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Title: Improved insulin sensitivity and secretion in pre-diabetic patients with adrenal insufficiency on dual-release hydrocortisone treatment: a 36-month retrospective analysis.

Running title: Adrenal insufficiency and pre-diabetes

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Summary

Objective: Dual-release hydrocortisone (DR-HC) provides physiological cortisol exposure, leading to an improvement of anthropometric and metabolic parameters. The aim of the study was to evaluate the effects of DR-HC on insulin secretion and sensitivity and cardio metabolic risk, indirectly expressed by the visceral adiposity index (VAI).

Design and patients: Retrospective analysis of 49 patients, 13 with primary and 36 with secondary adrenal insufficiency (AI) respectively, on conventional glucocorticoid treatment at baseline and switched to DR-HC for 36 months.

Overall, 24 patients had AI-pre-diabetes (impaired fasting glucose, impaired glucose tolerance and the combination) and 25 had AI-normal glucose tolerance (NGT).

Measurements: Clinical and metabolic parameters, including VAI, insulin secretion and sensitivity indexes [fasting insulinemia, $AUC_{2h\ insulinemia}$, oral disposition index (Dio) and ISI-Matsuda] were evaluated.

Results: In patients with AI-NGT and AI-pre-diabetes a significant decrease in BMI ($p= 0.017$ and $p< 0.001$), waist circumference ($p= 0.008$ and $p< 0.001$), HbA1c ($p= 0.034$ and $p= 0.001$) and a significant increase in HDL-C ($p= 0.036$ and $p= 0.043$) were respectively observed.

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In addition, in pre-diabetic patients only we found a significant decrease in insulinemia (p= 0.014), $AUC_{2h\ insulinemia}$ (p= 0.038) and VAI (p= 0.001), in concomitance with a significant increase in DIo (p= 0.041) and ISI-Matsuda (p= 0.038).

Conclusions: Long-term DR-HC therapy is associated with an improvement in insulin secretion and sensitivity in patients with pre-diabetes. However, all patients appear to benefit from the treatment in terms of improvement of metabolic and anthropometric parameters. Larger studies are required to confirm our preliminary data.

Key words: Dual-release hydrocortisone, diabetes, plenadren**,** adrenal insufficiency, insulin-secretion

Introduction

Adrenal insufficiency (AI), which can be due to a defect of steroid production from the adrenal glands, primary AI (PAI), or to inadequate secretion of ACTH by the pituitary gland, secondary AI (SAI), is a life-threatening disease, which requires chronic glucocorticoid (GC) replacement therapy (1). Currently patients with AI on conventional GC replacement therapy have more than double the standardized mortality ratio of the general population (2). Conventional GC treatment, hydrocortisone (HC) or cortisone acetate, requires two-three daily doses to maintain adequate serum cortisol levels, with the highest dose administered in the morning and a lower dose in the afternoon and, if required, in the evening (1). However, this conventional dosage scheme has been demonstrated to expose patients to supraphysiological levels of cortisol (3). Overexposure to GCs has been demonstrated to increase metabolic dysfunction, hypertension, sleep pattern disturbance and cardio metabolic risk, resulting in impaired quality of life and enhancing mortality and morbidity (3,4).

Few studies have been conducted aiming to improve health-related outcomes, through a decrease in the daily doses of GCs, only resulting in a weight decrease, without any favourable effects on hypertension and/or glucose metabolism (5). However, a quite recent study demonstrated that higher doses of hydrocortisone are associated with a better health-related quality of life than lower ones (6).

Recently, a novel once-daily dual-release HC (DR-HC) was approved for treatment of AI in adults, characterized by an immediate-release fraction of HC in the outer layer of the tablet and an extended-release fraction in the core, able to provide an adequate concentration of cortisol within 50 min after administration, half cortisol plasmatic concentration for 6 hours thereafter and a minimal cortisol level 18-24 hours after intake (7). This formulation tends to provide a cortisol exposure-time profile close to the physiological one, improving anthropometric parameters, HbA1c and questionnaire-related quality of life, and appears to be safe even in the long term (8-12).

The aim of the current study was to evaluate the effects of DR-HC (Plenadren, Shire Italy) in patients with normal glucose tolerance (NGT) and pre-diabetes [impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT)], on anthropometric parameters, insulin sensitivity and secretion and cardio metabolic risk indirectly estimated by a surrogate parameter, the visceral adiposity index (VAI), after switching from conventional GC treatment, during a 36-month follow-up.

Materials and methods

Study participants

We retrospectively recruited 49 patients, 25 with NGT and 24 with pre-diabetes, with chronic PAI and SAI, who were on stable GC replacement therapy before entering the study, consecutively referred to the Division of Endocrinology of Palermo University from January 2012 to December 2016. All patients had a disease duration of at least two years.

AI was diagnosed first assessing the function of adrenal cortex by serum cortisol in the morning. An early morning serum cortisol level lower than 3 μg/dL confirmed AI. Patients with cortisol levels more than 3 μg/dL and high clinical suspicion of AI were further evaluated by the corticotropin stimulation test at the standard dose of 250 μg. A normal response was a plasma cortisol concentration higher than 18 μg/dL at 30 minutes after the injection. Once AI was confirmed, we established the etiology by measurement of ACTH levels.

In patients with PAI, adrenal antibodies against the steroidogenetic enzyme 21-hydroxylase and other autoimmune polyendocrine insufficiencies (thyroid disease, premature ovarian failure, type 1 diabetes mellitus, hypoparathyroidism, candidiasis) were assessed as recently reported by international guidelines (13). In addition, a computed tomography scanning of adrenal glands was assessed to rule out infectious, hemorrhagic, or metastatic causes.

For patients with SAI magnetic resonance imaging of the pituitary gland was indicated.

Patients with adrenocortical carcinoma, overt diabetes mellitus, polyglandular autoimmune syndrome and pregnancy were excluded from the study.

Overall, 36 patients had SAI and 13 patients had isolated autoimmune PAI. Twenty patients with SAI had pre-diabetes (9 with IFG, 6 with IGT and 5 with IFG and IGT, respectively), while 16 had NGT. Four patients with PAI had pre-diabetes (2 with IFG and 2 with IGT), while 20 had NGT.

Among patients with SAI, 5 had an isolated disease, 3 had combined hypogonadism and hypocortisolism, 10 had combined hypogonadism, hypothyroidism and hypocortisolism, 10 had combined hypogonadism, hypothyroidism, GH-deficiency (GHD) and hypocortisolism, 4 had hypothyroidism and hypocortisolism, 1 had GHD and hypocortisolism and 3 had hypothyroidism and GHD. Patients with hypothyroidism were treated with levo-thyroxine at the average dose of 1 mcg/kg. Patients with GHD were treated with somatotropin at the average dose of 0.4 mg/die. Males with hypogonadism were treated with an average dose of monthly injection of testosterone enanthate 250 mg. Females were treated with a low dose of estroprogestinic therapy. All patients with SAI were on stable replacement treatment for the other deficiencies and maintained a good and stable hormonal control during the whole follow-up.

At baseline evaluation, among patients with AI-NGT, 10 were on cortisone acetate and 15 on HC treatment administered twice or three times a day. In patients with AI-pre-diabetes, 9 were on cortisone acetate and 15 on HC treatment, twice or three times a day. Patients with PAI were also on stable treatment with fludrocortisone (0.05-0.1 mg/day, once). All patients were switched to DR-HC, administered orally in the morning in a fasting state. All patients were instructed to double the dose of DR-HC during intercurrent illness or stress (according to severity of symptoms), at the normal administration time.

With regard to patients with pre-diabetes, no lifestyle changes or any drugs were suggested, in order to avoid any interference with glucose metabolism.

During the 36-month treatment, the DR-HC dose was changed based on the physician's judgement of a patient's need in both groups of patients (Table 1). Treatment with fludrocortisone was not changed during the study.

The study was carried out in accordance with the recommendations of the Policlinico Paolo Giaccone Ethics Committee, with written informed consent from all subjects, in accordance with the Declaration of Helsinki.

Study design

At baseline and after 12, 24 and 36 months of DR-HC treatment, patients underwent a complete clinical and metabolic evaluation, including a detailed assessment of insulin secretion and sensitivity.

Anthropometric parameters such as BMI, systolic and diastolic blood pressure and waist circumference (WC), measured at the midpoint between the lower rib and the iliac crest, were extracted. In addition, lipids [total cholesterol (TC), HDL cholesterol (HDL-C), LDL cholesterol (LDL-C) and triglycerides (TG)], HbA1c, glycaemia and insulinemia levels were obtained. The VAI was calculated according to gender, where TG levels were expressed in mmol/l and HDL levels were expressed in mmol/l:

- Males VAI= [WC/39.68 + (1.88 x BMI)] x (TG/1.03) x (1.31/HDL);

- Females VAI= [WC/36.58 + (1.89 x BMI)] x (TG/0.81) x (1.52/HDL) (14).

The Matsuda index of insulin sensitivity (ISI-Matsuda) [10000/glucose (mg/dl) x insulin (mU/ml) x glucose mean x insulin mean] (15), the oral disposition index (DIo) [(ΔInsulin0–30/ΔGlucose0–30) x (1/fasting insulin)] (16) and the area under the curve for insulin ($AUC_{2h\ insulinemia}$) and glucose ($AUC_{2h\ glycaemia}$) were derived from an oral glucose tolerance test (OGTT).

To evaluate the differences between patients with NGT and pre-diabetes during the 36 months of treatment with DR-HC, the change from baseline to 36 months of therapy (Δ value) of all parameters was calculated.

In addition, a comparison was made between patients, according to the etiology of AI (PAI and SAI).

Assays

Insulin, glycaemia, HbA1c and lipids were measured by standard methods (Modular P800, Roche, Milan). LDL-C levels were measured using the Friedewald formula [TC – (HDL + (TG/5)].

The conversion factors for the International System (SI) were as follows: glucose mg/dl vs. mmol/l: 0.0555; total cholesterol and HDL-C mg/dl vs. mmol/l: 0.0259; triglycerides mg/dl vs. mmol/l: 0.0113; HbA1c % vs. mmol/mol: 10.93 % - 23.5 .

Statistical analysis

The Statistical Packages for Social Science SPSS version 19 (SPSS, Inc., IBM, New York, USA) was used for data analysis. The normality of quantitative variables was tested with the Shapiro-Wilk test. Baseline characteristics of the groups were presented as mean ± SD for continuous variables, while rates and proportions were calculated for categorical data. The comparison between numerical variables at baseline, 12-, 24- and 36-month follow-up was performed with the Friedman analysis and $\chi 2$ for trend for categorical variables both in the total group and in the single groups (NGT and pre-diabetes and PAI and SAI). A p value <0.05 was considered statistically significant.

Results

Patients with SAI were on stable control for the other pituitary insufficiencies during the 36 months of treatment. At baseline and after 36 months of DR-HC, respectively they had IGF-1 levels 168.5 ± 45.4 and 178.5 ± 39.6 ng/ml, FT4 levels 1.47 ± 0.30 and 1.39 ± 0.32 ng/dl, total testosterone 5.4 ± 1.8 and 5.6 ± 1.65 ng/dl and total estrogens 98.2 ± 27.6 and 103 ± 30.1 pg/ml.

The effects of the different doses of DR-HC on metabolic parameters were also evaluated in all patients. However, only a minority of patients, 14/49 (28.6%) had their dose changed and no differences were found with the change in dosage (data not shown).

Comparison of patients according to glucose tolerance

The baseline characteristics of patients with AI grouped according to glucose tolerance, NGT and pre-diabetes are shown in Table 2.

At baseline, there were no significant differences regarding age, duration of disease, gender, type and dose of oral GC replacement therapy between the two groups (NGT and pre-diabetes) (Table 2). By contrast, patients with pre-diabetes showed significant differences regarding BMI, WC, HbA1c, $AUC_{2h\ glycaemia,}$ insulinemia, $AUC_{2h\ insulinemia}$, ISI-Matsuda, compared to patients with NGT, as reported in Table 2.

During the 36 months of DR-HC, in all patients a significant decrease in BMI ($p<0.001$), WC ($p<0.001$) and HbA1c ($p<0.001$) was observed. No significant differences were found for the other parameters (results not shown).

By contrast, in patients with NGT and pre-diabetes a significant decrease in BMI ($p=0.017$ and $p<0.001$), WC ($p=0.008$ and $p<0.001$), HbA1c ($p=0.034$ and $p=0.001$) and a significant increase in HDL-C ($p=0.036$ and $p=0.043$) were respectively observed during the 36 months of DR-HC (Figure 1). In addition, in pre-diabetic patients only we found a significant decrease in insulinemia ($p=0.014$), $AUC_{2h\ insulinemia}$ ($p=0.038$) and the VAI ($p=0.001$), in concomitant with a significant increase in DIo ($p=0.041$) and ISI-Matsuda ($p=0.038$) (Figure 1 and 2).

Comparing the Δ value from baseline to 36 months between patients with NGT and pre-diabetes, a significant decrease of Δ_BMI ($p=0.009$), Δ_insulinemia ($p=0.008$), Δ_ AUC2h

insulinemia (p=0.011) and Δ_ISI-Matsuda (p=0.040) was observed in patients with pre-diabetes.

Overall, after 36 months of DR-HC treatment, in the group of pre-diabetes, we had 8 patients with IFG, 6 with IGT and 3 with combined IFG and IGT, compared to 11 patients with IFG, 8 with IGT and 5 with combined IFG and IGT at baseline (data not shown). Interestingly, 7 patients with pre-diabetes at baseline had NGT after 36 months of treatment. By contrast, in the group of patients with NGT no changes in glucose tolerance were observed during treatment with DR-HC.

Comparison of patients according to adrenal insufficiency etiology

During the 36 months of DR-HC, no differences in electrolytes, clinical and metabolic parameters and insulin secretion and sensitivity indexes were found comparing patients with PAI and SAI (results not shown).

Discussion

The current study demonstrates that 36-month therapy with DR-HC is associated with an improvement of anthropometric and metabolic parameters and cardio metabolic risk, evaluated by a surrogate parameter, the VAI, in patients both with NGT and pre-diabetes. However, patients with pre-diabetes appear to have a stronger improvement of insulin secretion and sensitivity compared to those with NGT.

Decreased insulin sensitivity with impaired pancreatic β-cell function are the two key components in the pathogenesis of type 2 diabetes mellitus. Therefore, assessment of insulin sensitivity and secretion in patients with AI potentially at risk of glucose metabolism impairment, as during GC replacement treatment, is of crucial importance. As several studies have reported, cortisol plays an important role in glucose and insulin metabolism and an excess of substitutive GC therapy can have detrimental implications for glucose homeostasis (1). However, data on the effects of DR-HC treatment on insulin secretion and sensitivity in AI are still lacking.

In healthy subjects physiological levels of cortisol fluctuate from a peak of 136-720 nmol/L in the morning to 50-331 nmol/l in the night (17). Conventional GCs are administered twice-three times daily with the highest dose in the morning and a lower dose in the afternoon. These dosing regimens are designed to avoid overtreatment during the night-time nadir of physiological cortisol secretion and to reflect the peak release of cortisol at the time of waking (1). In stressful situations, such as high body temperature, trauma, surgery and during any intercurrent illness, the GC dose should be increased to maintain adequate cortisol levels.

Many studies have shown that conventional GC replacement therapy is associated with reduced life expectancy and increased morbidity in patients with AI, compared to the general population (18-19). In a recent survey on 1281 participants, conventional GC treatment was demonstrated to be associated with impaired quality of life, fatigue, high absenteeism from work or school and a high rate of hospitalization for adrenal crises, vomiting or acute infections (20). All of these GC treatment drawbacks are thought to be due to lack of adequate patient education and failure to individualize the dose, leading mainly to

overexposure, inability to achieve a near-physiological diurnal profile of cortisol exposure, and failure to provide adequate treatment in response to stress (1).

The recent formulation of DR-HC has been demonstrated to provide an effective plasma cortisol concentration with a peak of 400-600 nmol/l in the morning, a level of 200 nmol/l in the afternoon and a cortisol-free interval during the night, mimicking the physiological cortisol profile (7,21).

In a fairly recent Swedish multi-centre study comparing conventional hydrocortisone and DR-HC in 64 patients with primary AI, a significant reduction of weight, systolic and diastolic blood pressure and improvement of HbA1c, especially in patients with diabetes mellitus, was observed during DR-HC treatment (10). In addition, in a prospective study on 50 patients with PAI and SAI and congenital adrenal hyperplasia a significant reduction of BMI and HbA1c was observed in patients treated with DR-HC, compared with patients on conventional GC treatment (11).

A subsequent study, evaluating the switch from conventional HC to DR-HC in 19 patients with AI, showed a significant reduction of WC, HbA1c and LDL-cholesterol after 12 months of DR-HC therapy (12).

By contrast, in a Swedish 5-year study on 71 patients with PAI treated with DR-HC, an increase in fasting glucose and HDL-C was observed (22), without any change in anthropometric parameters.

In agreement with previous studies, we found a decrease in BMI, WC and HbA1c and an increase in HDL-C in all patients treated with DR-HC.

As expected, patients with pre-diabetes had higher baseline $AUC_{2h\ glycaemia}$, insulinemia, $AUC_{2h\ insulinemia}$ and lower ISI-Matsuda, than patients with NGT. In the group of patients with pre-diabetes we also found a significant improvement in insulin secretion and sensitivity, which to our knowledge has not been investigated previously.

Treatment with conventional GCs is known to affect glucose metabolism, inducing peripheral insulin resistance by impairing insulin signalling, resulting in decreased glucose disposal and increased glucose production (23). In response to GC-induced peripheral insulin resistance and trying to maintain normoglycaemia, β-pancreatic cells undergo several adaptations resulting in hyperinsulinemia (24). Patients receiving long-term GC replacement therapy develop abnormal glucose tolerance more frequently than healthy individuals (1), due to supraphysiological GC exposure in the early evening, which has asymptomatic but adverse metabolic effects (25).

In a study evaluating the effect of acute and chronic low doses of prednisolone in patients with rheumatoid arthritis, insulin sensitivity worsening and postprandial glucose increase were observed (26).

As expected, higher insulin requirement was shown in a study conducted on patients with type 1 diabetes mellitus and PAI, compared to patients with type 1 diabetes mellitus alone (25).

In the current study the improvement in insulin secretion in patients with pre-diabetes is demonstrated by the reduction of insulinemia and the increase in insulin secretion relative to insulin resistance (DIo). Indeed, DIo, which expresses the ability of β-cells to adequately compensate insulin resistance through increased insulin secretion, is a predictable factor of

diabetes development in adults (15) and for this reason it may be useful to evaluate this risk related to overtreatment with GC in AI patients.

On the other hand, ISI-Matsuda is a useful tool to evaluate the insulin resistance degree of the whole body. It derives from OGTT and more closely correlates with the M-value of the euglycaemic hyperinsulinemic clamp, which represents the gold standard of insulin sensitivity measurement (14). The increase in ISI-Matsuda during the 36 months of DR-HC in patients with pre-diabetes clearly shows an improvement in insulin sensitivity.

Interestingly, the variation of the daily oral HC dose was not demonstrated to affect insulin sensitivity and/or secretion (27), while the change in the scheme of administration provided discordant results. In a Norwegian study evaluating an HC infusion pump system, able to mime the physiological cortisol profile, used for 3 months in 7 patients with AI, reestablishment of the cortisol circadian rhythm and an improvement of quality of life (28) were observed. Conversely, another study conducted on 15 patients with SAI, receiving either a low dose of oral HC replacement therapy or a physiological hydrocortisone infusion, did not demonstrate any change in insulin sensitivity (29).

The term cardio metabolic risk was coined by the American Diabetes Association (30) and the American Heart Association (31) to describe the overall risk of developing type 2 diabetes and cardiovascular disease. To predict visceral adipose tissue-associated cardio metabolic risk, the VAI has been demonstrated to be a useful tool (13). Visceral fat is universally claimed to be more strongly associated with cardio metabolic risk than subcutaneous fat (32), due to its endocrine function. The VAI has been shown to correlate well with the adipokine profile in patients with type 2 diabetes (33) and to provide a good estimate of visceral adipose dysfunction, reflecting the cardio metabolic risk in many diseases (34).

In the current study, 36-month treatment with DR-HC in patients with pre-diabetes was associated with a significant improvement of cardio metabolic risk, indirectly estimated by the VAI. In addition, after 36 months of DR-HC, the VAI tended to be < 2, the cut-off to identify patients without visceral adipose dysfunction (35).

The main limitations of the present study are the retrospective design and the small number of patients enrolled. Patients were not blinded to the treatment and therefore their expectations on the new drug may have partially affected the results. Another limitation is the lack of evaluation of other factors that may impact on metabolic control in patients with pre-diabetes. Lastly, the cohort of patients is quite heterogeneous (patients with PAI and SAI) and is characterized by different metabolic alterations. Indeed, patients with SAI may have additional metabolic alterations due to other hormonal deficiencies. However, all patients enrolled in the study were on stable replacement treatment and maintained a good and stable hormonal control during the whole follow-up, so not affecting, in our opinion, the outcomes of the study.

Despite these limitations, we think that the strength of the study is that it represents the first study assessing insulin sensitivity and secretion and a surrogate index of cardio metabolic risk in patients with PAI and SAI treated with DR-HC.

In conclusion, long-term DR-HC therapy is associated with an improvement of insulin secretion and sensitivity in patients with pre-diabetes. However, both patients with NGT and pre-diabetes appear to benefit from treatment with DR-HC in terms of improvement of visceral adiposity, glycaemic control and HDL-C levels.

Further case-controlled studies performed in a larger cohort of patients are required in order to verify our preliminary data.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

References

1. Johannsson G, Falorni A, Skrtic S et al. Adrenal insufficiency: review of clinical outcomes with current glucocorticoid replacement therapy. *Clin Endocrinol (Oxf).* 2015; 82: 2–11

2. 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.* 2006; 91:4849–53.

3. Bleicken B, Hahner S, Loeffler M et al. Influence of hydrocortisone dosage scheme on health-related quality of life in patients with adrenal insufficiency. *Clin Endocrinol (Oxf).* 2010; 72:297–304.

4. Werumeus Buning J, van Faassen M, Brummelman P et al. Effects of Hydrocortisone on the Regulation of Blood Pressure: Results From a Randomized Controlled Trial. *J Clin Endocrinol Metab*. 2016; 101:3691-99.

5. Danilowicz K, Bruno OD, Manavela M, Gomez RM, Barkan A. Correction of cortisol overreplacement ameliorates morbidities in patients with hypopituitarism: a pilot study. *Pituitary* 2008; 11:279–85.

6. Werumeus Buning J, Brummelman P, Koerts J et al. Hydrocortisone Dose Influences Pain, Depressive Symptoms and Perceived Health in Adrenal Insufficiency: A Randomized Controlled Trial. *Neuroendocrinology* 2016; 103: 771-8.

7. Johannsson G, Bergthorsdottir R, Nilsson AG, Lennernas H, Hedner T, Skrtic S. Improving glucocorticoid replacement therapy using a novel modified-release hydrocortisone tablet: a pharmacokinetic study. *Eur J Endocrinol.* 2009; 161:119–30.

8. Johannsson G, Lennernas H, Marelli C, Rockich K. Achieving a physiological cortisol profile with once-daily dual-release hydrocortisone: a pharmacokinetic study. *Clin Endocrinol (Oxf).* 2016; 175:85-93.

9. Nilsson AG, Marelli C, Fitts D et al. Prospective evaluation of long-term safety of dual-release hydrocortisone replacement administered once daily in patients with adrenal insufficiency. *Eur J Endocrinol.* 2014; 171:369-77.

10. Johannsson G, Nilsson AG, Bergthorsdottir R et al. Improved cortisol exposure-time profile and outcome in patients with adrenal insufficiency: a prospective randomized trial of a novel hydrocortisone dual-release formulation. *J Clin Endocrinol Metab.* 2012; 97:473–81.

11. Quinkler M, Miodini Nilsen R, Zopf K, Ventz M, Øksnes M. Modified-release hydrocortisone decreases BMI and HbA1c in patients with primary and secondary adrenal insufficiency. *Eur J Endocrinol.* 2015; 172:619–26.

12. Giordano R, Guaraldi F, Marinazzo E et al. Improvement of anthropometric and metabolic parameters, and quality of life following treatment with dual-release hydrocortisone in patients with Addison's disease. *Endocrine* 2016; 51:360-68.

13. Bornstein SR, Allolio B, Arlt W, Barthel A, Don-Wauchope A, Hammer GD, Husebye ES, Merke DP, Murad MH, Stratakis CA, Torpy DJ. Diagnosis and Treatment of Primary Adrenal Insufficiency: An Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab. 2016 Feb;101(2):364-89

14. Amato MC, Giordano C, Galia M et al. Visceral adiposity index: a reliable indicator of visceral fat function associated with cardiometabolic risk. *Diabetes Care* 2010; 33:920–22.

15. Matsuda M, DeFronzo R. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care* 1999; 22:1462–70.

16. Utzschneider KM, Prigeon RL, Faulenbach MV et al. Oral disposition index predicts the development of future diabetes above and beyond fasting and 2-h glucose levels. *Diabetes Care* 2009; 32:335–41.

17. Horrocks PM, Jones AF, Ratcliffe WA et al. Patterns of ACTH and cortisol pulsatility over twenty-four hours in normal males and females. *Clin Endocrinol (Oxf).* 1990; 32:127-34.

18. Bensing S, Brandt L, Tabaroj F et al. Increased death risk and altered cancer incidence pattern in patients with isolated or combined autoimmune primary adrenocortical insufficiency. *Clin Endocrinol (Oxf).* 2008; 69: 697–704.

19. Hammarstrand C, Ragnarsson O, Hallén T et al. Higher glucocorticoid replacement doses are associated with increased mortality in patients with pituitary adenoma. *Eur J Endocrinol.* 2017; 177: 251-56

20. Forss M, Batcheller G, Skrtic S, Johannsson G. Current practice of glucocorticoid replacement therapy and patient-perceived health outcomes in adrenal insufficiency – a worldwide patient survey. *BMC Endocr Dis.* 2012; 12:1-8.

21. Johannsson G, Lennernas H, Marelli C, Rockich, Skrtic S. Achieving a phyisiological cortisol profile with once-daily dual-release hydrocortisone: a pharmacokinetic study. *Eur J Endocrinol.* 2016; 175:85-93.

22. Nilsson AG, Bergthorsdottir R, Burman P et al. Long-term safety of once-daily, dual-release hydrocortisone in patients with adrenal insufficiency: a phase 3b, open-label, extension study. *Eur J Endocrinol.* 2017; 176:715-25.

23. Malerbi D, Liberman B, Giurno-Filho A, Giannella-Neto D, Wajchenberg BL. Glucocorticoids and glucose metabolism: hepatic glucose production in untreated Addisonian patients and on two different levels of glucocorticoid administration. *Clin Endocrinol (Oxf).* 1988; 28:415-22.

24. Rafacho A, Ortsater H, Nadal A, Quesada I. Glucocorticoid treatment and endocrine pancreas function: implications for glucose homeostasis, insulin resistance and diabetes. *Eur J Endocrinol.* 2014; 223:R49-R62.

25. Elbelt U, Hahner S, Allolio B. Altered insulin requirement in patients with type 1 diabetes and primary adrenal insufficiency receiving standard glucocorticoid replacement therapy. *Eur J Endocrinol.* 2009; 160:919–24.

26. Radhakutty A, Mangelsdorf BL, Drake SM et al. Effects of acute and chronic glucocorticoid therapy on insulin sensitivity and postprandial vascular function. *Clin Endocrinol (Oxf).* 2016; 84:501-08.

27. Peterson CJ, Mangelsdorf B, Thompson CH, Burt MG. Acute effect of increasing glucocorticoid replacement dose on cardiovascular risk and insulin sensitivity in patients with adrenocorticotrophin deficiency. *J Clin Endocrinol Metab.* 2014; 99:2269-76.

28. Løvås K, Husebye ES. Continuous subcutaneous hydrocortisone infusion in Addison's disease. *Eur J Endocrinol.* 2007; 157:109–12.

29. McConnell EM, Bell PM, Ennis C et al. Effects of low-dose oral hydrocortisone replacement versus short-term reproduction of physiological serum cortisol concentrations on insulin action in adult-onset hypopituitarism. *Clin Endocrinol (Oxf).* 2002; 56:195-201.

30. Eckley ET. New ADA initiative moves beyond'metabolic syndrome'.'Cardiometabolic risk'proposed as umbrella term for diabetes risk factors. *DOC News* 2006; 3:1-3.

31. Eckel RH, Kahn R, Robertson RM, Rizza RA. Preventing cardiovascular disease and diabetes: a call to action from the American Diabetes Association and the American Heart Association. *Circulation* 2006; 113:2943-46.

32. Fox CS, Massaro JM, Hoffmann U et al. Abdominal visceral and subcutaneous adipose tissue compartments: association with metabolic risk factors in the Framingham Heart Study. *Circulation* 2007; 116:39-48.

33. Amato MC, Pizzolanti G, Torregrossa V, Misiano G, Milano S, Giordano C. Visceral adiposity index (VAI) is predictive of an altered adipokine profile in patients with type 2 diabetes. *PLoS One* 2014; 9: e91969

34. Amato MC, Guarnotta V, Forti D, Donatelli M, Dolcimascolo S, Giordano C. Metabolically healthy polycystic ovary syndrome (MH-PCOS) and metabolically unhealthy polycystic ovary syndrome (MU-PCOS): a comparative analysis of four simple methods useful for metabolic assessment. *Hum Reprod.* 2013; 28:1919–28.

35. Amato MC, Giordano C, Pitrone M, Galluzzo A. Cut-off points of the visceral adiposity index (VAI) identifying a visceral adipose dysfunction associated with cardiometabolic risk in a Caucasian Sicilian population. *Lipids Health Dis.* 2011; 10:183.

Legends to figures

Legend to figure 1

Changes in metabolic parameters during 36 months of DR-HC treatment in patients with AI-NGT (dark grey line) and pre-diabetes (light grey line). For trend: *p<0.05, **p <0.01; ***p <0.001. NS= not significant.

Legend to figure 2

Changes in insulin sensitivity and secretion parameters during 36 months of DR-HC treatment in patients with AI-NGT (dark grey line) and pre-diabetes (light grey line). For trend: *p<0.05, **p <0.01; ***p <0.001. NS= not significant.

Table 1. Dose adjustments according to the physician's judgement during the 36 months of dual-release hydrocortisone treatment

	Dose at 36 months				
Baseline dose	**20 mg/day**	**25 mg/day**	**30 mg/day**	**35 mg/day**	**40 mg/day**
20 mg/day (n= 28)	21	4	2	0	1
25 mg/day (n= 8)	1	3	2	1	1
30 mg/day (n= 3)	0	0	1	0	2
40 mg/day (n= 10)	0	0	0	0	10

Table 2. Baseline clinical and phenotypic features of patients grouped according to glucose tolerance in patients with normal glucose tolerance (NGT) and pre-diabetes

	Patients with NGT **N= 25**	**Patients with pre-diabetes** **N= 24**	
	Subjects (%)	***Subjects (%)***	***p***
Gender			
Male	10 (40%)	10 (42%)	0.789
Female	15 (60%)	14 (58%)	
Primary adrenal insufficiency (PAI)	4 (16%)	20 (83%)	0.001
Secondary adrenal insufficiency (SAI)	21 (84%)	4 (17%)	0.001
	Mean ± SD	***Mean ± SD***	
Age	52.4 ± 12.6	50.4 ± 15.9	0.838
Clinical parameters			
BMI (Kg/m^2)	25.1 ± 4.3	32.6 ± 5.3	0.001
Waist circumference (cm)	91.5 ± 12.1	108.9 ± 8.4	<0.001
Systolic blood pressure (SBP) (mmHg)	109.3 ± 17.5	120 ± 17.2	0.104
Diastolic blood pressure (DBP) (mmHg)	67.3 ± 9.6	72 ± 8.6	0.172
Electrolytes			
Na (mmol/L)	140.5 ± 2.6	139 ± 3.5	0.129
K (mmol/**L)**	4.3 ± 0.3	4.3 ± 0.3	0.744
Metabolic parameters			
Total cholesterol (mmol/l)	207.1 ± 33.6	235.1 ± 75.6	0.436
HDL cholesterol (mmol/l)	59.2 ± 18.2	53.6 ± 14.1	0.389
Triglycerides (mmol/l)	141.1 ± 77.5	172.8 ± 50.3	0.098
LDL cholesterol (mmol/l)	119.5 ± 35.9	146.8 ± 61.8	0.187
Fasting glycaemia (mmol/l)	4.3 ± 0.6	5 ± 1.1	0.161
HbA1c (mmol/mol)	36.6	39.8	0.029
HbA1c (%)	5.5 ± 0.4	5.8 ± 0.6	0.029
AUC 2h glycaemia (mmol/l 120 min)	749.6 ± 146.9	1063 ± 222.5	<0.001
VAI	2.1 ± 1.6	2.5 ± 0.8	0.089
Insulin secretion and sensitivity indexes			
Fasting insulinemia (uU/ml)	6.3 ± 3.9	13.3 ± 9.6	0.002
DIo	2.8 ± 1.8	2.7 ± 1.8	0.713
AUC 2h insulinemia (uU/ml 120 min)	5647.7 ± 3505.4	16980 ± 5613.1	<0.001
ISI-Matsuda	6.5 ± 3.3	2.4 ± 1.1	<0.001