Title: Life events, salivary cortisol, and cognitive performance in nondemented subjects: a population-based study.

Authors: Ouanes S, Castelao E, Gebreab S, von Gunten A, Preisig M, Popp J

Journal: Neurobiology of aging

Year: 2017 Mar

Issue: 51

Pages: 1-8

DOI: 10.1016/j.neurobiolaging.2016.11.014

Life events, salivary cortisol and cognitive performance in non-demented subjects: a population-based study

Sami Ouanes, Enrique Castelao, Sirak Gebreab, Armin von Gunten, Martin Preisig, Julius Popp

Department of Psychiatry – University Hospital of Lausanne

Cery Hospital – 1008 Prilly/Lausanne - SWITZERLAND

Abstract

Older people are particularly exposed to stressful events, known to activate the Hypothalamus-Pituitary-Adrenal axis resulting in increased cortisol levels. High cortisol has been associated with deleterious effects on cognition. We hypothesized that stressful life events could increase cortisol secretion leading to cognitive impairment.

A cross-sectional analysis was conducted using data from Colaus/PsyColaus, a longitudinal population-based study among Lausanne residents. Salivary cortisol samples were obtained from 796 non-demented subjects aged at least 65.

A neuropsychological battery was used to assess cognitive performance and determine the Clinical Dementia Rating Sum Of Boxes (CDRSOB). Lifetime life events and their subjective impact were assessed using a validated questionnaire.

The total impact of life events was associated neither with cortisol AUC nor with CDRSOB nor with any cognitive domain performance. The CDRSOB was associated with the cortisol AUC, controlling for age, sex, BMI, education, and depressive symptoms ($p=0.003$; $B=0.686[0.240;1.333]$; $r=0.114$). This association between CDRSOB and the cortisol AUC remained significant after controlling for life events total impact ($p=0.040$; $B=0.591[0.027;1.155]$; $r=0.106$).

These findings do not support the hypothesis that stressful life events increase cortisol secretion leading to cognitive impairment. The association of higher cortisol levels with poorer cognition might be not a mere reflection of stressful events but rather explained by other factors, yet to be elucidated.

Keywords: cognition; life events; cortisol; memory; dementia; stress.

1. Introduction:

Older people are particularly exposed to various stressful events. These might be acute (such as the death of a relative) or chronic (such as chronic medical conditions or financial difficulties) (Comijs, et al., 2011). Moreover, as the age advances, life events (including early and mid-life experiences) build up, thus leading to increased stressful life events load.

Cortisol is known to play a key role in stress. Stressful life events have been shown to activate the Hypothalamus-Pituitary-Adrenal (HPA) axis resulting in increased cortisol release (Comijs, et al., 2011).

Cortisol easily crosses the blood–brain barrier and binds to specific receptors throughout the brain, in particular in regions involved in cognitive functions (Daskalakis, et al., 2013,McEwen, 2007,Vogel, et al., 2016). Indeed, the hippocampus, mainly involved in memory, expresses both type I (Mineralocorticoid Receptors or MRs) and type II receptors (Glucocorticoid Receptors or GRs) and the prefrontal cortex, mainly involved in executive functions, expresses GRs (Lupien, et al., 2007,McEwen, 2007).

Several previous animal as well as clinical studies showed an association between increased cortisol and poorer overall cognitive performance (Geerlings, et al., 2015,Lee, et al., 2008,Lupien, et al., 2007,Tatomir, et al., 2014,Vogel, et al., 2016), declarative memory (Geerlings, et al., 2015,Lupien, et al., 2007,Tatomir, et al., 2014,Vogel, et al., 2016), language (Lee, et al., 2008), processing speed (Lee, et al., 2008), executive functioning (Geerlings, et al., 2015,Lupien, et al., 2007), spatial memory (de Quervain, et al., 1998) as well as social memory (Takahashi, et al., 2004). High levels of cortisol have been reported to exhibit neurotoxic effects on the hippocampus (Byers and Yaffe, 2011) and have been associated with hippocampal atrophy (Tatomir, et al., 2014). At the same time, since the hippocampus regulates the HPA axis activity through an inhibitory feed-back loop, hippocampal atrophy associated with elevated cortisol might further uninhibit the HPA axis leading to a vicious cascade (Geerlings, et al., 2015).

Elevated cortisol has also been associated with volume reductions in other regions of the brain involved in cognitive functions. Indeed, in a study involving 4244 patients without dementia, Geerlings *et al.* (2015) found that higher evening cortisol was associated with smaller total brain volume. Smaller volumes were observed in all brain regions, especially the gray matter (Geerlings, et al., 2015).

Furthermore, glucocorticoids have been shown to exacerbate oxidative injury and amyloid β peptide toxicity in cultured hippocampal neurons (Goodman, et al., 1996). High cortisol levels have also been associated with increased amyloid-β peptide and tau pathology in a mouse model of Alzheimer's Disease (AD) (Green, et al., 2006). Furthermore, increased Cerebro-Spinal Fluid (CSF) cortisol levels have been found in subjects with dementia and Mild Cognitive Impairment (MCI) due to AD compared to control subjects with normal cognition (Popp, et al., 2009,Popp, et al., 2015). In MCI due to AD, high CSF cortisol was also predictive of a more rapid cognitive decline (Popp, et al., 2015).

Studies investigating the possible effects of life events on cognitive performance are relatively scarce. Many studies suggested that life events might have negative effects on cognition (Lupien, et al., 2007,VonDras, et al., 2005) and might be associated with late-life brain atrophy and white matter lesions (Johansson, et al., 2012) as well as with an increased risk of dementia (Johansson, et al., 2010,Persson and Skoog, 1996).

Loss of one's partner has been associated with cognitive decline. Even though this association was reported to be thoroughly explained by depressive symptoms in the study by Ward et *al.* (2007) (Ward, et al., 2007), other studies found that this association was present above and beyond depressive symptoms (Aartsen, et al., 2005,van Gelder, et al., 2006,Xavier, et al., 2002). However, these links between life events and poorer cognitive performance have not been

found in other studies (Fountoulakis, et al., 2011,Sundstrom, et al., 2014). Using data from the Longitudinal Aging Study Amsterdam, Comijs *et al.* (2011) reported differential effects of different types of life events on cognition: “acute” events were associated with a higher rate of cognitive decline whereas “chronic” events were associated with better cognitive function (Comijs, et al., 2011).

In the light of these results highlighting that life events were commonly associated with increased cortisol, that high cortisol had possible detrimental cognitive effects and that life events might lead to cognitive decline, it has commonly been assumed that life events likely impair cognition through increased cortisol secretion. However, very few studies actually examined these assumed links between life events, cortisol and cognition.

In this study, we hypothesized that life events might increase cortisol secretion leading to cognitive impairment, in particular in episodic memory and executive functions. These effects might depend on the specific type of life events (acute vs chronic or physical vs social).

2. Methods:

2.1. Participants

A cross-sectional analysis was conducted using data from the first follow-up of the longitudinal population-based CoLaus/PsyCoLaus study designed to investigate the prevalence of cardiovascular risk factors and psychiatric disorders in the community and to identify their genetic determinants. The methodological features of this study were already described in detail (Firmann, et al., 2008,Preisig, et al., 2009). CoLaus/PsyColaus included a random sample of 6733 subjects (age range: 35-75 years) selected from the residents of the city of Lausanne (Switzerland) between 2003 and 2007. All subjects were also invited to participate at the first follow-up, which took place between 2009 and 2013. A total of 5064 and 4004 subjects accepted these new physical and psychiatric evaluations which also included salivary cortisol measures. In addition, all subjects aged 65 or older (n=1918) were invited to undergo a neuropsychological cognitive assessment. CoLaus/PsyCoLaus elicited Information on demographic, medical, and treatment history, life events as well as smoking and alcohol consumption using semi-structured interviews conducted by trained interviewers or self-rating questionnaires. Psychiatric/behavioral disorders were assessed using the Diagnostic Interview for Genetic Studies (DIGS) (Preisig, et al., 1999). Cardio-metabolic disorders were assessed clinically and with the use of biochemical measures. The distribution of age groups, gender and geographic distributions in CoLaus/Psycholaus participants at baseline were similar to the source population (Firmann, et al., 2008).

Of the 1918 subjects aged at least 65 at the first follow-up visit of CoLaus/PsyCoLaus, 1214 had a thorough cognitive assessment and 796 non-demented (CDR score <1) individuals also agreed to provide salivary samples (41.5% of all participants in this age range).

2.2. Cognitive assessment

A neuropsychological and clinical examination as well as an assessment of the participants' daily living activities were performed by trained master-level psychologists blinded for the salivary cortisol and life event scores.
The neuropsychological assessment included:

- The assessment of the global cognitive performance using the Mini Mental State Examination (MMSE), the most commonly used screening tool for global cognitive impairment. MMSE scores range from 0 to 30, a higher score indicating better performance.
- The assessment of *memory* using the Grober and Buschke Double Memory Test (DMT) (Buschke, et al., 1997).
- The assessment of *verbal fluency* using the DO40 picture-naming test (Deloche, 1997), the letter (phonemic) and the category (semantic) fluency tasks.
- The assessment of *executive functions* using the Stroop Test.
- The assessment of *visuo-spatial construction* using the figures from the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) neuropsychological test battery (Morris, et al., 1988).

All of these tests and scales have been validated and are widely used in the field.

Overall cognitive and functional status was assessed using the CDR scale, a widely used scale for the clinical staging of cognitive impairment. The CDR encompasses data about cognitive and functional performance in six domains: memory, orientation, judgment and problem solving, community affairs, home and hobbies, and personal care (Morris, 1993).
Participants with a global CDR>=1 (defining dementia) were excluded from the current study.
The CDR Sum Of Boxes (CDRSOB) was chosen as a main outcome measure.

2.3. Cortisol measures

Salivary cortisol has been established as a reliable indicator of circulating cortisol levels and HPA axis function (Gallagher, et al., 2006). Participants used Salivette sampling devices (Sarstedt, Rommeldsdorf, Germany) for saliva collection.
Four salivary samples were obtained from each consenting participant: upon waking, 30 minutes after waking, at 11 am and at 8 pm. Subjects were instructed not to brush their teeth and to refrain from eating, drinking, smoking and exercising 30 min prior to and during the sampling procedure (Kuehner, et al., 2007). Subjects were also instructed to keep a protocol where they would record adherence to the protocol including exact time of saliva collections. Until sampling had been completed, subjects stored the saliva samples at home in their freezers before returning them to the laboratory together with the saliva protocol, where they were stored at -20°C until biochemical analysis.
Free cortisol levels in the salivary samples were measured using a commercially available chemiluminescence assay (IBL, Hamburg, Germany). Inter- and intra-assay coefficients of variability were <9%.
In case of non-adherence to the protocol, missing values were analyzed using the expectation-maximization algorithm.
We calculated the cortisol Area Under the Curve (AUC) using the trapezoid formula (Pruessner, et al., 2003).
The AUC is a commonly used measure to estimate total hormonal output over a period of time. Unlike other summary measures such as mean cortisol, the AUC captures not only the cortisol

levels at the times of sampling but also changes over time (Pruessner, et al., 2003).
We also calculated the Cortisol Awakening Response (CAR) defined as the difference between cortisol 30 minutes after waking and cortisol upon waking.

2.4. Life events inventory

Impact of lifetime life events was assessed by the means of the Amiel-Lebigre event questionnaire (Amiel-Lebigre, 2004). This validated instrument consists of 52 items covering several domains (health, work, finance, interpersonal relationships, family, accidents, deaths, finance). For each lifetime event, the participants were instructed to specify the duration as well as the perceived negative impact (from 0 to 100, with higher scoring indicating greater impacts).
The total negative impact of life events was calculated by summing up the impact scores of all reported events. We also separately calculated the negative impact of acute/chronic and "social"/"physical" stressors as we hypothesized that different types of life events might have different effects on cognition and cortisol (Comijs, et al., 2011).

2.5. Other variables

Subjective cognitive decline was assessed using the Cognitive Complaint Questionnaire (QPC) (Thomas-Antérion, et al., 2003). The QPC is a French-language rater-administered instrument; consisting of 10 yes/no questions assessing subjective cognitive changes over the previous six months. According to the scoring method proposed by the authors, subjective cognitive decline is considered present if the participant gave at least three positive answers to the ten questions and/or a positive answer to question 5 and/or at least two positive answers to questions A,4,5,7,8.
Depressive symptoms were assessed at the same time as the physical evaluation (approximately one year before the cognitive assessment) using the Center for Epidemiologic Studies Depression scale (CES-D) in its French version (Morin, et al., 2011). CES-D scores range between 0 and 60 with higher scores indicating more depressive symptoms.
Level of formal *education* was categorized into two groups: primary/secondary school education and higher education.
Body Mass Index (BMI) was also included as a covariate since cortisol levels might depend on BMI (Odeniyi, et al., 2015).

2.6. Ethics

The CoLaus/PsyCoLaus study was approved by the Ethics Committee of the University of Lausanne and a written informed consent was obtained from all participants.

2.7. Statistical analysis

Statistical analysis was performed using SPSS v23.0 (IBM Corp., Armonk, NY, USA).

Descriptive statistics
Continuous variables were described as mean±standard deviation, while categorical variables were described through absolute and relative frequencies.
The scores of each cognitive domain (memory, fluency, executive functions and visuo-spatial construction) were standardized (z-scores were calculated for the sum of each cognitive domain score).

Associations between cognitive performance and cortisol
Hierarchical multiple linear regression models were constructed with the CDRSOB as an outcome variable and with the cortisol AUC then the CAR (respectively) as independent variables, controlling for age, sex, BMI, education and CES-D score.
Afterwards, each cognitive domain z-score was considered as a dependant variable with cortisol AUC then CAR (respectively) as independent variables, controlling for age, sex, BMI, education and CES-D score.

Associations between cognitive performance and life events
Hierarchical multiple logistic regression models were constructed with the CDRSOB, then with each cognitive domain z-score respectively as dependant variables, and with the negative impact of life events as an independent variable, controlling for age, sex, BMI, education and CES-D score.

Associations between cortisol and life events
Hierarchical multiple logistic regression models were constructed with the cortisol AUC then with the CAR respectively as dependant variables, and with the negative impact of life events as an independent variable, controlling for age, sex, BMI, education and CES-D score.

Associations between cognitive performance, cortisol and life events
A three-block hierarchical multiple regression model was constructed with the CDRSOB as a dependant variable. Block one included age, sex, BMI, education and CES-D score. Block two included the negative impact of life events score and the interaction between life events and the cortisol AUC and block three the cortisol AUC.

For each of these regression models, the unstandardized regression coefficients (B), their 95% confidence intervals (CIs), the partial correlation coefficients (r) and the p values are presented. For multiple comparisons, p values were adjusted according to Holm-Bonferroni's method.

3. Results:

3.1. Characteristics of the sample

Compared with the whole PsyCoLaus population aged at least 65 (n=1214), our sub-sample (n=796) had a comparable distribution by age, gender, education level, BMI and CES-D score (**Table 1**).
The results of the different cognitive scores, the life events inventory as well as the cortisol

measures are described in **Table 2.**
Among the salivary cortisol measures, 207 (6.5% of the total number of values) were missing.

Subjective cognitive decline was not associated with cortisol, cognition, depression or life events, and was not included in further statistical analysis.
The total impact of life events did not differ between genders and was not associated with the CES-D score. Moreover, separating life events into acute/chronic or physical/social did not show any significant associations with the CES-D score.

{INSERT Table 1}

{INSERT Table 2}

3.2. Life events and cortisol

Neither the cortisol AUC nor the CAR respectively were associated with life events total impact, controlling for age, sex, BMI, education and CES-D score. Separating life events into acute/chronic or physical/social did not modify these results.

3.3. Life events and cognitive performance

Neither the CDRSOB nor the MMSE were associated with life events total impact score, controlling for age, sex, BMI, education and depressive symptoms. None of the four cognitive domains was associated with life events total impact. Separating life events into acute/chronic or physical/social did not modify these results.

3.4. Cognitive performance and cortisol

Global cognitive performance as measured by the CDRSOB was positively associated with the cortisol AUC after controlling for age, sex, BMI, education and depressive symptoms (p=0.003; B=0.686[0.240;1.333]; r=0.114) **(Figure 1).** This association remained significant after excluding the outliers (the three individuals with the highest cortisol AUC, p=0.006; B=0.684[0.197;1.172]; r=0.105). Similarly, the MMSE score was negatively associated with the cortisol AUC, after controlling for age, sex, BMI, education and depressive symptoms although the association was weaker than with the CDRSOB (p=0.039; B=-1.079[-2.106;-0.052]; r=-0.079). Among the cognitive domains, only the memory domain z-score was significantly associated with cortisol AUC (p=0.025; B=-0.791[-1.485;-0.098]; r=-0.090). Neither the CDRSOB nor any of the cognitive domain z-scores was associated with CAR.
Among the individual cortisol measures, the 11 am value was significantly associated with the CDRSOB, after controlling for age, sex, BMI, education and depressive symptoms (p'=0.012; B=1.193[0.397;1.989]; r=0.112). The evening (8 pm) measure was also associated with the CDRSOB, but the association was no longer significant after applying the Holm-Bonferroni's method **(Table 3).**

{INSERT Table 3}

3.5. Are life events related to the cortisol AUC thereby affecting global cognitive performance?

3.6.

Global cognitive performance as measured by the CDRSOB remained associated with the cortisol AUC after controlling for life events total impact and for the interaction between life events and cortisol AUC (p=0.002; B=0.694[0.248;1.141]; r=0.116) **(Figure 1).**

This association between the CDRSOB and the cortisol AUC also remained after controlling for the negative impact of events subdivided into acute/chronic or physical/social and the interaction between the cortisol AUC and the negative impact of respectively acute/chronic, physical/social life events.

{INSERT Figure 1}

4. Discussion:

In this study, we analyzed the relationships between life events, cortisol levels and cognitive performance in a large sample of non-demented subjects aged >=65 years from the general population. We found that the subjectively rated total impact of life events was not associated with a poorer cognitive performance. In contrast, higher cortisol levels were associated with poorer global cognitive performance and more particularly with poorer episodic memory, regardless of life events.

4.1. Life events and cortisol

In our study, cortisol was not associated with the life events score, regardless of whether a lifetime score or scores distinguishing between acute/chronic or physical/social events were used. While it has been previously assumed that stressful life events are associated with increased cortisol, the link between life events and the HPA axis appears to be much more complex and still need to be elucidated. Stressful events have been shown to be associated with increased cortisol levels in some studies but with decreased cortisol levels in others (Daskalakis, et al., 2013,Miller, et al., 2007). It has been suggested that the effect of stressful events on the HPA axis might depend on several factors, including the nature of the stressor, the time elapsed since its onset, its controllability, the core emotions it is likely to elicit as well as its psychiatric sequelae (Miller, et al., 2007). Hence, while "social stressors" have been more commonly associated with increased cortisol, "physical" and traumatizing stressors have been rather associated with lower cortisol. Moreover, while a given stressor might acutely activate the HPA axis, cortisol levels tend to decrease with time, eventually reaching below-normal levels (Miller, et al., 2007). In our study, separating life events into acute/chronic or physical/social categories did not show any association between cortisol and the impact of any of these categories. The complexity of the link between life events and the HPA axis and the likely role of many other factors might explain this finding.

Moreover, although repeated exposure to stressors may disrupt the HPA axis (allostatic load), this does not necessarily occur in all individuals. Some individuals are indeed resilient and are able to adapt to adversity, likely through a better adaptation of their HPA axis functioning,

among other mechanisms (Garrido, 2011). Different coping styles may lead to different neuroendocrine responses, and thus to different vulnerabilities to stress-mediated disease (Koolhaas, et al., 1999).
This goes in line with the fact that we did not find, in our study, any link between depressive symptoms and the negative impact of all, acute/chronic or physical/social life events. Indeed, depression has been commonly associated with life events, in particular chronic and social ones (Miller, et al., 2007). The time elapsed since the occurrence of the life event, a variable which is not captured by our questionnaire, might explain these results.

4.2. Life events and cognitive performance

In our study, neither the global cognitive performance nor any cognitive subdomain score was associated with the life events score. Results of similar studies so far have been conflicting. Indeed, many studies reported that stressful events were associated with poorer cognitive performance, mainly affecting memory and/or executive functions (Lupien, et al., 2007,van Gelder, et al., 2006,VonDras, et al., 2005,Xavier, et al., 2002), and with an increased risk of late-life dementia (Johansson, et al., 2010), late-life brain atrophy and white matter lesions (Johansson, et al., 2012). Yet, other studies did not find any link between life events and cognition (Fountoulakis, et al., 2011,Sundstrom, et al., 2014,Ward, et al., 2007) and some even reported a possibly enhanced cognitive functioning following stressful events (Deeg, et al., 2005).
These differences might be due to methodological variance across studies, including the sample selection, the assessed type of life events and the inclusion of potential confounders in the statistical models such as depression, prior cognitive impairment or genetic predisposition. Indeed, life events have widely been associated with depression and given that some studies did not control for depressive symptoms, it remains unclear whether life events are actually associated with cognitive impairment beyond the effects attributable to depression (Comijs, et al., 2011). In our study, life events were not associated with cognitive impairment after controlling for different possible confounders including depressive symptoms. Moreover, the effects of life events might differ depending on the presence of prior cognitive impairment. Indeed, Peavy *et al.* (2009) reported that higher event-based stress ratings were associated with faster cognitive decline in subjects with MCI but not in cognitively intact individuals (Peavy, et al., 2009).
Different life events might also display different cognitive effects. Rosnick *et al.* (2007) found that experiencing the injury or illness of a friend was associated with better performance on recall, psychomotor speed and attention tasks. Yet, having less money to live on and being victim of a crime were associated with poorer performance on psychomotor speed tasks (Rosnick, et al., 2007). Comijs *et al.* (2011) found that acute stressors, such as the death of a (grand)child, were associated with a faster cognitive decline, whereas chronic stressors, such as the illness of a partner or relative, were associated with a slower cognitive decline (Comijs, et al., 2011). Fountoulakis *et al.* (2011) found that the association between life events and cognition might be falsely positive due to the inclusion of events that are causally related to dementia or secondarily related to the disease (Fountoulakis, et al., 2011).
Given the complexity of the inter-relationships between life events and cognition, our negative results should probably not dismiss the possible effects of cumulative stress on the cognitive performance in older age.

4.3. Cognitive performance and cortisol

The link found between higher cortisol levels and poorer cognitive performance is consistent with many previous studies. Indeed, most studies which focused on the link between cortisol levels and cognition in the older individuals without dementia found a negative association (Geerlings, et al., 2015,Lara, et al., 2015,Lee, et al., 2008,Li, et al., 2006,Lupien, et al., 1994). Similarly, increased cortisol levels were found in association with more rapid disease progression in subjects with very mild or mild AD dementia (Csernansky, et al., 2006), and in MCI due to AD (Popp, et al., 2015). Altered cortisol suppression after dexamethasone (DXT) administration, has also been described in AD. The prevalence of DXT non-suppressors among AD patients has been reported to range between 30% and 50%, with higher prevalences in the most cognitively affected patients (Murialdo, et al., 2000).

Yet, other studies reported opposite results with increased salivary cortisol being associated with a slower cognitive decline in individuals with MCI (Peavy, et al., 2009) and with better explicit memory in postmenopausal women using hormone replacement therapy (Hampson and Duff-Canning, 2016).

This discrepancy might be explained by different designs but also by different effects of cortisol on cognitive performance depending on its levels in the brain. This has been hypothesized to be due to differential effects of MRs and GRs. Thus, activation of MRs seems to enhance memory while activation of GRs seems to impair memory. MRs have a ten-fold higher affinity for cortisol so that mildly elevated cortisol levels mainly activate MRs in the hippocampus, thus resulting in enhancing effects. Higher levels result in an activation of both MRs and GRs in the hippocampus explaining the deleterious effects on declarative memory (Lupien, et al., 2007). This is consistent with the results of several previous animal as well as clinical studies showing that the effects of cortisol on declarative memory could be described by an inverted-U plot (de Kloet, et al., 1999,Lupien, et al., 2007,McEwen, 2007).

In the prefrontal cortex, where only GRs are expressed, elevated cortisol displays clearer detrimental effects on executive functions (Lupien, et al., 2007,McEwen, 2007).

In some cases, the link between cortisol and cognition may be accounted for by residual depressive symptoms. Indeed, depression may be associated with increased cortisol secretion and reduced feedback sensitivity of the HPA axis. In addition, depression commonly encompasses cognitive deficits (Wingenfeld and Wolf, 2015). In our study, since we controlled for the CES-D score, the link between cortisol and cognition seems to be independent of depressive symptoms. Previous studies reported the association between evening cortisol levels and worse performance in delayed paragraph recall (Li, et al., 2006), worse performance across all cognitive domains and smaller brain volumes (Geerlings, et al., 2015) in non-demented older subjects. In our study, the evening measure was also associated with the CDRSOB, but the association was no longer significant after the Holm-Bonferroni's correction. Rather, we found that the measure at 11 am was significantly associated with the CDRSOB. Since the 11am and the 8pm measures are the ones with the most important influence on the cortisol AUC, our results suggest that poorer cognition is likely to be associated with an overall increased cortisol secretion pattern rather than a specific change in the circadian rhythm.

Additionally, we did not find any link between the CAR and the cognitive performance. This contrasts with the findings of Hidalgo *et al.* who found that the CAR was negatively related to verbal and visual memory domains (Hidalgo, et al., 2016). This discrepancy might be due to the fact that the authors used three measures per day on two consecutive days to calculate the CAR, which may have provided a more accurate capture of the CAR (Hidalgo, et al., 2016).

4.4. Are life events related to the cortisol AUC thereby affecting global cognitive performance?

It has often been assumed that "stress" and "stressful events" might explain increased cortisol levels, eventually leading to impaired cognition (Lupien, et al., 2007,McEwen, 2007). Life events impact score was associated neither with cortisol nor with cognition. However, we observed that cortisol was associated with poorer cognitive performance independently of life events. There are several potential explanations for the lack of an association between life events and cortisol levels or poor cognitive functioning. First, the direction of the association between cortisol and poor cognitive functioning could be inverse: since the hippocampus inhibits the HPA axis, the observed high cortisol levels could be due to the hippocampal atrophy itself, with cortisol gradually increasing as the hippocampal atrophy advances (Geerlings, et al., 2015). Second, although cortisol is often referred to as the stress hormone (Lupien, et al., 2007) a series of other factors than life events determine its secretion, including metabolic and immune factors.

The established association between elevated cortisol and poorer cognitive functioning does not need to be direct. Given that cortisol plays a key role in metabolism it has been shown to be associated with the metabolic syndrome and insulin resistance, which, in turn, are tightly linked to vascular dementia and AD (Kim and Feldman, 2015).

4.5. Strengths and limitations

Major strengths of this study include its population-based design and the large sample size for this type of studies. Other strengths include the detailed neuropsychological examination. Moreover, in comparison with most other studies, we used an extensive life events inventory with a subjective rating of the impact of each event by the participant. We also controlled for a number of other potential confounders.

Nevertheless, some limitations are to be acknowledged. First, the cross-sectional design does not allow us to draw any conclusions about cause-to-effect relationships. Second, only 41.5% of the participants of 65 years and older underwent both the cognitive evaluation and the cortisol assessment. Nevertheless, it is not likely that selection bias significantly affected the established associations between cortisol or life events and cognitive functioning. Third, all participants were residents of an urban area in Switzerland, which reduces the generalizabity of the findings. Fourth, the cortisol measure was restricted to one day, which limits the accuracy of this assessment. There were four salivary cortisol time points. Hence a single spurious measurement deviating from the circadian rhythm could have biased the cortisol AUC. The use of the trapezoid formula to calculate the cortisol AUC may have underestimated the true value of cortisol output between 11am and 8pm. Nevertheless, most previous population-based studies had only one cortisol measure. Our cortisol measures were also static. Fifth, the CAR measures relied on two post-awakening samples, a protocol used in most population-based studies, providing a general approximation of the CAR. However, in some individuals, more samples might be needed to be sure to capture the CAR peak (Hidalgo, et al., 2016,Stalder, et al., 2016).

5. Conclusions:

In this population-based study, we found that elevated cortisol was associated with poorer

cognitive performance, in particular with poorer memory performance, independently of life events. We did not find a link between life events and both cortisol measures and global cognitive performance. Accordingly, the association between increased cortisol levels and poor cognition was independent of stressful life events. Thus, the link between life events and the HPA axis is likely to be much more complex than life events simply activating cortisol secretion. Increased cortisol levels may be not only a factor contributing to cognitive impairment but may also reflect damage and dysfunction of the brain structures related to both the HPA axis andcognitive performance. Longitudinal follow-up studies are necessary to better understand the complex interplay between life events, cortisol, and cognition.

Conflicts of interest:

None

Funding:

The CoLaus/PsyCoLaus study was and is supported by research grants from GlaxoSmithKline, the Faculty of Biology and Medicine of Lausanne, and the Swiss National Research Foundation (grants 3200B0–105993, 3200B0-118308, 33CSCO-122661, 33CS30-139468 and 33CS30-148401). JP received support from the Swiss National Research Foundation (grant 320030_141179).

Acknowledgements:

The authors would like to express their gratitude to the Lausanne inhabitants who volunteered to participate in the PsyCoLaus study. We would also like to thank all the investigators of the CoLaus study, which made the psychiatric PsyCoLaus study possible.

References:

Aartsen, M.J., Van Tilburg, T., Smits, C.H., Comijs, H.C., Knipscheer, K.C. 2005. Does widowhood affect memory performance of older persons? Psychological medicine 35(2), 217-26.

Amiel-Lebigre, F. 2004. Événements stressants de la vie: Méthodologie et résultats. EMC-Psychiatrie 1(1), 75-86.

Buschke, H., Sliwinski, M.J., Kuslansky, G., Lipton, R.B. 1997. Diagnosis of early dementia by the Double Memory Test: encoding specificity improves diagnostic sensitivity and specificity. Neurology 48(4), 989-97.

Byers, A.L., Yaffe, K. 2011. Depression and risk of developing dementia. Nature reviews Neurology 7(6), 323-31. doi:10.1038/nrneurol.2011.60.

Comijs, H.C., van den Kommer, T.N., Minnaar, R.W., Penninx, B.W., Deeg, D.J. 2011. Accumulated and differential effects of life events on cognitive decline in older persons: depending on depression, baseline cognition, or ApoE epsilon4 status? The journals of gerontology Series B, Psychological sciences and social sciences 66 Suppl 1, i111-20. doi:10.1093/geronb/gbr019.

Csernansky, J.G., Dong, H., Fagan, A.M., Wang, L., Xiong, C., Holtzman, D.M., Morris, J.C. 2006. Plasma cortisol and progression of dementia in subjects with Alzheimer-type dementia. The American journal of psychiatry 163(12), 2164-9. doi:10.1176/ajp.2006.163.12.2164.

Daskalakis, N.P., Lehrner, A., Yehuda, R. 2013. Endocrine aspects of post-traumatic stress disorder and implications for diagnosis and treatment. Endocrinology and metabolism clinics of North America 42(3), 503-13. doi:10.1016/j.ecl.2013.05.004.

de Kloet, E.R., Oitzl, M.S., Joels, M. 1999. Stress and cognition: are corticosteroids good or bad guys? Trends in neurosciences 22(10), 422-6.

de Quervain, D.J., Roozendaal, B., McGaugh, J.L. 1998. Stress and glucocorticoids impair retrieval of long-term spatial memory. Nature 394(6695), 787-90. doi:10.1038/29542.

Deeg, D.J., Huizink, A.C., Comijs, H.C., Smid, T. 2005. Disaster and associated changes in physical and mental health in older residents. European journal of public health 15(2), 170-4. doi:10.1093/eurpub/cki126.

Deloche, G., Hannequin, D. 1997. DO 80 : Epreuve de Dénomination Orale d'images. Editions du Centre de Psychologie Appliquée, Paris

Firmann, M., Mayor, V., Vidal, P.M., Bochud, M., Pecoud, A., Hayoz, D., Paccaud, F., Preisig, M., Song, K.S., Yuan, X., Danoff, T.M., Stirnadel, H.A., Waterworth, D., Mooser, V., Waeber, G., Vollenweider, P. 2008. The CoLaus study: a population-based study to investigate the epidemiology and genetic determinants of cardiovascular risk factors and metabolic syndrome. BMC cardiovascular disorders 8, 6. doi:10.1186/1471-2261-8-6.

Fountoulakis, K.N., Pavlidis, I., Tsolaki, M. 2011. Life events and dementia: what is the nature of their relationship? Psychiatry research 190(1), 156-8. doi:10.1016/j.psychres.2011.05.011.

Gallagher, P., Leitch, M.M., Massey, A.E., McAllister-Williams, R.H., Young, A.H. 2006. Assessing cortisol and dehydroepiandrosterone (DHEA) in saliva: effects of collection method. Journal of psychopharmacology 20(5), 643-9. doi:10.1177/0269881106060585.

Garrido, P. 2011. Aging and stress: past hypotheses, present approaches and perspectives. Aging and disease 2(1), 80-99.

Geerlings, M.I., Sigurdsson, S., Eiriksdottir, G., Garcia, M.E., Harris, T.B., Gudnason, V., Launer, L.J. 2015. Salivary cortisol, brain volumes, and cognition in community-dwelling elderly without dementia. Neurology 85(11), 976-83. doi:10.1212/WNL.0000000000001931.

Goodman, Y., Bruce, A.J., Cheng, B., Mattson, M.P. 1996. Estrogens attenuate and corticosterone exacerbates excitotoxicity, oxidative injury, and amyloid beta-peptide toxicity in hippocampal neurons. Journal of neurochemistry 66(5), 1836-44.

Green, K.N., Billings, L.M., Roozendaal, B., McGaugh, J.L., LaFerla, F.M. 2006. Glucocorticoids increase amyloid-beta and tau pathology in a mouse model of Alzheimer's disease. The Journal of neuroscience : the official journal of the Society for Neuroscience 26(35), 9047-56.

doi:10.1523/JNEUROSCI.2797-06.2006.
Hampson, E., Duff-Canning, S.J. 2016. Salivary cortisol and explicit memory in postmenopausal women using hormone replacement therapy. Psychoneuroendocrinology 64, 99-107. doi:10.1016/j.psyneuen.2015.11.009.
Hidalgo, V., Almela, M., Pulopulos, M.M., Salvador, A. 2016. Memory performance is related to the cortisol awakening response in older people, but not to the diurnal cortisol slope. Psychoneuroendocrinology 71, 136-46. doi:10.1016/j.psyneuen.2016.05.019.
Johansson, L., Guo, X., Waern, M., Ostling, S., Gustafson, D., Bengtsson, C., Skoog, I. 2010. Midlife psychological stress and risk of dementia: a 35-year longitudinal population study. Brain : a journal of neurology 133(Pt 8), 2217-24. doi:10.1093/brain/awq116.
Johansson, L., Skoog, I., Gustafson, D.R., Olesen, P.J., Waern, M., Bengtsson, C., Bjorkelund, C., Pantoni, L., Simoni, M., Lissner, L., Guo, X. 2012. Midlife psychological distress associated with late-life brain atrophy and white matter lesions: a 32-year population study of women. Psychosomatic medicine 74(2), 120-5. doi:10.1097/PSY.0b013e318246eb10.
Kim, B., Feldman, E.L. 2015. Insulin resistance as a key link for the increased risk of cognitive impairment in the metabolic syndrome. Experimental & molecular medicine 47, e149. doi:10.1038/emm.2015.3.
Koolhaas, J.M., Korte, S.M., De Boer, S.F., Van Der Vegt, B.J., Van Reenen, C.G., Hopster, H., De Jong, I.C., Ruis, M.A., Blokhuis, H.J. 1999. Coping styles in animals: current status in behavior and stress-physiology. Neuroscience and biobehavioral reviews 23(7), 925-35.
Kuehner, C., Holzhauer, S., Huffziger, S. 2007. Decreased cortisol response to awakening is associated with cognitive vulnerability to depression in a nonclinical sample of young adults. Psychoneuroendocrinology 32(2), 199-209. doi:10.1016/j.psyneuen.2006.12.007.
Lara, V.P., Caramelli, P., Teixeira, A.L., Barbosa, M.T., Carmona, K.C., Guimaraes, H.C., Carvalho, M.G., Fernandes, A.P., Gomes, K.B. 2015. Cortisol, HDL-c, VLDL-c, and APOE Polymorphisms as Laboratorial Parameters Associated to Cognitive Impairment No Dementia (CIND) and Dementia. Journal of clinical laboratory analysis. doi:10.1002/jcla.21865.
Lee, C.M., Huxley, R.R., Wildman, R.P., Woodward, M. 2008. Indices of abdominal obesity are better discriminators of cardiovascular risk factors than BMI: a meta-analysis. Journal of clinical epidemiology 61(7), 646-53. doi:10.1016/j.jclinepi.2007.08.012.
Li, G., Cherrier, M.M., Tsuang, D.W., Petrie, E.C., Colasurdo, E.A., Craft, S., Schellenberg, G.D., Peskind, E.R., Raskind, M.A., Wilkinson, C.W. 2006. Salivary cortisol and memory function in human aging. Neurobiology of aging 27(11), 1705-14. doi:10.1016/j.neurobiolaging.2005.09.031.
Lupien, S., Lecours, A.R., Lussier, I., Schwartz, G., Nair, N.P., Meaney, M.J. 1994. Basal cortisol levels and cognitive deficits in human aging. The Journal of neuroscience : the official journal of the Society for Neuroscience 14(5 Pt 1), 2893-903.
Lupien, S.J., Maheu, F., Tu, M., Fiocco, A., Schramek, T.E. 2007. The effects of stress and stress hormones on human cognition: Implications for the field of brain and cognition. Brain and cognition 65(3), 209-37. doi:10.1016/j.bandc.2007.02.007.
McEwen, B.S. 2007. Physiology and neurobiology of stress and adaptation: central role of the brain. Physiological reviews 87(3), 873-904. doi:10.1152/physrev.00041.2006.
Miller, G.E., Chen, E., Zhou, E.S. 2007. If it goes up, must it come down? Chronic stress and the hypothalamic-pituitary-adrenocortical axis in humans. Psychological bulletin 133(1), 25-45. doi:10.1037/0033-2909.133.1.25.
Morin, A.J., Moullec, G., Maiano, C., Layet, L., Just, J.L., Ninot, G. 2011. Psychometric properties of the Center for Epidemiologic Studies Depression Scale (CES-D) in French clinical and nonclinical adults. Revue d'epidemiologie et de sante publique 59(5), 327-40. doi:10.1016/j.respe.2011.03.061.
Morris, J.C. 1993. The Clinical Dementia Rating (CDR): current version and scoring rules. Neurology 43(11), 2412-4.
Morris, J.C., Mohs, R.C., Rogers, H., Fillenbaum, G., Heyman, A. 1988. Consortium to establish a registry for Alzheimer's disease (CERAD) clinical and neuropsychological assessment of Alzheimer's disease. Psychopharmacology bulletin 24(4), 641-52.
Murialdo, G., Barreca, A., Nobili, F., Rollero, A., Timossi, G., Gianelli, M.V., Copello, F., Rodriguez, G.,

Polleri, A. 2000. Dexamethasone effects on cortisol secretion in Alzheimer's disease: some clinical and hormonal features in suppressor and nonsuppressor patients. Journal of endocrinological investigation 23(3), 178-86. doi:10.1007/BF03343703.

Odeniyi, I.A., Fasanmade, O.A., Ogbera, A.O., Ohwovoriole, A.E. 2015. Body mass index and its effect on serum cortisol level. Nigerian journal of clinical practice 18(2), 194-7. doi:10.4103/1119-3077.151040.

Peavy, G.M., Salmon, D.P., Jacobson, M.W., Hervey, A., Gamst, A.C., Wolfson, T., Patterson, T.L., Goldman, S., Mills, P.J., Khandrika, S., Galasko, D. 2009. Effects of chronic stress on memory decline in cognitively normal and mildly impaired older adults. The American journal of psychiatry 166(12), 1384-91. doi:10.1176/appi.ajp.2009.09040461.

Persson, G., Skoog, I. 1996. A prospective population study of psychosocial risk factors for late onset dementia. International Journal of Geriatric Psychiatry 11(1), 15-22. doi:10.1002/(SICI)1099-1166(199601)11:1<15::AID-GPS262>3.0.CO;2-5.

Popp, J., Schaper, K., Kolsch, H., Cvetanovska, G., Rommel, F., Klingmuller, D., Dodel, R., Wullner, U., Jessen, F. 2009. CSF cortisol in Alzheimer's disease and mild cognitive impairment. Neurobiology of aging 30(3), 498-500. doi:10.1016/j.neurobiolaging.2007.07.007.

Popp, J., Wolfsgruber, S., Heuser, I., Peters, O., Hull, M., Schroder, J., Moller, H.J., Lewczuk, P., Schneider, A., Jahn, H., Luckhaus, C., Perneczky, R., Frolich, L., Wagner, M., Maier, W., Wiltfang, J., Kornhuber, J., Jessen, F. 2015. Cerebrospinal fluid cortisol and clinical disease progression in MCI and dementia of Alzheimer's type. Neurobiology of aging 36(2), 601-7. doi:10.1016/j.neurobiolaging.2014.10.031.

Preisig, M., Fenton, B.T., Matthey, M.L., Berney, A., Ferrero, F. 1999. Diagnostic interview for genetic studies (DIGS): inter-rater and test-retest reliability of the French version. European archives of psychiatry and clinical neuroscience 249(4), 174-9.

Preisig, M., Waeber, G., Vollenweider, P., Bovet, P., Rothen, S., Vandeleur, C., Guex, P., Middleton, L., Waterworth, D., Mooser, V., Tozzi, F., Muglia, P. 2009. The PsyCoLaus study: methodology and characteristics of the sample of a population-based survey on psychiatric disorders and their association with genetic and cardiovascular risk factors. BMC psychiatry 9, 9. doi:10.1186/1471-244X-9-9.

Pruessner, J.C., Kirschbaum, C., Meinlschmid, G., Hellhammer, D.H. 2003. Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. Psychoneuroendocrinology 28(7), 916-31.

Rosnick, C.B., Small, B.J., McEvoy, C.L., Borenstein, A.R., Mortimer, J.A. 2007. Negative life events and cognitive performance in a population of older adults. Journal of aging and health 19(4), 612-29. doi:10.1177/0898264307300975.

Stalder, T., Kirschbaum, C., Kudielka, B.M., Adam, E.K., Pruessner, J.C., Wust, S., Dockray, S., Smyth, N., Evans, P., Hellhammer, D.H., Miller, R., Wetherell, M.A., Lupien, S.J., Clow, A. 2016. Assessment of the cortisol awakening response: Expert consensus guidelines. Psychoneuroendocrinology 63, 414-32. doi:10.1016/j.psyneuen.2015.10.010.

Sundstrom, A., Ronnlund, M., Adolfsson, R., Nilsson, L.G. 2014. Stressful life events are not associated with the development of dementia. International psychogeriatrics / IPA 26(1), 147-54. doi:10.1017/S1041610213001804.

Takahashi, T., Ikeda, K., Ishikawa, M., Tsukasaki, T., Nakama, D., Tanida, S., Kameda, T. 2004. Social stress-induced cortisol elevation acutely impairs social memory in humans. Neuroscience letters 363(2), 125-30. doi:10.1016/j.neulet.2004.03.062.

Tatomir, A., Micu, C., Crivii, C. 2014. The impact of stress and glucocorticoids on memory. Clujul medical 87(1), 3-6. doi:10.15386/cjm.2014.8872.871.at1cm2.

Thomas-Antérion, C., Ribas, C., Honoré-Masson, S., Berne, G., Ruel, J.H., Laurent, B. 2003. Le questionnaire de plainte cognitive (QPC): Un outil de recherche de plainte suspecte d'évoquer une maladie d'Alzheimer? L'année Gérontologique 17(1), 56-65.

van Gelder, B.M., Tijhuis, M., Kalmijn, S., Giampaoli, S., Nissinen, A., Kromhout, D. 2006. Marital status and living situation during a 5-year period are associated with a subsequent 10-year cognitive decline in older men: the FINE Study. The journals of gerontology Series B, Psychological sciences

and social sciences 61(4), P213-9.
Vogel, S., Fernandez, G., Joels, M., Schwabe, L. 2016. Cognitive Adaptation under Stress: A Case for the Mineralocorticoid Receptor. Trends in cognitive sciences. doi:10.1016/j.tics.2015.12.003.
VonDras, D.D., Powless, M.R., Olson, A.K., Wheeler, D., Snudden, A.L. 2005. Differential effects of everyday stress on the episodic memory test performances of young, mid-life, and older adults. Aging & mental health 9(1), 60-70.
Ward, L., Mathias, J.L., Hitchings, S.E. 2007. Relationships between bereavement and cognitive functioning in older adults. Gerontology 53(6), 362-72. doi:10.1159/000104787.
Wingenfeld, K., Wolf, O.T. 2015. Effects of cortisol on cognition in major depressive disorder, posttraumatic stress disorder and borderline personality disorder - 2014 Curt Richter Award Winner. Psychoneuroendocrinology 51, 282-95. doi:10.1016/j.psyneuen.2014.10.009.
Xavier, F.M., Ferraz, M.P., Trentini, C.M., Freitas, N.K., Moriguchi, E.H. 2002. Bereavement-related cognitive impairment in an oldest-old community-dwelling Brazilian sample. Journal of clinical and experimental neuropsychology 24(3), 294-301. doi:10.1076/jcen.24.3.294.983.

Annexes

Table 1: General characteristics of the studied population

	PsyCoLaus population aged at least 65	Studied population	p
n	1214	796	
Age, years (m ±SD)	71.6±4.7	71.5±4.6	0.51
Gender, % women	57.9	58.3	0.80
Education level, % higher education	40.5	38.8	0.27
BMI, Kg/m^2 (m ±SD)	26.9±4.7	26.8±4.6	0.51
CES-D score (m ±SD)	10.3±8.2	10.1±7.9	0.45

m: Mean ; SD: Standard Deviation; BMI : Body Mass Index ; CES-D: Center for Epidemiologic Studies Depression scale

Table 2: Cognitive and cortisol profile of the studied population

CDRSOB (m ±SD)	0.9±0.7
CDR score, n(%)	
0^	379 (47.6)
0.5	417(52.4)
QPC, subjective cognitive decline present, n(%)	53(21.9)
MMSE score (0-30) (m ±SD)	29.2±1.6
DMT (m ±SD)	
Immediate recall (0-16)	15.8±1.1
Total free recall (0-48)	29.8±6.9
Total cued recall (0-48)	18.0±9.2
Identification (0-16)	15.9±0.4
Recognition (0-48)	44.8±9.4
Delayed free recall (0-16)	11.6±2.6
Delayed cued recall (0-16)	4.9±3.7
DO40 picture-naming test (0-40) (m ±SD)	39.7±1.2
Semantic verbal fluency (m ±SD)	29.5±8.1
Phonemic verbal fluency (m ±SD)	20.9±7.8
Stroop test (m ±SD)	
Stroop dots condition (0-24)	23.9±0.6
Stroop words condition (0-24)	23.9±0.4
Stroop interference condition (0-24)	23.2±1.9
CERAD figures (0-11) (m ±SD)	10.5±1.0
Life events	
Number of life events (m ±SD)	7.9±4.8
Mean impact (0-100) (m ±SD)	27.5±14.2
Cortisol AUC, in µmol.h/L (m ±SD)	0.28±0.11
CAR, in nmol/L (m ±SD)	6.7±12.1

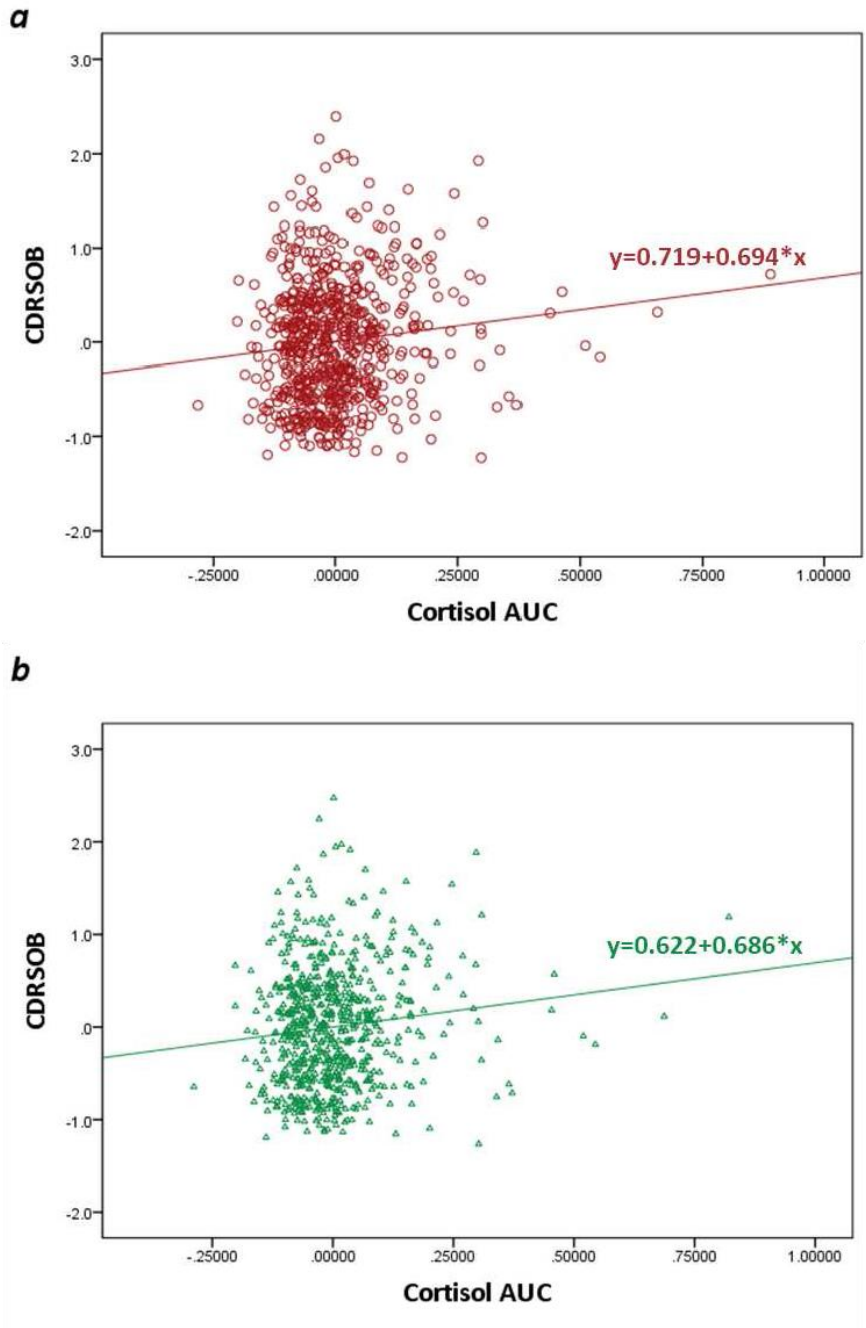
m: Mean ; SD: Standard Deviation; CDR: Clinical Dementia Rating; CDRSOB: Clinical Dementia Rating Sum Of Boxes; QPC: Cognitive Complaint Questionnaire; MMSE: Mini Mental State Examination; DMT: Grober and Buschke Double Memory Test; DO40: CERAD: Consortium to Establish a Registry for Alzheimer's Disease;
AUC : Area Under the Curve; CAR: Cortisol Awakening Response

Table 3: Associations between the CDR Sum Of Boxes and the individual cortisol measures

Cortisol measure	B[95% CI]	r	*p*	*p '*
Waking	0.937[-0.119;1.992]	0.066	0.082	0.164
30 minutes after waking	0.458[-0.931;1.846]	0.025	0.518	0.518
11 am	1.193[0.397;1.989]	0.112	0.003	0.012*
8 pm	0.533[0.036;1.030]	0.080	0.036	0.108

p' corresponds to the p value after adjustment for multiple comparisons using the Holm-Bonferroni's method
** p'<=0.05*

Figure 1: Scatter plot graphs of partial regression analysis results for the association between the CDR Sum Of Boxes and Cortisol AUC before (*a*) and after (b) controlling for life events total impact and for the interaction between life events and cortisol AUC*



** Associations are adjusted for age, sex, education, BMI and depressive symptoms*
AUC: Area Under the Curve; CDR: Clinical Dementia Rating scale