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Higher body mass index (BMI) is associated with reduced glucocorticoid inhibition of inflammatory cytokine production following acute psychosocial stress in men

Petra H. Wirtz [a,*], **Ulrike Ehlert** [a], **Luljeta Emini** [a], **Tobias Suter** [b]

[a] *Department of Clinical Psychology and Psychotherapy, University of Zurich, Switzerland*
[b] *Clinical Immunology, Department of Internal Medicine, University Hospital, Zurich, Switzerland*

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Summary
Background: Body mass index (BMI) and mental stress seem to exert part of their cardiovascular risk by eliciting inflammation. However, the adverse effects of stress on inflammatory activity with BMI are not fully understood. We investigated whether higher BMI is associated with reduced glucocorticoid inhibition of inflammatory cytokine production following stress in men while controlling for age and blood pressure. We measured glucorticoid inhibition of lipopolysaccharide (LPS)-stimulated release of the proinflammatory cytokine tumor necrosis factor (TNF)-α.
Methods: Forty-two men (age range 21—65 years; BMI range 21—34 kg/m^2) underwent the Trier Social Stress Test (combination of mock job interview and mental arithmetic task). Whole blood samples were taken immediately before and after stress, and during recovery up to 60 min post-stress. Glucocorticoid sensitivity of LPS-stimulated TNF-α expression was assessed in vitro with and without coincubating increasing doses of dexamethasone. Moreover, salivary cortisol was measured during the experiment and on a normal day for assessment of baseline circadian cortisol.
Results: Higher BMI was associated with lower glucocorticoid sensitivity of monocyte TNF-α production after stress (main effect of BMI: $p < 0.001$) and with more pronounced decreases of glucocorticoid sensitivity following stress (interaction of stress-by-BMI: $p = 0.002$). Neither LPS-stimulated TNF-α release nor baseline glucocorticoid sensitivity were associated with BMI. Similarly, BMI was not associated with salivary cortisol, either in reaction to stress or in circadian cortisol secretion.
Conclusions: Our data suggest that with increasing BMI, glucocorticoids are less able to inhibit TNF-α production following stress. This might suggest a new mechanism linking BMI with elevated risk for adverse cardiovascular outcomes following stress.

* Corresponding author. Department of Clinical Psychology and Psychotherapy, University of Zurich, Binzmühlestrasse 14/Box 26, CH-8050 Zurich, Switzerland. Tel.: +41 44 635 7367; fax: +41 44 635 7359.
E-mail address: p.wirtz@psychologie.uzh.ch (P.H. Wirtz).

1. Introduction

The risk of cardiovascular disease and the underlying process of atherosclerosis increases with increasing body mass index (BMI) (Jee et al., 2006). Particularly obesity, the severe form of overweight, is a main independent risk factor for atherosclerosis and heart failure (Kenchaiah et al., 2002).

Atherogenesis is an inflammatory process occurring in the vessel wall (Ross, 1999). Monocytes infiltrate the vessel, produce cytokines and chemokines and thereby attract more leukocytes and propagate the inflammation (Ross, 1999). There is evidence that elevated inflammatory activity with increasing BMI plays an important role in linking higher BMI with increased incidence of atherosclerosis: animal and human studies suggest that obesity and obesity-related parameters like BMI are associated with elevated levels of inflammatory cardiovascular risk markers including monocyte-derived inflammatory cytokines like tumor necrosis factor (TNF)-α (Yamakawa et al., 1995; Visser et al., 1999; Ridker et al., 2000; Elkind et al., 2002; Laimer et al., 2002; Bray, 2004; Pai et al., 2004; Pickup, 2004; Vendrell et al., 2004; Gonzalez et al., 2006). Indeed, a very recent study found elevated inflammatory activity in obese men with acute myocardial infarction (Piestrzeniewicz et al., 2007). The mechanisms that link higher BMI with the presence of a low-grade systemic inflammation and thereby with atherosclerotic risk are beginning to be understood. In addition to genetic predispositions (Esteve et al., 2006), human adipose tissue seems to be involved in up-regulation of inflammation as it expresses and releases proinflammatory cytokines (Hotamisligil et al., 1995; Creely et al., 2007).

An additional mechanism involved in the association between BMI and inflammation might be related to mental stress, a further risk factor for cardiovascular disease and particularly for acute coronary syndromes (ACS) (Hemingway and Marmot, 1999; Strike and Steptoe, 2004; Strike and Steptoe, 2005; Bhattacharyya and Steptoe, 2007). A recent review by Steptoe and co-workers suggests that acute mental stress induces increases in circulating inflammatory factors within less than 2 h (Steptoe et al., 2007). Such increased inflammatory activity might in turn be involved in mediation of ACS risk following acute stress especially in at-risk persons (Gidron et al., 2002; Steptoe et al., 2006; Bhattacharyya and Steptoe, 2007; Steptoe et al., 2007). Indeed, elevated cytokine and CRP levels were found in patients with preinfarction unstable angina within 3 h of symptom onset (Liuzzo et al., 1999).

However, mental stress also induces activation of the hypothalamus-pituitary-adrenal (HPA) axis, leading to glucocorticoid (GC) secretion which, especially in high doses, effectively down-regulates inflammatory activity (Sapolsky et al., 2000). The effects of endogenously released GCs on target tissues strongly depend on the sensitivity of these tissues to GCs (Rohleder et al., 2003). Several studies demonstrated that the GC sensitivity of pro-inflammatory cytokine production is dynamically regulated by psychosocial stress (Rohleder, 2003; Rohleder et al., 2003). Therefore, a potential GC down-regulation of an elevated inflammatory status following psychosocial stress (e.g. following an acute emotional ACS trigger) depends not only on the endogenous GC level but also on stress-induced changes in the sensitivity of inflammatory target tissues to GCs.

Whereas most studies found basal cortisol secretion and cortisol circadian rhythm to be usually normal even in obese persons, some studies report higher cortisol excretion in abdominally obese individuals (Pasquali et al., 2006). Thus, basal HPA activity and cortisol release are not strongly positively related to BMI, if at all. However, the secretion of cortisol after experimental stress induction is elevated with increasing obesity (Epel et al., 1999; Pasquali et al., 2006). Therefore, one would rather expect similar or even lower inflammatory activity with increasing BMI. However, to the best of our knowledge, associations between BMI and stress-induced changes in the ability of GCs to down-regulate inflammatory activity have not yet been studied.

We therefore wondered whether, with increasing BMI within the normal to mildly overweight range, the inflammatory status would be altered following acute stress through modulation of the GC-induced suppression of the monocyte proinflammatory response (i.e. monocyte GC sensitivity). Such an alteration might provide a new biological mechanism linking BMI with elevated levels of inflammatory markers and thereby with elevated risk of ACS following stress. We assessed the GC sensitivity of lipopolysaccharide (LPS)-induced TNF-α production in whole blood samples of men with a BMI ranging broadly from 21 to 34 kg/m^2. Study participants underwent a standardized psychosocial stress test (Trier Social Stress Test, TSST) (Kirschbaum et al., 1993) and we measured GC sensitivity of monocyte TNF-α production at different time points before and after the stressor. We decided to measure TNF-α because it plays a crucial role in the initial activation of inflammatory changes such as stimulation of C-reactive protein production by the liver thought to sustain atherosclerosis development (Plutzky, 2001). Moreover, it contributes to the entry of inflammatory cells into the arterial wall by induction of adhesion molecule expression (Pober and Cotran, 1991; Plutzky, 2001) and it mediates T-cell activation as well as foam cell formation (Ross, 1993; Ross, 1999). To obtain a single measure to assess GC sensitivity, we determined the amount of dexamethasone required to suppress the LPS-stimulated release of TNF-α by 50%. We additionally measured salivary cortisol before and several times after stress to assess the amount of stress-induced GC secretion, as well as a circadian cortisol profile.

2. Materials and methods

2.1. Study participants

The study was part of a larger project (Wirtz et al., 2006; Wirtz et al., 2007a; Wirtz et al., 2007b). We obtained complete GC sensitivity data from 42 men who provided written informed consent and fulfilled the inclusion criteria outlined below. Study participants varied in age (range 21—65 years), screening systolic (range 108—167 mmHg) and diastolic (range 66—114 mmHg) blood pressure (BP), and BMI (range 21—34 kg/m^2). Recruitment was carried out through advertisement at the University of Zurich, and with the help of the Swiss Red Cross of the State of Zurich. The Ethics Committee of the State of Zurich, Switzerland formally approved the study protocol.

All participants were required not to take any regular or occasional medication, to be non-smokers, and to be in excellent physical and mental health as confirmed by an

extensive health questionnaire and telephone interview. We excluded subjects who reported any clinical psychosomatic and psychiatric diseases, allergies and atopic diathesis, heart disease, varicosis or thrombotic diseases, elevated cholesterol, elevated blood sugar and diabetes, liver and renal diseases, chronic obstructive pulmonary disease, rheumatic diseases, current infectious diseases and regular heavy physical exercise, alcohol and illicit drug abuse. The general practitioner was contacted if the personal or medication history was inconclusive. All eligible subjects provided three seated BP assessments after a 15-min rest using a fully automated sphygmomanometry device (Omron 773, Omron Healthcare Europe B.V. Hoofddorp, The Netherlands) on three different days. We calculated the average mean arterial BP (MAP) by the formula (2/3 × diastolic BP) + (1/3 × systolic BP) (screening BP). BMI was calculated as the ratio of weight in kilograms to height in square meters.

2.2. Stress protocol

Subjects reported to the lab between 1400 and 1600 h after having abstained from physical exercise, alcohol, and caffeinated beverages since the previous evening. We applied the standard protocol of the widely used TSST (Kirschbaum et al., 1993), which combines a short introduction followed by a 5-min preparation phase, a 5-min mock job interview, and a 5-min mental arithmetic task in front of an audience (Kirschbaum et al., 1993). After the TSST, subjects remained seated in a quiet room to recover.

Blood samples were obtained via an indwelling catheter under resting conditions 1 min before subjects were introduced to the TSST and immediately after completion of the TSST as well as 20 and 60 min after completion of the TSST. To assess cortisol stress reactivity, samples of saliva were taken by Salivette collection devices 1 min before subjects were introduced to the TSST in order to assess resting levels as well as immediately thereafter, and 10, 20, 30, 40, 50 and 60 min after completion of the TSST.

Circadian profiles of cortisol secretion during the day were also assessed. Subjects were instructed to collect saliva samples within 1 week after the TSST using the Salivette system (see below) during a normal work day. Sampling intervals were at 0800, 1100, 1600, and 2000 h, as previously described (Gaab et al., 2005). Subjects were asked to refrain from eating and drinking for 30 min before each sample was taken. All instructions were given verbally as well as through detailed written information accompanying the sampling tubes. Self-reports of the exact sampling time as well as electronic monitoring devices (MEMS Track Cap, Aardex, Switzerland) were used to control the sampling time. No subject had to be excluded from the analysis because of sampling time. All saliva samples were stored in a refrigerator until completion of sampling and were then sent to our laboratory and stored at −20 °C until biochemical analysis.

2.3. Glucocorticoid sensitivity assay

Using heparinized syringes (Vacutainer, Becton Dickinson), 5 ml of venous blood were drawn for the whole blood GC sensitivity assay, and 2.7 ml of blood were drawn into EDTA monovettes (Sarstedt, Rommelsdorf, Germany) for a differential blood count on an automated hematology system (Advia 120, Bayer Diagnostics). Heparinized whole blood of each subject was diluted 10:1 with saline and dispensed into 24-well cell culture plates (Becton Dickinson, San Diego, California) at 400 μl per well. All aliquots were incubated with equal amounts of the bacterial endotoxin lipopolysaccharide (LPS, *Escherichia coli*, 055:B5, L-2880, Sigma, Deisenhofen, Germany) to stimulate TNF-α production by monocytes. Each sample was co-incubated with various concentrations of dexamethasone (DEX, D-8893, Sigma, Deisenhofen, Germany). The final concentrations in the respective well were 30 ng/mL LPS, and 0, 10^{-9}, 10^{-8}, 5×10^{-8}, 10^{-7}, 10^{-6} M DEX, respectively. After 18 h of incubation at 37 °C and 5% CO_2, the plates were centrifuged for 10 min at 2000 × *g* and 4 °C and the supernatant was collected and stored at −80 °C until analysis at completion of the study.

Concentrations of TNF-α were measured by commercial enzyme-linked immunosorbent assay (ELISA; BD Pharmingen, San Diego, California). The detection limit of the ELISAs was 7.8 pg/ml for TNF-α. All cytokine measurements were done in duplicates. Plates were read by a microplate reader (Synergy HT Multi-Detection Microplate Reader, BioTek Instruments, Vinooski, Vermont, USA), and absorbance was transformed to cytokine concentration (ng/ml) using a standard curve computed by KC4 software (BioTek Instruments, Winooski, Vermont, USA). Cytokine levels were corrected for number of monocytes in the respective blood sample because monocytes are the main source of TNF-α in LPS-stimulated whole blood (Wright et al., 1990). As an index for GC sensitivity, we calculated the inhibitory concentration 50 (IC50) of each individual dose-response curve for DEX inhibition of LPS-induced cytokine production. The IC50 reflects the specific DEX concentration ($\times 10^{-8}$ M) required for 50% inhibition of the maximum cytokine production observed after LPS-stimulation without DEX. The IC50 is inversely related to GC sensitivity of the respective cytokine production.

2.4. Cortisol assay

For assessment of salivary free cortisol levels, saliva was collected using Salivette collection devices (Sarstedt, Rommelsdorf, Germany) and stored at −20 °C until biochemical analysis. Saliva samples were thawed and spun at 3000 rpm for 10 min yielding low-viscosity saliva. Cortisol concentrations were measured using a commercially available competitive chemiluminiscence immunoassay with high sensitivity of 0.16 ng/ml (LIA, IBL Hamburg). Intra- and inter-assay variability were <7.7 and 11.5%, respectively.

2.5. Statistical analyses

Data were analyzed using SPSS statistical software package version 12.0 (SPSS Inc., Chicago, IL, USA). IC50 values were calculated using Graphpad Prism version 4.0 (GraphPad Software Inc., San Diego, CA, USA). All testing was two-tailed with the significance level set at $p \leq 0.05$. Normal distribution of data was verified by the Kolmogorov-Smirnov test. Missing data were excluded listwise.

To test for an association between BMI and baseline GC sensitivity, we calculated an ANCOVA with IC50 of TNF-α at baseline (IC50 −1 min) as dependent variable and BMI as *continuous* independent variable while controlling for age

and MAP. We controlled for MAP, since we found previously that hypertensives show a higher TNF-alpha release upon LPS-stimulation and a lower GC sensitivity (Wirtz et al., 2004). To assess whether GC sensitivity following stress differs with increasing BMI, we performed general linear models with repeated measurement for GC sensitivity of LPS-stimulated TNF-α secretion. We used the IC50s of TNF-α as repeated dependent variable and BMI as *continuous* independent variable. As covariates, we controlled for baseline GC sensitivity (IC50 −1 min), age, and MAP (Rohleder et al., 2002; Wirtz et al., 2004). Associations between BMI and LPS-stimulated release of TNF-α without dexamethasone inhibition (0 M DEX) were calculated accordingly. As post-hoc tests of GC sensitivity results, we used partial correlations between BMI and each IC50 post-stress time point controlling for age and MAP. To assess cortisol stress reactivity and circadian cortisol secretion, we also performed general linear models with repeated measurement. For cortisol stress reactivity, we used the cortisol measures before and after stress as repeated dependent variable and controlled for age, MAP, and the cortisol baseline levels, while BMI served as continuous independent variable. For cortisol daytime profiles, daytime cortisol levels were used as dependent and BMI as continuous independent variable, while we controlled for age and MAP. To test whether cortisol stress reactivity is associated with stress-induced changes in GC sensitivity and LPS-stimulated release of TNF-α, we recalculated all stress reactivity analyses and additionally included measures of cortisol. As measures of cortisol stress reactivity, we used a previously published formula to compute the area under the curve with respect to increase (AUCi) (Pruessner et al., 2003) and calculated a maximum change score for cortisol from baseline to 20 min post-stress. To test whether LPS-stimulated TNF-α levels were associated with changes in GC sensitivity, we again recalculated general linear models for IC50s. We separately included LPS-stimulated TNF-α levels of each time point, as well as TNF-α AUCi and maximum change scores from baseline to immediately after stress.

The Huynh-Feldt correction for repeated measures was applied.

For illustrative purposes, we categorized the study group by median split based on their BMI into two groups of subjects with lower and higher BMI.

3. Results

3.1. Subjects' characteristics

As depicted in Table 1, the mean BMI of the 42 men studied was 25.8, the mean age was 43.3, and mean MAP was 101.7. Four persons were obese (BMI $\geq$ 30).

3.2. Glucocorticoid sensitivity

Means ± SEM for IC50s of TNF-α were 1.11 ± 0.10 (−1 min), 1.52 ± 0.11 (+1 min), 1.33 ± 0.12 (+20 min), and 1.67 ± 0.09 (+60 min).

3.2.1. Glucocorticoid sensitivity at rest

Baseline GC sensitivity (IC50 −1 min) was not associated with BMI ($F(1/38) = 0.028$, $p = 0.87$). We controlled for age and MAP as covariates.

Table 1 Characteristics of the 42 subjects studied

Body mass index (kg/m^2)	25.8 ± 0.5 (20.7–34.3)
Age (years)	43.3 ± 2.2 (21–65)
Mean blood pressure (mmHg)	101.7 ± 1.9 (82.8–131.6)
Normal weight (*n*, %)	19, 45.2%
Overweight (*n*, %)	19, 45.2%
Obese (*n*, %)	4, 9.5%

Values are given as means ± SEM (range), normal weight: BMI < 25, overweight: BMI 25–29.9, obese: ≥30.

3.2.2. Stress changes

To test whether BMI is associated with changes in GC sensitivity of stimulated TNF-α release following stress, we applied general linear models with repeated measures of IC50s of TNF-α as dependent variables and BMI as continuous independent variable. Baseline GC sensitivity, age and MAP were controlled for. Higher BMI was associated with higher IC50s of TNF-α (i.e. lower GC sensitivity) after stress (main effect of BMI: $F(1/37) = 19.6$, $p < 0.001$, $f = 0.68$) and with higher IC50 increase following stress (interaction of stress-by-BMI: $F(3.0/111) = 5.2$, $p = 0.002$, $f^2 = .37$). Exclusion of the four obese participants did not significantly change the results. Post-hoc tests revealed that we did not capture the full time course for IC50s of TNF-α, as compared to the baseline they were still significantly elevated 60 min after stress cessation ($p < 0.001$). Post-hoc tests using partial correlations controlling for age and MAP revealed that BMI significantly correlated with IC50 of TNF-α immediately ($r = 0.42$, $p = 0.007$) as well as 60 min after stress ($r = 0.42$, $p = 0.007$) but not 20 min after stress ($r = 0.21$, $p = 0.19$).

Our analysis with BMI entered as continuous variable is statistically strong but does not allow a graphical representation of our results. In order to visualize the data (but not used for modeling and testing), we grouped our study subjects by median split into two groups of subjects with either higher BMI (individual BMI above median BMI, $n = 21$, mean ± SEM: 27.99 ± 0.59) or lower BMI (individual BMI below median BMI, $n = 21$, mean ± SEM: 23.65 ± 0.26). Fig. 1 shows the mean IC50s of TNF-α before and in response to the TSST in the two groups of subjects with lower and higher BMI.

3.3. Stimulation of cytokine production

Means ± SEM for LPS-stimulated release of TNF-α without GC inhibition were 5540 ± 380 (−1 min), 3905 ± 266 (+1 min), 4450 ± 272 (+20 min), and 4967 ± 374 (+60 min) (ng/10^5 monocytes).

We tested associations between BMI and LPS-stimulated release of TNF-α without dexamethasone inhibition according to calculations on IC50s. There were no associations between BMI and LPS-stimulated release of TNF-α without dexamethasone inhibition, either at rest ($p = 0.63$) or in reaction to stress (main effect BMI: $p = 0.98$; interaction stress-by-BMI: $p = 0.84$).

Next, we tested whether LPS-stimulated TNF-α levels were associated with changes in GC sensitivity. We recalculated general linear models for IC50s and separately included LPS-stimulated TNF-α levels of each time point, as well as AUCi and maximum change scores. LPS-stimulated TNF-α measures were not associated with repeated measures of

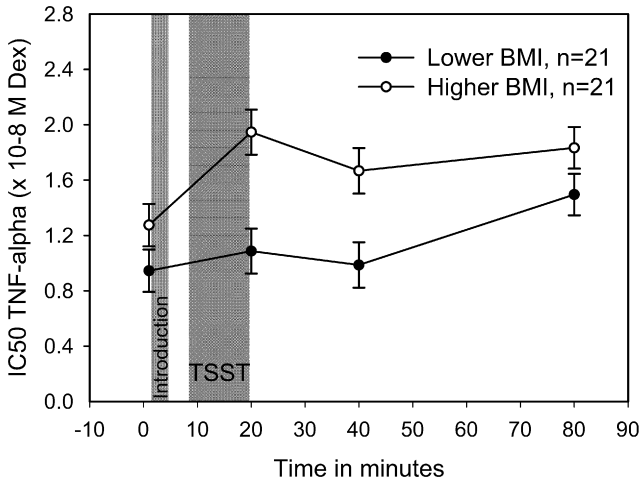

Figure 1 Glucocorticoid sensitivity in subjects with lower and higher BMI before and after stress. We calculated general linear models with repeated measures of IC50s of TNF-α as dependent variables and BMI as continuous independent variable while controlling for baseline glucocorticoid sensitivity, age, and MAP. Higher BMI was associated with higher IC50s of TNF-α (i.e. lower glucocorticoid sensitivity) after stress (main effect of BMI: $F(1/37) = 19.6$, $p < 0.001$, $f = 0.68$, $n = 42$) and with higher IC50 increase following stress (interaction of stress-by-BMI: $F(3.0/111) = 5.2$, $p = 0.002$, $f^2 = 0.37$, $n = 42$). IC50 is given as 10^{-8} mol/l. Values are means $\pm$ SEM.

IC50 of TNF-α (main effects and interaction effects: p's > 0.23), nor did they significantly change the reported associations between BMI and GC sensitivity.

3.4. Cortisol stress reactivity and daytime profile

To test whether BMI is associated with changes in cortisol release in response to stress, we applied general linear models with repeated measures of cortisol as dependent variables and BMI as continuous independent variable. Baseline cortisol, age and MAP were controlled as covariates. BMI was not associated with cortisol levels, either before ($p = 0.88$) or after stress ($p = 0.42$), or in response to stress ($p = 0.60$). Similarly, there were no associations between BMI and circadian cortisol profiles (p's > 0.90).

Fig. 2A shows cortisol levels before and after the TSST in two groups of subjects with lower ($n = 19$) and higher BMI ($n = 20$). Fig. 2B shows cortisol levels during the day in two groups of subjects with lower ($n = 17$) and higher BMI ($n = 19$).

We tested whether cortisol stress reactivity was associated with changes in GC sensitivity or stimulated TNF-α responses without GC inhibition. In terms of GC sensitivity, cortisol AUCi and MaxInc scores were neither associated with stress reactivity (main effects and interaction effects: p's > 0.20), nor did inclusion of cortisol stress reactivity measures change the significance of the reported associations between BMI and GC sensitivity. In terms of LPS-stimulated TNF-α release, cortisol stress reactivity was associated with lower TNF-alpha levels before and after stress (main effect cortisol: AUCi: $F(1/33) = 4.0$, $p = 0.05$; MaxInc: $F(1/33) = 4.1$, $p = 0.05$) but not with altered stress reactivity (interaction effects: p's > 0.40).

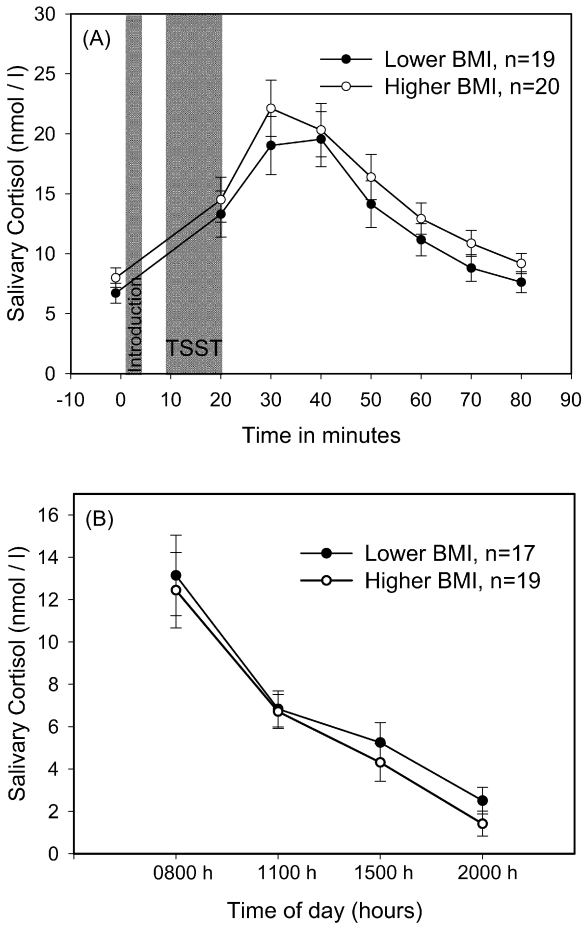

Figure 2 (A and B) Cortisol secretion in subjects with lower and higher BMI over time. ANCOVA analyses with age and MAP as covariates did not reveal associations between BMI (entered as continuous variable) and cortisol secretion (p's > 0.42), either with respect to stress (Fig. 2A, $n = 39$) or with respect to circadian secretion (Fig. 2B, $n = 36$). Values are means $\pm$ SEM.

4. Discussion

This is the first study to examine associations between BMI and stress-induced changes in the ability of GCs to down-regulate monocyte inflammatory activity. We were interested in whether following acute stress, stimulated release of TNF-α would be altered with increasing BMI through modulation of the GC sensitivity of blood monocytes.

The main finding of our study was that following acute psychosocial stress, increasing BMI was associated with higher IC50 of monocyte TNF-α production and thereby lower GC sensitivity. These results showed a large effect size. Post-hoc analyses indicate that this association is carried by the time points immediately after and 60 min after stress. In other words, the higher the BMI, the more dexamethasone was needed to suppress stimulated cytokine release after stress, particularly immediately after and 60 min after stress. This suggests that with increasing BMI, inflammatory activity following stress is less effectively down-regulated by GCs. Baseline GC sensitivity, however, was not associated with BMI. Moreover, as BMI was associated neither with altered cortisol stress reactivity nor with differences in

circadian cortisol secretion, it seems unlikely that increased endogenous GC release could compensate the less effective GC action. Thus, the net effect of GC inhibition on monocyte inflammatory activity following stress seems to be reduced with increasing BMI. There were no associations between BMI and LPS-stimulated release of TNF-α without dexamethasone inhibition, either at baseline or in response to stress.

To the best of our knowledge, our study is the first to investigate associations between GC down-regulation of inflammatory activity and BMI or other obesity-related parameters, both at baseline and in reaction to psychosocial stress. Our finding of a non-significant association between circardian cortisol secretion is in line with previous studies (Pasquali et al., 2006). However, in contrast to previous research, we could not find elevated cortisol stress reactivity with increasing obesity or BMI, respectively (Rosmond et al., 1998; Epel et al., 1999; Pasquali et al., 2006). One explanation for this difference might relate to the fact that most of our subjects were within the normal to mildly obese range, whereas most of the other studies mainly included severely obese participants. In addition, we recruited apparently healthy, unmedicated men who reported reasonable health behavior.

Our results might have important clinical implications. With respect to cardiovascular risk, one might assume that mental stress increases the BMI-related risk of long-term progression of atherosclerosis in healthy stress-responsive people as it is likely that people who are more stress responsive in the laboratory are also more responsive to stress encountered in daily life. Moreover, mental stress might increase cardiovascular risk in overweight persons by lowering the capacity of GCs to down-regulate monocyte cytokine release. An increasing line of evidence indeed indicates that emotional events including acute stress trigger ACS (Bhattacharyya and Steptoe, 2007), but the underlying biological mechanisms are only partly understood (Strike et al., 2006). The mechanism of stress-induced reduction in the capacity of GCs to inhibit inflammation might be involved in stress-induced ACS triggering: ACS patients exhibit increased inflammatory activity, thought to trigger ACS onset (Brueckmann et al., 2004; Pasqui et al., 2005), and heightened inflammatory activity was shown to correlate with increasing BMI (Yamakawa et al., 1995; Visser et al., 1999; Ridker et al., 2000; Elkind et al., 2002; Laimer et al., 2002; Bray, 2004; Pai et al., 2004; Pickup, 2004; Vendrell et al., 2004; Gonzalez et al., 2006). Via such a mechanism, stress might potentiate the cardiovascular and ACS risk in overweight people. Notably, diseased patients' responses often differ from those of healthy people. Therefore, our results in apparently healthy persons should be interpreted with caution.

The molecular mechanism leading to reduced GC sensitivity of inflammatory cytokine production after stress remains to be investigated. Obesity and thereby a high BMI is related to a number of mechanisms responsible for reduced GC sensitivity of inflammatory activity (Bamberger et al., 1996; Rohleder et al., 2003). In a very elegant animal study, the development of obesity was related to overexpression of 11β-hydroxysteroid dehydrogenase (11-β-HSD-1) (Masuzaki et al., 2001). This enzyme reduces intracellular GC availability and thereby lowers GC sensitivity (Bamberger et al., 1996). In addition, mental stress might increase 11-β-HSD activity (Hardy et al., 2002). However, it remains open whether potential stress-induced up-regulation of 11-β-HSD activity correlates with increasing BMI. A second mechanism underlying our finding of lower GC sensitivity with stress might relate to lowered expression of GC receptors with increasing overweight. Central GC receptors seem to be down-regulated in obesity, as evidenced by cortisol suppression after oral dexamethasone intake (Ljung et al., 1996). Although GC sensitivity of the HPA axis feedback loop and GC sensitivity of inflammatory activity seem to be unrelated (Ebrecht et al., 2000), it cannot be ruled out that GC receptors on leukocytes are also down-regulated in overweight subjects. Indeed, adipose tissue produces excess GCs (Pasquali et al., 2006), which in turn could down-regulate GC receptor expression (Burnstein et al., 1991). However, as BMI was not related to altered cortisol stress reactivity, it is unlikely that the stress-induced changes in GC sensitivity are due to excess cortisol secretion following stress. A further mechanism known to down-regulate GC receptors relates to elevated levels of inflammatory markers, such as observed in obesity. Inflammatory cytokines are capable of inducing relative over-expression of the dominant-negative GC receptor β isoform, which competes with the ligand-binding GC receptor α in DNA binding (Rohleder et al., 2003). Indeed, acute mental stress rapidly induces activation of the nuclear factor κB (NF-κB) in macrophages, probably via stress-induced norepinephrine release (Bierhaus et al., 2003; Black, 2006). NF-κB in turn induces inflammatory cytokine production (Auphan et al., 1995), but the role of BMI needs to be elucidated. Genetic variations associated with obesity as a part of the metabolic syndrome may also play a role (Di Blasio et al., 2003; Buemann et al., 2005). An additional mechanism which might down-regulate GC receptors relates to chronic psychological stress or depression, as depression has been associated with BMI and HPA dysregulations (Kloiber et al., 2007). However, our data gave no indication of modulating effects of depression or vital exhaustion (questionnaires are described elsewhere (Wirtz et al., 2007a)) on the observed association between BMI and GC sensitivity (data not shown).

Our study has several strengths, including the recruitment of apparently healthy, non-smoking, drug-free subjects within the normal to mildly obese range. This is important as it allows us to assess the linear impact of BMI levels within a more "normal" range, which has a higher prevalence in the general Swiss population as compared to obese BMI levels. In addition, we excluded confounding by smoking or by medication intake such as the use of GCs. Moreover, potential confounding factors were reduced in the analyses by a priori controlling for age, blood pressure, and baseline GC sensitivity (Rohleder et al., 2002; Wirtz et al., 2004) without statistical overcontrolling given our sample size (Babyak, 2004). Furthermore, we used a statistical approach allowing assessment of the linear associations between BMI entered as continuous variable and each of the sampling time points. This approach extends multiple linear regression analyses: it does not require data reduction of the dependent variables to a single integrated measure but allows full inclusion of all sampling time points even accounting for repeated measurement. Thus, we achieved a high statistical power given our data set. However, the study also has limitations. First, the cross-sectional design of our study does not allow us to draw

conclusions about the direction of the association between BMI and altered GC sensitivity after stress. GC resistance and heightened inflammatory stress responses may contribute to increases in BMI. For example, chronic stress exposure and elevated GCs can result in an increased risk of weight gain (Brunner et al., 1997; Bjorntorp, 2001; Brunner et al., 2007). Moreover, inflammatory cytokines such as TNF-α have profound effects on adipose tissue, insulin resistance, lipid metabolism and adipogenesis (Cawthorn and Sethi, 2008).

Prospective designs are needed to elucidate whether stress-induced alterations in GC sensitivity are a cause, a consequence, or a concomitant phenomenon of higher BMI. Second, our findings cannot be generalized to populations other than apparently healthy men within the normal to mildly obese range, such as women, severely obese persons or persons with overt cardiovascular disease. Third, we did not measure any obesity-related parameters other than BMI, such as fat distribution, percentage of body fat, waist—hip ratio, and waist circumferences. Additional assessment of these parameters could provide more detailed information as to whether there are BMI subgroups with higher or lower decreases in GC sensitivity of inflammatory cytokine release following stress. Finally, the underlying biological mechanisms and the clinical implications of our findings need to be elucidated.

In conclusion, our findings suggest that with increasing BMI, and thereby with obesity, GCs are less able to downregulate inflammatory activity following acute psychosocial stress. Future research should include measures of fat distribution and address whether this has implications for ACS following emotional stress in overweight people.

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Conflict of interest

There are no conflicts of interest.

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