

Brief report

An investigation into the relationship between salivary cortisol, stress, anxiety and depression

Kav Vedhara^{a,b,*}, Jeremy Miles^c, Paul Bennett^d,
Sue Plummer^e, Deborah Tallon^b, Emily Brooks^a,
Lone Gale^a, Katherine Munnoch^a, Christa Schreiber-
Kounine^f, Clare Fowler^f, Stafford Lightman^g,
Alistair Sammon^f, Zenon Rayter^f, John Farndon^f

^a *Department of Experimental Psychology, Human Stress Research Unit, University of Bristol, 8 Woodland Road, Clifton, Bristol BS8 1TN, UK*

^b *MRC Health Services Research Collaboration, Department of Social Medicine, University of Bristol, Canynge Hall, Whiteladies Road, Clifton, Bristol BS8 2PR, UK*

^c *Department of Health Sciences, University of York, York YO10 5DD, UK*

^d *Health Services Research Focus, University of Wales College of Medicine, Cardiff CF14 4XN, UK*

^e *Diagnostech, York Chambers, York Street, Swansea SA1 3NJ, UK*

^f *Department of Surgery, University of Bristol, Bristol Royal Infirmary, Bristol BS2 8HW, UK*

^g *Department of Medicine, University of Bristol, Bristol Royal Infirmary, Bristol BS2 8HW, UK*

Received 22 April 2002; accepted 8 September 2002

Abstract

This study examined the relationship between indices of self-reported emotional distress and absolute versus change in cortisol levels. Fifty-four women attending a diagnostic breast clinic completed scales measuring stress, anxiety and depression and provided five saliva samples over the course of a single day for the measurement of cortisol. No significant relationships were evident between absolute cortisol levels and the distress measures. Analysis of the change in cortisol levels revealed a non-linear interaction effect between stress and anxiety and time of day. There was a non-linear relation between time of day and cortisol levels, but the extent of the non-linearity was dependent upon levels of stress and anxiety, not depression. A relationship was apparent between indices of distress and change in cortisol levels, but not absolute levels of the hormone.

© 2002 Elsevier Science B.V. All rights reserved.

* Corresponding author. Tel.: +44-117-928-7243; fax: +44-117-928-7236.

E-mail address: k.vedhara@bristol.ac.uk (K. Vedhara).

Keywords: Salivary cortisol; Stress; Anxiety; Depression

1. Introduction

The use of plasma and salivary cortisol as an index of hypothalamic–pituitary–adrenal axis activity and, therefore, emotional distress is widespread (Melamed et al., 1999; Buchanan et al., 1999). There are, however, several issues concerning the nature of the relationship between cortisol and emotional distress that are worthy of further enquiry. One of the most keenly debated issues concerns the apparent equivocal nature of the relationship between cortisol and self-reported distress. While there is a substantial literature that has identified increased cortisol levels in populations reporting increased distress (Schulz et al., 1998; Melamed et al., 1999); several investigations have failed to replicate this association (Semple et al., 1988; Kirschbaum et al., 1995a; Marshall et al., 1998).

A number of methodological considerations suggest that this inconsistency may be more apparent than real. These include: the inherent variability in cortisol levels both between and within individuals (Cummins and Gevirtz, 1993; Bohnen et al., 1991); effects of time of day, medication, or food in-take (Smyth et al., 1997; Kirschbaum et al., 1995b) and differences in analytical approach (e.g. area under the curve, single versus multiple measures, delta values etc). These factors increase the likelihood of variance and inaccuracy in the determination of cortisol, potentially contributing to the apparent equivocal relationship between emotional distress and cortisol.

In the presence of such variability it may be necessary to consider alternative techniques to examining the relationship between emotional distress and cortisol. Thus, we compared the more conventional approach using absolute cortisol levels with analyses designed with examine the relationship between emotional distress (anxiety, depression, and stress) and the ‘rate of change in cortisol levels’.

2. Method

2.1. Participants

Women attending a one-stop diagnostic breast clinic over a 6 months period with suspected breast disease were approached regarding this study. Such clinics involve patients receiving all diagnostic tests and their test results in a single appointment. Previous research has shown that attendance at these clinics is associated with increased emotional distress (Harcourt et al., 1998).

Of the 158 women who were recruited and who completed the study, 55 agreed to provide saliva samples for the measurement of cortisol. Eighteen of these women reported current medication use, however, only one participant was on a medication that could affect the cortisol data. This participant’s data were excluded from the analyses. Thus, the final sample consisted of 54 women. The mean age of this sub-sample (cortisol group) was 44 years (rest of sample = 41 years) and all these women

eventually went on to receive a benign diagnosis (two women in the remainder of the sample received a diagnosis of malignant disease). The majority of the cortisol group (41 of 54) did not report any previous medical history (cf. 72 of 153 of the remainder). Of those who did, the most common complaint was asthma ($n = 2$). *T*-tests confirmed that the cortisol group did not differ from the rest of the sample in levels of stress, anxiety and depression (data not shown).

2.2. Procedure

Patients were contacted by letter regarding the study and then telephoned 7–10 days before their clinic appointment to determine whether they wished to participate. Women who agreed were sent a consent form and questionnaires to capture demographic details and pertinent psychosocial variables including indices of emotional distress i.e. stress, anxiety and depression. Participants completed these questionnaires in advance of their appointment and returned them during their scheduled visit to the clinic.

Patients were also asked to provide five saliva samples over the course of a single day for the measurement of cortisol. Patients were instructed to provide the samples on one of the days immediately before their appointment, but not on the appointment day, and between the following times: 07:00–08:00 h; 60 min after the first sample (08:00–09:00 h); 12:00–13:00 h; 16:00–17:00 and 23:00–24:00 h. Participants were asked to avoid meals within 60 min of each sample and to avoid caffeine-containing products during the day. Participants kept samples refrigerated until their appointment.

2.3. Indices of emotional distress

2.3.1. Stress

Global measure of perceived stress scale (Cohen et al., 1983) measures self-reported levels of stress over the past month.

2.3.2. Anxiety and depression

Hospital anxiety and depression scale (Zigmond and Snaith, 1983) measures anxiety and depression

2.4. Salivary cortisol

Saliva samples for the determination of cortisol were collected using salivettes. Cortisol remains stable in saliva for several days and so is suited to investigations in which the participant is required to provide multiple samples away from the study site (Kahn et al., 1988). Levels of cortisol were determined by using a modification of the Cortisol Elisa Kit (Neogen Corporation, KY, USA). A full description of the methods has been reported previously (Vedhara et al., 2000).

2.5. Analytical approach

The distribution of cortisol level at each time point was positively skewed, and so a logarithmic transformation (base 10, no constant added) was conducted to ensure that scores approximated a normal distribution. The relationship between the measures of emotional distress and absolute log cortisol levels was analysed using Pearson's product moment correlation coefficients. A GLM based approach was used to analyse the rate of change in cortisol levels over time, and examine predictors of the rate of change in cortisol levels (Rutherford, 2001). This technique allows us to examine consistent changes over time, using a longitudinal model, while retaining the continuous nature of time i.e. it is not treated as a categorical (or nominal) variable. Criterion scaling was employed, as described by Pedhazur (1982). This involves entering the mean cortisol level over the whole day as a predictor variable. The other predictor variables were:

- Time: recorded as the number of hours from when the first sample was taken, plus 1. Thus, the first measure (07:00–08:00 h) was recorded as time = 1; the second measure (08:00–09:00 h) was recorded as time = 2 etc. This predictor assesses linear change in cortisol levels over time.
- Time²: evaluates the quadratic effect of time and assesses non-linear change in cortisol levels over time. If this predictor is found to significantly improve the fit (R^2) of the model, we can conclude that the change in level of cortisol over the day is not linear—i.e. it is better described as a curve than as a straight line.
- Emotional distress measure (i.e. stress, anxiety or depression) \times time interaction effect: assesses whether the linear change in cortisol level over time varies according to levels of the distress measure. If this predictor is found to significantly improve the fit (R^2) of the model, we can conclude that the linear change in the level of salivary cortisol over the day is affected by the selected distress measure. The standardised form of the distress predictor was used to create multiplicative interaction effects (Aiken and West, 1996; Miles and Shevlin, 2001).
- Psychological measure \times time² interaction: measures whether the non-linear effect of change in cortisol levels over time varies according to levels of the distress measure. If this predictor is found to significantly improve the fit of the model, this is interpreted as meaning that the degree of curvature of the curve that describes the level of cortisol over the day is dependent, in part, upon levels of the selected distress variable.

The predictors were entered hierarchically in the following order: Mean cortisol score over the day, plus emotional distress variable (i.e. anxiety, depression or stress) (model 1); Time (model 2); time² (model 3); distress variable \times time interaction (model 4); distress variable \times time² interaction (5). The models were tested hierarchically i.e. model 1 was estimated and the R^2 determined. Model 2 was then estimated, and the improvement in R^2 was tested for statistical significance, continuing to Model 5.

3. Results and discussion

3.1. Description of cohort

Consistent with previous research with this patient group (Harcourt et al., 1998) mean scores revealed the presence of modest levels of stress (24.32 ± 9.7) and anxiety (8.46 ± 4.28) and lower levels of depression (4.28 ± 3.65). Mean cortisol levels revealed the expected diurnal pattern of cortisol production (07:00–08:00 h = 16.16 ± 9.69 ; 08:00–09:00 h = 12.35 ± 7.10 ; 12:00–13:00 h = 6.24 ± 3.87 ; 16:00–17:00 h = 4.78 ± 2.74 ; 23:00–24:00 h = 2.61 ± 3.24 (all nM/l)).

3.2. Relationship between absolute cortisol levels and emotional distress

Correlations were computed to examine the relationship between absolute cortisol levels obtained at each sampling point and stress, anxiety and depression. After controlling for the number of correlations conducted using Bonferroni corrections, all correlations emerged as non-significant (data not shown).

3.3. Relationship between rate of change in cortisol levels and emotional distress

The analyses shown in Table 1 reveal that the linear effect of time is highly predictive of cortisol levels (showing a decrease in levels over the day). This is evident from the change in R^2 from model 1 to 2 in all three regressions. The quadratic effect of time, although smaller, is also significant. This indicates that the change in cortisol levels is best described with a non-linear effect. When the simple interaction effects

Table 1
Model statistics for anxiety, depression and stress

Model	R^2	Change statistics			
		R^2	F	df	P
1 (Criterion plus anxiety)	0.213	0.213	35.4	2, 262	< 0.001
2 (Time)	0.772	0.559	639.2	1, 261	< 0.001
3 (Time ²)	0.778	0.006	7.6	1, 260	0.006
4 (Anxiety \times time)	0.780	0.002	1.8	1, 259	0.178
5 (Anxiety \times time ²)	0.785	0.005	6.3	1, 258	0.013
1 (Criterion plus depression)	0.211	0.211	34.4	2, 262	0.000
2 (Time)	0.770	0.559	622.3	1, 261	0.000
3 (Time ²)	0.778	0.008	8.9	1, 260	0.003
4 (Depression \times time)	0.778	0.000	0.0	1, 259	0.862
5 (Depression \times time ²)	0.782	0.004	5.1	1, 258	0.025
1 (Criterion plus stress)	0.213	0.213	35.4	2, 257	< 0.001
2 (Time)	0.772	0.559	638.5	1, 256	< 0.001
3 (Time ²)	0.778	0.006	7.6	1, 255	0.006
4 (Stress \times time)	0.778	0.000	0.1	1, 254	0.736
5 (Stress \times time ²)	0.781	0.003	3.6	1, 253	0.058

are examined (model 4 in the regressions), which test whether the rate of change of cortisol level is dependent upon the selected distress measure (i.e. stress, anxiety or depression), the effects are not significant. When the non-linear interaction effects are tested (model 5 in the regressions) we find a significant, but small, effect of stress and anxiety, and a probability value approaching significance for depression.

To interpret these findings we present the lines of best fit for stress and anxiety where the non-linear time \times distress variable was significant (Figs. 1 and 2). The two lines in these figures represent the predicted curve for a person with high (1.5 S.D.'s above the mean) and low (1.5 S.D.'s below the mean) levels of stress or anxiety. The graphs show that rate of linear drop in cortisol level is unaffected by levels of stress or anxiety, but the non-linear rate of drop (i.e. the degree of curvature of the line) is affected by these distress indices.

These findings are consistent with other recent work (Sephton et al., 2000; Turner-Cobb et al., 2000) which found that mortality in women with breast cancer was predicted by the extent to which patients' diurnal cortisol profile deviated from the 'normal' pattern of cortisol release. This association was not upheld when absolute cortisol levels (as expressed by area under the curve) were examined. These, and the present findings, suggest it may be appropriate to consider how emotional distress influences the pattern of change in cortisol levels, rather than just absolute levels. However, it should be noted that we cannot determine whether these results indicate that the relationship between cortisol and indices of distress is more readily apparent when patterns of change in cortisol are considered, or whether emotional distress actually affects the pattern of change in cortisol levels.

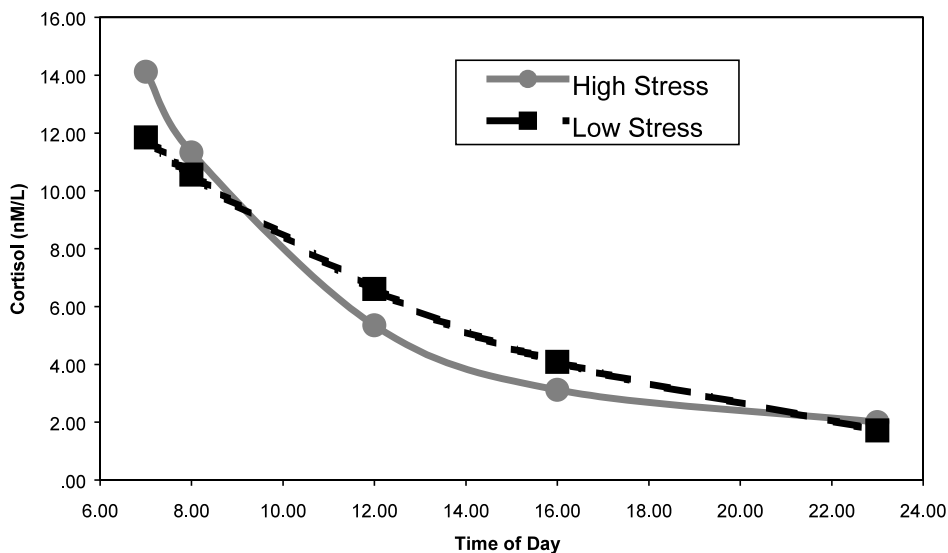


Fig. 1. Lines of best fit for high stress (1.5 S.D.'s above the mean; solid line) and low stress (1.5 S.D.'s below the mean; dashed line), with mean cortisol level.

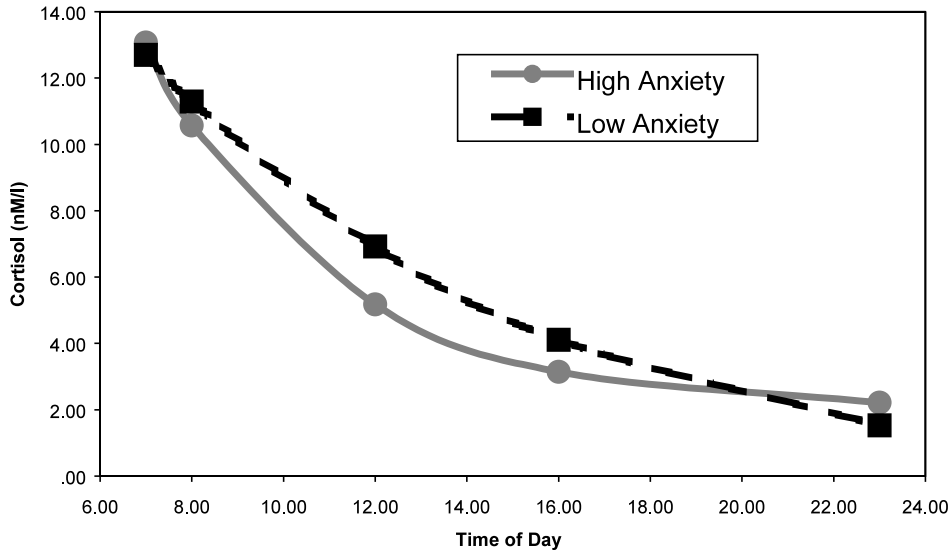


Fig. 2. Lines of best fit for high anxiety (1.5 S.D.'s above the mean; solid line) and low anxiety (1.5 S.D.'s below the mean; dashed line), with mean cortisol level.

In conclusion, the present results advocate the measurement of patterns of change in cortisol levels when examining the relationship between self-reported emotional distress and cortisol; and suggest that differing indices of distress exhibit differing relationships with cortisol. This interpretation is, however, limited by: the absence of a control group, no control for confounders such as sleep quality and caffeine withdrawal, and no assessment of how imprecision and variability in the measurement of emotional distress may affect the relationship between cortisol and distress.

Acknowledgements

Bristol is the lead centre of the MRC Health Service Research Collaboration.

References

- Aiken, L., West, S.G., 1996. *Multiple Regression: Testing and Interpreting Interactions*. Sage, London.
- Bohnen, N., Nicolson, N., Sulon, J., Jolles, J., 1991. Coping style, trait anxiety and cortisol reactivity during mental stress. *Journal of Psychosomatic Research* 35, 141–147.
- Buchanan, T.W., Al'Absi, M., Lovallo, W.R., 1999. Cortisol fluctuates with increases and decreases in negative affect. *Psychoneuroendocrinology* 24, 227–241.
- Cohen, S., Kamarack, T., Mermelstein, R., 1983. A global measure of perceived stress. *Journal of Health and Social Behavior* 24, 385–396.
- Cummins, S.E., Gevirtz, R.N., 1993. The relationship between daily stress and urinary cortisol in a normal population: an emphasis on individual differences. *Behavioral Medicine* 19, 129–134.

- Harcourt, D., Ambler, N., Rumsey, N., Cawthorn, S.J., 1998. Evaluation of a one-stop breast lump clinic: a randomised controlled trial. *Breast* 7, 314–319.
- Kahn, J.-P., Rubinow, D.R., Davis, C.L., Kling, M., Post, R.M., 1988. Salivary cortisol: a practical method for evaluation of adrenal function. *Biological Psychiatry* 23, 335–349.
- Kirschbaum, C., Klauer, T., Filipp, S.-H., Hellhammer, D.H., 1995a. Sex-specific effects of social support on cortisol and subjective responses to acute psychological stress. *Psychosomatic Medicine* 57, 23–31.
- Kirschbaum, C., Pirke, K.-M., Hellhammer, D.H., 1995b. Preliminary evidence for reduced cortisol responsiveness to psychological stress in women using oral contraceptive medication. *Psychoneuroendocrinology* 20, 509–514.
- Marshall, G.D., Jr, Agarwal, S.K., Lloyd, C., Cohen, L., Henninger, E.M., Morris, G.J., 1998. Cytokine dysregulation associated with exam stress in healthy medical students. *Brain, Behavior and Immunity* 12, 297–307.
- Melamed, S., Ugarten, U., Shirom, A., Kahana, L., Lerman, Y., Froom, P., 1999. Chronic burnout, somatic arousal and elevated salivary cortisol levels. *Journal of Psychosomatic Research* 46, 591–598.
- Miles, J.N.V., Shevlin, M.E., 2001. *Applying Regression and Correlation*. Sage, London.
- Pedhazur, E.J., 1982. *Multiple Regression in Behavioral Research: Explanation and Prediction*. Harcourt Brace Jovanovich, New York.
- Rutherford, A., 2001. *Introducing ANOVA and ANCOVA: A GLM Approach*. Sage, London.
- Schulz, P., Kirschbaum, C., Prussner, J., Hellhammer, D., 1998. Increased free cortisol secretion after awakening in chronically stressed individuals due to work overload. *Stress Medicine* 14, 91–97.
- Semple, C.G., Gray, C.E., Borland, W., Espie, C.A., Beastall, G.H., 1988. Endocrine effects of examination stress. *Clinical Science* 74, 255–259.
- Sephton, S.E., Sapolsky, R.M., Kraemer, H.C., Spiegel, D., 2000. Diurnal cortisol rhythm as a predictor of breast cancer survival. *Journal of the National Cancer Institute* 92, 994–1000.
- Smyth, J.M., Ockenfels, M.C., Gorin, A.A., Catley, D., Porter, L.S., Kirschbaum, C., Hellhammer, D.H., Stone, A.A., 1997. Individual differences in the diurnal cycle of cortisol. *Psychoneuroendocrinology* 22, 89–105.
- Turner-Cobb, J.M., Sephton, S.E., Koopman, C., Blake-Mortimer, J., Spiegel, D., 2000. Social support and salivary cortisol in women with metastatic breast cancer. *Psychosomatic Medicine* 62, 337–345.
- Vedhara, K., Hyde, J., Gilchrist, I.D., Tytherleigh, M.Y., Plummer, S., 2000. Acute stress, memory, attention and cortisol. *Psychoneuroendocrinology* 25, 535–549.
- Zigmond, A.S., Snaith, R.P., 1983. The hospital anxiety and depression scale. *Acta Psychiatrica Scandinavica* 67, 361–370.