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Plasma cortisol levels, brain volumes and cognition in healthy elderly men

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Summary *Purpose*. In ageing animals, exposure to chronic high levels of glucocorticoids is associated with cognitive impairment and hippocampal atrophy. However, there are few studies examining relationships among glucocorticoids, brain volumes and cognitive function in healthy older humans. This study examined the hypotheses that higher plasma cortisol levels and altered sensitivity to glucocorticoids are associated with worse cognition and more brain atrophy in elderly men.

Materials and methods. Ninety-seven healthy men aged 65–70 had plasma cortisol measured at 09:00, 14:30 h, and post-dexamethasone (0.25 mg, 09:00 h), and had dermal sensitivity to glucocorticoids measured. They also underwent cognitive testing, with scores adjusted for estimated prior mental ability, and had MRI measurements of intracranial area (a validated estimate of intracranial capacity), and hippocampus, temporal lobe and frontal lobe volumes.

Results. Plasma cortisol levels at 09:00 h were significantly and negatively correlated with a summary General Cognitive Factor accounting for 51% of the variance of cognitive function (rho $= -0.22$, $p = 0.035$), and specific cognitive tests: delayed paragraph recall (rho $= -0.28$, $p = 0.036$) and processing speed (rho $= -0.23$, $p = 0.026$). Regional brain volumes adjusted for intracranial area generally did not correlate with cortisol levels. Tissue glucocorticoid sensitivity did not correlate with any measure of cognition or brain volume.

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Conclusions. In healthy older men, higher plasma cortisol levels are associated with worse ageing-related overall cognitive change but not ageing-related brain atrophy.

1. Introduction

There is remarkable heterogeneity in changes in cognitive ability with ageing, with some people showing little or no change, others showing moderate decline and some developing dementia (Christensen et al., 1999). One hypothesis is that this heterogeneity is partly due to variations in exposure to glucocorticoids (cortisol, corticosterone) over the lifespan. This notion was spawned by observations, both in vitro and in vivo, that glucocorticoids potentiate the adverse effects of a range of neuronal insults, including excess NMDA (Armanini et al., 1990), reactive oxygen species (Patel et al., 2002) and disruption of neuronal calcium homeostasis (Nair et al., 1998). Moreover, in vivo prolonged elevation of glucocorticoid levels causes deleterious structural changes in neurons as well as neuronal electrophysiological and metabolic dysfunction, leading to decrements in cognitive function (Seckl and Olsson, 1995; Lupien et al., 1998; Porter and Landfield, 1998; Starkman et al., 1999; Lyons et al., 2000). The hippocampus is particularly vulnerable to such effects as it has a high density of intracellular receptors for glucocorticoids (De Kloet et al., 1998), though increasing evidence suggests that the medial prefrontal cortex, also rich in glucocorticoid receptors, is also vulnerable to the effects of prolonged high glucocorticoids (Lyons et al., 2000; Cook and Wellman, 2004). Crucially, a subset (around one-third) of ageing animals and humans show higher baseline levels of glucocorticoids, slower return to basal glucocorticoid levels after a stressor, and reduced suppression of glucocorticoids after dexamethasone (Gust et al., 2000; Issa et al., 1990; Lupien et al., 1996; Seeman and Robbins, 1994) and this is associated with both the development of atrophy of brain regions rich in glucocorticoid receptors, such as the hippocampus, and concomitant deficits in cognitive function (Landfield, 1981; Issa et al., 1990; Lupien et al., 1998). Glucocorticoid excess appears causative, at least in rodents, since treatments that maintain low glucocorticoid levels (mid-life adrenalectomy and low-dose corticosterone replacement (Landfield, 1981), neonatal handling (Vallee et al., 1999), antidepressant therapy from midlife (Yau et al., 2002), knockout of the gene for of 11β-hydroxysteroid dehydrogenase type 1 which amplifies local glucocorticoid action (Yau and Seckl, 2001)) all prevent the emergence of cognitive deficits with ageing and may even abolish ageing-related hippocampal atrophy (Landfield, 1981).

In humans the strongest evidence for a deleterious effect of high glucocorticoids on cognitive ability is from studies of patients with Cushing's syndrome in whom partially reversible cognitive impairments (Starkman et al., 2001) and hippocampal atrophy are observed (Starkman et al., 1999; Bourdeau et al., 2002). Higher cortisol levels in relation to lower hippocampal volumes have been observed in Alzheimer's disease (De Leon et al., 1988; O'Brien et al., 1996); in mild cognitive impairment salivary cortisol levels have been found to be negatively related to paragraph recall (Wolf et al., 2002a). In *healthy* elderly humans, some of the few published studies suggest that, as in ageing rodents, higher cortisol is associated with declining cognitive function (Lupien et al., 1996; Seeman et al., 1997; Kalmijn et al., 1998) and that strategies to reduce neuronal exposure to glucocorticoids may enhance cognitive function (Sandeep et al., 2004). There is little work examining regional brain volumes and cortisol levels in healthy individuals. Wolf et al. (2002b) did not find a correlation between hippocampal volume and cortisol output in 11 men aged 59–76, but did find a negative correlation when younger subjects were included. The one published study in healthy ageing subjects to examine cortisol levels in relation to cognition and neuroimaging variables found that individuals with rising cortisol levels (measured yearly, over a 24-h period) showed hippocampal atrophy and decline in cognitive function. This study also found a negative correlation between cross-sectional cortisol levels and hippocampal volume in the 11 subjects analysed (Lupien et al., 1998).

Previously we have shown that in a cohort of 97 healthy, unmedicated, elderly men, higher overall cognitive ability was associated with larger overall brain volumes and intracranial area (MacLullich et al., 2002). Here, in the same cohort, we enquire whether higher cortisol levels and reduced central and peripheral glucocorticoid sensitivity are related to (a) worse overall cognitive function and (b) overall brain atrophy. We were also interested

in testing hypotheses that glucocorticoid variables were negatively associated with individual cognitive tests and brain regions, such as the hippocampus.

2. Methods

2.1. Subjects

The study was approved by the Lothian Health Ethics Committee. Subjects were healthy male volunteers aged 65–70 who were living in Edinburgh, Scotland. They were recruited through an invitation letter and interview and gave informed consent. All were recruited with the assistance of their local General Practitioners. Each subject was interviewed by a physician (AM) to screen for symptoms suggestive of significant illness, including dementia, cerebrovascular disease, ischaemic heart disease and depressive illness. Those with symptoms were excluded. Subjects with a history of cancer, heart disease, respiratory disease, diabetes, neurological disease and other significant disease were excluded. Venous blood was analysed for urea and electrolytes, calcium, liver function tests, thyroid function tests, glucose, glycosylated haemoglobin; haemoglobin, white cell count, platelet count, B_{12} and folate levels, and any subject with abnormalities according to standard clinical criteria was excluded. No subject was on regular medication at the time of cognitive testing or imaging. One hundred subjects were entered into the study following the screening process.

2.2. Cognitive testing

Subjects underwent a set of tests designed to assess several domains of cognitive functioning. All testing was carried out in the morning by the same tester (AM) who was blind to the results of brain imaging and laboratory results. Cognitive testing started 60 min after venepuncture. Non-verbal reasoning was evaluated with Raven's Standard Progressive Matrices (Raven et al., 1977) using the number correct in 20 min. Verbal memory was evaluated with the Logical Memory (immediate and 30-min delayed) subtest of the Wechsler Memory Scale (Wechsler, 1987) and the Rey Auditory-Verbal Learning Test (AVLT) (Lezak, 1995). Testing of delayed paragraph recall, in which subjects returned for retesting (Logical Memory-24 h delayed), was performed in 58 subjects. Visuospatial memory was evaluated with the Visual Reproduction (immediate and 30-min delayed) subtest of the Wechsler Memory Scale (Wechsler, 1987) and Administration A of the Benton Visual Retention Test (BVRT) (Sivan, 1992). Because the immediate and delayed components of both Logical Memory and Visual Reproduction are highly correlated ($r = 0.83$ and 0.75, respectively) the summed standardized scores from each component were entered into the analysis. Verbal fluency was assessed with the Controlled Word Association Test using the letters C, F and S (Lezak, 1995). Attention and processing speed were evaluated with the Digit-Symbol Substitution Test from the Wechsler Adult Intelligence Scale (WAIS) (Wechsler, 1981). Prior intelligence was estimated with the National Adult Reading Test (NART) (Nelson and Willison, 1991).

2.3. Magnetic resonance imaging and analysis

Brain imaging was performed in an Elscint Prestige MR scanner operating at 1.9 T. Structural image age acquisition followed a three-view localiser and consisted of a coronal T1-weighted three-dimensional gradient-echo sequence covering the entire brain and skull (echo time = 9.254, repetition time = 28.5, tip angle = 25°, slice thickness = 1.5 mm [no interslice gap] × 1 × 1, 18-cm field of view, matrix = 180 × 180). The subjects were also scanned for white matter and spectroscopic analyses, the details of which are published elsewhere (MacLullich et al., 2004; Ferguson et al., 2002).

Image analysis. Image analysis was carried out on Sun workstations using Analyze software (Mayo Clinic, Rochester, MN). An intensity threshold separating the brain from the meninges was imposed for semiautomated analysis. Hippocampal formation was defined as subiculum, hippocampus proper, and dentate gyrus with the alveus and fimbria. The hippocampus was measured bilaterally using manual tracing from the first slice in which it appeared until the full extent of the crus fornicis appeared. In the posterior head of the hippocampus, an arbitrary judgment of the boundary between the hippocampus and the amygdala was made from the position of the temporal horn and the alveus or by comparing a slice with subsequent slices in which the division was more obvious (Jack et al., 1989, 1990). A line was drawn at the angle between the superior and medial surfaces of the parahippocampal gyrus (Watson et al., 1992) to separate the subiculum from the parahippocampal gyrus. Intrarater error was ± 0.03% for left and ±0.04% for right hippocampal volumes. The prefrontal lobe was measured from the slice in which the frontal pole could be

distinguished from the meninges. Measurements were made using automated methods with manual tracing to separate the lobes through the interhemispheric fissure. The last slice was that before the appearance of the genu of the corpus callosum. The anterior part of the temporal lobe was measured semiautomatically. Subsequently, a line was drawn manually along the lateral fissure to the Sylvian point and then diagonally to the superior-most point of the amygdalohip-pocampal complex and along the superior boundary of this structure. The last slice of the temporal lobe was the same as for the hippocampus. The intracranial area (ICA) was measured in the midline sagittal slice of the sagittal localiser by manually tracing round the inner table of the cranial vault, along the superior surface of the floor of the frontal fossa, and across the pituitary fossa to the dorsum sella. Tracing continued down the posterior surface of the clivus and completed by a line joining the anterior and posterior rims of the foramen magnum. ICA correlated at $r=0.88$ ($p<0.001$) with intracranial volume in 40 subjects in this cohort and is used as an estimate of intracranial volume (Ferguson et al., 2005).

2.4. Glucocorticoid measures

Blood for cortisol levels was drawn at 09:00 and 14:30 h on the same day. Central sensitivity to dexamethasone was assessed with a very low dose dexamethasone suppression test, in which 0.25 mg of dexamethasone taken at 23:00 h and blood was taken at 09:00 h the following morning. In each case 10 ml of blood was taken and immediately spun at 3000 rpm at 4 °C. Then 2–3 ml of plasma was extracted and stored at −20 °C until analysis. Cortisol concentrations were measured with an in-house radioimmunoassay. The mean intra-assay coefficient of variation was 6.2%. The interassay coefficient of variation (calculated through quality control samples included in each batch) was 9.2%. There are few studies of the within-subjects variability of basal plasma cortisol levels. Huizenga et al. (1998) found that in 76 elderly males cortisol levels taken between 08:00 and 09:00 h on two occasions 2.5 years apart showed a Spearman correlation of rho = 0.53 ($p<0.001$) and in 20 males aged 21–29 Coste et al. (1994) found an intraclass correlation of $r=0.54$ (confidence interval 0.32–0.70) among three measurements of 08:00 plasma cortisol. Other studies have reported broadly similar findings (Burleson et al., 2003).

Dermal sensitivity to glucocorticoids was assessed with the skin vasoconstrictor assay in which differences in the intensity of dermal blanching after overnight topical application of beclomethasone dipropionate are measured using a reflectance spectrophotometer and also by a visual estimate (Walker et al., 1997). This analysis gave two variables, the blanching index and the visual score.

2.5. Statistical analysis

Descriptive statistics, bivariate correlations and principal components analyses were performed using SPSS for Windows 11.5. Data from each domain of measurement were first explored. Because we had previously found that there were substantial significant positive intercorrelations among regional brain volumes, principal components analysis was used to derive a factor reflecting the shared variance of the regional brain volumes. This factor was designated the Fronto-temporal Volumes Factor (FTVF). Then an estimate of brain atrophy was provided by adjusting each regional volume and the FTVF for intracranial area (this was done by saving residuals from a linear regression). In young adulthood intracranial capacity is closely associated with brain volume (Sgouros et al., 1999), so controlling for intracranial capacity in older adults provides an estimate of ageing-related decline in brain volumes. The adjusted brain volumes and adjusted FTVF were then correlated with the glucocorticoid and cognitive test variables.

Because scores in diverse cognitive tests are also known to be substantially positively intercorrelated, with data reduction procedures yielding a factor explaining between 40 and 60% of the variance (Carroll, 1993; Deary, 2001) the cognitive test data were also subjected to principal components analysis, excluding the NART. The NART is an estimate of prior mental ability rather than a measure of current ability and was used to adjust current scores on the other cognitive tests for prior intelligence, using linear regression. The adjusted scores therefore are an estimate of ageing-related change in cognitive function (Deary et al., 2000; Crawford et al., 2001; Leaper et al., 2001).

3. Results

Of the 100 subjects scanned, three were excluded when unexpected pathology was discovered: two because of congenital arachnoid cysts, and one because of a pituitary macroadenoma. Data from the remaining 97 patients were included in the analyses.

Table 1 Descriptive data for the cortisol levels and skin vasoconstrictor assay.

	N	Minimum	Maximum	Mean	SD
09:00 h cortisol (nmol/l)	95	105	898	444.1	171.0
14:30 h cortisol (nmol/l)	88	23	369	168.1	66.0
Post-dex cortisol (nmol/l)	89	38	632	233.5	142.0
Blanching index (arbitrary units)	87	−13	552	231.4	123.6
Visual score	87	2	14	8.5	3.0

3.1. Glucocorticoids: descriptive data

Descriptive data for 09:00, 14:30 h and post-dexamethasone plasma cortisol levels, and the blanching index and visual scores from the skin vasoconstrictor assay are shown in Table 1. Of the 97 possible 09:00 h cortisol levels, two were unavailable for reasons of technical failure. Eighty-eight and 89 subjects, respectively, underwent testing of 14:30 h cortisol levels and post-dexamethasone cortisol levels. Post-dexamethasone cortisol levels showed a positive skew (that is, more subjects had lower cortisol levels); the other cortisol levels showed a near-normal distribution. Cortisol levels showed some positive intercorrelations, with 09:00 h cortisol correlating at rho = 0.26 ($p = 0.017$) with 14:30 h cortisol and rho = 0.22 ($p = 0.039$) with post-dexamethasone cortisol, and 14:30 h cortisol correlating at rho = 0.06 ($p = 0.579$) with post-dexamethasone cortisol. Eighty-seven subjects completed the skin vasoconstrictor assay.

3.2. Cortisol levels and cognition

Data reduction of cognitive test scores (excluding NART scores) with principal components analysis was performed. Complete data from 93 subjects were available. The first unrotated component accounted for 51% of the variance. This was termed the 'General Cognitive Factor' and a score on this factor was computed for each individual.

The General Cognitive Factor and the individual cognitive tests, except the NART, were adjusted for estimated prior intelligence using NART scores; these adjusted cognitive variables were then correlated with glucocorticoid measures. Spearman's correlations were used because some of the variables were not normally distributed. Cognitive test data were incomplete for the following tests: Raven's Matrices ($n = 95$), where two subjects were excluded because of incorrect completion of the answer sheets, Logical Memory ($n = 96$), where one subject's score was invalid in this test because of interruption of the testing session, Visual Reproduction ($n = 96$), where another subject also had a the test session interrupted, and for Logical Memory 24 h delayed, in which 58 subjects returned for testing of paragraph recall the following day.

Spearman correlations are shown in Table 2. Cortisol levels at 09:00 h correlated negatively and significantly with the General Cognitive Factor

Table 2 Spearman correlations between cortisol levels, the General Cognitive Factor, and cognitive test scores.

	09:00 h cortisol	14:30 h cortisol	Post-dex cortisol
General Cognitive Factor	−0.22 ($p = 0.035$)	−0.15 ($p = 0.158$)	−0.13 ($p = 0.157$)
Verbal Fluency	−0.09 ($p = 0.412$)	0.04 ($p = 0.634$)	−0.11 ($p = 0.301$)
DSST	−0.23 ($p = 0.026$)	−0.15 ($p = 0.129$)	−0.07 ($p = 0.072$)
AVLT	−0.08 ($p = 0.446$)	−0.10 ($p = 0.305$)	−0.06 ($p = 0.576$)
Logical Memory	−0.17 ($p = 0.104$)	−0.04 ($p = 0.655$)	−0.02 ($p = 0.823$)
Logical Memory 24 h	−0.28 ($p = 0.036$)	−0.15 ($p = 0.300$)	−0.07 ($p = 0.592$)
Visual Reproduction	−0.12 ($p = 0.270$)	−0.07 ($p = 0.663$)	0 ($p = 0.981$)
Benton Visual Retention Test	−0.10 ($p = 0.353$)	−0.04 ($p = 0.513$)	−0.10 ($p = 0.366$)
RSPM	−0.17 ($p = 0.099$)	−0.06 ($p = 0.489$)	−0.18 ($p = 0.100$)

DSST, Digit-Symbol Substitution Test; AVLT, Rey Auditory-Verbal Learning Test; RSPM, Raven's Standard Progressive Matrices. For the cognitive test scores, $N = 93$–95 for 09:00 h cortisol levels, $N = 86$–88 for 14:30 h cortisol levels and $N = 87$–89 for post-dexamethasone cortisol levels, except Logical Memory 24 h delayed, for which $N = 58$ for 09:00 h cortisol levels, $N = 55$ for 14:30 h cortisol levels and $N = 54$ for post-dexamethasone cortisol levels. For the correlations with the Cognitive Factors, $N = 91$ for 09:00 h cortisol levels, $N = 84$ for 14:30 h cortisol levels and $N = 85$ for post-dexamethasone cortisol levels.

(rho = −0.22, $p=0.035$), DSST (rho = −0.23, $p=0.026$), and with Logical Memory 24 h delayed (rho = −0.29; $p=0.027$, $N=58$). Since other studies have not adjusted for NART, we also report here that no cortisol levels were significantly correlated with the unadjusted General Cognitive Factor (09:00 h: rho = −0.167, $p=0.114$; 14:30 h: rho = −0.177, $p=0.108$; post-dex: rho = −0.070, $p=0.523$).

3.3. Cortisol levels and adjusted brain volumes

Descriptive data for regional brain volumes and intracranial area are reported elsewhere (MacLullich et al., 2002). As mentioned above, because of positive intercorrelations among the brain regions, data reduction was performed with principal components analysis. The first component (the FTVF) explained 58.0% of the variance. A score on the FTVF was computed for each individual. All brain volumes and the FTVF were adjusted for intracranial area, to give estimates of ageing-related atrophy. Cortisol levels were tested for correlations with adjusted regional brain volumes and the FTVF. All correlations were non-significant, apart from that between adjusted left temporal lobe volumes and 14:30 h cortisol (rho = −0.25, $p=0.018$, $N=95$). The correlations are shown in Table 3.

In a previous paper we reported that, in this subject group, the shared variance among the regional brain volumes was significantly associated with overall cognitive ability (MacLullich et al., 2002). Here we wished to determine whether the significant and negative correlation between cortisol levels and overall adjusted cognitive function was reduced after adjusting for brain volumes; this is a test of the hypothesis that the association between cortisol and cognitive function was mediated through variations in brain volumes. The Spearman correlation between 09:00 h cortisol and the General Cognitive Factor, adjusted for brain volume and prior intelligence scores, was rho = −0.18, $p=0.088$. The other cortisol correlations were nonsignificant. This is a very small reduction from the raw General Cognitive Factor (adjusted for prior intelligence scores) versus 09:00 h cortisol correlation (rho = −0.22), suggesting that the relationship between cortisol levels and cognitive function is not significantly mediated by differences in brain volumes.

Additionally, to enable comparison with other studies which have employed cut-off points in dexamethasone suppression tests, we compared the mean (adjusted) General Cognitive Factor and FTVF scores against post-dexamethasone cortisol levels in the first three quartiles ($N=64$, cortisol range 38–310 nmol/l) versus the fourth quartile ($N=21$, cortisol range 312–638 nmol/l). Mann–Whitney U tests showed (a) $U=433$, $p=0.015$ for General Cognitive Factor, and (b) $U=520$, $p=0.121$ for the Fronto-Temporal Volumes Factor.

3.4. Skin vasoconstrictor assay

There were no significant correlations between either the blanching index or the visual score and plasma cortisol levels, brain volumes or cognitive function (data not shown).

4. Discussion

We found that in a cohort of healthy elderly men higher plasma cortisol levels at 09:00 h were associated with worse cognitive function, as represented by a summary General Cognitive Factor which accounted for 51% of the variance in cognitive function. The association was not mediated via differences in brain volumes. Higher

Table 3 Spearman correlations between cortisol levels, and the Fronto-temporal Volumes Factor and regional brain volumes.

	09:00 h cortisol	14:30 h cortisol	Post-dex cortisol
Fronto-temporal Volumes Factor	−0.14 ($p=0.187$)	−0.07 ($p=0.491$)	0.02 ($p=0.888$)
Left hippocampus	−0.14 ($p=0.165$)	−0.05 ($p=0.633$)	−0.06 ($p=0.594$)
Right hippocampus	−0.12 ($p=0.241$)	0.01 ($p=0.927$)	−0.17 ($p=0.121$)
Left temporal lobe	−0.17 ($p=0.095$)	−0.25 ($p=0.018$)	0.12 ($p=0.254$)
Right temporal lobe	−0.16 ($p=0.126$)	−0.04 ($p=0.736$)	−0.05 ($p=0.632$)
Left frontal lobe	0.06 ($p=0.579$)	−0.20 ($p=0.063$)	0.14 ($p=0.178$)
Right frontal lobe	0.10 ($p=0.327$)	0.08 ($p=0.445$)	0.03 ($p=0.812$)

Factor and volumes adjusted for intracranial area. $N=95$ for 09:00 h cortisol levels, $N=88$ for 14:30 h cortisol levels and $N=89$ for post-dexamethasone cortisol levels.

09:00 h cortisol levels were also significantly associated with two individual tests of cognition, the Digit-Symbol Substitution Test and delayed paragraph recall. Only the correlation between left temporal lobe volumes and 14:30 h cortisol levels was significant among the correlations between adjusted brain volumes and cortisol levels. Therefore, we did not find that, in general, brain volumes adjusted for intracranial area were associated with cortisol levels. There were no significant correlations between a measure of peripheral glucocorticoid sensitivity, the skin vasoconstrictor assay, and neuroimaging variables or cognitive function. Similarly, a measure of 'central' (mainly pituitary) glucocorticoid sensitivity, dexamethasone suppression of cortisol, was not associated with neuroimaging variables or cognition.

Our findings provide some support for the hypothesis that higher glucocorticoids are associated with worse *general* cognitive functioning. The notion of a general effect of high glucocorticoids, in both acute and chronic situation is supported by several lines of evidence. Glucocorticoid receptors are widespread in the brain (Patel et al., 2000; Sanchez et al., 2000; Watzka et al., 2000). Acutely, high glucocorticoids have been shown to be associated with various cognitive impairments, including deficits in attention (Wolkowitz, 1994), working memory (Lupien et al., 1999), and declarative memory (Newcomer et al., 1999). Patients with Cushing's syndrome demonstrate multiple cognitive deficits (Whelan et al., 1980; Starkman et al., 2001). With ageing, higher glucocorticoids are associated with deficits in memory and selective attention (Lupien et al., 1994; Seeman et al., 1997). In terms of direct effects on brain structures crucial to cognitive function, high glucocorticoids are associated with atrophy of the hippocampus in animals and humans (Seckl and Olsson, 1995; Lupien et al., 1998). Other brain regions may also be compromised by high glucocorticoids, for example, the temporal lobes (van der Beek et al., 2004), and the medial prefrontal cortex, an area which is involved in both regulation of the HPA axis, and multiple cognitive functions, notably attention (Lyons et al., 2000; Herman et al., 2003; Cook and Wellman, 2004). In terms of the significant correlations with the individual tests, it is unclear as to why 09:00 h cortisol levels were significantly negatively correlated with delayed paragraph recall, but not short-term paragraph recall. It is possible that the delayed test is more sensitive to the effects of higher cortisol levels on functioning of structures important for delayed memory, such as the hippocampus. The Digit-Symbol Substitution Test is highly correlated with general cognitive ability (Carroll, 1993) and the positive correlation with 09:00 h cortisol here may reflect a general cognitive deficit. However, it is also plausible that acute elevations in cortisol occurring in the experimental setting might have affected performance on this test in the short term.

Basal cortisol levels show large and moderately consistent individual differences across the lifespan (Petrides et al., 1994; Huizenga et al., 1998; Stone et al., 2001; Burleson et al., 2003). These long-term individual differences are believed to be partly genetic (Bartels et al., 2003) and partly environmental, for instance due to HPA axis 'programming' by intrauterine/early postnatal events such as maternal 'stress' (Welberg and Seckl, 2001). Thus, lower birth weight is associated with higher cortisol levels (Shenkin et al., 2001; Reynolds et al., 2001) and worse cognitive function across the lifespan (Richards et al., 2002). Further important sources of variance are individual differences in the response to stress (Roy et al., 2001) and in tissue sensitivity due to polymorphisms of the intracellular glucocorticoid receptor (Derijk et al., 2001).

However, any differences might also occur with ageing. There is good evidence that some older animals and humans show an increase in basal glucocorticoid levels (Lupien et al., 1996), a slower return to basal levels of glucocorticoids after a stressor (Seeman and Robbins, 1994; Ferrari et al., 2001), and decreased sensitivity to glucocorticoid suppression by dexamethasone (Huizenga et al., 1998). Thus, rising cortisol levels might cause decrements in cognitive function over shorter periods of time in later life. Studies examining this are few but are mostly consistent with the hypothesis that rising glucocorticoid levels are associated with cognitive decline (Issa et al., 1990; Seeman et al., 1997; Greendale et al., 2000). Here, in healthy elderly men, in whom we assume any disease process is mild or latent at most, basal plasma cortisol levels associated with cognitive function. There was no evidence of abnormal tissue sensitivity to glucocorticoids or of defective HPA feedback suggesting that fundamental differences in these variables do not underpin the link between elevated cortisol and cognitive decline. Interestingly, it was the diurnal peak morning cortisol levels that associated with cognitive function, rather than the afternoon levels, suggesting that perhaps exposure to repeated high levels of cortisol which activates the lower affinity GR may be most important; this contention fits with animal data on the link between activation of this receptor and neuronal dysfunction (Kerr et al., 1992). Naturally, cross-sectional studies such as this

cannot determine the lifecourse of such subtle but important relationships.

In this cross-sectional study with healthy elderly men we did not find a clear pattern of significant correlations between plasma cortisol levels and brain volumes (adjusted for intracranial area), other than between 14:30 h cortisol levels and left temporal lobe volume. The finding of an association with the left temporal lobe, and not the right (albeit with only one measure of cortisol), may be of interest given that others have also found indirect evidence of laterality on cortisol effects on the brain, with the left side being more often affected in conditions with abnormalities of cortisol levels (Hull, 2002; Campbell et al., 2004; van der Beek et al., 2004). There were no significant correlations between hippocampal volumes and cortisol levels. However the cross-sectional design and method of cortisol measurements means that the present findings do not preclude relationships between rising cortisol levels and hippocampal volume, as reported by Lupien et al. (1998). Observation of our cohort sequentially over time will determine whether there is an emerging association between brain volume loss, cognitive decline and elevated cortisol levels. Alternatively, if higher cortisol levels are indeed associated with relative cognitive impairment, the effects of cortisol might be mediated by subtle changes in the central nervous system, such as changes in neuronal or synaptic function rather than volume changes measurable with structural MRI.

There were no significant correlations with results from the skin vasoconstrictor assay. This may be because individuals may show differing sensitivities to glucocorticoids and that peripheral sensitivity to glucocorticoids does not relate to central sensitivity (Ebrecht et al., 2000). This contention is indeed borne out by the lack of correlation between the skin vasocontrictor assay and the dexamethasone suppression test in the present study. In fact this finding is consistent with the cell-specific regulation of glucocorticoid receptor gene expression (McCormick et al., 2000; Seckl and Walker, 2001). Additionally, the lack of an association may indicate that the adverse effects of higher cortisol levels on the brain operate through direct actions on neurons rather than through vascular effects.

Some methodological limitations should be noted. Subjects attended hospital and underwent venepuncture and cognitive testing. The cognitive testing occurred 1 h after measurement of 09:00 h cortisol and testing was punctuated with frequent breaks so as to minimise stress. However, as with any set of novel events, involvement in the study is likely to have had some effect on cortisol levels, although the normal diurnal fall of cortisol in the afternoon was apparent; it is also important to note that all subjects underwent the same procedures. The possibility does exist that acute rises in cortisol relating to the test procedures are related to performance on the cognitive tests, rather than reflecting longer-term relationships. Related to this is that single measurements of plasma cortisol are a limited measure of the HPA axis activity (other studies have employed other methods such as 24 h urine collections and frequent salivary cortisol measurements). All but one of the previous studies which have examined the reliability of 09:00 h plasma cortisol levels have involved periods between measurements of greater than 1 year (Coste et al., 1994; Thomas et al., 1994; Huizenga et al., 1998). The differing protocols of these studies make an exact calculation impossible but taking an approximate estimate of a reliability of 0.5 from these studies, and assuming a reliability of 0.9 for cognitive tests (Lezak, 1995) correction for attenuation would increase the correlation between, for example, 09:00 h cortisol and the General Cognitive Factor from rho = 0.22 to 0.3 (Glass and Hopkinson, 1991). This is a cross-sectional, observational study and therefore the direction of causation, or any causation at all, cannot be determined. The subjects were all males, and this is important because several studies have shown that females may show increased dysregulation of the HPA axis with ageing (Otte et al., 2005), and also increased cognitive decrements with rising cortisol levels relative to males (Seeman et al., 1997).

The subjects are all volunteers and this is therefore not a population sample. As all were between 65 and 70 and in good health, any effects of higher cortisol might be mild and difficult to detect. However, because of the lack of disease, the association is more likely to be genuine. Multiple analyses were carried out and this increases the risk of Type I statistical error. However, although for completeness we reported all the correlations between cortisol levels and cognitive tests, it is essential to note that the main cognitive outcome measure was the General Cognitive Factor, which represents the *empirically derived* shared variance, which amounted to 51%, amongst *all* the cognitive tests. The correlation between the General Cognitive Factor and 09:00 h plasma cortisol is a much more robust finding than correlations between cortisol levels and a single cognitive test, and suggests that the association between cortisol and cognitive function is valid. It is also important, since cognitive test results are not independent outcomes

(Deary, 2001; MacLullich et al., 2002), to observe patterns in the correlation matrix. All correlations between 09:00 h cortisol and individual cognitive test scores are in the same direction, which would be unlikely if there is no relationship between 09:00 h cortisol and cognitive function.

In this cross-sectional study we examined relationships among measurements of glucocorticoids, regional brain volumes and cognitive function in a large group of healthy elderly men. The findings suggest that single, cross-sectional measurements of basal plasma cortisol levels are not associated with brain atrophy in healthy older men. However, the findings are supportive of the hypothesis that there is a small but significant cross-sectional association between higher plasma cortisol levels and worse overall cognitive ability. Follow-up studies with this cohort will examine the relationships between rising cortisol levels and both brain volumes and cognitive function.

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