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Short Communication: Post-awakening changes in salivary cortisol in veterans with and without PTSD

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Summary
Cortisol shows a well-documented circadian rhythm with peak levels observed shortly after awakening and steadily decreasing values thereafter. This is a relatively robust diurnal pattern that is displayed by most non-ill individuals, but recently it has been shown that in the general population some 10–15 per cent of individuals have 'flat' circadian cycles of cortisol. We have analyzed post-awakening variations in levels of salivary cortisol in veterans without post-traumatic stress disorder (PTSD), veterans with diagnosed PTSD that were not hospitalized, and hospitalized veterans with PTSD. 'Flat' circadian cycles of salivary cortisol were observed in 6 per cent of veterans without PTSD, 29 per cent of non-hospitalized veterans with PTSD, and 42 per cent of veterans hospitalized for PTSD. Copyright © 2004 John Wiley & Sons, Ltd.

Key Words
PTSD; salivary cortisol; circadian cycle; war-stress

Introduction

Participation in a war is an intensive stressor that has significant short- and long-term consequences on both physical and psychological health. Statistical evidence depicts a very gruesome picture of the effects of the 1991/1992 war on the health of the population in Croatia. Several diseases that are known to be associated with stress, like diabetes, tumors, cardiovascular diseases and gastric ulcers increased sharply during and immediately after the war (Flögel & Lauc, 2000). Most other diseases displayed a slight decrease in 1991/1992 (as a consequence of temporary population decrease at the peak of the war in Croatia), and subsequently returned to the pre-war values. During all this time infant mortality was continuously declining, indicating that there was no significant decrease in the general quality of the health-care system.

War veterans are an especially vulnerable group frequently associated with long-lasting consequences of exposure to traumatic stress. One of the prominent consequences is the post-traumatic

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stress disorder (PTSD) (Vukšić-Mihaljević, Mandić, Benšić, & Mihaljević, 2000).

Although the level of knowledge of stress endocrinology is impressive, key molecular mechanism that could explain how and why corticosteroids and other stress hormones cease to be beneficial, and start to cause damage are still not known. Cortisol is one of the main hormones that coordinate numerous physiological functions both in stress and non-stress conditions. In stress, cortisol is an important factor that mediates suppression of immune system and other adverse consequences of excessive stress. There are significant variation in cortisol levels during the day that follow more or less regular diurnal cycles. Especially large variations in cortisol levels occur in the first 60 minutes after awakening, and they are considered to be a reliable marker for the assessment of adrenocortical activity (Pruessner et al., 1997). Here we report results of our study of post-awakening changes in salivary cortisol in veterans with and without PTSD.

Materials and methods

Saliva samples were collected from 12 veterans with diagnosed PTSD that were hospitalized in the Clinical Hospital Osijek (mean age 38.1 ± 8.3 years), 14 veterans with diagnosed PTSD that were not hospitalized (mean age 34.2 ± 4.6 years) and 16 veterans without diagnosed PTSD (mean age 33.6 ± 1.1 years). All veterans were White males and have been actively involved in the 1991/92 war in Croatia for at least 6 months. All examines were without positive diagnosis and history of any endocrine disorders and they were not addicted to psychoactive substances. Patients were under individual drug therapy. PTSD was diagnosed according to Diagnostic and Statistical Manuals of Mental Disorder (DSM-III Criteria). The initial number of examinees was larger, but a significant number of them were excluded from the study because they did not obey the predetermined schedule of sampling, or did not have sufficient saliva at more than one sampling point.

From each subject, six saliva samples were collected strictly obeying the following time schedule: 0, 15, 30 and 45 minutes after awaking, then at noon and 6 p.m. All samples were taken before meals and in non-smoking conditions. Saliva samples were collected by salivettes (Sartstadt Ltd., Leciester, UK) and saliva was recovered by centrifugation at 3000 × *g* for 10 minutes. Samples were stored at –20°C until analyzed. Salivary cortisol was determined by indirect ELISA method using biotin/streptavidin detection systems (Dressendorfer, Kirschbaum, Rohde, Stahl, & Strasburger, 1992).

Results

We have analyzed post-awakening variations in levels of salivary cortisol in veterans without PTSD, veterans with diagnosed PTSD that were not hospitalized, and hospitalized veterans with PTSD. Saliva samples were collected as described earlier at six pre-determined time points: at the time of awakening, 15, 30 and 45 minutes after awakening, at noon and at 6 p.m. Concentration of cortisol was determined using competition assay with biotin-labeled cortisol as described in the Materials and methods section.

As expected, individual differences in absolute levels were quite high, but the cortisol levels observed in veterans with PTSD were generally lower than in veterans without diagnosed PTSD. The difference was notably larger in earlier than in later sampling times (Figure 1).

Circadian cycles of salivary cortisol of each individual was plotted against sampling time and according to the shape of the curve classified as 'normal', or 'flat'. Examples of each of these curves are shown in Figure 2. The incidence of 'flat' cortisol cycles in the population of veterans without PTSD was 6 per cent, which is approximately the level that is reported to occur in the general population. However, this type of irregular cortisol cycle was found in 29 per cent of veterans with PTSD that were not hospitalized at the time of analysis. In the group of hospitalized veterans with PTSD 'flat' cortisol cycles were found in 42 per cent of examined individuals.

Discussion

Molecular mechanisms underlying the link between the response to stress and the development of disease appear to be exceedingly complex and are only partly understood. Though hormonal changes are key mediators of the physiological changes in stress, other factors appear to be decisive in the development of stress-associated disorders (Lauc & Flögel, 2000). Regulatory mechanisms of the HPA axis are of considerable

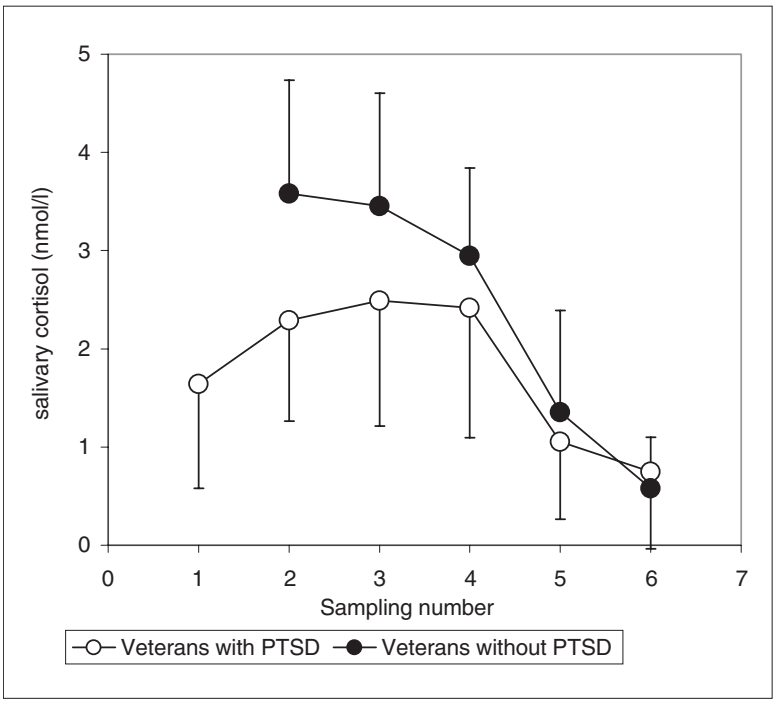

Figure 1. Average circadian cycles of salivary cortisol in veterans with and without diagnosed PTSD. First saliva sample was collected immediately after awakening. The following three 15, 30 and 45 minutes later. Fifth sample was collected at noon, and sixth at 6 p.m. Cortisol was assayed as described in the Materials and methods section.

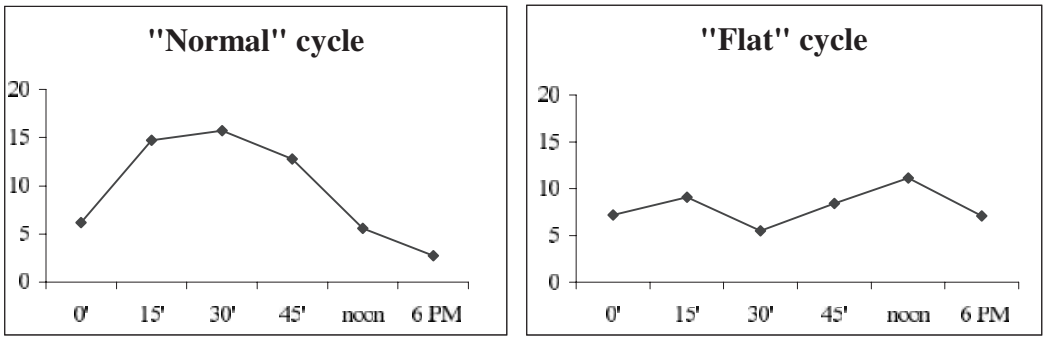

Figure 2. Examples of representative 'normal' and 'flat' circadian cycles of salivary cortisol. First saliva sample was collected immediately after awakening. The following three 15, 30 and 45 minutes later. Fifth sample was collected at noon, and sixth at 6 p.m.

concern because they may be related to HPA axis dysfunction. HPA axis hormones show a well-documented circadian rhythm resulting with peak cortisol levels observed shortly after awakening and steadily decreasing values thereafter in the absence of significant external stimulation. This is a relatively robust diurnal pattern that is being displayed by most non-ill individuals experiencing a typical daily life (Stone et al., 2001). However, it has been reported that some individuals lack this pattern (Smyth et al., 1997). Recent studies estimate that in the general population some 10–15 per cent of individuals have 'flat' circadian cycles of cortisol (Stone et al., 2001).

In this study we found that the incidence of 'flat' circadian cycles of salivary cortisol is increased to 29 per cent in veterans with PTSD, compared to veterans that did not develop PTSD. In veterans with PTSD that were hospitalized at the time of analysis the percentage of 'flat' cortisol cycles was 42 per cent, indicating that the appearance of 'flat' circadian cycles of cortisol might be in correlation with the severity of the disease, but since only a limited number of individuals have been studied, further studies are needed to evaluate this hypothesis.

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