Decrease in Cortisol Reverses Human Hippocampal Atrophy following Treatment of Cushing's Disease

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Background: *Decreased hippocampal volume is observed in patients with Cushing's syndrome and other conditions associated with elevated cortisol levels, stress, or both. Reversibility of hippocampal neuronal atrophy resulting from stress occurs in animals. Our study investigated the potential for reversibility of human hippocampal atrophy.*

Methods: *The study included 22 patients with Cushing's disease. Magnetic resonance brain imaging was performed prior to transsphenoidal microadenomectomy and again after treatment.*

Results: *Following treatment, hippocampal formation volume (HFV) increased by up to 10%. The mean percent change (3.2 ± 2.5) was significantly greater (p < .04) than that of the comparison structure, caudate head volume (1.5 ± 3.4). Increase in HFV was significantly associated with magnitude of decrease in urinary free cortisol (r = −.61, p < .01). This relationship strengthened after adjustments for age, duration of disease, and months elapsed since surgery (r = −.70, p < .001). There was no significant correlation between caudate head volume change and magnitude of cortisol decrease.*

Conclusions: *Changes in human HFV associated with sustained hypercortisolemia are reversible, at least in part, once cortisol levels decrease. While many brain regions are likely affected by hypercortisolemia, the human hippocampus exhibits increased sensitivity to cortisol, affecting both volume loss and recovery.* Biol Psychiatry 1999;46:1595–1602 © 1999 Society of Biological Psychiatry

Key Words: Magnetic resonance imaging, hippocampal formation volume, caudate head volume, reversibility, cortisol, Cushing's Disease

Introduction

The hippocampus as a target of glucocorticoid (GC) activity is an area of increasing scientific and clinical interest (Sapolsky 1996). Animal studies in rodents and primates indicate that chronic exposure to elevated glucocorticoid concentrations or chronic stress results in dendritic atrophy in hippocampal CA3 neurons (Watanabe et al 1992) and can result in hippocampal pyramidal cell loss (Uno et al 1990).

Patients with spontaneous Cushing's syndrome (CS) represent a powerful model in which to investigate the association of hypercortisolemia with brain structure and function in humans. These patients experience high levels of endogenous cortisol, the naturally occurring GC, for periods of months to years. Using magnetic resonance imaging (MRI) of the brain, we previously showed that patients with active CS had decreased hippocampal formation volume (HFV). In the same study, HFV was negatively correlated with plasma cortisol concentrations and positively correlated with scores for verbal learning and recall (Starkman et al 1992). Since that time, decreased hippocampal volume has been observed in other conditions associated with elevated cortisol levels or stress, such as the normal elderly (Lupien et al 1998), recurrent depressive disorder (Sheline et al 1996), and PTSD (Bremner et al 1995).

The potential for reversibility of glucocorticoid-induced alterations of hippocampal structure and function has important theoretical and clinical implications. In animal models, such reversibility has already been demonstrated; exposure to severe and prolonged stress or to high GC concentrations causes irreversible loss of pyramidal cells (Sapolsky et al 1990), but a less severe stress leads to reversible morphologic changes (Sapolsky 1994). Whether reversibility can occur in humans remains an unanswered question.

CS provides a unique opportunity to study reversibility in humans. After patients experience chronic stress-level elevations of cortisol for several years before diagnosis, their cortisol concentrations revert to normal following successful treatment. In the present study, MRI was used to investigate the reversibility of cortisol-induced changes

in HFV in 22 patients with Cushing's disease (CD) (pituitary ACTH-dependent CS) who were examined prior to and then following treatment. We also tested the hypothesis that following sustained normalization of cortisol levels, the magnitude of increase in HFV is associated with the magnitude of decrease in cortisol.

Methods and Materials

Subjects

We limited the study group to patients with CD, the most common form of spontaneous CS. CD results from hypersecretion of pituitary ACTH. Although other etiologic types of CS, such as adrenal adenomas, also exhibit elevated cortisol, they differ in potentially confounding variables, such as the suppression of pituitary ACTH. The study included 22 patients with CD. Of the 22 participants, 17 were women and 5 were men, approximating the gender ratio seen in CD. Eighteen of the 22 patients had not participated in our previous studies; pretreatment HFV was reported in four of the patients in an earlier paper (Starkman et al 1992).

The study was approved by the University of Michigan's Institutional Review Board for Medical Experimentation, and all patients provided informed consent. Mean age ($\pm$ 1 SD) for patients at the time of diagnosis was 38.7 $\pm$ 14.8 years. Mean estimated duration of illness was 2.6 $\pm$ 2.3 years, based on an assessment of the patient's history and old photographs. Patients were admitted to the University of Michigan General Clinical Research Center (GCRC) for diagnostic studies and were restudied at the same facility following clinical and biochemical remission.

All patients met standard clinical and biochemical diagnostic criteria for CD. Clinical criteria included a disease-compatible history and physical findings (e.g., truncal obesity, skin and muscle atrophy, and "moon facies"). Biochemical criteria included high basal cortisol production (increased cortisol secretion rates, 24-hour urinary free cortisol and mean total plasma cortisol levels), high plasma ACTH levels, lack of normal cortisol circadian rhythm, lack of normal suppression with a low dose (2 mg), but $>$ 50% suppression with a high dose (8 mg) of dexamethasone. Normal circadian rhythm, evaluated by measuring ACTH and cortisol levels every 2 hours for 24 hours, is defined as a fluctuation in plasma total cortisol of greater than 5.0 μg/dL across time. Normal 24-hour urinary free cortisol (UFC) is 20–90 μg/day. Normal mean plasma total cortisol (protein-bound plus free) is 5.4–10 μg/dL. Mean values ($\pm$ 1 SD) for these patients were: UFC, 365.6 $\pm$ 252.6 μg/day and mean total plasma cortisol, 21.2 $\pm$ 4.0 μg/dL. Inferior petrosal sinus sampling was performed to confirm pituitary origin of the excessive ACTH secretion. All patients received an MRI at the time of diagnosis to determine the presence of a pituitary tumor and to obtain volumetric measurements of the brain structures of interest.

Following diagnosis, patients underwent transsphenoidal pituitary surgery with resection of a pituitary microadenoma. Surgical access to the pituitary utilized an incision between the lip and the gum, avoiding any invasion of the brain. One patient received a total hypophysectomy because a defined tumor could not be identified. Time elapsed between diagnosis and treatment was 62.6 $\pm$ 13.9 days. Immediately following surgery, all patients exhibited the expected complete suppression of ACTH and cortisol secretion and required replacement therapy with cortisol for at least 6 months. This replacement therapy was adjusted at regular intervals to ensure that cortisol levels remained normal until recovery of spontaneous hypothalamic-pituitary-adrenal function occurred. The patient who had a total hypophysectomy also received thyroid hormone replacement.

Patients received a repeat MRI of the brain after an interval of 16 $\pm$ 9.3 months following surgery. The mean time period between the pretreatment and posttreatment imaging was l7.2 $\pm$ 10.1 months (minimum 5 months, maximum 52 months). Because there is individual variability in the rate at which HPA function recovers, at the time of their reimaging, 19 patients were maintaining normal cortisol spontaneously, and three were still receiving replacement therapy with cortisol. For analyses evaluating the extent of brain volume changes, the full sample of 22 subjects was used. For analyses examining the relationship of these volume changes with changes in cortisol concentrations, the three patients receiving replacement were excluded. Of the remaining 19 patients, laboratory values for UFC were unavailable for two participants ($n = 17$), and mean plasma cortisol was unavailable for one different participant ($n = 18$).

Cortisol Measurement

24-hour urinary free cortisol and total plasma cortisol levels (bound plus free) were determined by radioimmunoassay using the Coat-a-Count Diagnostic Products Corporation (Los Angeles) kits. This assay has a detection limit of 0.2 μg/dL. The antiserum used is highly specific for cortisol with an extremely low ($<$ 1.4%) cross-reactivity to other naturally occurring steroids. Intraassay and interassay coefficients of variability are 2% and 5%, respectively. For plasma cortisol concentration, the mean of 12 samples taken every 2 hours during a 24-hour period was used in the analyses.

Magnetic Resonance Brain Imaging

All MRI imaging was performed on a 1.5 Tesla superconducting MR unit (General Electric, Milwaukee). Daily use of quality control phantoms, biweekly calibrations for magnetic field homogeneity, and system stability are regular features for the MR units. While we presently use contiguous 3D gradient echo technology for current research with new CD patients, in the studies reported here, we utilized a T1-weighted, off-axis, spin-echo sequence to use consistent methodology before and after treatment in patients with this rare disease who were entered into the study over a period of years. Technical details of the sequence were TR of 600 msec, TE of 20 msec, 256 × 256 pixel matrix, 2 NEX, 24-cm field of view, 4-mm slice thickness and a 0.5-mm interslice gap. The plane of acquisition was set for each patient to be perpendicular to the long axis of the HF by obtaining a "scout" saggital acquisition, locating the HF, and individually setting the off-axis coronal plane of acquisition. This T1-weighted, off-axis acquisition imaged the entire brain and
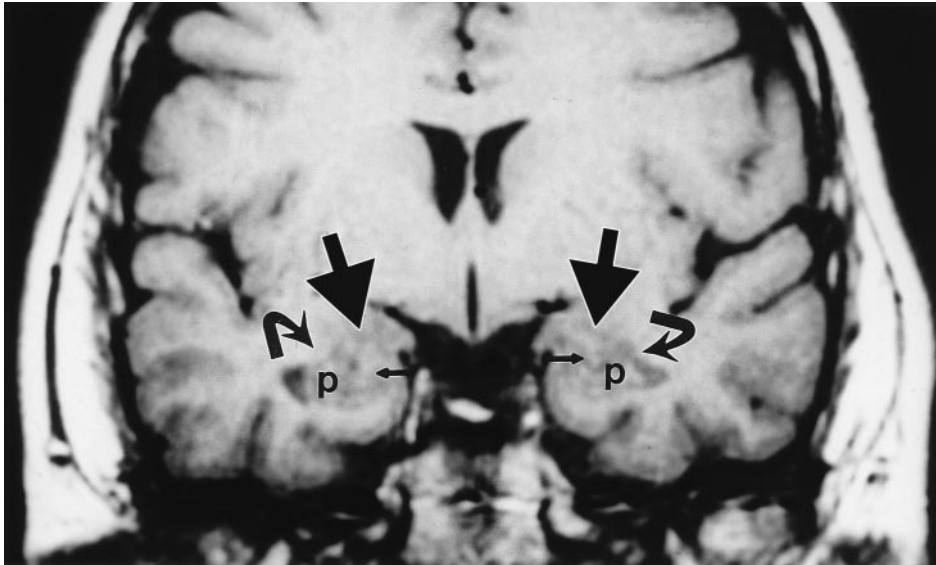
Figure 1. Off-axis coronal T1-weighted MR image through the junction of the amygdala and hippocampal pes. Although the posterior aspect of the amygdala and the anterior aspect of the pes are difficult to separate on this image, a notch at the amygdala-hippocampal junction (curved arrows) and white matter signal (small black arrows) helps to separate the amygdala (large black arrows) from the hippocampal pes (p).

skull. At reimaging, coronal acquisitions were obtained in the same plane as was used for the subjects' pretreatment scan.

Image Processing and Analysis

One neuroradiologist (SSG) analyzed all the images without knowledge of the patient's clinical or treatment status or endocrine test results. In addition to HFV, caudate head volume (CHV) was measured as a comparison. The caudate head was selected because it is also a gray matter nucleus, contains nearly the same neuronal density as the HFV, and has a concentration of GC receptors comparable to the remainder of the brain (Reul et al 1985, 1986). Because HFV and CHV are proportional to overall head size, total intracranial volume (ICV) was also determined.

Volume measurements of the HF and caudate head (CH) were made by manually tracing outlines of the structures on each serial coronal image using the track ball and light pen systems of the analysis software on the radiologist's display console. The volumes contained within the tracings were then manually summed, taking slice thickness into account and adding the interslice gap. The HF was defined as the dentate gyrus and hippocampus proper, also known as Ammon's Horn. The HFV was measured from its most anterior aspect, the pes, back to the body and tail tissue included on the most posterior coronal section, which includes the posterior commissure. The HFV included that portion of the subiculum along the roof of the parahippocampal gyrus, as this portion of the subiculum is contiguous with the gray matter signal of the HF and cannot be reliably separated from the HF. The hippocampal pes was disarticulated from the amygdala, excluding as much amygdala tissue as is possible. Such disarticulation is difficult because the amygdala's posterior border is cup-shaped and fits over the ball-shaped anterior border of the hippocampal pes, separated by a thin band of entorhinal white matter. Nonetheless, it was usually possible to discriminate between the two by locating the well-defined notch of gray matter and the thin band of entorhinal

white matter and using this as a cleavage plane (Figure 1). Although the white matter band itself may be about 1 mm in thickness, its signal will affect the pixels even in a 4 mm section, causing them to be somewhat lighter in intensity than the gray matter next to them. Thus, the influence of the structure can be seen even when the structure itself cannot be resolved. In subjects where the notch and white band were less well delineated, the shape of the anterior-most pes was predicted from the shape of the pes on the immediately adjacent, more posterior section where the amygdala is totally separate from the pes. This technique has been previously shown to have excellent reproducibility (Jack et al 1989, 1990).

The technique described by Jack et al (1989, 1990) was followed to delineate both the anterior and posterior portions of the hippocampus. The remaining borders in the gray matter HF are well defined with white matter outlining it inferiorly and laterally, and ventricular/cisternal cerebrospinal fluid (CSF) outlining it superiorly and medially. Based on each patient's anatomy and symmetry in the MR gantry, 12 to 19 slices were obtained and used for the HF volume calculation. The CH was defined as all caudate head tissue from its most anterior point to the abrupt posterior narrowing where the almond-shaped cross section of CH joins the disk-shaped caudate body. For CH measurement, a plane of cleavage was set at the abrupt change in caliber of the caudate gray matter signal. The remaining borders of the CHV were delineated by white matter inferiolaterally and ventricular CSF superioromedially. For the CHV calculations, 5 to 10 slices were obtained. Total intracranial volume was defined as the volume found by tracing the inner table of the entire skull. This provides a reliable means for normalizing volumes of individual brain structures (Free et al 1995).

In order to test the reproducibility of MRI measurements, 10 HFV images were each read on two separate occasions. No significant difference in means between the two measurements was observed (paired $t = .01$, $p > .3$). The Pearson product moment correlation coefficient between the pairs of measure-

Table 1. Comparisons of Volumes (cm^3) of Hippocampal Formation and Caudate Head in 22 Patients with Cushing's Disease before and after Treatment

	Pretreatment		Posttreatment			
	Mean	SD	Mean	SD	Paired t	$p <$
Hippocampal formation						
Absolute values						
Right	2.72	0.31	2.79	0.30	7.13	.0001
Left	2.53	0.29	2.61	0.27	6.05	.0001
Mean	2.62	0.30	2.70	0.28	6.89	.0001
ICV corrected values[a]						
Right	1.83	0.16	1.89	0.15	7.85	.0001
Left	1.70	0.15	1.76	0.15	6.37	.0001
Mean	1.77	0.15	1.82	0.15	7.41	.0001
Caudate head						
Absolute values						
Right	5.44	0.72	5.52	0.77	2.77	.01
Left	5.50	0.85	5.57	0.79	1.11	NS
Mean	5.47	0.77	5.55	0.77	1.9	NS
ICV corrected values[a]						
Right	3.67	0.44	3.73	0.48	2.98	.007
Left	3.71	0.49	3.76	0.49	1.4	NS
Mean	3.69	0.46	3.75	0.48	2.25	.04
Intracranial volume	1485.45	122.40	1484.32	121.63	1.14	NS

[a]For clarity of table presentation, ICV-corrected values have been multiplied by 1000.

ments was .996, and the concordance correlation coefficient (Lin 1989) between these pairs of measurements was .99. Both of these correlations indicate excellent reproducibility.

Data Analysis

In preliminary (paired t test) statistical analyses, pre–post comparisons were completed on the right, left, and mean (right and left) HFV and CHV. Table 1 summarizes these findings. Because of the consistency of these results for right, left, and mean volumes, all subsequent analyses reported in this paper utilized the mean score. The volumes of HF and CH in normal subjects differ substantially from each other, and therefore change scores were expressed as percent change of the pretreatment value. Relationships between the changes in brain volume and cortisol, as well as other key variables, were examined initially using paired t tests and χ^2 tests, then using partial correlation and multiple regression analyses to adjust for covariates. A sign test was used to examine whether the direction of changes (1% or greater) in HFV and in CHV were significantly different from chance (Remington and Schork 1985). The χ^2 test was used to determine whether the HFV and CHV changed independently of each other. Because cortisol values are characteristically not normally distributed, analyses using log-transformed hormone values were also performed. The statistical results using transformed and nontransformed data were comparable, therefore analyses and figures are presented using the nontransformed hormonal data for clarity. The two measures of cortisol, mean total plasma cortisol (bound plus free) and 24-hour urinary free cortisol, were not significantly correlated with each other before or after treatment ($r = .30$, $p > .17$ and $r = .21$, $p > .43$,

respectively). Thus, the following analyses were performed using each of the two measures of cortisol separately.

Results

We first examined whether there was a change in the brain volumes after treatment had reduced cortisol to normal levels. These analyses were completed for the mean (right and left), as well as the right and left volumes separately (Table 1). There was a highly significant difference between pre- and posttreatment HFV, using the mean of the right and left absolute values (difference = .08, paired $t = 6.89$, $p < .0001$) or values corrected for intracranial volume (ICV) (difference = 5.6×10^{-5}, paired $t = 7.4$, $p < .0001$). Using absolute values, there was a trend for a change in CHV (difference = .08, paired $t = 1.90$, $p < .08$), and this change reached significance after correction for ICV (difference = 5.9×10^{-5}, paired $t = 2.2$, $p < .04$). Similar findings were noted for right and left volumes separately (Table 1). Although there was a change in the volume for both structures, the change was significantly greater for HFV than CHV (mean percent change in HFV = 3.2 ± 2.5; mean percent change in CHV = 1.5 ± 3.4; paired $t = 2.20$, $p < .04$).

The sign test was used to investigate whether the direction of change in volumes observed after treatment was statistically different from chance. For the HFV, 18 of 22 patients (82%) showed an increase in volume of 1% or greater; 4 showed no change. This distribution in favor of improvement, instead of the expected 50% by chance, was highly statistically significant ($p = .0004$). In contrast, for CHV, 11 of 22 patients (50%) showed an increase in volume, while 11 were either unchanged (7) or had a decrease in volume (4). This distribution for increase in volume for CHV was not significantly different from chance ($p = .42$). A χ^2 test assessing the patterns of change in the two brain regions (HFV and CHV) indicated that these two patterns were statistically significantly different ($p = .02$).

Before investigating whether these changes in HFV or CHV were associated with changes in cortisol levels, we evaluated whether variables other than cortisol decrease could predict the change in HFV after treatment. Age was negatively correlated with the percent change in HFV ($r = -.43$, $p < .04$). Neither estimated duration of disease pretreatment nor the number of months elapsed since treatment were significantly related to percent change in HFV.

We then examined whether there was an association between changes in brain volumes and changes in cortisol levels. There was a significant relationship between the magnitude of the decrease in UFC concentration following treatment and the increase in HFV: the greater the de-
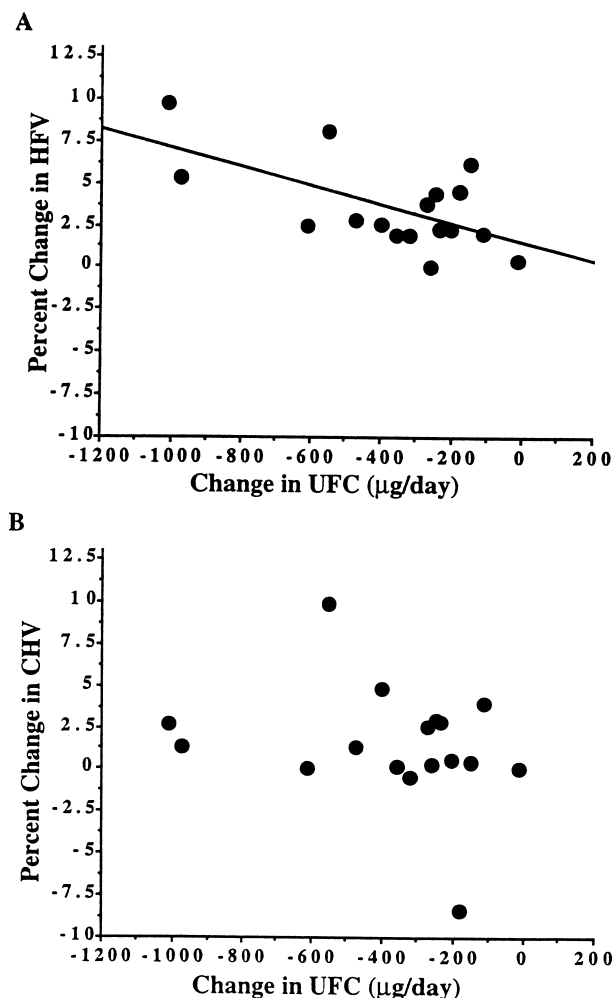
Figure 2. The relationship between change in HFV and CHV with change in UFC following treatment. Changes in brain volume are expressed as percent change posttreatment minus pretreatment. Change in UFC is expressed as the difference (decrease) in concentration posttreatment minus pretreatment. **(A)** The regression line is shown for HFV ($r = -.61$, $p < .01$). **(B)** The relationship for CHV was not significant.

crease in UFC concentration, the greater the increase in HFV ($r = -.61$, $p < .01$) (Figure 2A). In contrast to the findings for HFV, there was no significant relationship between percent change in CHV and the reduction in UFC ($r = -.24$, $p > .35$) (Figure 2B).

Unlike the significant relationship between the reduction in UFC and increase in HFV, no significant relationships were found between the reduction in total plasma cortisol and percent change of either HFV or CHV ($r = .02$, $p > .9$ and $r = .05$, $p > .8$, respectively).

To assess whether any of the covariates (age, duration of illness, or months since surgery) could impact these findings, general linear multiple regression models were completed separately for the HFV and CHV percent

change values. These models controlled for possible effects of the three covariates and included the primary variable, change in cortisol. First, we used a model including change in UFC, then a model including change in total plasma cortisol. The purpose of these models was to assess if the relationships of change in UFC or change in plasma cortisol with change in HFV and change in CHV were altered by these adjustments.

There was a significant percent change (increase) in HFV when either set of covariates was included. For the model including change in UFC, $F = 15$, $p < .0001$; for the model including change in plasma cortisol, $F = 8.2$, $p < .0005$. The contribution of change in UFC was a significant predictor (partial $t = 3.42$, $p = .004$): greater declines in UFC were associated with larger percent change increases in HFV. For the model that included the change in total plasma cortisol, none of the predictors including change in total plasma cortisol were individually predictive of change in HFV (partial t for change in plasma cortisol $= .39$, $p = .70$).

Based on these multiple regressions, the correlation between change in UFC and change in HFV adjusting for age, duration of illness, and months since surgery remained significant and strengthened ($r = -.70$, $p < .001$). In a secondary analysis, we examined the laterality of change. The relationship between the decrease in UFC and increase in volume was significant for both the left HFV ($r = -.50$, $p < .04$) and right HFV ($r = -.68$, $p < .002$). After adjustment, for the covariates, these relationships remained significant ($r = -.63$, $p < .002$ and $-.73$, $p < .003$, respectively).

For change in CHV, the multiple regression model, which includes change in UFC, was significant overall ($F = 3.6$, $p = .02$), but the change in UFC was not an individually significant predictor (partial $t = 1.5$, $p = .16$). After adjustment for age, duration of illness, and months since surgery, the correlation between percent change in CHV and the reduction in UFC remained nonsignificant ($r = -.36$, $p > .19$). The overall model for change in CHV including change in plasma cortisol was not significant ($F = 2.6$, $p = .07$), and there was no significant individual contribution of change in cortisol (partial $t = 1.25$, $p = .24$).

Informative volumetric data were also obtained from one CD patient not included in the present study whose hypercortisolemia continued after unsuccessful pituitary surgery and medical treatment with ketoconazole. Her brain MRI analysis was performed together with the image analyses from successfully treated patients, maintaining the study's design to keep the neuroradiologist unaware of any patient's clinical and endocrine status. Unlike the successfully treated patients whose HFV increased at the posttreatment reimaging, this patient had a 2.4% decrease

in HFV when reimaged after 13 months of continuing hypercortisolemia. Her CHV showed a decrease of 0.55%.

Discussion

These results indicate that the human hippocampal formation is able to increase in volume following sustained reduction of previously elevated cortisol concentrations. With remission of Cushing's disease, HFV increased in individual patients up to 10%. The percent increase in HFV was significantly correlated with the magnitude of change in urinary free cortisol level, a relationship that was region specific, as there was no significant association between change in cortisol and change in CHV.

We do not have brain imaging of the patients prior to the onset of CD. It is, therefore, not possible to know whether the increase in volume we observed represents partial or complete reversibility of the decrease in HFV that had occurred as a result of exposure to high levels of cortisol. Our studies currently in progress include comparisons with normal control participants studied over time to help shed light on this question. Because there are no published studies in human subjects that examine the reversibility of hippocampal formation atrophy to our knowledge, we cannot compare the magnitude of volume increase in our patients to other populations. There are, however, studies that examine the degree of reduction in HFV in conditions other than CD that are associated with elevated cortisol. In elderly healthy volunteers, for example, the degree of hippocampal atrophy correlated strongly with both the degree of cortisol elevation over time and the currently prevailing basal cortisol levels. The elderly subjects whose cortisol levels had increased over a period of 5 to 6 years and had reached levels of 12.8 μg/dL had a 14% decrease in hippocampal volume compared to aged subjects whose cortisol levels declined over time and reached lower levels of 9.1 μg/dL (Lupien et al 1998). Other studies have examined subjects exposed to severe stress and/or possible prior cortisol elevations. Compared to normal subjects, there was a 12% reduction in hippocampal volume in adults with posttraumatic stress disorder (PTSD) secondary to childhood abuse (Bremner et al 1995). Compared to normal subjects, patients with a history of recurrent major depression, a disease often associated with increased HPA activity, had 11% (right) and 15% (left) reductions of hippocampal volume (Sheline et al 1996). The increase in HFV observed in patients in our study, up to 10%, is consistent with the magnitude of changes of HFV reported in these other human study populations.

Our findings also suggest that increased age in humans may affect hippocampal plasticity adversely. In the present study, younger age was associated with greater increase in HFV after cortisol declined. An age-dependent difference in the magnitude of cortisol decrease after treatment did not explain this result, as there was no significant correlation between age and decrease in UFC ($r = .11$, $p = .63$). The effects on hippocampus of increased age and exposure to elevated cortisol levels may be synergistic. The frequency of occurrence of HF atrophy is strongly related to increasing age in the normal human elderly (de Leon et al 1997a). In addition, as shown in animals, glucocorticoids increase the rate of age-dependent cell loss in the hippocampus (Kerr et al 1992).

As we have noted previously (Starkman et al 1992), it is likely that multiple regions of the brain are affected to some degree by hypercortisolemia. Glucocorticoid receptors are distributed widely throughout the brain. A computerized tomography study in Cushing's disease reported a high incidence of cortical atrophy of the cerebrum and cerebellum (Momose et al 1971). In the present study, we did find a small but significant mean increase in caudate head volume following treatment; however, the direction of change in volume for this structure was not consistently positive, and the change in volume was not significantly correlated with the degree of UFC decrease after treatment.

There are several possible mechanisms that may underlie the increase in volume of brain regions following treatment of CD. Some may be nonspecific for the entire brain, others specific to the HF. The following speculations, based on current knowledge, are neither mutually exclusive nor exhaustive. 1) The water content of gray and white matter throughout the brain, including the CH as well as the HF, may increase as a result of the reduction in cortisol levels (Andersen et al 1993; James 1978). 2) More specific to the HF, atrophy of human hippocampal pyramidal cell dendritic structure may be reversible, at least in part, once glucocorticoid levels decrease, as occurs in animals (Sapolsky 1994). 3) Glial cells may increase in number or morphology in response to normalization of cortisol levels. 4) There may be reversal of cortisol-suppressed granule cell neurogenesis in the dentate gyrus. The HF in several species, including adult primates, is able to produce new granule cell neurons in adulthood (Gould et al 1998). These new cells originate by division from granule cell precursors in the dentate gyrus of the HF, and mature to participate in synapses. Production of new hippocampal neurons has recently been demonstrated in human adults (Eriksson et al 1998). In animal species, corticosterone or acute social stressors attenuate the proliferation of the granule cell precursors (Cameron et al 1994; Gould et al 1998). Thus, once cortisol levels are decreased in the CD patients posttreatment, enhanced neurogenesis in the dentate gyrus may contribute to the increase in HFV. In humans, there is also evidence that

MRI-determined volumes do reflect cellular profiles. That is, when both of these were examined postmortem in patients with Alzheimer's Disease, MRI-determined HFV and the number of hippocampal neurons were highly correlated, $r = .9$ (de Leon, unpublished data).

At a neurochemical level, the decline in GC following treatment reverses the exposure of hippocampal neurons to the chronic effects of increased GC concentrations. Exposure to increased GC has been shown in animals to increase release of serotonin and excitatory amino acids (glutamate), increase extracellular glutamate concentration and increase intracellular calcium (McEwen 1997). Many of these alterations are deleterious to neurons. GC also inhibits glucose utilization of the brain, particularly in the hippocampus (Kadekaro et al 1988). In humans, there is evidence of similar alterations elicited by glucocorticoids: a pharmacologic dose of cortisol induced a reduction in glucose utilization specific to the hippocampus in healthy elderly subjects (de Leon et al 1997b).

The present results indicate that after sustained normalization of cortisol, HFV increases to a greater degree than CHV, and the change in HFV, but not CHV, is correlated with the magnitude of decrease in cortisol concentration. These results are consistent with observations in animals and demonstrate that hippocampal volume change in response to sustained elevations in cortisol is reversible, at least in part, once cortisol levels have decreased to normal. The results support the hypothesis that the hippocampus exhibits enhanced sensitivity to cortisol, which affects both volume loss and recovery. Our findings are also relevant to aging and to medical and psychiatric conditions in which glucocorticoids, either endogenous or exogenously administered, are elevated. They further support our view that elevated cortisol is more than simply a downstream biomarker in psychiatric illness reflecting central processes, such as CRH activation. Cortisol elevation, disturbances in its circadian rhythm, or both likely contribute actively to neurobehavioral abnormalities.

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