Positive affective states are associated with favorable health outcomes, but the underlying mechanisms are poorly understood. The authors assessed associations between positive affect, cortisol sampled over the day, and inflammatory markers (C-reactive protein and interleukin-6) among 2,873 healthy members of the Whitehall II study. Data for this study were collected in 2002–2004 in London, United Kingdom. Saliva free cortisol was assessed on waking, 30 minutes later, and four times over the day and evening. Positive affect was indexed by aggregating ecological momentary assessments of positive mood over the day. Salivary cortisol averaged over the day was inversely associated with positive affect after controlling for age, gender, income, ethnicity, body mass index, waist/hip ratio, smoking, paid employment, time of waking in the morning, and depression ($p = 0.003$). There was no association with cortisol responses to waking. The adjusted odds of C-reactive protein $\geq 3.00$ mg/liter was 1.89 (95% confidence interval: 1.08, 3.31) in low- compared with high-positive-affect women, and plasma interleukin-6 was also inversely related to positive affect in women ($p = 0.016$). Neither inflammatory marker was related to positive affect in men. These results confirm findings from smaller studies relating cortisol with positive affect while suggesting that in women, positive affect is associated with reduced levels of inflammatory markers.

adrenal cortex hormones; affect; C-reactive protein; happiness; interleukin-6

Abbreviations: CES-D, Center for Epidemiologic Studies Depression; CRP, C-reactive protein; IL-6, interleukin-6; SD, standard deviation.
other studies (13), although effects have not all been consistent (14, 15). In a recent study of younger working men, positive affect was related to reduced cortisol increases following waking in the morning, but not to values over the rest of the day (16). The cortisol awakening response and cortisol over the day are regulated differently, having distinct psychosocial and health correlates and patterns of heritability (17).

The first aim of the present study was to investigate associations between positive affect assessed by aggregating ecological momentary assessments and both cortisol over the day and the cortisol awakening response in a large sample of middle-aged and older men and women from the Whitehall II cohort. We also measured depression with the Center for Epidemiologic Studies Depression (CES-D) Scale to discover whether associations between positive affect and cortisol were independent of depressed mood.

The second aim of these analyses was to investigate the relation between positive affect and two measures of chronic inflammation: C-reactive protein (CRP) and interleukin-6 (IL-6). Both markers have previously been associated with depressed mood (18, 19), but associations with positive affect are not known. We hypothesized that if the protective effects of positive affective states on future health are mediated in part through reduced chronic inflammation, then inverse associations between positive affect, CRP, and IL-6 would be observed in this healthy population.

MATERIALS AND METHODS

Study population

The data reported here were collected in 2002–2004 during phase 7 of the Whitehall II study. The Whitehall II study was initiated in 1985 to investigate demographic, psychosocial, and biologic risk factors for coronary heart disease, and 10,308 men and women aged 35–55 years from London-based civil service departments in the United Kingdom were recruited (20). The number who took part in physical examinations at phase 7 was 6,483. We asked 5,115 persons who consented to provide salivary cortisol assessments, and sample tubes were returned by 4,609 (90.1 percent). Participants were aged 50–74 years at the time of testing. The study was approved by the Joint University College London/University College London Hospitals Ethics Committee, and all participants gave informed consent.

Procedure

Participants attended a physical examination session during which clinical and cognitive measures were obtained (refer to Marmot and Brunner (20) for details). Height, weight, and waist and hip circumference were measured, from which body mass index and waist/hip ratio were calculated. Blood was drawn for the assessment of CRP and IL-6. A questionnaire containing socioeconomic, demographic, and lifestyle items was administered, from which measures of marital status, socioeconomic status, paid employment, and smoking status were obtained. Income was used as a marker of socioeconomic status. Participants were asked to classify their income into one of eight categories. Income tended to be lower among women than men, so respondents were classified into gender-specific tertiles: low (<£20,000 for men and <£15,000 for women), medium (£20,000–£34,999 for men and £15,000–£24,999 for women), and high (£35,000 for men and £25,000 for women) (1 British pound (£) = approximately US $0.50). Participants also completed the CES-D Scale, a well-established 20-item checklist that assesses the presence and frequency of depressive symptoms in the preceding 7 days (21). They were instructed how to measure salivary cortisol and complete the logbook and were asked to complete cortisol assessments on a weekday within the next few days.

Assessment of cortisol and positive affect

Participants were requested to collect six saliva samples over the day by using salivettes (Sarstedt, Leicester, United Kingdom) at the following times: immediately after waking; 30 minutes after waking (waking þ 30); 2.5 hours, 8 hours, and 12 hours after waking; and bedtime. They were asked to record their time of waking and the time of each sample collection and not to drink caffeinated beverages before the waking þ 30-minute sample. Immediately after collecting each sample, participants were asked how happy, excited, or content they felt at that moment, with four response options: not at all, somewhat, very much, and extremely. The salivettes and logbooks were returned by post.

Positive affect shows marked variation over the day and evening (22). In our previous investigation relating positive affect with physiologic responses (11), we measured positive affect by computing the proportion of measures over the day and evening in which respondents rated themselves as very much or extremely happy. To replicate this measure, we computed the proportion of day and evening samples (i.e., samples 3–6) rated as very much or extremely. The positive affect ratings on waking and 30 minutes later were excluded; the waking measure is difficult to interpret, while the 30-minute postwaking measure is related to the specific experiences of the early morning period (23). The average times at which these samples were obtained were 9:27 hours (standard deviation (SD), 62 minutes), 15:02 hours (SD, 69 minutes), 19:02 hours (SD, 68 minutes), and 23:14 hours (SD, 59 minutes). Because there were four ratings, each person could be categorized as being in a positive affective state on 0, 25, 50, 75, or 100 percent of occasions. These categories were collapsed into three: low (0), moderate (up to 50 percent), and high (75–100 percent) positive affect.

Biologic assays

Cortisol was assayed from saliva samples by using a commercial high-sensitivity chemiluminescence assay (IBL–Hamburg, Hamburg, Germany). Inter- and intraassay coefficients of variance were less than 8 percent. Cortisol was successfully assayed from at least one of the six samples in 4,474 cases. CRP was measured by using a high-sensitivity immunologic assay on a ProSpec nephelometer (Dade-Behring, Milton Keynes, United Kingdom), and IL-6 was assayed with a commercial high-sensitivity, two-site,
enzyme-linked immunosorbent assay kit (R&D Systems, Oxford, United Kingdom). CRP was analyzed in 2,853 persons and IL-6 in 2,519.

Cortisol data reduction

Separate analyses were carried out on the cortisol awakening response and cortisol over the day. Data were screened and high values excluded as potentially due to hemolysis. The cortisol awakening response was defined as the difference between the waking +30 and the waking samples. Participants who reported a delay of more than 10 minutes between waking and taking the first cortisol sample, or an interval of more than 45 minutes between waking and the waking +30 sample, were excluded, since it has previously been shown that the cortisol awakening response is not accurately assessed when sampling is delayed (24). Only those persons for whom data for samples 3–6 were complete were included in the analysis of cortisol over the day. The total sample with satisfactory positive affect data and cortisol over the day was 4,162 and was 3,519 for the cortisol awakening response. Additionally, we excluded from all analyses participants who were taking any steroid, antihypertensive, or cardiac medication and those with a history of coronary heart disease or stroke. The result was that data for 2,873 were analyzed for cortisol over the day and 2,444 for the cortisol awakening response. Positive affect levels did not differ among participants with and without satisfactory cortisol data.

Statistical analysis

The three positive affect categories (low, moderate, and high) were compared regarding all participant characteristics with analysis of variance for continuous variables and $\chi^2$ tests for categorical variables. The associations between positive affect and cortisol averaged over the day and the cortisol awakening response were analyzed by using analysis of covariance, with age, gender, income, ethnicity, body mass index, waist/hip ratio, smoking, paid employment, and time of waking in the morning as covariates, since these variables have previously been related to cortisol (17, 25, 26). A second analysis included the CES-D Scale as an additional covariate to determine whether associations between cortisol and positive affect were independent of negative mood states. Plasma CRP values were divided into $<3.0$ mg/liter and $\geq3.0$ mg/liter, the criterion for high risk of cardiovascular disease as defined by the Centers for Disease Control and Prevention/American Heart Association joint statement (27). Logistic regression was used to analyze the odds of high CRP in relation to positive affect, adjusting for the same covariates, with the high positive affect group as the reference category; odds ratios with 95 percent confidence intervals are presented in this paper. IL-6 was analyzed as a continuous variable because a high-risk cutoff for the general population has not been established. Interactions between gender and positive affect were also tested, and, when the relation between positive affect and biomarkers differed in men and women, the genders were analyzed separately.

RESULTS

More than half the participants (52.9 percent) reported no high levels of positive affect at any of the sampling points, so they were designated low positive affect. However, 23.5 percent reported high positive affect on one or two samples (moderate positive affect) and 23.6 percent reported high positive affect on three or four samples (high positive affect). The characteristics of these three groups are summarized in table 1. The high positive affect group was an average 2 years older than the low and moderate groups ($p < 0.001$). They were also more likely to be from an ethnic minority, have a low income, be married, and not be in paid employment (all $p < 0.001$). These differences remained significant once age had been taken into account, except for the association with employment, since the large majority (89.6 percent) of those not in paid employment were retired. As might be expected, depression scores on the CES-D Scale were inversely related to positive affect ($p < 0.001$), being twice as great in the low than high positive affect groups. Participants experiencing high positive affect tended to wake slightly later in the morning, but this effect was no longer significant once age had been included in the model. The mean positive affect rating over the four samples was 1.19 (SD, 0.57) on the 0–3 rating scale, indicating that participants were on average somewhat happy, excited, or content. As might be expected from the categorization of participants, these mean ratings varied systematically across the low, moderate, and high positive affect groups.

Salivary cortisol over the day and evening averaged 5.44 (SD, 2.61) nmol/liter. As predicted, average cortisol over the day was inversely associated with positive affect, after controlling for age, gender, income, ethnicity, body mass index, waist/hip ratio, smoking, paid employment, and time of waking in the morning ($p = 0.003$). As shown in figure 1, cortisol was an average 7 percent higher in the low positive affect group after adjustment for covariates. This association was unchanged when the CES-D Scale was included as an additional covariate ($p = 0.004$), indicating that the association was independent of psychological distress. There was no interaction with gender. Similar results emerged when household income was used instead of personal income as an indicator of socioeconomic status. Independently of positive affect, cortisol over the day was greater in women ($p = 0.005$), smokers ($p < 0.001$), participants with greater body mass index and waist/hip ratios ($p < 0.001$), and persons who woke later in the morning ($p < 0.001$).

The associations between positive affect, cortisol on waking, and the cortisol awakening response were also analyzed. Mean cortisol on waking was 16.08 (SD, 8.27) nmol/liter, and the cortisol awakening response averaged 8.19 (SD, 11.29) nmol/liter. However, neither measure was related to positive affect.

Plasma CRP averaged 1.72 (SD, 1.80) mg/liter, and 13.7 percent of men and 20.3 percent of women had values of $\geq3.0$ mg/liter. CRP was not correlated with cortisol over the day. There were significant interactions between gender and positive affect in the analyses of inflammatory markers ($p < 0.001$), so men and women were analyzed separately. The likelihood of high CRP was associated with low positive
affect in women but not men. As can be seen in table 2, 23.2 percent of low positive affect women had high CRP values of \( \geq 3.0 \text{ mg/liter} \) compared with 17.2 percent of high positive affect women. The odds ratio adjusted for covariates including CES-D Scale scores was 1.89 (95 percent confidence interval: 1.08, 3.31). The corresponding proportions of low, moderate, and high positive affect men with high CRP values were 13.2, 14.3, and 14.2 percent, and the odds ratio for men was 1.09 (95 percent confidence interval: 0.73, 1.61).

The mean plasma IL-6 concentration was 2.71 (SD, 1.31) pg/ml. Plasma IL-6 was positively correlated with cortisol over the day in both men \((r = 0.065, p = 0.005)\) and women \((r = 0.081, p = 0.042)\). There was an inverse relation between positive affect and IL-6 in women but not men. IL-6 averaged 2.00 (SD, 1.48), 1.84 (SD, 1.22), and 1.71 (SD, 1.03) pg/ml in the low, moderate, and high positive affect groups, respectively \((p = 0.016)\), after adjustment for age, income, ethnicity, body mass index, waist/hip ratio, smoking, paid employment, and time of waking. The relation was preserved after additional control for CES-D Scale scores, showing that the association with positive psychological states is not secondary to the absence of depression. Additionally, we found that positive affect was inversely associated with plasma CRP and IL-6 levels in women, after controlling for potential confounders. The cortisol findings

**DISCUSSION**

The results of this study indicate that salivary cortisol averaged over the day and evening was inversely associated with positive affect independently of age, gender, income, ethnicity, body mass index, waist/hip ratio, smoking, paid employment, and time of waking. The relation was preserved after additional control for CES-D Scale scores, showing that the association with positive psychological states is not secondary to the absence of depression. Additionally, we found that positive affect was inversely associated with plasma CRP and IL-6 levels in women, after controlling for potential confounders. The cortisol findings

**TABLE 1.** Characteristics associated with positive affect in three groups* of study participants from whom data were collected in 2002–2004 in London, United Kingdom

<table>
<thead>
<tr>
<th></th>
<th>Low positive affect ( (n = 1,520) )</th>
<th>Moderate positive affect ( (n = 675) )</th>
<th>High positive affect ( (n = 678) )</th>
<th>( p ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (men)</td>
<td>1,139 ( 74.9 )</td>
<td>490 ( 72.6 )</td>
<td>497 ( 73.3 )</td>
<td>0.36</td>
</tr>
<tr>
<td>Ethnicity (non-White)</td>
<td>69 ( 4.3 )</td>
<td>45 ( 6.7 )</td>
<td>57 ( 8.4 )</td>
<td>0.001</td>
</tr>
<tr>
<td>Income (high tertile)</td>
<td>571 ( 38.9 )</td>
<td>277 ( 42.6 )</td>
<td>192 ( 29.7 )</td>
<td>0.009</td>
</tr>
<tr>
<td>Married</td>
<td>1,091 ( 73.0 )</td>
<td>530 ( 80.1 )</td>
<td>544 ( 82.2 )</td>
<td>0.001</td>
</tr>
<tr>
<td>Paid employment</td>
<td>868 ( 58.4 )</td>
<td>386 ( 58.4 )</td>
<td>298 ( 45.4 )</td>
<td>0.001</td>
</tr>
<tr>
<td>Smoker</td>
<td>163 ( 10.8 )</td>
<td>57 ( 8.5 )</td>
<td>70 ( 10.4 )</td>
<td>0.59</td>
</tr>
<tr>
<td>Age (years)</td>
<td>59.9 ( 5.7 )</td>
<td>59.8 ( 5.6 )</td>
<td>61.8 ( 5.7 )</td>
<td>0.001</td>
</tr>
<tr>
<td>Body mass index ( \text{kg.m}^2 )</td>
<td>26.2 ( 4.2 )</td>
<td>26.1 ( 4.2 )</td>
<td>26.3 ( 3.9 )</td>
<td>0.69</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.923 ( 0.08 )</td>
<td>0.917 ( 0.08 )</td>
<td>0.924 ( 0.08 )</td>
<td>0.21</td>
</tr>
<tr>
<td>CES-D Scale score</td>
<td>9.34 ( 8.5 )</td>
<td>7.47 ( 6.4 )</td>
<td>4.77 ( 5.5 )</td>
<td>0.001</td>
</tr>
<tr>
<td>Time of waking</td>
<td>6:43 ( 1:05 )</td>
<td>6:44 ( 0:57 )</td>
<td>6:50 ( 1:00 )</td>
<td>0.049</td>
</tr>
<tr>
<td>Positive affect (mean)</td>
<td>0.78 ( 0.33 )</td>
<td>1.30 ( 0.20 )</td>
<td>1.99 ( 0.27 )</td>
<td>0.001</td>
</tr>
</tbody>
</table>

* Refer to the Materials and Methods section of the text for a definition of these groups.

† SD, standard deviation; CES-D, Center for Epidemiologic Studies Depression.

**FIGURE 1.** Mean levels of cortisol averaged over the day in low, medium, and high positive affect groups from the Whitehall II cohort (refer to the Materials and Methods section of the text for a definition of these groups). Data were collected in 2002–2004 in London, United Kingdom. All values were adjusted for age, gender, income, ethnicity, body mass index, waist/hip ratio, smoking, paid employment, and time of waking. The solid bars represent additional adjustment for Center for Epidemiologic Studies Depression Scale scores. Error bars, standard error of the mean.
confirm, in a much larger sample, the earlier results obtained from the Whitehall II cohort (11) and other studies (13). Polk et al. (14) found that trait positive affect, estimated by averaging seven sets of readings obtained over a 6-week period, was associated with lower cortisol over the day in only men and not women. These data were collected while participants were living in a hotel before carrying out a virus challenge study, so they may not reflect cortisol output over a typical day. More recently, Jacobs et al. (15) found no association between positive affect and cortisol in 556 women studied over 5 days. The participants in this study were aged 27 years on average, much younger than in our investigation. In a study of men with a mean age of 33.6 years, Steptoe et al. (16) found that positive affect was associated with a reduced cortisol awakening response but not with cortisol over the day and evening. It is possible that there are age differences in associations between cortisol and positive affect, with differences in output over the day evolving with increasing age.

These findings were obtained with positive affect measured by aggregating ecological momentary assessment ratings, not with a standard questionnaire measure of positive well-being. Questionnaire measures typically require the person to describe mood over a prolonged period such as 1 week or 1 month, and they have been criticized for recall bias, memory distortions, the dominant influence of current mood, and other biases such as “focusing” illusions (28, 29). Aggregation of ecological momentary assessments may provide a more valid estimate of typical affective states (30), even though such assessments depend on the events and activities that the person experiences over the monitoring period. In a recent study, more consistent associations were found between biologic variables and affect assessed with ecological momentary assessments than with questionnaire measures of positive and negative affect (16). In the present study, we had only four ecological momentary assessment ratings, so the observation of a consistent association with cortisol is striking. Although ecological momentary assessment ratings were collected over 1 day only, aggregate values appear quite stable and may index more general affective experience. We previously found that positive affect across working and weekend days correlated at 0.65 ($p < 0.001$), and the association over a 3-year interval was 0.62 ($p < 0.001$) (11, 12).

It is interesting that high positive affect was inversely associated with income (table 1). In a related analysis of a subgroup from the Whitehall II cohort, we reported that negative affect over the day was greater in more educated persons, although CES-D Scale depression levels were lower (31). It appears that on a moment-to-moment basis, people of higher social status may experience greater demands that adversely influence their affective states (32).

Elevated salivary cortisol over the day is an indicator of activation of the hypothalamic-pituitary-adrenocortical axis and may be relevant to a range of health outcomes. Cortisol is implicated in abdominal adiposity (33), depression (34), and cognitive dysfunction (35). It has a range of effects on cardiovascular and metabolic processes (36), and an elevated cortisol/testosterone ratio predicted incident coronary heart disease in the Caerphilly Study (37).

Cortisol shows marked diurnal variation, rising after waking and then declining progressively over the day and evening. To estimate average cortisol output over the day, it is desirable to obtain several samples at intervals over the day (38). We asked participants to take samples at fixed intervals following waking in the morning, instead of specifying particular times. The reason was to standardize the timing of each sample in relation to the cortisol decline after waking up. A similar procedure has been used in the CARDIA study (39).

This investigation also showed that higher positive well-being was associated with lower levels of CRP and plasma IL-6 in women. There was an 89 percent increase in the odds of elevated CRP in women in the lowest compared with the highest positive affect group after adjusting for covariates including the CES-D Scale. IL-6 was an average 17 percent higher in the low than the high positive affect group. These effects have not been described before. Previously, we noted an association between positive affect and reduced fibrinogen responses to acute mental stress, but not with resting fibrinogen levels (11). However, the findings add to the evidence that inflammatory markers are sensitive to psychosocial factors including acute stress (40), childhood maltreatment (41), chronic stress experience (42), and social isolation (43).

The hypothalamic-pituitary-adrenocortical axis is thought to down-regulate proinflammatory cytokines, whereas IL-6 activates hypothalamic-pituitary-adrenocortical and cortisol secretion (44). The blood samples from which CRP and IL-6 were measured were collected several days before cortisol assessment, but it cannot therefore be assumed that decreases in IL-6 were driving the cortisol effects. Many acute stimuli, such as physical exertion and mental stress, elicit simultaneous increases in both IL-6 and cortisol (40, 45). Chronic psychosocial stress is also associated with increased concentrations of cortisol and inflammatory markers.

This study adds to the evidence for gender differences in associations between inflammatory processes and psychosocial factors. For example, the relation between elevated CRP and depression appears to be stronger in men than in table 2:

**TABLE 2. Positive affect and plasma CRP* levels in women study participants from whom data were collected in 2002–2004 in London, United Kingdom**

<table>
<thead>
<tr>
<th>Positive affect†</th>
<th>Proportion (%) of women with CRP levels of ≥3.0 mg/liter</th>
<th>Odds ratio‡</th>
<th>95% CI*</th>
</tr>
</thead>
<tbody>
<tr>
<td>High ($n = 180$)</td>
<td>17.2 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate ($n = 181$)</td>
<td>17.1 0.995 0.52, 1.90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low ($n = 379$)</td>
<td>23.2 1.89 1.08, 3.31</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* CRP, C-reactive protein; CI, confidence interval.
† Refer to the Materials and Methods section of the text for a definition of these groups.
‡ Adjusted for age, ethnicity, income, body mass index, waist/hip ratio, smoking, paid employment, time of waking, and Center for Epidemiologic Studies Depression Scale score.
women (18, 46), whereas chronic inflammation may be more significant for type 2 diabetes development in women than in men (47). Some studies have shown larger IL-6 responses to acute stress in women than in men (48). Rohleder et al. (49) demonstrated that acute stress induces similar cortisol responses in men and women, but an upregulated lipopolysaccharide-induced IL-6 response in women and down-regulation in men. The possibility that positive affective states also show differential neuroendocrine-inflammatory associations in men and women deserves further investigation.

Our study was carried out with a large, well-characterized sample, with careful exclusion of persons with clinical conditions or those using medications that might affect neuroendocrine or inflammatory activity. There are a number of limitations. Both cortisol and positive affect were assessed over a single day, and more robust evidence for stable individual differences in neuroendocrine function may emerge with repeated measurements. Our study did not include any objective checks on the reliability of timing of cortisol samples. Inaccurate timing can be especially problematic in assessing the cortisol awakening response, since delays between waking and the “waking” sample lead to reduced cortisol increases (17). To redress this problem, we excluded those persons from the cortisol awakening response analysis who reported a delay of more than 10 minutes between waking and collecting the first sample, since it has previously been shown that such delays predict a low cortisol awakening response (24). Despite these limitations, the findings add to the evidence that positive affect is associated with a pattern of hypothalamic-pituitary-adrenocortical activity that may contribute to reduced risk of chronic disease, while suggesting that, for women, a happier state of mind is related to reduced levels of inflammatory markers.

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Conflict of interest: none declared.

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