily, modified-release hydrocortisone on metabolic outcomes in patients with adrenal insufficiency were found, although two non-randomised studies had shown a metabolic advantage with shifting from conventional glucocorticoid to a once-daily regimen. An observational study in March, 2017, reported impaired function of natural killer cells in patients with primary adrenal insufficiency treated with conventional therapy multiple times a day, suggesting a predisposition to infections in these patients.

Added value of this study

This study is the first non-sponsored, randomised controlled trial to investigate the effect of once-daily, modified-release hydrocortisone versus standard therapy on the primary outcome of metabolism in both patients with primary and secondary adrenal insufficiency. We showed that patients with adrenal insufficiency on standard therapy had impaired cell immunity and increased frequency of infections, probably due to disruption of the circadian rhythm of cortisol by use of a regimen of multiple daily doses of glucocorticoids. We also showed that two of the most deleterious effects of glucocorticoid overtreatment—weight gain and increased HbA_{1c}—can be prevented by changing the timing of administration without altering the dose. Additionally, switching from multiple-daily to once-daily glucocorticoid administration restored immune-cell homoeostasis and reduced the frequency of infections. We have provided a molecular mechanism for the observed findings: the presence of low-grade inflammation leads to selective depletion of the immunoglobulin receptor, CD16, from the surface of natural killer cells. Such low-grade inflammation could also account for some of the unexplained symptoms reported by patients with adrenal insufficiency (eg, fatigue, impaired quality of life) and the increased risk of atherosclerosis in these patients in the absence of the usual predisposing factors.

Implications of all available evidence

Glucocorticoids are not just immunosuppressive, but could increase inflammation if given without consideration of the circadian rhythm. Because prevention of the complications of glucocorticoids cannot be achieved by simply reducing the dose, new effective approaches are needed. Changing the timing of glucocorticoid administration can normalise the immune profiles of patients with adrenal insufficiency. In the absence of objective indicators to assess the appropriateness of glucocorticoid replacement therapy, our data support the use of leucocyte profiling as a novel tool to monitor adrenal insufficiency. Since the observed immune changes were correlated with a reduced frequency of infections, once-daily, modified-release hydrocortisone might help prevent life-threatening adrenal crises in patients prone to infections. Further research is needed in patients who are adrenally sufficient and receiving steroids for various reasons (ie, rheumatoid, neoplastic, or autoimmune disorders) to confirm whether more careful timing of glucocorticoid administration could reduce the detrimental metabolic effects.

unexplored. Dose reduction has been advocated to counteract two of the most deleterious effects of glucocorticoid overtreatment: weight gain and metabolic impairment. However, most epidemiological studies[3–6] have not shown an association between total daily glucocorticoid dose and metabolic status. Administration of multiple doses of glucocorticoids a day has been associated with bone loss and reduced quality of life,[7,8] probably due to exposure to higher concentrations of glucocorticoids in the evening than is normal.[9]

Modified-release preparations have been developed to mimic the physiological cortisol rhythm and improve compliance.[10,11] The once-daily, modified-release hydrocortisone tablet was developed to prevent the afternoon peaks seen with conventional therapies given in multiple daily doses. Use of once-daily, modified-release hydrocortisone has been shown to improve cardiovascular risk factors, glucose metabolism, and quality of life in controlled trials.[12–14] However, the mechanism involved remains unexplained.

The immune system, which is highly sensitive to glucocorticoids, has seldom been investigated in patients with adrenal insufficiency receiving lifelong glucocorticoid replacement therapy. The immunosuppressive effects of high doses of glucocorticoids are known. However, the subtle long-term changes occurring with multiple daily doses of low amounts of glucocorticoids are largely unknown.

Glucocorticoids are endogenous synchronisers of the circadian rhythm, including that of blood cells. The effects of multiple daily peaks of glucocorticoid, as occurring in patients with adrenal insufficiency receiving standard therapy, had not been assessed until 2017.[15] No previous study has investigated changes to the immune system in patients with primary or secondary adrenal insufficiency switching from multiple doses a day to a once-daily regimen. Additionally, no previous study has assessed bodyweight change as the primary endpoint, despite adiposity being considered sensitive to glucocorticoid treatment and some trials showing changes in bodyweight associated with different steroid-delivery regimens. Bodyweight has also been shown to be less dependent than HbA_{1c} on the prevalence of diabetes in the study population.[11,12]

Therefore, we aimed to investigate how bodyweight, metabolic parameters, and immune cell profiles of patients with adrenal insufficiency are affected by the circadian rhythm of glucocorticoid administration.

Methods

Study design and participants

We did this 24 week, randomised, active comparator, non-intervention, parallel-group, controlled, clinical trial at two reference university hospitals in Italy (one in Rome and the other in Naples). Eligible patients were aged 18–80 years, had primary or secondary adrenal insufficiency, were taking conventional glucocorticoid therapy (hydrocortisone or cortisone two or three times a day plus daily doses of fludrocortisone as needed), had been stable for at least 3 months before enrolment, and were willing to change their regimen according to random allocation. An age-matched and sex-matched parallel group of healthy volunteers (non-intervention control group) who were adrenally sufficient—recruited in a 1:4 ratio to patients with adrenal insufficiency—was followed during the same period. Exclusion criteria are listed in the appendix.

This study was approved by the ethics review board at Sapienza University and done in accordance with the Declaration of Helsinki and Good Clinical Practice. All patients provided written informed consent before enrolment.

Randomisation and masking

All data were anonymised using a four-digit code. Patients with adrenal insufficiency were randomly assigned (1:1) to continue multiple daily doses of conventional glucocorticoids (standard treatment group) or to switch to once-daily, modified-release hydrocortisone tablet (switch treatment group) with a computer-generated random sequence. Randomisation was stratified by adrenal insufficiency type (primary *vs* secondary) and BMI (<26·5 kg/m² *vs* 26·5–30·0 kg/m² *vs* >30·0 kg/m²). Outcome assessors (independent) were masked to treatment allocation, and the success of masking was tested by questioning. Patients were not masked to treatment allocation.

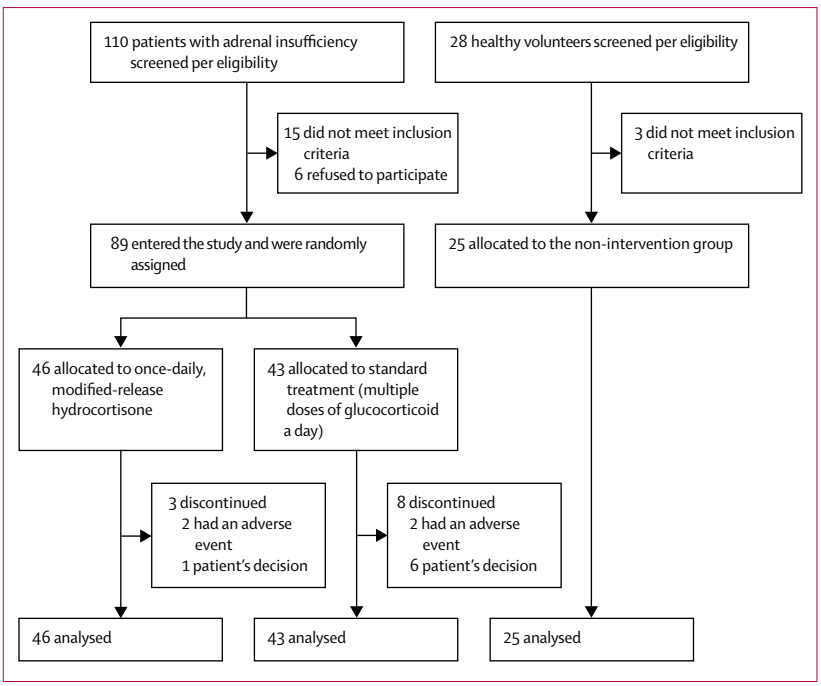

Figure 1: **Trial profile**

Procedures

Patients assigned to continue standard therapy were instructed to take the first dose on waking before leaving their bed and subsequent doses according to their established schedule (two or three times a day), but with the last dose no later than 1700 h. For all participants, blood samples were collected in the morning (between 0800 h and 0900 h) by research nurses after overnight fasting; participants had to take their usual morning dose 2 h before blood sampling. Patients allocated to once-daily, modified-release hydrocortisone were instructed to take the dose on waking, before leaving their bed. Patients previously on multiple doses of hydrocortisone a day received the same total daily dose, whereas patients previously on cortisone received 0·8 mg of hydrocortisone per 1 mg of cortisone, as recommended by the European Medicines Agency drug fact sheet.[16] Intermediate doses were rounded up to the nearest 5 mg (eg, 22·5 mg to 25·0 mg) to avoid any potential dangerous reduction in total daily dose. No change was allowed in any other medication or in glucocorticoid dose or timing, except for in cases of intercurrent illnesses requiring upscaling of the dose (involved administration of an immediate-release hydrocortisone tablet in both groups), and only after consultation with the study team. Patients and controls were interviewed about their dietary habits and all were on a standard Mediterranean diet. General nutritional recommendations were provided.

See Online for appendix

All investigations were done by a central laboratory (Sapienza University). Peripheral blood mononuclear cells (PBMCs) were isolated from fresh whole blood and gated (appendix). Briefly, the monocyte and lymphocyte gate was analysed for CD14 and CD16 expression to identify CD14+CD16– monocytes, CD14+CD16+ monocytes, and CD16+CD14– cells. Absolute cell counts were derived from the total cell counts provided by the haematological analyser (SYSMEX Roche, Indianapolis, IN, USA). The lymphocyte gate was also analysed for CD3 and CD56 expression to identify CD56+CD3– natural killer cells, CD56–CD3+ T lymphocytes, and CD3+CD56+ cells. Natural killer cells were defined as CD14–CD19–CD3–CD56+ (appendix) and re-analysed for CD16 expression to identify CD16+ natural killer cells. Natural killer cells were further divided into two subsets on the basis of CD56 density: CD56dim cells and CD56bright cells. B lymphocytes were quantified by analysis of CD19 expression in the lymphocyte gate.

The Human Fc Fragment of IgG Low Affinity IIIa Receptor ELISA Kit (MyBioSource, San Diego, CA, USA) was used to estimate the amount of soluble CD16 in the serum. The Human TACE ELISA Kit (ADAM17; Abcam, Cambridge, MA, USA) was used to estimate the amount of disintegrin and metalloproteinase domain-containing protein 17 (ADAM17) in the serum.

	Controls (n=25)	Adrenally insufficient (n=89)	
		Switch group (n=46)	Standard treatment group (n=43)
Age (years)	42 (35–49)	48 (43–52)	49 (44–54)
Sex			
Female	14 (56%)	25 (54%)	22 (51%)
Male	11 (44%)	21 (46%)	21 (49%)
Type of adrenal insufficiency			
Primary	..	22 (48%)	22 (51%)
Secondary	..	24 (52%)	21 (49%)
Duration of adrenal insufficiency (months)*	..	42 (24–108)	48 (24–132)
Comorbidities			
Diabetes	0 (0%)	8 (17%)	7 (16%)
Other autoimmune disorders	3 (12%)	12 (26%)	12 (28%)
Pituitary tumour or surgery	0 (0%)	22 (48%)	20 (47%)
Other hypothalamic-pituitary failure	0 (0%)	2 (4%)	1 (2%)
Adrenalectomy	0 (0%)	2 (4%)	2 (5%)
Glucocorticoid replacement			
Hydrocortisone	..	20 (43%)	17 (40%)
Cortisone	..	26 (57%)	26 (60%)
Baseline hydrocortisone equivalent dose (mg/m^2 per day)†	..	16 (14–18)	18 (15–21)
BMI (kg/m^2)	23 (22–24)	27 (25–28)	26 (24–27)
Bodyweight (kg)	68 (64–72)	75 (69–81)	70 (63–76)
Waist circumference (cm)	90 (86–94)	90 (84–95)	87 (80–93)
Fasting blood glucose (mg/dL)	84 (78–90)	89 (80–98)	79 (74–84)
Insulin (μU/mL)	7 (5–8)	10 (8–12)	9 (7–12)
HOMA index	1·51 (1·12–1·90)	2·14 (1·65–2·63)	1·87 (1·34–2·40)
HbA_{1c} (%)	5·2% (4·9–5·4)	5·5% (5·2–5·8)	5·6% (5·3–5·9)
HbA_{1c} (mmol/mol)	33 (28–37)	36 (33–39)	38 (34–41)
Total cholesterol (mg/dL)	184 (169–199)	215 (202–229)	205 (190–221)
HDL cholesterol (mg/dL)	52 (48–55)	64 (56–71)	69 (60–78)
LDL cholesterol (mg/dL)	120 (104–135)	128 (118–139)	118 (107–130)
Triglycerides (mg/dL)	82 (68–96)	138 (103–173)	124 (90–158)
Red blood cell count (×10^{12}/L)	5·01 (4·86–5·17)	4·91 (4·70–5·12)	4·68 (4·45–4·92)
Haemoglobin (g/dL)	14·4 (13·9–15·0)	14·0 (13·5–14·5)	13·6 (13·0–14·3)
Haematocrit (%)	45% (44–46)	43% (42–45)	42% (40–44)
White blood cell count (×10^3/μL)	6·13 (5·69–6·56)	7·34 (6·59–8·08)	7·85 (6·94–8·76)
Neutrophils (%)	58% (55–61)	55% (51–58)	55% (52–58)
Lymphocytes (%)	30% (28–32)	35% (31–38)	33% (31–36)
Monocytes (%)	6·6% (5·8–7·4)	6·5% (5·9–7·2)	6·5% (6·0–7·1)
Eosinophils (%)	2·8% (1·7–3·9)	2·4% (1·9–2·9)	2·3% (1·9–2·7)
Basophils (%)	0·5% (0·4–0·7)	0·5% (0·4–0·6)	0·6% (0·5–0·7)

(Table 1 continues on next page)

Outcomes

The primary efficacy outcome was bodyweight change from baseline to 24 weeks. Secondary outcomes were changes from baseline to 12 weeks and 24 weeks in metabolic profile (assessed by measurement of fasting blood glucose, insulin, HbA_{1c}, BMI, waist circumference, and serum lipids and use of the homoeostatic model assessment [HOMA] index); immune profile (assessed by immunophenotyping of PBMCs and measurement of concentrations of soluble CD16 and ADAM17, full blood cell count, C-reactive protein, erythrocyte sedimentation rate, fibrinogen, and immunoglobulin; rate, duration, and severity of infections (assessed with an adaptation of the German National Cohort Questionnaire;[17–19] appendix); and quality of life (assessed with the updated Addison's disease-specific quality-of-life [AddiQoL] questionnaire).[20] Other prespecified secondary outcomes were bone metabolism, bone mineral density, hepatic steatosis, epicardial fat thickness, and expression of clock genes in PBMCs; these will be reported in a future publication.

Safety was monitored throughout the study and included recording of adverse events, adrenal crises, syncope, fainting, dizziness, hospital admissions, requests for medical assistance, laboratory analyses, and vital signs. Adverse events were classified according to Common Terminology Criteria for Adverse Events version 4.0. An independent local data monitoring committee monitored adverse events, routine blood biochemistry, and full blood counts on data sheets. The local data monitoring committee had no involvement in the planning or reporting of this trial.

Statistical analysis

Assuming a standard deviation of 1·3 for bodyweight change—on the basis of published data[12]—and 1·0 kg as the minimal clinically relevant difference between treatments, we estimated that a sample size of 72 would

be needed to provide 90% power to detect a 1·0 kg difference in the primary outcome, with a two-sided significance level of 0·05. In establishing the sample size, we also assumed that 20% of patients would drop out of the study. Change from baseline was calculated as the value at 12 weeks or 24 weeks minus the baseline value.

Efficacy analyses included data from all patients who had received at least one dose of study drug. We assessed normality of distribution for all interventions at all timepoints using the Shapiro-Wilk's test (p>0·05). We used log transformation or reciprocal transformation to correct for skewed data and a mixed-model analysis to assess changes in outcomes with accommodation for repeated measurements. In the mixed-model analysis, the patient was a random effect and treatment, time, and treatment-by-time interaction were fixed effects. We analysed the differences in change from baseline to week 12 and week 24 between the groups using an ANCOVA model that included baseline outcome as a covariate and treatment as a fixed effect and used the last-observation-carried-forward principle. Other covariates were sex, BMI, age, smoking, type and duration of adrenal insufficiency, diabetes, and white blood cell count. Standardised residuals were tested for normality with Shapiro-Wilk's test. Homoscedasticity and homogeneity of variances were assessed by visual inspection and with Levene's test. Multicollinearity was assessed by calculation of the variance inflation factor. We calculated least-squares mean estimates with 95% CIs of treatment differences between the groups using Bonferroni correction. Subgroup analysis was done to report the significance of treatment-by-subgroup interaction. A two-sided p value of less than 0·05 was considered significant.

Discrete secondary endpoints were analysed using χ^2 analysis or Fisher's exact test in cases of few events. Odds ratios (ORs) and 95% CIs were calculated with a logistic regression model. We estimated correlations between circulating protein concentrations and blood-cell subtypes using Pearson correlation. Statistical analyses were done with SPSS version 20.0.

This study is registered with ClinicalTrials.gov, NCT02277587.

	Controls (n=25)	Adrenally insufficient	
		Switch group (n=46)	Standard treatment group (n=43)
(Continued from previous page)			
Immune cells			
CD14+CD16– monocytes (cells per μL)	540 (478–601)	1102 (979–1225)	993 (811–1175)
CD16+CD14+ monocytes (cells per μL)	82 (69–95)	111 (96–126)	113 (87–139)
CD16+CD14–cells (cells per μL)	250 (213–288)	136 (109–163)	147 (108–186)
CD3–CD56+ natural killer cells (cells per μL)	243 (214–273)	179 (143–214)	173 (143–203)
CD56bright natural killer cells (%)	7·1% (5·9–8·2)	11·4% (8·7–14·2)	14·0% (9·9–18·2)
CD56dim natural killer cells (%)	93% (92–94)	89% (86–91)	86% (82–90)
CD16+ natural killer cells (cells per μL)	228 (201–254)	130 (96–164)	127 (99–155)
CD56–CD3+T lymphocytes (cells per μL)	1212 (1122–1302)	1411 (1243–1579)	1647 (1437–1857)
CD19+ B lymphocytes (cells per μL)	199 (179–219)	248 (193–303)	308 (247–369)
Infections			
Total score (IN1–IN5)	4·1 (3·6–4·8)	6·9 (6·1–7·6)	7·2 (6·3–8·1)
Flu or flu-like events in 6 months	0·5 (0·0–1·0)	1·8 (1·3–2·2)	1·8 (1·3–2·4)
AddiQoL score	..	82 (78–86)	83 (76–89)
Medications			
Fludrocortisone	..	21 (46%)	20 (47%)
Thyroid hormones	..	20 (43%)	16 (37%)
Sex steroids	..	10 (22%)	6 (14%)
Vitamin D	..	6 (13%)	8 (19%)
Lipid-lowering drugs	..	13 (28%)	9 (21%)
Anti-hypertensives	..	5 (11%)	4 (9%)
Antidiabetics	..	5 (11%)	4 (9%)
Insulin	..	3 (7%)	3 (7%)
Cabergoline	..	5 (11%)	4 (9%)
Somatostatin analogues	..	2 (4%)	1 (2%)
Antacids	..	2 (4%)	4 (9%)
Aspirin	..	1 (2%)	0 (0%)
Growth hormone	..	1 (2%)	1 (2%)
Antidepressants	..	1 (2%)	1 (2%)

Data are mean (95% CI) or n (%) unless otherwise specified. HOMA=homoeostatic model assessment. AddiQoL=Addison's disease-specific quality of life. *Data are median (IQR). †Adjusted for body surface area.

Table 1: **Baseline characteristics**

Role of the funding source

The funder of the study had no role in the study design, data collection, data analysis, data interpretation, writing of the report, or in the decision to submit for publication. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

From March 1, 2014, to June 30, 2016, 138 individuals were screened for eligibility, including 110 patients with adrenal insufficiency and 28 healthy volunteers (figure 1). Overall, 89 patients with adrenal insufficiency were randomly assigned to the standard treatment group (n=43) or to the switch treatment group (n=46). All 89 patients were included in the primary analysis. 25 healthy volunteers were assigned to the non-intervention control group. 11 (12%) of 89 patients with adrenal insufficiency discontinued the study, eight of whom were receiving standard treatment. Reasons for dropout were patient's decision (n=7) and adverse events (n=4), including arthritis (n=1), presence of a mammary nodule requiring biopsy (n=1), and worsening of chronic kidney disease (n=2).

Baseline characteristics were similar for the two intervention groups, whereas the healthy volunteers in the control population had lower BMIs and lipid concentrations at baseline than did patients with adrenal

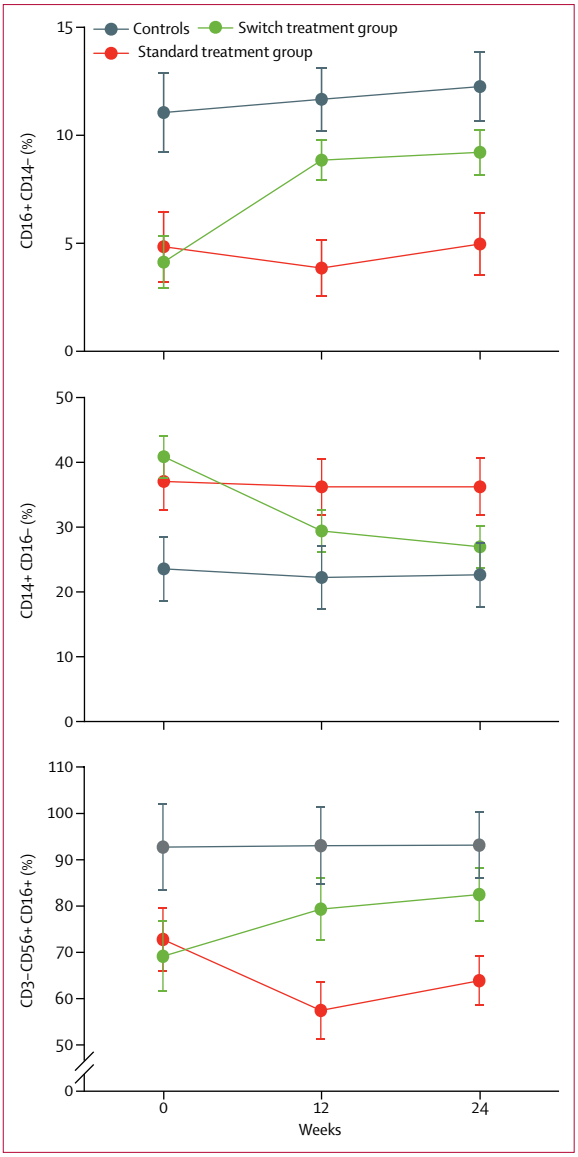

Figure 2: **Counts of circulating monocytes and natural killer cells over time**
Data are estimated marginal means and 95% CIs. Cell counts were measured at baseline (n=114), 12 weeks (n=108), and 24 weeks (n=103). Estimates were adjusted for age, sex, BMI, type of adrenal insufficiency, diabetes, and smoking.

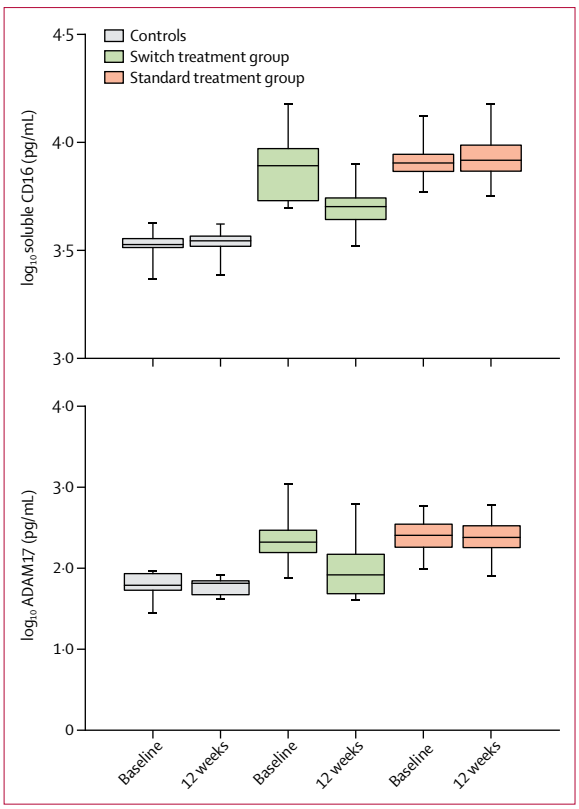

Figure 3: **Concentrations of soluble CD16 and ADAM17**
Circulating concentrations of soluble CD16 and ADAM17 were measured at baseline (n=114) and 12 weeks (n=108). The bottom of the boxplot indicates the 25th percentile and the top the 75th percentile. Horizontal lines in the boxes are medians. The lower end of the whiskers represents the minimum observation and the upper end the maximum. Treatment differences were derived from a mixed-effects analysis of covariance after adjusting for age, sex, BMI, type of adrenal insufficiency, diabetes, and smoking. ADAM17=disintegrin and metalloproteinase domain-containing 17.

insufficiency (table 1). Patients with adrenal insufficiency had similar full blood counts to healthy volunteers, but higher T lymphocyte (CD3+) and B lymphocyte (CD19+) counts, higher numbers of classic pro-inflammatory monocytes (CD14+CD16–), and lower numbers of CD16+CD14– cells and CD16+ natural killer cells at baseline (figure 2; appendix). Additionally, patients with adrenal insufficiency had higher concentrations of soluble CD16 and ADAM17 at baseline than did healthy volunteers (figure 3).

Of the 89 patients with adrenal insufficiency, 44 had primary adrenal insufficiency and 45 had secondary adrenal insufficiency. The cause of most cases of secondary adrenal insufficiency was post-surgical hypopituitarism for non-functioning pituitary macroadenomas. The total daily dose of glucocorticoids was well balanced between the intervention groups at randomisation and was not different between the groups at study end (appendix).

The mean bodyweight at 24 weeks was 72 kg (95% CI 67 to 78) in the switch treatment group and 71 kg (62 to 79) in the standard treatment group (table 2), which was a mean change from baseline of –2·1 kg (95% CI –4·0 to –0·3) in the switch treatment group and 1·9 kg (–0·1 to 3·9) in the standard treatment group (table 3). With adjustment for covariates, the estimated difference in mean bodyweight change between the two interventions at week 24 was –4·0 kg (–6·9 to –1·1; p=0·008; table 3; appendix). Bodyweight reduction had a significant treatment-by-time interaction (p=0·001) in the switch treatment group, starting from week 12 and improving further at week 24, but not in the standard treatment group.

The estimated treatment difference between the intervention groups at week 24 was –1·7 kg/m² (95% CI

	Switch treatment group		Standard treatment group	
	12 weeks (n=43)	24 weeks (n=43)	12 weeks (n=40)	24 weeks (n=35)
Anthropometrics*				
BMI (kg/m^2)	26·1 (24·4 to 27·8)	25·3 (23·7 to 26·9)	25·7 (23·8 to 27·6)	26·1 (23·5 to 28·7)
Bodyweight (kg)	74 (69 to 80)	72 (67 to 78)	70 (64 to 77)	71 (62 to 79)
Waist circumference (cm)	88 (83 to 94)	87 (81 to 92)	88 (81 to 95)	88 (80 to 96)
Glucose metabolism†				
HbA_{1c} (%)	5·3% (5·1 to 5·6)	5·3% (5·0 to 5·5)	5·5% (5·2 to 5·9)	5·7% (5·3 to 6·0)
HbA_{1c} (mmol/mol)	35 (34 to 36)	34 (33 to 36)	37 (35 to 38)	38 (37 to 39)
Fasting blood glucose (mg/dL)	97 (85 to 108)	96 (85 to 106)	82 (77 to 88)	84 (75 to 93)
Insulin (μIU/mL)	10 (8 to 12)	10 (8 to 13)	9 (7 to 11)	9 (6 to 13)
HOMA index	2·3 (1·8 to 2·8)	2·4 (1·6 to 3·2)	1·9 (1·4 to 2·4)	2·0 (1·2 to 2·8)
Lipids†				
Total cholesterol (mg/dL)	205 (190 to 219)	210 (196 to 225)	212 (194 to 229)	209 (192 to 226)
HDL cholesterol (mg/dL)	58 (51 to 65)	55 (50 to 60)	71 (61 to 82)	68 (60 to 76)
LDL cholesterol (mg/dL)	123 (115 to 130)	129 (120 to 138)	126 (117 to 135)	122 (111 to 133)
Triglycerides (mg/dL)	126 (90 to 162)	115 (96 to 134)	127 (89 to 165)	120 (90 to 150)
Immune cells†				
Lymphocytes and monocytes (cells per μL)	2491 (2289 to 2692)	2539 (2337 to 2742)	2947 (2638 to 3256)	3288 (2859 to 3716)
CD14+CD16– monocytes (cells per μL)	715 (605 to 825)	681 (603 to 759)	1086 (910 to 1262)	1106 (867 to 1345)
CD16+CD14+ monocytes (cells per μL)	96 (82 to 110)	105 (83 to 126)	115 (92 to 138)	121 (92 to 150)
CD16+CD14– cells (cells per μL)	223 (189 to 257)	239 (206 to 272)	129 (104 to 154)	146 (106 to 186)
CD3–CD56+ natural killer cells (cells per μL)	182 (152 to 212)	216 (186 to 245)	188 (159 to 217)	177 (142 to 212)
CD56bright natural killer cells (%)	9·4% (7·2 to 11·5)	8·7% (7·6 to 9·8)	11·3% (9·2 to 13·5)	13·7% (10·6 to 16·7)
CD56dim natural killer cells (%)	91% (89 to 93)	91% (90 to 92)	89% (87 to 91)	86% (83 to 89)
CD16+ natural killer cells (cells per μL)	147 (118 to 176)	170 (141 to 199)	105 (83 to 127)	117 (88 to 146)
CD3+ T lymphocytes (cells per μL)	1311 (1152 to 1470)	1344 (1229 to 1459)	1643 (1462 to 1824)	1744 (1493 to 1995)
CD19+ B lymphocytes (cells per μL)	246 (201 to 291)	234 (196 to 272)	292 (227 to 357)	300 (223 to 378)
Infections†				
Total score (IN1–IN5)	..	5·6 (4·8 to 6·3)	..	7·6 (6·7 to 8·4)
Flu or flu-like events in 6 months	..	0·6 (0·3 to 0·9)	..	1·4 (0·8 to 2·0)
AddiQoL†				
Total score	..	88 (85 to 92)	..	84 (78 to 91)

Data are mean (95% CI). HOMA=homoeostatic model assessment. AddiQoL=Addison's disease-specific quality of life. *Adjusted for age, sex, type of adrenal insufficiency, diabetes mellitus, smoking, and outcome at baseline. †Adjusted for age, sex, BMI, type of adrenal insufficiency, diabetes, smoking, and outcome at baseline.

Table 2: **Characteristics at 12 weeks' and 24 weeks' follow-up**

–3·0 to –0·5; p=0·008) for BMI and –2·5 cm (95% CI –4·3 to –0·5; p=0·016) for waist circumference (table 3). The estimated treatment difference between the groups for HbA_{1c} was –0·3% (95% CI –0·5 to –0·1; p=0·001) at week 24; no significant change was seen in fasting blood glucose, insulin, or HOMA index among patients without diabetes. LDL cholesterol and triglycerides were unaffected by treatment, whereas HDL cholesterol concentration was decreased in the switch treatment group compared with the standard treatment group (table 3). The estimated treatment difference between the intervention groups for HDL cholesterol at week 24 was –9 mg/dL (95% CI –15 to –3; p=0·002). In three (7%) of 46 patients allocated to the switch treatment group, HDL cholesterol concentrations were decreased from normal at baseline to less than 35 mg/dL after treatment; no such decrease was seen in the standard treatment group.

Subgroup analysis for type of adrenal insufficiency revealed no treatment-by-subgroup interaction for waist circumference (p=0·508), HbA_{1c} (p=0·328), fasting blood glucose (p=0·722), insulin (p=0·304), HOMA index (p=0·280), total cholesterol (p=0·957), HDL cholesterol (p=0·905), and triglycerides (p=0·509). A borderline interaction was observed for BMI (p=0·081) and bodyweight (p=0·074), with a tendency toward greater reduction in patients with secondary adrenal insufficiency.

Numbers of classic pro-inflammatory monocytes (CD14+CD16–) were reduced at 12 weeks and 24 weeks in the switch treatment group, with the baseline count almost reduced by 50% at week 24 (table 3 and figure 2). The estimated treatment difference between the groups for CD14+CD16– monocytes was –351 cells per μL (95% CI –543 to –159; p=0·00054) from baseline to week 12 and –481 cells per μL (–701 to –261; p<0·0001)

	Switch treatment group (n=43)		Standard treatment group (n=40)		Treatment-related difference	
	12 weeks (n=43)	24 weeks (n=43)	12 weeks (n=40)	24 weeks (n=35)	Baseline to 12 weeks	Baseline to 24 weeks
Anthropometrics*						
BMI (kg/m²)	−0·6 (−1·2 to 0·0)	−0·9 (−1·7 to −0·1)	0·2 (−0·1 to 0·4)	0·7 (−0·1 to 1·5)	−0·7 (−1·4 to 0·0; p=0·060)	−1·7 (−3·0 to −0·5; p=0·008)
Bodyweight (kg)	−1·3 (−2·5 to 0·0)	−2·1 (−4·0 to −0·3)	0·5 (−0·2 to 1·2)	1·9 (−0·1 to 3·9)	−1·5 (−3·0 to 0·0; p=0·047)	−4·0 (−6·9 to −1·1; p=0·008)
Waist circumference (cm)	−1·8 (−3·1 to −0·6)	−2·2 (−3·6 to −0·9)	0·0 (−0·7 to 0·8)	0·6 (−0·7 to 1·8)	−1·7 (−3·2 to −0·3; p=0·020)	−2·5 (−4·3 to −0·5; p=0·016)
Glucose metabolism†						
HbA_{1c} (%)	−0·1% (−0·2 to 0·0)	−0·2% (−0·3 to −0·1)	0·0% (−0·1 to 0·1)	0·1% (0·0 to 0·2)	−0·1% (−0·3 to 0·0; p=0·077)	−0·3% (−0·5 to −0·1; p=0·001)
HbA_{1c} (mmol/mol)	1·5 (−2·5 to −0·4)	−2·1 (−3·2 to −0·9)	0·0 (−1·2 to 1·3)	1·4 (0·0 to 2·8)	−1·5 (−3·1 to 0·2; p=0·077)	−3·5 (−5·3 to −1·6; p=0·001)
Fasting blood glucose (mg/dL)	9 (5 to 12)	7 (3 to 10)	3 (1 to 5)	5 (0 to 11)	5 (1 to 9; p=0·011)	3 (−2 to 9; p=0·239)
Insulin (μIU/mL)	0 (−2 to 1)	0 (−2 to 2)	0 (−2 to 1)	0 (−3 to 3)	0 (−2 to 2; p=0·928)	0 (−4 to 4; p=0·987)
HOMA index	0·1 (−0·2 to 0·4)	0·3 (−0·4 to 0·9)	0·0 (−0·2 to 0·3)	0·3 (−0·4 to 0·9)	0·2 (−0·2 to 0·5; p=0·402)	0·1 (−0·8 to 1·1; p=0·785)
Lipids†						
Total cholesterol (mg/dL)	−10 (−20 to −1)	−1 (−11 to 10)	5 (−3 to 12)	0 (−9 to 9)	−12 (−24 to 0; p=0·056)	0 (−16 to 15; p=0·962)
HDL cholesterol (mg/dL)	−5 (−8 to −1)	−7 (−13 to −2)	2 (−1 to 6)	0 (−5 to 6)	−8 (−13 to −2; p=0·006)	−9 (−15 to −3; p=0·002)
LDL cholesterol (mg/dL)	−1 (−9 to 7)	7 (−2 to 16)	2 (−7 to 10)	0 (−11 to 12)	−3 (−15 to 9; p=0·589)	7 (−8 to 21; p=0·364)
Triglycerides (mg/dL)	−12 (−38 to 13)	−21 (−49 to 7)	0 (−18 to 19)	−4 (−23 to 15)	−7 (−34 to 21; p=0·614)	−7 (−35 to 21; p=0·605)
Immune cells†						
Lymphocytes and monocytes (cells per μL)	−240 (−448 to −33)	−177 (−403 to 49)	48 (−196 to 291)	172 (−183 to 528)	−359 (−645 to −72; p=0·015)	−488 (−897 to −79; p=0·020)
CD14+CD16− monocytes (cells per μL)	−404 (−563 to −244)	−440 (−580 to −301)	83 (−160 to 327)	90 (−226 to 405)	−351 (−543 to −159; p=0·001)	−481 (−701 to −261; p<0·0001)
CD16+CD14+ monocytes (cells per μL)	−16 (−35 to 2)	−7 (−31 to 18)	7 (−23 to 38)	3 (−30 to 36)	−19 (−43 to 5; p=0·124)	−17 (−52 to 18; p=0·328)
CD16+CD14− cells (cells per μL)	100 (52 to 149)	119 (79 to 159)	−16 (−59 to 28)	0 (−48 to 48)	99 (57 to 142; p<0·0001)	87 (41 to 134; p=0·0004)
CD3−CD56+ natural killer cells (cells per μL)	6 (−39 to 50)	24 (−15 to 63)	14 (−7 to 35)	4 (−35 to 43)	−5 (−46 to 36; p=0·818)	30 (−23 to 83; p=0·258)
CD56bright natural killer cells (%)	−2·2% (−5·8 to 1·5)	−1·8% (−5·1 to 1·5)	−2·6% (−6·1 to 0·9)	−0·3% (−4·6 to 4·0)	−1·0% (−2·2 to 4·2; p=0·546)	−2·8% (−6·5 to 0·9; p=0·135)
CD56dim natural killer cells (%)	2·2% (−1·5 to 5·8)	1·8% (−1·5 to 5·0)	−2·6% (−0·9 to 6·1)	0·3% (−4·0 to 4·6)	1·0% (−4·2 to 2·2; p=0·546)	2·8% (−0·9 to 6·5; p=0·135)
CD16+ natural killer cells (cells per μL)	19 (−21 to 60)	32 (−2 to 66)	−18 (−47 to 10)	−9 (−42 to 23)	39 (4 to 73; p=0·030)	42 (3 to 81; p=0·037)
CD3+ T lymphocytes (cells per μL)	−85 (−263 to 93)	−11 (−198 to 175)	−1 (−161 to 159)	97 (−116 to 310)	−172 (−382 to 38; p=0·106)	−182 (−469 to 105; p=0·209)
CD19+ B lymphocytes (cells per μL)	0 (−40 to 39)	−17 (−60 to 26)	−18 (−69 to 34)	−8 (−67 to 52)	3 (−59 to 66; p=0·917)	−18 (−90 to 53; p=0·608)
Infections†						
Total score (IN1–IN5)	..	−1·3 (−2·1 to −0·5)	..	0·4 (−0·4 to 1·1)	..	−1·7 (−2·6 to −0·8; p=0·0002)
Flu or flu-like events in 6 months	..	−1·2 (−1·7 to −0·7)	..	−0·4 (−0·9 to 0·2)	..	−1·0 (−1·6 to −0·4; p=0·001)
AddiQoL†						
Total score	..	7 (4 to 10)	..	2 (−1 to 5)	..	5 (1 to 9; p=0·027)

Data are mean (95% CI). HOMA=homoeostatic model assessment. AddiQoL=Addison's disease-specific quality of life. *Adjusted for age, sex, type of adrenal insufficiency, diabetes mellitus, smoking, and outcome at baseline. †Adjusted for age, sex, BMI, type of adrenal insufficiency, diabetes, smoking, and outcome at baseline.

***Table 3*: Change between baseline and follow-up and treatment-related differences**

from baseline to week 24. Conversely, the number of CD16+CD14– cells increased from baseline to week 12 and week 24 in the switch treatment group but not in the standard treatment group, reaching a similar level to the control population (table 3 and figure 2). Although non-classic CD16+CD14+ monocyte counts were unaffected by treatment, the number of natural killer cells expressing CD16 increased from baseline to week 12 and week 24 in the switch treatment group (table 3 and figure 2). Subgroup analysis revealed no treatment-by-subgroup interaction for any of the immune outcomes CD14+CD16– (p=0·563), CD16+CD14+ (p=0·760), CD16+CD14– (p=0·368), CD3–CD56+ natural killer cells (p=0·547), CD16+ natural killer cells (p=0·554), total infections score (IN1–IN5; p=0·737), flu or flu-like events (p=0·156), and AddiQoL total score (p=0·121; appendix).

The dynamics of changes in circulating PBMCs are shown in the appendix. A lag of about 8 weeks was observed before the effect of the treatment switch became detectable, excluding acute cell redistribution.

Concentrations of soluble CD16 and ADAM17 normalised at 12 weeks in the switch treatment group (figure 3). The estimated treatment difference at 12 weeks was –0·26 ng/mL (95% CI –0·33 to –0·19; p<0·0001) for ADAM17 and –4·99 ng/mL (–6·21 to –3·74; p<0·0001) for soluble CD16.

The distribution of the total infection score, measuring the cumulative incidence of different infections over 6 months, is reported in the appendix. The number of mild infections reported by patients allocated to the switch treatment group after 24 weeks of treatment was only slightly higher than the number reported by healthy volunteers (table 2; appendix). Similarly, the frequency of flu or flu-like events decreased from baseline to 24 weeks in the switch treatment group (table 3). Post-hoc analyses showed significant associations between improvements in CD16+CD14– cell counts or infection scores and reductions in concentrations of soluble CD16 and ADAM17 (appendix). An increase in the AddiQoL score was observed at week 24 in the switch treatment group only, with an estimated treatment difference of 5 points (95% CI 1 to 9; p=0·027; table 3).

163 adverse events were recorded; most were mild (n=157), not requiring admission to hospital. No difference was found in the number of adverse events between the two treatment groups (table 4). A higher cumulative number of recurrent upper respiratory tract infections was found in the standard treatment group than in the switch treatment group (38 *vs* 17; p=0·016). Three moderate-to-severe adverse events occurred in the switch treatment group, including diagnosis of papillary thyroid cancer, detection of a mammary nodule, and arthritis, of which only arthritis could be considered drug related. Two serious adverse events (two cases of renal impairment) and one moderate-to-severe adverse event (urinary tract obstruction) occurred in the standard treatment group. These patients discontinued the study, but continued corticosteroid treatment.

	Switch treatment group	Standard treatment group	Odds ratio (95% CI)	p value
Mild adverse events				
Cardiovascular and general symptoms (fatigue, dizziness, fainting, vertigo, shortness of breath)	26 (16)	37 (20)	1·63 (0·69–3·82)	0·287
Respiratory tract symptoms (rhinopharyngitis, sore throat, sinusitis, cough, upper and lower respiratory tract)	23 (21)	46 (26)	1·82 (0·78–4·20)	0·204
Gastrointestinal symptoms (abdominal pain, diarrhoea, nausea)	8 (8)	17 (13)	2·06 (0·75–5·61)	0·213
Moderate-to-severe adverse events				
Papillary thyroid cancer (diagnosis)	1 (1)	0 (0)	..	..
Mammary nodule requiring biopsy (diagnosis)	1 (1)*	0 (0)	..	..
Prostatic hyperplasia with urinary tract obstruction	0 (0)	1 (1)	..	..
Arthritis	1 (1)*	0 (0)	..	..
Serious adverse events				
Acute or progressive renal impairment in patient with chronic kidney disease	0 (0)	2 (2)*	..	..

Numbers of patients affected are shown in parentheses. *Patients discontinued the study.

Table 4: **Number of adverse events**

Discussion

We did a head-to-head comparison of hydrocortisone or cortisone delivered two or three times a day and once-daily, modified-release hydrocortisone. The once-daily regimen improved bodyweight, waist circumference, and HbA_{1c} concentration. Compared with a control population, patients with adrenal insufficiency on conventional replacement therapy had unexpected abnormalities in circulating PBMCs, which were not evident on a routine full blood count. An increased number of classic monocytes and a reduced number of CD16+ natural killer cells were found at baseline in these patients, which reversed after switching from the multi-dose regimen to the once-daily regimen. Quality of life and frequency of infections also improved in patients who switched to once-daily administration. Our results suggest that some of the most detrimental effects of glucocorticoid therapy—ie, weight gain, impaired metabolism, and recurrent infections, which are all associated with low-grade inflammation—can be reversed by changing the circadian timing of glucocorticoid administration.

It is generally accepted that conventional treatment of adrenal insufficiency is far from ideal. Patients taking multiple, divided daily doses of glucocorticoids are advised to take the last dose no later than 4–6 h before bedtime.[3]

In reality, patients will often take hydrocortisone late in the evening. Experimental studies have shown that night-time exposure to glucocorticoids contributes to their detrimental effects on metabolism, independently of the cumulative 24 h dose.[9] In a large cohort of patients with adrenal insufficiency in France,[4] hydrocortisone doses marginally contributed to dyslipidaemia but not to glucose impairment. In a study in Germany,[6] no difference in BMI was seen between groups on higher or lower doses of hydrocortisone. In other real-life surveys,[4] no correlation was found between the total daily dose of hydrocortisone and fasting blood glucose or insulin. By contrast, modified-release hydrocortisone has been shown to slightly reduce bodyweight, BMI, and HbA_{1c},[10,14] which is consistent with our findings. Eve Van Cauter showed that hydrocortisone administered in the evening produced a more pronounced hyperglycaemic effect,[21] attributed to increased night-time sensitivity to glucocorticoids. In this trial, we observed a reduction in HbA_{1c}, a transient increase in morning fasting glucose, and no change in insulin resistance in the group receiving the once-daily regimen. These findings suggest an effect on liver metabolism, which functions under strict circadian control. The result is clinically relevant, because improved gluconeogenesis could help counteract night-time hypoglycaemia in patients with adrenal insufficiency and type 1 diabetes.

Early studies[22–24] described increased and premature mortality in patients with adrenal insufficiency, which was attributed to cardiovascular disease, neoplasms, and infections.[22,23] However, those studies were biased by their retrospective nature and higher glucocorticoid doses than recommended. More recent prospective data from patients with pituitary disease in Europe showed an increased standardised mortality ratio from infectious disease in patients who were adrenocorticotropic hormone insufficient compared with those who were adrenocorticotropic hormone sufficient.[24] A survey[25] in Australia found disproportionate use of hospital services by patients with adrenal insufficiency due to comorbidities[22,26] and predisposition to complications.[26,27]

Respiratory or gastrointestinal tract infections are the most common factors causing admission to hospital[2] in patients with adrenal insufficiency. However, the incidence of infectious disease not resulting in hospital admission remained unaddressed until Smans and colleagues[27] observed a four to five times increased use of antimicrobial drugs in patients with adrenal insufficiency compared with controls. The increased incidence of viral infections (up to 7·4%) and use of antifungal and antiviral drugs in these patients suggested that they had weakened innate immunity.[27] Using a validated questionnaire,[17–19] we observed more frequent reporting of events attributed to mild, recurrent infections—mainly of the upper respiratory and gastrointestinal tracts—in patients with adrenal insufficiency than in controls, consistent with impairment of adaptive cellular immunity. That respiratory tract infections in these patients are more common than previously thought[27] reflects the resizing of the number of cases of gastroenteritis because of awareness that nausea and vomiting could simply reflect hypoadrenalism.

The susceptibility of patients with adrenal insufficiency to infections has been attributed to the immunosuppressive effects of glucocorticoids, despite patients often being treated with low doses. Bancos and colleagues[15] reported that patients with adrenal insufficiency receiving hydrocortisone replacement therapy three times a day had selective impairment of natural killer cell cytotoxicity. Natural killer cells provide innate defence against virally infected and transformed cells, but also participate in immune regulation.[28,29]

By recognition of IgG-opsonised cells through the CD16 receptor, natural killer cells can activate antibody-dependent cell-mediated cytotoxicity and, hence, interface with adaptive immunity. We found significant depletion of CD16+ natural killer cells in patients with adrenal insufficiency, suggesting prolonged activation.[30] This pattern is similar to that observed in severely obese individuals with low-grade inflammation, who display hypo-responsive natural killer cells with downregulation of CD16.[31] Such low-grade inflammation could also account for some of the unexplained symptoms reported by patients with adrenal insufficiency (eg, fatigue, impaired quality of life) and their increased risk of atherosclerosis, even in the absence of visceral fat accumulation.[32] Thus, a dissociation between adiposity and the classic cardiovascular risk factors seems to exist in patients with adrenal insufficiency.

We found that patients with adrenal insufficiency on conventional therapy had an increased number of classic monocytes (CD14+CD16–). Switching from multiple to once-daily glucocorticoid administration reduced CD14+CD16– counts and restored counts of CD16+CD14– cells and CD16+ natural killer cells. The absence of CD16 impairs activation of antibody-dependent cell-mediated cytotoxicity by natural killer cells, thus favouring infections. To investigate whether the Fc receptor was lost or simply not expressed, we measured the soluble form of CD16. The soluble CD16 concentration was elevated in patients with adrenal insufficiency compared with controls and normalised after patients switched to the once-daily regimen. In support of Fc-receptor cleavage, we found elevated ADAM17 concentrations in these patients, which is the main metalloproteinase responsible for CD16 shedding from natural killer cells and whose concentration increases during inflammation. We established that CD16 shedding did not affect the overall cell number because the CD14+CD16+ monocyte count remained unaltered. Thus, we documented a pro-inflammatory state and a mechanistic explanation for CD16 cleavage from natural killer cells in patients with adrenal insufficiency treated with multiple doses of glucocorticoids a day, which reverted on switching to once-daily administration.

Shedding of CD16 from natural killer cells due to chronic inflammation has been associated with the so-called exhausted phenotype observed in patients with cancer.[33] Exhaustion of natural killer cells could explain the reported susceptibility of patients with adrenal insufficiency to neoplastic disorders.[10,15] Two of the patients in our study were diagnosed with cancer during or immediately after study completion.

The mechanism through which timing of glucocorticoid doses modifies the characteristics of circulating PBMCs is beyond the scope of this trial. However, our time-course of cell profiles, the reduction in intercurrent illnesses 3 months after switching to once-daily hydrocortisone observed in other trials,[34] and the fact that the total leucocyte count did not change over 6 months in our study all suggest that our findings do not only reflect cell recirculation. By contrast, the function of natural killer cells shows circadian rhythmicity. In humans, natural killer cells peak during early wakefulness, through an anticipatory mechanism designed to counteract pathogens when the risk of infection is highest.[35] During subsequent rest, when the risk is lessened, resolution of inflammation and tissue repair occur.[36] Glucocorticoids are crucial synchronisers of the central and peripheral clock genes, including those in immune cells.[9,35] An altered circadian rhythm has been linked to increased incidence of metabolic, inflammatory, and neoplastic diseases.[37] Desynchronisation of the clock can affect immune function, leading to an uncoordinated inflammatory response known as over-reaction.[36] Adrenal crisis and immune overreaction have similar features, suggesting that the crisis might develop not because glucocorticoid concentrations are low at the time of the precipitating factor, but because exposure to many preceding lows (or lows and highs) have hampered the defence or response mechanisms. Therefore, our findings could have broader implications outside adrenal insufficiency, for all patients taking steroids chronically.[36]

This trial confirms the hypothesis that the effects of cortisol are more immune modulatory than overall suppressive; whether glucocorticoids increase or decrease inflammation depends on the dose, timing, duration, and exposure type.[38] Previous studies in patients who were adrenally sufficient were limited by the feedback mechanism of the hypothalamic-pituitary-adrenal axis, requiring supra-physiological doses or adrenolytic drugs to block endogenous cortisol. Our recruitment of patients with adrenal insufficiency allowed us to show the impact of circadian administration of glucocorticoids without confounding effects. In the absence of objective indicators to assess the appropriateness of glucocorticoid replacement therapy, our data support the use of PBMC profiling as a novel approach to monitor adrenal insufficiency.

Most adrenal insufficiency guidelines have focused on reducing the overall glucocorticoid dose[3] rather than on the timing of administration. Our patients were on the standard replacement dose for cohorts in Italy,[39] which is slightly lower than that used in other countries,[40] reinforcing the hypothesis that they were not immune suppressed. The total daily glucocorticoid dose was similar between the groups and had no effect on the findings. Although time exposure in the evening might have been lower with the once-daily regimen than with the conventional regimen, to consider our findings the result of an overall dose effect would be too simplistic because concentrations of glucocorticoids in the evening, even with multiple dosing, are quite low. Additionally, previous attempts to improve therapy by only reducing the total daily intake have been unsuccessful, and our immune findings do not favour this hypothesis.

Mimicking a more circadian rhythm also improved quality of life compared with conventional regimens.[41] Hydrocortisone given in fractionated daily doses to recreate the curve of cortisol rhythm is associated with considerable variability in concentrations,[4] with a substantial proportion of patients being undertreated and overtreated in the same day.[4] Some previous studies[14] have found no change in AddiQoL scores in patients who have switched to once-daily, modified-release hydrocortisone, but a deterioration in patients who remained on conventional hydrocortisone. We found that quality of life, assessed with the most recent update of the AddiQoL questionnaire,[20] was improved with the once-daily regimen.

Transition to once-daily, modified-release hydrocortisone appeared to be safe, with no significant differences in adverse events between the treatment groups. However, this trial is the first to describe a reduction in HDL cholesterol concentration associated with switching to the once-daily regimen, which merits further investigation.

This study has several advantages, including the sample size, random allocation, masking of the assessor, duration, use of strict inclusion criteria, non-crossover design, and inclusion of a control group. However, one limitation was that participants were not masked to treatment allocation, risking recollection bias. This decision was motivated by previous negative experiences with adherence of patients with adrenal insufficiency to double-blind studies. Any bias would probably have increased vigilance and reporting in patients receiving the new formulation; patients with adrenal insufficiency are often resistant to changing their life-saving treatment. Moreover, data were collected prospectively; therefore, any recollection bias should have operated on an individual basis at baseline and after treatment.

Another limitation was the use of a self-reported questionnaire to measure infection rates. Mild infections could not be verified against medical records. Therefore, we cannot exclude possible information bias. However, the 6 month observation is likely to have minimised any bias that, in any case, would probably produce a non-differential misclassification (both groups scoring higher or lower consensually). Finally, we were unable to do an

ex-vivo assay to assess the cytotoxicity of natural killer cells and 24 h sampling to investigate correlations with the different pharmacokinetics of the two regimens.

In conclusion, once-daily glucocorticoid administration improved the immune and metabolic functions of patients with adrenal insufficiency compared with multiple daily doses. In patients with recurrent infections, poor metabolic control, and impaired quality of life, replacement therapy that better mimics the circadian rhythm of endogenous glucocorticoids offers measurable benefits. Whether the observed immunoregulatory effect of the timing of glucocorticoid administration could be transferred outside adrenal insufficiency deserves further study, given that it has important potential clinical implications.

Contributors

AMI, MAV, CG, and DF contributed to the study design, data acquisition and interpretation, and drafting of the report. VH, CS, ES, DG, CP, PP, SM, AS, FN, AC, RP, and AL contributed to the study design, data acquisition and interpretation, and reviewing and editing of the report.

Declaration of interests

AMI reports grants and personal fees from Shire and Novartis, personal fees from Otsuka and Menarini, and personal fees and non-financial support from Ipsen, outside the submitted work. AC reports personal fees from Novartis, outside the submitted work. RP reports grants and personal fees from Novartis, Pfizer, HRA Pharma, Viropharma, Shire, and Ipsen, and personal fees from Ferring and Italfarmaco, outside the submitted work. AL reports personal fees from MSD, Novartis, Shire, Novo Nordisk, and Aegerion, outside the submitted work. All other authors declare no competing interests.

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