A two-year follow-up study of salivary cortisol concentration and the risk of depression

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Cortisol;
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Summary Stress is a suspected cause of depression. High cortisol concentration, a biomarker of an activated stress response, has been found in depressed patients. The aim of this study was to determine if a high level of salivary cortisol is a risk factor of depression. In 2007, we enrolled 4467 public employees. Morning and evening salivary cortisol concentration were measured for each participant. Participants reporting high levels of depressive, burnout, or stress symptoms, assessed by questionnaires were assigned to a psychiatric interview. In this interview 98 participants were diagnosed with depression and subsequently excluded. Two years later in 2009, 2920 participants who had provided at least one valid saliva cortisol measurement at baseline participated at follow up. The psychiatric interviews were repeated and 62 cases of newly onset depression were diagnosed. Odds ratios of depression were estimated for every 1.0 nmol/l increase in morning, evening, and daily mean cortisol concentration, as well as for the difference between morning and evening cortisol concentration. The risk of depression decreased by increasing daily mean cortisol concentration and by increasing difference between morning and evening concentrations, while morning and evening cortisol concentrations were not significantly associated with depression. The adjusted odds ratios for 1.0 nmol/l increase in morning, evening, and daily mean cortisol concentration were 0.69 (95% CI: 0.45, 1.05), 0.87 (95% CI: 0.59, 1.28), and 0.53 (95% CI: 0.32, 0.90), respectively. The adjusted odds ratio for 1.0 nmol/l increase in morning cortisol concentration were 0.69 (95% CI: 0.45, 1.05), 0.87 (95% CI: 0.59, 1.28), and 0.53 (95% CI: 0.32, 0.90), respectively.
1. Introduction

Stress and stressful life events are often implicated in the causation of depression and numerous other diseases (Maddock and Pariante, 2001; Risch et al., 2009), although there are unresolved questions about the causal mechanisms (Hammen, 2005). Sudden and intense stressors cause an acute increase in cortisol secretion, while it has been suggested that long-term and less intense stressors may cause a lower-level increase as well as a lowered cortisol secretion after several years (Yehuda et al., 1996; Rosmond and Bjorntorp, 2000). Abnormalities in the HPA axis have therefore been speculated to play a key role in the development and recurrence of depression (Hammen, 2005).

Increased cortisol level and thus hyperactivity of the HPA axis has repeatedly been reported in cross-sectional studies of patients diagnosed with depression (Brown et al., 2004; Pariante and Lightman, 2008; Knorr et al., 2010; Stetler and Miller, 2011; Jonsdottir et al., 2012). However, it is unclear whether this reflects a causal mechanism leading to depression or mechanisms that are secondary to the inception of the disease. The few longitudinal studies conducted so far show that different measures of increased cortisol level at baseline predict depression at follow up 1 to 6 years later (Goodyer et al., 2000; Harris et al., 2000; Halligan et al., 2007; Adam et al., 2010; Goodyer et al., 2010; Ellenbogen et al., 2011; Vrshek-Schallhorn et al., 2012). Harris et al. (2000) examined 116 adult women screened to have a high risk of depression and observed that a high morning cortisol concentration was associated with depression during 13 months of follow up, but did not find any association with evening cortisol concentration. Goodyer et al. (2000) and Halligan et al. (2007) found similar results during 1 year and 3 years of follow up that included 180 and 57 adolescents, respectively. Goodyer et al. (2010) in a later study examined 401 adolescents and found high concentrations of morning cortisol to be associated with depression 3 years later. Ellenbogen et al. (2011) showed that a high mean concentration of cortisol across the day among 59 adolescents predicted depression during 1–6 years of follow up. Adam et al. (2010) observed no association between morning-to-evening slope or mean cortisol concentration across the day and depression in 230 adolescents during 1 year of follow up. But the cortisol awakening response was a significant predictor of depression. Vrshek-Schallhorn et al. (2012) examined 270 adolescents and showed that the cortisol awakening response predicted depression up to 2½ year after baseline, but not thereafter. They observed no relation between morning-to-evening slope or mean cortisol concentration across the day and depression.

Thus, results from longitudinal studies are equivocal and based on relatively few observations. Studies are mainly conducted among adolescents and include no healthy adult populations. We recruited a large, healthy working population and measured the HPA activity by saliva cortisol concentration and analysed the risk of new onset depression two years later. We hypothesised that a high level of cortisol increases the risk of depression.

2. Methods

2.1. Design

This follow-up study is based on the Danish PRISME cohort established in 2007 and re-examined in 2009 (Kolstad et al., 2011; Grynderup et al., 2012). The purpose of the PRISME study is to examine to what extent psychological work factors and increased HPA axis activity are risk factors of depression, burnout, or stress symptoms. We measured salivary cortisol in all participants in 2007 and analyzed if morning concentration, evening concentration, mean of morning and evening concentration, or the morning-to-evening slope (difference between morning and evening concentration) predicted new-onset of depression at follow up in 2009. Cases of depression were identified in 2007 and 2009 by a two-step procedure: First, we identified participants reporting mental symptoms (symptoms of depression, perceived stress, or burn-out) in a questionnaire. Second, these participants were invited to a standardized psychiatric interview to identify cases with depression.

2.2. Population

In 2007, we approached 10,036 public employees from the municipal and hospital sector in Aarhus, Denmark for participation in the Danish PRISME cohort. Of these 4467 employees (45%) participated by collecting saliva samples and filling in a short questionnaire on sleep, medication, and alcohol intake the day of sampling. Participants with a clinical diagnosis of depression at baseline according to ICD-10 (n = 98) and pregnant women (n = 138) were excluded leaving 4231 participants for follow up. In 2009, all participants from 2007 were approached, and a total of 3031 participated. A total of 2920 of these participants provided a valid salivary cortisol measurement, as described later, and thus comprised the final study population.

2.3. Collection of saliva samples

All participants received Salivette® cotton swabs that they were instructed to keep in the mouth until thoroughly saturated. The saturated swabs were kept in a tube and stored in a refrigerator until they were returned by mail. The average time from date of sampling to date of receiving the samples at the National Research Centre for the Working Environment were 5 days (SD = 3 days). The samples were then stored at −20 °C and analyzed within 6 months. Participants sampled...
saliva during a workday (90.0%) or during a day off work (10.0%), and were instructed to collect the samples 30 min after awakening, and at 8 PM. Morning samples were considered valid if they were collected within 2 h of awakening, and evening samples if they were collected between 5 PM and 4 AM. In this paper we only included valid saliva samples.

The choices of sampling times were based on several factors. For the morning sample, the aim was to detect the morning cortisol peak that is expected to occur about 30 min after awakening (Pruessner et al., 1997; Edwards et al., 2001). Because cortisol concentration is stable during the evening (Ranjit et al., 2005; Kudielka et al., 2007) sampling time is less important and we decided on a fixed time for feasibility reasons. Our funding only allowed two samples per participant and furthermore we expected that more samples would decrease compliance in a field study like this.

2.4. Measurement of cortisol in saliva

Determination of cortisol in saliva was carried out with a competitive radioimmunoassay (RIA) designed for quantitative in vitro measurement of cortisol in serum, plasma, urine, and saliva, the Spectria Cortisol Coated Tube RIA (Orion Diagnostica, Espoo, Finland) according to the manufacturer’s specifications. The sample volume was 150 μl, the range of the standard solutions prepared was 1.0–100.0 nmol/l, and the incubation time was 30 min at 37 °C. The specifications given by the manufacturer were a sensitivity of twice the standard deviation of the zero binding value in saliva (0.8 nmol/l), a bias of 10% (3–15%), an intra-assay variation of 5.4%, and an inter-assay variation of 7.3%. Cross-reactivity to cortisone was <0.2%. A 1470 Wizard gamma counter (Wallac, Turku, Finland) was used for measurement of radioactivity. A method evaluation of certified reference material in water performed by our laboratory showed no bias of the method, with recovery being 97% [95% CI: 94.0–100.9]. Limit of detection was 1.59 nmol/l. Between-run coefficients of variation were 19% at 11.5 nmol/l and 16% at 49.2 nmol/l (Hansen et al., 2003).

To show equivalence between different runs, natural saliva samples (5.9 nmol/l and 18.5 nmol/l) were used as control materials and analyzed together with the samples. WESTgard control charts were used to document that the trueness and the precision of the analytical methods remained stable (WESTgard et al., 1981). The performance of the methods has been further validated by participation in interlaboratory comparison schemes (Garde et al., 2003; Hansen et al., 2003).

2.5. Measures of mental symptoms

We assessed depressive symptoms by the Common Mental Disorder Questionnaire subscale for depression (six items) (Christensen et al., 2005), stress symptoms by the Perceived Stress Scale (four items) (Cohen et al., 1983), and burn-out by the Copenhagen Burn-Out Inventory (six items) (Kristensen et al., 2005). All questions concerned the last four weeks and responses were given on 5-point scales (scores 1–5).

At baseline, participants were selected for the psychiatric interview if their point score was 3 or higher on three or more of the six items on the subscale for depression, the mean score was 2.5 or more on the Perceived Stress Scale, or the mean score was 4 or more on the Copenhagen Burn-Out Inventory.

At follow-up in 2009 we redefined the selection criteria for the psychiatric interviews based on tabulation of the frequency of diagnosed depression by different cut-off levels of depressive, stress, and burn-out scores in the baseline data. We did this in order to identify the largest number of depression cases. We selected participants with high scores in at least two of the three mental symptom scales (depressive scores of 3 or higher on two or more of the six questions, average stress and burn-out scores of 2.5 or higher).

In 2007, we invited 715 workers to participate in the psychiatric interviews and 552 participated (77%). In 2009, 671 workers were invited and 426 participated (63%).

2.6. Diagnosis of depression

Diagnoses of depression were obtained by the Schedules for Clinical Assessment in Neuropsychiatry (SCAN) interview (version 2.1 part I, sections 6, 7, 8, and 10) (Wing et al., 1990) according to the ICD-10 classification of mental and behavioral disorders: diagnostic criteria for research (ICD-10-DCR) and referred to the previous three months. The interviews were conducted by 10 students of medicine or psychology, who were trained during a one week course given by a WHO certified trainer (OM). Inter-rater reliability on item level was satisfactory (κ = 0.71).

In 2007, a total of 100 participants were diagnosed with depression and excluded from the study. The ICD-10-DCR diagnostic criteria for a mild, moderate, and severe depressive episode were fulfilled for 40, 43 and 17 participants, respectively. Of these, 98 depressed participants had collected baseline saliva samples. In 2009, a total of 62 among the 2920 participants were diagnosed with depression. The ICD-10-DCR diagnostic criteria for a mild, moderate, and severe depressive episode were fulfilled for 19, 31 and 12 participants, respectively.

2.7. Statistical analyses

Odds ratios of depression were analysed by logistic regression. Diagnosis of depression was categorized as a dichotomous variable including mild, moderate, and severe cases of depression. Logarithmic transformation was used to normalize the cortisol distribution. The morning-to-evening slope was calculated as the difference between morning and evening cortisol concentration in valid saliva samples divided by the number of hours between the collections of the two samples, and was also analyzed on a logarithmic scale. The daily mean concentration of cortisol was calculated as the mean of morning and evening cortisol concentration of valid saliva samples. In the analyses of daily mean cortisol concentration and morning-to-evening slope, we only included participants with both valid morning and evening sample times, and where the evening sample were collected at least 9 h after the morning sample. Analyses of morning, evening, and daily mean cortisol concentrations as well as the morning-to-evening slope were performed on a continuous-scale and with tertile categorization. Linearity of the
relation between the continuous cortisol measures and depression were tested using likelihood-ratio tests comparing linear models to models including both linear and quadratic terms as covariates.

We included the following potential confounders as measured at baseline in all models: gender (male, female), age (<34, 35–44, 45–54, ≥55), previous episodes of depression (yes, no), family history of depression (yes, no), income (continuous), and years of education beyond primary or high school (<3, 3–4, >4). We included the following lifestyle factors as potential confounders in some models: alcohol consumption (≤14, >14 g/week), body mass index (continuous), and smoking (never, up to 20 years, 20 or more years). The selection of these potential confounders was based upon a review of the literature (Kessler, 1997; Hasin et al., 2005; Burcusa and Iacono, 2007; Andersen et al., 2009; Boden et al., 2010).

Few participants collected the saliva samples exactly at 8 PM and 30 min after awakening. We therefore performed sub-analyses to examine the effect of sampling time. We excluded the 10% of the participants who collected their morning sample earliest (5%) and latest (5%) during the day and calculated the odds ratio of depression by cortisol level for the remaining 90% of the population. We did the same for 80% and 70% of the population after we had excluded the 10% and 15%, respectively, who collected their samples earliest and latest. Similar sub-analyses were performed for evening and daily mean cortisol concentration and morning-to-evening slope. All analyses were conducted using the STATA 11 statistical software (StataCorp LP, College Station, Texas).

3. Results

Nurses (30%), social workers (18%), teachers (11%), managers (7%), and medical doctors (6%) were the most prevalent professions among the participants. The mean age of the participants were 45.5 years, 78% were women, 82% had 3 or more years of professional education beyond primary or high school, and 13% reported a history of depression before enrolment in the study.

The mean morning cortisol concentration was 12.7 nmol/l based on 2615 valid samples, the mean evening cortisol concentration was 2.1 nmol/l based on 2856 valid samples, the mean daily mean cortisol concentration was 7.44 nmol/l based on 2517 valid morning and evening samples, and the mean morning-to-evening slope was 0.79 nmol/l decrease for every hour based on 2517 valid morning and evening samples. Previous depression, income, and smoking at baseline all predicted depression at follow-up (Table 1). Non-depressed participants at follow up, at baseline collected the morning sample on average 43.2 min after awakening on average at 7.04 AM and the evening sample on average at 8.37 PM. These participants had geometric mean morning and evening cortisol concentrations of 10.61 (95% CI: 10.35, 10.88) and 1.44 (95% CI: 1.40, 1.48), respectively. The depressed participants at follow up, at baseline collected the morning sample on average 46.8 min after awakening on average at 7.20 AM and the evening sample at 8.49 PM. These participants had geometric mean morning and evening cortisol concentrations of 9.28 (95% CI: 7.62, 11.31) and 1.36 (95% CI: 1.09, 1.71), respectively (Fig. 1).

Table 1 Baseline characteristics of 2920 public employees with or without a diagnosis of depression at follow-up.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Non-depressed (n = 2858)</th>
<th>%</th>
<th>Depressed (n = 62)</th>
<th>%</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;35 years</td>
<td>515</td>
<td>18.0</td>
<td>14</td>
<td>22.6</td>
<td>1</td>
<td>0.33, 1.50</td>
</tr>
<tr>
<td>35–44 years</td>
<td>682</td>
<td>23.9</td>
<td>13</td>
<td>21.0</td>
<td>0.70</td>
<td>0.42, 1.60</td>
</tr>
<tr>
<td>45–54 years</td>
<td>1076</td>
<td>37.7</td>
<td>24</td>
<td>38.7</td>
<td>0.82</td>
<td>0.42, 1.60</td>
</tr>
<tr>
<td>≥55 years</td>
<td>585</td>
<td>20.5</td>
<td>11</td>
<td>17.7</td>
<td>0.69</td>
<td>0.31, 1.54</td>
</tr>
<tr>
<td>Previous depression</td>
<td>339</td>
<td>12.3</td>
<td>27</td>
<td>46.6</td>
<td>6.24</td>
<td>3.68, 10.58</td>
</tr>
<tr>
<td>Family history of depression</td>
<td>752</td>
<td>26.9</td>
<td>21</td>
<td>34.4</td>
<td>1.56</td>
<td>0.89, 2.71</td>
</tr>
<tr>
<td>Professional education beyond primary or high school</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;3 years</td>
<td>505</td>
<td>17.8</td>
<td>11</td>
<td>18.0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>3–4 years</td>
<td>1979</td>
<td>69.7</td>
<td>45</td>
<td>73.8</td>
<td>1.04</td>
<td>0.54, 2.03</td>
</tr>
<tr>
<td>&gt;4 years</td>
<td>355</td>
<td>12.5</td>
<td>5</td>
<td>8.2</td>
<td>0.65</td>
<td>0.22, 1.88</td>
</tr>
<tr>
<td>Income &gt; 300,000 Dkr</td>
<td>1401</td>
<td>51.2</td>
<td>21</td>
<td>36.8</td>
<td>0.56</td>
<td>0.32, 0.96</td>
</tr>
<tr>
<td>Alcohol consumption above 14 g/week</td>
<td>701</td>
<td>24.9</td>
<td>14</td>
<td>23.0</td>
<td>0.90</td>
<td>0.49, 1.65</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never smoked</td>
<td>1350</td>
<td>52.2</td>
<td>23</td>
<td>41.8</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>0–19 years of smoking</td>
<td>599</td>
<td>23.2</td>
<td>10</td>
<td>18.2</td>
<td>0.98</td>
<td>0.46, 2.07</td>
</tr>
<tr>
<td>20 or more years of smoking</td>
<td>637</td>
<td>24.6</td>
<td>22</td>
<td>40.0</td>
<td>2.03</td>
<td>1.12, 3.66</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;18.5</td>
<td>48</td>
<td>1.7</td>
<td>2</td>
<td>3.3</td>
<td>2.09</td>
<td>0.49, 8.93</td>
</tr>
<tr>
<td>18.5–25</td>
<td>1805</td>
<td>64.0</td>
<td>36</td>
<td>60.0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>&gt;25</td>
<td>967</td>
<td>34.3</td>
<td>22</td>
<td>36.7</td>
<td>1.14</td>
<td>0.67, 1.95</td>
</tr>
</tbody>
</table>
The risk of depression decreased by increasing daily mean cortisol concentration and by increasing morning-to-evening slope (Table 2). The fully adjusted odds ratio for 1.0 nmol/l increase on the logarithmic scale in morning, evening, and daily mean cortisol concentration were 0.69 (95% CI: 0.45, 1.05), 0.87 (95% CI: 0.59, 1.28), and 0.53 (95% CI: 0.32, 0.90), respectively. The fully adjusted odds ratios for the highest tertile compared with the lowest tertile were 0.48 (95% CI: 0.22, 1.04) for morning cortisol concentration, 1.29 (95% CI: 0.60, 2.76) for evening cortisol concentration, and 0.48 (95% CI: 0.22, 1.05) for daily mean cortisol concentration. The adjusted odds ratio for a 1.0 nmol/l increase in morning-to-evening slope on the logarithmic scale was 0.64 (95% CI: 0.45, 0.90) and the adjusted odds ratio of the highest tertile compared with the lowest tertile was 0.50 (95% CI: 0.22, 1.12) (Table 2). Models with quadratic terms of cortisol concentration included as covariates did not perform significantly better than the simple linear models of morning, evening, or daily mean cortisol; or morning-to-evening slope.

The effect of measuring time was examined in sub-analyses where only the 90%, 80% and 70% of the population that collected their saliva samples closest to the intended time of sampling were included (Fig. 2). These analyses showed even stronger inverse relations between salivary cortisol level and odds ratio of depression. 90% of the participants collected their morning samples between 9 and 102 min after awakening, 80% between 19 and 73 min after awakening, and 70% between 26 and 59 min after awakening. 90% of the participants collected their evening samples between 7.25 PM and 10.56 PM, 80% between 7.48 PM and 10.18 PM, and 70% between 7.58 PM and 9.59 PM.

4. Discussion

We found that participants with a high daily mean concentration of cortisol or a steep morning-to-evening slope had a decreased risk of depression two years later. From our hypothesis we had expected that a high concentration of salivary cortisol showed an increased risk of depression. However, we found the opposite pattern. Thus the hypothesis...
<table>
<thead>
<tr>
<th>Exposure</th>
<th>Mean (range) nmol/l</th>
<th>Depressed</th>
<th>Non-depressed</th>
<th>Crude OR 95% CI</th>
<th>Adjusted(^a) OR 95% CI</th>
<th>Adjusted(^b) OR 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Continuous</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Morning cortisol(^c)</td>
<td>12.7 (0.2—99.6)</td>
<td>53</td>
<td>2562</td>
<td>0.75</td>
<td>0.52: 1.10</td>
<td>0.49: 1.09</td>
</tr>
<tr>
<td>Evening cortisol(^c)</td>
<td>2.1 (0.1—83.7)</td>
<td>61</td>
<td>2795</td>
<td>0.91</td>
<td>0.66: 1.26</td>
<td>0.65: 1.32</td>
</tr>
<tr>
<td>Daily mean cortisol(^c)</td>
<td>7.4 (0.25—52.6)</td>
<td>52</td>
<td>2465</td>
<td>0.61</td>
<td>0.38: 0.97</td>
<td>0.36: 0.96</td>
</tr>
<tr>
<td>Slope(^d)</td>
<td>0.8 (−4.28—7.53)</td>
<td>52</td>
<td>2465</td>
<td>0.65</td>
<td>0.48: 0.89</td>
<td>0.47: 0.90</td>
</tr>
<tr>
<td><strong>Categorical</strong></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Low morning cortisol</td>
<td>6.0 (0.2—8.9)</td>
<td>24</td>
<td>830</td>
<td>1</td>
<td>—</td>
<td>1</td>
</tr>
<tr>
<td>Medium morning cortisol</td>
<td>11.6 (9—14.4)</td>
<td>15</td>
<td>864</td>
<td>0.60</td>
<td>0.31: 1.15</td>
<td>0.27: 1.09</td>
</tr>
<tr>
<td>High morning cortisol</td>
<td>22.0 (14.5—99.6)</td>
<td>14</td>
<td>868</td>
<td>0.56</td>
<td>0.29: 1.09</td>
<td>0.28: 1.17</td>
</tr>
<tr>
<td>Low evening cortisol</td>
<td>0.7 (0.1—1.0)</td>
<td>17</td>
<td>885</td>
<td>1</td>
<td>—</td>
<td>1</td>
</tr>
<tr>
<td>Medium evening cortisol</td>
<td>1.4 (1.1—1.8)</td>
<td>21</td>
<td>958</td>
<td>1.14</td>
<td>0.60: 2.18</td>
<td>0.66: 2.77</td>
</tr>
<tr>
<td>High evening cortisol</td>
<td>5.6 (1.9—83.7)</td>
<td>23</td>
<td>952</td>
<td>1.26</td>
<td>0.67: 2.37</td>
<td>0.67: 2.80</td>
</tr>
<tr>
<td>Low mean cortisol</td>
<td>3.8 (0.25—5.34)</td>
<td>24</td>
<td>796</td>
<td>1</td>
<td>—</td>
<td>1</td>
</tr>
<tr>
<td>Medium mean cortisol</td>
<td>6.8 (5.35—8.25)</td>
<td>13</td>
<td>840</td>
<td>0.51</td>
<td>0.26: 1.02</td>
<td>0.21: 0.92</td>
</tr>
<tr>
<td>High mean cortisol</td>
<td>13.3 (8.3—52.6)</td>
<td>15</td>
<td>829</td>
<td>0.60</td>
<td>0.31: 1.15</td>
<td>0.30: 1.25</td>
</tr>
<tr>
<td>Low slope</td>
<td>0.1 (−4.28—0.54)</td>
<td>24</td>
<td>815</td>
<td>1</td>
<td>—</td>
<td>1</td>
</tr>
<tr>
<td>Medium slope</td>
<td>0.7 (0.55—0.94)</td>
<td>15</td>
<td>824</td>
<td>0.62</td>
<td>0.32: 1.19</td>
<td>0.29: 1.19</td>
</tr>
<tr>
<td>High slope</td>
<td>1.5 (0.95—7.53)</td>
<td>13</td>
<td>826</td>
<td>0.53</td>
<td>0.27: 1.06</td>
<td>0.26: 1.15</td>
</tr>
</tbody>
</table>

\(^a\) Adjusted for age, gender, income, educational level, previous episodes of depression, and family history of depression.

\(^b\) Adjusted for age, gender, income, educational level, previous episodes of depression, family history of depression, body-mass index, smoking, and alcohol consumption.

\(^c\) Increase in odds ratio for every increase of 1.0 nmol/l cortisol on the logarithmic scale.

\(^d\) Increase in odds ratio for every 1.0 nmol/l per hour increase in morning-to-evening slope on the logarithmic scale.
that high cortisol concentration is a risk factor of depression was rejected.

Consequently, the results of this study were not in line with results from the few other longitudinal studies of cortisol concentration and the risk of depression (Goodyer et al., 2000, 2010; Harris et al., 2000; Halligan et al., 2007; Adam et al., 2010; Ellenbogen et al., 2011; Vrshek-Schallhorn et al., 2012). The populations investigated in Adam et al. (2010), Ellenbogen et al. (2011), Goodyer et al. (2000), Goodyer et al. (2010), Halligan et al. (2007) and Vrshek-Schallhorn et al. (2012) were much younger than in our study (average age 17.0, 17.5, 13.5, 13.6, 13.0 and 17.1 years, respectively). Increased morning and daily mean cortisol concentrations and a high cortisol awakening response have been shown among young adults with depression compared to young non-depressed adults. Among older adults there were no such difference in cortisol measurements between the depressed and non-depressed (Heaney et al., 2010). This may explain the different results in these studies compared to ours, since the association between depression and diurnal cortisol vary with age, and these studies examined children and adolescents, while our study examined adults. Compared to the participants in the study by Harris et al. (2000), which also examine adults, the participants in our study were older, more educated, were all employed, had a far less frequent history of depression, and were not selected because they were likely to develop depression.

Adam et al. (2010), Ellenbogen et al. (2011), Harris et al. (2000), Halligan et al. (2007) and Vrshek-Schallhorn et al. (2012) selected study populations that had higher risk of developing depression due to personality traits or a familial disposition compared to the population in average. This may also affect the comparability between these studies and ours, since we examined a healthy working population. Less severe depression has shown weaker association with cortisol levels than more severe cases (Stetler and Miller, 2011), and cases of depression are likely to be less severe in our healthy working population.

The 2 years of follow-up in our study were not comparable to Goodyer et al. (2000, 2010) and Harris et al. (2000) with 1 year of follow-up, or Adam et al. (2010) with 13 months of follow-up. Halligan et al. (2007) and Vrshek-Schallhorn et al. (2012) had 3 and 4 years of follow-up, respectively. Ellenbogen et al. (2011) had a follow-up period of 1–6 years (average of 2.5 years). There may be differences between those participants who are not depressed at baseline, but who are depressed 1 year later, those who are depressed 2 years later, and those who develop depression later than that. The duration of a depressive episode has been found to vary widely, with median durations between 3 and 12 months, and around 20% of depressive episodes last longer than 2 years (Spijker et al., 2002). It is possible that several participants in our study have developed and recovered from depression during the 2-year period. It is a limitation of our study that we were not able to identify those participants and we may have oversampled cases of prolonged or chronic depression. However, chronicity does not seem to affect cortisol concentration of the depressed beyond the effect of symptom severity and hospitalization (Stetler and Miller, 2011).

Cortisol concentration exhibits diurnal variation and due to differences in cortisol awakening response among depressed and non-depressed participants the exact time of sampling could be important. We measured morning cortisol concentration 30 min after awakening, which is not comparable to the measurements at 8 AM by Harris et al. (2000), Goodyer et al. (2000), Goodyer et al. (2010), and Halligan et al. (2007), or the measurements 1 h after awakening by Ellenbogen et al. (2011). Adam et al. (2010) and Vrshek-Schallhorn et al. (2012) collected saliva samples 40 min after awakening, and did not find any significant association between morning cortisol concentration and subsequent depression. Morning cortisol concentration is affected more by the time of awakening than by the time of the day (Puressner et al., 1997; Edwards et al., 2001). Thus, it is possible that the 8 AM samples do not reflect the morning cortisol peak, but the capacity for recovery following the morning peak.

Depression is associated with a blunted cortisol response when exposed to an acute stressor and an impaired recovery (Burke et al., 2005). If a similar pattern is present at the causal path leading to depression this could explain the low morning cortisol among the depressed participants of our study as well as the high 8 AM cortisol concentration among the depressed in the studies by Harris et al. (2000), Goodyer et al. (2000, 2010), and Halligan et al. (2007). We do, however, find no indication of a higher evening cortisol concentration, as would be expected due to the impaired recovery among the depressed.

To account for the fact that all participants did not collect the saliva samples at the exact time they were instructed to; we performed sub-analyses based on sub-groups of participants who collected their samples closest to the instructed time. This sub-analyses showed lower odds ratio of depression by increasing cortisol concentration compared to the entire study population, and indicate that our results are biased towards the null and even stronger inverse association between cortisol level and depression.

The study included only 62 cases of depression. This limits the statistical power. Furthermore, the low number of cases limits the ability to adjust thoroughly for all potential confounders. The similarity between the crude and the two differently adjusted results does however indicate no strong confounding.

The baseline participation rate was low (45%), which could have biased results, if participation was associated with cortisol concentration as well as depression. To assess selective participation we obtained registry information on both responders and non-responders at baseline (Kaerlev et al., 2011). Compared to non-responders, participants were more often women, were older, had higher social class, were less frequently on sick leave, and were less often prescribed antidepressant medicatiion. We did, however, have no way to assess cortisol levels of non-responders, but we would not expect participation to be related to cortisol levels that hardly were known by the candidates for the study. The prevalence of depression in this study population was lower than in the general Danish population. Based on previously reported prevalence and recurrence rates of depression we had expected twice the number of cases (Olsen et al., 2004; Burcusa and Iacono, 2007). Our lower-than-expected number of cases may be due to a healthy worker effect.

During follow up the participation rate was higher (72%) but selection may still have biased our findings. However,
selection bias is unlikely because cortisol level has no strong perceivable correlates in a healthy, employed population that may have influenced participation. Furthermore, we found that the relation between cortisol concentration and depressive symptoms at baseline did not differ between participants and non-participants at follow up and thus does not indicate strong selection bias. Participants had mean morning and evening cortisol concentrations of 12.7 nmol/l and 2.1 nmol/l, respectively compared to non-participants with mean morning and evening concentrations of 12.2 nmol/l and 2.3 nmol/l, respectively.

The odds ratios of depression for morning-to-evening slope, morning, and daily mean cortisol concentration are strongly correlated ($r > 0.9$). Evening cortisol is correlated to mean cortisol concentration ($r = 0.4$) but are not significantly correlated to morning-to-evening slope. The four cortisol measures do not reflect four independent factors but are strongly related, especially morning-to-evening slope, mean, and morning concentration.

To conclude, this study did not support our hypothesis that high salivary cortisol concentration is a risk factor for depression, but indicate that a low mean salivary cortisol concentration and a flat morning-to-evening curve may be risk factors of depression.

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The funding sources had no role in the design or conduct of the study; collection, management, analysis, or interpretation of the data; or preparation, review, or approval of the manuscript.

**Conflict of interest**

All authors declare no conflicts of interest.

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