Perceived stress and cortisol levels predict speed of wound healing in healthy male adults

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\textit{Summary} The main purpose of the present study was to investigate the association between perceived stress and impaired cutaneous wound healing in humans using a novel wound assessment technique, and taking into account putative mediating factors such as cortisol levels, health behaviours, and personality factors.

The study made use of a prospective, within-subjects design in which 24 male non-smokers participated. Every subject received a standard 4mm-punch biopsy, and the healing progress was monitored via high-resolution ultrasound scanning. Participants completed questionnaires on perceived stress, health behaviours, and personality factors, and sampled saliva for cortisol assessment after awakening at 2 weeks prior, directly after, and 2 weeks after the biopsy.

The overall results showed a significant negative correlation between speed of wound healing, and both Perceived Stress scale (PSS) scores ($r = -0.59; p < 0.01$), and General Health Questionnaire (GHQ) scores ($r = -0.59; p < 0.01$) at the time of the biopsy. The area under the morning cortisol response curve was negatively correlated with speed of wound healing ($r = -0.55; p < 0.05$), indicating a clear elevation in the morning cortisol slope of those whose wounds were slowest to heal. A median split of the complete sample yielded that the ‘slow healing’ group showed higher stress levels (PSS $t = 3.93$, $p < 0.01$, GHQ $t = 2.50$, $p < 0.05$), lower trait optimism ($t = 3.25$, $p < 0.05$), and higher cortisol levels to awakening ($F = 5.60$, $p < 0.05$) compared with the ‘fast healing’ group. None of the health behaviours investigated (i.e. alcohol consumption, exercise, healthy eating, and sleep) were correlated with healing speed at any time point.

Our data hint at a considerable influence of stress on wound healing, and suggests that elevated cortisol levels, rather than altered health behaviours, play a role in this effect.

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1. Introduction

Cutaneous wound healing in mammals can be subdivided into three successive overlapping phases: acute inflammation, proliferation and granulation tissue formation, and tissue remodelling. During the early inflammatory phase, platelet aggregation and blood coagulation leads to the formation of a clot. The extravasational part of the blood clot provides a provisional matrix for the migration of cells such as polymorphonuclear leukocytes, lymphocytes, monocytes, fibroblasts, endothelial cells and pericytes (Dyson, 1997). Platelets secrete PDGF, TGF-alpha, and TGF-beta, which promote tissue generation, while pro-inflammatory agents enhance recruitment of leukocytes such as granulocytes and macrophages to the wound (Wolpe and Cerami, 1989).

During granulation tissue formation, new blood vessels grow into the wound bed. Granulation tissue is a well-vascularised, soft connective tissue in which the key cells are macrophages, fibroblasts and endotheliocytes. Cytokines derived from macrophages and fibroblasts, such as IL-1α, IL-1β, IL-8 and TNF-α orchestrate the formation of a fibroblast matrix, which replaces the provisional matrix provided by the blood clot (Hubner et al., 1996; Schaffer and Barbul, 1998; Agaiby and Dyson, 1999). In parallel, wound contraction, re-epithelisation, and angiogenesis occur. The remodelling phase of wound repair is characterised by collagen fibrillogenesis and development of extracellular matrix. The cellularity and vascularity of the reparative tissue decreases until finally the granulation tissue is replaced by scar tissue (Dyson, 1997).

Recent studies have confirmed a negative effect of stress on the rate of wound healing in animals (Padgett et al., 1998) and humans. One human study examined levels of chronic stress and wound healing in a group of Alzheimer’s caregivers and compared them with matched controls (Kiecolt-Glaser et al., 1995). Carers who exhibited high scores on the Perceived Stress Scale (PSS) showed slower wound healing rates and this difference was particularly marked at 14 days after a standardised punch biopsy. Likewise, in a sample of elderly people, individuals scoring high on the Hospital Anxiety and Depression Scale (HADS) exhibited significantly impaired healing of chronic wounds (Cole-King and Harding, 2001). Another study reported an intra-individual comparison of medical students during a phase of high psychosocial stress (i.e. during the exam period) versus a phase of relatively low stress (i.e. holiday period) (Marucha et al., 1998). In this study, standardised wounds to the dental palate during a holiday period healed significantly faster than equivalent wounds incurred during an exam period.

While these findings provide evidence for a clear relationship between heightened perceived stress and delayed wound healing in humans, some putative mediating factors have not been taken into account in previous studies. For example, health-risk behaviours such as smoking, alcohol consumption, poor sleep and lack of physical exercise have been associated with psychological distress (Hellerstedt and Jeffery, 1997; Baum and Posluszny, 1999; Vitaliano et al., 2002). The wound healing progress is highly dependent on the host’s nutritional status; especially glucose, polyunsaturated fatty acids, protein, and the vitamins A, C, E, and Zinc are essential dietary components during the healing progress (Russell, 2001; Scholl and Langkamp-Henken, 2001). Therefore, a lack of intake of these substances due to unhealthy eating habits (i.e. high saturated fat/low protein and vitamins), or vitamin depletion due to increased smoking and alcohol consumption could compromise wound healing (van den Berg et al., 2002). Additionally, disturbed sleep patterns due to stress could result in reduced growth hormone release and further downregulation of tissue repair processes (Lee and Stotts, 1990; Rose et al., 2001). Therefore, it is possible that the reported correlations between psychological distress and impaired wound healing could be secondary to stress-induced changes in health behaviours, which suppress the individual’s immune functions, thus delaying the repair process of the wound.

Most of the previous studies involved the use of macrophotography and hydrogen peroxide foaming to assess wound healing. However, hydrogen peroxide interacts with non-epithelial tissues causing tissue damage, and macrophotography only records the surface appearance of the wound. It has recently been demonstrated, that high resolution ultrasound (HRUS) scanning of a standard punch biopsy wound is a more valid measure of healing activity in deeper tissue layers than surface photography (Dyson et al., in press). Measures obtained by photography of the wound diameter were influenced by variable contractions of the wound scab, and especially by increases in wound diameter after the scab detaches. In contrast, non-invasive HRUS scans obtained at the base (i.e. at the level of the dermal/hypodermal junction) of a standard punch biopsy wound yielded measures documenting a more stable wound healing progress unaffected by changes in surface contractions (Dyson et al., in press).

Another putative mediating factor between psychological stress and wound healing are gluco-
corticoid levels. Glucocorticoid-induced suppression of the wound healing process has been documented in humans and animals (Goforth and Gudas, 1980; Gupta et al., 1999). This down-regulating effect might be due to inhibition of cytokines such as IL-1, IL-6, IL-8 and TNF-α (Sapolsky et al., 2000) or growth factors, such as keratinocyte growth factor (KGF) 1 (Brauchle et al., 1995; Chedid et al., 1996), all of which play a pivotal role during the inflammatory-, re-epithelialisation-, and fibroblast matrix-formation phase of wound regeneration (Dyson, 1997; Schaffer and Barbul, 1998). This hypothesis is supported by animal data demonstrating that glucocorticoid-induced immunosuppression leads to impaired dermal wound healing (Gupta et al., 1999). Studies in humans have shown that IL-1β levels were downregulated in individuals who exhibited slow wound healing (Kiecolt-Glaser et al., 1995; Marucha et al., 1998). Further, blister chamber fluid levels of the cytokines IL-1α and IL-8 were lower in participants with high perceived stress levels, and participants with the lowest levels of both cytokines at the induced blister wound site showed elevated cortisol levels in saliva (Glaser et al., 1999).

The cortisol response to awakening has been established as a reliable and stable marker of HPA axis activity in humans (Pruessner et al., 1997). Since this response has been found to be associated with levels of reported psychological distress (Schulz et al., 1998; Steptoe et al., 2000; Wüst et al., 2000), we investigated cortisol levels directly after awakening in relation to wound healing speed and levels of perceived stress.

In sum, the present study aimed to confirm and extend previous reports on positive associations between stress on wound healing. The effects of recent life stress during the preceding month on wound healing in a young adult sample were investigated using high resolution ultrasound (HRUS) scans to assess wound healing, controlling for health behaviours and cortisol levels. The goal of the study was to examine the relationship between perceived stress, emotional distress and wound healing over 21 days, using a longitudinal within-groups design.

2. Materials and Methods

2.1. Participants

Participants consisted of staff and students recruited via circular e-mails and posters at Kings College London. The mean age of the 24 participants was 29.42 (minimum 19, maximum 59, standard deviation 11.53 years). To minimise inter-individual variation in cortisol levels due to gender (Kirschbaum et al., 1992), only males were recruited. Participants were screened for the following exclusion criteria via short interviews: Smoking, intake of glucocorticoid medication during the last month, chronic inflammatory disorders, allergies, clinical depression, acute illness such as infections, allergic reactions to local anaesthetics, bleeding disorders, and risk of keloid scarring.

2.2. Experimental procedure

On the first day of the experiment, participants reported to the Unit of Psychology for an introductory meeting. The experimental procedure was explained and written informed consent was obtained from each participant. The following questionnaires were completed on the first visit: the Perceived Stress Scale (PSS, Cohen et al., 1983), the General Health Questionnaire (GHQ, Goldberg, 1992), a health behaviour questionnaire to assess reported health behaviours such as alcohol consumption, diet, sleep, and exercise as described previously in (Odgen and Mbandabari, 1997), the Life Orientation Test (LOT, Scheier and Carver, 1985), the Short-form Social Support Scale (SSS, Sarason et al., 1987), the Rosenberg Self Esteem Scale (RSE, Rosenberg, 1989), and the UCLA Loneliness Scale (UCLA-LC, Russell, 1996). Participants received a batch of saliva sampling devices (Salivettes®) to assess their cortisol levels after awakening and throughout the day on the following day (see below for a detailed description of the sampling procedure).

On the second visit two weeks later, participants were seen at the Dermatology Unit to have their biopsies performed. Each participant completed the PSS, the GHQ and the Health Behaviour Questionnaire a second time. After this, participants received a local anaesthetic of Lidocaine, and a standard 4mm punch biopsy was performed at the inner aspect of the upper non-dominant arm by a trained dermatologist. The wound site was sealed with a standard plaster, and participants received their second batch of Salivettes® to sample saliva for the analysis of cortisol levels on the following day.

Seven, 14, and 21 days after the biopsy, participants attended follow-up visits at the Unit of Psychology. During these visits, short (10 min) ultrasound scans of the wound sites were performed to monitor wound-healing progress (see below for detailed description). At the 14-day follow-up visit, participants filled in the PSS, GHQ and Health Behaviour questionnaire a third time, and received a third batch of Salivettes® to collect saliva samples on the following day. After the 21-day follow-up visit, participants received mon-
etary compensation for travel expenses and inconveniences. This study protocol was approved by the Guy’s Hospital Research Ethics Committee.

2.3. Ultrasound scanning of the wound site

At days 7, 14, and 21 after the punch biopsy, the participant’s wounds were scanned using high resolution ultrasound (HRUS). We used a prototype of the EPISCAN™ HRUS-Scanner (Longport Intl. Ltd., Silchester, UK), operating at a frequency of 20 MHz. The scanner consists of a handheld ultrasound probe, a custom designed proprietary amplifier/analogue to digital converter (ADC) board, standard PC components (motherboard, display, disk drives, communication ports) and the operating software (Windows Skin Scanner V. 2.05, © 2000 Longport Intl. Ltd.).

Eight mm deep, and 15 mm wide 2D digitised scans were taken through the centre of the wound bed and the adjacent intact skin. Using the scanner’s calibrated measurement capability, the wound width was measured at its base, defined as being level with the dermal/hypodermal junction. Scanning of the wound base by HRUS has been shown to be a more accurate marker of wound healing progress, when compared to surface photography (Dyson et al., in press). HRUS has previously been used for assessment of surgical wounds in renal transplant patients (Calvin et al., 1997).

2.4. Cortisol sampling and assay

Free cortisol levels after awakening have been reported to reliably reflect the individual’s adrenocortical activity. Normative data for the early morning cortisol response to awakening is available (Wüst et al., 2000).

Participants were instructed to sample saliva for cortisol assessment on three days (the day after the first visit, the day after the biopsy and the day after the 14-day follow-up visit). In order to safeguard against any influence of anticipatory stress on cortisol levels on the morning of the biopsy day, we scheduled the cortisol sampling to take place on the morning after the biopsy day. The participants obtained saliva samples using Salivette® sampling devices (Sarstedt, Rommelsdorf, Germany). The first sample on each day was collected immediately after awakening. Four additional samples were collected 10, 20, 30 and 60 minutes later. Saliva samples were stored at −20°C until assay.

After thawing, saliva samples were centrifuged at 3000 rpm for 5 minutes, which resulted in a clear supernatant of low viscosity. A 50μl sample of saliva was used for duplicate analysis. Cortisol levels were determined employing a time-resolved immunoassay with fluorometric end point detection (DELFIA, Wallac, Turku, Finland) with an intra assay coefficient of variance below 10% as described in detail elsewhere (Dressendorfer et al., 1992). This assay has a lower detection limit of 0.78 nmol/l, an intra assay variation of 4.0% to 6.7% and an inter assay variation of 7.1 to 9.0%. To reduce error variance caused by intra assay inaccuracies all samples of one participant were analysed in the same run.

2.5. Questionnaires

2.5.1. Perceived Stress

The Perceived Stress Scale (PSS, Cohen et al., 1983) is a 14-item scale, which has been shown to possess test-retest reliability, adequate internal consistency and concurrent and predictive validity (Cohen et al., 1983). Participants were asked to indicate how often they felt or thought a certain way in the past month. Scores range from 0 to 40, with higher scores indicating more perceived stress. Perceived stress scores were calculated for each participant, at baseline, the day of biopsy and 14 days after the biopsy, creating the variables ‘PSS1’, ‘PSS2’ and ‘PSS3’. The internal consistency was Cronbach’s α = .72 for PSS1, α = .66 for PSS2, and α = .49 for PSS3. Obtaining repeated PSS scores in 14 days intervals leads to high intercorrelations since the retrospective measurement periods (1 month) overlap (see also results section). However, we did not favour changing the instructions of the PSS to cover the past 2 weeks (instead of 4 weeks) since we did not want to alter this frequently used and well known scale. Thus we maintained high comparability of our results at the expense of enhanced intercorrelation between repeated measures on this scale.

2.5.2. Emotional Distress

The General Health Questionnaire (GHQ-12, Goldberg, 1992), a shortened version of the well-validated full version, was used to detect the degree of emotional distress in participants. Each of the 12 items asks whether the respondent has experienced a particular symptom or item of behaviour over the past few weeks and whether this is usual. Scores range from 0 to 36 with higher scores indicating a greater probability of clinical disorder. GHQ scores were calculated for each participant, at baseline, the day of biopsy and 14 days after the biopsy, creating the variables ‘GHQ1’ (Cronbach’s α = .90), ‘GHQ2’ (α = .78) and ‘GHQ3’ (α = .91).
2.5.3. Health Behaviours
Fourteen days before the biopsy, on the day of the biopsy and 14 days after the biopsy participants were asked to complete questions about four health related behaviours: alcohol consumption; sleep; exercise and eating behaviour. Based on a similar format to that used by Odgen and Mtandabari, (1997) participants were required to indicate how many alcoholic drinks they consumed per week (variables 'Drink1', 'Drink2' and 'Drink3'); on average, how many hours they had slept per night (variables 'Sleep1', 'Sleep2' and 'Sleep3'); and how many hours they had spent exercising per week (variables 'Exercise1', 'Exercise2' and 'Exercise3'). Participants were also asked to indicate how often they had practised the following eating behaviours on a scale from 'never' to 'all the time': Eat three meals; Eat fruit; Eat vegetables; Eat snack foods between meals; Eat high fat foods; Have a healthy diet. These items formed an eating behaviour score (variables 'Eat1'; Cronbach’s α = .70, 'Eat2'; α=.69 and 'Eat3'; α = .65) that ranged from 6 to 30, with high scores indicating healthier eating behaviour.

2.5.4. Dispositional Optimism
Dispositional optimism (the habitual style of anticipating favourable outcomes) was assessed by the 8-item Life Orientation Test (LOT) designed by Scheier and Carver (1985). The questionnaire requires participants to report how much they agree with each statement. For instance, 'In uncertain times, I usually expect the best'. The LOT has been shown to have satisfactory internal consistency and test-retest reliability (Scheier and Carver, 1985). Scores range from 0 to 32 with higher scores indicating higher dispositional optimism. Cronbach’s α for the sample reported here was .41.

2.5.5. Social Support
To measure social support, the short form Social Support Questionnaire (SSQ6, Sarason et al., 1987) was administered. This six-item questionnaire consists of two sub-scales: a quasi-structural measure (number of social supports, variable SSSa, Cronbach’s α = .86) by asking participants which people in their environment provide help or support, and a global functional measure (satisfaction with support, variable SSSb, Cronbach’s α = .93) by asking how satisfied they are with the support that they receive. The score for number of social supports sub-scale ranges from 0 to 54 with higher score indicating more support. The satisfaction with support score ranges from 6 to 36 with higher scores indicating higher satisfaction with support.

2.5.6. Self-Esteem
The Rosenberg Self-Esteem Scale (RSE, Rosenberg, 1989) is one of the most widely used measures of self-esteem/self-worth. The scale requires participants to indicate the extent to which they agree with statements dealing with their general feelings about themselves such as 'At times I think I am no good at all'. Scores range from 10 to 40 with low scores indicating high self-esteem. Cronbach’s α for the sample reported here was .40.

2.5.7. Loneliness
To assess loneliness, the UCLA Loneliness Scale (Version 3), developed by (Russell, 1996), was implemented. Analyses have indicated that this 20-item measure is highly reliable and has convergent and construct validity (Russell, 1996). The UCLA Loneliness Scale asks participants to indicate how often they feel the feeling described in each item e.g. 'How often do you feel that you are no longer close to anyone?'. Scores range from 20 to 100. High scores indicate greater degree of loneliness. Cronbach’s α for the sample reported here was .45.

2.6. Statistical analysis
Cumulative measures for the cortisol responses and the healing progress were established as follows: The area under the reaction curve (AUC) for the saliva samples 0, 10, 20, 30, and 60 minutes after awakening on the three days was computed employing the trapezoid formula (AUC1 for the response on the baseline day, AUC2 for the day after the biopsy and AUC3 for the 14-days follow-up after the biopsy). The rate of healing was calculated as the difference in wound diameter at the base of the wound between the 7-day follow up and the 21-day follow up visit (=HEAL).

A median split of the total sample was performed employing the variable HEAL to create a group of fast healing and a group of slow healing individuals. ANOVAs for repeated measures were computed to detect differences in cortisol responses after awakening between groups defined by the median split, with Greenhouse-Geisser corrections applied for repeated measures factors. Correlations between biological, psychometric and other markers were computed using Pearson correlations. Due to the relatively small sample size, and to control for the effect of outliers, nonparametric Spearman correlations were computed in addition. To account for the influence of a third variable on a correlation between two variables, partial correlations were calculated. To compare group differences in the questionnaire data, t-tests for...
independent samples were calculated. Since the data presented here was analysed to test specific hypotheses rather than in an exploratory way, Bonferroni correction of α-levels were not applied when more than one comparison of means, or more than one correlation was computed at a time. Data are presented as mean ± standard error of the mean (SEM) unless stated otherwise. The statistical package SPSS v.10 was used for the calculation of all statistical procedures.

3. Results

All participants showed a significant progression of wound healing over the time between day 7 and day 21 after the biopsy. Average diameters of the wound base changed from 4.38 mm (std. dev. 0.58 mm) on day 7 to 3.52 mm (std. dev. 0.60 mm) on day 14 and to 2.80 mm (std. dev. 0.74 mm) on day 21. A repeated measures ANOVA confirmed a highly significant within subjects reduction in wound base diameter over time (F = 49.9, p < .001).

Table 1 summarises the correlations between speed of wound healing and the psychological variables measured in this experiment. We observed high negative correlations between the participants’ scores on the PSS and on the GHQ 14 days before the biopsy, at the day of the biopsy, and 14 days after the biopsy and the total amount of wound healing measured between days 7 and 21 after the biopsy. The strongest correlation was found between the amount of healing and the scores on the PSS and the GHQ on the day of the biopsy. Scores on both subscales of the SSS were not correlated with any of the wound healing measures. Similarly, participant’s scores on the UCLA-LC, and the RSE were not found to be significantly associated with wound healing in this sample. There was a nonsignificant trend towards a positive correlation between high scores on the LOT and faster wound healing between days 7 and 21 after the biopsy.

Table 2 shows the correlations between the aggregated cortisol responses on the three test days and the healing progress between the days 7 and 21 after the biopsy. There was no association between the cortisol responses 14 days before the biopsy and any measure of healing progress. Also no association was observed between the cortisol

<table>
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<tr>
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<td>.014</td>
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<td>-.593 **</td>
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<td>p</td>
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<tr>
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<td>.149</td>
</tr>
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<tr>
<td>p</td>
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<td>.072</td>
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*Correlation is significant at the .05 level (2-tailed).
**Correlation is significant at the .01 level (2-tailed).
response 14 days after the biopsy and any of the healing measures. However, strong negative correlations emerged between the cortisol response directly after awakening one day after the biopsy and any of the relations emerged between the cortisol response 14 days after the biopsy (PSS1), on the day of the biopsy (PSS2) and 14 days after the biopsy (PSS3) when compared with the ‘fast healing’-group. Likewise, scores on the GHQ were significantly elevated on the day of the biopsy (GHQ2) and 14 days after the biopsy (GHQ3) in the ‘slow healing’-group. There was a nonsignificant trend towards a more diverse social network (SSSx-score), a more satisfactory perceived social support (SSSy-score), and higher self-esteem (RSE-score) in the ‘fast healing’-group when compared with the ‘slow healing’-group. Further, participants in the ‘fast healing’-group scored significantly higher than the ‘slow healing’-group on the LOT (e.g. life optimism scale).

An ANOVA for repeated measures was calculated comparing the cortisol levels to awakening between the ‘slow healing’ and ‘fast healing’ groups. On the first cortisol measurement 14 days before the biopsy, both groups showed a significant rise in cortisol levels in the morning (F = 10.39, p < .01), but the groups did not differ in the magnitude of the total response, and the interaction was not significant. On the second measurement (in the morning after the biopsy), both groups showed a significant elevation in cortisol levels (F = 14.71, p < .01), and the ‘slow healing’ group exhibited significantly higher overall cortisol levels than the ‘fast healing’ group (significant group effect: F = 5.60, p < .05, see Fig. 1). The interaction was not significant in this analysis. On the third cortisol measurement 14 days after the biopsy, both groups showed a significant rise in cortisol levels in the morning (F = 4.09, p < .05), but the groups did not differ in the magnitude of the total response, and the interaction was not significant.

Pearson and Spearman correlations were calculated for health behaviours measured by the ‘Health Behaviour Questionnaire’ at 14 days prior to the biopsy, on the day of the biopsy, and 14 days after the biopsy. The scores on the four scales (‘exercise’, ‘sleep’, ‘alcohol consumption’

Table 2  Pearson and Spearman correlations between aggregated cortisol responses 14 days prior, 1 day after, and 14 days after the biopsy (AUC1,2,3) and the healing progress between the days 7 and 21 after the biopsy (HEAL).

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<thead>
<tr>
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<th>HEAL Spearman</th>
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<td>AUC1</td>
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<td>-.510 *</td>
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<td>-.180</td>
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<tr>
<td>p</td>
<td>.923</td>
<td>.461</td>
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The cortisol responses to awakening (AUC1,2,3) were not correlated with PSS scores obtained on any of the test days. However, the cortisol response after the biopsy day was significantly correlated with the GHQ score obtained at the day of the biopsy (r = .49, p < .05). Social support (SSS), loneliness (UCLA-LC) and optimism (LOT) showed no correlation with the AUC of the cortisol responses measured on any of the test days. Although there was a significant negative correlation between the participants’ self esteem-scores (RSE) and cortisol responses after awakening at day 14 after the biopsy (r = -.44, p < .05), cortisol responses on the other two test days were not associated with RSE scores. As expected, intra-individual Pearson correlations were mostly significant between the 2 measures of distress (PSS and GHQ) and repeated scores on the same questionnaire. Further, a consistent negative correlation was found between measures of distress and dispositional optimism scores (LOT) (see Table 3).

A median split of the total sample was performed employing the variable HEAL. Participants showing less than 1.5mm wound healing progress between days 7 and 21 after the biopsy were defined as the ‘slow healing’-group, whereas those healing 1.5mm or more during this period were defined as the ‘fast healing’-group. Table 4 shows the comparison of means of the cortisol responses to awakening and various psychological variables between the ‘slow healing’, and the ‘fast healing’ group.

T-test analysis revealed a significantly higher cortisol response to awakening on the day after the biopsy in the ‘slow healing’-group when compared to the ‘fast healing’-group (AUC2). Further, the ‘slow-healing’ group yielded significantly higher scores on the PSS scale 14 days before the biopsy (PSS1), on the day of the biopsy (PSS2) and 14 days after the biopsy (PSS3) when compared with the ‘fast healing’-group. Likewise, scores on the GHQ were significantly elevated on the day of the biopsy (GHQ2) and 14 days after the biopsy (GHQ3) in the ‘slow healing’-group. There was a nonsignificant trend towards a more diverse social network (SSSx-score), a more satisfactory perceived social support (SSSy-score), and higher self-esteem (RSE-score) in the ‘fast healing’-group when compared with the ‘slow healing’-group. Further, participants in the ‘fast healing’-group scored significantly higher than the ‘slow healing’-group on the LOT (e.g. life optimism scale).
and ‘healthy eating’) were not significantly correlated with the variable HEAL.

The means for the variable age and all health behaviours were compared between the ‘fast’ and ‘slow’ healing groups. Table 5 shows a series of 13 t-tests, out of which only the variable EAT 2 was significant, suggesting that participants in the ‘fast healing’ group reported significantly more healthy eating behaviour on the day of the biopsy, but none of the other group comparisons for health behaviours reached statistical significance.

### 4. Discussion

In the sample reported here, wound healing assessed by repeated ultrasound scans was negatively correlated with perceived stress measured by the PSS and the GHQ, and positively with dispositional optimism. Further, the cortisol response in the morning of the day after the biopsy was negatively correlated with speed of wound healing. However, only inconsistent associations were observed between levels of cortisol after awakening and levels of perceived stress. Similarly, vari-

### Table 3 Pearson correlations between measures of distress and dispositional optimism (PSS 1-3, GHQ 1-3, and LOT)

<table>
<thead>
<tr>
<th></th>
<th>PSS1</th>
<th>PSS2</th>
<th>PSS3</th>
<th>GHQ1</th>
<th>GHQ2</th>
<th>GHQ3</th>
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<td>.704 **</td>
<td>.920 **</td>
<td>.318</td>
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<td></td>
<td></td>
</tr>
<tr>
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<td>.000</td>
<td>.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>PSS3</td>
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<td>.380</td>
<td>.067</td>
<td>.547 **</td>
<td>.533 **</td>
<td>.613 **</td>
</tr>
<tr>
<td>p</td>
<td>.001</td>
<td>.000</td>
<td>.007</td>
<td>.000</td>
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<td>GHQ1</td>
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<td>.140</td>
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<tr>
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<tr>
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<td>.515</td>
<td>.070</td>
<td>.547 **</td>
<td>.533 **</td>
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<tr>
<td>p</td>
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<td>.007</td>
<td>.000</td>
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<tr>
<td>LOT</td>
<td>-.773 **</td>
<td>-.862 **</td>
<td>-.770 **</td>
<td>-.426 **</td>
<td>-.437 **</td>
<td>-.310</td>
</tr>
<tr>
<td>p</td>
<td>.000</td>
<td>.000</td>
<td>.000</td>
<td>.038</td>
<td>.033</td>
<td>.150</td>
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</table>

*Correlation is significant at the .05 level (2-tailed).

**Correlation is significant at the .01 level (2-tailed).
ous health behaviours such as diet, alcohol consumption, sleep, and healthy eating were not consistently correlated with healing speed, suggesting that a moderating influence of these behaviours on the healing progress was absent in our sample. Thus, the present findings replicate the well-documented association between psychosocial stress and impaired wound healing in animals (Padgett et al., 1998), as well as in human studies using cross sectional- (Kiecolt-Glaser et al., 1995), and within groups-designs (Marucha et al., 1998). Moreover, our data extends those comparisons between extreme groups or conditions to a sample which can be regarded as normal in terms of perceived stress levels.

As described in the introduction, the successive phases of wound healing are each dependent on the respective preceding phase. Therefore, one could speculate that a slowing of the initial inflammatory phase delays onset of the following phases thus slowing the overall healing process. It has been demonstrated, that topical glucocorticoids slow down the healing process of dermal wounds (Goforth and Gudas, 1980).

Further, there is abundant evidence, that production of inflammatory cytokines such as IL-1, IL-6, IL-8 and TNF-α can be suppressed by glucocorticoids in vivo and in vitro (Sapolsky et al., 2000). These findings have been replicated in various wound healing paradigms. In an immunocompromised host model, rats were treated with 40mg intramuscular hydrocortisone (HC). In contrast to control animals, the HC treated rats exhibited signs of immunosuppression and significantly impaired healing of a 8mm punch biopsy wound (Gupta et al., 1999). Also, wound healing is greatly delayed in IL-6 deficient (IL-6 KO) mice. Treatment with recombinant murine IL-6 restored the wound healing capacity not only in IL-6 KO mice, but also in wild type animals previously immunocompromised by dexamethasone (DEX) treatment (Gallucci et al., 2000).

![Cortisol levels](image)

**Fig. 1.** Cortisol levels directly after awakening at the day after the biopsy in the groups ‘slow healing’ and ‘fast healing’, created by a median split of the sample using the variable HEAL. Repeated measures ANOVA yielded an overall significant main effect for group and time, but not a significant interaction (see text for details).

**Table 5** Comparison of means for the groups ‘slow healing’ vs. ‘fast healing’ based on a median split employing the variable HEAL

<table>
<thead>
<tr>
<th>Variables</th>
<th>‘slow healing’-group</th>
<th>‘fast healing’-group</th>
<th>t²</th>
<th>df²</th>
<th>p²</th>
</tr>
</thead>
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<tr>
<td>Mean¹ SEM</td>
<td>Mean² SEM</td>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
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<td>3.17</td>
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<td>4.01</td>
<td>0.25</td>
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<td>3.90</td>
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<td>.91</td>
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<td>6.45</td>
<td>.31</td>
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<tr>
<td>Sleep 3</td>
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<td>1.02</td>
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<td>Eat 2</td>
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<td>.91</td>
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<tr>
<td>Eat 3</td>
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<tr>
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<td>.29</td>
<td>2.00</td>
<td>.33</td>
<td>0.19</td>
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<tr>
<td>Drink 2</td>
<td>2.00</td>
<td>.25</td>
<td>2.09</td>
<td>.37</td>
<td>−0.20</td>
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<tr>
<td>Drink 3</td>
<td>2.00</td>
<td>.28</td>
<td>1.82</td>
<td>.35</td>
<td>0.41</td>
</tr>
</tbody>
</table>

¹arbitrary units except for age (years)
²Adjusted for equal variances not assumed,
³difference is significant at the .05 level (2-tailed).
In a human blister chamber paradigm, it has been shown that blister fluid levels of the cytokines IL-1Rx and IL-8 were lower in participants with high perceived stress levels. Participants with the lowest levels of both cytokines at the wound site showed elevated cortisol levels in saliva (Glaser et al., 1999). Reduced responses of IL-1β to LPS stimulation in whole blood were associated with poorer wound healing in two other human studies (Kiecolt-Glaser et al., 1995; Marucha et al., 1998). It should be pointed out, however, that other ways in which glucocorticoids could affect dermal wound healing might exist. For example, the inhibition of keratinocyte growth factor (KGF) by glucocorticoids has been demonstrated both in vitro and in vivo (Brauchle et al., 1995; Chedid et al., 1996).

It appears likely that stress-induced elevation of glucocorticoid levels are responsible for the slowing of the cutaneous healing process. However, in our sample there was only an inconsistent correlation between the cortisol response in the morning and scores on questionnaires measuring perceived stress, with significant positive correlations between GHQ scores and morning cortisol responses, and nonsignificant correlations between PSS scores and morning cortisol responses. Likewise, findings from other groups provide a mixed picture of the influence of stress on diurnal HPA axis activity. For example, school teachers with high job strain according to the demand/control model of work stress, exhibited higher levels of cortisol early during a working day (0800 h – 0830 h), compared with their less stressed colleagues (Steptoe et al., 2000). In this study, cortisol was only sampled once in the early morning with no reference to the time of awakening. In contrast, other studies have found lower levels of cortisol in the morning in women with high work and home demands (Adam and Gunnar, 2001), and in workers in the retail industry who reported high job strain (Steptoe et al., 1998). However, individuals with high chronic burnout seem to exhibit elevated salivary cortisol levels in the morning (0800 h) and afternoon (1600 h) (Malamut et al., 1999).

Employing a sampling procedure similar to the one reported in our study, Schulz et al. (1998) compared cortisol levels directly after awakening in students reporting high and low chronic work overload. In this study, one hundred students were divided into two groups based on a median split of their scores on the ‘chronic work overload’ scale of the ‘Trier Inventory of Chronic Stress’ (TICS; Schulz and Schlotz, 1999). The students reporting more chronic work overload showed significantly higher cortisol levels directly after awakening, when arithmetic means of the morning cortisol response over three separate days were compared between groups (Schulz et al., 1998).

These findings have been supported by results obtained in a sample of 104 twin pairs (Wüst et al., 2000). Scores on three different scales of the (TICS), namely 'worries', 'social stress', and 'lack of social recognition' were all significantly associated with an elevated morning salivary cortisol response after awakening. The studies by Schulz et al. (1998), and Wüst et al. (2000) used a stress questionnaire assessing chronic stress and specifically work overload over a time span of 1 year, and it is possible that abnormalities of HPA axis after awakening are only associated with long term stressors. Therefore, in the study presented here, it may not have been possible to differentiate morning cortisol responses using scores on the Perceived Stress Scale (PSS), which only assessed levels of perceived stress during the previous month. This hypothesis is supported by a finding of Pruessner et al. (1999), who reported no differences in morning cortisol levels between school teachers with high and low PSS scores using the same cortisol sampling technique as described here. It is possible that a questionnaire focusing on more work-related, chronic stress would have differentiated between participants with high and low morning cortisol responses.

In contrast to a number of previous studies in the literature on stress and wound healing in humans, our study attempted to control for the possible mediating effects of health behaviours. Since stress can lead to a less healthy lifestyle, with increased alcohol consumption, poorer sleep, and a less healthy diet, this can indirectly compromise bodily functions such as wound healing. However, even though two distinct measurements of reported health behaviours were correlated with the healing progress in our sample, partial correlations showed that they did not account for the association between perceived stress and wound healing. It is of course possible, that longer term changes in health behaviours associated with chronic stress may have stronger effects on wound healing. Further, more accurate outcome measures of health behaviours might have yielded group differences on variables such as blood levels of nutrients or detailed analysis of sleep quality between the "high" and "low stress" group. Another limitation of our study is the relatively small sample size. Even though the sample size provides the power to detect an effect comparable to the ones reported in previous studies which have compared high- and low-
stressed individuals on wound healing speed, this might have been insufficient to uncover more subtle group differences or correlations in the other variables investigated (e.g. cortisol, health behaviours). Finally, our data provides a hint to a mediation of the effect of stress on wound healing by cortisol, even though correlations between measures of psychosocial stress and cortisol proved inconsistent in our sample.

In our study we were able to demonstrate the usefulness of ultrasound B-scans for the monitoring of wound healing over time. Digitised ultrasound scans have proven superior to surface photography as demonstrated recently (Dyson et al., in press). The use of a ultrasound imaging in combination with measurement software can be regarded as a promising new method to obtain quantifiable data in an in vivo human wound healing paradigm.

In conclusion, our study supports the hypothesis of a relation between perceived stress and wound healing in humans. This effect has previously been demonstrated by comparing extremely stressed individuals with controls, and our data extend these findings in a sample of healthy individuals who exhibit a normal distribution of perceived stress. From our data, it appears that this association is less likely to be caused by compromising health behaviours, but by increased cortisol levels, which could influence healing processes by well documented physiological mechanisms.

Due to the longitudinal design of this study, a cautious interpretation can be drawn in terms of a causal link between perceived stress and wound healing, since the perceived stress levels measured before the event of wounding were predictive for the healing process over the three weeks after the biopsy. Considerable implications for the health sector arise from our findings, particularly for interventions aiming at reducing psychosocial stress before surgical procedures. An influence of psychosocial stress on post-surgical recovery has been described (George and Scott, 1982; Kiecolt-Glaser et al., 1998), and beneficial effects of interventions reducing state anxiety and cortisol levels have been reported in clinical trials (Field et al., 1998; Holden-Lund, 1988; Whitney and Heitkemper, 1999).

Acknowledgements

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References


