

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

IMPROVEMENT OF ANTHROPOMETRIC AND METABOLIC PARAMETERS, AND QUALITY OF LIFE FOLLOWING TREATMENT WITH DUAL-RELEASE HYDROCORTISONE IN PATIENTS WITH ADDISON'S DISEASE

This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/1522098> since 2019-04-26T00:25:49Z

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

This is the author's final version of the contribution published as:

Giordano, R; Guaraldi, F; Marinazzo, E; Fumarola, F; Rampino, A; Berardelli, R; Karamouzis, I; Lucchiari, M; Manetta, T; Mengozzi, G; Arvat, E; Ghigo, E. IMPROVEMENT OF ANTHROPOMETRIC AND METABOLIC PARAMETERS, AND QUALITY OF LIFE FOLLOWING TREATMENT WITH DUAL-RELEASE HYDROCORTISONE IN PATIENTS WITH ADDISON'S DISEASE. ENDOCRINE. 51 (2) pp: 360-368.

When citing, please refer to the published version.

Link to this full text:

<http://hdl.handle.net/2318/1522098>

**IMPROVEMENT OF ANTHROPOMETRIC AND METABOLIC PARAMETERS, AND QUALITY OF LIFE
FOLLOWING TREATMENT WITH DUAL-RELEASE HYDROCORTISONE IN PATIENTS WITH
ADDISON'S DISEASE**

Roberta Giordano¹, Federica Guaraldi², Elisa Marinazzo², Federica Fumarola², Alessia Rampino¹, Rita Berardelli⁴,
Ioannis Karamouzis², Manuela Lucchiari³, Tilde Manetta³, Giulio Mengozzi³, Emanuela Arvat⁴, Ezio Ghigo²

¹Department of Clinical and Biological Sciences, University of Turin, 10126 Turin, Italy

²Division of Endocrinology, Diabetes and Metabolism, Department of Medical Sciences, University of Turin, 10126
Turin, Italy

³Clinical Biochemistry Laboratory, "Città della Salute e della Scienza di Torino" University Hospital, 10126 Turin, Italy

⁴Division of Oncological Endocrinology, Department of Medical Sciences, University of Turin, 10126 Turin, Italy

Abbreviated Title: Dual-release hydrocortisone treatment in patients with Addison's disease

Key words: Addison's disease, dual-release hydrocortisone, metabolism, QoL

Word count: 2958

Number of figures and tables: 8

Grant support: the study was supported by a University of Turin grant (ex 60%, year 2013).

Corresponding Author and person to whom reprint should be addressed:

Roberta Giordano, M.D., Ph.D.

Division of Endocrinology, Diabetes and Metabolism

Department of Medical Sciences

University of Turin

Corso Dogliotti 14

10126 Turin, ITALY

Telephone (office): (+39) 011 6709586

Fax (office): (+39) 011 6647421

E-mail: roberta.giordano@unito.it

Disclosure statement: The Authors have nothing to disclose.

Abstract

Purpose: In patients with Addison's disease (AD), a dual-release preparation of hydrocortisone (Plenadren, PLEN) has been demonstrated to maintain cortisol levels in a more physiological range than conventional glucocorticoid therapy, and to exert positive effects. This study aimed to assess variations of anthropometric, metabolic and hormonal parameters in patients with AD after switching from conventional hydrocortisone (HC) treatment to PLEN.

Methods: in nineteen AD patients (15 F and 4 M, age 27-65 yr) treated with HC 20 mg/day thrice daily, body weight, BMI, waist circumference, fasting glucose, HbA1c, serum lipids, plasma renin activity (PRA), electrolytes and blood pressure were evaluated at baseline, and 1, 3, 6 and 12 months after switching from HC to PLEN. At baseline, and after 1 and 12 months of PLEN, blood ACTH and cortisol (at 0800 h at fasting, and 30, 60, 90, 120 and 240 minutes after drug ingestion), and health-related quality of life (HRQoL), using 30-AddiQoL questionnaire, were evaluated.

Results: During PLEN, waist and serum lipid progressively decreased. After 12 months of PLEN, a significant difference was observed in waist circumference ($P=0.007$), HbA1c ($P=0.002$), total and LDL-cholesterol levels ($P<0.05$). ACTH levels at 240 minutes and the area under the curve (AUC) were lower ($P<0.05$) during PLEN than HC, while cortisol peaks and AUC were similar. 30-AddiQoL total score also improved ($P=0.04$) during PLEN.

Conclusions: In Addison's disease patients, PLEN reduces central adiposity, and improves glucose and metabolism parameters and HRQoL.

Introduction

In patients with Addison's disease (AD) glucocorticoids (GC) are insufficiently secreted, so that replacement therapy is essential for health and, indeed, life [1-5].

Primary aim of GC replacement therapy is to reproduce the physiological circadian pattern of cortisol secretion by the normal adrenal gland as much as possible. At the same time, the pharmacokinetic of available oral fast-release GC, hydrocortisone or cortisone, makes impossible to fully mimic physiological cortisol secretion rhythm [4,6].

Moreover, despite GC replacement therapy patients with adrenal insufficiency present higher mortality rates, typically from cardiovascular diseases, and increased morbidity, mainly obesity and glycometabolic alterations, as compared to the general population [7-13]. Likely explanations are supra-physiological GC maintenance doses [14], poor diurnal GC exposure-time profile [15], and inadequate rescue therapy in response to intercurrent illnesses [16,17]. Finally, patients with AD also present impaired health-related quality of life (HRQoL) [18-20].

A dual-release preparation of hydrocortisone (PLENADREN; PLEN; ViroPharma, Maidenhead, UK) was developed to maintain cortisol levels in a more physiological range [21]. A Swedish multicenter study conducted in AD, has demonstrated that, compared to conventional GC treatment with hydrocortisone, PLEN reduces body weight and blood pressure, and improves glucose metabolism and HRQoL [22].

Based on these premises, we designed a clinical prospective study aimed at evaluating the anthropometric, metabolic and hormonal profile, as well as HRQoL in a group of patients with AD, under conventional hydrocortisone treatment and after 1, 3, 6 and 12 months of PLEN.

Subjects and Methods

Subjects

Nineteen patients with autoimmune Addison's disease (F:M=15:4; mean age 47.3 ± 3.2 yr, range 27-65) were enrolled. Ten patients had normal weight, 7 were overweight (BMI 25-30 kg/m²) and 2 were obese (BMI>30 kg/m²). Two patients presented with isolated autoimmune AD, 17 were affected by autoimmune-poliendocrine syndrome (APS), 14 of type 2 (APS 2), 2 of type 1 (APS 1), and 1 of type 4 (APS 4).

The diagnosis of autoimmune Addison's disease was based on the presence of circulating adrenal autoantibodies against the steroidogenic enzyme 21-hydroxylase (21OHAb), determined in radio binding assay that uses in vitro translated recombinant human 35S-21OH, and expressing 21OHAb levels as a relative index (21OH index) based on the analysis of one positive and two negative standard sera included in each assay, with the upper level of normal for the 21OH index that was 0.06 [23].

Exclusion criteria were clinical or laboratory signs of severe cerebral, cardiovascular, respiratory, hepatobiliary or pancreatic disease; renal dysfunction; gastrointestinal emptying or motility disturbances, and immunosuppressive steroid therapy.

All pre-menopausal women were studied in the early follicular phase; the two patients with primary ovary insufficiency (case 6 and 11) were under appropriate hormonal replacement therapy (HRT) at the time of the study, and were evaluated in the estrogen phase (days 5–10 of HRT).

Patients with autoimmune hypothyroidism were under appropriate treatment with l-thyroxin at the time of the study.

At baseline evaluation, all the patients had been treated with hydrocortisone (HC, 20 mg/day, thrice/daily), and fludrocortisone (0.025-0.1 mg/day, once/daily), for at least 6 months. They were then switched to a dual-release hydrocortisone (PLEN, 20 mg/day), administered orally in the morning at fasting. All patients were instructed to add a rescue dose of HC during an intercurrent illness or stress (5 or 10 mg dose according to severity of stress and symptoms).

None of the patients was affected by arterial hypertension, whereas 3 of them were affected by type 1 (case 8, 9 and 11) or type 2 (case 7) diabetes mellitus.

The main clinical patients features at baseline are reported in **Table 1**.

All patients gave their written informed consent to participate in the study, which had been approved by the Ethical Committee of the University of Turin, in agreement with the principles of the Declaration of Helsinki.

The patients were recruited among patients affected by autoimmune Addison's disease, consecutively evaluated at the Division of Endocrinology, Diabetes and Metabolism, fitting specific inclusion criteria, and that agreed to switch from conventional hydrocortisone therapy to Plenadren.

The study was conducted between September 2012 and September 2014.

Evaluation of clinical parameters and health-related quality of life

Body weight, body mass index (BMI), waist circumference and blood pressure were measured using standard methods at baseline, and after 1, 3, 6 and 12 months of treatment with PLEN.

Health-related quality of life (HRQoL) was evaluated by a new 30-item questionnaire (AddiQoL), purposely developed and validated in patients with Addison's disease [24, 25], administered in the morning. The algebraic sum of the various items scores was calculated: a higher score indicated a better HRQoL.

Biochemical evaluation

Blood samples were collected from all patients at fasting, between 0800 and 0900 h, before and after 30,60, 90, 120 and 240 minutes after drug ingestion, and at least 30 minutes after venous cannulation kept patent by slow infusion of isotonic saline.

Blood fasting glucose, HbA1C, total cholesterol, total and high density lipoprotein (HDL) cholesterol, triglycerides, sodium (Na), potassium (K), ACTH, cortisol (F) and plasma renin activity (PRA) levels were directly measured, while low density lipoprotein (LDL) cholesterol was calculated by Friedewald equation.

Serum glucose levels (mg/dl; 1 mg/dl=18 mmol/l) were measured by enzymatic method based on hexokinase reaction, traceable according to isotope dilution/mass spectrometry (ID/MS) standards.

Serum total cholesterol (mg/dl; 1 mg/dl=38.6 mmol/l), and triglycerides (mg/dl; 1 mg/dl=88.5 mmol/l) levels were measured by enzymatic colorimetric assay, standardized according to ID/MS standards.

Serum HDL cholesterol levels (mg/dl; 1 mg/dl=38.6 mmol/l) were measured by enzymatic colorimetric homogeneous test (immunoseparation followed by enzymatic cholesterol assay), standardized according to the US national reference system for cholesterol measurement, Cholesterol Reference Method Laboratory Network – CRMLN.

HbA1C levels (mmol/mol) were measured by gold standard ion-exchange high-performance liquid chromatography (HPLC, Tosoh Bioscience, Inc., San Francisco, CA, USA).

Serum Na and K were measured using ion-selective electrode system (Cobas ISE module), fully automated on routine clinical biochemistry analyzers.

All the above mentioned analysis were performed on Roche/Hitachi Cobas c automated platforms (Roche Diagnostics), being coefficient of variations <1.9% for both within-run and between-run evaluations.

Serum cortisol (F) levels (µg/dl; 1 µg/dl=27.59 nmol/l) were measured by automated immunoassay on Cobas e601 instrument (Roche Diagnostics GmbH, Mannheim, Germany) based on a competitive electrochemiluminescence immunoassay, with an analytical sensitivity of 0.018 µg/dl, and intra- and inter-assay precision ranging from 3.0% to 5.7%, and from 2.4% to 6.2%, respectively.

Plasma ACTH levels (pg/ml; 1 pg/ml=4.5 pmol/l) were measured by solid-phase, two-site sequential chemiluminescent immunometric assay (Immulite 2000, Siemens Healthcare Diagnostics Inc., USA), with an analytical sensitivity of 5 pg/ml, and intra- and inter-assay precision ranging from 6.7 to 9.5%, and from 6.1% to 10.0%, respectively.

Plasma Renin Activity (PRA; ng/ml/h; 1 ng/ml/h=3.6 ng/(l*s)) levels were measured by radioimmunoassay of angiotensin I (Beckman Coulter), after its generation in plasma samples as the result of the enzymatic cleavage of the renin substrate, angioteninogen, in the presence of ACE inhibitor, by radioimmunological competition assay, using anti-angiotensin I polyclonal antibody-coated tubes. Analytical and functional sensitivity were 0.07 ng/ml/h and 0.20 ng/ml/h, respectively, with an intra- and inter-assay variation coefficient ranging from 11.3 to 20.9%, respectively.

Statistical analysis

Results are expressed as median and range.

The various parameters were compared by non-parametric Wilcoxon matched-pairs signed-rank test, using Statistical Package for the Social Science (IBM SPSS 21.0 for Windows).

A P value <0.05 was considered significant.

Results

During PLEN treatment, body weight, BMI and waist circumference progressively decreased, although as compared to baseline during HC, a significant reduction ($P=0.007$) was observed only for waist circumference after 12 months of PLEN treatment (median/range, 12 months vs. baseline: 78.0/60.0-103.0 vs. 82.0/65.0-110.0 cm), with no differences between normal-weight and overweight/obese patients (data not shown). (**Fig. 1**)

No significant differences of fasting glucose or HbA1c levels were detected up to 6 month treatment, while HbA1c levels significantly decreased at 12 months in the whole group of patients (42.0/23-61 vs. 44.0/24-74 mmol/mol; $P=0.002$; **Fig. 2**) and in the subgroup of 15 non-diabetic patients (40.0/23-46 vs. 42.0/24-49 mmol/mol; $P=0.004$; data not shown). Considering the subgroup of 4 diabetic patients, HbA1C showed a progressive reduction since the sixth month of treatment in all cases (**Table 2**), with a consequent reduction of insulin requirement in type 1 diabetic patients (data not shown).

A progressive decrease of total (baseline vs. 1, 3, 6 and 12 months: 208/145-271 vs. 182/128-272, 180/149-273, 178/139-234 and 172/120-229 mg/dl; $P<0.05$ vs. baseline from time 3 months) and LDL cholesterol levels (107.4/59.6-173.6 vs. 95.8/45-165.6, 67.2/65-162.2, 89/51-164.4 and 85.6/51.4-152.0 mg/dl; $P<0.05$ vs. baseline from time 1 month) was observed during PLEN treatment; on the contrary, triglycerides and HDL cholesterol levels did not significantly change (**Fig. 3**).

PLEN induced a decrease in ACTH levels more marked than HC, being ACTH levels at 240 minutes after drug ingestion and AUC curve lower during PLEN than HC ($P<0.05$), despite similar F peaks and AUC. (**Table 3, Fig. 4**)

No significant changes in PRA, blood pressure and electrolytes were observed during PLEN treatment (data not shown).

The total AddiQoL total score increased during PLEN treatment, with a reported significant improvement of QoL at 12 months ($P=0.04$). Interestingly, fatigue scores increased, although not significantly ($P=0.052$), while scores for the other item categories were similar at baseline during HC and after PLEN treatment (**Table 4**).

The most commonly reported adverse events were fatigue (5 out of 19 patients) and influenza (2 out of 19 patients), both occurring in the first 3 months of treatment. No severe adverse event was reported.

The reported number of days during PLEN in which patients had to increase HC dosage for intercurrent illnesses or physical stresses was similar to that reported during previous conventional HC.

Discussion

Our data clearly showed that in patients with Addison's disease, the switch from conventional hydrocortisone replacement treatment to dual-release hydrocortisone replacement therapy is associated with a significant reduction in central adiposity, together with an improvement of glucose and lipid metabolism and HRQoL.

Studies performed in 1960s suggested a normalization of life expectancy and quality in patients with Addison's disease during appropriate replacement treatment [26]. Afterwards, it has become increasingly clear that, on the contrary, despite glucocorticoid replacement therapy, mortality rate and morbidity remain increased in patients with adrenal insufficiency as compared to the general population [7-10].

In particular, there is a more than a two-fold increase in relative risk of death in Swedish patients with AD, predominantly due to cardiovascular, malignant, and infectious diseases [7,8]. The same for Norwegian patients with AD, particularly males, presenting with an increased risk of premature death due to infections, sudden death and acute adrenal failure [9].

Moreover, a higher prevalence of central adiposity, impaired glucose tolerance and dyslipidemia, recognized as independent risk factors for cardiovascular disease, have been demonstrated in several studies during treatment [10-13].

A recent cross-sectional study performed by Ross et al. [10] in the population of South Africa demonstrated an increase in cardiovascular risk factors, especially dyslipidemia, in AD patients as compared to healthy controls matched by age, gender, ethnicity and BMI. A second study from the same group [11] compared cardiovascular risk factors in AD patients from South Africa and from Sweden, demonstrating a significant presence of hypertension and diabetes in both groups, but a worse lipid profile and higher levels of inflammation markers in the first group, despite the lower replacement doses of hydrocortisone. Overall, these results support the hypothesis of AD as an independent risk factor for cardiovascular disorders, although the overall cardiovascular risk derives from the association of AD and several other environmental and genetic factors.

Since the conventional glucocorticoid replacement therapy often exceed the normal endogenous cortisol production rate, it could be expected that using supra-physiological doses of glucocorticoids may increase the prevalence of risks for cardiovascular diseases. Indeed, traditional synthetic glucocorticoids did not fully mimic physiological circadian cortisol rhythm, as serum cortisol levels were abundantly above the expected physiological levels right after tablet intake, with a rapid decrease in the following hours, often reaching undetectable levels [4, 15].

Based on these premises, a number of studies have explored different hydrocortisone formulations trying to identify the best doses and patterns of replacement treatment.

In particular, a dual-release preparation of hydrocortisone (Plenadren) was developed to maintain cortisol levels in a more physiological range, as clearly demonstrated in a phase I study [21].

To date, a single phase II multicenter study has assessed the effect of Plenadren on metabolic parameters and HRQoL in patients with AD. This study, performed in 64 Swedish patients with AD, demonstrated that, compared to conventional HC, Plenadren significantly reduced body weight and blood pressure, and improve glucose metabolism and HRQoL after 12-week treatment [22].

In agreement with Johannsson's group [22], we found a remarkable improvement in glucose metabolism. Indeed, Plenadren was associated with an important, and early (in the subgroup of diabetic patients) reduction of HbA1c levels, while fasting glycemia was similar before and after treatment. Interestingly type 1 diabetes mellitus patients have reduced their insulin requirement during Plenadren treatment.

Conventional replacement therapy results in large fluctuations of daily cortisol levels, directly influencing glucose homeostasis, which makes insulin treatment difficult to manage in diabetic patient. Specifically, increased evening exposure to cortisol has been shown to reduce glucose tolerance, insulin secretion and insulin sensitivity in healthy subjects [27]. In addition, an increased insulin requirement, together with a higher frequency of severe hypoglycemia episodes have been reported in patients with type 1 diabetes associated to AD, as compared with patients with type 1 diabetes only [28].

Differently from a previous study [22], although a trend towards weight reduction was observed in our patient, mean weight at 12 month follow-up was not significantly reduced from baseline. The lower mean weight and BMI in our patients at baseline could explain, at least partially, this difference.

Interestingly, we firstly reported a significant reduction in waist circumference after 12 months of Plenadren treatment, that was independent from BMI. The absence of a body composition study could represent a minor study limitation as it would have helped in better characterizing differences in fat distribution, before and after treatment.

With regards to lipid profile, we demonstrated a positive effect of Plenadren on levels of total and LDL cholesterol, not detected in the Swedish study [22].

Glucocorticoids have complex, still not fully elucidated effects on lipid metabolism, including direct and indirect actions on lipolysis, free fatty acid production and turnover, very-low density lipoproteins synthesis and fatty accumulation in liver [29]. Our data are supported by previous studies performed in patients with secondary hypoadrenalism, suggesting that long-term glucocorticoid replacement therapy can cause dyslipidemia, with a clear relation between glucocorticoid dose and levels of total cholesterol and LDL-cholesterol [14, 30]. Differently from previous observations that found a positive correlation between hydrocortisone dose and HDL-cholesterol [10], we did not find any modification on HDL-cholesterol levels during PLEN.

As central adiposity, together with impaired glycolipid metabolism, are well recognized risk factors of cardiovascular diseases, our results suggest that patients treated with Plenadren have a safer cardiovascular profile with respect to conventional HC treatment.

Considering that the total dose of Plenadren and HC administered in our study is similar, our results suggest that a more physiological daily cortisol profile is associated with an improvement of waist anthropometric and metabolic parameters. The reduction in 24-hour hydrocortisone exposure with Plenadren may explain the reduction in waist, HbA1c and lipid parameters.

Although we did not perform complete daily serum cortisol curves, lower ACTH levels at 240 minutes after Plenadren ingestion, as compared to HC, suggest a more physiologic serum cortisol levels during the day. Plasma ACTH concentrations have not been usually considered for the definition of the adequacy of glucocorticoid replacement therapy. More recently, it has been suggested that ACTH profiles could be useful to assess the variable sensitivity to glucocorticoid activity in patients with Addison's disease [29] and, therefore, should be used as additional parameter for the monitoring of glucocorticoid replacement [30]. The evaluation of 24-hours urinary free cortisol or salivary cortisol could add further information on cortisol exposure during the day.

Differently from the Swedish study [22], we did not observe a reduction of arterial pressure associated with Plenadren treatment. This could be due to the fact that none of our patient was hypertensive at enrollment.

Finally, we confirmed the previously reported improvement in quality of life associated with Plenadren, with special regards to reduction, of fatigue, although not significant (this could be due to the low numbers of patients enrolled, so that longitudinal studies in larger cohorts of patients could clarify this aspect).

A similar prevalence of minor adverse events was recorded between HC and Plenadren, being fatigue and influenza the most common, especially in the first month of treatment. We suppose this phenomenon could be related more to an increased awareness of associated signs and symptoms, than to a real change in cortisol regimen. At the same time, changes in cortisol exposure time can not be ruled out.

Our study present some limitations. First, patients were not blinded to the treatment; therefore, their expectations on the efficacy of the new drug could have, at least partially, affected results. Secondly, the small number of patients, due to rarity of the disease, as well as the predominance of women, increases the risk of type 2 error and do not allow definitive conclusions. A case-controlled study, performed in a larger patient cohort and with a longer follow-up is mandatory in order to verify and explain these preliminary observations-

Despite these limitations, the strength of our study is that is the first Italian study assessing anthropometric, metabolic and quality of life in a homogeneous cohort of patients with autoimmune Addison's disease enrolled in a single tertiary care Italian center, after a relatively long (1 year)-period of treatment with Plenadren.

In conclusion, our study provides further evidence that dual-release hydrocortisone preparation is efficacious in reducing central adiposity, improving glucose and lipids metabolism, as well as quality of life, in patients with Addison's disease.

References

1. Arlt, W., Allolio, B.: Adrenal insufficiency. *Lancet* 361, 1881-1893 (2003)
2. Besser, G.M., Jeffcoate, W.J.: Endocrine and metabolic diseases. Adrenal diseases. *Br. Med. J.* 1, 448-451 (1976)
3. Oelkers, W., Diederich, S., Bahr, W.: Therapeutic strategies in adrenal insufficiency. *Ann. Endocrinol.* 62, 212-216 (2001)
4. Falorni, A., Minarelli, V., Morelli, S.: Therapy of adrenal insufficiency: an update. *Endocr.* 43, 514-528 (2013)
5. Husebye, E.S., Allolio, B., Arlt, W., Badenhop, K., Bensing, S., Betterle, C., Falorni, A., Gan, E.H., Hulting, A.L., Kasperlik-Zaluska, A., Kämpe, O., Løvås, K., Meyer, G., Pearce, S.H.: Consensus statement on the diagnosis, treatment and follow-up of patients with primary adrenal insufficiency. *J. Intern. Med.* 275, 104-115 (2014)
6. Derendorf, H., Möllmann, H., Barth, J., Möllmann, C., Tunn, S., Krieg, M. Pharmacokinetics and oral bioavailability of hydrocortisone. *J. Clin. Pharmacol.* 31, 473-476 (1991)
7. Bergthorsdottir, R., Leonsson-Zachrisson, M., Odén, A., Johannsson, G.: Premature mortality in patients with Addison's disease: a population-based study. *J. Clin. Endocrinol. Metab.* 91, 4849-4853 (2006)
8. Bensing, S., Brandt, L., Tabaroj, F., Sjöberg, O., Nilsson, B., Ekbom, A., Blomqvist, P., Kämpe, O.: Increased death risk and altered cancer incidence pattern in patients with isolated or combined autoimmune primary adrenocortical insufficiency. *Clin. Endocrinol.* 69, 697-704 (2008)
9. Erichsen, M.M., Løvås, K., Fougner, K.J., Svartberg, J., Hauge, E.R., Bollerslev, J., Berg, J.P., Mella, B., Husebye, E.S.: Normal overall mortality rate in Addison's disease, but young patients are at risk of premature death. *Eur. J. Endocrinol.* 160, 233-237 (2009)
10. Ross, I.L., Bergthorsdottir, R., Levitt, N.S., Schatz, D.A., Johannsson, G., Marais, A.D.: Increased cardiovascular risk in south african patients with Addison's disease. *Horm. Metab. Res.* 45, 905-910 (2013)
11. Ross, I.L., Bergthorsdottir, R., Levitt, N., Dave, J.A., Schatz, D., Marais, A.D., Johannsson, G., Marais, A.D.: Cardiovascular risk factors in patients with Addison's disease: a comparative study of south african and swedish patients. *PLoS ONE* 9(3): e90768.
12. Giordano, R., Marzotti, S., Balbo, M., Romagnoli, S., Marinazzo, E., Berardelli, R., Migliaretti, G., Benso, A., Falorni, A., Ghigo, E., Arvat E.: Metabolic and cardiovascular profile in patients with Addison's disease under conventional glucocorticoid replacement. *J. Endocrinol. Invest.* 32: 917-923 (2009)
13. Leelarathna, L., Breen, L., Powrie, J.K., Thomas, S.M., Guzder, R., McGowan, B., Carroll, P.V.: Co-morbidities, management and clinical outcome of auto-immune Addison's disease. *Endocr.* 38,113-117 (2010)
14. Filipsson, H., Monson, J.P., Koltowska-Hägström, M., Mattsson, A., Johannsson, G.: The impact of glucocorticoid replacement regimens on metabolic outcome and comorbidity in hypopituitary patients. *J. Clin. Endocrinol. Metab.* 91, 3954-3961 (2006)

15. Dallman, M.F., Akana, S.F., Bhatnagar, S., Bell, M.E., Strack, A.M.: Bottomed out: metabolic significance of the circadian trough in glucocorticoid concentrations. *Int. J. Obes. Relat. Metab. Disord.* 24 (Suppl 2), S40–S46 (2000)
16. Hahner, S., Loeffler, M., Bleicken, B., Drechsler, C., Milovanovic, D., Fassnacht, M., Venz, M., Quinkler, M., Allolio, B.: Epidemiology of adrenal crisis in chronic adrenal insufficiency: the need for new prevention strategies. *Eur. J. Endocrinol.* 162, 597–602 (2010)
17. Hahner, S., Spinnler, C., Fassnacht, M., Burger-Stritt, S., Lang, K., Milovanovic, D., Beuschlein, F., Willenberg, H.S., Quinkler, M., Allolio B.: High incidence of adrenal crisis in educated patients with chronic adrenal insufficiency – a prospective study. *J. Clin. Endocrinol. Metab.* 100, 407–416 (2015)
18. Lovas, K., Loge, J.H., Husebye, E.S.: Subjective health status in Norwegian patients with Addison's disease. *Clin. Endocrinol.* 56, 581–588 (2002).
19. Hahner, S., Loeffler, M., Fassnacht, M., Weismann, D., Koschker, A.C., Quinkler, M., Decker, O., Arlt, W., Allolio, B.: Impaired subjective health status in 256 patients with adrenal insufficiency on standard therapy based on cross-sectional analysis. *J. Clin. Endocrinol. Metab.* 92, 3912–3922 (2007)
20. Bleicken, B., Hahner, S., Loeffler, M., Venz, M., Allolio, B., Quinkler, M.: Impaired subjective health status in chronic adrenal insufficiency: impact of different glucocorticoid replacement regimens. *Eur. J. Endocrinol.* 159, 811–7 (2008).
21. Johannsson, G., Bergthorsdottir, R., Nilsson, A.G., Lennernas, H., Hedner, T., Skrtic, S.: Improving glucocorticoid replacement therapy using a novel modified-release hydrocortisone tablet: a pharmacokinetic study. *Eur. J. Endocrinol.* 161, 119–130 (2009)
22. Johannsson, G., Nilsson, A.G., Bergthorsdottir, R., Burman, P., Dahlqvist, P., Ekman, B., Engström, B.E., Olsson, T., Ragnarsson, O., Ryberg, M., Wahlberg, J., Biller, B.M., Monson, J.P., Stewart, P.M., Lennernas, H., Skrtic, S.: Improved cortisol exposure time profile and outcome in patients with adrenal insufficiency: a prospective randomized trial of a novel hydrocortisone dual release formulation. *J. Clin. Endocrinol. Metab.* 97, 473–481 (2012)
23. Falorni, A., Nikoshkov, A., Laureti, S., Grenbäck, E., Hulting, A.L., Casacci, G., Santeusano, F., Brunetti, P., Luthman, H., Lernmark, A.: High diagnostic accuracy for idiopathic Addison's disease with a sensitive radiobinding assay for autoantibodies against recombinant human 21-hydroxylase. *J. Clin. Endocrinol. Metab.* 80, 2752–2754 (1995)
24. Løvås, K., Curran, S., Oksnes, M., Husebye, E.S., Huppert, F.A., Chatterjee, V.K.: Development of a disease-specific quality of life questionnaire in Addison's disease. *J. Clin. Endocrinol. Metab.* 95, 545–551 (2010)
25. Øksnes, M., Bensing, S., Hulting, A.L., Kämpe, O., Hackemann, A., Meyer, G., Badenhoop, K., Betterle, C., Parolo, A., Giordano, R., Falorni, A., Papierska, L., Jeske, W., Kasperlik-Zaluska, A.A., Chatterjee, V.K.,

- Husebye, E.S., Løvåsm K.: Quality of life in European patients with Addison's disease: validity of the disease-specific questionnaire AddiQoL. *J. Clin. Endocrinol. Metab.* 97, 568–576 (2012)
26. Mason, A.S.: Epidemiological and clinical picture of Addison's disease. *Lancet* 2, 744-747 (1968)
 27. Plat, L., Leproult, R., L'Hermite-Baleriaux, M., Fery, F., Mockel, J., Polonsky, K.S., Van, C.E.: Metabolic effects of short-term elevations of plasma cortisol are more pronounced in the evening than in the morning. *J. Clin. Endocrinol. Metab.* 84, 3082-3092 (1999)
 28. Elbelt, U., Hahner, S., Allolio, B.: Altered insulin requirement in patients with type 1 diabetes and primary adrenal insufficiency receiving standard glucocorticoid replacement therapy. *Eur. J. Endocrinol.* 160, 919–924 (2009)
 29. Arnaldi, G., Scandali, V.M., Trementino, L., Cardinaletti, M., Appolloni, G., Boscaro, M.: Pathophysiology of dyslipidemia in Cushing's syndrome. *Neuroendocrinology* 92 (Suppl 1), 86-90 (2010)
 30. Ekman, B., Blomgren, J., Andersson, P.O., Carlsson, M., Arnqvist, H.J.: Variable sensitivity to the glucocorticoid activity of cortisol in patients with primary adrenal insufficiency: assessment with ACTH profiles. *Horm. Metab. Res.* 42, 961-966 (2010)
 31. Ekman, B., Bachrach-Lindström, M., Lindström, T., Wahlberg, J., Blomgren, J., Arnqvist, H.J.: A randomized, double-blind, crossover study comparing two- and four-dose hydrocortisone regimen with regard to quality of life, cortisol and ACTH profiles in patients with primary adrenal insufficiency. *Clin. Endocrinol.* 77, 18-25 (2012)

Table 1. Clinical data of patients with Addison's disease

Case	Gender	Age	Etiology	Associated diseases	Disease duration
(n)		(yr)			(yr)
1	F	58	APS2	BD, CAG	2
2	M	39	APS2	HYPOTH	29
3	F	49	APS2	HYPOTH	10
4	M	54	APS2	HYPOTH	4
5	F	62	APS2	HYPOTH, CAG	16
6	F	27	APS1	AL, POI, HYPOPTH	10
7	F	61	APS2	HYPOTH, CAG, DMT2	11
8	F	29	APS2	HYPOTH, CAG, DMT1	7
9	M	20	APS1	HYPOPTH, DMT1	11
10	F	49	APS2	HYPOTH	3
11	F	45	APS2	HYPOTH, POI, DMT1	6
12	F	50	APS2	HYPOTH	7
13	F	57	APS2	HYPOTH	33
14	F	59	APS2	HYPOTH, CAG	7
15	F	59	APS2	HYPOTH	42
16	M	29	AI		11
17	M	56	APS4	CAG, VIT	18
18	F	65	AI		4
19	F	30	APS2	HYPOTH	23

AI: autoimmune isolated; APS: autoimmune polyendocrine syndrome type 1 (APS 1), type 2 (APS 2) or type 4 (APS 4); AL: Alopecia; BD: Basedow's disease; CAG: chronic atrophic gastritis; DMT1: diabetes mellitus type 1; DMT2: diabetes mellitus type 2; HYPOPTH: hypoparathyroidism; HYPOTH: hypothyroidism; VIT: vitiligo; POI: primary ovary insufficiency.

Table 2. HbA1c (mmol/l) levels in 4 patients with Addison's disease and diabetes mellitus

Case	HC	PLEN 1M	PLEN 3M	PLEN 6M	PLEN 12M
(n)					
7	55.0	57.0	57.0	55.0	47.0
8	58.0	62.0	68.0	53.0	53.0
9	74.0	65.0	65.0	64.0	61.0
11	62.0	57.0	59.0	58.0	56.0
<i>median</i>	<i>60.0</i>	<i>59.5</i>	<i>62.0</i>	<i>57.0</i>	<i>55.0</i>
<i>lower</i>	<i>55.0</i>	<i>57.0</i>	<i>57.0</i>	<i>53.0</i>	<i>47.0</i>
<i>upper</i>	<i>74.0</i>	<i>65.0</i>	<i>68.0</i>	<i>64.0</i>	<i>61.0</i>

Table 3. (Median/range) ACTH levels (at 240 minutes after drug ingestions), cortisol (F) levels (peaks after drug ingestions), ACTH and F AUCs in patients with Addison’s disease during conventional hydrocortisone treatment (HC), and after 1 (1M) and 12 (12M) months of Plenadren (PLEN)

	HC	PLEN 1 M	PLEN 12 M
ACTH levels (pg/ml)	140.0/170.0-600.0	24.0*/5.0-298.0	26.0*/5.0-306.0
ACTH AUC (pg/ml/h)	32110.0/18170.0-194746.0	28307.0*/1072.0-151448.0	23322.0*/1844.0-115615.0
F levels (µg/dl)	25.0/13.2-33.3	21.4/10.4-32.8	19.0/10.5-26.8
F AUC (µg/dl/h)	4092.1/2246.4-5428.3	4149.3/2423.1-6521.4	3915.3/2487.6-6400.6

*P< 0.05 vs. HC

Table 4. (Median/range) AddiQoL scores in patients with Addison’s disease during conventional hydrocortisone treatment (HC), and after 3 (3M) and 12 (12M) months of Plenadren (PLEN)

AddiQoL	Fatigue	Emotions	Symptoms	Miscellaneous	Total
HC	20.0/18.0-27.0	21.0/17.0-28.0	23.0/19.0-32.0	12.0/6.0-18.0	76.0/68.0-100.0
PLEN 3M	21.0/16.0-28.0	20.0/18.0-30.0	24.0/20.0-30.0	13.0/7.0-18.0	77.0/67.0-102.0
PLEN 12M	22.0/17.0-28.0	21.0/17.0-30.0	24.0/20.0-29.0	13.0/10.0-18.0	80.0/70-104.0*

*P= 0.04 vs. HC

Fatigue: questions 1-5, 23, 26 and 27

Emotions: questions 11-15, 24, 25 and 30

Symptoms: questions 6, 9 and 16-22

Miscellaneous: questions 7, 8, 10, 28 and 29

Legend to figures

Fig. 1: body weight (Kg), BMI (kg/m^2) and waist circumference (cm) (expressed as median, upper and lower limits) during hydrocortisone (HC) regimen, and after 1 (1M), 3 (3M), 6 (6M) and 12 months (12M) of Plenadren (PLEN), in patients with Addison's disease. * $P < 0.05$ vs. HC

Fig. 2: fasting glucose (mg/dl) and HbA1c (mmol/mol) (expressed as median, upper and lower limits) during hydrocortisone (HC) regimen, and after 1 (1M), 3 (3M), 6 (6M) and 12 months (12M) of Plenadren (PLEN), in patients with Addison's disease. * $P < 0.05$ vs. HC

Fig. 3: Total cholesterol (TC), triglycerides (TG), HDL-cholesterol and LDL-cholesterol (mg/dl; expressed as median, upper and lower limits) during hydrocortisone (HC) regimen, and after 1 (1M), 3 (3M), 6 (6M) and 12 months (12M) of Plenadren (PLEN), in patients with Addison's disease. * $P < 0.05$ vs. HC

Fig. 4: ACTH (pg/ml) and cortisol ($\mu\text{g}/\text{dl}$) levels and AUCs (expressed as median, upper and lower limits) during hydrocortisone (HC) regimen, and after 1 (1M) and 12 months (12M) of Plenadren (PLEN), in patients with Addison's disease. * $P < 0.05$ vs. HC







