EFFECT OF CORTISOL LEVELS ON WORKING MEMORY PERFORMANCE IN ELDERLY SUBJECTS WITH ALZHEIMER'S DISEASE

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Abstract – **Background:** Subjects with Alzheimer's disease (AD) have elevated cortisol levels as a result of hypothalamic-pituitary-adrenal (HPA) axis dysfunction. Acute administration of hydrocortisone has been associated with working memory (WM) performance in young adults. **Objective:** To investigate whether cortisol levels are associated with WM performance in subjects with AD. **Method:** Eighty subjects were included, comprising 40 patients with mild AD and 40 healthy elderly controls. WM was assessed using the Digit Span Backward test (DSB). Saliva samples were collected to determine cortisol levels. **Results:** AD subjects had poorer performance on the DSB than controls (p=0.002) and also presented higher levels of cortisol than control group (p=0.04). No significant correlation was observed between the DSB and cortisol levels in both groups (r= −0.29). **Conclusion:** In this study, elevated cortisol levels were not associated with poorer WM performance in patients with AD or in healthy elderly subjects.

KEY WORDS: cortisol levels, working memory, Alzheimer disease, aged.

Efeito dos níveis de cortisol no desempenho da memória operacional de idosos com doença de Alzheimer

Resumo – **Introdução:** Idosos com doença de Alzheimer (DA) apresentam elevados níveis de cortisol como resultado de uma disfunção no eixo hipotálamo-pituitária-adrenal (HPA). A administração aguda de hidrocortisona tem sido associada ao desempenho da memória operacional (WM) em adultos jovens. **Objetivo:** Investigar se os níveis de cortisol estão associados com o desempenho na WM em pacientes com DA. **Método:** Oitenta indivíduos foram incluídos, sendo 40 pacientes com DA leve e 40 idosos saudáveis controles. WM foi avaliada a partir do teste de Extensão de Dígitos na ordem direta (DSB). Amostras de saliva foram coletadas para determinar os níveis de cortisol. **Resultados:** Indivíduos com DA apresentaram pior desempenho no DSB (p=0,002) e maiores níveis de cortisol (p=0,04) do que os controles. Não foi observada correlação significativa entre DSB e níveis de cortisol em nenhum dos grupos (r= −0,29). **Conclusão:** Neste estudo, níveis de cortisol elevados não estão associados com pior desempenho da WM em pacientes com DA ou em idosos cognitivamente saudáveis.

PALAVRAS-CHAVE: níveis de cortisol, memória operacional, doença de Alzheimer, idoso.

Alzheimer's disease (AD) is a chronic neurodegenerative disorder which implies cognitive impairment including memory and executive dysfunction[1]. There is evidence that neuroendocrine dysfunctions may also be involved in the disease process, given that stress hormones affect neuronal survival[2]. Moreover, elderly subjects prone to psychological distress are more likely to develop AD than non-stressed individuals[3]. In the presence of stress stimuli, the hypothalamic-pituitary-adrenal (HPA) axis is stimulated and glucocorticoids (cortisol in primates, corticosterone in mice and rats) are released. Some studies have demonstrated elevated cortisol levels in AD subjects resulting from HPA axis dysfunction, particularly in the earlier stages of the disease[4,5]. Further, cortisol levels have been associated with more rapid disease progression[6] and increased β-amyloid and tau pathology in a mouse model of AD[7].

Considering the glucocorticoid receptors in the hippocampus and prefrontal cortex[8], cortisol levels can af-

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fect both declarative memory performance[9], dependent on hippocampal activity, and working memory (WM)[10], dependent on frontal lobe functions.

WM is the cognitive mechanism that allows the human brain to keep a limited amount of information active for a certain period[11]. Studies in nonhuman primates have demonstrated that mild stress exposure impairs WM performance[12]. In young adults, WM is more sensitive than declarative memory to the acute administration of hydrocortisone[8,13]. However, the effect of endogenous cortisol levels on both healthy elderly subjects and individuals with AD remains unclear.

Given the presence of glucocorticoid receptors in the prefrontal cortex of humans and its relationship to WM, this study aimed to investigate whether endogenous cortisol levels are associated with WM performance in patients with AD and in healthy elderly subjects.

METHOD

Participants

Eighty subjects were included in the study, subdivided into two groups: the Control Group, composed of 40 fully independent elderly subjects with normal cognitive function, randomly chosen from a group of subjects participating in cultural activities at the university campus (mean age±SD: 72.2±6.3 years; mean educational level±SD: 6.0±4.2 years), and the AD Group, composed of 40 elderly patients with mild AD (mean age±SD: 80.1±6.0 years; mean educational level±SD: 4.7±2.6 years), who fulfilled NINCDS-ADRDA criteria[14] for probable AD, having mild dementia according to the DSM-III-R criteria[15], randomly chosen from the group of outpatients followed at the Behavioral and Cognitive Neurology Unit (BCNU) of the Hospital das Clínicas of the University of São Paulo School of Medicine (HC-FMUSP). All data were collected after approval of the study by the Ethics and Research Committee of the HC-FMUSP and School of Nursing of the University of São Paulo and written informed consent by all participants and their relatives.

For the Control Group, exclusion criteria were elderly individuals diagnosed with any other neurological or psychiatric disease, with evidence of cognitive alterations incompatible with the norm for their age, having history of alcohol or drug abuse in the preceding year or for a previous prolonged period, individuals using psychoactive medication, as well as illiterate individuals. Cognitive impairment was ruled out based on a combination of cognitive and functional evaluation instruments (Mini-Mental State Examination – MMSE[16,17] and Informant Questionnaire on Cognitive Decline – IQCODE[18,19]). The MMSE[16] was employed as a global measure of cognitive function and the following education-adjusted cut-off scores were adopted for elderly individuals without cognitive impairment: ≥28 for subjects with more than seven years of formal education, ≥24 for subjects with 4 to 7 years and ≥23 for subjects with 1 to 3 years of schooling[17]. The IQCODE was employed as a functional evaluation tool and a cut-off score ≤3.40 was adopted for individuals without cognitive impairment[19]. The combination of these two screening tools can increase the diagnostic accuracy of dementia[19].

All individuals participating in the study were submitted to the study protocol evaluation, which included demographic data (gender, age and education level), working memory assessment (Digit Span test) and endogenous cortisol measurement.

Cognitive assessment

Working memory was evaluated using the Digit Span backward (DSB) together with the difference between the Digit Span Forward (DSF) and backward scores (Δ DSF–DSB)[20]. The Digit Span test in the Wechsler batteries (the intelligence and memory scales) is the most commonly used measure of immediate verbal recall. In these batteries it comprises two different tests, the DSF and DSB, each of which involves different mental activities[20]. The DSF test is more closely related to attention efficiency, while the DSB test is related to working memory performance[20]. Both tests consist of six pairs of random digit sequences that the examiner reads aloud at the rate of one per second. In the DSF the subject has to repeat each sequence of digits exactly as is given, whereas in the DSB the subject has to repeat each sequence of digits in exact reverse order[20]. When a sequence is repeated correctly, the examiner reads the next longer digit sequence, continuing until the subject fails a pair of sequence or repeats the highest sequence correctly[20]. For each digit repeated correctly on either the DSF or DSB, the subject scores one point giving a maximum score of seven.

All evaluation instruments were administered by the same researcher (JNST) through individual interviews with the elderly subjects.

Cortisol measurement

Saliva samples were obtained from all subjects during the study to assay cortisol concentrations. This was done within two hours of waking in the next day after cognitive evaluation. Immediately after sampling, saliva was placed on chipped ice and centrifuged within 2,200 rpm for 15min at 4ºC. Saliva was drawn off with pipette and transferred to a sterile tube and then distributed to samples of 0.5 mL that were stored at –20ºC until assayed.

Saliva cortisol levels were determined with a radioimmunoassay kit (Amersham, Fleury S.A, São Paulo, BR) with [1,2,6,7-^{3}H] cortisol as tracer (Kendall's Compound F), and cortisol F-79-I antibody. The intra and inter assay coefficients of variation were 6.8% and 3.9%, respectively.

Statistical analysis

Initially, all variables were analyzed through descriptive statistics, with determination of means, standard deviations, and minimum/maximum values of the quantitative data (age, schooling, DSF and DSB scores). For the categorical variable gender, relative and absolute frequencies were calculated. Non-parametric

Table. Demographic characteristics and digit span scores in AD and control subjects.

Variables	AD (n=40) N (%) or Mean (±SD)	Control (n=40) N (%) or Mean (±SD)	Statistical significance p (value)*
Sex (female)	27.0 (67.5)	35.0 (85.4)	0.31[a]
Age (years)	80.1±6.0	72.2±6.3	<0.01[b]
Education level (years)	4.7±2.6	6.0±4.2	0.34[b]
Ds forward	6.1±0.9	6.5±0.7	0.09[b]
Ds backward	3.2±0.9	3.8±1.2	0.02[b]
Δ forward and backward	2.9±1.2	2.6±1.1	0.32[b]

*Value of less than 0.05 indicates significance; [a]Chi square test; [b]Mann-Whitney test.

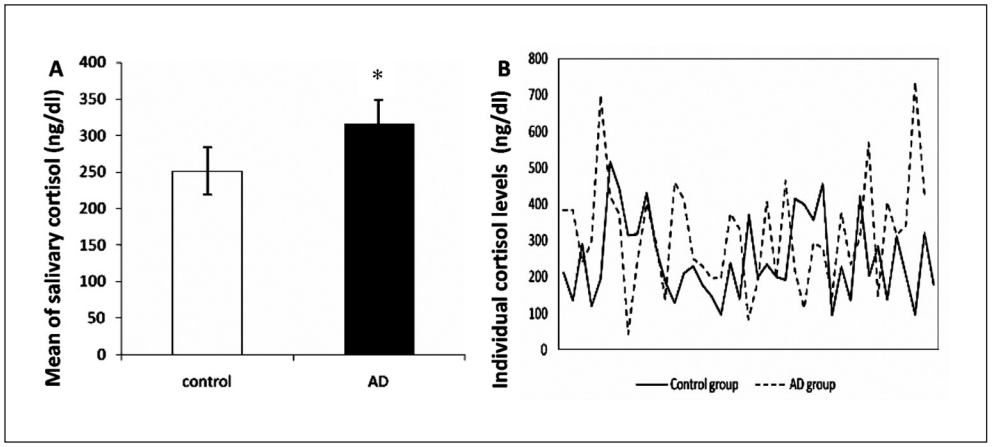

*Fig 1. Distribution and means of cortisol levels in AD and control subjects. *The p value of less than 0.05 indicates significance, Mann-Whitney test. The error bars represent the standard error of the mean.*

tests were used for those variables not presenting normal distribution. The Mann-Whitney test was used to compare two independent groups (AD and control), Chi square to compare gender between control and AD groups, whereas Spearman's correlation coefficient was used to study the correlation between DS test scores and cortisol levels, where the level of significance used for the test was 5% ($p<0.05$, 95% confidence interval).

RESULTS

In relation to demographic data, the two groups were similar in gender and educational level but showed a significant difference regarding age. Demographic data are shown in Table.

Cognitive assessment

AD subjects presented a mean score of 6.1 (ranging from 4 to 7) on the DSF and a mean score of 3.2 (ranging from 6 to 2) on the DSB. The maximum difference between DSF and DSB was 5 points in these subjects. The Control Group presented better performance than AD subjects in all digit span tests, but a significant difference was obtain only on the DSB test. Therefore, AD subjects presented poorer working memory performance than control individuals. Cognitive assessments are shown in Table.

Cortisol measurement

AD subjects presented higher cortisol levels than control subjects (p=0.004). Although saliva samples were obtained at slightly different times in AD subjects, with a mean of 1.46 hours from waking (ranging from 0.15 to 2.45 hours) versus a mean of 1.51 hours (ranging from 0.30 to 2.30 hours) in controls, no significant difference was observed between the two groups (p=0.536; Mann-Whitney). Therefore, the different timings in saliva samples collection was not consider a confounder variable in the present study, considering that both groups presented no difference in the interval between awaking time and saliva sample collections. Endogenous cortisol levels are shown in Figure 1.

Cortisol levels and working memory

Those AD subjects who exhibited higher cortisol levels presented worse DSB score, but this correlation did not attain statistical significance (p=0.062). No significant correlations were obtained between the DSF test and cor-

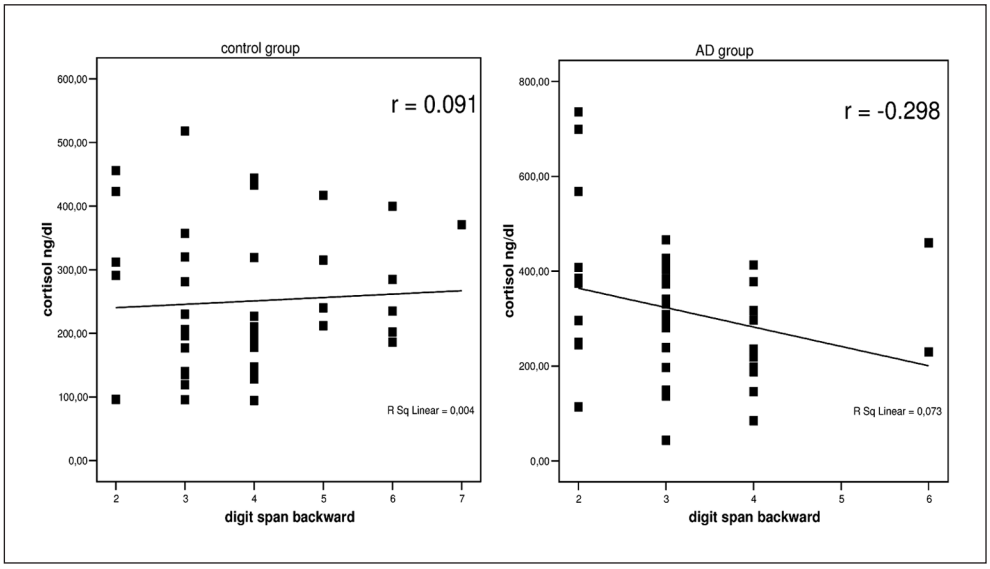

Fig 2. Lack of correlation between cortisol levels and digit span backward in AD and control subjects.

tisol levels, or between the difference between the DSF and DSB tests and cortisol levels in both groups. Lack of correlation is shown in Figure 2.

DISCUSSION

The results of this study revealed no significant effect of endogenous cortisol levels on WM performance in healthy elderly and AD subjects. Although AD subjects presented higher cortisol levels than controls, there was no significant association between elevated endogenous cortisol levels and WM performance. Elevated cortisol levels in AD individuals were consistent with previous literature supporting the hypothesis that such elevation is a consequence of neuronal injury in the hippocampus blocking inhibition of HPA axis activity and producing a repetitive cycle of increasing HPA dysfunction and ongoing hippocampal injury with consequent increase in cortisol levels[21].

The fact that no significant correlation was observed between endogenous cortisol levels and the DSB in both groups is consistent with the possibility that WM is not sensitive to endogenous cortisol levels without an acute stressful situation. Several studies have demonstrated a significant correlation between administrations of hydrocortisone and memory performance, which are dependent on frontal lobe functions in young subjects. In one such study, a significant impairment in the attention task after acute administration of 100 mg of hydrocortisone was reported[22]. Another study demonstrated WM deficits after 600 μg/kg/hr administration of hydrocortisone in young men, corresponding to an average dose of 42 mg for an individual weighing 70kg[8], while visuo-spatial memory was impaired after chronic administration of 20 mg of hydrocortisone for 10 days. In all these studies mean cortisol levels after hydrocortisone administration were higher than 530 ng/dL.

Thus, one possible interpretation of our results is that endogenous cortisol levels were not sufficiently high to sensitize WM performance in our sample of AD subjects who presented a mean of 316 ng/dL of cortisol. This notion is supported by a previous study which showed no association between WM impairment and a mean lower than 400 ng/dL of cortisol levels after acute administration of 30 mg of hydrocortisone[23]. It may be possible that the lack of association between endogenous cortisol levels and WM is due to different sensitivities of this type of memory to cortisol rather than an absence of an effect of glucocorticoids on WM, whose interaction has also been demonstrated in previous studies[24,25]. Another point that has to be considered in the lack of correlation is related to small differences on WM scores and cortisol levels between control and AD group. Correlations are more likely to be observed in small groups when there is a substantial variation in the range of the variables observed.

In addition, it should be raised that the cortisol levels obtained in the present study represents basal conditions. Maybe in an acute stressful situation which triggers the HPA axis resulting in relevant increases in cortisol levels would produces a different result. In previous literature, negative correlation was observed between cortisol levels induced by psychosocial stressor and WM performance[26]. Thus, on future studies should be interesting investigate the effect of cortisol levels on WM exposed to acute stressful situation.

There is evidence that mild stress exposure and increases in corticosterone levels impair WM performance

mediated by high levels of dopamine in the prefrontal cortex[27]. Based on this interpretation, our results suggest that endogenous cortisol levels are not sufficient to sensitize dopamine receptors, and consequently no effect on WM was produced. Additional studies examining glucocorticoid and dopamine induced changes in prefrontal cortex using functional brain imaging are now necessary to support this hypothesis.

Another interpretation for these results relates to age of the subjects. The effect of glucocorticoid on WM performance had been demonstrated in young individuals, while our study evaluated elderly subjects with and without cognitive impairment. Hence, it may be possible, considering the changes in dopamine activity which occur with aging, that glucocorticoids and dopamine receptor sensitivity differs between in young and elderly subjects[28].

Despite the dissociation observed between endogenous cortisol levels and WM, it is important to consider that only a single cortisol measurement was obtained from subjects. Although no statistical difference was shown in the timing of saliva sample collection, the reliable standard for analyzing basal cortisol tends to include five measurements taken within 24 hours, preferably obtained from subjects at the same time during the day. This can avoid *bias* due to cortisol peaks in the morning compared with levels at other times of day.

Furthermore, considering that changes in sleep patterns are commonly observed in elderly subjects, this variable may have played a role on our results. Sleep disturbances associated with poor cognitive performance and cortisol levels has been reported in elderly healthy controls, being increased cortisol levels associated with poor sleep patterns and sleep-quality self-evaluation[29]. Therefore, cortisol effects on cognitive performance covariate with sleep patterns could have revealed different results.

The fact that this study not replicated the association between cortisol levels and WM reported in previous literature[8,24,25] raised the question whether the cognitive performance observed in both AD and control groups truly represented presence or absence of dementia process. Although, we have carefully selected eligible control subjects and AD based on diagnosis criteria widely described in the literature[14,15], cases previously classified as "cognitively normal" presenting neuropathological profile for AD or cerebrovascular disease (CVD) have been reported, being "cognitively normal" subjects proportionately more frequent in CVD (45%) than in AD (4%)[30]. In contrast, the same authors also reported subjects previously classified as "cognitively impaired" or "demented" with absence of neuropathological changes compatible with dementia process[30]. Therefore, the lack of association between cortisol and WM observed in our study may be partially explained to the fact that control subjects and AD patients in early stages of disease could be mislead classified, since an infallible criteria remains under investigation.

In addition, the groups were different in relation to age and the sample generally small, and therefore, not so clinically meaningful. Future studies involving elderly in different age clusters and with a higher number of individuals are warranted, since these may show more consistent results.

In conclusion, despite the limitations of the present study, our findings confirm the occurrence of elevated cortisol levels in AD subjects together with the absence of WM sensitivity to this endogenous glucocorticoid. However, subjects with high levels of stress should undergo cortisol level assessment, given the negative effect that higher doses of this glucocorticoid may have on WM.

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