Exercise Reduces Salivary Morning Cortisol Levels in Patients with Depression

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Keywords

Hydrocortisone · Saliva · Hypothalamus · Pituitary-adrenal system

Abstract

Purpose of the Study: Cortisol hypersecretion plays a role in depression pathophysiology. Internet-based cognitive behavioural therapy (ICBT) and physical exercise (PE) are new treatment alternatives for depression, and their long-lasting effect on cortisol is unknown. We investigated cortisol level changes after 12 weeks of ICBT, PE or treatment as usual (TAU). **Procedures:** The present pre-post repeated measure study analysed data derived from a randomised controlled trial evaluating the effects of 12 weeks' interventions of ICBT, PE and TAU in depressed primary care patients (Sweden 2011–2013) and aimed at prospectively evaluating the within-group effects of ICBT, PE and TAU on diurnal salivary cortisol levels in a small representative subsample ($n = 56$, 38 and 27, respectively). **Results:** We found a marked flattening of the diurnal cortisol slope ($p = 0.004$) and a reduced

cortisol level at awakening ($p = 0.017$) after 12 weeks of PE treatment. No apparent effects of ICBT or TAU interventions were seen on diurnal cortisol levels. **Conclusions and Message:** PE reduced the rate of cortisol level decline across the day in depressed adults. ICBT and TAU treatments had no detectable effects on diurnal cortisol levels. Larger samples are required for the detection and comparison of smaller effects of PE, ICBT and TAU on diurnal cortisol levels.

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Introduction

There are several evidence-based therapeutic options for depression, such as pharmacotherapy and various forms of psychotherapy [1, 2], but many patients do not have access to, or respond adequately to, the offered treatments [3]. There are new promising treatments, such as Internet-based cognitive behavioural therapy (ICBT) and physical exercise (PE), proven to be effective in several reviews [4–8]. No established biomarker for

depression exists, but cortisol levels in blood plasma are estimated to be elevated in 20–40% of the outpatients with depression and in 60–80% of hospitalized patients with severe depressive symptoms [9]. The cortisol levels in humans follow diurnal cycles, peaking in the early morning and hitting bottom 3–5 h after the onset of sleep [10]. Most cortisol in plasma is protein bound; only a small percentage is free and biologically active. Saliva and urine testing evaluate only free cortisol, which correlates with the levels of free and biologically active cortisol in the blood. On a neurobiological level, PE modulates the hypothalamic-pituitary-adrenal axis [11], which is a part of the stress response system. Studies have shown that plasma cortisol levels are increased immediately after PE [12, 13] ($n = 46$ and 13 , respectively) and that the plasma and urine cortisol levels decrease after repeated long-term PE [14, 15] ($n = 21$ and 49 , respectively) in healthy and depressed patients. Also, a few traditional cognitive-behavioural therapies have been reported to decrease salivary cortisol levels [16] ($n = 26$), but no study has been performed on the effects of ICBT on cortisol levels. Salivary cortisol level is highly correlated with serum cortisol level and suitable for the clinical assessment of adrenocortical function [17, 18]. The present study aimed to explore if there was a within-group effect of 12 weeks' treatment with ICBT, PE and treatment as usual (TAU) on diurnal salivary cortisol levels in patients with depression. Cortisol levels were measured in 3 batches defined by the 3 treatment groups. Batch differences in cortisol levels could not be excluded; therefore, the present study did not aim to compare cortisol levels between treatment groups.

Methods

Study Design and Participants

The study utilized data from a randomized controlled single-blinded trial in Sweden called Regassa, which evaluated the relative effectiveness of 3 interventions for mild to moderate depression, including PE, ICBT and TAU [19, 20]. Primary care patients (≥ 18 years) scoring >9 on the Patient Health Questionnaire (PHQ-9) [21] were recruited from 6 counties in Sweden (Stockholm, Skåne, Västra Götaland, Kronoberg, Blekinge and Västmanland) from February 2011 to December 2012. Patients with severe somatic illness, a primary alcohol or drug use disorder or a psychiatric diagnosis that required specialist treatments and non-Swedish speakers (ICBT was only available in Swedish) were excluded. No data were available on the number of patients who were invited but declined to participate. At baseline, 945 subjects were included and randomised to the 3 intervention arms: ICBT ($n = 317$), PE ($n = 316$) and TAU ($n = 312$). In the present study, only participants from the ICBT ($n = 56$), PE ($n = 38$) and TAU ($n = 27$) arms who

had contributed saliva according to the protocol of salivary collection were included. Of all Regassa participants, one-third were taking antidepressants (primarily selective serotonin reuptake inhibitors) ahead of and during the trial. In this cortisol study sample, the use of antidepressants at baseline within the PE, ICBT and TAU arms was 31.6, 32.1 and 22.2%, respectively, and did not differ statistically. All participants gave written informed consent, and the Stockholm regional ethical review board approved the trial. All procedures were conducted in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000.

Interventions

Physical Exercise

Participants were requested to take part in a 60-min exercise session at “Friskis and Svettis,” a modern fitness centre with multiple locations throughout Sweden, 3 times per week for 3 months (12 weeks). All patients were provided with free fitness centre memberships. Weekly meetings with a qualified personal trainer were used to assess adherence. If patients did not attend these meetings, they were contacted by phone or text message and encouraged to continue the intervention. The patients who failed to attend the meetings were reminded and encouraged via telephone calls or text messages. In this study, we have analysed the PE arm regardless of frequency and intensities of exercise.

Internet-Based Cognitive Behavioural Therapy

ICBT was delivered through a secure web-based system and executed similar to ICBT in routine care but with an individualized composition of treatment modules to fit different diagnostic profiles of the individual patient. The weekly treatment modules consisted of text, images and sound clips and included 3 kinds of modules. The basic modules addressed general depression-related problems. Subsequent modules targeted patient-specific symptoms other than depression, such as panic attacks, stress, insomnia and pain. All patients had contact with active therapists/psychologists who monitored symptoms and coached the patients. Patients received ICBT free of charge and were contacted by their psychologist if they stopped using the service for more than 1 week or if they expressed suicidal ideations.

Treatment as Usual

The TAU group received usual care in primary care settings, which included either pharmacotherapy or psychotherapy. Patients attended on average 8.2 (SD = 6.4) face-to-face counselling sessions during the 12-week trial. A total of 25% of patients in this group had no treatment recorded.

Baseline and Follow-Up Assessments

Both at baseline and follow-up, the level of depressive symptoms was assessed using the Montgomery-Åsberg Depression Rating Scale (MADRS) [22]. Each item yields a score ranging from 0 to 6, and total scores range from 0 to 60. The MADRS score mentioned in this paper represents the total MADRS score. The severity categorisation was based on the following ranges: 35–60 = severe, 20–34 = moderate, 7–19 = mild and 0–6 = remission [19]. Within 1 week after the 12-week intervention, the patients were invited to a follow-up assessment in order to evaluate the MADRS score and collect saliva samples under the same conditions as at baseline.

Saliva Collection and Cortisol Measurement

Before and after the interventions, participants were asked to provide saliva samples at home in Salivette tubes (polypropylene/low-density polyethylene; Sarstedt, Leicester, UK), with samples taken immediately upon awakening, 40 min after awakening and at bedtime over 2 consecutive work-free days. In these tubes, cortisol levels are reported to remain stable at ambient temperature for at least 4 days [23]. Within 4 days at ambient temperature, each sample was frozen for storage at -20°C . All who had provided samples from at least 1 day before and 1 day after intervention, where the time interval between the awakening and the 40 min after awakening samples was within 30–60 min after awakening, were included in this study ($n = 56$ in ICBT, $n = 38$ in PE and $n = 27$ in TAU). There could be many reasons why few participants provided valid saliva samples (e.g., participants did not remember or misunderstood the information provided by nurses or could not adhere to the rules of not taking breakfast between 2 saliva collections in the morning). Before measuring cortisol, Salivette tubes were centrifuged at 1,000 g for 2 min; upon reaching room temperature, the salivary cortisol level was measured using the enzyme-linked immunosorbent assay (ELISA) (Salivary Cortisol ELISA kit, Salimetrics, UK) according to the manufacturer's instructions. No saliva sample had a bad smell, suggesting no considerable microbiotic growth. Each sample was run in duplicate. All samples from the same participant were run on the same 96-well plate. Each plate contained a cortisol standard curve and 2 inter-plate control samples with known cortisol concentrations already prepared by the manufacturer; the latter 2 control samples were used to determine intra- and inter-assay precisions being less than 7 and 11%, respectively.

Statistical Analyses

The present study explored the within-group effect of 12 weeks' treatment with ICBT, PE and TAU on diurnal salivary cortisol levels in patients with depression. Cortisol levels were measured in 3 batches defined by the 3 treatment groups. Because batch differences in cortisol levels could not be excluded, the present study did not compare cortisol levels between treatment groups.

The descriptive statistics for the participants in the 3 treatment arms are presented as proportions (for categorical variables), as median and interquartile range (for non-normally distributed variables), and as mean and standard deviation (for normally distributed variables). A paired t test was performed to explore the within-group differences in MADRS scores between baseline and follow-up in the 3 treatment arms. The distribution of cortisol levels was normal. In order to assess the effects of treatments on cortisol levels throughout the day, 2 measures were studied: (1) the diurnal cortisol level slope between awakening and bedtime and (2) areas under the curve with respect to ground (AUC) of the cortisol levels from awakening to bedtime, i.e., daytime cortisol exposure [24], using a paired t test. Here, a two-tailed p value $< 0.05/6 = 0.0083$ (2 measures $\times$ 3 treatments = 6 tests) was considered statistically significant. For the AUC, all individuals' bedtime cortisol concentration measurements were brought to a comparative base (similar duration of time being awake). The adjusted cortisol levels at bedtime were set at 500 min after awakening assuming a linear cortisol concentration slope between the original cortisol level at 40 min after awakening and that at bedtime (Fig. 1B). Using this adjusted cortisol level at bedtime, the standardised AUC (sAUC) was calculated. The differences of

sAUCs between before and after treatment were tested using a paired t test. Detected effects on cortisol were further explored by examining the individual time points underlying the diurnal slope and AUC, i.e., cortisol levels at morning awakening, 40 min after awakening and at adjusted bedtime, using paired t tests. In these post hoc analyses, two-tailed $p < 0.05$ was regarded statistically significant. Next, we explored if detected PE effects (on diurnal slope and awakening level) were specific for a depression severity category by using a paired t test stratified for baseline depression severity category (mild and moderate). Finally, the association between reduction in awakening cortisol level and reduction in MADRS score in the PE arm was assessed using linear regression adjusting for baseline depression severity, age, gender, baseline use of antidepressants and tobacco use. All analyses were performed on absolute values. All statistical analyses were performed in IBM SPSS Statistics version 25.

Results

The characteristics of the participants (who provided saliva at adequate dates and time points before and after intervention) and non-participants (who did not provide adequate saliva samples but completed follow-up assessment) are displayed in Table 1. In each of the 3 arms, de-

Fig. 1. Overview of results. **A** Mean values for diurnal cortisol slope (between awakening and bedtime) before and after treatment: PE (**a**); ICBT (**b**); TAU (**c**). Corresponding bars indicate 95% confidence intervals. $**p = 0.004$. RQ, relative quantity using the mean baseline diurnal cortisol slope as a reference. **B** Standardisation of bedtime cortisol levels. Red solid lines represent the original diurnal cortisol level curve. Blue solid lines represent the measured original cortisol levels. Blue dashed line represents the adjusted cortisol level at bedtime. AUC (nmol/L min) was calculated as follows: $\text{AUC} = [(\text{Cort}_{40} + \text{Cort}_{\text{aw}})/2] \times \text{time 1} + [(\text{Cort}_{\text{sleep}} + \text{Cort}_{40})/2] \times \text{time 2}$ (Pruessner et al. [38]). $\text{sAUC} = [(\text{Cort}_{40} + \text{Cort}_{\text{aw}})/2] \times \text{time 1} + [(\text{Cort}_{\text{adjusted}} + \text{Cort}_{40})/2] \times 500$. **C** Mean values for AUC and sAUC before and after treatment: PE (**a**); ICBT (**b**); TAU (**c**). Blue line indicates AUC, red spotted line indicates sAUC, and corresponding bars indicate 95% confidence interval. **D** Diurnal cortisol curves (mean values) at baseline and post-treatment follow-up of patients receiving PE (**a**), ICBT (**b**), and TAU (**c**). Blue line indicates before treatment measurements, red spotted line represents after treatment measurements, and corresponding bars indicate 95% confidence interval. $^{\#}$ Adjusted cortisol level by standardising the time interval from 40 min after awakening to bedtime. $^*p = 0.017$; $^{\dagger}p = 0.073$. PE, physical exercise; ICBT, Internet-based cognitive behavioural therapy; TAU, treatment as usual; RQ, relative quantity using the mean baseline cortisol level at awakening as a reference; Cort_{aw} , cortisol levels at awakening; Cort_{40} , cortisol levels at 40 min after awakening; $\text{Cort}_{\text{sleep}}$, cortisol levels at bedtime; $\text{Cort}_{\text{adjusted}}$, adjusted cortisol levels at bedtime; AUC, area under the curve with respect to the ground; sAUC, standardised area under the curve with respect to the ground.

(For figure see next page.)

pression severity was significantly lowered after 12 weeks of intervention when compared to baseline. To assess if there was an effect of treatment on cortisol level, we assessed diurnal cortisol profiles ($n = 121$) by calculating the

diurnal cortisol level slope between cortisol level at awakening and that at bedtime. The analysis of effect of treatment on cortisol slope was stratified by treatment arm. In the PE group, there was a reduced slope by PE ($p = 0.004$),

Table 1. Characteristics of the participants and non-participants in the 3 treatment arms (Sweden 2011–2013)

	Participants ^b (<i>n</i> = 121)			Non-participants ^c (<i>n</i> = 613)		
	ICBT (<i>n</i> = 56)	PE (<i>n</i> = 38)	TAU (<i>n</i> = 27)	ICBT (<i>n</i> = 201)	PE (<i>n</i> = 208)	TAU (<i>n</i> = 204)
Female, %	76.8	71.1	77.8	69.6	67.3	77.9
Median age (IQR), years	49 (21)	51 (16)	48 (21)	44 (19)	41 (18)	43 (19)
Mean BMI (SD)	25.3 (4.4)	25.8 (3.9)	25.4 (4.6)	25.7 (4.6)	25.4 (3.9)	25.6 (4.8)
Born in Sweden, yes, %	85.7	73.7	85.2	77.6	79.8	82.1
Baseline use of antidepressants, yes, %	32.1	31.6	22.2	32.8	31.7	25.1
Follow-up use of antidepressants, yes, %	32.1	27.8	23.8	35.9	26.4	32.1
Daily tobacco use at baseline, yes, %	16.1	7.9	18.5	26.9	20.2	19.1
Mean MADRS score at baseline (SD)	21.1 (6.1)	22.1 (5.9)	19.0 (5.7)	19.8 (6.1)	20.1 (6.2)	19.4 (7.0)
Mean MADRS score at follow-up (SD) ^a	10.4 (6.1)*	11.0 (6.3)*	14.7 (8.5)*	11.4 (7.7)*	11.4 (8.2)*	13.9 (9.0)*

ICBT, Internet-based cognitive behavioural therapy; PE, physical exercise; TAU, treatment as usual; IQR, interquartile range; MADRS, Montgomery-Åsberg Depression Rating Scale; BMI, body mass index; SD, standard deviation. * *p* value of the difference in MADRS score between baseline and follow-up within each treatment arm (ICBT, *p* < 0.001; PE, *p* < 0.001; TAU, *p* = 0.003). ^a MADRS scores at follow-up were missing for 1 participant in both the ICBT and PE arms, respectively. ^b Provided saliva before and after PE, ICBT and TAU intervention. ^c Did not provide saliva but completed follow-up MADRS assessment after PE, ICBT and TAU intervention.

whereas in the ICBT group there was a trend (*p* < 0.10) for a reduced slope (*p* = 0.091), and in the TAU group, there was no slope difference (*p* = 0.90) (Fig. 1A). To assess if this cortisol level slope difference by PE resulted in a PE-associated change in cortisol exposure, the AUC was calculated. For comparative AUC between individuals with different bed times, we standardised the bed times and the corresponding cortisol levels and calculated a standardised total daily cortisol exposure value (sAUC) (Fig. 1B). Accordingly, only the PE treatment showed a trend for effect of treatment on cortisol exposure sAUC (*p* = 0.099; Fig. 1Ca). The mean change in cortisol exposure was similar in AUC and sAUC. As expected, the variation in sAUC was lower than in AUC since the length of sleeping time was standardised.

Because of the PE effect on diurnal cortisol slope and the trend of a PE effect on sAUC we tested for cortisol level differences by treatment at the individual cortisol measure points in the PE group. We found that at wakening, the cortisol level was significantly reduced by PE treatment (*p* = 0.017), and the level 40 min after wakening was suggestively reduced (*p* = 0.071; Fig. 1Da). Further exploration of the ICBT and TAU arms found, as expected from the cortisol slope and sAUC data, no apparent effect on cortisol levels at 3 time points (Fig. 1Db, Dc; *p* > 0.17). Therefore, a further investigation of cortisol effects was performed for the PE arm only.

We explored if the baseline depression severity category affected the change in diurnal cortisol slope or awaken-

ing cortisol level by PE. Those with mild depression (*n* = 9) at baseline had 34.5% (*p* = 0.27) lowering of the diurnal slope and a tendency of lowering the awakening cortisol level following PE treatment (*p* = 0.092). Those with moderate depression (*n* = 26) at baseline had a 41.2% reduction in diurnal cortisol slope (*p* = 0.016) and a tendency for reduction of the awakening cortisol level (*p* = 0.078) by PE, whereas the group with severe depression at baseline was too small to analyse (*n* = 3). At this point, we assessed if these changes in awakening cortisol level due to PE had an effect on changes in depression level due to PE treatment. No association between the changes in diurnal cortisol slopes or awakening cortisol levels with regard to changes in depression severity was found (*p* > 0.57) in our sample after adjusting for age, gender, baseline depression severity, and baseline use of antidepressants and tobacco. Except for age, there was no difference in the parameters presented in Table 1 (in all 3 arms) between the participants and the non-participants.

Discussion

In the present study, the 12 weeks' PE intervention attenuated depression and the rate of salivary cortisol level decline across the day. The latter reflected reduced salivary cortisol levels at morning awakening following PE intervention. A tendency of a similar PE effect on cortisol was noticeable in the cortisol level 40 min after awaken-

ing. Furthermore, the total daily cortisol exposure, sAUC, tended to be reduced by PE. These PE effects seemed to be independent of whether the person had mild or moderate depression at baseline. However, no apparent relationship was found according to treatment between the reduction in cortisol level (diurnal cortisol slope or awakening cortisol level) and the reduction in depression severity. ICBT and TAU interventions attenuated depression severity, but no change in cortisol level following the ICBT and TAU interventions was observed.

A sharp decline in cortisol level from morning to late evening, or bedtime, has been considered to be associated with improved psychosocial and physical health [24]. Hypersecretion of cortisol plays a role in depression pathophysiology [25]. Also, cortisol has been suggested to be a biochemical marker of exercise-induced affective responses [26–28]. An antidepressive effect of PE is probably achieved through changes in the levels also of other hormones (e.g., thyroid hormones), as well as neuropeptides (e.g., brain-derived neurotrophic factor), neurotransmitters and inflammatory cytokines, previously proposed to be associated with both PE, hypothalamic-pituitary-adrenal axis activity and depression [29, 30], although evidence is still missing [31, 32]. Previous studies reported that long-term PE (8–16 weeks) reduces not only depression but also cortisol levels in morning plasma ($n = 21$) and 24-h urine ($n = 49$) of young and middle-aged adults [14, 15]. In agreement with those PE effects, we found a PE-induced reduction of awakening cortisol levels as well as a trend for a PE effect on total daily cortisol exposure (sAUC). Moreover, we found that the diurnal cortisol slope significantly flattened following PE intervention. The effect of long-term PE on the diurnal cortisol profile in depressed patients has, to our knowledge, not been previously explored.

Despite having a baseline depression severity similar to the PE group as well as a similar reduction in the depression score following 12 weeks' treatment, the reductions of cortisol levels in the ICBT arm were not statistically significant. This indicates that basal cortisol level is unlikely to be influenced by ICBT intervention. This was supported by findings of a recent systematic review and meta-analysis that investigated if cortisol level could be a predictor of treatment response to psychological therapy in depression [33]. However, because of the limited sample size, our findings should be considered as preliminary. Future studies are needed to establish why PE but not ICBT may have an attenuating effect on cortisol levels in addition to the effect on depression. If our findings are corroborated by future research, this might imply that PE

and ICBT (and most likely traditional CBT) affect depression through partly different mechanisms and, therefore, they might in combination further improve symptom reduction.

A main limitation of this study is, as stated earlier, the small sample size. Only 38, 56 and 27 participants in the PE ($n = 316$), ICBT ($n = 317$) and TAU ($n = 312$) arms had provided diurnal saliva for cortisol measurement with adequate date and time marks both at baseline and follow-up, allowing inclusion in this study. However, these 38, 56 and 27 participants showed a similar distribution of MADRS scores at baseline and follow-up as those who completed follow-up assessment for MADRS after PE ($n = 208$), ICBT ($n = 201$) and TAU ($n = 204$), respectively, and did not participate in the present study. However, the age distribution was shifted upwards 5–10 years in all arms.

Further, the cortisol levels could not be compared between treatment arms because the ELISA analyses of the cortisol levels in the ICBT, PE and TAU arms were done at different time points, in February 2016, December 2014 and June 2017, respectively. However, all samples for each individual patient were assessed under identical laboratory analysis conditions on the same 96-well plate, and all ELISA measurements were done using adequate standard curves and control reactions. Also, the coefficient of variation (standard deviation_{group}/mean_{group}) did not differ more between the PE arm and the other treatment arms than between baseline and follow-up within the PE arm. This suggests that the lack of a statistically significant effect of ICBT and TAU on cortisol levels in this study was not because of analysis batch effects.

It is known that the concentration of cortisol decreases if stored for long periods of time [34]. Another limitation includes that the present study could not account for intensities, duration and frequencies of exercise, which affect excretion of cortisol [35–37]. The Regassa study had an intention-to-treat protocol, hence the exact frequency and intensity of PE for each patient are unknown. However, interviews and pulse watches were used to control that the training sessions at the fitness centre were according to protocol. A strength of the study is that data come from a randomised controlled trial. Also, research nurses rated depression in the study and were masked at follow-up regarding treatment allocation. Studies addressing biological aspects of cognitive behavioural therapy in depression are limited; thus, our study adds knowledge to this unexplored area of psychiatry research.

Conclusions

Twelve weeks of PE reduced the rate of cortisol level decline across the day in adults suffering from depression. ICBT and TAU treatments had no detectable effects on diurnal cortisol levels. Larger samples are required for the detection and comparison of smaller effects of PE, ICBT and TAU on diurnal cortisol levels.

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Statement of Ethics

All participants gave written informed consent, and the Stockholm regional ethical review board approved the trial. All procedures were conducted in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000.

Disclosure Statement

All authors declare that they have no conflict of interest.

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