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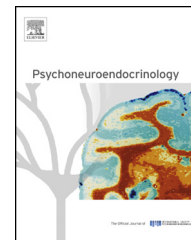
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# Blunted glucocorticoid and mineralocorticoid sensitivity to stress in people with diabetes



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## KEYWORDS

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**Summary** Psychological stress may contribute to type 2 diabetes but mechanisms are still poorly understood. In this study, we examined whether stress responsiveness is associated with glucocorticoid and mineralocorticoid sensitivity in a controlled experimental comparison of people with type 2 diabetes and non-diabetic participants. Thirty-seven diabetes patients and 37 healthy controls underwent psychophysiological stress testing. Glucocorticoid (GR) and mineralocorticoid sensitivity (MR) sensitivity were measured by dexamethasone- and prednisolone-inhibition of lipopolysaccharide (LPS)-induced interleukin (IL) 6 levels, respectively. Blood pressure (BP) and heart rate were monitored continuously, and we periodically assessed salivary cortisol, plasma IL-6 and monocyte chemotactic protein (MCP-1). Following stress, both glucocorticoid and mineralocorticoid sensitivity decreased among healthy controls, but did not change in people with diabetes. There was a main effect of group on dexamethasone ( $F_{(1,74)} = 6.852, p = 0.013$ ) and prednisolone ( $F_{(1,74)} = 7.295, p = 0.010$ ) sensitivity following stress at 45 min after tasks. People with diabetes showed blunted stress responsiveness in systolic BP, diastolic BP, heart rate, IL-6, MCP-1, and impaired post-stress recovery in heart rate. People with Diabetes had higher cortisol levels as measured by the total amount excreted over the day and increased glucocorticoid sensitivity at baseline. Our study suggests that impaired stress responsiveness in type-2 diabetes is in part due to a lack of stress-induced changes in mineralocorticoid and glucocorticoid sensitivity.

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## 1. Introduction

Patients with type 2 diabetes have a substantially increased risk of developing coronary heart disease (CHD) as well as poorer long-term prognosis following myocardial infarction

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(Haffner et al., 1998; Mukamal et al., 2001; Yusuf et al., 2004). Recently, some mechanisms on how inflammation could play a role in diabetes have been described, and could play a role in CHD risk. For example, low-grade local and systemic inflammation in  $\beta$ -cells of pancreatic islets and peripheral tissues to induce  $\beta$ -cells' dysfunction and apoptosis, insulin resistance, and ultimately, overt type 2 diabetes mellitus (T2DM) (Akash et al., 2013a). Despite this, the mechanisms underlying the association between diabetes and CHD are not completely clear. Psychological stress may contribute to risk of developing CHD in people with diabetes. Longitudinal studies have also confirmed an increased risk of developing diabetes in people with high levels of stress (Eriksson et al., 2008; Chida and Hamer, 2008; Nyberg et al., 2014). People who experience depression also present increased risk of future diabetes (Rotella and Mannucci, 2013).

Psychological stress has been linked to production of pro-inflammatory markers and these effects may mediate the influence of psychosocial factors on cardiovascular risk in people with diabetes (Steptoe et al., 2007). There is evidence that elevated inflammatory responses in people with diabetes play an important role in linking diabetes with increased incidence of atherosclerosis (Ray et al., 2009). Diabetes patients with raised levels of C-reactive protein are 30% more likely to have cardiovascular events than those without inflammation (Akash et al., 2013b). Treatment with anti-inflammatory drugs are currently being investigated to dampen the effects of T2DM inducers (Akash et al., 2012, 2013c).

Apart from traditional risk factors, stress-induced inflammation may be an additional mechanism in people with diabetes. Psychological stress activates the hypothalamus–pituitary–adrenal (HPA) axis that regulates inflammation. Hyperactivation of the HPA axis has been reported in patients with diabetes (Cameron et al., 1984; Roy et al., 1990, 1993), and is associated with coronary heart disease and its risk factors (Reynolds et al., 2010). Patients with diabetes present alterations of the HPA axis negative feedback (Bruehl et al., 2007) suggestive of an impairment of corticosteroid receptor sensitivity. HPA axis disturbance seems to be particularly important in people with diabetes since the degree of cortisol secretion is related to the presence and number of diabetes complications (Chiodini et al., 2007).

Psychosocial stress regulates the effect of corticosteroids on target tissues via dynamic modulation of corticosteroid sensitivity (Rohleder et al., 2003). Two distinct intracellular sensitivity subtypes mediate the effect of cortisol: the type I or mineralocorticoid sensitivity (MR) and the type II or glucocorticoid sensitivity (GR) (de Kloet et al., 1998). Dexamethasone activates human-GR-mediated gene transcription, but even at the highest concentrations is unable to fully activate human-MR-mediated gene transcription (Rupprecht et al., 1993). On the other hand, prednisolone is a synthetic glucocorticoid that is more similar to cortisol in its capacity to bind and activate the GR and the MR, especially when compared with dexamethasone. Thus, the effect of corticosteroids on the regulation of inflammation during stress will depend not only on the actual basal levels of cortisol, but also on stress-induced changes in the sensitivity of both glucocorticoid and mineralocorticoid receptors.

As part of a larger study of stress processes in type 2 diabetes participants, we assessed stress-related modulation of GR and MR sensitivity in a subset of patients randomly selected. Such alterations may provide a new biological mechanism linking diabetes with elevated levels of inflammation and the elevated risk of acute cardiovascular syndrome following stress. We analysed the glucocorticoid and mineralocorticoid sensitivity of lipopolysaccharide (LPS)-induced IL-6 production in whole blood of people with diabetes and healthy controls. All participants underwent a standardized acute psychological stress test (Zalli et al., 2014), and we measured MR and GR sensitivity of IL-6 production at different time points before and after stress. We measured IL-6 because it seems to play an important role in how psychological stress contributes to physical and mental illness. IL-6 is also involved in the activation of the inflammatory cascade that leads to the production of C-reactive protein by the liver. To obtain each measure of glucocorticoid and mineralocorticoid sensitivity we calculated the amount of dexamethasone and prednisolone, respectively that was necessary to suppress LPS-induced IL-6 levels by 50%. Moreover, we measured blood pressure, heart rate, salivary cortisol, and inflammatory cytokines such as IL-6 and MCP-1 before and after stress to assess the amount of stress induced by the behavioural tasks.

## 2. Methods

### 2.1. Participants

This is a sub-study of a larger cohort of type 2 diabetic patients (T2DM) recruited from primary care clinics in the London area who participated in a mental stress protocol (Steptoe et al., 2001, 2002a). For this particular study, cells were collected from thirty-seven diabetes participants and 37 healthy controls by simple random sampling. Glucocorticoid sensitivity function was analysed in a subgroup of 20 people from each group. Participants gave fully informed written consent to participate in the study and ethical approval was obtained from the National Research Ethics Service. We limited enrolment to diabetes participants without a history or previous diagnosis of coronary heart disease (CHD), inflammatory diseases, allergies or mood disorders, but had no other exclusion criteria. Healthy controls were recruited from the subsample of the Whitehall II epidemiological cohort recruited between 2006 and 2008 to investigate socioeconomic and psychosocial factors, physiological stress responsiveness, and subclinical coronary artery disease (Hamer et al., 2010). Healthy participants were white European origin with no history or objective signs of CHD, no previous diagnosis or treatment for hypertension, diabetes, inflammatory diseases, or allergies. The absence of diabetes was confirmed by low glycated haemoglobin (HbA1c) levels ( $\leq 6.5\%$ ) and negative oral glucose tolerance tests over the previous 20 years. Participants underwent identical mental stress testing to that carried out with the diabetes group.

### 2.2. Mental stress tasks

Mental stress was induced in the laboratory using two 5-min behavioural tasks: the Stroop colour-word interference

and mirror tracing. These tasks were selected because they have been shown to stimulate cardiac responses (Stepptoe et al., 2002a), and have been used in a number of previous studies in our laboratory (Stepptoe et al., 2001, 2002a; Zalli et al., 2014). Cardiovascular activity was monitored throughout tasks, and subjective stress, blood and saliva samples were taken immediately after tasks. Monitoring of the post-task recovery period continued for 75 min and additional blood samples and stress ratings at 45 and 75 min.

### 2.3. Biological measures

Blood samples were collected in EDTA tubes and centrifuged immediately at 2500 rpm for 10 min at room temperature. Plasma was separated and stored at  $-80^{\circ}\text{C}$  until analysis. Plasma IL-6 was assayed using a high sensitivity Quantikine two-site enzyme-linked immunosorbent assay (ELISA) from R&D Systems (Oxford, UK). The sensitivity of the assay ranged from 0.016 to 0.110 pg/ml and the intra and inter assay coefficient of variations (CVs) of 7.3% and 7.7% respectively. MCP-1 was assayed in duplicate using the fluorokine MAP profiling from R&D (Oxford, UK), and concentrations were determined with Luminex technology from Bio Rad (Bio-Plex, Hercules, CA, US). For MCP-1 the limit of detection was 1.2 pg/ml and the mean intra and inter assay CVs were 6.1% and 12% respectively. Plasma IL-6 and MCP-1 were assayed from all four blood samples i.e. baseline, task, 45 and 75 min post-task, whereas HbA1c was analyzed from the baseline sample alone.

### 2.4. Glucocorticoid function assay reagents

PBS Gibco, 500 ml, ref. 2012-019, sterile (Invitrogen); RPMI 1640 medium (Sigma), 500 ml, sterile, R8758; DEX (Sigma), D4902; PRED (Sigma), P-6004; C7291; penicillin/streptomycin (Sigma), 500 ml, sterile, P4458; LPS (Gibco, catalog 20012-019, Lot L-2880); Fetal Calf Serum (Gibco 10270).

### 2.5. Glucocorticoid function assay protocol

The protocol was based on a published method (Carvalho et al., 2008, 2010). Glucocorticoid function was measured by glucocorticoid inhibition of LPS-stimulated IL-6 levels. Whole blood was diluted tenfold with RPMI 1640 medium, supplemented with 10% foetal calf serum. All solutions were prepared in pyrogene-free sterile saline (NaCl 0.9%) to achieve final concentrations in the cultures of 0, 1, 10, 100, 1000 nM for DEX; 0, 1, 10, 100, 1000 nM for PRED. A total of 540 ml of diluted blood (in RPMI 1640 medium with L-glutamine supplemented with 100 IU/ml penicillin and 100 mg/ml streptomycin) was added onto 48-well cell culture plates (Falcon, no. 3078). LPS and glucocorticoids were subsequently added to the wells. Samples were incubated for 24 h in a humidified atmosphere containing 5%  $\text{CO}_2$ . After the incubation, plates were centrifuged ( $1000 \times g$ , 20 min) and supernatant carefully collected and kept at  $-70^{\circ}\text{C}$  until analysis. Each concentration for each drug was performed in duplicates and always by the same researcher.

### 2.6. Stimulated IL-6 production and GC sensitivity

All analysis for IL-6 production and GC sensitivity was carried out using a commercially available Luminex technology kit for IL-6 from Bio-RAD®. The coefficient of variation (CV) for IL-6 analysis was 4.7% within run and 6.5% in between runs, and the detection limit was 2 pg/ml.

Glucocorticoid (GC) inhibition was calculated by considering LPS-stimulated IL-6 levels in the absence of glucocorticoids as 100%. Specifically, the calculation of the percentage inhibition in glucocorticoids condition was as conducted as LPS-induced IL-6 levels in the presence of glucocorticoids, divided by LPS-induced IL-6 levels in the absence of glucocorticoids  $\times 100$ .

Percentage inhibition from each glucocorticoid concentration were inserted in GraphPad Prism (GraphPad Software Inc., San Diego, CA, USA) to calculate one log  $\text{IC}_{50}$  for each time-point. We estimated log  $\text{IC}_{50}$  (instead of raw  $\text{IC}_{50}$ ) due to superior statistical properties. Log  $\text{IC}_{50}$  values are inversely proportional to glucocorticoid sensitivity. Higher  $\text{IC}_{50}$  values indicate that more dexamethasone or prednisolone is needed to suppress cytokine production by 50%, and thus white blood cells are viewed as more resistant to anti-inflammatory signals.

### 2.7. Salivary cortisol over the day

Saliva was collected over the course of a single day within the seven days following the laboratory testing. Participants were asked to provide five samples in salivettes over the course of a normal weekday at waking, at waking plus 30 min, 10–10.30 am, 4–4.30 pm, and 8–8.30 pm. They were instructed not to brush their teeth or eat or drink anything for 15 min prior to sample collection. Information on the day of sampling was recorded in a booklet, which included information on time of waking, and the time each sample was taken. The salivettes and booklet were posted back in a freepost envelope.

### 2.8. Statistical analysis

We computed mean systolic BP, diastolic BP and heart rate for five 5-min periods across the mental stress testing session: baseline (the last 5 min of the 30 min baseline period), the two task periods, and post-stress recovery minutes 40–45 and 70–75. The two task trials were subsequently averaged. We carried out generalized linear regression to assess patterns of change across the session, with group and time of session (morning or afternoon) as between-person factors and trial as the within-person factor. Differences between groups in stress reactivity for subjective stress, cardiovascular variables, cortisol and MCP-1 were assessed by computing difference scores between task and baseline. Stress responses in IL-6, and glucocorticoid sensitivity are delayed in comparison with other measures, so we assessed reactivity as differences between values recorded at 45 min and 75 min compared with baseline. Post-stress recovery in cardiovascular measures, cortisol and MCP-1 was measured using difference scores between task and recovery means. Cortisol was analyzed by computing cortisol area under the curve (AUC) using procedures described by (Pruessner et al.,

2003) to investigate total cortisol output over the day. Units for AUC: (nmol/l)-h.

We log transformed cortisol, IL-6 and MCP-1 to normalize the distribution. All analyses of physiological data were adjusted for BMI and time of session. There were no associations with  $IC_{50}$  and medication types and thus, as covariates in GC sensitivity analysis we controlled for BMI and time of session.

Repeated measures analyses were conducted using all measures (baseline, stress, 45 min post-stress, 75 min post-stress). Our protocol was designed to assess both reactivity and recovery processes. To capture this dynamics the contrasts we reported quadratic trends. Finally, for each outcome we report a within-subjects contrast for the effect of time, which reflects the extent of change for the whole sample over the lab session. We also report a contrast for the group  $\times$  time interaction.

Because of occasional technical difficulties with venepuncture and laboratory equipment, reported degrees of freedom vary slightly from analysis to analysis. All data are reported as mean  $\pm$  SEM unless otherwise noted. Data were analysed using SPSS statistical software package version 22.0 (SPSS Inc., Chicago, IL, USA). All testing was two-tailed with the significance level set at  $p=0.05$ .

### 3. Results

#### 3.1. Participant characteristics

The diabetes group consisted of 23 men and 14 women with an average age of  $64.8 \pm 1.2$  years. The diabetes and healthy control groups did not differ in sex distribution, or the likelihood of being smokers, marital status, or paid employment (Table 1). As expected, HbA1c, BMI and waist to hip ratio were greater in people with diabetes than controls. The majority of diabetic participants were taking oral medications (87%) such as metformin, hypertensive medications (75%) and statins (80%). None of the participants in the non-diabetic control group were taking any medication. Number of participants seen in each time of session did not differ between groups (morning: HC = 12, DM = 15, and afternoon: HC = 25, DM = 22,  $\chi^2 = 0.315$ ).

#### 3.2. Stress changes

##### 3.2.1. Regulation of inflammatory cytokines

There was no difference in plasma IL-6 levels at baseline between diabetes and control groups ( $F_{(1,74)} = 0.506$ ,  $p = 0.48$ ). Analysis revealed that there was an effect of group at 45 min after tasks ( $F_{(1,74)} = 9.005$ ,  $p = 0.005$ ) demonstrating an increase in IL-6 levels following stress only in healthy controls, but no change in diabetes (Table 2). There was no main effect of group on stress reactivity immediately after tasks or at 75 min. However, healthy controls had higher levels of IL-6 at 45 min ( $F = 6.87$ ,  $p = 0.011$ ) and at 75 min after tasks ( $F = 5.56$ ,  $p = 0.022$ ) than at baseline (Fig. 1).

There was no difference in plasma MCP-1 at baseline between diabetes and control groups ( $F_{(1,74)} = 0.737$ ,  $p = 0.39$ ). There was a significant effect of group on MCP-1 stress recovery at 75 min ( $F = 5.367$ ,  $p = 0.024$ ). After stress, MCP-1 levels decreased in diabetes group, but increased

in healthy controls. There were no other significant differences.

##### 3.2.2. IL-6 production in response to stress

Whole blood cells from diabetes participants were less capable of producing IL-6 at baseline after LPS stimulation, when compared with healthy controls at baseline ( $F_{(1,49)} = 5.755$ ,  $p = 0.022$ ). There was no effect of group on stress reactivity or recovery of IL-6 production, when controlled for BMI, age, baseline values and time of session (Fig. 1). The healthy control group showed a decrease in IL-6 production (Mean difference  $-326 \pm 151$ ,  $p = 0.042$ ) in the 45 min recovery compared with baseline, which returned to baseline levels at 75 min recovery (Mean difference  $-9.2 \pm 190$ ,  $p = 0.96$ ). Exposure to stressor did not change IL-6 production in diabetes participants (Fig. 1).

##### 3.2.3. Glucocorticoid sensitivity: Dexamethasone- and prednisolone- inhibition of LPS-induced IL-6 production

The diabetes group had greater dexamethasone ( $F_{(1,39)} = 9.586$ ,  $p = 0.004$ ), but similar prednisolone sensitivity ( $F_{(1,39)} = 1.914$ ,  $p = 0.175$ ) at baseline (Fig. 2). Exposure to the stress tasks influenced the sensitivity of white blood cells to the anti-inflammatory properties of glucocorticoids. Following exposure to the stressor, dexamethasone and prednisolone sensitivity did not change among the diabetes group, whereas it decreased among controls (Fig. 2).

Repeated measures ANOVA revealed the contrasts for time were not significant for dexamethasone ( $F = 0.94$ ,  $p = 0.338$ ), but were significant for prednisolone ( $F = 4.214$ ,  $p = 0.047$ ). There was a significant time vs group interaction for dexamethasone ( $F = 4.011$ ,  $p = 0.050$ ), and for prednisolone ( $F = 4.998$ ,  $p = 0.031$ ), supporting a significant difference in stress reactivity of prednisolone and dexamethasone sensitivity over time between diabetes participants and healthy controls. In healthy controls, there was a progressive desensitization of glucocorticoid and mineralocorticoid sensitivity after stress which reached significance during 45 min of the recovery period compared with baseline (dexamethasone mean difference 45 min,  $-0.18$ ,  $p = 0.012$ ; prednisolone mean difference 45 min,  $-0.39$ ,  $p = 0.034$ ); at 75 min glucocorticoid and mineralocorticoid sensitivity had returned to baseline values (dexamethasone, mean difference  $-0.07$ ,  $p = 0.35$ ; prednisolone mean difference  $-0.16$ ,  $p = 0.39$ ) in healthy controls. There was no change in glucocorticoid or mineralocorticoid sensitivity after stress in the diabetes group.

##### 3.2.4. Physiological responses to mental stress

The mental stress tasks elicited substantial subjective stress responses with increases from  $1.68 \pm (\text{SEM}) 0.19$  to  $4.86 \pm 0.19$  in the diabetes and  $1.38 \pm (\text{SEM}) 0.10$  to  $4.89 \pm 0.18$  in controls. There were no differences in subjective stress at baseline between diabetes and healthy controls ( $F = 0.018$ ,  $p = 0.892$ ). The increase in subjective stress ratings did not differ between groups. In sum, there was no difference in task engagement between groups.

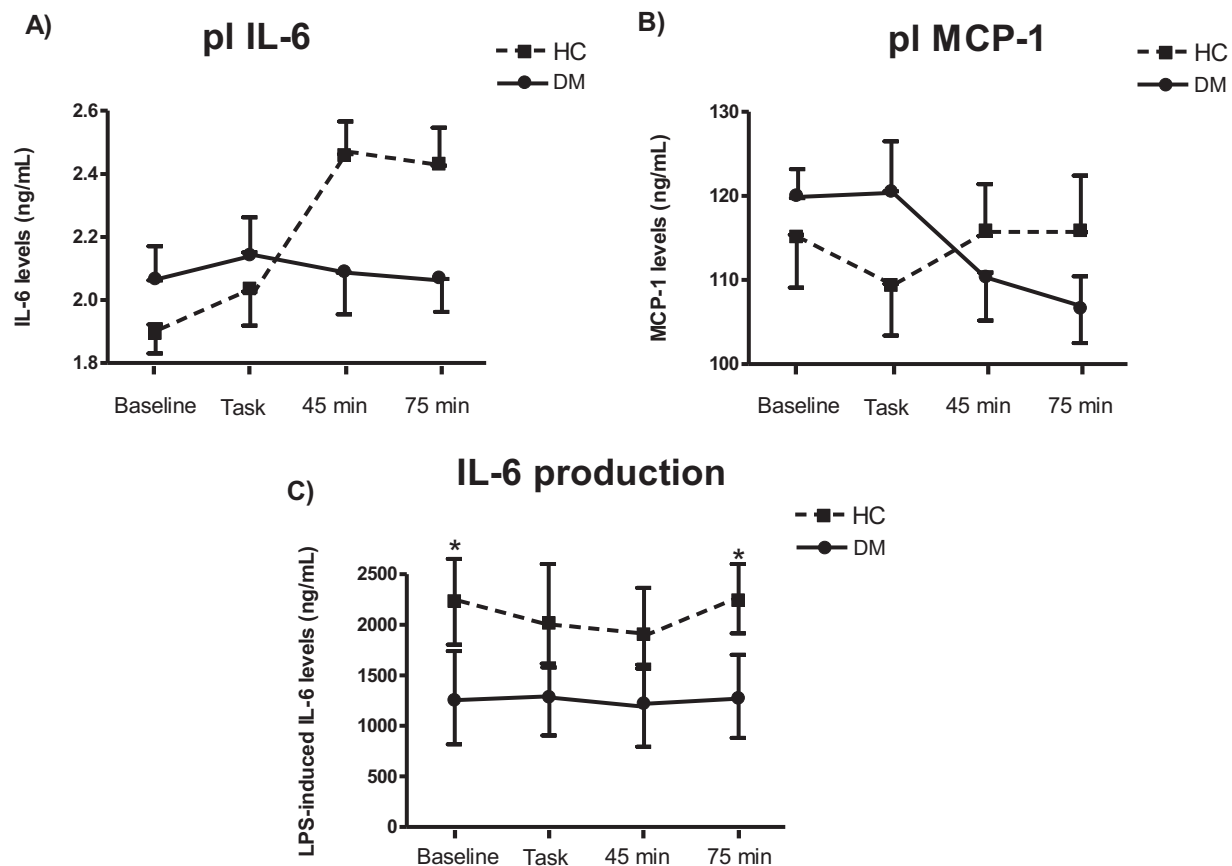
Systolic diastolic BP and heart rate rose during stress tasks, returning towards baseline over the post-stress



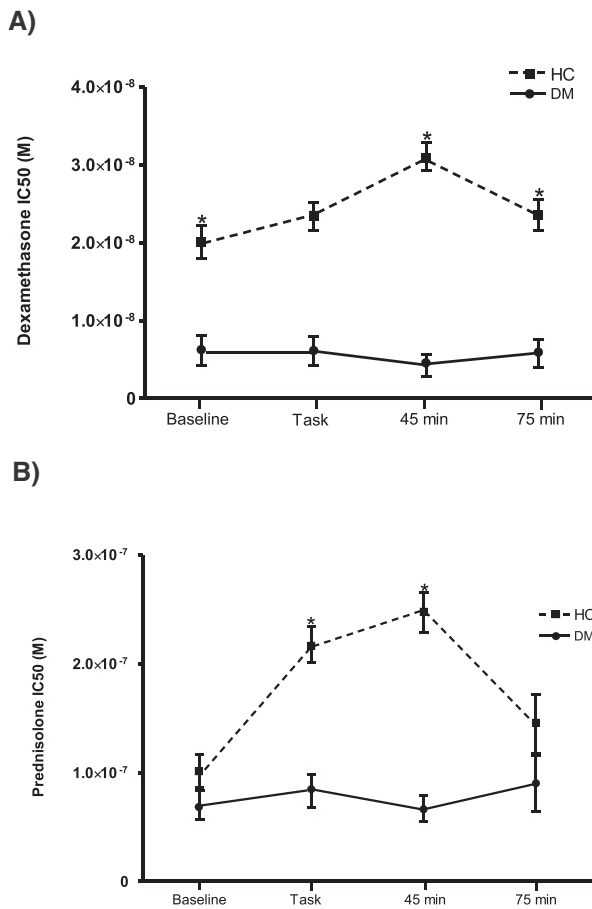
**Table 1** Clinical and demographics characteristics of healthy controls and diabetes.

Demographic characteristics	Diabetes (N= 37)	Control (N= 37)	P value/ $\chi^2$
Age (years)	64.8 ± 1.23	67.53 ± 0.77	0.063
Sex (male, N (%))	23 (64%)	20 (54%)	0.638
Paid employment N (%)	12 (32%)	9 (25%)	0.607
Marital status (single/divorced)	16(43%)	8(22%)	0.041
Income (<40k)	28(76%)	12(32%)	<0.001
Current smokers	3 (8%)	0 (0%)	0.120
HbA1c (%)	7.13 ± 0.18	5.68 ± 0.048	<0.001
Body mass index (kg/m <sup>2</sup> )	26.7 ± 0.7	31.6 ± 1.9	<0.001
WHR	1.01 ± 0.01	0.88 ± 0.01	<0.001
Medication—N (%)			
Statins	31 (84%)	—	
Oral diabetic medication	31 (84%)	—	
Insulin, other diabetic medication	2 (0.05%)	—	
Aspirin	15 (40%)	—	
Beta-blockers	3 (0.08%)	—	
Other anti-hypertensive medication	28 (76%)	—	

Values are mean ± SEM or number of participants (% of sample). CES-D—centre for epidemiologic studies depression (Scale); WHR: waist-to-hip ratio; HbA1c—glycated haemoglobin.



**Fig. 1** Mean IL-6, MCP-1 and IL-6 production in the diabetes (circles) and no diabetes (square) groups sampled at baseline, immediately after tasks, and 45 and 75 min later. Values are adjusted for sex, BMI, and time of testing. Error bars are S.E.M. pl—Plasma; IL—interleukin; LPS—lipopolysaccharide; MCP-1—monocyte chemotactic protein 1; HC—healthy controls; DM—diabetes mellitus.

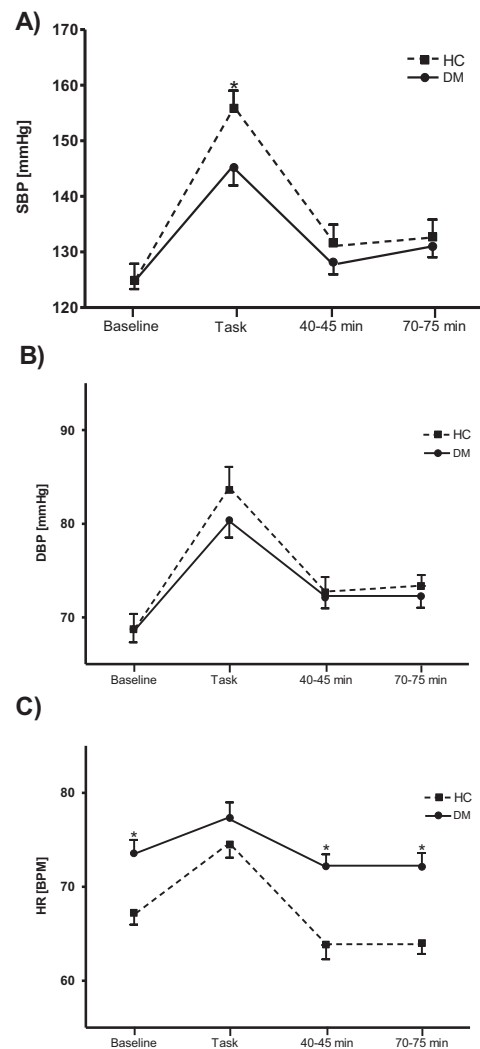


**Fig. 2** Mean IC<sub>50</sub> values for dexamethasone (A) and prednisolone (B) in diabetes (circles) and healthy controls (square). This figure shows that diabetes moderates the impact of acute stress on the immune system's sensitivity to the anti-inflammatory properties of glucocorticoids via GR and MR. IC<sub>50</sub> was calculated by dexamethasone and prednisolone inhibition of LPS-induced IL-6 levels. HC—healthy controls; DM—diabetes mellitus.

recovery period in both groups (Fig. 3). The diabetes group showed a reduced pattern of cardiovascular response to stress. Notably, systolic BP stress reactivity ( $F=5.007$ ,  $p=0.029$ ), and heart rate stress reactivity ( $F=3.327$ ,  $p=0.039$ ) were blunted in the diabetes compared with healthy controls group. Heart rate post-stress recovery at 45 min ( $F=13.624$ ,  $p<0.001$ ) and at 75 min ( $F=8.116$ ,  $p=0.006$ ) were blunted in the diabetes compared with the control (Fig. 3).

### 3.2.5. Cortisol stress reactivity

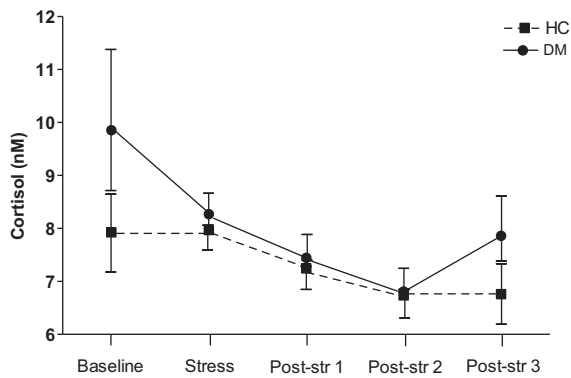
There was no difference in baseline cortisol levels in diabetes compared with healthy controls ( $F_{(1,70)}=1.174$ ,  $p=0.283$ ) when controlled for age, gender, BMI and time of session. There was a main effect of group on cortisol stress response immediately following tasks ( $F=4.214$ ,  $p=0.044$ ), since only in the diabetes group cortisol levels fell. Cortisol levels went back to baseline levels during the recovery period (Fig. 4). There were no main effect of group on cortisol levels measured repeatedly over the session ( $F_{(1,66)}=0.465$ ,  $p=0.49$ ).



**Fig. 3** Mean systolic BP (upper panel), diastolic BP (center panel) and heart rate (lower panel) during baseline, task trials, and 40–45 min and 70–75 min post-tasks in the diabetes (circles) and no diabetes (square) groups. BP data are adjusted for sex, BMI, smoking status and beta-blocker use, and heart rate for sex, BMI, smoking status, oral diabetic medication and beta-blocker use.  $P$  values indicate group differences in stress reactivity (baseline—task difference) and post-stress recovery (task—post-task differences). Error bars are standard errors of the mean (s.e.m.). HC—healthy controls; DM—diabetes mellitus.

### 3.3. Cortisol samples over the day

Diabetes participants had increased cortisol levels when compared to healthy controls as shown by higher AUC over the day (DM  $99.19 \pm 12.62$ , HC  $75.47 \pm 6.04$ ,  $F_{(1,62)}=35.392$ ,  $p<0.001$ ). Diabetes participants had blunted levels of cortisol at awakening (DM  $16.69 \pm 1.72$ , HC  $19.82 \pm 1.53$ ,  $F_{(1,66)}=3.812$ ,  $p=0.05$ ), when controlled for age, gender and BMI. There was no group difference in the cortisol awakening response.



**Fig. 4** Mean salivary cortisol in the diabetes (circles) and no diabetes (square) groups. Cortisol sampled at baseline, immediately after tasks, and 20, 45 and 75 min later. Values are adjusted for sex, BMI, and time of testing. Error bars are s.e.m. HC—healthy controls; DM—diabetes mellitus; post-str1—20 min after task; post-str2—40–45 min after task and post-str3—70–75 min after task.

### 3.4. Correlations

Baseline  $\text{LogIC}_{50}$  was negatively associated with HbA1c levels in the diabetes group (DEX,  $\beta = -0.847$ ,  $t = -6.79$ ,  $p < 0.001$ ; PRED,  $\beta = -0.724$ ,  $t = -5.85$ ,  $p < 0.001$ ) and healthy controls (DEX  $\beta = -0.942$ ,  $t = -8.58$ ,  $p < 0.001$ ; PRED  $\beta = -0.577$ ,  $t = -5.27$ ,  $p < 0.001$ ). Therefore, at baseline resistance to anti-inflammatory signals is negatively associated to poor glycaemic control in both groups. HbA1c inversely associated to MCP-1 stress response at 75 min in diabetic participants ( $\beta = -0.081$ ,  $t = -0.39$ ,  $p = 0.026$ ), but not in healthy controls ( $\beta = -0.054$ ,  $t = -0.24$ ,  $p = 0.142$ ). Thus, the lower MCP-1 stress response in diabetes the worse the glucose regulation. The dexamethasone  $\text{IC}_{50}$  stress response was positively associated to MCP-1 stress response at 45 min in healthy controls ( $\beta = 0.492$ ,  $t = 2.28$ ,  $p = 0.034$ ), but not in diabetes ( $\beta = -0.043$ ,  $t = -0.17$ ,  $p = 0.865$ ). Thus, the higher MCP-1 after stress the higher the resistance to anti-inflammatory signals in healthy controls. The prednisolone  $\text{IC}_{50}$  stress response was positively associated with the IL-6 stress response at 45 min in the diabetes group ( $\beta = 0.518$ ,  $t = 2.37$ ,  $p = 0.032$ ), but the association in controls did not reach significance ( $\beta = 0.527$ ,  $t = 0.186$ ,  $p = 0.081$ ).

## 4. Discussion

This study investigated whether psychological stress, manifested in alterations in dynamic physiological responses to standardized mental stress is associated with impairment of glucocorticoid and mineralocorticoid sensitivity in type 2 diabetes. The diabetes group were more sensitive to the immunosuppressive actions of glucocorticoids in the beginning of the session. Following exposure to stress, both glucocorticoid and mineralocorticoid sensitivity decreased among controls whilst no change was observed in diabetes participants. Glucocorticoid sensitivity was inversely associated with HbA1c indicating that people with higher glucocorticoid sensitivity had poorer control of blood glucose levels. We also found that the diabetes group showed

blunted stress reactivity in systolic BP, heart rate, IL-6, and impaired post-stress recovery in MCP-1 and heart rate. Taking together, diabetes is associated with attenuated responsiveness to psychological stress, and a lack of stress-induced modulation of glucocorticoid and mineralocorticoid sensitivity.

At baseline, diabetes patients had increased glucocorticoid, but normal mineralocorticoid sensitivity in the presence of hypercortisolemia. The action of dexamethasone—which exclusively binds to GR—and of prednisolone—which binds to both GR and MR—is respectively a proxy for glucocorticoid and mineralocorticoid sensitivity (Jurueña et al., 2009). Upon ligand binding, corticosteroid receptors are activated and translocate to the nucleus where it transmits the anti-inflammatory property of corticosteroids. In healthy participants, subsequent GR and MR down-regulation occurs. In the presence of chronic hormone exposure decreased glucocorticoid sensitivity is expected in order to maintain overall effect of cortisol in peripheral tissue. This was not the case in diabetes participants. Contrary to our study, Hudson et al. (1984) showed decreased response in the dexamethasone suppression test in type 1 and type 2 diabetes participants (Hudson et al., 1984). Dexamethasone suppression was also analysed in another large group of diabetes, but without a group of healthy controls direct comparison cannot be made (Kaye et al., 1992). Despite the well-known relationship between circulating aldosterone with insulin resistance (Lastra-Gonzalez et al., 2008; Briet and Schiffrin, 2011), diabetes participants showed normal mineralocorticoid sensitivity at baseline when compared to healthy controls. Similar pattern have been observed in people with post-traumatic stress disorder (Yehuda et al., 2006), and in people with mild depression (Miller et al., 2005), but are in contrast to people with severe major depression (Bauer et al., 2003). Such disturbance in the regulation of the HPA axis may contribute to deterioration in diabetes by enhancing the effect of cortisol and its anti-insulin actions, including the inhibition of glucose uptake in adipocytes and fibroblasts, increasing hepatic gluconeogenesis, sensitizing the liver to catecholamines and glucagon, and elevating blood glucose (Chan et al., 2003). In accordance, we found increased baseline corticosteroid sensitivity associated with poorer glucose control.

Our study suggests that in diabetes the ability to match the body's response to a stressor involves modulation of corticosteroid receptor sensitivity, an evidence of insufficient corticosteroid signalling. Reduced corticosteroid receptor stress responsiveness has been previously shown in the hippocampus of diabetic mice (Chan et al., 2002, 2003, 2005). Reduced glucocorticoid responses to a laboratory stressor have been shown previously in middle-aged men with a high body mass index (Ljung et al., 2000). Insufficient corticosteroid signalling may release inflammatory markers previously under inhibitory glucocorticoid-mediated control and contribute to altered glucose metabolism (Raison and Miller, 2003). In accordance, it was observed by this study and by others that pro-inflammatory cytokines are increased in patients with diabetes, obesity and insulin resistance (Fernandez-Real and Ricart, 1999; Fernandez-Real et al., 2001; Kern et al., 2001). Moreover, insufficient corticosteroid signalling may also increase arousal and thus play a



role in stress-related pathology. Glucocorticoid sensitivity can be activated by  $\beta$ -adrenergic agonists via a cortisol-independent mechanism (Eickelberg et al., 1999). In our study, there was a limited cortisol response to psychological stress even in healthy controls. Nevertheless, corticosteroid sensitivity decreased after stress in healthy controls, but not in diabetes participants. It is thus possible that the reduced corticosteroid stress responsivity in diabetes participants involves impairment of the  $\beta$ -adrenergic pathways.

In accordance to an impairment of  $\beta$ -adrenergic pathways, diabetes participants showed blunted stress reactivity in systolic BP, IL-6 and impaired post-stress recovery in MCP-1 and heart rate. Blunted stress reactivity in diabetes was confirmed by us in a recent larger study (Step toe et al., 2014) where this sub-group came from. Other types of stressor than the one used in this study – such as socially-evaluative threat – are associated with bigger activation of the HPA axis (Dickerson and Kemeny, 2004; Gruenewald et al., 2004). Biological responses to stress in healthy controls were of similar magnitude than what has been previously described (Step toe and Cropley, 2000; Step toe et al., 2002b, 2007; Hamer et al., 2010; Hackett et al., 2012).

This study has several limitations. It was cross sectional in nature and no causal conclusions can be drawn. Our study did not greatly alter salivary cortisol levels possibly due to the small sample size, and results may differ if other types of stressors were used. Our interpretation must take into account that cortisol plasma levels responses were not reflected in absolute increases in levels, but in differences in rates of decline. This is likely due to the overlay of diurnal cortisol rhythms. Cortisol stress reactivity might have been different if all experiments were conducted in the afternoon, when cortisol diurnal rhythm is not so obvious, though this factor is unlikely to have influenced our results since all analysis were corrected for time of session. In any case, corticosteroid sensitivity function seems to be more sensitive to stress-related changes than actual cortisol levels. Although studies have shown that measuring cortisol profile over one day only is reliable (Kraemer et al., 2006), measuring cortisol profile over two days might better reflect actual HPA axis disturbances. Nevertheless, these findings shed additional light on stress-related mechanisms in diabetes participants.

## 5. Conclusion

We have shown that diabetes is associated with a lack of stress-induced modulation of glucocorticoid and mineralocorticoid sensitivity. Emphasis on modulating glucocorticoids in stress-related pathology encourages development of therapeutic strategies to modulate glucocorticoid-signalling pathways.

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## Conflict of interest statement

The authors have no conflicts of interest to declare.

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