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Cortisol responses to mild psychological stress are inversely associated with proinflammatory cytokines

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Abstract

Glucocorticoids can down-regulate immune activity, but acute stress has been reported to increase both cortisol and levels of plasma cytokines. We investigated individual differences in cortisol responses and their associations with proinflammatory cytokines, such as interleukin-6 (IL-6), interleukin-1 receptor antagonist (IL-1ra), cardiovascular activity, and mental health. Saliva samples and blood were taken from 199 healthy middle-aged participants of the Whitehall II cohort at baseline, immediately after stress and 45 min later. We defined the 40% of participants with the highest cortisol response to stress as the cortisol responder group and 40% with the lowest response as the cortisol non-responder group. Plasma IL-6 was higher and the IL-1ra response to stress was greater in the cortisol non-responder group. The cortisol non-responders showed lower heart rate variability than the cortisol responders. The cortisol responder group experienced more subjective stress during the tasks and reported more impaired mental health than the non-responders. We conclude that individual variations in neuroendocrine stress responsivity may have an impact on proinflammatory cytokines, and that both high and low cortisol stress responsiveness has potentially adverse effects.

Keywords: Salivary cortisol; Cytokines; Interleukin-6; Interleukin-1 receptor antagonist; Heart rate variability; Mental stress; Mental health

1. Introduction

The physiological stress response consists of an activation of the sympathetic nervous system (SNS), a parasympathetic withdrawal, and increased activity of the hypothalamic–pituitary–adrenal (HPA) axis, with wide individual differences and consequences. As these systems interact with the immune systems via different pathways (Felten and Felten, 1994), stress also influences immune functions (Abraham, 1991; Maier and Watkins, 1998). Impaired immune competence has been shown in individuals experiencing chronic stress and may lead to increased risk of infectious disease (Herbert and Cohen, 1993; Cohen et al., 2002). In contrast to chronic stress, acute stress not only activates the SNS and HPA axis, but also parts of the immune system (Abraham, 1991; Maier and Watkins, 1998). Recent findings suggest that proinflammatory cytokine production increases in response to acute psychological stress in humans (Maes et al., 1998; Steptoe et al., 2001). The first part of the acute stress response characterized by an activation of the SNS is accompanied by an increase in immune cell numbers, such as B-cells (Landmann et al., 1984) and natural killer cells and their

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activity (Benschop et al., 1996), but is also marked by an increase in IL-1ra and IL-6 that may take several minutes to evolve (Shephard, 2002). Glucocorticoids modulate cytokine production and receptor expression (Wiegers and Reul, 1998). It is thought that glucocorticoids play a beneficial role during acute stress, but that during chronic stress the effects of glucocorticoids on immune functions are detrimental (Van den Berghe et al., 1998).

There is an important feedback loop between cytokines and glucocorticoids: proinflammatory cytokines, such as interleukin-1 (IL-1) and interleukin-6 (IL-6) are potent activators of the HPA axis (Turnbull and Rivier, 1999). Glucocorticoids in turn negatively control cytokine production and by this mechanism are able to shut down inflammatory processes to prevent host destruction due to prolonged immune activity (Besedovsky and del Rey, 2000; Sapolsky et al., 2000). IL-6 is secreted from a number of different cells, such as activated macrophages, lymphocytes, adipocytes, and skeletal muscle (Febbraio and Pedersen, 2002; Mohamed-Ali et al., 2001). It is negatively controlled by glucocorticoids and positively controlled by catecholamines (Baker et al., 2001), and in turn regulates immune responses, acute phase protein synthesis, and hematopoiesis (Turnbull and Rivier, 1999). Interleukin-1 receptor antagonist (IL-1ra) plays an important role in regulating the physiological responses to endogenous IL-1 as it binds the IL-1 receptor, but lacks intrinsic biological activity. IL-1ra is secreted by similar cell types as those which produce IL-1, and in response to similar stimuli (Turnbull and Rivier, 1999). It can therefore also be looked at as a marker for IL-1 activity. IL-1ra is positively controlled by IL-6 and there are findings that IL-1ra is also an acute phase protein (Gabay et al., 1997). IL-1ra, seems to be sensitive to chronic and acute stress and has been found to be raised in individuals with high anxiety levels (Song et al., 1999) and high perceived stress (Maes et al., 1998).

Studies in patients with clinical depression have shown elevated levels of IL-1ra and Il-6 (Maes et al., 1997; Musselman et al., 2001; Seidel et al., 1995). Depression is often associated with higher levels of cortisol (Holsboer, 2000), which seems to contradict the findings of higher cytokine levels in depressed patients. However, within the clinical picture of depression, different subtypes with specific cortisol secretion patterns have been described (Anisman et al., 1999; Gold and Chrousos, 2002). Lower feedback sensitivity of the HPA axis has been shown in major depression (Holsboer, 2000), and this may explain the dysfunction of inflammatory mediators in depression (Licinio and Wong, 1999). It is also possible that individual differences in disturbances of cortisol regulation influence the extent to which elevated levels of inflammatory cytokines are observed.

The secretion of cortisol after stress has been shown to be a reliable marker of psychological stress (Biondi and Picardi, 1999). However, individuals differ in the level of response (Kirschbaum et al., 1995) and in the rate of post-stress recovery (Roy et al., 2001). Gender differences (Kirschbaum et al., 1992), effects of age (Ershler and Keller, 2000; Lutgendorf et al., 1999), personality factors (Pruessner et al., 1997; Van Eck et al., 1996), and mood (Smyth et al., 1998) influence cortisol responses. Persistent cortisol responses without adaptation are thought to be related to susceptibility to infectious disease (Mason, 1991), particularly in individuals with high glucocorticoid sensitivity (Rohleder et al., 2001). On the other hand, blunted cortisol responses to stress could be related to risk of the development of autoimmune disorders (Sternberg, 2001), especially if combined with low glucocorticoid sensitivity (Rohleder et al., 2001). These findings suggest that both high and low cortisol responsivity might be associated with potentially adverse effects.

The aim of the present study was to assess individual differences in salivary cortisol responses to acute mild psychological stress in healthy individuals, and to investigate associations between different types of responses and cytokine levels. We hypothesized that cortisol responses would be inversely associated with proinflammatory cytokines. We also assessed whether cortisol responder types would differ in blood pressure and heart rate stress responses. In addition, we hypothesized that poorer mental health would be related to a stronger cortisol response to stress.

2. Materials and methods

2.1. Participants

Data were collected from 109 women and 123 men, part of a sample of 240 drawn from participants in the Whitehall II cohort. The Whitehall II study was set up in 1985 to investigate demographic, psychosocial and biological risk factors for cardiovascular diseases in 10,308 London-based civil servants (Marmot et al., 1991). Participants for this substudy were all white Caucasian and recruited on the following criteria: aged 45–59 years, still based in the London area and not planning to retire for at least three years. They were drawn systematically from higher, intermediate, and lower occupational grades, so as to investigate psychobiological factors related to socioeconomic status (Steptoe et al., 2002a). Since cortisol responsivity did not vary with grade of employment, this factor is not included in the present analysis (Steptoe et al., 2002b). The response rate was 55%. The following exclusion criteria were applied: history of coronary heart disease and/or previous diagnosis or treatment for hypertension. As we were investigating levels of cortisol and cytokines, we also excluded participants with inflammatory diseases or

allergies. In addition, volunteers were not allowed to use any anti-histamine or anti-inflammatory medication for 24 h before testing. Participants who had colds or other infections on the day of testing were rescheduled. All participants gave written informed consent before the testing. Adequate data for assessing cortisol stress responses were available from 199 individuals, since the remainder had insufficient saliva for analysis of some samples, or had values outside the acceptable range. The study was approved by the UCL/UCLH Committee on the Ethics of Human Research.

2.2. Behavioural tasks

Mental stress was induced by two behavioural tasks. The first was a computerized version of the Stroop colour-word interference task, involving the successive presentation of target colour words (e.g., green, yellow) printed in another colour (Muldoon et al., 1995). At the bottom of the computer screen were four names of colours printed in incongruous colours. The task consisted in pressing the computer key that corresponded to the position at the bottom of the screen of the name of the colour in which the target word was printed. The rate of presentation of stimuli was adjusted to the performance of the participant, to ensure sustained demands.

The second task was mirror tracing, involving the tracing of a star with a metal stylus, which could only be seen in a mirror image (Lafayette Instruments, Lafayette, IN, USA). A loud beep was emitted by the apparatus each time the stylus came off the star to signal a mistake and the number of mistakes was recorded in front of the participants. Both tests were introduced with a short and standardized explanation also to induce feelings of time pressure and of competition.

2.3. Psychosocial and subjective measures

Psychological well-being was assessed with three measures. First, the 30-item General Health Questionnaire (GHQ) was administered (Goldberg and Williams, 1988). This measure has been widely used to assess psychological well-being in the adult population. Conventional scoring of the questionnaire was employed, and a threshold of 5/6 used to define psychological distress. The Cronbach α in this population was 0.93. Second, the mental health subscale from the SF36 was administered (Ware, 1993). This consists of five items, each of which is rated on a 6-point scale, and scores are scaled so that 100 represents the maximum possible mental health. The Cronbach α of the scale was 0.81. Third, hopelessness, defined as negative expectancies about oneself and the future, was measured by the 2-item Hopelessness Scale that has been shown in Finnish studies to predict hypertension (Everson et al., 2000) and the progression of carotid atherosclerosis (Everson et al., 1997).

Participants rated their physical activity ('How many times a week do you engage in vigorous physical activity enough to make you out of breath?') on a 7-point scale (none to seven times a week). Drinking habits were assessed in terms of the number of units of alcohol consumed over the past week. Smoking and the use of hormone replacement therapy was assessed by questionnaires. Subjective responses to the tasks were assessed ratings on a scale from 1 (low) to 7 (high) stress.

2.4. Anthropometry

Height was measured using the Frankfort plane to standardize the measurement. Body weight was measured to the nearest 0.1 kg. Body Mass Index (BMI) was calculated as weight in kilograms divided by height in meters squared. The waist circumference was measured midway between the lowest rib and iliac crest, and the hip circumference was measured at the level of the great trochanters.

2.5. Haemodynamic measures

Blood pressure and heart rate were monitored continuously from the finger using a Portapres-2, a portable version of the Finapres device that shows good reproducibility and accuracy in a range of settings. Details of this measure are described elsewhere (Steptoe et al., 2002b). To assess heart rate variability, we used the impedance-based Ambulatory Monitoring System (VU-AMS, Free University, Amsterdam, NL) with electrocardiographic signals checked on a beat-to-beat basis (Willemsen et al., 1996). Ensemble averages based on 60-s epochs that were scored and edited off line using an interactive software program. As an index of heart rate variability, square root of the mean of the sum of the squared successive differences (RMSSD) was calculated with the following formula: $\mathrm{RMSSD}\ (\mathrm{ms}) = \sqrt{(1/n \sum(\mathrm{IBI}_i - \mathrm{IBI}_{i-1}))}$, where IBI = inter-beat-interval, I = current IBI, n = number of IBI in the epoch.

2.6. Procedure

The experimental procedure has been detailed previously (Steptoe et al., 2002a). Briefly, the experiments were carried out in the morning (10 a.m.) or the afternoon (2 p.m.) in a light and temperature controlled laboratory. Participants were instructed not to have drunk any caffeinated beverages, or to have smoked for at least two hours prior to the testing, and not to have consumed alcohol or exercised on the evening before or the day of testing. After anthropometric measurements and instrumentation, an initial saliva sample was obtained (initial sample). A venous cannula was then

inserted for the periodic collection of blood samples, the participant rested for 30 min during which blood pressure, heart rate and heart rate variability were monitored continuously. A second saliva sample (post-adaptation baseline sample) was obtained, a subjective stress rating taken, and the baseline blood sample was drawn for the analysis of cytokines and catecholamines.

The two tasks were then administered in random order. Each lasted for 5 min during which blood pressure and heart rate were recorded continuously. Immediately after each task, the participant filled in the five rating scales. The whole stress period lasted for 12 min including the rating scales. After the two tasks, the third saliva (stress) sample was taken and the second blood sample (post-stress) for the analysis of insulin, glucose, and catecholamines. Following the tasks, the participant rested for 45 min, with blood pressure and heart rate monitoring continuing throughout. Saliva samples were taken after 20 and 45 min (post-stress 1 and 2), and a post-stress subjective rating after 45 min. A third blood sample was also taken after 45 min. The results of studies of mental and physical stress indicate that plasma cytokine responses may not emerge immediately, but evolve over 30–60 min (Altemus et al., 2001; Febbraio and Pedersen, 2002; Steptoe et al., 2001). We therefore decided to analyze cytokines from blood drawn 45 min post-stress. Haematocrit was also analysed from all three blood samples, since changes in haemoconcentration may account for some increases in the concentration of plasma proteins (Patterson et al., 1998).

2.7. Cortisol assays

Saliva samples were collected using Salivettes (Sarsted, Leicester, UK), which were stored at −30 °C until analysis. Salivary cortisol was analyzed with a time-resolved immunoassay with fluorescence detection as described in detail elsewhere (Dressendorfer et al., 1992). Intra- and interassay variability of the assay was less than 10 and 12%, respectively.

2.8. Cytokine assays

Peripheral blood was collected in EDTA coated tubes and spun at +4 °C. All blood samples were frozen at −20 °C until assay. Unstimulated levels of IL-6 and IL-1ra in plasma were determined: IL-6 levels were analyzed using the high-sensitivity two-site ELISA from R&D Systems (Oxford, UK). The limit of detection was 0.09 pg/ml, and the intra- and interassay variability was 5.3 and 9.2%, respectively. IL-1ra levels were measured using a commercial ELISA (R&D Systems) with a limit of detection of 15 pg/ml, and intra- and interassay coefficients of variation of less than 10%. Haematocrit was assessed using a micro-haematocrit centrifuge (Hawksley Gelman, Lancing, W.Sussex, UK).

2.9. Statistical analysis

Blood pressure and the impedance electrocardiogram were measured continuously throughout the whole session, but only shorter periods were analyzed. The analysis of BP and heart rate involved five 5-min trial periods: baseline, task1, task2, post-stress 1 (minutes 15–20), and post-stress 2 (minutes 40–45). Heart rate variability was analysed from the baseline, the two 5-min task periods, and two 10-min post-stress periods: minutes 10–20 (post-stress 1) and minutes 30–40 (post-stress 2). The two task periods were averaged for analysis. There were five samples for salivary cortisol (initial sample, baseline, stress, post-stress 1, and post-stress 2). An area under the curve (AUC) measure with reference to zero was calculated which had been used in other studies (Wust et al., 2000). This AUC includes the area starting from the baseline measurement to the immediate post-task (= stress) sample (S): $\mathrm{AUC} = (S1 * 15 + (S2 - S1) * 15/2) + (S2 * 20 + (S3 - S2) * 20/2)$ with S1 = baseline sample, S2 = stress sample, and S3 = post-task 1. The cytokines IL-6 and IL1-ra were analysed from the baseline and 45 min post-stress blood samples.

Cortisol stress reactivity was defined as the change between the baseline level and the higher of the stress and post-stress 1 samples, since some individuals (38.2%) showed a delayed response. The distribution of cortisol responses was symmetrical, averaging + 0.4 nmol/l (standard deviation 3.62), and with a range from −14.3 to +12.8 nmol/l. To test the hypotheses concerning individual differences in cortisol response, we defined two groups by selecting the upper 40% and lower 40% responders. This 40% division was chosen for two reasons: a binary split would include a number of individuals on the borderline between the two categories who are not highly distinct in cortisol response profile, but would nevertheless add error variance to the two groups. A more extreme division would reduce the sample size and therefore the power of the study. There were 80 individuals in the cortisol responder and 80 in the cortisol non-responder groups.

Comparisons between groups in demographic, anthropometric, behavioural, and psychological variables were assessed using analysis of variance with cortisol responder group and gender as between-subject factors, or with χ^2 tests and logistic regression for categorical variables. Physiological data were analysed with repeated measures analysis of variance using the General Linear Model (GLM), with Greenhouse–Geisser adjustments employed where appropriate, and adjusted degrees of freedom are presented. In these analyses, cortisol responder group and gender were the between–subject factors, and trial (initial, baseline, task, post-stress 1, post-stress 2 for cortisol, and baseline, task, post-stress 1, post-stress 2 for blood pressure and heart rate) was the within-subject factor. Haematocrit was

included as a covariate in all cytokine analyses to guard against changes being secondary to alterations in haemoconcentration (Patterson et al., 1998). Recent findings suggest that cytokine responses may vary with blood drawing techniques (Haack et al., 2000), although the evidence for this is inconclusive (Altemus et al., 2001; Mohamed-Ali et al., 2001). The venous cannula did not remain patent throughout the protocol for some individuals, so a proportion of blood samples was taken by separate venipunctures. The method of blood sampling was therefore included as a covariate in IL-6 analyses. Other covariates were introduced as appropriate, as detailed in Steptoe et al. (2002a). All data analysis was carried out using SPSS 10.0 software and results are presented as means ± standard deviation.

3. Results

3.1. Definition of cortisol responder groups

There was no difference between morning and afternoon testing in cortisol responses, $F(1/197) = 0.43$, $p = .51$, and the proportion of participants in cortisol responder and non-responder groups was the same for people tested in the morning and afternoon. Analyses were therefore carried out without differentiating between times of testing.

The cortisol stress response in the cortisol responder group averaged 3.45 ± 2.7 nmol/l, compared with a mean decrease of 2.55 ± 2.7 nmol/l in the non-responder group. The profile of cortisol responses over the session in the two groups is summarized in Fig. 1. There was a significant effect of trial, $F(2/295) = 33.6$, $p < .001$, and a significant cortisol responder group by trial interaction effect, $F(2/295) = 11.4$, $p < .001$. There was in addition a main effect of gender, $F(1/131) = 18.8$, $p < .001$, since men had higher cortisol overall, but no interaction between gender and cortisol responder group, or gender and trial. It can be seen in Fig. 1 that the two groups did not differ in initial cortisol level, but that the cortisol responder group showed greater adaptation to the situation than non-responders, with a larger decline in cortisol between initial and baseline samples, $F(1, 141) = 7.08$, $p = .01$. Subsequently, the responses to tasks differed, with an increase in the cortisol responder group, and continued decline in the non-responder groups. There was no significant correlation between the blood sampling method and the cortisol AUC.

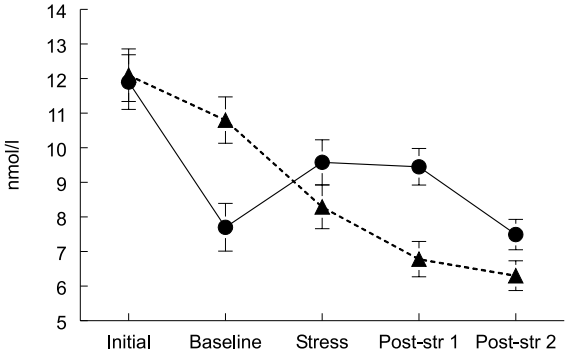

Fig. 1. Mean levels of salivary cortisol in cortisol responder (solid line) and non-responder (dotted line) groups. Error bars are standard errors of mean.

3.2. Demographic, anthropometric, psychological, and behavioural results

Demographic, anthropometric, and psychological characteristics of the participants in this study are presented in Table 1. The two cortisol responder groups did not differ in the proportion of men and women, age, or proportion of women taking hormone replacement therapy. Concerning anthropometric measures, there were no cortisol group differences in BMI or waist/hip ratio for the female participants. Among men, we found a significant group difference for waist/hip ratio, since cortisol non-responders had greater central obesity than cortisol responders, $F(1/97) = 4.53$, $p = .036$. There were differences as predicted in all three measures of psychological well-being. The proportion of individuals with GHQ scores exceeding threshold for psychological distress was greater in cortisol responder than non-responder groups ($\chi^2 = 50$, $p = .040$). In logistic regression, the odds ratio for having a GHQ score above threshold in the cortisol responder compared with non-responder groups was 2.25 (95% confidence intervals 1.05–4.82), after adjustment for gender and age. Ratings on the SF36 mental health scale were significantly higher in cortisol non-responders than responders, $F(1/155) = 4.69$, $p = .032$, indicating poorer mental health in the latter. In addition, the cortisol responders scored significantly higher on the hopelessness scale, $F(1/153) = 5.99$, $p = .016$. There were no interactions between cortisol responder group and gender in these analyses.

The cortisol responder groups did not differ in physical activity or alcohol intake. The proportion of current smokers was different in the two groups with more smokers among the cortisol nonresponders ($\chi^2 = 4.52$, $p = .033$). As effects of smoking on HPA-responses have been reported (Kirschbaum et al., 1994), comparisons were carried out between smokers and non-smokers, but no differences were found in this study in cortisol stress responses, $F(1, 195) = 2.14$, $p = .15$, or in cortisol levels throughout the session. This apparent discrepancy is likely due to the fact that the number of smokers was small, totaling 16.

The cortisol responder groups did not differ in subjective stress ratings at baseline. However, the increase in

Table 1
Characteristics of the two cortisol response groups

	Cortisol responders	Cortisol non-responders	p
Men/women	49/31	49/31	ns
Age	52.4 ± 2.9	52.2 ± 2.6	ns
Hormone replacement therapy (%)	32.3%	25.8%	ns
Current smokers (%)	5.1%	15%	.033*
No physical activity (%)	39.7%	43.4%	ns
Alcohol intake (units/week)	8.04 ± 9.3	10.4 ± 10.4	ns
GHQ psychological distress (%)	30.4%	16.3%	.040*
SF36 (mental health)	71.8 ± 16	76.8 ± 13.2	.032*
Hopelessness	2.40 ± 1.1	2.02 ± 0.8	.016*
Women			
BMI (kg/m^2)	24.8 ± 4.0	25.6 ± 4.5	ns
WHR	0.79 ± .14	0.81 ± .11	ns
Men			
BMI (kg/m^2)	24.9 ± 3.1	25.8 ± 3.0	ns
WHR	0.89 ± .07	0.92 ± .06	.036*

Values are means ± standard deviation.
* $p < .05$.

subjective stress was greater in the cortisol responders than non-responders, $F(1/154) = 3.87$, $p = .05$. Subsequently, stress ratings fell to low levels during the post-stress period.

3.3. Haemodynamic responses

The analyses of systolic and diastolic pressure revealed significant effects of trial, $F(2/291) = 190.6$ and $F(2/370) = 197.1$ $p < .001$, respectively, since there were large increases in blood pressure during tasks (Table 2), followed by return towards baseline during the post-stress period, as described previously (Steptoe et al., 2002b). The cortisol responder groups did not differ in blood pressure responses.

A similar pattern was observed for heart rate, with increases in response to tasks that did not differ between cortisol responder groups. However, in the analysis of heart rate variability, the cortisol responder group by trial interaction was significant, $F(2/247) = 3.82$, $p = .016$. These results are illustrated in Fig. 2. The groups did not differ at baseline, $F(1/102) = 2.47$, $p = .12$, although there was a tendency for heart rate variability to be greater in the cortisol responders. However, the cortisol responder group showed a larger stress-induced inhibition of heart rate variability than the non-responders, $F(1/102) = 5.54$, $p = .021$. Subsequently, the post-stress increase in heart rate variability was greater in the cortisol responder group, $F(1/101) = 6.52$, $p = .012$. There were no interactions involving gender in any of these analyses.

3.4. Cytokine responses

The number of participants with satisfactory IL-6 data was 69 in the cortisol responder and 74 in the

Table 2
Haemodynamic, neuroendocrine, and subjective responses to mental stress in the two cortisol responder groups

Parameter		Baseline	Tasks	Post-stress 1	Post-stress 2
Heart rate (bpm)	Cortisol responders	63.3 ± 8.7	70.3 ± 10.2	61.8 ± 8.1	62.1 ± 7.9
	Cortisol non-responders	64.9 ± 10.3	71.3 ± 10.8	62.3 ± 9.7	62.6 ± 9.5
Systolic BP (mmHg)	Cortisol responders	114.7 ± 12.7	138.2 ± 19.7	120.6 ± 15.3	121.6 ± 15.7
	Cortisol non-responders	114.9 ± 12.2	135.3 ± 19.1	119.6 ± 12.1	119.6 ± 14.4
Diastolic BP (mmHg)	Cortisol responders	70.4 ± 10.1	83.9 ± 11.4	75.0 ± 10.3	75.0 ± 10.7
	Cortisol non-responders	69.5 ± 9.1	82.5 ± 11.1	74.0 ± 9.7	75.6 ± 10.3
Hematocrit (%)	Cortisol responders	39.7 ± 3.0	40.5 ± 3.1	—	40.2 ± 3.0
	Cortisol non-responders	39.2 ± 2.9	39.8 ± 3.0	—	39.6 ± 2.8
Subjective stress (1–7)	Cortisol responders	1.39 ± .65	4.14 ± 1.3		1.39 ± .72
	Cortisol non-responders	1.41 ± .80	3.63 ± 1.6		1.30 ± .59

Values are means ± standard deviation.

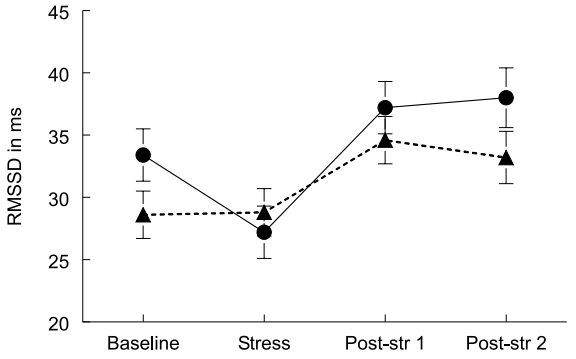

Fig. 2. Mean levels of heart rate variability in cortisol responder (solid line) and non-responder (dotted line) groups. Error bars are standard errors of mean.

non-responder groups. The analysis of IL-6 with concurrent haematocrit as a covariate showed significant effects of cortisol responder group, $F(1/137) = 5.55$, $p = .02$, and trial $F(1/137) = 4.60$, $p = .034$, with no interaction between the two, $F(1/137) = 1.17$, $p = .28$. As shown in Fig. 3, there were higher levels of plasma IL-6 at both time points in the cortisol non-responder group, while the trial effect reflects the increase between baseline and post-stress measures. The stress-induced change in IL-6 did not differ between groups. However, the partial correlation between cortisol AUC and change in IL-6 was significant, after controlling for age, gender, BMI, waist/hip ratio, change in haematocrit, and method of blood sampling ($r = -.18$, $p = .023$). Participants with greater cortisol output as defined by AUC showed smaller IL-6 stress responses.

The analysis of IL-1ra involved 70 cortisol responders and 72 non-responders. In repeated measures analysis of covariance, there was a significant effect of cortisol responder group, $F(1/136) = 5.93$, $p = .016$, since levels were greater in the cortisol non-responders. The group by trial interaction was not reliable, $F(1/138) = 3.17$, $p = .077$. However, analysis of the change in IL-1ra between baseline and post-stress samples revealed a main effect for cortisol responder group, $F(1/132) = 4.12$, $p = .044$, after controlling for gender, BMI, waist/hip ratio, time of day, the use of hormone replacement therapy by women, and change in haematocrit. The cortisol non-responders showed an adjusted change of 10.4 ± 42.5 pg/ml, compared with an average decrease of -2.76 ± 30.3 pg/ml in cortisol responders (Fig. 3). Thus for both IL-6 and IL-1ra, there was evidence that cortisol stress responsivity was inversely associated with cytokine levels and changes.

4. Discussion

The increases in cortisol of our participants were relatively small in comparison with those observed in some experimental studies (Biondi and Picardi, 1999; Kirschbaum et al., 1995). The rather mild stressors could explain the small responses observed. Both groups showed a reduction in cortisol from the initial sample to the baseline measurement. The initial sample was obtained prior to inserting the venous cannula, so might reflect an anticipatory response to this procedure. Alternatively, the cortisol reduction between initial and baseline samples might be due to habituation to the unfamiliar laboratory setting. We allowed 30 min quiet rest following cannula insertion, so that responses to the behavioural tasks were not confounded with neuroendocrine responses to cannulation. The low levels of blood pressure, heart rate, and subjective stress recorded at baseline suggest that participants were relaxed by this time. Individual differences in cortisol responses to the mental stress tests were then associated with distinctive psychological and biological characteristics.

As hypothesized, the cortisol responders experienced more subjective distress during the laboratory session, and also reported poorer mental well-being than the non-responders. The relationship between psychological characteristics and cortisol responses to acute stress has

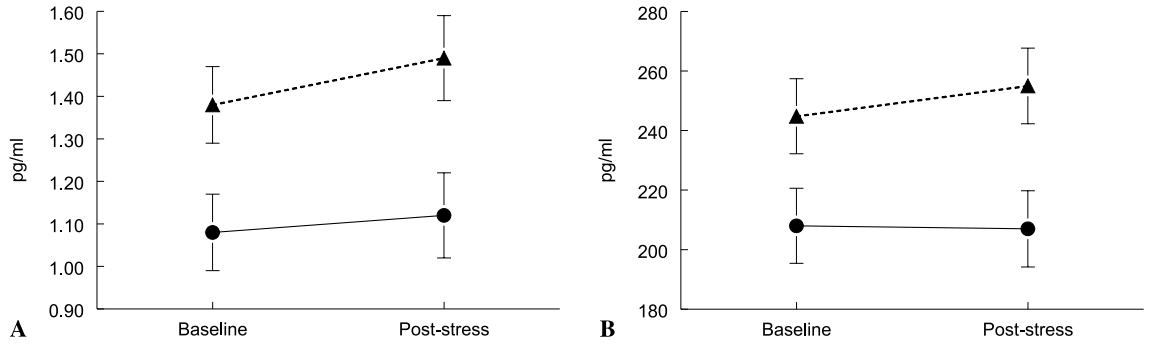

Fig. 3. Mean levels of interleukin-6 (A) and interleukin-1 receptor antagonist (B) in cortisol responder (solid line) and non-responder (dotted line) groups. Error bars are standard errors of mean.

been inconsistent. For example, no associations between cortisol responsivity and anxiety, depression or anger were recorded by Van Eck et al. (1996) in a sample of working men, while Schommer et al. (1999) showed no relationship with personality. But individuals with higher depression and lower self-esteem were found to be more responsive in a study involving repeated stress testing (Kirschbaum et al., 1995), and a relationship between low self-esteem and acute cortisol responsivity has been observed by others (Pruessner et al., 1997; Seeman et al., 1995). In naturalistic settings, negative affect has been related to higher cortisol increases after stress (Smyth et al., 1998). The two groups experienced the tasks differently, with larger subjective stress responses in cortisol responders. The higher levels of hopelessness in the cortisol responder group may reflect associations between depression and dysregulation of the HPA axis.

There is evidence in this study that individual differences in cortisol responsivity were associated with disturbances of autonomic function. Although the two groups did not differ in blood pressure or heart rate stress responses, differences in heart rate variability were observed (Fig. 2). Low heart rate variability suggests increased sympathetic tone and vagal withdrawal (Stein and Kleiger, 1999), and many authorities would cautiously interpret heart rate variability as a marker of vagal activity (Berntson et al., 1997). Acute reductions in heart rate variability are typically seen during mental stress tests. Reduced heart rate variability has also been associated with increased cardiovascular disease risk and psychosocial stress factors in a number of studies (Hemingway et al., 2001). In the present investigation, the cortisol non-responders appeared to demonstrate less flexibility in heart rate variability, with smaller reductions during stress and less increase during the post-stress period than the cortisol responders. The general effects of mental stress on cytokine levels in this study have been described previously in this journal (Steptoe et al., 2002a). Previous literature on stress-induced changes in unstimulated cytokine levels has been inconsistent (Altemus et al., 2001; Dugué et al., 1993). Evidence from the related field of exercise stress indicates that increases in cytokine levels are not immediate, but take several minutes to evolve (Febbraio and Pedersen, 2002; Shephard, 2002). Cytokine stress responses may not therefore be revealed in samples obtained immediately post-stress. In a preliminary study, we observed that increases in IL-6 and IL-1ra had emerged by 45 min post-stress, but were greater after two hours (Steptoe et al., 2001). It was not possible to obtain two hour samples in the present study, so responses were based on samples obtained 45 min post-stress.

As hypothesized, there was an inverse relationship between cytokines and cortisol. There were higher levels of both IL-6 and IL-1ra in the cortisol non-responder than responder groups. The cortisol AUC was negatively associated with IL-6 increases between baseline and post-stress after adjusting for covariates, while the IL1-ra increase was greater in the cortisol non-responders. These results are consistent with the evidence that glucocorticoids down-regulate cytokine levels and receptor expression (Wiegers and Reul, 1998). Since glucocorticoids also act on cytokine receptors the result of the CR group could also be explained by the interference of glucocorticoids with the activation of the IL-6 receptor. The higher cortisol in the CR group might have affected both the IL-1ra and the IL-6 receptor (Wiegers and Reul, 1998). However, the fact that cytokine levels were greater even at baseline in the cortisol non-responders suggests that the reverse pattern might be operating, with cytokines regulating the HPA response (Rivest, 2001). The overall pattern of increased cortisol and cytokine levels in response to stress may disguise subtle individual variations in response profile.

One study has shown that glucocorticoids in physiological doses suppressed IL-1β and TNF-α, but not IL-6 (DeRijk et al., 1997). On the other hand, Rohleder and colleagues (2002) showed that IL-6 was dose dependently inhibited by dexamethasone. It has also been noted that catecholamines affect IL-6 release (Mohamed-Ali et al., 2001; Papanicolaou et al., 1996). In a related analysis, it has been found that levels of IL-6 post-stress were associated with heart rate (Owen and Steptoe, in press). This may reflect a sympathetically driven response in those individuals who do show increases in IL-6.

Repeated episodes of acute or chronic stress might initiate down-regulation of glucocorticoid receptors and lead to the development of a chronic inflammatory state. The glucocorticoid–cytokine feedback loop seems to be altered in chronic inflammatory diseases. A variety of studies have shown elevated levels of IL-1 and IL-6 in patients with rheumatoid arthritis (Ridderstad et al., 1991), atherosclerosis (Yudkin et al., 2000), and inflammatory bowel disease (Papadakis and Targan, 2000). IL-6 levels were found to be a predictor of mortality after stroke (McCarty, 1999). Two studies have shown an increase of IL-6 levels after stress in patients with juvenile rheumatoid arthritis (Hirano et al., 2001; Voort et al., 2000). However, it is still unclear if these cytokines are elevated because of ongoing disease processes or as trait markers of the disease. Our findings are consistent with the hypothesis that a hypoactive HPA axis might contribute to the development of inflammatory conditions, since increases in proinflammatory cytokines were only found in individuals who did not increase cortisol in response to mild psychological stress.

The limitations of this study should be recognized. The investigation was carried out with healthy middle-aged men and women, and results may not generalize to other populations. The mental stress tests were not effective in inducing substantial increases in cortisol, so

the cortisol responder and non-responder groups were not as distinct as would be desirable. We were only able to assess cytokine changes over 45 min, and larger responses may emerge with longer time periods. The study was cross-sectional, so causal conclusions cannot be drawn. Nevertheless, the results suggest that high and low cortisol responsivity are associated with distinctive psychobiological responses. Cortisol responders showed greater psychological distress and poorer mental well-being. Lack of cortisol response was associated with heightened cytokine levels, and with cardiovascular responses suggestive of vagal withdrawal. One reason why both cortisol and inflammatory cytokines increase in response to stress in groups of participants may be that different individuals show different patterns of response. Those who are cortisol responsive may show little cytokine response, while in people who are unresponsive in terms of cortisol, autonomic pathways may stimulate greater cytokine changes.

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