Dual-release hydrocortisone improves hepatic steatosis in patients with secondary adrenal insufficiency: a real-life study

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Abstract

Background: Conventional glucocorticoid treatment has a significant impact on liver in patients with adrenal insufficiency. Dual-release hydrocortisone (DR-HC) provides physiological cortisol exposure, leading to an improvement in anthropometric and metabolic parameters. We aimed to evaluate the effects of 12-month DR-HC treatment on the hepatic steatosis index (HSI), a validated surrogate index of hepatic steatosis, in patients with secondary adrenal insufficiency (SAI).

Methods: A total of 45 patients with hypopituitarism, 22 with hypogonadism, hypothyroidism, ACTH, and GH deficiencies, and 23 with hypogonadism, hypothyroidism, and ACTH deficiency, on replacement therapy for all the pituitary deficiencies, were switched from conventional hydrocortisone to DR-HC. At baseline and after 12 months, glucose and insulin levels, surrogate estimates of insulin sensitivity, and hepatic steatosis were evaluated through ultrasonography and HSI.

Results: At diagnosis, ultrasonography documented steatosis in 31 patients (68.8%) while 33 (73.3%) showed high HSI. Hydrocortisone (HC) dose ($\beta = 1.231$, $p = 0.010$), insulin resistance index (HOMA-IR) ($\beta = 1.431$, $p = 0.002$), and insulin sensitivity index (ISI)-Matsuda ($\beta = -1.389$, $p = 0.034$) were predictors of HSI at baseline. After 12 months of DR-HC, a significant decrease in body mass index (BMI) ($p = 0.008$), waist circumference (WC) ($p = 0.010$), fasting insulin ($p = 0.041$), HOMA-IR ($p = 0.047$), HSI ($p < 0.001$) and number of patients with HSI $\geq 36$ ($p = 0.003$), and a significant increase in sodium ($p < 0.001$) and ISI-Matsuda ($p = 0.031$) were observed. HOMA-IR ($\beta = 1.431$, $p = 0.002$) and ISI-Matsuda ($\beta = -9.489$, $p < 0.001$) were identified as independent predictors of HSI at 12 months.

Conclusions: In adults with SAI, DR-HC is associated with an improvement in HSI, regardless of the dose used, mainly related to an improvement in insulin sensitivity.

Keywords: dual-release hydrocortisone, hepatic steatosis, hypopituitarism, insulin sensitivity, secondary adrenal insufficiency

Introduction

Nonalcoholic fatty liver disease (NAFLD) is characterized by high serum levels of free fatty acids (FFA) and fatty infiltration of the liver. It is frequently associated with metabolic syndrome, obesity, diabetes mellitus, and insulin resistance, and can be a predictor of cardiovascular mortality.\textsuperscript{1–5} Hypopituitarism is generally characterized by the presence of metabolic syndrome and hepatic steatosis.\textsuperscript{6} Growth hormone (GH) deficiency has been considered the main contributing factor involved in the regulation of hepatic lipid metabolism.\textsuperscript{7,8} Glucocorticoids (GCs) also contribute to lipid and glucose metabolism, favoring obesity,
diabetes mellitus, hepatic steatosis and dyslipidemia, as observed in patients with hypercortisolism and in animal models.9–12

Dual-release hydrocortisone (DR-HC) is characterized by an immediate-release fraction of hydrocortisone (HC) in the outer layer of the tablet and an extended-release fraction in the core. This formulation provides a peak of cortisol within 50 min of administration, half cortisol plasmatic concentration for 6 h thereafter, and a minimal cortisol level 18–24 h after intake, leading to a cortisol exposure-time profile close to the physiological one.13 DR-HC has been reported to improve anthropometric and metabolic parameters, appearing to be safe even in the long term.14–19

However, to our knowledge, the effects of DR-HC on hepatic steatosis have not yet been evaluated. Evaluation of the degree of hepatic steatosis is important for predicting the progression and severity of histological features. The most reliable noninvasive technique for quantifying hepatic steatosis is magnetic resonance imaging (MRI). However, MRI is quite expensive and not immediately available in clinical routine. Currently, liver ultrasound is the method most widely used to assess hepatic steatosis, even though its utility as screening tool is unproven and it is not perfectly adequate at quantifying hepatic fat.2 The hepatic steatosis index (HSI) is quite an accurate screening measure of NAFLD that has demonstrated good performance in different populations with and without diabetes mellitus, and has been shown to correlate with the fatty liver grade measured by ultrasonography.20,21

Based on these assumptions, we aimed to evaluate the HSI in patients with secondary adrenal insufficiency (SAI) treated with DR-HC for 12 months, and to investigate whether HSI would be a good predictor of insulin sensitivity in this population.

Materials and methods

Study participants

In the current observational retrospective study, data collected routinely from 45 consecutive patients (26 women and 19 men aged 48.4 ± 11.1 years, range 29–70) with hypopituitarism referred to the Division of Endocrinology of Palermo University from January 2015 to December 2017, were evaluated. Of these 45 patients, 20 were naïve to treatment, while 25 were on treatment with conventional HC.

Of the total of 45 patients with hypopituitarism, 22 had combined hypogonadism, hypothyroidism, ACTH, and GH deficiencies (GHD), while 23 had combined hypogonadism, hypothyroidism, and ACTH deficiency.

SAI was first diagnosed by assessing the function of the adrenal cortex on the basis of serum cortisol in the morning. An early morning serum cortisol level lower than 3 μg/dl confirmed AI. When cortisol levels were higher than 3 μg/dl, in presence of clinical suspicion of AI, the endovenous injection of corticotropin stimulation test at the standard dose of 250 μg was performed. A normal response was a plasma cortisol concentration higher than 18 μg/dl 30 min after injection. Once AI was confirmed, ACTH levels were measured. GHD was documented by appropriate stimulation tests.22 Secondary hypogonadism was defined as low luteinizing hormone/follicle-stimulating hormone (LH/FSH) in amenorrheic women of fertile age range and with inappropriately low gonadotropin levels for their age in postmenopausal women. In men, low testosterone level with inappropriately low LH/FSH documented secondary hypogonadism. Secondary hypothyroidism was defined as low free T4 (FT4) levels with inappropriately low serum TSH.

The switch to DR-HC was judged to be appropriate on clinical grounds in those patients who complained of fatigue and weakness, presented hyponatremia (≤134 mmol/l) or hypoglycaemia (≤2.78 mmol/l), or showed more than two comorbidities such as diabetes, osteoporosis, hypertension, and central obesity. The replacement therapy prescribed was as follows: levo-thyroxine at an average dose of 1 μg/kg for hypothyroidism, somatropin at an average dose of 0.4 mg/day for GHD, injected testosterone enanthate at a monthly dose of 250 mg for male hypogonadism, and low dose of estrogen and progesteron therapy for premenopausal female hypogonadism.

Patients maintained good and stable replacement treatment and hormonal control evaluated by FT4, IGF-1, and estrogen or testosterone levels, which were kept within normal ranges, during the entire follow up.
During the 12-month treatment period, the DR-HC doses were modified according to the physician’s judgement of patient need in both groups of patients (Table 1). Each patient received instructions for treatment in special or emergency situations. Patients were instructed to double the dose of DR-HC during an intercurrent illness or stress.

This study was carried out in accordance with the recommendations of the Paolo Giaccone Policlinico ethics committee with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Paolo Giaccone Policlinico ethics committee (04/19).

### Study design
At baseline, and after 12 months of DR-HC treatment, patients underwent a clinical and metabolic evaluation.

Anthropometric parameters such as body mass index (BMI), systolic and diastolic blood pressure (SBP and DBP) and waist circumference (WC), measured at the midpoint between the lower rib and the iliac crest, were evaluated.

After an overnight fast, total, high-density lipoprotein (HDL), and low-density lipoprotein (LDL), cholesterol and triglycerides (TG), HbA1c, alanine (ALT) and aspartate (AST) aminotransferase, γ-glutamyl-transferase (GGT), alkaline phosphatase (ALP), total bilirubin, IGF-1, total testosterone, estrogens, and FT4 levels were measured. An oral glucose tolerance test (OGTT) was performed in nondiabetic patients by measuring plasma blood glucose and insulin every 30 min for 2 h after a 75-g oral glucose load. Basal insulin sensitivity was evaluated by the homeostasis model assessment of insulin resistance (HOMA-IR) index, while the stimulated insulin sensitivity was measured using the insulin sensitivity index (ISI), a composite index derived from the OGTT and validated by Matsuda and DeFronzo.

Metabolic syndrome is defined, according to Adult Treatment Panel III, by the presence of at least three or more of the following criteria: waist circumference over 102 cm in males or 88 cm in females, high blood pressure of ≥130/≥85 mmHg, high fasting TG level of ≥1.7 mmol/l, low fasting HDL cholesterol level <1.04 mmol/l in males and <1.30 mmol/l in females, and hyperglycaemia as fasting glucose ≥6.1 mmol/l. Hepatic steatosis was evaluated by an abdominal ultrasound, routinely performed in all patients to assess the hepatic structure. Liver ultrasound was done in fasting patients before starting the DR-HC, by a radiologist expert in ultrasound techniques and particularly dedicated to liver examination who was unaware of the patients’ history. A real-time Hitachi H21 apparatus with a 2–5 MHz, convex, multifrequency probe was used.

We defined visceral obesity as the presence of WC over 102 cm in males or 88 cm in females. We calculated the HSI with the following formula: HSI = 8 × ALT/AST ratio + BMI (+2 if female; +2 if diabetic). Patients were considered to have steatosis if HSI ≥ 36. Fatty liver index (FLI) was also evaluated as previously reported. Patients with FLI ≥ 60 were considered to have hepatic steatosis.

### Assays
Lipids, serum glucose, ALT, AST, GGT, ALP, and bilirubin were measured in our centralized laboratory with standard methods. Serum insulin and FT4 were measured by electro chemiluminescence (ECLIA, Elecsys Insulin, Roche, Milan, Italy). Serum IGF-I levels were measured by a chemiluminescent immunometric assay (Immule

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**Table 1. Dose adjustments according to the physician’s judgement during the 12 months of dual-release hydrocortisone treatment.**

| Baseline dose | 20 mg/day | 25 mg/day | 30 mg/day | 35 mg/day | 40 mg/day |
|--------------|-----------|-----------|-----------|-----------|-----------|
| 20 mg/day (n=43) | 34 | 4 | 4 | 0 | 1 |
| 25 mg/day (n=2) | 0 | 1 | 1 | 0 | 0 |
2000; Diagnostic Products Corp., Los Angeles, CA) using murine monoclonal anti-IGF-I antibodies. The standards were calibrated against the World Health Organization second IS 87/518. The sensitivity was 1.9 μg/l. The intra- and interassay CVs were 2.3–3.9% and 3.7–8.1%, respectively, as previously reported.²⁷

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

Statistical analysis
The Statistical Packages for Social Science SPSS version 19 (SPSS, Inc., IBM, New York, NY, USA) was used for data analysis. The normality of quantitative variables was tested with the Shapiro-Wilk test. The baseline characteristics of the groups were presented as mean ± SD for continuous variables, while the rates and proportions were calculated for categorical data. The differences between paired continuous variables (before and after 12 months of treatment) were analyzed using paired t test. Relations between the outcome variables and continuous variables were evaluated using univariate Pearson correlation coefficients. Multiple linear regression analysis was performed to identify independent predictors of the dependent variable HSI at baseline and at 12 months. The decision to keep the variables in the multivariate model was based on clinical and statistical significance. Variables having a potential clinical impact on HSI levels and significantly associated with HSI on univariate analysis (Pearson correlation) were included (i.e. WC, HDL, TG, LDL, fasting insulin, HOMA-IR, ISI-Matsuda, HbA1c, and HC dose at baseline, and WC, fasting insulin, HOMA-IR, and ISI-Matsuda at 12 months). A p value of 0.05 was considered statistically significant.

Results

Baseline
At baseline, 15 patients (33.3%) had arterial hypertension, 27 (60%) had osteoporosis/osteopenia, 43 (95.6%) had visceral obesity, 8 (17.8%) had dyslipidemia, 8 (17.8%) had diabetes mellitus, and 31 (68.8%) had hepatic steatosis documented by ultrasound. No significant difference in the prevalence of hypertension, osteoporosis/osteopenia, visceral obesity, dyslipidemia, diabetes mellitus, and hepatic steatosis, evaluated by ultrasound, was found from baseline to 12 months.

Dividing patients in two groups with and without GHD, no significant differences were found at baseline.

At baseline, 33 patients had HSI ≥ 36 and 31 had FLI ≥ 60 (p = 0.765). HSI at baseline was correlated with WC (r = 0.674; p < 0.001), HDL (r = –0.445; p = 0.003), TG (r = 0.438; p = 0.003), LDL (r = 0.361; p = 0.017), HOMA-IR (r = 0.576; p < 0.001), ISI-Matsuda (r = –0.677; p < 0.001), HbA1c (r = 0.396; p = 0.019), and HC dose (r = 0.595; p = 0.009) (Table 2). In addition, patients with HSI ≥ 36 were also taking a significantly higher daily dosage of HC, than those with HSI < 36 (20.1 ± 4.08 versus 13.7 ± 2.31 mg/day; p = 0.001) (data not shown).

To assess independent predictors of HSI, a multivariate regression model using backward stepwise removal was used, entering HSI as a dependent variable and average daily dose of HC, WC, HDL, TG, LDL, HOMA-IR, ISI-Matsuda, and HbA1c as independent variables. In this model, the only independent predictors were HC dose (β = 1.231, p = 0.010), HOMA-IR (β = 1.431, p = 0.002) and ISI (β = –1.389, p = 0.034) (Figure 1).

Twelve months of DR-HC
After 12 months of treatment, a significant decrease in BMI (p = 0.008), WC (p = 0.010), fasting insulin (p = 0.041), HOMA-IR (p = 0.047), HSI (p < 0.001), and number of patients with HSI ≥ 36 (p = 0.003), and a significant increase in sodium (p < 0.001) and ISI-Matsuda (p = 0.031) were observed (Table 3). Comparing patients with and without GHD, no significant differences were observed after 12 months of treatment with DR-HC; 11 patients had HSI ≥ 36 while 9 had FLI ≥ 60 (p = 0.837).

HSI at 12 months (HSI₁₂m) was correlated with WC₁₂m (r = 0.503; p = 0.010), TG₁₂m (r = 0.493; p = 0.012), fasting insulin (r = 0.435; p = 0.039), HOMA-IR₁₂m (r = 0.389; p = 0.035), and ISI₁₂m (r = –0.601; p = 0.035) (Table 2).

To assess independent predictors of HSI₁₂m, a multivariate regression model using backward stepwise
Table 2. Correlation between HSI and clinical, hormonal and metabolic parameters (univariate analysis) in patients with hypopituitarism at baseline and after 12 months of DR-HC treatment.

|            | HSI | Baseline | r  | p    | 12 months | r  | p    |
|------------|-----|----------|----|------|-----------|----|------|
| Gender     |     |          | -0.113 | 0.470 | 0.197     | 0.346 |
| Age (years)|     |          | 0.231 | 0.136 | 0.410     | 0.058 |
| Waist circumference (cm) |     |          | 0.674 | <0.001 | 0.503     | 0.010 |
| Na (mmol/l) |     |          | -0.343 | 0.124 | 0.253     | 0.222 |
| K (mmol/l)  |     |          | 0.188 | 0.277 | 0.234     | 0.261 |
| Total cholesterol (mmol/l) |     |          | 0.266 | 0.085 | 0.237     | 0.253 |
| HDL cholesterol (mmol/l) |     |          | -0.445 | 0.003 | -0.314     | 0.127 |
| Triglycerides (mmol/l) |     |          | 0.438 | 0.003 | 0.493     | 0.012 |
| LDL cholesterol (mmol/l) |     |          | 0.361 | 0.017 | 0.226     | 0.278 |
| Fasting glycaemia (mmol/l) |     |          | 0.005 | 0.973 | 0.347     | 0.089 |
| Fasting insulin (UI/ml) |     |          | 0.656 | <0.001 | 0.435     | 0.039 |
| HOMA-IR |     |          | 0.576 | <0.001 | 0.389     | 0.035 |
| ISI-Matsuda |     |          | -0.677 | <0.001 | -0.601     | 0.005 |
| HbA1c (mmol/mol) |     |          | 0.396 | 0.019 | 0.333     | 0.104 |
| HC/DR-HC dose |     |          | 0.595 | 0.009 | 0.645     | 0.079 |
| FT4 (pmol/l) |     |          | -0.218 | 0.161 | -0.213     | 0.395 |
| IGF-1 (µg/dl) |     |          | 0.278 | 0.117 | 0.034     | 0.901 |
| Total testosterone (nmoL/l) |     |          | 0.068 | 0.751 | 0.298     | 0.128 |
| Estrogens [pg/ml] |     |          | 0.098 | 0.761 | 0.123     | 0.896 |
| GH deficiency |     |          | -0.126 | 0.420 | -0.013     | 0.952 |

DR-HC, dual-release hydrocortisone; HSI, hepatic steatosis index.

removal was used, entering HSI_{12m} as a dependent variable, WC_{12m}, TG_{12m}, fasting insulin_{12m}, HOMA-IR_{12m} and ISI-Matsuda_{12m} as independent variables. In this model, the only independent predictors were HOMA-IR_{12m} (β=1.431, p=0.002) and ISI_{12m} (β=-9.489, p<0.001) (Figure 1).

**Discussion**

This study shows that DR-HC treatment is associated with a significant improvement of HSI as a marker of NAFLD, concomitant with an improvement in insulin sensitivity, in patients with SAI.

Hypopituitarism is frequently associated with a condition of hepatic steatosis, due mainly to the presence of visceral obesity and metabolic syndrome. Hepatic steatosis is generally more common in hypopituitary patients with GHD than in patients without it. Indeed, GH has a strong lipolytic effect, preferentially on visceral adipose tissue, with a lesser effect on subcutaneous
adipose tissue.29 Some studies have reported a high prevalence of NAFLD in patients with untreated GHD, documenting a significant improvement in terms of liver histology and enzymes in a subgroup of patients receiving GH,8,30 though other studies did not show any improvement in hepatic fatty content during GH replacement therapy.31 GCs are also implicated in intrahepatic lipid accumulation. Indeed, GCs increase adipose tissue lipolysis, resulting in enhanced FFA release into the circulation. FFA are subsequently taken up by the liver, leading to increased TG synthesis and hepatic steatosis.32 Excess of GCs, as in Cushing syndrome (CS), has been associated with NAFLD.9 In a study on active CS that evaluated the prevalence of NAFLD by computed tomography, NAFLD was present in 20% of patients, and its presence correlated with both total abdominal and visceral fat.33 A quite recent study evaluating FLI as a marker of NAFLD in patients with controlled CS in comparison to patients with nonfunctioning pituitary adenomas revealed that the average daily HC intake was an independent predictor of FLI. In addition, patients with higher FLI were receiving a significantly higher daily HC dosage.34 In the current study, no significant differences were found in detecting hepatic steatosis by HSI or FLI. These data are discordant from those recently reported in a cohort of patients with type 1 diabetes, where FLI had higher sensitivity and specificity than HSI, compared with hepatic MRI.35 However, MRI is not considered the gold standard for hepatic steatosis diagnosis, even though it is superior to ultrasonography, and the cohort of patients in these two studies is quite different. In our opinion, further studies are needed to better clarify which of these two indexes is more accurate and reliable in detecting hepatic steatosis.

DR-HC has been demonstrated to have a more physiological profile, and the switch from conventional GCs to DR-HC has shown an improvement in metabolism. In a prospective study on 50 patients with primary AI (PAI) and SAI and congenital adrenal hyperplasia, a significant reduction in BMI and HbA1c was observed in patients treated with DR-HC compared with patients on standard GCs.15 However, this was an open, non-randomized study in which the patients decided whether they wanted to change to modified release HC or to stay on conventional HC. A subsequent study, evaluating the switch from conventional HC to DR-HC in 19 patients with AI, showed a significant reduction in WC, HbA1c, and LDL-cholesterol, and a significant improvement of the AddiQoL questionnaire after 12 months of DR-HC therapy.16 However, in this latter study,
**Table 3.** General characteristics of all patients at baseline and 12 months.

|                                | Baseline     | 12 months    | p      |
|--------------------------------|--------------|--------------|--------|
|                                | Subjects (%) | Subjects (%) |        |
| HSI ≥ 36                       | 33 (73.3%)   | 11 (24.4%)   | 0.003  |
|                                | Mean ± SD    | Mean ± SD    |        |
| **Anthropometric parameters**  |              |              |        |
| BMI [Kg/m²]                    | 27.1 ± 5.1   | 26.1 ± 4.9   | 0.008  |
| Waist circumference (cm)       | 97.3 ± 12.2  | 94.9 ± 11.7  | 0.010  |
| SBP [mmHg]                     | 114.7 ± 14.9 | 116.8 ± 23.1 | 0.586  |
| DBP [mmHg]                     | 69.7 ± 8.86  | 69.8 ± 11.1  | 0.959  |
| **Electrolytes**               |              |              |        |
| Na [mmol/l]                    | 138.1 ± 3.88 | 141.1 ± 3.21 | <0.001 |
| K [mmol/l]                     | 4.36 ± 0.35  | 4.23 ± 0.37  | 0.054  |
| **Metabolic parameters**       |              |              |        |
| Total cholesterol [mmol/l]     | 5.39 ± 1.12  | 5.13 ± 0.86  | 0.175  |
| HDL cholesterol [mmol/l]       | 1.42 ± 0.43  | 1.41 ± 0.48  | 0.890  |
| Triglycerides [mmol/l]         | 1.59 ± 0.78  | 1.51 ± 0.58  | 0.330  |
| LDL cholesterol [mmol/l]       | 3.16 ± 0.85  | 3.05 ± 0.83  | 0.532  |
| Fasting glycaemia [mmol/l]     | 4.52 ± 0.75  | 4.45 ± 0.85  | 0.325  |
| Fasting insulin [UI/ml]        | 8.64 ± 5.82  | 6.53 ± 2.29  | 0.041  |
| HOMA-IR                        | 1.71 ± 1.23  | 1.32 ± 0.48  | 0.047  |
| ISI-Matsuda                    | 7.94 ± 5.78  | 10.74 ± 9.8  | 0.031  |
| HbA1c [mmol/mol]               | 5.82 ± 0.87  | 5.61 ± 0.58  | 0.133  |
| AST [UI/L]                     | 29.3 ± 17.1  | 22.6 ± 8.43  | 0.069  |
| ALT [UI/L]                     | 32.8 ± 24.8  | 24.3 ± 7.81  | 0.116  |
| GGT [UI/L]                     | 23.1 ± 16.9  | 18.3 ± 10.1  | 0.189  |
| Alkaline phosphatase [UI/l]    | 51 ± 30.1    | 45 ± 20.7    | 0.285  |
| Total bilirubin [µmol/l]       | 0.81 ± 0.17  | 1.01 ± 0.25  | 0.055  |
| HSI                            | 37.2 ± 5.9   | 31.6 ± 5.3   | <0.001 |

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; DBP, diastolic blood pressure; DR-HC, dual-release hydrocortisone; GGT, \( \gamma \)-glutamyl-transferase; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance index; HSI, hepatic steatosis index; ISI, insulin sensitivity index; LDL, low-density lipoprotein; SBP, systolic blood pressure.
patients were not blinded to treatment, and the number of patients included was quite small. In a retrospective nonrandomized study by our group, an improvement in insulin secretion and sensitivity in patients with prediabetes and normal glucose tolerance was demonstrated, even though the cohort of patients evaluated was heterogeneous (patients with PAI and SAI). Another study evaluated the effects of DR-HC on glycemic profile and quality of life, showing a significant improvement of HbA1c and quality of life after only 3 months of therapy. This study evaluated both patients with PAI and SAI, some of whom were naive. A quite recent single-blind randomized controlled study showed that the switch to DR-HC was associated with a decrease in body weight, normalization of immune profile, reduction of infections, and improvement of quality of life.

In the present study, only patients with hypopituitarism were included. Indeed, the combination of pituitary deficiencies contributes at different levels to hepatic steatosis that can be already present at the diagnosis. On the other hand, patients with PAI, who have isolated cortisol deficiency, develop hepatic steatosis as a consequence of GC overtreatment.

Interestingly, in the current study, the HC dose at baseline was an independent predictor of HSI as a marker of NAFLD. The average HC dose before the switch to DR-HC was also significantly higher in those patients with an HSI score \( \geq 36 \), showing that increased hepatic exposure to HC is associated with increased hepatic lipid accumulation.

A significant decrease in HOMA-IR and increase in ISI-Matsuda, a reduction in fasting insulin and improvement in insulin sensitivity, respectively, were observed concurrently with a change of HSI. The multivariate analysis showed a significant negative correlation between HSI and ISI Matsuda and positive correlation between HSI and HOMA-IR, supporting a relation between the HSI and insulin sensitivity. This finding confirms the hypothesis that insulin resistance represents a pathogenic factor of steatosis, and, notably, that the metabolic improvement during DR-HC therapy has a great impact on it. Indeed, as recently reported, conventional GCs treatment deteriorates metabolic parameters, favoring metabolic syndrome and hepatic steatosis, compared with innovative therapy (DR-HC).

In our study, no differences between patients with and without GHD were found at baseline, or after 12 months of DR-HC. In addition, no correlation between HSI and IGF-1 was found, showing that the replacement therapy with GH does not seem to have a significant influence on HSI.

Interestingly, WC was not an independent predictor of HSI, although a correlation between WC and HSI was previously reported in patients with type 1 diabetes and WC has been frequently shown to be related to visceral fat and abdominal obesity.

The improvement in HSI as a marker of NAFLD in hypopituitary patients with ACTH deficiency treated with DR-HC is an interesting and innovative finding that may be mediated by direct and indirect effects (improvement in insulin sensitivity). In our opinion, it may be explained by a more physiological GC profile, while the dose of DR-HC does not seem to be involved. Indeed, during the 12-month treatment, no patients reduced the DR-HC dose, while, by contrast, an increase in dose was seen in 10 patients.

The main limitation of our study is the lack of liver biopsy, the gold standard method for the diagnosis of hepatic steatosis. Although the HSI has been proven to be quite accurate in providing measurements of increased liver fat, the lack of direct hepatic data based on histological examination of liver biopsy specimens is a strong limitation. However, the widespread use of liver biopsy is limited by the risk associated with an invasive procedure, cost, and sampling error, and this
approach is justified only if clinical history, examination, and hepatic ultrasound show severe NAFLD or NASH.\textsuperscript{2} Other limitations are the small number of patients enrolled, and those typical of real-life studies such as the lack of patient selection, which makes it impossible to avoid unmeasured confounding factors,\textsuperscript{42} and the absence of blinding and randomization, which does not always allow factors potentially influencing the outcomes to be properly balanced.\textsuperscript{43} This results in the patient being aware of the treatment they are assigned to. However, this ‘knowledge effect’ can be interpreted as a component of the treatment response that is normally present in clinical practice.\textsuperscript{44}

In conclusion, our data, extracted from real-life clinical practice, suggest that 12 months’ treatment with DR-HC is associated with an improvement in HSI, regardless of the dose used. The improvement in insulin sensitivity leads to an improvement in HSI, a surrogate index of hepatic steatosis, which should always be evaluated in the follow up of hypopituitary patients with SAI.

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\section*{Conflict of interest statement}
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\section*{Research ethics and patient consent}
Written informed consent was obtained from all individual participants included in the study.

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