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Interperson Variability but Intraperson Stability of Baseline Plasma Cortisol Concentrations, and Its Relation to Feedback Sensitivity of the Hypothalamo-Pituitary-Adrenal Axis to a Low Dose of Dexamethasone in Elderly Individuals*

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ABSTRACT

In the present study, we investigated whether the negative feedback action of glucocorticoids (GCs) on the hypothalamo-pituitary-adrenal (HPA) axis changes with age. We performed a 1-mg dexamethasone (DEX) suppression test in 216 healthy elderly individuals. To investigate individual variability of feedback sensitivity in more detail, 2.5 yr later a 0.25-mg DEX suppression test was carried out in 164 of the same individuals. We investigated whether there was an effect of age or gender on both basal and post-DEX cortisol levels, as well as on the concentration of DEX. Furthermore, we examined whether the reactions to the two doses of DEX differed, and whether indications for an intraperson stability of baseline cortisol levels could be found.

Neither the pre- nor the post-1-mg DEX plasma cortisol concentrations showed a correlation with age, and there were no differences between men and women. The same was true for the pre- and post-0.25-mg DEX cortisol concentrations. In reaction to 1 mg DEX, over 90% of the subjects investigated showed a cortisol suppression to levels below 50 nmol/L. After the administration of 0.25 mg DEX, there was a much wider range in post-DEX cortisol concentrations. After the administration of 1 mg DEX, there was a significant correlation between liver function parameters and plasma DEX concentrations in males, and there was a correlation between body mass index and plasma DEX concentration in females. Plasma DEX concentrations after the administration of 1 mg and 0.25 mg DEX were closely correlated within subjects ($P < 0.001$).

There was an intraindividual stability of serum cortisol levels determined at an interval of 2.5 yr. Furthermore, the individuals with the highest baseline cortisol concentrations also had the highest post–0.25-mg DEX cortisol concentrations, indicating a close relationship between basal cortisol levels and the feedback sensitivity of the HPA axis to a low dose of DEX. These observations suggest a genetic influence on the set point of the HPA axis. Aging does not seem to lead to a change in HPA activity as measured by early morning total cortisol levels. Also, no changes in the sensitivity of the feedback system to DEX were observed with age. DEX metabolism is influenced by liver function (in males) and by body mass index (in females). (*J Clin Endocrinol Metab* **83:** 47–54, 1998)

GLUCOCORTICOIDS (GCs) are necessary for the normal functioning of most tissues. Even small changes in the concentrations of circulating GCs may result in a wide spectrum of physiological and biochemical changes throughout the body. Under basal conditions, the concentration of plasma cortisol and the secretion of pituitary ACTH constitute a negative feedback system. Because of the diurnal rhythm in the rate of ACTH secretion, plasma cortisol levels are higher in the morning than at night. Both negative feedback and diurnal rhythm may be overcome by stress, which causes increases in ACTH output resulting in plasma cortisol levels considerably above those found in basal conditions.

Basal concentrations of plasma cortisol exhibit a wide variation between normal subjects (1). Little is known concerning the question of whether cortisol levels are stable within subjects. Interperson variation and intraperson stability of plasma cortisol levels have not been extensively investigated. For the intraperson constancy, as reported in twins, genetic factors may be of importance (2, 3). The variation in hypothalamo-pituitary-adrenal (HPA) activity between individuals is thought to be a function of many influences, including the individual's feedback sensitivity, circadian rhythm, and episodic secretion, whereas cortisol binding globulin (CBG) levels also affect cortisol concentrations (3). Over the years, the effects of age and gender on the function of the pituitary-adrenocortical axis have been extensively investigated. These studies, however, have resulted in contradictory results. Both circadian patterns of cortisol and ACTH secretion and random morning plasma cortisol concentrations were

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reported to be unaltered in the elderly (4–7). On the other hand, an age-related trend towards increased levels of evening plasma cortisol was reported (8), and increased mean cortisol levels derived from 24-h frequent sampling were found in elderly men but not in women (9). Finally, Waltman *et al.* (10) reported that aging in humans is not accompanied by a significant increase in spontaneous ACTH or cortisol secretion or by a decrease in HPA sensitivity to GC feedback suppression.

Another controversy in the literature concerns the influence of age and gender on the dexamethasone (DEX) suppression test (DST). The 1-mg overnight DST is a convenient screening procedure for patients with Cushing's syndrome. Administration of 1 mg of DEX at 2300 h to normal subjects suppresses the nocturnal surge in ACTH production and, as a consequence, cortisol levels are low when measured the next morning (11). In patients suffering from endogenous Cushing's syndrome of any etiology, cortisol levels are less sensitive to DEX. Another application for this test was found in depressed patients, who may show an insufficient cortisol suppression in reaction to the 1-mg DST as well (12). To interpret this test in geriatric patients, many studies were done to investigate the effect of age per se on the duration and pattern of cortisol suppression by DEX. Also, many drugs and medical conditions may affect the outcome of the DST (13). In some studies (14, 15) there were significant correlations between age and post-DEX cortisol concentrations, whereas in other studies no such correlations could be demonstrated (16, 17). Irrespective of psychiatric disturbances, a decreased sensitivity to GC feedback may be prevalent in the very elderly (>80 yr) (18).

In the present study we investigated whether the autoregulatory negative feedback action of GCs on the HPA axis changes with age. We performed a 1-mg DST in 216 healthy elderly individuals, and 2.5 yr later a 0.25-mg DST was carried out in 164 out of these 216 subjects. In addition, it was investigated whether there was an effect of age or gender on basal and post-DEX cortisol, as well as on the metabolism of DEX. Furthermore it was examined whether there were differences in response to the two doses of DEX administered, and whether the suppression of cortisol was related to baseline cortisol levels. Finally, the two baseline cortisol concentrations in the 164 individuals who underwent two examinations were compared to get an impression about the intraperson stability of these concentrations.

Subjects, Methods, and Materials

For the present study, a sample of participants from the Rotterdam Study was invited for an additional examination. The Rotterdam study is a population-based cohort study of the determinants of chronic disabling diseases in the elderly. All approximately 10,000 inhabitants of a suburb of Rotterdam, age 55 yr and over were invited to participate as described elsewhere (19). The population for the present study included 216 individuals age 55–80 yr (102 men and 114 women with mean ages of 67.7 ± 5.6 and 65.8 ± 6.1 yr, respectively) who had completed the baseline visit for the Rotterdam Study not more than 6 months earlier. Subjects with acute, psychiatric, or endocrine diseases, including diabetes mellitus treated with medication, were not invited. Three of the patients used antiepileptic drugs, whereas one, who showed the lowest pre-DEX cortisol concentration, was treated with GCs. None of them was treated with estrogens. Compared with the other participants of the Rotterdam Study of the same age without diabetes mellitus, there were no differences in age and gender distribution. Written informed consent was obtained from all subjects, and the study was approved by the medical ethics committee of the Erasmus University Medical School.

1-mg DST

Participants were seen at the research center after an overnight fast. Between 0800 and 0900 h blood was drawn by venapuncture to determine the serum cortisol concentrations (pre-DEX concentrations), using RIA kits obtained from DPC (Los Angeles, CA). Intra- and interassay variations were below 8.0% and 9.5%, respectively. Also, serum concentrations of the liver enzymes alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), and γ-glutamyltransferase (γ-GT) were determined, using standard methods. At the same time, the body weight and height of the volunteers were measured, and the body mass index (BMI, kg/m^2) was calculated to be 26.4 ± 0.29 for the men and 26.4 ± 0.39 for the women (mean ± SE).

In the evening following the first visit to the research center, a 1-mg DST was carried out. Participants were given a tablet of 1 mg DEX and instructed to ingest it at 2300 h. The next morning, fasting blood samples were obtained by venapuncture at the same time as the previous morning. In these samples, cortisol and DEX concentrations were measured. DEX levels were estimated by RIA using an antiserum supplied by IgG Corp. (Nashville, TN) and [^{3}H]-DEX from Amersham (Little Chalfont, UK). Intra- and interassay variations were below 8.5 and 14.2%, respectively. Sensitivity of this assay, defined as mean result of assay of non-DEX containing plasma + 3 SD, was 0.13 nmol/L.

0.25-mg DST

To get more insight into individual variability of feedback sensitivity to GCs, all 216 participants were invited for a second DST 2.5 yr later. Out of this original group, 11 subjects had died and 6 subjects could not be reached. Eventually, 164 individuals (76 men and 88 women with mean ages of 69.1 ± 5.9 and 67.6 ± 5.6 yr, respectively), agreed to participate in a second test. The same procedures were used as described for the 1-mg DST but using 0.25 mg DEX. The samples obtained from the first and the second baseline visit as well as the pre- and post-1- and 0.25-mg DEX samples were determined in separate assays. To investigate possible differences in cortisol concentrations caused by interassay variation, baseline samples obtained at the first and second examination from 20 randomly selected subjects were determined in a single cortisol assay.

0.25-, 0.50-, and 1-mg DST in healthy laboratory personnel

A group of nine healthy laboratory workers (age 26–49 yr) agreed to participate in a series of DSTs with increasing doses of DEX. At intervals of 2 weeks (to ensure the absence of interference with DEX effects from the previous test) we performed three DSTs, using 0.25, 0.50, and 1 mg DEX as described above. None of these individuals were using medication or oral contraceptives.

Statistical analysis

All results are reported as the mean ± SE. Serum cortisol concentrations in men and women were compared using the two-sample Wilcoxon rank-sum test. The Wilcoxon matched pairs signed rank sum test was used to compare serum cortisol concentrations before and after DEX administration and between the two examinations in each person.

To assess the relationships between cortisol and age and between DEX and liver enzymes, BMI, and age, linear regression analysis was used. To calculate the degree of association between these parameters, the Pearson correlation coefficient (r) was calculated. Partial correlation coefficients were used to adjust for several parameters.

Spearman's rank correlation coefficient (r) was used to assess the association between the DEX concentrations after the administration of the two different doses and between basal cortisol concentrations at the two examinations.

Results

Baseline hormone determinations

Table 1 shows the baseline concentrations of serum cortisol in the whole group of 216 elderly subjects and in males and females separately. There was no difference in cortisol concentrations between men and women. Using linear regression analysis, no significant correlation could be demonstrated between the baseline serum cortisol concentration and age ($P > 0.2$).

TABLE 1. Cortisol concentrations (nmol/L) before and after administration of 1 mg DEX in 216 elderly individuals (114 females and 102 males)

	Total	Females	Males	P^a
Before DEX	521.7 ± 10.4	524.8 ± 14.5	517.4 ± 15.4	0.89
After DEX	35.5 ± 4.40	32.0 ± 4.70	38.9 ± 8.1	0.55

Values are mean ± SE.
[a] Test for difference between males and females.

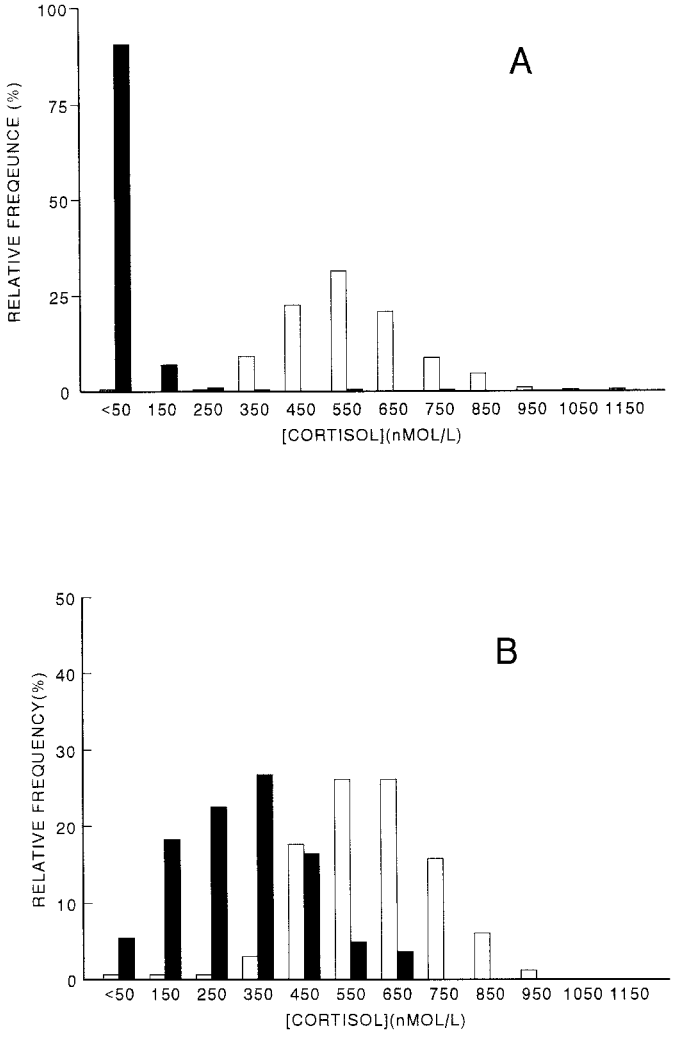

FIG. 1. Cortisol concentrations before and after DEX administration. A, Relative distribution of cortisol concentrations before (*open bars*) and after (*solid bars*) 1 mg DEX in 216 healthy elderly individuals. B, Relative distribution of cortisol concentrations before (*open bars*) and after (*solid bars*) 0.25 mg DEX in 164 healthy elderly individuals. Blood samples were drawn between 0800–0900 h after 1 or 0.25 mg DEX was administered at 2300 h on the previous night. Period between two doses was approximately 2.5 yr.

1-mg DST

Figure 1A shows the distribution of cortisol concentrations before and after 1 mg DEX. Out of the 216 healthy individuals, 196 (90.7%) showed a post-DEX cortisol concentration below 50 nmol/L. Only five individuals (2.3%) had post-DEX cortisol concentrations above 140 nmol/L, which is the cutoff point for a normal test result when the DST is used as a screening procedure for Cushing's syndrome in our clinic (20). These five individuals have been further investigated for cortisol resistance and are reported separately (21).

As shown in Table 1, there was no difference between men and women with regard to their post-DEX cortisol concentrations. There was no correlation between post-DEX cortisol concentrations and age ($P = 0.3$).

Figure 2A shows the distribution of the early morning plasma DEX concentrations after the administration of 1 mg DEX. None of the individuals investigated had an unmeasurably low circulating DEX level, indicating that all participants indeed had ingested the DEX tablets. Six individuals had DEX concentrations below 1.8 nmol/L. Three of them were using antiepileptic drugs.

Table 2 shows the results of the linear regression analysis for the relationship between DEX and several factors that might influence the metabolism of DEX. In males, there was a positive correlation between concentrations of DEX and the liver enzymes ALAT, ASAT, and γ-GT. After deletion of the results of the 35 men who had supranormal levels of at least one of the enzymes, the relationship was no longer significant. In females, these correlations were not significant, despite the fact that in 21 of them increased levels of at least one of the enzymes was found. In women there was a significant correlation between DEX concentrations and BMI that was not present in men. Neither in men nor in women was there a relation between the DEX concentration and age. Baseline cortisol concentrations were not correlated with liver function tests in either sex.

There was no significant relation between cortisol concentrations before and after 1 mg DEX ($P > 0.1$). The post-DEX cortisol concentration and the DEX concentration itself demonstrated a low but significant negative relationship ($r = -0.17$, $P < 0.01$). This was because of the six individuals with the lowest DEX concentrations (<1.8 nmol/L); when these individuals were excluded from the analysis, the association was no longer significant. Also, there was no significant relationship between the decrease in cortisol concentrations in reaction to DEX administration (Δ cortisol) and the DEX concentration ($P > 0.4$).

0.25-, 0.50-, and 1-mg DST in healthy laboratory personnel

From the results of the 1-mg DST, it became clear that more than 90% of the subjects investigated demonstrated a suppression of cortisol to levels below 50 nmol/L. Therefore 1 mg DEX might be too high a suppressive dose to allow the detection of individual differences in sensitivity of the HPA axis to GCs. A pilot study with several doses of DEX was subsequently carried out in nine healthy volunteers. Figure

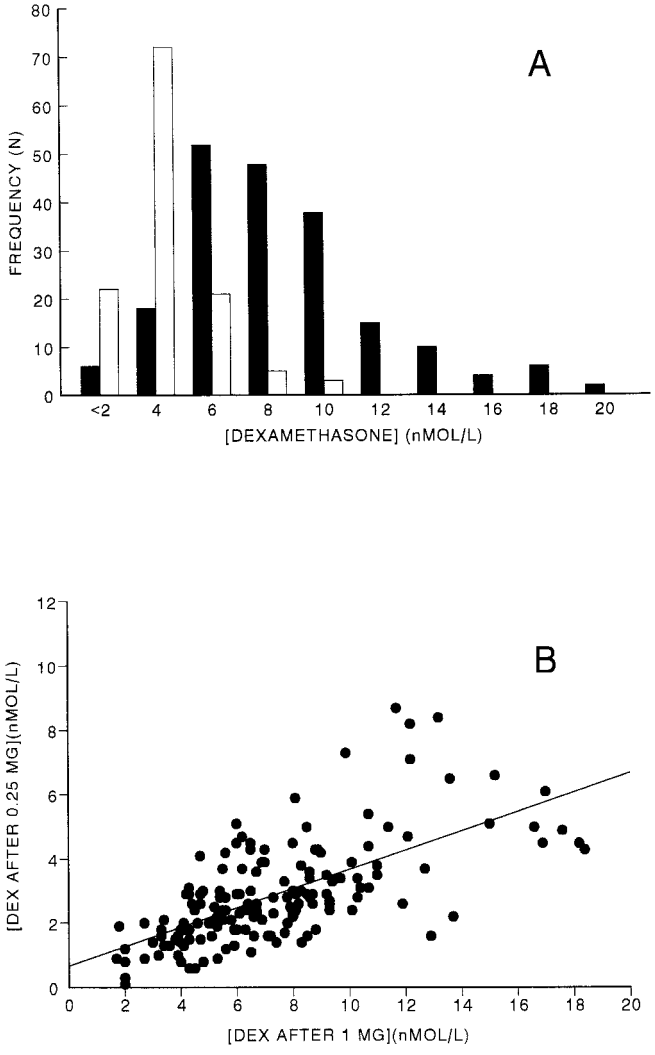

FIG. 2. DEX concentrations after 1 or 0.25 mg DEX. A, Distribution of DEX concentrations after 1 mg (n = 216, *solid bars*) or 0.25 mg (n = 164, *open bars*) DEX. B, Relationship between between DEX concentrations after 1 mg and 0.25 mg DEX; r = 0.66, $P < 0.001$ in 164 subjects in whom both doses were tested. Blood samples were drawn between 0800–0900 h after 1 or 0.25 mg DEX were administered at 2300 h on the previous night. Period between two doses was approximately 2.5 yr.

3A shows the cortisol and DEX levels after the administration of 0.25, 0.50, and 1 mg DEX. There was a considerable variability in baseline serum cortisol concentrations. The administration of increasing doses of DEX resulted in a dose-dependent increase in mean circulating DEX levels and a dose-dependent decrease of cortisol levels. The administration of 0.25 mg DEX even enlarged the range of serum cortisol levels: in one subject the serum cortisol level was suppressed to a level below 50 nmol/L, whereas in three individuals post-DEX cortisol levels remained above 200 nmol/L. After 0.50 mg DEX, the range became more narrow, and after the administration of 1 mg DEX, all individuals had a cortisol concentration below 50 nmol/L.

As shown in Fig. 3B, there was a considerable individual variability in cortisol suppression in reaction to a given DEX concentration. This variability was not caused by individual differences in CBG concentrations (data not shown). Using linear regression analysis, there was no significant relationship between the individual DEX concentrations and the individual degree of cortisol suppression or the actual post-DEX cortisol levels (all P values > 0.8). This suggests that different individuals suppress the HPA axis to a variable

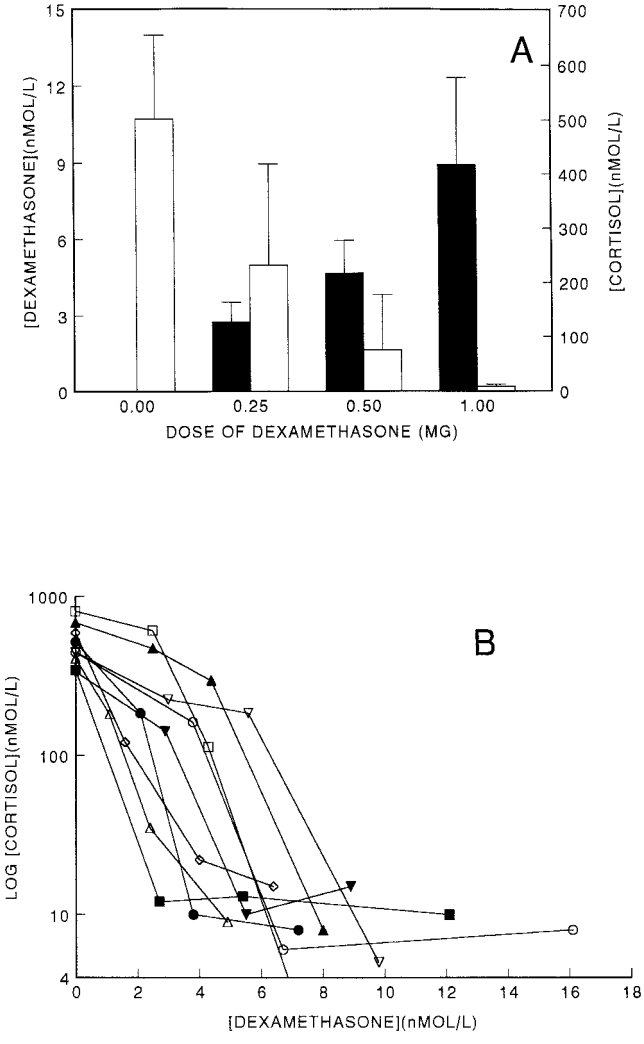

FIG. 3. Effects of various doses of DEX on cortisol suppression. A, Cortisol (*open bars*) and DEX (*solid bars*) concentrations after administration of 0.25, 0.50, and 1 mg DEX in nine healthy individuals. Blood samples were drawn between 0800–0900 h. DEX (0.25, 0.50, or 1 mg) were administered at 2300 h on the previous night at intervals of 2 weeks. Data are means and SD of concentrations in nine individuals. B, Individual relations between logarithm of cortisol concentration and DEX concentration.

degree in reaction to similar circulating DEX concentrations.

0.25-mg DST

Anticipating that a lower dose of DEX would indeed allow a better insight into the individual variability of the feedback sensitivity of the HPA axis, all subjects who originally participated in the 1-mg DST were invited to join in a 0.25-mg DST approximately 2.5 yr later.

Table 3 shows the mean baseline levels of serum cortisol

TABLE 2. Relations between DEX concentrations and liver enzymes, BMI, and age

	Total		Females		Males	
	r	*P*	r	*P*	r	*P*
ALAT (U/L)	0.19	<0.01	0.08	0.38	0.28	<0.01
ASAT (U/L)	0.22	<0.01	0.14	0.13	0.25	<0.02
γGT (U/L)	0.06	0.36	−0.1	0.33	0.24	<0.02
BMI (kg/m^2)	0.12	0.07	0.24	<0.01	−0.03	0.78
Age (yr)	0.09	0.15	0.14	0.14	0.03	0.78

TABLE 3. Cortisol concentrations (nmol/L) before and after administration of 0.25 mg DEX in 164 elderly individuals (88 females and 76 males)

	Total	Females	Males	*P*[a]
Before DEX	547.8 ± 10.9	568.4 ± 14.2	523.6 ± 17.2	<0.04
After DEX	260.5 ± 10.9	243.5 ± 15.2	281.3 ± 16.0	0.08

Values are mean ± SE.
[a] Test for difference between males and females.

in 164 elderly subjects. The second baseline mean serum cortisol concentrations turned out to be significantly higher than those before the 1-mg DST. The original cortisol levels of these 164 subjects amounted to 503.0 ± 11.3 nmol/L, whereas the follow up baseline cortisol levels were 547.8 ± 1.9 nmol/L ($P < 0.001$). Furthermore, in these 164 individuals, there was a significant difference in cortisol levels between men and women ($P < 0.04$). Linear regression analysis for baseline cortisol levels and age showed no significant correlation ($P > 0.06$). Random samples from 20 individuals from both groups of baseline samples were determined in a single assay. Mean cortisol concentrations in both groups were identical, and there was no difference between the sexes (data not shown). This suggests that there was no age-related change in cortisol concentrations, but that the differences found should be explained by the interassay variation.

Figure 1B shows the distribution of cortisol concentrations before and after 0.25 mg DEX. There was a much wider range in post-DEX cortisol concentrations than observed after 1 mg DEX (*cf.* Fig. 1A). A small number of individuals suppressed their cortisol levels to below a level of 140 nmol/L (n = 34, 20.7%), whereas only nine individuals (5.5%) suppressed to levels below 50 nmol/L.

Table 3 shows the serum cortisol concentrations after the administration of 0.25 mg DEX. There are no differences between men and women.

The distribution of DEX concentrations after 0.25 mg DEX is shown in Fig. 2A. Mean DEX concentrations after this low dose of DEX were significantly lower than after 1 mg DEX ($P < 0.001$). On the other hand, as shown in Fig. 2B, subjects who showed the highest DEX concentrations after the administration of 1 mg DEX, also showed the highest levels after the administration of 0.25 mg DEX (r = 0.66, $P < 0.001$). Figure 2B shows only two out of the six individuals with DEX concentrations below 1.8 nmol/L, because four of those showing the lowest DEX concentrations after 1 mg DEX did not participate in the 0.25-mg DST.

Figure 4, A and B show the linear regression analysis for the relationship between pre- and post-0.25-mg DEX cortisol concentrations (r = 0.39, $P < 0.001$), and the DEX concentration and the post-DEX cortisol concentrations (r = −0.24, $P < 0.002$). Adjusting the relationship between pre- and post-DEX cortisol concentrations for the DEX concentration gives rise to a partial correlation coefficient of 0.43, $P < 0.001$. This indicates that those individuals with the highest baseline cortisol levels have also higher post-0.25-mg DEX cortisol levels in response to a given DEX concentration.

Figure 4C shows the linear regression analysis for the DEX concentration and Δ cortisol (decrease in cortisol concentration after 0.25 mg DEX), (β = 30.7, r = 0.31, $P < 0.001$). Although there was no correlation between δ cortisol and the DEX concentration in the pilot study (n = 9), there was an association between these parameters in this much larger group of individuals. The finding of a regression coefficient of 30.7 confirms that the biological activity of DEX is about 30 times higher than the biological activity of cortisol. Figure 4C also shows that in four subjects, there was a low δ cortisol (<230 nmol/L) at DEX levels above 7 nmol/L. All four subjects showed post-1-mg DEX cortisol concentrations below 50 nmol/L. This indicates again that the 0.25-mg DST indeed gives a better insight into individual sensitivity to GCs than the 1-mg DST.

Individual stability of serum cortisol and CBG concentrations

To investigate whether plasma cortisol concentrations were stable within each person after an interval of 2.5 yr, Spearman's rank correlation coefficient (r) was calculated for the baseline cortisol concentrations that were obtained at the two examinations. Table 4 shows that there are highly significant correlations between the concentrations determined at an interval of 2.5 yr in both sexes. It can be concluded that subjects with high cortisol levels at the first examination also had a high level 2.5 yr later. The small size of the coefficient is probably because of the interassay variation. The coefficient within the 20 samples measured in one assay was much larger (r = 0.59). Therefore there is indeed an intraperson stability in the baseline early morning concentrations of these parameters.

Discussion

Individual feedback sensitivity of the HPA axis

1 mg DST

In man, the major circulating GC hormone is cortisol. Cortisol production in an unstressed individual follows a nycthemeral rhythm, with peak cortisol concentrations shortly before waking and a decline through the day, reaching a nadir at bedtime. Large individual variations both in baseline and stimulated cortisol levels have been reported

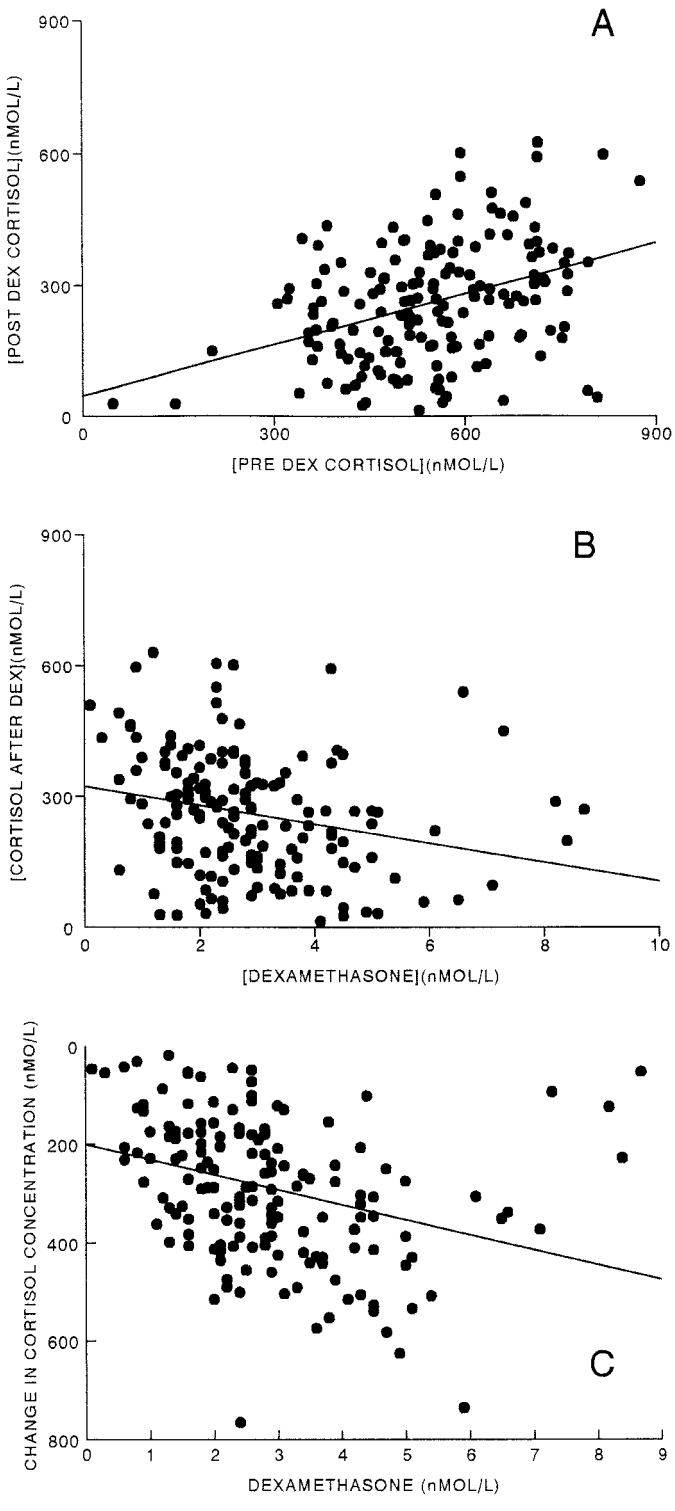

FIG. 4. Relationships between cortisol and DEX concentrations in 0.25-mg DST. A, Linear regression analysis for relationship between pre- and post-0.25-mg DEX cortisol concentrations in 164 elderly subjects; $r = 0.39$, $P < 0.001$. B, Linear regression analysis for relationship between DEX concentrations and post-0.25-mg DEX cortisol concentrations in 164 elderly subjects; $r = -0.24$, $P < 0.002$. C, Linear regression analysis for relationship between DEX concentrations and change in cortisol concentration in response to administration of 0.25 mg DEX in 164 elderly subjects; $r = 0.31$, $P < 0.001$.

TABLE 4. Spearman's rank correlation coefficient between cortisol concentrations determined at an interval of 2.5 yr

	Total		Females		Males	
	r_s	*P*	r_s	*P*	r_s	*P*
Cortisol (nmol/L)	0.45	<0.001	0.34	<0.001	0.53	<0.001

(1). One of the goals of the present study was to gain more insight into the interindividual variability of feedback sensitivity of the HPA axis, as well as into the intraindividual stability of circulating cortisol values. In 216 healthy elderly individuals, a 1-mg overnight DST was carried out to investigate the variability in feedback sensitivity. Only 5 out of the 216 individuals (2.3%) showed post-DEX cortisol concentrations above 140 nmol/L, which is the cut-off point for a normal test result in our clinic when the DST is used as a screenings procedure for Cushing's syndrome (20). These results are in accordance with previous studies in which nonsuppression was reported in 4% (14), 9.1% (16), and 5% (17) of subjects. Our data demonstrate that 1 mg DEX is too high a dose to detect individual differences in feedback sensitivity within a normal population, because near total suppression of cortisol levels was observed in most individuals.

Another aspect of this study was to gain more insight into the role of the metabolism of DEX in its suppressive effects on the HPA axis. Synthetic steroid hormones are metabolized in the liver before being eliminated from the organism (22). It is well known that a number of drugs influence the outcome of a DST by altering the metabolism of DEX: *e.g.* sodium phenytoin induces hepatic enzyme activity, thereby increasing the metabolic clearance of GCs including DEX (23). In our study population, there were six subjects with a DEX concentration below 1.8 nmol/L, three of whom were indeed on antiepileptic drugs. In the other subjects, there was a wide variation in DEX levels (ranging from 2.0–18.4 nmol/L). Also, obesity might influence the outcome of a DST. In a study by Crapo *et al.* (24), nonsuppression of cortisol after 1 mg DEX was reported in 1.1% of lean outpatient controls and in 13% of obese controls. Cronin *et al.* (25) reported that the degree of adiposity did not differ between patients with false-positive responses in a DST and patients with normal responses. The data from our study shows that in males, the capacity of the liver to metabolize DEX is related to liver function parameters (ALAT, ASAT, and γ-GT). In women, DEX metabolism was not related to liver enzyme levels. This sex difference may be caused by the larger proportion of men with liver function disturbances and the larger range of BMI values in women. Baseline and post-DEX cortisol levels were not related to liver function parameters and/or BMI in either sex. Still, the influence of liver function disturbances and BMI on the metabolism of DEX might contribute to the occurrence of false-positive or false-negative outcomes of the 1-mg DST in the endocrine clinic in more severely affected individuals. In studies performed by Meikle (26) and by Weiner (27), it was found that plasma DEX concentrations of 5.6 nmol/L and 5.1 nmol/L, respectively, at 0800 h postadministration are required to reliably suppress HPA function. In another study (28) in psychiatric patients, abnormal results of the DST were closely associated with low plasma levels of DEX. In a study comparing patients with endogenous depression

and normal controls, it was shown that serum DEX levels were significantly lower in nonsuppressors than in suppressors (29).

0.25-, 0.50-, and 1-mg DST in healthy laboratory personnel

From the data obtained from the 1-mg DST, it became clear that this dose might suppress the HPA axis too much to allow the detection of individual differences of its feedback regulation. A pilot study with 0.25, 0.50, and 1 mg DEX in nine healthy laboratory workers showed that these lower doses indeed allowed a better discrimination in HPA axis feedback sensitivity. The broadest range of cortisol concentrations was present after the administration of 0.25 mg DEX. This suggests that the 0.25-mg DST may give a good insight into the individual feedback sensitivity of the HPA axis.

0.25-mg DST

Out of the original group of 216 subjects, 164 persons participated in a 0.25-mg DST. The results from the 0.25-mg DST showed a wide range of post-DEX cortisol concentrations from 14–630 nmol/L. It also became clear that there was a close relationship between pre- and post-DEX cortisol concentrations, a relation that was absent in the 1-mg DST. The association became even stronger when, by calculating a partial correlation coefficient, it was adjusted for the actual DEX concentration. These results show that subjects with the highest baseline cortisol concentrations also have the highest post-0.25-mg DEX cortisol concentrations for a given DEX concentration, and indicate that the sensitivity of the feedback regulation of the HPA axis is related to the baseline cortisol concentration of the individual. This means that the set point of the HPA axis baseline levels is related to its feedback sensitivity.

Individual stability of cortisol concentrations

In this study, we were able to measure early morning cortisol concentrations in a large group of healthy subjects twice, at an interval of approximately 2.5 yr. Serum cortisol showed an individual stability, indicating that the HPA axis is set at a stable and reproducible set point for a given individual. As described above, it became clear from the data of the 0.25-mg DST that subjects with the highest baseline cortisol concentrations also had the highest post-DEX cortisol concentrations. It seems that within an individual, there is a set point for HPA activity, which is defined before as well as after a low dose of DEX. A dose of 1 mg DEX has too much suppressive effect to demonstrate this phenomenon, but a dose of 0.25 mg DEX, which results in a subtotal suppression of cortisol levels, leaves the influence of the individual's set point of the HPA axis intact. Previous studies showed that genetic factors may play a role in the regulation of cortisol levels. Maxwell *et al.* (2) reported similar unstimulated plasma cortisol levels in a sample of female monozygotic twins. However, this was not found in male monozygotic twins. Meikle *et al.* (3) observed evidence for a moderate genetic impact on early morning plasma cortisol levels. A study by Kirschbaum *et al.* (30) investigated cortisol responses to three different stimulation procedures (a CRH test, a physical stimulation procedure, and a psychological stress test), with the focus on the contribution of genetic factors. A decided influence of genetic factors was observed for baseline cortisol levels, as well as for the response to CRH, but heredity appeared to play a minor role in the adrenocortical response to physiological stress. One of the differences between this last study and the present results is that Kirschbaum and co-workers demonstrated a significant intraindividual stability of baseline cortisol concentration in females, whereas levels in males were much less stable. In our study, however, there was a highly significant individual stability of baseline cortisol concentrations in both sexes. Furthermore, the post-0.25-mg DEX cortisol levels were significantly correlated to the baseline cortisol concentrations, both in male and in female elderly individuals.

Effect of age on pre- and post-DEX cortisol concentrations

Studies of age-related changes in basal, nonstimulated HPA function have produced varying results (31). Most studies have demonstrated no change or nonsignificant increases in mean baseline ACTH (6, 8, 10) and serum cortisol levels at older ages (32, 33). The consistent lack of age and gender differences in basal cortisol levels across these various studies strengthens the conclusion that age does not have a major impact on basal nonstimulated cortisol concentrations (31). In the present study, there was no relation between age or gender and basal total cortisol concentrations, neither in the first nor in the second examination. Furthermore, there was no difference between these baseline cortisol concentrations, which were determined at an interval of approximately 2.5 yr. However, it must be stated that our study population included elderly people only, so we were not able to compare baseline cortisol concentrations between young and old subjects.

We found no correlation between post-DEX cortisol concentrations and age, after neither 1 mg nor 0.25 mg DEX administration. Furthermore, there was no difference between males and females in their post-DEX cortisol concentrations using either one of doses of DEX. Two previous studies of healthy individuals have also demonstrated no age-related differences in cortisol suppression after 1 mg DEX (16, 17). In two other studies (14, 15), however, a decreased suppression was reported in older subjects after the 1-mg DST.

The most important conclusions from our study are the observation of an individual stability of baseline cortisol levels, which is closely related to the feedback sensitivity of the HPA axis in response to a low dose of DEX. These observations suggest a genetic influence on the set point of the HPA axis.

References

1. **Krieger DT, Allen W, Rizzo F, Krieger HP.** 1971 Characterization of the normal temporal pattern of plasma corticosteroid levels. J Clin Endocrinol Metab. 32:266–284.
2. **Maxwell JD, Boyle JA, Greig WR, Buchanan WW.** 1969 Plasma corticosteroids in healthy twin pairs. J Med Genet. 6:294–297.
3. **Meikle AW, Stringham JD, Woodward MG, Bishop DT.** 1988 Heritability of variation of plasma cortisol levels. Metabolism. 37:514–517.
4. **Colucci CF, D'Alessandro B, Bellastella A, Montalbetti N.** 1975 Circadian rhythm of plasma cortisol in the aged (Cosinor method). Gerontol Clin. 17:89–95.

5. **Touitou Y, Sulon J, Bogdan A, et al.** 1982 Adrenal circadian system in young and elderly human subjects: a comparative study. J Endocrinol. 93:201–210.
6. **Blichert-Toft M.** 1971 Assessment of serum corticotrophin concentration and its nyctohemeral rhythm in the aging. Gerontol Clin. 13:215–220.
7. **Friedman M, Green MF, Sharland DE.** 1969 Assessment of hypothalamic-pituitary-adrenal function in the geriatric age group. J Gerontol. 24:292–297.
8. **Pavlov EP, Harman SM, Chrousos GP, Loriaux DL, Blackman MR.** 1986 Responses of plasma adrenocorticotropin, cortisol, and dehydroepiandrosterone to ovine corticotropin-releasing hormone in healthy aging men. J Clin Endocrinol Metab. 62:767–772.
9. **Zumoff B, Fukushima DK, Weitzman ED, Kream J, Hellman L.** 1974 The sex difference in plasma cortisol concentration in man. J Clin Endocrinol Metab. 39:805–808.
10. **Waltman C, Blackman MR, Chrousos GP, Riemann C, Harman SM.** 1991 Spontaneous and glucocorticoid-inhibited adrenocorticotropic hormone and cortisol secretion are similar in healthy young and old men. J Clin Endocrinol Metab. 73:495–502.
11. **Nugent CA, Nichols T, Tyler FH.** 1965 Diagnosis of Cushing's syndrome. Single dose dexamethasone suppression test. Arch Int Med. 116:172–176.
12. **Carroll BJ.** 1984 Dexamethasone suppression test for depression. Adv Biochem Psychopharmacol. 39:179–188.
13. **Guthrie S.** 1991 The impact of dexamethasone pharmacokinetics on the DST: a review. Psychopharmacol Bull. 27:565–576.
14. **Georgotas A, McCue RE, Kim OM, et al.** 1986 Dexamethasone suppression in dementia, depression, and normal aging. Am J Psychiatry. 143:452–456.
15. **Weiner MF, Davis BM, Mohs RC, Davis KL.** 1987 Influence of age and relative weight on cortisol suppression in normal subjects. Am J Psychiatry. 144:646–649.
16. **Ansseau M, von Frenckell R, Simon C, Sulon J, Demey-Ponsart E, Franck G.** 1986 Prediction of cortisol response to dexamethasone from age and basal cortisol in normal volunteers: a negative study. Psychopharmacology. 90:276–277.
17. **Tourigny-Rivard MF, Raskind M, Rivard D.** 1981 The dexamethasone suppression test in an elderly population. Biol Psychiatry. 16:1177–1184.
18. **Sapolsky RM, Krey LC, McEwen BS.** 1986 The neuroendocrinology of stress and aging: the glucocorticoid cascade hypothesis. Endocr Rev. 7:284–301.
19. **Hofman A, Grobbee DE, de Jong PT, van den Ouweland FA.** 1991 Determinants of disease and disability in the elderly: the Rotterdam Elderly Study. Eur J Epidemiol. 7:403–422.
20. **Lamberts SWJ, De Jong FH, Birkenhager JC.** 1977 Evaluation of diagnostic and differential diagnostic tests in Cushing's syndrome. Neth J Med. 20:267–274.
21. **Koper JW, Stolk RP, de Lange P, et al.** 1997 Lack of association between five polymorphisms in the human glucocorticoid receptor gene and glucocorticoid resistance. Hum Genet. 99:663–668.
22. **Kutemeyer S, Schurmeyer TH, von zur Muhlen A.** 1994 Effect of liver damage on the pharmacokinetics of dexamethasone. Eur J Endocrinol. 131:594–597.
23. **Jubiz W, Meikle AW, Levinson RA, Mizutani S, West CD, Tyler FH.** 1970 Effect of diphenylhydantoin on the metabolism of dexamethasone. N Engl J Med. 283:11–14.
24. **Crapo L.** 1979 Cushing's syndrome: a review of diagnostic tests. Metabolism. 28:955–977.
25. **Cronin C, Igoe D, Duffy MJ, Cunningham SK, McKenna TJ.** 1990 The overnight dexamethasone test is a worthwhile screening procedure. Clin Endocrinol. 33:27–33.
26. **Meikle AW.** 1982 Dexamethasone suppression tests: usefulness of simultaneous measurement of plasma cortisol and dexamethasone. Clin Endocrinol. 16:401–408.
27. **Weiner MF.** 1989 Age and cortisol suppression by dexamethasone in normal subjects. J Psychiatr Res. 23:163–168.
28. **Arana GW, Workman RJ, Baldessarini RJ.** 1984 Association between low plasma levels of dexamethasone and elevated levels of cortisol in psychiatric patients given dexamethasone. Am J Psychiatry. 141:1619–1620.
29. **Poland RE, Rubin RT, Lesser IM, Lane LA, Hart PJ.** 1987 Neuroendocrine aspects of primary endogenous depression. II. Serum dexamethasone concentrations and hypothalamic-pituitary-adrenal cortical activity as determinants of the dexamethasone suppression test response. Arch Gen Psychiatry. 44:790–795.
30. **Kirschbaum C, Wust S, Faig HG, Hellhammer DH.** 1992 Heritability of cortisol responses to human corticotropin-releasing hormone, ergometry, and psychological stress in humans. J Clin Endocrinol Metab. 75:1526–1530.
31. **Seeman TE, Robbins RJ.** 1994 Aging and hypothalamic-pituitary-adrenal response to challenge in humans. Endocr Rev. 15:233–260.
32. **Vermeulen A, Deslypere JP, Schelfhout W, Verdonck L, Rubens R.** 1982 Adrenocortical function in old age: response to acute adrenocorticotropin stimulation. J Clin Endocrinol Metab. 54:187–191.
33. **West CD, Brown H, Simons EL, Carter DB, Kumagai LF, Englert E.** 1961 Adrenocortical function and cortisol metabolism in old age. J Clin Endocrinol Metab. 21:1197–1207.