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Effects of Major Depression, Aging and Gender upon Calculated Diurnal Free Plasma Cortisol Concentrations: a Re-Evaluation Study

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Depression, aging and female gender are associated with increased diurnal concentrations of total plasma cortisol. For the physical effects of hypercortisolemia, however, it is generally assumed that free rather than total plasma cortisol concentrations are of importance. Herein, we report a mathematical approach to determine free plasma cortisol concentrations on the basis of total cortisol, corticosteroid binding-globulin (CBG) and albumin plasma concentrations. This approach was used to re-evaluate two sets of data in order to estimate the effect of depression as well as the effect of aging and gender upon free plasma cortisol concentrations. Comparing male depressed patients with healthy controls, we found 24-hour free cortisol minima (MIN: 4.1 ± 1.8 vs. 1.6 ± 1.1 nmol/l, $p < 0.0001$), mean (MEAN: 25.5 ± 6.7 vs. 10.4 ± 2.7 nmol/l, $p < 0.0001$) and maximal (MAX: 85.3 ± 23.3 vs. 45.2 ± 15.8 nmol/l, $p < 0.0001$) concentrations to be significantly increased in depressed patients. In general, the impact of depression upon total plasma cortisol were not only maintained, but stronger regarding free plasma cortisol. Also, age was associated with free plasma cortisol MIN ($F_{1,30}$= 10.8, $p < 0.003$) and free plasma cortisol MEAN ($F_{1,30} = 8.9$, $p < 0.006$). All effects of age upon total plasma cortisol were generally also found in free plasma cortisol, though with less impact. No effect of gender upon any of the given free plasma cortisol outcome variables was found. Taken together, our re-evalution clearly shows not only depression but also aging to be associated with increases in free plasma cortisol concentrations. This finding is in line with the observation that in both conditions medical problems triggered and/or maintained by glucocorticoids (e.g. osteoporosis) are frequently seen.

Keywords: free cortisol, diurnal, depression, aging, gender

INTRODUCTION

There is general agreement that depression is associated with an increase in the activity of the hypothalamus-pituitary-adrenal-(HPA)-system leading to elevated diurnal plasma concentrations of cortisol (Deuschle et al., 1997a; Mortola et al., 1987; Linkowski et al., 1987, 1985; Halbreich et al., 1985; Pfohl et al., 1985; Branchey et al., 1982; Sachar et al., 1973). Furthermore, there is accumulating evidence that with aging the diurnal activity of the HPA-system increases (Deuschle et al., 1997b; Van Cauter et al.,

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1996). With regard to depression, studies on urinary (for review: Schlechte and Coffman, 1985) and saliva cortisol (Guechot et al., 1987; Galard et al., 1991) also show an increase in free plasma cortisol. Also, free cortisol as measured by frequent urine sampling (Touitou et al., 1983) and single-point saliva measurements (Nicolson et al., 1997) was found to be increased in the elderly. So far, however, in both conditions the diurnal activity of free cortisol has not been reported.

Although there is some evidence that albumin-bound, but not corticosteroid-binding globulin (CBG) bound corticosteroids enter the brain (Pardridge et al., 1981; 1983), it is generally assumed that the biological activity of a hormone is determined by its unbound rather than its protein-bound fraction in the plasma (Mendel, 1989). This hypothesis is generally refered to as "free hormone hypothesis". With regard to the physiological effects of HPA system activation under conditions of stress, depression or aging, it seems reasonable to study free rather than total plasma corticosteroid concentrations. However, since CBG is saturable at physiological concentrations there is no linear correlation between free and total plasma corticosteroid concentrations (Dunn et al., 1981). Thus no simple inference as to free hormone concentrations can be made from plasma corticosteroid measurements.

Three approaches to determine diurnal free plasma cortisol concentrations have been described. First, measuring free plasma hormones directly by equilibrium dialysis, ultrafiltration or gel filtration, secondly, frequent saliva sampling which is known to reflect free plasma cortisol (for review: Kirschbaum and Hellhammer, 1989) and third, calculating free plasma hormone concentrations from total plasma concentrations and concentrations of the respective binding proteins (Coolens et al., 1987; Angeli et al., 1977). The first approach is hampered by several methodological problems while frequent saliva sampling has some procedural shortcomings since sampling is not possible when subjects are asleep. In our case, we decided to re-evaluate two recently published sets of data (Deuschle et al., 1997a; 1997b) using a mathematical approach in order to estimate free plasma cortisol concentrations.

METHODS

Transformation of total plasma cortisol in calculated free plasma cortisol concentrations

We used the approach of Feldman et al. (1972) to transform total plasma cortisol concentrations in calculated free plasma cortisol concentrations:

$$R = \frac{k_{CBG}[CBG]}{1+k_{CBG}[\text{free cortisol}]} + \frac{k_{Alb}[Alb]}{1+k_{Alb}[\text{free cortisol}]}$$

with:

R = relation of plasma concentrations of bound to free cortisol

k_{CBG} = association constant of CBG to cortisol

K_{Alb} = association constant of albumin to cortisol

On the other hand, the relation of plasma concentrations of bound to free cortisol is total plasma cortisol concentration minus concentrations of bound cortisol:

$$R = \frac{[\text{total cortisol}]}{[\text{free cortisol}]} - 1$$

Therefore, the following equation results:

$$0 = \frac{k_{CBG}[CBG]}{1+k_{CBG}[\text{free cortisol}]} + \frac{k_{Alb}[Alb]}{1+k_{Alb}[\text{free cortisol}]} - \frac{[\text{total cortisol}]}{[\text{free cortisol}]} + 1$$

Knowing the association constants, albumin concentration, CBG concentration and total cortisol concentrations the equation allows the estimation of the concentration of free plasma cortisol. The calculation was done using the program MAPLE™.

The following association constants were used:

$K_{CBG} = 7.6\ 10^7\ M^{-1}$ (Westphal, 1971)

$K_{Alb} = 0.3\ 10^4\ M^{-1}$ (Dunn et al., 1981)

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Association between calculated free cortisol and saliva cortisol concentrations

In ten healthy controls total plasma cortisol and saliva cortisol was measured in samples drawn every 60 minutes from 14.00 h to 23.00 h and from 7.00 h to 14.00 h. In this assay, the cross-reactivity with cortisone was less than 1 %. Plasma total cortisol concentrations were transformed to free plasma cortisol concentrations as described above. Linear regression was used to test the association between calculated free plasma cortisol and saliva cortisol concentrations in these 10 individuals and in the combined group.

Effect of depression upon calculated free cortisol concentration in male patients

Details about subject and methods are given elsewhere (Deuschle et al., 1997a). In summary, 15 depressed (Hamilton Depression Scale 30.4 ± 6.7) male patients (age: 47.7 ± 14.8) and 22 age-matched male controls (age: 53.1 ± 18.2) were studied. One patient (52 yrs) of the original analysis could not be included due to missing albumin data. Twenty-four hour blood sampling from 0800 h to 0800 h with 30-min sampling intervals was performed. Total cortisol plasma concentrations were estimated by commercial RIA (ICN Biomedicals, Costa Mesa, CA). CBG plasma concentrations were determined at 0800, 1400, 2000 and 2400 hours by RIA. Also, albumin plasma concentrations were determined from the first 0800 h sample. Total plasma cortisol concentrations were transformed, as described above, to free plasma cortisol concentrations using albumin and mean CBG plasma concentrations. The free plasma cortisol profile was condensed in minimal (MIN), mean (MEAN) and maximal (MAX) concentrations. Also, the diurnal amplitude DELTA (MAX-MIN) and the diurnal amplitude relative to MEAN (VAR = DELTA divided by MEAN) were calculated. The following intervals have been analyzed separately: 0800–1330 h, 1400–1930 h, 2000–0130 h and 0200–0730 h. The condensed data were analyzed by ANCOVA using 'diagnosis' as grouping variable and 'age' as covariate.

Effects of age and gender upon calculated free cortisol concentrations in healthy controls

Details about subjects and methods are given elsewhere (Deuschle et al., 1997b). Briefly, eleven healthy females (age: 24 – 81 yrs, mean 47.9 ± 21.6) and twenty-two healthy male volunteers (age: 23 – 85 yrs, mean 53.1 ± 18.3 yrs) participated in the study. One subject of the original analysis could not be included due to missing albumin data. Blood samplings, hormone assays and transformation of total cortisol values in free plasma cortisol values were done according to the forementioned study. ANCOVA analysis with 'gender' as grouping variable and `age' as covariate was performed.

RESULTS

Reliability and validity of the mathematical approach

In all subjects there was a strong association between calculated free plasma cortisol and saliva cortisol concentrations (range of r^2: 0.73 – 0.92, mean of r^2: 0.84). The same was true when the groups were combined ($r^2 = 0.834$). The slope of the regression lines was similar in all subjects (range of slope: 1.421 – 2.393, mean of slope: 1.989). When all subjects were combined to one group the slope was 1.88 (fig. 1).

Effect of depression upon calculated free cortisol concentration in male patients

Major depression was significantly associated with increased free cortisol MIN (4.1 ± 1.8 vs. 1.6 ± 1.1 nmol/l, $F_{1,33} = 32.8$, $p < 0.0001$), free cortisol MEAN (25.5 ± 6.7 vs. 10.4 ± 2.7 nmol/l, $F_{1,33} = 111.4$, $p < 0.0001$), free cortisol MAX (85.3 ± 23.3 vs. 45.2 ± 15.8 nmol/l, $F_{1,33} = 39.5$, $p < 0.0001$) and free cortisol DELTA (81.3 ± 22.2 vs. 43.6 ± 15.8 nmol/l, $F_{1,33} = 36.2$, $p < 0.0001$) (see fig.2). Regarding free cortisol MEAN, we found an 145 % increase in depressed patients compared to healthy controls. Also, free cortisol VAR was significantly reduced in

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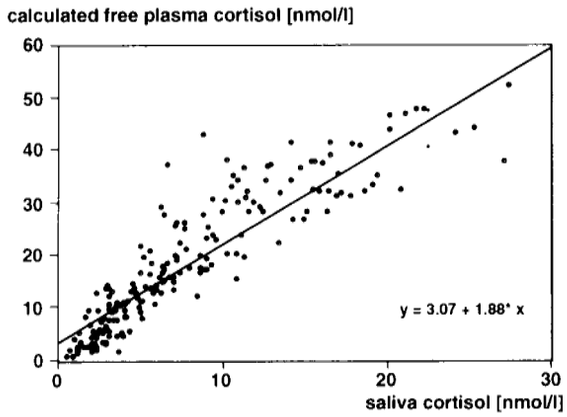

FIGURE 1 Relationship between calculated free and measured saliva cortisol: linear regression of 10 pooled diurnal profiles of healthy controls

depressed patients (3.3 ± 0.8 vs. 4.3 ± 1.4, $F_{1,33} = 7.5$, $p < 0.01$). In all time windows free plasma cortisol was strongly increased in depressed patients (0800 – 1330 h: $F_{1,33} = 101.2$; 1400 – 1930 h: $F_{1,33} = 41.3$; 2000 – 0130 h: $F_{1,33} = 32.8$; 0200 – 0730 h: $F_{1,33} = 77.6$). In general, all effects of depression upon total plasma cortisol were also observed in free plasma cortisol with some effects appearing much stronger (free cortisol MEAN ($F_{1,33} = 111.4$ vs. $F_{1,34} = 93.5$); free cortisol MAX ($F_{1,33} = 39.5$ vs. $F_{1,34} = 21.3$); free cortisol DELTA ($F_{1,33} = 36.2$ vs. $F_{1,34} = 7.8$)).

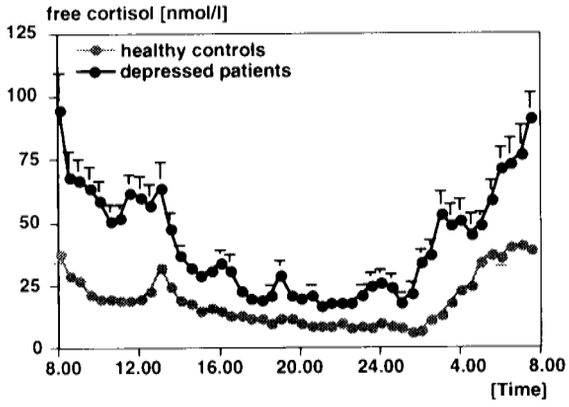

FIGURE 2 Diurnal profile of calculated free cortisol (mean ± SEM) in male depressed patients (n=15) and age-matched healthy controls (n=22)

Effects of age and gender upon calculated free cortisol concentrations in healthy controls

Age was significantly and positively correlated with free plasma cortisol MIN ($F_{1,30} = 10.8$, $p < 0.003$) and free plasma cortisol MEAN ($F_{1,30} = 8.9$, $p < 0.006$). Also, age was negatively associated with free plasma cortisol VAR ($F_{1,30} = 4.3$, $p < 0.05$). Furthermore, age was positively associated with nighttime free plasma cortisol (2000 – 0130 h: $F_{1,30} = 22.8$, $p < 0.0001$; 0200 – 0730 h: $F_{1,30} = 6.6$, $p < 0.02$). There was no significant effect of age upon free plasma cortisol MAX and DELTA. Furthermore, daytime free plasma cortisol levels showed no association with age (all F values below 2.2). Comparing young subjects (<30 years, n=9) with elderly subjects (>60 years, n=10) there was a 23 % increase in free cortisol MEAN (20.7 ± 3.3 vs. 16.8 ± 4.5 nmol/l) (see fig. 3). In general, all effects of age upon total plasma cortisol were also found in free plasma cortisol. However, these effects had less impact upon free than upon total plasma cortisol (cortisol MIN: $F_{1,30} = 10.8$ vs. $F_{1,31} = 19.5$; cortisol MEAN: $F_{1,30} = 8.9$ vs. $F_{1,31} = 13.1$; cortisol VAR: $F_{1,30} = 4.3$ vs. $F_{1,31} = 9.6$; 2000 – 0130 h: $F_{1,30} = 22.8$ vs. $F_{1,30} = 25.8$; 0200 – 0730 h: $F_{1,30} = 6.6$ vs. $F_{1,31} = 9.0$). There was no effect of gender upon any of the given free plasma cortisol outcome variables. This finding is in contrast to the effect of gender upon total plasma cortisol in the original analysis which showed females there to have increased total plasma cortisol MEAN ($F_{1,31} = 7.5$), cortisol 0800- 1330 h ($F_{1,31} = 11.4$) and cortisol 2000–0130 h ($F_{1,31} = 7.0$).

DISCUSSION

The main findings of this study can be summarized as follows: First, the high correlation between saliva cortisol and calculated free plasma cortisol concentrations suggests the mathematical approach to be a reliable and applicable method for estimating free plasma cortisol. Second, the impact of depression upon free plasma cortisol concentrations is equivalent or even stronger than the impact upon total plasma

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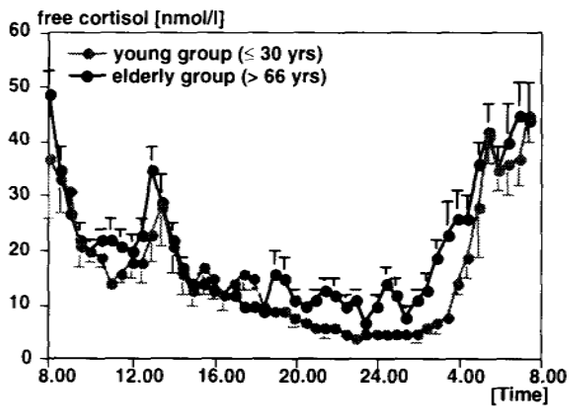

FIGURE 3 Diurnal profiles of calculated free cortisol (mean ± SEM) in young (n=9, age 23–29, mean 26.7 ± 2.0 yrs) and elderly (n=10, age 67–85, mean 73.2 ± 6.0 yrs)

concentrations. Third, the effects of aging upon total plasma cortisol are not completely "buffered" by CBG, reflected by increased free plasma cortisol concentrations. Fourth, increased total plasma cortisol concentrations in females are not tightly correlated to derived free plasma cortisol concentrations.

On a cautionary note, several factors may influence our model: the precision of the model is affected by assay-variabilities of the three assays carried out and by the association constants of cortisol binding to CBG and albumin, which have been used from the literature. The regression models to study the reliability of this approach uncovered roughly 17% unexplained variance in the calculated free plasma cortisol concentrations, which might be attributed to the above-mentioned assay-variabilities. Although the saliva cortisol concentration parallels that of free plasma cortisol, the absolute values are roughly 50 % lower in saliva due to 11-beta-hydroxysteroid dehydrogenase being present in saliva (Hellhammer and Kirschbaum, 1989). This difference between saliva and free plasma cortisol concentrations explains the slope of our regression lines. We can not fully exclude that depression or aging-related changes in the concentrations of other steroids binding to CBG, like DHEA or cortisone, might interfere with the model when applied to profiles of respective subjects. Also, diurnal variations of CBG concentrations are reported to be similar in depressed and healthy subjects (Deuschle et al., 1997c). Therefore, this possible intervening factor was not incorporated in our model. Yet, the strong association of calculated free plasma cortisol with saliva cortisol, which is generally assumed to reflect free plasma cortisol, indicates that the method may be applied to data sets of total plasma cortisol in order to estimate diurnal free plasma cortisol concentrations, when direct estimation is not available.

Marked variations in the percent of free cortisol occur with physiological fluctuations of total cortisol concentration since CBG becomes saturated in low to medium total cortisol plasma concentrations (Dunn et al., 1981). Therefore, increases in total cortisol may be buffered by CBG in a low concentration range, but may lead to a linear and strong increase in free cortisol in the medium to high concentration range. These kinetic specifities also explain that free plasma cortisol is mainly affected by changes in CBG plasma concentrations.

We found the HPA system activating effect of depression much better reflected by free in comparison to total cortisol concentration. This can mainly be attributed to two facts. First, increases in total cortisol concentrations occur over the complete diurnal cycle and especially those elevations occuring at the diurnal peak can no longer be bound due to saturation effects. Second, some (Maes et al., 1996; Ktiouet et al., 1984), but not all (Deuschle et al., 1997c; Leake et al., 1989) authors found CBG concentrations to be decreased in depressed patients. Obviously, even the non-significant decrease in plasma CBG in our patients (Deuschle et al., 1997c) had a strong impact upon free cortisol MEAN and MAX.

Major depression is not only a disorder of mood and behavior, but also affects a wide range of metabolic functions. There is good evidence that hypercortisolemic depressed patients suffer from insulin resistance (Weber et al., 1995), disturbances in the GH-IGFs system (Deuschle et al., 1997d) and in the hypothalamus-pituitarygonadal system (Schweiger et al., submitted). Increased HPA system activity is the most likely force to drive these changes and which, in turn, directly or indirectly affects body composition,

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such as reduced bone mass density (Schweiger et al., 1995) or increased visceral fat (Thakore et al., 1997). Furthermore, glucocorticoids can have a broad range of deleterious effects upon neurons, including neuronal atrophy, neurotoxicity and neuroendangerment (review: Sapolsky, 1996). Thus, the finding that free plasma cortisol in major depression is significantly elevated suggests relevant, albeit probably untoward consequences upon physical health and neurobiological integrity in these patients.

The observed age-associated increase of total cortisol occurs mainly in the low concentration range (van Cauter et al., 1996; Deuschle et al., 1997b). Therefore, one might speculate that these elevations are being "buffered" by CBG. Our analysis, however, does not support this hypothesis: all effects of aging upon free plasma cortisol, although being somewhat less strong, were similar to those upon total cortisol concentrations. This result leads us to assume that, similar to depression, untoward sequelae of glucocorticoid exposure must be expected in healthy elderly individuals as well.

All effects of female gender upon total cortisol concentrations could not be replicated with the analysis of free cortisol concentrations. Obviously, this is due to the strong increase of CBG in females (Deuschle et al., 1997c). This finding supports the view that a comparison of HPA system activity of males and females can not be reliable without considering CBG concentrations. Along the same line, data from several earlier studies reporting gender effects upon HPA system activity (van Cauter et al., 1996; Heuser et al., 1994; Keitner et al., 1992) have to be reconsidered.

Taken together, our re-evalution clearly shows that depression but also aging to be associated with increases in free plasma cortisol concentrations. This finding is in line with the assumption that in both conditions medical problems caused by glucocorticoids are prevalent. Additionally, these findings underscore the possibility of underestimating the activity of the HPA system when only total plasma cortisol concentrations are being examined.

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