Verbal and Visual Memory Performance and Hippocampal Volumes, Measured by 3-Tesla Magnetic Resonance Imaging, in Patients with Cushing’s Syndrome

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Context: Cushing’s syndrome (CS) affects cognition and memory.

Objective: Our objective was to evaluate memory and hippocampal volumes (HV) on 3-tesla magnetic resonance imaging (3T MRI) in CS patients and controls.

Patients and Methods: Thirty-three CS patients (11 active, 22 cured) and 34 controls matched for age, sex, and education underwent Rey Auditory Verbal Learning Test and Rey-Osterrieth Complex Figure memory tests. Gray matter and HV were calculated on 3T MRI, using FreeSurfer image analyses software.

Results: No differences in HV were observed between active and cured CS or controls. Memory performance was worse in CS patients than controls (P < 0.04 in active; P < 0.03 in cured CS) but did not differ among CS groups, which were therefore analyzed together; they performed worse for verbal (P = 0.02) and visual memory (P = 0.04) than controls. In 12 CS patients, memory was below normative cutoff values for verbal (n = 6, cured), visual memory (n = 10, six cured) or both (n = 4); these patients with severe memory impairments showed smaller HV compared with their matched controls (P = 0.02 with verbal impairment; P = 0.03 with visual impairment). They were older (P = 0.04), had shorter education (P = 0.02), and showed a trend toward longer duration of hypercortisolism (P = 0.07) than the remaining CS patients. Total (P = 0.004) and cortical (P = 0.03) brain gray matter volumes were decreased in CS compared with controls, indicating brain atrophy, whereas subcortical gray matter (which includes HV) was reduced only in the 12 patients with severe memory impairment.

Conclusion: Verbal and visual memory is worse in CS patients than controls, even after biochemical cure. HV was decreased only in those whose memory scores were below normative cutoff values. (J Clin Endocrinol Metab 97: 663–671, 2012)
Chronic exposure to elevated glucocorticoid (GC) levels in Cushing’s syndrome (CS) is associated with deficits in concentration and memory (1, 2) and cognitive impairment (2–6). The hippocampus, critical for learning and memory, is rich in GC receptors and therefore particularly vulnerable to GC excess (7). Animal studies show that the hippocampus is sensitive to both deficiency (8, 9) and elevation of GC (10, 11). Administration of exogenous GC or exposure to endogenous hypercortisolism due to chronic social and experimental stress induces changes in hippocampal pyramidal cell morphology and loss (10–12), impairing memory performance in animals (13). CS is a good human model to characterize relationships between cognitive performance and chronic exposure to elevated levels of GC (14).

Using a 1.5-tesla magnetic resonance imaging (MRI), memory dysfunction and hippocampal atrophy were described in CS patients compared with reference values from the literature (14, 15). A decrease in cortisol partially reversed human hippocampal atrophy after treatment of Cushing’s disease (CD) (15) and improved learning of unrelated words with a structural volumetric increase in hippocampal formation volume (16). But a recent study demonstrated that patients with long-term cure of CD still had impaired memory scores compared with both controls and patients with nonfunctioning pituitary macroadenomas, not exposed to hypercortisolism (17), pointing to possible damage in the hippocampus. Children with CS experience a significant decline of cognitive function despite reversibility of cerebral atrophy (18).

Thus, reversibility of brain abnormalities after resolution of hypercortisolism is questioned. In fact, greater cardiovascular risk, typical of active CS, is still present after remission (19), as well as persistent accumulation of central fat also in cured patients, associated with an unfavorable adipokine profile (20). Cognition (21) and impaired health-related quality of life (22, 23) do not normalize after endocrine cure either, suggesting that these changes are not fully reversible.

Several neuropsychiatric conditions, like major depressive and posttraumatic stress disorders, are associated with dysregulation of the hypothalamic-pituitary-adrenal axis, resulting in elevated cortisol (1, 24). Elucidating pathophysiological mechanisms through which cortisol determines cognitive dysfunction would be particularly useful to determine the specific pattern of neuropsychological deficits that accompany GC elevations.

With the advent of structural and functional neuroimaging techniques, the role of different nervous system structures and the hypothalamic-pituitary-adrenal axis can be investigated directly. MRI allows noninvasive assessment of gray matter structures, both cortical and subcortical, which includes the hippocampus. High-field MRI, such as 3T, is an even more powerful tool. Using dedicated acquisition protocols, and sophisticated postprocessing software, precise measurements of hippocampal volume (HV) on brain 3T MRI has proven invaluable in patients with memory impairment, such as Alzheimer’s disease (25, 26). However, these techniques have rarely been used in patients with endocrine diseases. To the best of our knowledge, this is the first comparative study including both a high-resolution 3T MRI, together with a specific calculation of HV as well as cortical and subcortical gray matter, and an evaluation of verbal and visual memory in CS patients compared with controls.

The aim of our study was to evaluate verbal and visual memory performance and measure HV in CS patients. We hypothesized that in CS patients, impaired memory performances and decreased HV would be found.

Subjects and Methods

Subjects

In this cross-sectional study, 33 right-handed CS patients (six males, age 44.8 ± 11.8 yr, 13.2 ± 3.6 yr of education) clinically followed in our institution and 34 healthy controls matched for age, sex, and years of education (nine males, age 41.4 ± 12.1 yr, 13.7 ± 3.6 yr of education) were evaluated with Rey Auditory Verbal Learning Test (RAVLT) and Rey-Osterrieth Complex Figure (ROCF) memory test and underwent a 3T MRI of the brain.

Control subjects recruited among right-handed healthy volunteers had previously participated in clinical studies in our institution. They had no history of GC exposure and were free of medications; one was taking oral contraceptives.

At the time of the study, 11 CS patients were hypercortisolemic (active), seven of pituitary and four of adrenal origin. One of pituitary origin was naïve to treatment. The remaining 10 were on medical therapy (four metyrapone, six ketoconazole). Eight of the 10 were awaiting surgery (four adrenal and four pituitary). The remaining two had previously undergone unsuccessful pituitary transphenoidal neurosurgery.

Twenty-two patients were in remission (biochemically cured), 18 of pituitary, three of adrenal origin, and one case of ectopic ACTH secretion. All patients cured of CS of pituitary origin had undergone transphenoidal surgery and five also pituitary radiotherapy. Unilateral adrenalectomy was performed in patients of adrenal origin. Four were adrenal insufficient at the time of study on hydrocortisone replacement (median 20 mg/d in two or three doses), whereas the remaining 29 presented transient hypercortisolism after surgery but did not require substitution therapy. Patients with CS due to adrenal carcinoma or exogenous treatment with GC were excluded.

Duration of hypercortisolism was considered as the time from symptom onset until remission of hypercortisolism after treatment and was assessed by the endocrinologist in charge. At diagnosis, the duration of hypercortisolism was estimated by personal interview and detailed revision of medical records and...
photographs of patients. All information was written or kept in clinical records, together with data regarding the achievement of the biochemical cure. Mean duration of hypercortisolism was 5.5 ± 3.7 yr.

CS was considered in remission if patients achieved adrenal insufficiency or morning cortisol suppression (<50 nmol/liter; <1.8 μg/dl) after 1 mg dexamethasone overnight and repeated normal 24-h urinary free cortisol (measured with a commercial RIA after a previous urine extraction with an organic solvent; normal <280 nmol/24 h). Mean time of biochemical cure at study date was 7.3 ± 2.4 yr.

CS patients with diabetes mellitus and GH deficiency were excluded because cognitive deficits and hippocampal atrophy have been described in these conditions (27–31). Information on pituitary function was also evaluated (GH, IGF-I, TSH, free T₄, prolactin, gonadotropins, and testosterone in males and menstrual history in females). Two CS patients required L-T₄ replacement, one for primary and one for secondary hypothyroidism.

Two patients were on oral contraceptives and one on testosterone, one for primary and one for secondary hypothyroidism. Taking tranquilizers.

Six cured and six active patients were hypertensive on medical treatment. Two cured and two active patients also had dyslipidemia and were on statin therapy.

All patients and controls signed an informed consent after study approval by the Hospital Ethics Committee. None of the participants had a past medical history of head injury, cerebrovascular disease, mental illness, or psychiatric disorders or were taking tranquilizers.

**Memory tests**

**Rey Auditory Verbal Learning Test**

RAVLT assesses immediate and delayed verbal memory, learning capacity, and recognition. It was administered in a standard manner (32, 33) consisting of 1) presentation of a 15-word list (list A) in five learning trials (referred to as Rey1 for the first trial, Rey2 for the second, Rey3 for the third, Rey4 for the fourth, Rey5 for the fifth), with the score for each trial being the number of words correctly recalled; 2) a single presentation of an interference 15-word list (list B); 3) two postinterference recall trials (one immediately after list B, referred to as retention index, one delayed after 15 min, referred to as recall index); 4) after an extended delay (30 min), subjects are asked to recognize the words of list A (recognition-A) and of list B (recognition-B) presented at the beginning; and 5) total score (the sum of trials 1–5, referred to as total recall score) was also calculated, 75 being the maximum score.

**ROCf memory test**

ROCf assesses visual memory (33, 34) and consists of three steps: copying the complex figure on paper (referred to as Rey-copy) while the figure is in front of the patient and then drawing the figure without the model, immediately (referred to as FIG-Rey-lm) and after 20 min (referred to as Fig-Rey-delayed). The score evaluates the number of figure parts correctly recalled and drawn, 36 being the maximum score.

Normative data were used to define cognitive performance in each test (32); patients with a score below 2 SD for RAVLT or below percentile 10 for the ROCf test were defined as having severe memory impairments.

The selection of test variables to be analyzed was based on their relation with hippocampal functioning. Rey5, total recall score, retention index, recognition-A, and recognition-B (verbal memory) are related to left hippocampal function, whereas Fig-Rey-lm and Fig-Rey-delayed (visual memory) are related to right hippocampal function.

**3T MRI**

MRI was obtained using a 3T Philips Achieva facility (software version 2.1.3.2) and a dedicated acquisition protocol: 3D-MPRAGE whole brain sequence (repetition time = 6.7 msec; echo time = 3.1 msec, 170 slices; voxel size = 0.889 × 0.889 × 1.2).

Volumetric segmentation was performed automatically using FreeSurfer version 4.3.1 image analysis software (http://surfer.nmr.mgh.harvard.edu/), previously implemented in the Port d’Informació Científica (PIC) of the Universitat Autònoma de Barcelona, composed of 170 HP blades with two quad-cores CPU (Hewlett Packard, Palo Alto, CA), each one with 16 GB of RAM, running over Scientific Linux version 5 (https://www.scientificlinux.org/). Postprocessing was launched using the PICNIC tool (www.neuroweb01.pic.es).

MRI were processed in parallel and launched through a batch system, considerably reducing processing time for the dataset. Postprocessing procedures to obtain total brain gray matter (i.e., cortical and subcortical areas, the latter including the HV) includes motion correction, removal of nonbrain tissue using a hybrid watershed/surface deformation procedure (35), automated Talairach transformation, and segmentation of the subcortical white matter and deep gray matter volumetric structures (36, 37). All HV were then normalized to the estimated total intracranial volume of each individual, also provided by FreeSurfer, to correct for differences in individual cranial volumes, using the covariance estimate method (38), an established and accepted procedure for volumetric assessment of brain structures (25).

FreeSurfer provides automated measurements of cortical and subcortical gray matter volumes, the latter including the hippocampi (http://surfer.nmr.mgh.harvard.edu/), compared with older, manual volumetric methods. Validity of this method has been demonstrated in healthy elderly individuals, mild cognitive impairment, and Alzheimer’s disease patients (25). To assure validity of automated hippocampal measurements, an interreliability analysis was performed with manual hippocampal measures, showing an α of Cronbach value of 0.84; furthermore, the Bland-Altman analysis showed acceptable interchangeability of methods.

**Statistical analyses**

Data analyses were carried out with the statistical package SPSS for Windows, version 19.0 (SPSS Inc., Chicago, IL). Quantitative data are expressed as mean ± SD (Gaussian distribution). Data distribution was analyzed by the Kolmogorov-Smirnov test. Comparison of sociodemographic differences among groups was accomplished with ANOVA, and nonparametric tests were used as convenient. For the main analyses, multivariate ANOVA (MANOVA) were performed, one for HV and another for memory variables, with group as the between-subjects factor. If necessary, differences between groups were analyzed with post hoc tests. Bivariate correlations (Pearson) were analyzed between memory performances, HV, duration of hypercortisolism, and 24-h urinary free cortisol. A multiple linear regression analysis was performed to determine predictors for HV. $P < 0.05$ was considered significant.
Results

Comparison of active CS patients, cured CS patients, and healthy controls

There were no differences in age, years of education, or gender between active CS patients, cured CS patients, and healthy controls (Table 1). Regarding MANOVA, no group effect was shown on HV ($F = 0.95; df = 4, 128; P = 0.4$). Memory performance did not show a group effect either ($F = 1.25; df = 14, 94; P = 0.2$). Because this approach of analyzing three groups showed no differences, a new approach comparing CS patients (active and cured) with healthy controls was adopted.

HV and memory performance in CS patients and healthy controls

When CS patients were compared with controls, MANOVA revealed no group effect on HV ($F = 1.16; df = 2, 64; P = 0.3$). By contrast, MANOVA of memory performance showed a significant group effect ($F = 2.88; df = 7, 47; P = 0.01$). Additionally, all memory variables of CS patients were significantly worse than in healthy controls (with the exception of immediate visual recall) (Table 2).

Correlation analyses showed that right and left HV were, respectively, related to visual and verbal memory tasks (Fig. 1) but not to cortisol-related variables, with the exception of the verbal recognition task, known to be hippocampal dependent, which showed a positive correlation with duration of hypercortisolism and a negative correlation with 24-h urinary free cortisol (Table 3). The multiple linear regression model showed that only age was predictive for both left and right HV ($r = 0.64; P < 0.01$) (older age predicting a smaller HV).

HV in CS patients with memory scores below normative cutoff values

Six CS patients (18%, all cured) scored below normative cutoff values for verbal memory and were considered to have severe verbal memory impairment. Four of these six also scored below normative cutoff values for visual memory. Reanalyzed, the left HV in this subgroup of six patients with severe verbal memory impairment was smaller than that of their six matched controls (3405 ± 541 and 4074 ± 488 mm$^3$, respectively, $P = 0.02$). Moreover, these six CS patients had smaller left HV than the CS patients whose verbal memory tests were within normative values (3781 ± 423 mm$^3$, respectively, $P = 0.01$).

Ten CS patients (30%, six cured and four active) scored below normative cutoff values for visual memory and were considered to have severe visual memory impairment. On reanalysis, their right HV was also smaller than

### TABLE 1. Sociodemographic variables in CS patients and controls

<table>
<thead>
<tr>
<th>CS patients (n = 33)</th>
<th>Controls (n = 34)</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td><strong>Active (n = 11)</strong></td>
<td></td>
<td></td>
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<tr>
<td>Age</td>
<td>42.64 ± 10.11</td>
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<tr>
<td>Years of education</td>
<td>14.45 ± 2.28</td>
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<tr>
<td>Gender (male/female)</td>
<td>2/9</td>
<td></td>
</tr>
<tr>
<td><strong>Cured (n = 22)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>45.95 ± 12.70</td>
<td></td>
</tr>
<tr>
<td>Years of education</td>
<td>12.64 ± 4.06</td>
<td></td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>4/22</td>
<td></td>
</tr>
<tr>
<td><strong>Controls (n = 34)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>41.38 ± 12.09</td>
<td>NS</td>
</tr>
<tr>
<td>Years of education</td>
<td>13.71 ± 3.65</td>
<td>NS</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>9/25</td>
<td>NS</td>
</tr>
</tbody>
</table>

P values represent significance of the between-subjects differences. NS, Not significant.

### TABLE 2. Memory performances and HV in CS patients and controls

<table>
<thead>
<tr>
<th></th>
<th>CS patients (n = 33)</th>
<th>Controls (n = 34)</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td><strong>Left HV (mm$^3$)</strong></td>
<td>3781.34 ± 423.94</td>
<td>3924.53 ± 405.97</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Right HV (mm$^3$)</strong></td>
<td>3893.32 ± 357.94</td>
<td>4025.63 ± 357.29</td>
<td>NS</td>
</tr>
<tr>
<td>Rey5</td>
<td>12.09 ± 1.89</td>
<td>13.23 ± 1.15</td>
<td>0.01</td>
</tr>
<tr>
<td>Retention index</td>
<td>9.12 ± 2.93</td>
<td>11.14 ± 2.39</td>
<td>0.01</td>
</tr>
<tr>
<td>Total recall score</td>
<td>46.97 ± 8.46</td>
<td>52.32 ± 6.06</td>
<td>0.01</td>
</tr>
<tr>
<td>Recognition-A</td>
<td>12.85 ± 1.75</td>
<td>13.95 ± 1.33</td>
<td>0.01</td>
</tr>
<tr>
<td>Recognition-B</td>
<td>7.36 ± 3.71</td>
<td>9.50 ± 2.87</td>
<td>0.02</td>
</tr>
<tr>
<td>Fig-Rey-Im</td>
<td>18.86 ± 7.07</td>
<td>22.23 ± 6.43</td>
<td>NS</td>
</tr>
<tr>
<td>Fig-Rey-delayed</td>
<td>18.50 ± 6.90</td>
<td>22.34 ± 6.62</td>
<td>0.04</td>
</tr>
</tbody>
</table>

P values are between-subjects differences of the MANOVA. NS, Not significant.

HV were determined using FreeSurfer after 3T MRI of the brain. Results for normalized left and right HV are shown. For RAVLT test (verbal memory), Rey5 is number of words of the list A correctly recalled at the fifth trial; retention index is the number of words of the list A correctly recalled after the interference list; total recall score is total score, the sum of words correctly recalled during the five trials; recognition-A is the number of recognized words of list A after 30 min; and recognition-B is the number of recognized words of list B after 30 min. For ROCF test (visual memory), Fig-Rey-Im is the immediate visual memory, measured by ROCF test, and Fig-Rey-delayed is the delayed visual memory, measured by ROCF test. P values are between-subjects differences of the MANOVA. NS, Not significant.

FIG. 1. Correlation between normalized left HV and total recall score (RAVLT) in cured (gray dots) and active (black squares) CS patients and healthy controls (black triangles).
that of their 10 matched controls (3755 ± 273 and 4015 ± 365 mm³, respectively, \(P = 0.03\)). However, no differences in right HV were observed between these 10 patients and the remaining CS patients with normal visual memory results.

Globally, these 12 CS patients with severe memory impairments (six with severe verbal memory impairment, 10 with severe visual memory impairment, and four with both) had fewer years of education (10.5 ± 3.9 vs. 14.8 ± 2.0 yr, respectively, \(P = 0.02\)) and were older (50.2 ± 12.3 vs. 41.7 ± 10.6 yr, respectively, \(P = 0.04\)) than the remaining 21 CS patients and showed a trend toward a longer duration of hypercortisolism (6.7 ± 5.2 vs. 4.4 ± 2.4 yr, respectively, \(P = 0.07\)).

**Brain cortical and subcortical gray matter**

Because the hippocampus is part of the subcortical gray matter, brain gray matter was investigated further. Total gray matter volume (cortical and subcortical together) was found to be decreased in CS compared with controls (416,924 ± 41,354 vs. 446,999 ± 40,465 mm³, \(P = 0.004\)). This was also found for cortical gray matter (CS 364,230 ± 37,430 vs. 392,002 ± 36,318 mm³, \(P = 0.03\)). This indicates brain atrophy in CS patients. In fact, when routine neuroradiological reports of brain MRI were reviewed retrospectively, greater brain atrophy than expected for the patients’ age had been reported in 17 of 33 CS patients but only in two of 34 healthy controls.

In contrast, no differences in subcortical gray matter volume were observed between CS patients and controls (55,274 ± 18,837 vs. 52,491 ± 19,440 mm³). There was a reduction in subcortical gray matter volume in the 12 patients with severe memory impairment compared with the remaining 21 CS patients (49,552 ± 3,245 vs. 54,502 ± 6,196 mm³, \(P = 0.005\)), similarly to that observed for HV in this subset of patients. They showed no difference in cortical gray matter compared with the remaining 21 CS patients, compatible with previous clinical and experimental evidence of GC-induced brain atrophy, persistent to some degree despite endocrine control of hypercortisolism. A negative correlation was observed between duration of hypercortisolism and subcortical gray matter volume in CS patients (\(r = -0.48; P = 0.005\); Fig. 2), suggesting that longer exposure to hypercortisolism was associated with morphological and functional hippocampal decline.

### TABLE 3. Correlations between HV, memory tests, 24-h urinary free cortisol, and duration of hypercortisolism in CS patients and all studied subjects

<table>
<thead>
<tr>
<th></th>
<th>Verbal memory</th>
<th>Visual memory</th>
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<tr>
<td></td>
<td>Retention index</td>
<td>Total recall score</td>
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<tr>
<td>CS patients (n = 33)</td>
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<tr>
<td>Left HV</td>
<td></td>
<td></td>
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<tr>
<td>(P)</td>
<td>0.009</td>
<td>0.002</td>
</tr>
<tr>
<td>(r)</td>
<td>0.45</td>
<td>0.51</td>
</tr>
<tr>
<td>Right HV</td>
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<tr>
<td>(P)</td>
<td>0.03</td>
<td>0.04</td>
</tr>
<tr>
<td>(r)</td>
<td>0.29</td>
<td>0.34</td>
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<tr>
<td>Duration</td>
<td></td>
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<tr>
<td>(P)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>(r)</td>
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<tr>
<td>UFC</td>
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<tr>
<td>(P)</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>(r)</td>
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<tr>
<td>All studied subjects</td>
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<tr>
<td>(n = 67, 33) CS plus 34 controls</td>
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<tr>
<td>Left HV</td>
<td></td>
<td></td>
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<tr>
<td>(P)</td>
<td>0.004</td>
<td>0.001</td>
</tr>
<tr>
<td>(r)</td>
<td>0.43</td>
<td>0.49</td>
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<tr>
<td>Right HV</td>
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<tr>
<td>(P)</td>
<td>0.01</td>
<td>0.001</td>
</tr>
<tr>
<td>(r)</td>
<td>0.39</td>
<td>0.45</td>
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<tr>
<td>UFC</td>
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<tr>
<td>(P)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>(r)</td>
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Duration is duration of hypercortisolism. For explanation of memory tests, see Table 2. NA, Not applicable; NS, not significant; UFC, 24-h urinary free cortisol.
Discussion

In CS patients with either active or biochemically cured disease, matched for age, gender, and years of education with healthy controls, we found no differences in HV between the three groups, but memory was worse in both the CS patient groups. Because no differences were seen between the two CS groups, we reanalyzed them together and confirmed that previous exposure to hypercortisolism did not affect HV but did impair memory function. Furthermore, we observed a correlation of left HV with verbal memory tasks and of right HV with visual memory tasks in patients and controls, as previously described in normal subjects and other patient groups, confirming that RAVLT and ROCF are good tests to evaluate hippocampal-related memory. The search for predictors of HV reduction with multiple linear regression analysis only identified older age, as described in normal population.

No correlations between memory and cortisol-related variables, namely 24-h free urinary cortisol and duration of hypercortisolism, were evidenced except for the verbal recognition task known to be hippocampal dependent, weakly correlated with duration hypercortisolism and negatively with urinary cortisol. The exact implications of these findings are currently unknown.

Because our initial hypothesis, namely that chronic exposure to hypercortisolism affected both memory and HV, was not confirmed, we reanalyzed results and observed that although the majority of memory scores for CS patients fell within normal values, 12 patients were below normative cutoff values (2 SD for RAVLT or percentile 10 for ROCF) (32); this subgroup with severe memory impairment did have a reduced HV, of the left HV in those with severe verbal memory impairment and of the right HV in those with visual memory results below normative cutoff values. They were older (on average 9 yr) and had fewer years of education (4 yr fewer) than the other 21 CS patients who scored within normative values for memory tests. Whether longer duration of exposure to hypercortisolism also negatively affected their HV and severe memory impairment, as suggested by a nonsignificant trend, could not be statistically confirmed. Interestingly, most were biochemically cured of their hypercortisolism, pointing to a not completely reversible effect of previous exposure to cortisol on memory and HV. This finding further supports the concept that resolution of hypercortisolism is not followed by complete normalization of morbidity (19–22).

Total and cortical gray matter volumes were decreased in CS patients compared with controls, indicating brain atrophy. In fact, using a similar subjective approach described by Bourdeau et al. (39) for estimation of the degree of apparent cerebral atrophy on MRI reports, 17 of 33 CS patients were reported to have more atrophy than expected for their age, contrasting with only 6% of controls (two of 34). Subcortical gray matter volume (which includes the hippocampus) did not differ between CS and controls and was smaller only in the 12 patients with severe memory impairment, who also had a decreased HV compared with the other 21 CS patients. This would suggest that GC exposure initially determines cortical gray matter atrophy, persistent after control of hypercortisolism, and after longer exposure to GC would also determine HV reduction, especially in older subjects.

CS patients often complain of memory problems, in particular verbal, and perceive this as an important problem. They may indeed have lost part of their previous performance over the years, which would explain their perception of loss, despite performance being mostly still within normative ranges. Given the cross-sectional design of this study, previous memory performance before the diagnosis of CS was not known.

Together these findings support the concept that hypercortisolism negatively affects HV, memory, and cortical structures, the latter being evidenced earlier. The 3T MRI has a high spatial and contrast resolution and allows a high-quality assessment of brain gray matter including the hippocampus, ensuring accurate determination of cortical, subcortical, and HV.

The mechanism through which memory is impaired in CS is still unclear. Memory is a complex function in humans, involving numerous brain areas (including frontal and temporal lobes and white matter connections) and modulated by different cognitive functions (i.e., attention, visuospatial abilities, etc.). The hippocampal-dependent memory may explain only part of the memory process and reduced HV are evident only in
severe memory impairment. Cortisol excess would appear to trigger premature aging of the hippocampus. Cumulative exposure to GC over the lifespan has been associated with cognitive impairment and may contribute to physical and cognitive degeneration in aging, underlining that hypersecretion of GC has aging-like effects on cognitive performance (40). Hippocampal degeneration could appear in severe physical or psychological stress as evidenced in rats (41).

Previous studies related excess cortisol of CS with memory dysfunction and HV reduction, comparing with reference values from the literature (14) or in a group of 22 CD patients studied before and after transsphenoidal surgery (15). Recently, verbal memory was evaluated in 15 active female CS patients, compared with controls using Luria’s Memory Word-Revised test (a 10-word list repeated in 10 consecutive trials and a delayed recall after 30 min) (6). Globally, patients’ verbal memory performance was worse than that of controls, but no specification of how many did not reach the normative score value cutoff was given.

Another study evaluated memory and executive functions in long-term cured CD, compared with nonfunctioning pituitary macroadenoma patients and controls. They described impairment in cured CD patients, again pointing to irreversible effects of previous hypercortisolism on cognitive function and the central nervous system (17), as described at other levels (17, 19–23, 42, 43), supporting that previous exposure to hypercortisolism induces partially irreversible residual morbidity.

A weakness of our study is that it included patients with pituitary-dependent and adrenal CS; even though they share clinical complaints and comorbidities of chronic exposure to endogenous hypercortisolism, we cannot exclude certain differences, such as duration of GC exposure, usually shorter in adrenal CS. The number of patients is relatively small, a problem that is practically unavoidable in rare diseases, especially if followed up long-term over years; despite these limitations, we have identified differences, as described. Additionally, duration of endogenous hypercortisolism before diagnosis can never be exact, because it depends on patients’ recollections of when complaints began. However, this was prospectively and systematically approached in all new patients over the years and collected in the clinical file, and we therefore believe it is valid clinical information.

Whether medical therapy such as ketoconazole or metyrapone used in active CS plays any role in the results is unknown. We have not found any reports relating these drugs to memory or cognitive functioning in clinical practice. Furthermore, we found no differences in memory performance or HV between patients taking these medications or not.

Finally, a negative effect of radiotherapy, known to affect cognitive function, cannot be excluded, although it is unlikely to have influenced our findings significantly, because only five patients had been irradiated. Only large longitudinal epidemiological studies of patients with a previous diagnosis of CS, with and without radiotherapy, could answer this and other questions related to their long-term prognosis.

In conclusion, CS patients had lower performances in verbal and visual memory tests compared with matched controls, but only those with severe memory impairments (below normative cutoff scores) also had reduced HV. Brain atrophy and reduction in total and cortical gray matter volumes were observed in CS patients compared with controls, but subcortical gray matter reduction was seen only in those with severe memory impairment, in parallel to the findings of reduced HV. The negative effect of GC excess on memory and HV was not totally reversible after biochemical cure. Whether less exposure to hypercortisolism by earlier diagnosis and successful treatment of CS would avoid the progression of memory problems and the reduction of HV remains to be confirmed.

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