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Original Research

Effects of metyrapone in patients with mild hypercortisolism

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Abstract

Background: Mild Hypercotisolism (mH) is associated with increased cardiometabolic risk despite limited clinical signs. Surgery is the first-line treatment, with medical therapies reserved when surgery is not feasible. However, data on its efficacy in mH, particularly for blood pressure (BP) control, remain limited. The aim of the present study was to evaluate the impact of low-dose evening metyrapone on 24-hour ambulatory BP, glucose metabolism, and cortisol circadian rhythm in patients with mH.

Methods: In this prospective, single-centre observational study (NCT05255900), 20 patients with mH were treated with evening metyrapone (250-500 mg/day) for 24 weeks. Inclusion criteria included cortisol >1.8 µg/dL after 1 mg dexamethasone suppression, <2 specific Cushing syndrome related symptoms (ie, easy bruising, facial plethora, proximal myopathy, purple striae) and presence of hypertension or glucose metabolism impairment. BP control, glucometabolic profile, and salivary cortisol rhythms were assessed at baseline, 12, and 24 weeks.

Results: At 24 weeks, 40% of patients (8/20) achieved a predefined clinically significant BP improvement (≥5 mmHg reduction in mean 24-h systolic BP without therapy escalation). This effect was more pronounced in patients with elevated baseline systolic BP. Glucometabolic control improved in 4 patients, notably in those with type 2 diabetes, with a reduction in HbA1c ≥ 0.5% in two patients without changes in antidiabetic therapy. Metyrapone was well tolerated, with no adverse events or adrenal insufficiency reported.

Conclusions: Low-dose evening metyrapone appears to be a safe and potentially effective option for improving BP control in patients with mH, especially those with higher baseline systolic pressure.

Keywords: metyrapone, hypercortisolism, blood pressure, cortisol circadian rhythm

Introduction

Cushing syndrome (CS) is a disease characterized by excessive cortisol exposure leading to an increased risk of cortisol excess-related comorbidities (ie,. bone frailty, diabetes mellitus, hypertension, risk of thrombotic events, infections, mood disorders) and of mortality. It can be caused by exogenous glucocorticoids administration or by an increased endogenous production (for example caused by an adrenal adenoma, adrenocortical carcinoma, pituitary adenoma, neuroendocrine neoplasia producing ACTH).[1-9] Weight gain, moon facies, striae rubrae, buffalo hump, skin frailty, easy bruising and muscular hypotrophy are the most common clinical signs at presentation, and they can be considered sensitive but not specific findings.[8]

In the last decade, the arising awareness on hypercortisolism impact on health and the use of more sensitive and systematic screening tools brought to clinical attention patients affected by milder degrees of excessive cortisol secretion (mild Hypercortisolism, mH). Even if this phenotype can present with few or no clinical signs, an increased risk of all cortisol related comorbidities and mortality has been reported also for these patients.[10-13] Recent works from different groups outlined the possible phenotypical and clinical overlap between Cushing syndrome and mH, making clear that cortisol production, and resulting phenotypes, should be considered and managed as a continuum.[14-16] Surgical intervention is considered the best therapeutical approach for CS but some data outline also its potential benefit for milder degrees of cortisol secretion.[17-19] When surgical intervention is not feasible for clinical contraindications or patient's preference, medical therapy with steroidogenesis inhibitors is a treatment option.

Metyrapone is an inhibitor of adrenal steroidogenesis acting mainly against adrenal 11-β hydroxylase, a crucial enzyme for the conversion from the 11-deoxycortisol to cortisol. The metyrapone half-life is short (2 hours), a pharmacokinetic that renders this drug very manageable. Importantly, the available data on CS have suggested that this drug has good efficacy and high safety,[20-23] leading to its approval for the management of endogenous CS in US and Europe.

Interestingly, some data suggested the possible benefit of using steroidogenesis inhibitors in the form of chronotherapy as late-afternoon/evening daily administration.[24,25] The clinical efficacy on blood pressure (BP), glucose metabolism and cortisol circadian profile alterations of long-term use of metyrapone in mH have still to be investigated.

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Therefore, the aim of the present study was to evaluate the impact of low-dose evening metyrapone on 24-hour ambulatory BP, glucose metabolism, and cortisol circadian rhythm in patients with mH.

Patients, methods and predefined outcomes

Patients

This is a prospective, single-centre, observational study (registered with ClinicalTrial.gov: NCT05255900). The study was carried out between April 21st, 2022, and May 31st, 2025. Patients referring to the general endocrinological outpatient-clinic were referred to the second-level outpatient clinics for "Cushing's syndrome" due to the presence of multiple and/or progressive features (among hypertension, osteoporosis, diabetes), which are known to be possibly linked to cortisol excess. Twenty-two patients were enrolled according to the following criteria: i) age 50-85 years; ii) cortisol after 1 mg overnight dexamethasone suppression test (F-1mgDST) > 1.8 µg/dL confirmed on at least two separate determinations; iii) < 2 Cushing's syndrome related symptoms (ie, easy bruising, facial plethora, proximal myopathy, purple striae > 1 cm wide); iv) initiation of metyrapone therapy (250 mg/day) less than one week earlier; v) urinary free cortisol (UFC) < 1.5× upper limit of normal range; vi) ≥ 1 among: hypertension, impaired fasting glucose, glucose intolerance, type 2 diabetes mellitus.

Exclusion criteria were: i) malignant hypertension and/or BP <200/120 mmHg and/or severe hyperglycaemia (ie, FG >350 mg/dL); ii) presence of pheochromocytoma or primary hyperaldosteronism; iii) possible adrenal metastases or radiological features suggestive for adrenal malignancy (ie, not homogeneous pattern, necrosis, calcifications, irregular margins, local invasion and high density at computed tomography); iv) congenital adrenal hyperplasia; v) intake of drugs influencing cortisol metabolism and/or secretion; vi) women in child-bearing age; vii) patients with body mass index (BMI) > 35 kg/m^2.

Two patients dropped out at week 4 complaining of vague non-specific malaise and decided to stop the therapy with metyrapone. These symptoms did not require medical attention and biochemical data of cortisol secretion excluded adrenal insufficiency. Twenty patients completed the study period.

The study was conducted in accordance with the ethical standards of the Declaration of Helsinki and was approved by the Istituto Auxologico Italiano Ethical Committee on October 19th, 2021. All study participants provided written informed consent.

The Figure 1 reports the flow diagram of patient selection and treatment allocation.

Methods

Patients were evaluated at baseline, 12, and 24 weeks according to an internal hospital protocol for good clinical practice used to follow-up patients taking steroidogenesis inhibitors. Twenty patients completed the study (13 females, 7 males). In all patients, assessments included a comprehensive clinical examination and laboratory tests for hypothalamic–pituitary–adrenal axis evaluation, such as morning serum cortisol, adrenocorticotroph hormone (ACTH), UFC, and androgens (testosterone, dehydroepiandrosterone sulphate, 17-hydroxy-progesterone), as well as electrolytes, renal and liver function, . Patients with low-suppressed ACTH values underwent abdominal CT scan resulting in a unilateral (14 patients) or bilateral adrenal adenoma (4 patients). For patients with normal-high ACTH values, additional dynamic tests were made to ascertain hypercortisolism aetiology, in particular desmopressin stimulation test and corticotropin stimulation test. In two patients dynamic tests were indicative of ACTH dependent aetiology and a pituitary microadenoma (larger than 6 mm) as found by magnetic resonance imaging. These patients did not present features suggestive for pseudo-Cushing (psychiatric disorders, alcohol use disorder, polycystic ovary syndrome). None of the patients was a shift worker. Cortisol, ACTH, testosterone and dehydroepiandrosterone sulphate were measured by electrochemiluminescence (Cobas, Elecysis II, Roche Diagnostics GmbH, Mannheim, Germany), while 17-hydroxy-progesterone by chemiluminescence (Technogenetics, Milan, Italy). Glucometabolic assessment included fasting glucose (FG) and glycated haemoglobin (HbA1c) levels, and, in non-diabetic patients, a 2-hour oral glucose tolerance test (OGTT). Insulin resistance was calculated using homeostatic model assessment (HOMA-IR) ([fasting insulin × fasting glucose]/405), with a threshold of ≥2.5 indicating resistance.[26] The homeostatic model assessment β (HOMA-B: [fasting insulin × 20]/[(fasting glucose/18)−3.5])[27] was used as β-cell function index. Cortisol circadian rhythm was evaluated through salivary cortisol sampling at 08:00, 12:00, 16:00, 20:00 and 23:00 and expressed as total and partial area under the curve (AUC).

All patients underwent 24-hour ambulatory blood pressure monitoring (ABPM) at baseline, week 12, and week 24. Metyrapone was administered as a single evening dose (250 mg) at 20:00, with dose adjustments based on clinical response and cortisol secretion.

Following the protocol used at our centre for subjects with mild hypercortisolism treated with metyrapone, in 12 patients with no signs of hypoadrenalism (ie, severe asthenia, hypotension, hypoglycaemia), an additional dose (250 mg) was administered at 1 PM, during mealtime, after week 12 (mean ± SD dose 400 mg/day, min 250 mg/day, max 500 mg/day) in the presence of ≥ 1 following criteria: i) daytime and/or night-time systolic and/or diastolic hypertension at ABPM (for daytime mean BP > 135/85 mmHg; for night-time mean BP > 120/70 mmHg); ii) HbA1c > 7% in diabetic patients; iii) fasting glucose >100 mg/dL and/or glucose after OGTT >140 mg/dL in non-diabetic patients.[28,29]

Predefined outcomes

The primary endpoint was the effect of metyrapone at 24 weeks on blood pressure control, defined as the proportion of patients achieving a reduction in mean 24-hour systolic BP of ≥5 mmHg without any increase in antihypertensive medication dosage and/or in the number of medications.

The secondary endpoints included: i) the proportion of patients without type 2 diabetes achieving normalization of FG (<100 mg/dL) and/or the reduction of 2-hour glucose levels below 140 mg/dL after OGTT; ii) the proportion of patients with type 2 diabetes achieving HbA1c < 7% among those with baseline HbA1c ≥ 7%; iii) the modification of salivary cortisol circadian rhythm.

Statistical analysis

Considering a probability of 25% cases with BP amelioration during the treatment period, we needed to include 17 patients

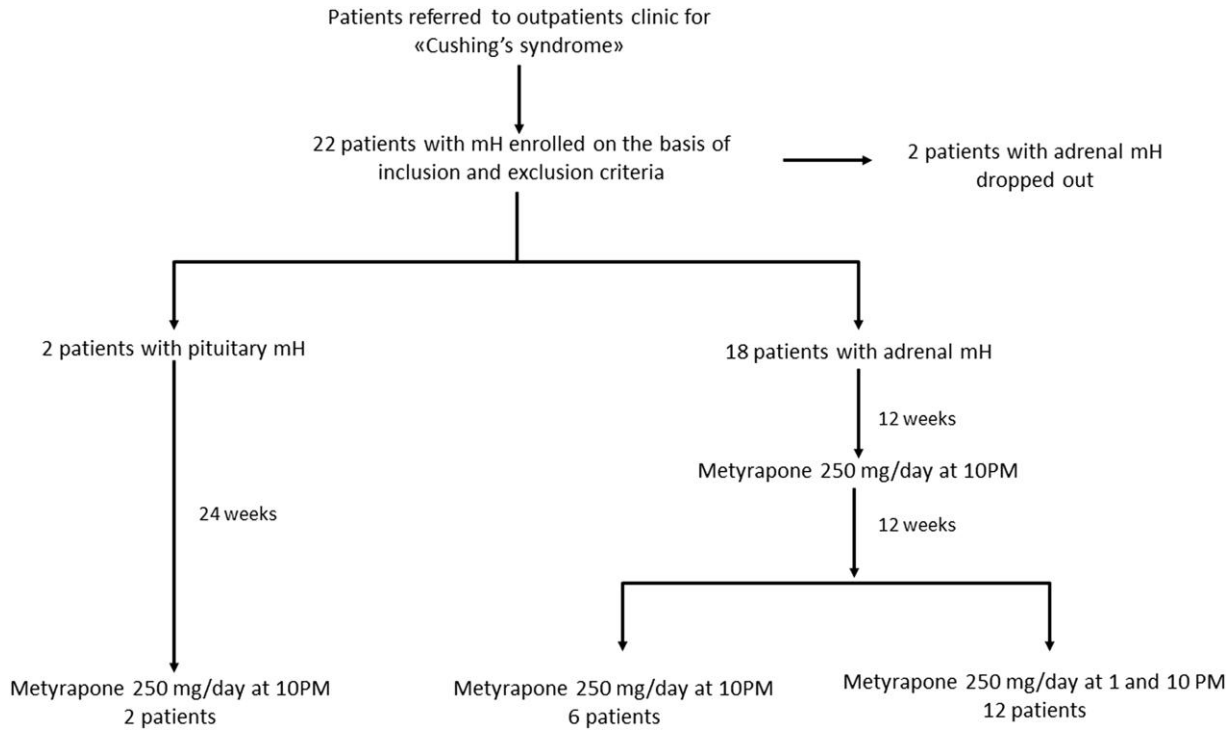

Figure 1. Flow diagram of patient selection and treatment allocation. Footnotes: In compliance with the active protocol established at our institution, patients with multiple and/or progressive features among hypertension, diabetes and osteoporosis are referred to the outpatients clinic for "Cushing's syndrome". Inclusion criteria: i) age 50-85 years; ii) cortisol after 1 mg overnight dexamethasone suppression test (F-1mgDST) > 1.8 μg/dL confirmed on at least two separate determinations; iii) < 2 Cushing's syndrome related symptoms (ie, easy bruising, facial plethora, proximal myopathy, purple striae > 1 cm wide); iv) initiation of metyrapone therapy less than one week earlier; v) urinary free cortisol (UFC) < 1.5× upper limit of normal range; vi) presence of ≥1 among: hypertension, impaired fasting glucose, glucose intolerance, type 2 diabetes mellitus. Exclusion criteria: i) malignant hypertension and/or BP <200/120 mmHg and/or severe hyperglycaemia (ie, FG >350 mg/dL); ii) presence of pheochromocytoma or primary hyperaldosteronism; iii) possible adrenal metastases or radiological features suggestive for adrenal malignancy (ie, not homogeneous pattern, necrosis, calcifications, irregular margins, local invasion and high density at computed tomography); iv) congenital adrenal hyperplasia; v) intake of drugs influencing cortisol metabolism and/or secretion; vi) women in child-bearing age; vii) patients with body mass index (BMI) > 35 kg/m2. Two patients dropped out as they refused to follow the scheduled visits. An additional dose (250 mg) of metyrapone was administered at 1 PM, during mealtime, after week 12 in the presence of ≥1 following criteria: i) daytime and/or night-time systolic and/or diastolic hypertension at ABPM (for daytime mean BP > 135/85 mmHg; for night-time mean BP > 120/70 mmHg); ii) HbA1c > 7% in diabetic patients; iii) fasting glucose >100 mg/dL and/or glucose after OGTT >140 mg/dL in non-diabetic patients. mH: mild hypercortisolism.

(type 1 error 0.01, power 0.8) in the study. Considering a potential 10% drop-out we aimed to enrol 20 patients.

Statistical analysis was performed by SPSS version 28.0 statistical package (IBM Corporation), JMP (JMP® Pro, Version 18. SAS Institute Inc., Cary, NC, 1989-2021), and GraphPad Prism version 10 (GraphPad Software). Sankey diagrams were created through the open-source SankeyMATIC (sankeymatic.com). Figures have been done by Python software.

Continuous variables are presented as median and interquartile range (IQR), while categorical variables are reported as counts and percentages. Comparisons of non-parametric continuous data were performed using the Wilcoxon rank-sum test for independent groups and the Wilcoxon signed-rank test for paired data. Categorical variables were compared using the χ^2 test or Fisher's exact test, as appropriate. For paired categorical data, the McNemar test was applied.

A *P*-value <.05 was considered statistically significant.

Results

Eighteen patients were affected with ACTH-independent mH due to adrenal adenomas, while 2 patients were diagnosed with ACTH secreting pituitary adenomas. The demographic, clinical, and biochemical characteristics of patients are reported in Table 1 and Table S1. UFC was completely normal in 16 patients.

The behaviour of BP and glucometabolic control after the initiation of the therapy with metyrapone is summarized in Table 2. At baseline, 19/20 patients already had a diagnosis of hypertension and were on medical treatment. No patient had to increase their therapy in terms of dosage or the number of medications during the follow-up. A clinically significant improvement in blood pressure control (≥ 5 mmHg reduction in mean 24-hour systolic blood pressure without treatment intensification) was observed in 8 out of 20 patients at 24 weeks. This improvement remained statistically significant when daytime and night-time systolic BP were analysed separately. The normalization of the dipping pattern at 24 weeks was noted in 2 out of 8 patients with a non-dipper blood pressure profile at baseline. The grouped data and individual data on the behaviour of BP during the study period are depicted in Figures 2 and 3, respectively. At 24 weeks, in 8 (40%), 10 (50%) and 2 (10%) patients, the BP control was ameliorated, stable and worsened, respectively (Figure 2). Interestingly, the BP amelioration was more evident in the 6 patients with elevated systolic BP at baseline (Figure 3). A summary of detailed data on systolic and diastolic BP measured by 24-hour ABPM

Table 1. Clinical and biochemical characteristics of patients (n = 20) with mild hypercortisolism at baseline.

mCS etiology (adrenal/pituitary, n.)	18/2 (90/10%)
Sex (F/M n.)	13/7 (65/35%)
Age (years)	70.5 (59.5-70.2)
Body weight (kg)	78 (69.5-84.8)
Waist Circumference (cm)	102.5 (91.5-114)
Body Mass Index, BMI (kg/m2)	29.3 (25.8-32)
Hypertension (n.)	19 (95%)
IFG/IGT (n.)	7 (35%)
Diabetes mellitus (n.)	8 (40%)
ACTH (pg/mL)	8.8 (6.5-20.8)
Cortisol (µg/dL)	15.3 (13.1-19.3)
UFC (µg/24 h)	49.7 (29.3-101.5)
LNSC (µg/dL)	0.1 (0.06-0.14)
MSC (µg/dL)	0.4 (0.2-0.55)
1 mg DST (µg/dL)	2.9 (2.6-4.6)
17OHP (ng/mL)	0.31 (0.31-0.56)
DHEA-s (µg/dL)	0.43 (0.27-0.86)
Testosterone—women (ng/mL)	0.22 (0.09-0.85)
Testosterone—men (ng/mL)	16.2 (11.2-17.9)
Testosterone—overall (ng/mL)	0.85 (0.12-13)
24-h SBP (mmHg)	128 (116-140)
24-h DBP (mmHg)	75.5 (71-81)
Daytime SBP (mmHg)	122 (115-134)
Daytime DBP (mmHg)	72 (67.5-79)
Nighttime SBP (mmHg)	116 (107-124)
Nighttime DBP (mmHg)	64 (61-73)
HbA1c in pts. without T2D (%)	6.3 (5.7-6.9)
HbA1c in pts with T2D (%)	7.2 (6-8.4)
Blood fasting glucose (mg/dL)	121.2 (105.2-137.2)
Cushing syndrome clinical signs	
Easy bruising (n.)	3 (15%)
Facial plethora (n.)	5 (25%)
Purple striae (n.)	0 (0%)
Proximal muscle myopathy (n.)	4 (20%)
Skin and hair frailty (n.)	3 (15%)
Central obesity (n.)	11 (55%)
Dorsocervical fat pad (n.)	6 (30%)

Data are shown as median with range in brackets or number with percentage in brackets. BMI: body mass index; IFG: impaired fasting glucose; IGT: impaired glucose tolerance; UFC, urinary free cortisol; MSC: salivary cortisol measured at 08:00; LNSC: salivary cortisol measured at 23:00. HbA1c: glycated haemoglobin ACTH: adrenocorticotroph hormone (normal values 5-55 pg/mL); 17OHP: 17 hydroxyprogesterone (normal values: males 0.32-3.32 ng/mL, post-menopausal women 0.32-2.72 ng/mL). DHEA-s: dehydroepiandrosterone sulphate (normal values: males 0.8-5.6 µg/dL, women 0.35-4.3 µg/dL). UFC: urinary free cortisol (normal values: 11.5-102.0 µg/24 hour); MSC: morning (8:00 AM) salivary cortisol (normal values 0.1-0.35 µg/dL); LNSC: late night (11:00 PM) salivary cortisol (normal values: < 0.35 µg/dL). Testosterone normal values: men 9.9-27.8 nmol/L, women 0.2-2.9 nmol/L) HbA1C: glycated haemoglobin; 1 mg DST: cortisol post 1 mg dexamethasone suppression test (normal values: < 1.8 µg/dL).

and of HbA1C levels at the different time-points is reported in Table 3.

We observed an improvement of the glucometabolic control at 24 weeks in 4 out of 20 patients, 3 of whom presenting with type 2 diabetes at baseline. In patients without type 2 diabetes, no statistically significant differences were observed in glucometabolic control between baseline and week 24 (Table 4).

Interestingly, the 37.5% of patients with type 2 diabetes at baseline showed an improvement of glucometabolic control, even though the statistical significance was not reached, possibly due to the small sample size (Table 2). A general trend towards HbA1c reduction was also observed over the treatment period (Table 4). The two patients with the highest baseline HbA1c values experienced the greatest benefit from steroidogenesis inhibitor therapy, showing a reduction of more than one percentage point in HbA1c (Figure 4). These patients achieved a HbA1c reduction of at least 0.5% without any increase in antidiabetic medications.

We did not observe any difference between patients that experienced the amelioration of BP and/or the glucometabolic control and those who did not, in terms of mH aetiology, metyrapone dosage or hypothalamic-pituitary-adrenal axis parameters at baseline.

No significant changes were observed in hormonal, metabolic and anthropometric parameters between baseline and week 24, except for testosterone levels in women. However, at no time point androgen levels exceeded sex- and age-specific reference ranges (Table 4).

As far as the circadian rhythm of cortisol, is concerned, after 24 weeks of treatment, no statistically significant differences were observed in salivary cortisol AUC compared to baseline, either for total AUC or for the partial intervals, even though a trend towards a more physiological circadian rhythm (ie, slightly higher and lower cortisol levels at morning and evening, respectively) was understandable (Figure 5, Table 4).

Finally, no patient experienced symptoms of adrenal insufficiency, and the treatment was well tolerated with no side-effects.

Discussion

Cushing's syndrome encompasses a wide spectrum of clinical manifestations and complications, with cardiovascular disease representing the leading cause of mortality.[30] Effective control of cortisol excess is therefore essential to mitigate these risks, even in patients with mH, who present with subtle clinical features but still experience increased morbidity and mortality.

To our knowledge, this is the first prospective study specifically designed to evaluate the effect of low dose metyrapone on BP control in patients with mH. Our findings showed that patients on 24 weeks metyrapone treatment were able to reach the primary endpoint of a reduction in mean 24-hour systolic BP of ≥5 mmHg in 40% of cases, without requiring intensification of antihypertensive therapy. These results suggest that metyrapone may provide clinically meaningful cardiovascular benefits in patients with mH. However, we did not observe a significant reduction in mean blood pressure levels measured with ABPM, outlining the potential blood pressure slight increase in some patients and an absent reduction in others counterbalancing the effects seen in ameliorated ones. On the basis of the present data we could not identify features predictive for lack of effectiveness.

Interestingly, patients with higher baseline systolic BP exhibited the greatest reductions, and the two individuals with poorly controlled diabetes achieved the most pronounced glycaemic improvements, despite reductions in antidiabetic therapy. These observations suggest that the clinical benefit of metyrapone may be greatest in patients with uncontrolled comorbidities, regardless of the degree of cortisol excess.

The cortisol-lowering effect of metyrapone was first described in 1958 by Liddle et al.[31] Despite its long-standing use, available data on metyrapone remain limited and are mostly derived from retrospective studies. The largest study to date[23] was a retrospective analysis of 195 patients, demonstrating that metyrapone effectively reduced UFC, but without evaluating the impact of the drug on clinical complications or surrogate markers such as BP or glycaemic control. Even prospective data are scarce and primarily come from the results of

Table 2. Prevalence of patients experiencing blood pressure and glucometabolic parameters amelioration over time.

	Week 12		Week 24	
SBP amelioration 24 h n. (%)	6 (30)	**$P = .031$**	8 (40)	**$P = 0.008$**
SBP amelioration daytime n. (%)	6 (30)	**$P = .031$**	9 (45)	**$P = .004$**
SBP amelioration nighttime n. (%)	7 (35)	**$P = .016$**	7 (35)	**$P = .016$**
DBP amelioration 24 h n. (%)	6 (30)	**$P = .031$**	4 (20)	$P = .125$
DBP amelioration daytime n. (%)	1 (5)	$P = 1.0$	4 (20)	$P = .125$
DBP amelioration nighttime n. (%)	1 (5)	$P = 1.0$	3 (15)	$P = .25$
DM patients with HbA1c reduction n. (%)	2 (25)	$P = .5$	3 (37.5)	$P = .25$
Glycometabolic control amelioration[a] n. (%)	2 (16.7)	$P = .5$	1 (8.30)	$P = 1.0$
Glycometabolic control amelioration n. (%)	4 (20)	$P = .125$	4 (20)	$P = .125$

Data are absolute number with percentage in square. SBP: systolic blood pressure; DPB: diastolic blood pressure; DM: diabetes mellitus; HbA1c: glycated haemoglobin.
[a]In non-DM patients.

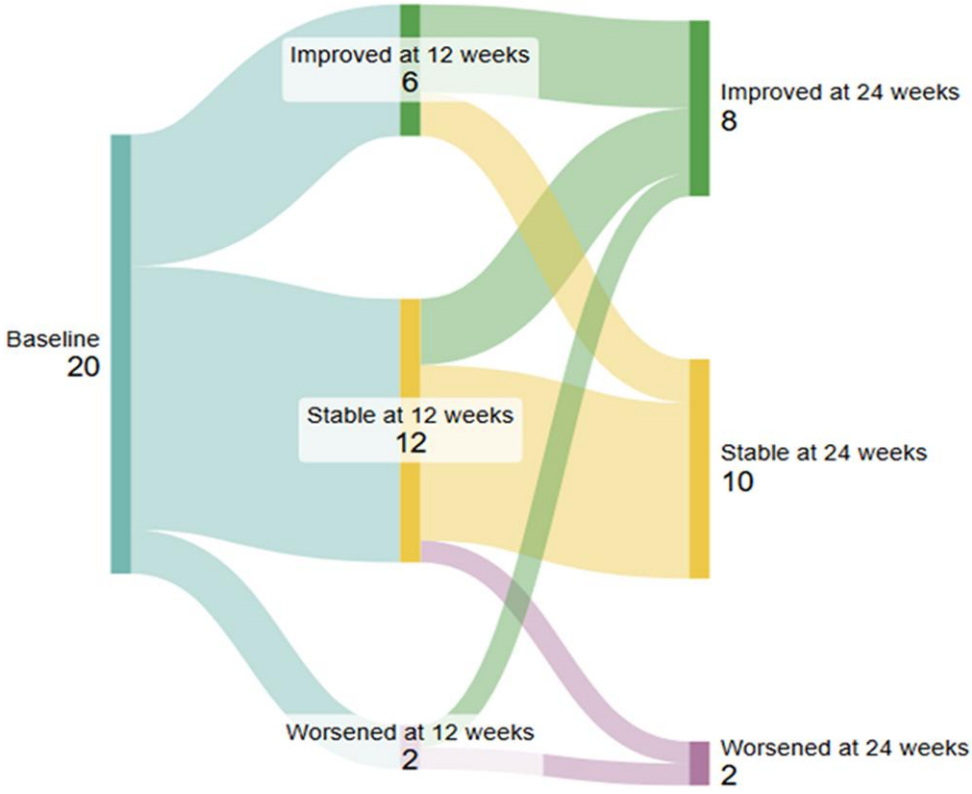

Figure 2. The individual data on the behaviour of blood pressure during the study period. Footnotes: The number represents the number of patients at each time point evaluation. At 24 weeks, two patients showed a worsening of BP control. One patient had stable BP after 12 weeks of treatment, while the second experienced a worsening at 12 weeks that partially improved by week 24, though BP levels remained elevated compared to baseline.

the PROMPT study at 36 weeks,[20] which found a normalization of UFC in 80.9%, 77.5%, and 71.4% at weeks 12, 24, and 36, respectively. It is important to note, however, that the primary endpoint of this study was the proportion of patients achieving UFC normalization, and that 96% of participants had moderate-to-severe hypercortisolism (eg, UFC 2-5× or >5x upper limit of normal range, respectively).[20]

Several lines of evidence suggest that normalization of UFC levels may not accurately reflect the optimal control of hypercortisolism. Indeed, UFC measures total cortisol secretion over 24 hours, which does not necessarily correspond to a physiologic secretion pattern. In fact, UFC can return to normal while the circadian rhythm of cortisol remains disrupted.[32] This is clinically relevant because the complications of hypercortisolism have been observed even in patients with normal UFC but persistent rhythm alterations, including those with mH.[32] At this regard, a recent pilot study in patients with CS further highlighted the importance of treatment timing, showing that the administration schedule of steroidogenesis inhibitors can influence circadian rhythm restoration and potentially improve disease control, even in patients with UFC values within the normal range.[25] Indeed, in our study some patients improved clinical outcomes even in the presence of normal UFC at baseline, outlining the need to focus attention not only on biochemical markers but also on the clinical aspects in patients with hypercortisolism.

Metyrapone, due to its short half-life, is particularly suitable for such chronotherapy approaches. In a pivotal study by Debono et al., metyrapone successfully restored an abnormal cortisol circadian rhythm and reduced interleukin-6, a marker

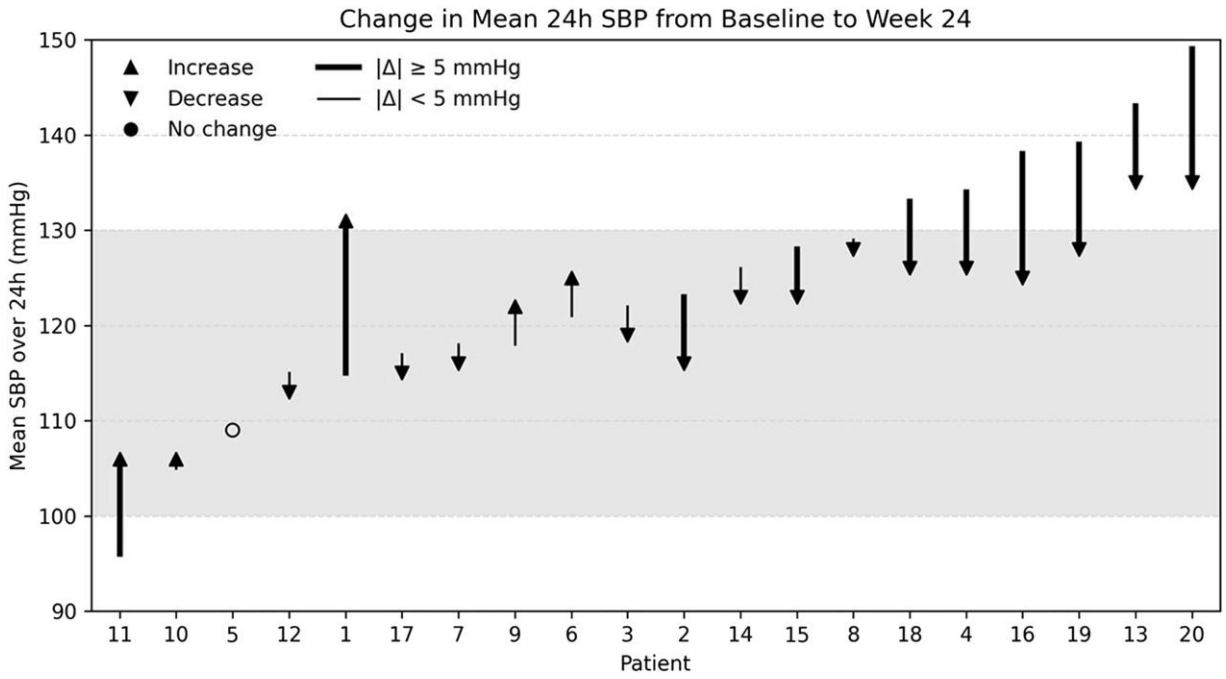

Figure 3. Change in median 24-hour systolic blood pressure (SBP) from baseline to week 24. Footnotes: Each arrow represents the individual variation in median 24-hour SBP between baseline and follow-up. Up arrows indicate an increase in SBP, while down arrows indicate a reduction. Patients 5, whose SBP remained unchanged, is represented by a black dot.

Table 3. Blood pressure and glucometabolic control parameters over time.

	Baseline	Week 12	Week 24	*P*-value
24-h SBP (mmHg)	128 (116-140)	124 (115-137)	127 (119-132)	.88
24-h DBP (mmHg)	75.5 (71-81)	71.5 (68-81)	74 (68-78)	.66
Daytime SBP (mmHg)	122 (115-134)	120 (114-131)	124 (116-128)	.81
Daytime DBP (mmHg)	72 (67.5-79)	70.5 (66-76)	70 (65-76)	.84
Nighttime SBP (mmHg)	116 (107-124)	113 (108-121)	115 (109-123)	.77
Nighttime DBP (mmHg)	64 (61-73)	64 (59-70)	65 (58-70)	.9
HbA1c in pts. without T2D (%)	6.3 (5.7-6.9)	6.2 (5.4-5.8)	6.1 (5.7-6.4)	.09
HbA1c in pts with T2D (%)	7.2 (6.0-8.4)	7.0 (5.9-8.5)	6.8 (6.3-8.4)	.25

Data are median with range in parentheses. Pts: patients.
SBP: systolic blood pressure; DPB: diastolic blood pressure measured by 24-hour ambulatory blood pressure monitoring.

of endothelial dysfunction, in six patients with mild clinical and biochemical Cushing's phenotype after six weeks of treatment, underscoring its potential utility in this setting.[24] In the present study, although no statistically significant changes were observed in salivary cortisol area under the curve, we noted a trend towards restoration of the circadian rhythm, with higher morning and lower evening cortisol levels (Figure 5). This pattern is consistent with the pharmacodynamic profile of metyrapone and its evening administration. The lack of statistical significance may be due to the small sample size, to the use of immunoassay to measure salivary cortisol, potentially leading to an overestimation of cortisol levels due to interference induced by cortisol precursors.

However, based on the present data, we cannot conclude that the trend towards restoration of cortisol circadian rhythm is responsible for outcomes improvement. Indeed, we cannot exclude that improved adherence to antihypertensive and antidiabetic medications, or lifestyle changes occurred during study time have contributed to our findings. On the other hand, we acknowledge the lack of evidence of amelioration in several endpoints (ie, number of patients with DBP amelioration, glucometabolic control amelioration). This could be due to the insufficient power of the study for those endpoints and/or to a possible therapy underdosage in therapy. Moreover, the additional dose timing (1 PM, at mealtime rather than in late afternoon), that was decided to improve gastro-intestinal tolerability, may have influenced the efficacy of the therapy. However, it is not possible to exclude that some cardiovascular system alterations are irreversible and cannot be cured even after the recovery from hypercortisolism[6] even due to the delay in the diagnoses.[9]

The strengths of our study include its prospective design and monocentric setting, which allowed for consistent methodology and minimized inter-operator variability. This facilitated reliable longitudinal comparisons and enhanced the internal validity of our findings. Moreover, the inclusion of patients with mH, who were either ineligible for or declined surgery, reflects a real-world clinical scenario, highlighting the potential role of metyrapone as a therapeutic alternative in this subset of patients.

Nonetheless, several limitations must be acknowledged. The treatment duration was relatively short, and longer-term

Table 4. Metabolic and hormonal parameters at baseline and week 24.

	Baseline	Week 24	*P*-value
Weight (kg)	78 (69.5-84.8)	76.4 (68.2-83.8)	.67
Waist circumference (cm)	102.5 (91.5-114)	103 (94-110.5)	.70
Sodium (mmol/L)	141 (139-143)	141 (139-142)	.28
Potassium (mmol/L)	4.3 (4.0-4.6)	4.2 (3.9-4.6)	.43
BMI (kg/m²)	29.3 (25.8-32.0)	28.7 (26.0-30.4)	.74
Morning serum cortisol (μg/dL)	15.3 (13.1-19.3)	13.1 (11.9-22.2)	.29
ACTH (pg/mL)	8.8 (6.5-20.7)	10.8 (6.9-17.5)	.65
17OHP (ng/mL)	0.31 (0.31–0.56)	0.42 (0.31–0.59)	.37
DHEA-s (μg/dL)	0.43 (0.27–0.86)	0.4 (0.25–0.79)	.87
Testosterone—women (ng/mL)	0.22 (0.09–0.85)	0.82 (0.38-1.59)	.04
Testosterone—men (ng/mL)	16.2 (11.2-17.9)	14.8 (10.3-18.9)	.85
Testosterone—overall (ng/mL)	0.85 (0.12-13)	1.59 (0.49-11.4)	.49
UFC (μg/24 h)	49.7 (29.3-101.5)	55.8 (29.1-80.7)	.67
MSC (μg/dL)	0.4 (0.2–0.55)	0.45 (0.28–0.63)	.61
LNSC (μg/dL)	0.1 (0.06–0.14)	0.075 (0.05–0.12)	.39
AUC salivary cortisol 8-16	134 (105-164)	142 (113-171)	.71
AUC salivary cortisol 16-23	120 (84-157)	104 (80-127)	.43
AUC salivary cortisol 24 h	202 (152-252)	179 (143-215)	.43
AUC insulin in patients without T2D	5929 (4257-7601)	8992 (4929-13055)	.22
AUC glucose in patients without T2D	20 787 (17206-24369)	20 495 (17879-23110)	.32
HbA1c in pts. without T2D (%)	6.3 (5.7-6.9)	6.1 (5.7-6.4)	.09
HbA1c in pts with T2D (%)	7.2 (6-8.4)	6.8 (6.3-8.4)	.25
Blood fasting glucose (mg/dL)	121.2 (105.2-137.2)	114.4 (104.9-123.8)	.45

Abbreviations: BMI, body mass index; UFC, urinary free cortisol; 8 AM Salivary Cortisol, salivary cortisol measured at 08:00; 11 PM Salivary Cortisol, salivary cortisol measured at 23:00. HbA1c: glycated haemoglobin. ACTH: adrenocorticotroph hormone (normal values 5-55 pg/mL); 17OHP: 17 hydroxyprogesterone (normal values: males 0.32-3.32 ng/mL, post-menopausal women 0.32-2.72 ng/mL). DHEA-s: dehydroepiandrosterone sulphate (normal values: males 0.8-5.6 μg/dL, women 0.35-4.3 μg/dL). UFC: urinary free cortisol (normal values: 11.5-102.0 μg/24 hour); MSC: morning (8:00 AM) salivary cortisol (normal values 0.1-0.35 μg/dL); LNSC: late night (11:00 PM) salivary cortisol (normal values: < 0.35 μg/dL). Testosterone normal values: men 9.9-27.8 nmol/L, women 0.2-2.9 nmol/L) HbA1C: glycated haemoglobin.

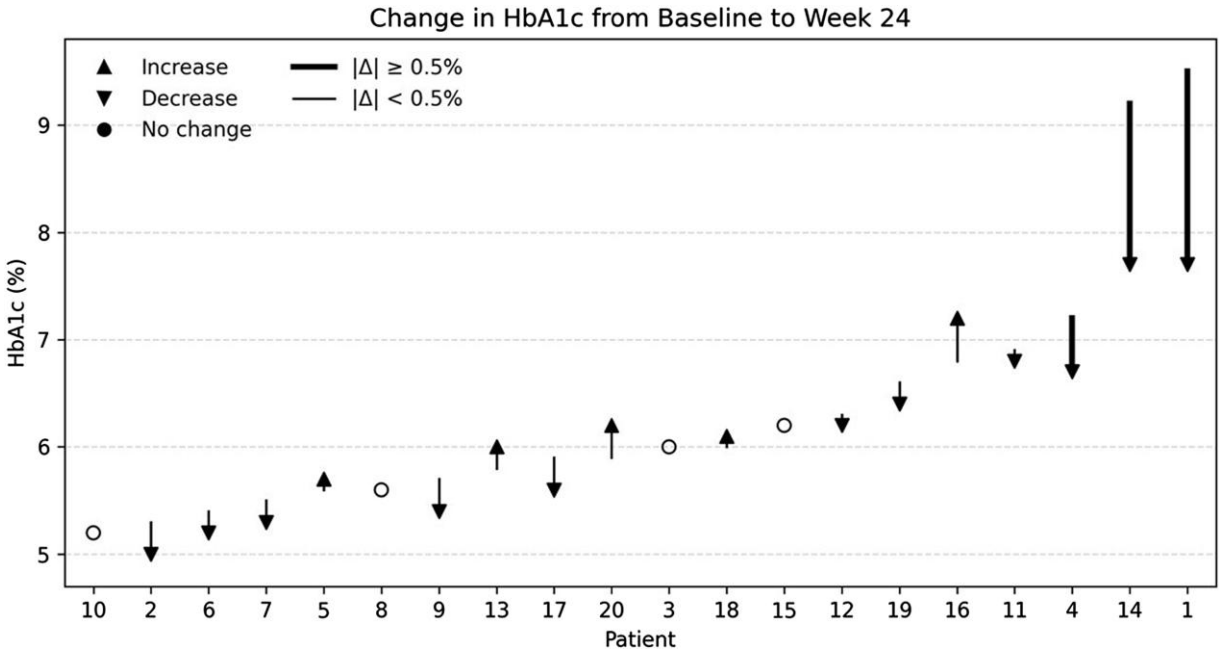

Figure 4. Change in HbA1c from baseline to week 24. Footnotes: Each arrow represents the individual variation in median 24-hour SBP between baseline and follow-up. Up arrows indicate an increase in SBP, while down arrows indicate a reduction. Patients whose HbA1c remained unchanged, are represented by a black dot.

studies are needed to assess the sustainability of the observed effects and to determine whether they persist, improve, or attenuate over time. In addition, as stated above, we cannot exclude the potential role of adherence bias. Moreover, our cohort mean age was 70.5 years, higher than the mean age reported in similar studies, probably because patients underwent hypercortisolism screening in the presence of multiple progressive comorbidities, whose number is higher in elderly subjects and because they were not considered suitable for surgery in many cases. This can restrain the generalizability of our data. The sample size, though adequate for the primary endpoint, was anyway limited, reflecting both the rarity of

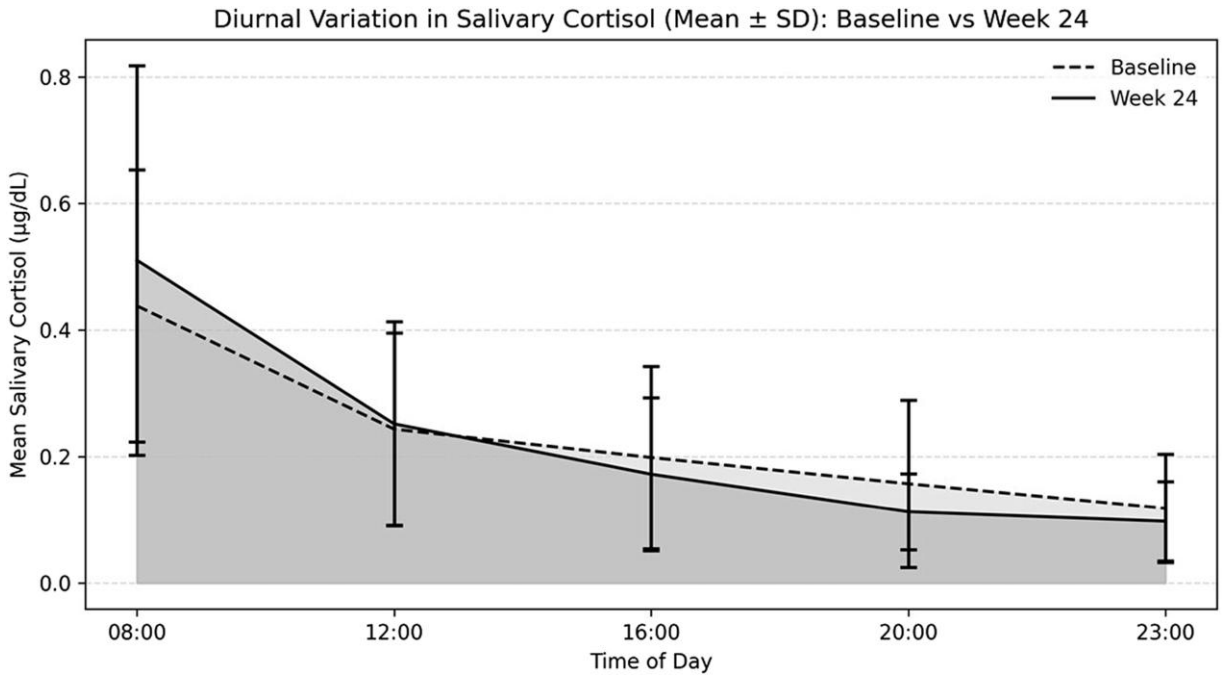

Figure 5. Diurnal variation of salivary cortisol: baseline vs 24 weeks. Salivary cortisol values are expressed as median values for each collection timepoint. Footnotes: a trend towards a more physiological circadian rhythm (ie, slightly higher and lower cortisol levels at morning and evening, respectively) was understandable.

the condition and the strict inclusion criteria. Finally, the absence of a control group limits our ability to definitively attribute the observed improvements to metyrapone therapy. Future studies should aim to include larger cohorts, longer follow-up periods, and ideally randomized controlled designs to strengthen the evidence base.

In conclusion, our findings support the notion that metyrapone may offer clinical benefits in patients with mH, particularly those with uncontrolled comorbidities. The observed improvements in BP and glycaemic control, despite minimal changes in UFC levels, underscore the need to re-evaluate traditional therapeutic targets and to adopt a more holistic approach to disease management. Further research is warranted to validate these findings and to explore the broader implications of circadian rhythm restoration in endocrine disorders.

Supplementary material

Supplementary material is available at *European Journal of Endocrinology* online.

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Authors' contributions

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Iacopo Chiodini has received speaker and consultancy fees from Corcept Therapeutics. Vittoria Favero has received honoraria from Corcept Therapeutics as a speaker. The other authors have no conflict of interest concerning this work.

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