Increased Cortisol Awakening Response after Completing the Summer Treatment Program in Children with ADHD

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Abstract

OBJECTIVE: Little is known about the cortisol awakening response (CAR) in children with attention deficit hyperactivity disorder (ADHD). Here, we examined the CAR in children with ADHD and their mothers before, immediately after, and 4 months after an intensive summer treatment program (STP). METHODS: Participants were 37 children aged 7–12 years who completed the STP in 2009 and 2010, and their mothers. Daily saliva samples for cortisol measurement were collected twice daily at awakening and 30 min afterwards at pre-STP, post-STP, and during a follow-up measurement period. ADHD symptom scores were evaluated by parents, and participants completed the Kid-KINDL^{R} QOL questionnaire. RESULTS: CAR was low in children with ADHD before the STP, and increased to the control range 4 months after STP. Maternal CAR also tended to increase after STP. Changes in the CAR in children tended to correlate with an improved ADHD inattention scores (p = 0.091), physical health (p = 0.070), and school life subscales scores in the Kid-KINDL^{R} (p = 0.079). CONCLUSION: We demonstrated that STP improved the behavior and QOL of children with ADHD. Our results indicate that STP could lead to improvements in HPA axis function, as reflected by increased CAR after STP.

Key words

Attention deficit hyperactivity disorder, cortisol awakening response, summer treatment program, QOL, psychosocial treatment

Introduction

Attention-deficit/hyperactivity disorder (ADHD) is one of the most common childhood psychiatric disorders. Globally, it affects about 5% of children and about 2.5% of adults [1]. Children with ADHD show symptoms of inattention, hyperactivity, and impulsivity that is inappropriate for their developmental level [1]. In the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5), ADHD is categorized as a neurodevelopmental disorder involving brain dysfunction.

Based on differing symptoms, ADHD type has been classified according to three clinical presentations: predominantly inattentive, predominantly hyperactive-impulsive, and combined. Comorbid disorders, such as autism spectrum disorders (ASD), learning disorders (LD), anxiety disorders (AxD), oppositional defiant disorders (ODD), and conduct disorders (CD) are frequently observed. ADHD can affect all aspects of a child's life, and can also have a strong influence on the lives of parents and siblings.

The hypothalamic-pituitary-adrenal (HPA) axis is a major homeostatic system that is involve in the maintenance of equilibrium between an organism and its environment [2]. The HPA axis is the primary mammalian system associated with stress responses, and the endpoint of HPA-axis activation is the release of glucocorticoid cortisol [3].

87 Cortisol secretion is governed by a diurnal rhythm such that levels are at their highest in
88 the morning and gradually decrease during the night. In addition to circadian variation,
89 an acute increase in cortisol secretion occurs after awakening [4]. Although cortisol is
90 often used as a measure of HPA activation, several studies have indicated that this
91 cortisol awakening response (CAR) may be a more appropriate measure for assessing
92 HPA activation in relation to psychosocial factors [4, 5].

93 Our research team has conducted multiple experiments in which we sampled and
94 measured salivary cortisol in individuals ranging in age from neonates to adults [5-7]. In
95 these previous studies, we reported that salivary CAR was associated with mood status
96 in healthy female children [5] and that plasma cortisol levels were linearly correlated
97 with salivary cortisol in neonates [7]. These findings indicate that salivary cortisol is a
98 useful surrogate marker for plasma cortisol that can be collected in a non-invasive way.

99 Salivary cortisol levels and HPA axis activity in individuals with ADHD have been
100 previously assessed within the framework of the behavioral inhibition system [8].
101 Previous studies have reported an altered cortisol response [9] or lower CAR in children
102 with ADHD compared with healthy control children [9]. Compared with control
103 children, children with ADHD had lower levels of cortisol both when they awoke and

104 30 min thereafter, and a smaller CAR [10, 11]. However, these findings have not been
105 replicated in other studies. Corominas et al. conducted a large-scale review of studies
106 that examined the involvement of cortisol in ADHD [12], and observed two diverse
107 patterns: 1) dampened cortisol responses to stress were associated with comorbid
108 disruptive behavior disorder (DBD), and 2) high cortisol responses were associated with
109 comorbid AxD [12, 13]. These results remain controversial. Discrepancies between
110 studies may be due to different participant characteristics (e.g., medication,
111 comorbidities, age) or different assessment methods (e.g., saliva, plasma).

112 There are three evidence-based treatments for ADHD: medication with a central
113 nervous system stimulant, behavioral modification, such as the summer treatment
114 program (STP) and parent training programs, and a combination of medication and
115 behavioral modification [14]. Numerous studies in North America have reported on the
116 efficacy of the STP over the past decade. These studies have documented substantial
117 STP-related improvements in multiple domains, including peer relationships,
118 compliance with adult requests, and classroom functioning, as assessed via direct
119 behavioral observation and rating scales [15]. We modified the American STP into a 2-
120 week program, which we have administered since 2005 in Kurume, Japan. In a previous
121 report, we demonstrated that our program had a short-term effect on behavioral and

122 cognitive function that lasted up to 4 months after completion [16]. However, previous
123 study lacked objective evaluation techniques, such as measurements of salivary cortisol
124 as a biomarker. Therefore, in the present study, we sought to clarify the influence of
125 STP on the CAR in both children with ADHD and their mothers. We hypothesized that
126 the CAR would be lower in children with ADHD prior to the treatment program, and
127 that it would increase after intensive STP, with specific improvements in behavioral
128 function and QOL.

129 Participants and Methods

130 Participants

131 Participants included 48 school children (41 boys and 7 girls) aged 7–12 years who
132 participated in the STP in 2009 and 2010. Diagnoses for these patients were based on
133 the criteria of the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition
134 Text Revision (DSM-IV-TR) [17] and were determined by three experienced pediatric
135 neurologists (YY, SN, and TM) who, together with multiple clinical psychologists,
136 reviewed detailed participant data [16]. Each participant met the criteria for IQ level,
137 i.e., an IQ greater than 70, as per the Wechsler Intelligence Scale for Children-Third
138 edition.

As a control group, 16 age and sex matched children were recruited from an elementary school in the same area. We obtained information about the developmental history and medical status of the children via questionnaires completed by their parents. The parents also completed a questionnaire focused on the symptoms of ADHD based on the DSM-IV-TR [17]. This enabled us to rule out the possibility that members of our control group had ADHD. The control group did not participate in the STP.

We obtained both oral and written informed consent from each participant and his/her parents after the details of the study had been fully explained. The ethical committee at Kurume University School of Medicine approved the present study.

Saliva sampling

Figure 1 shows the detailed study protocol. We collected saliva samples from each child and his/her mother on 2 consecutive Saturdays during weeks without school days or special occasions prior to the STP (pre-STP) and following the STP (post-STP). We also conducted follow-up evaluations of 20 randomly selected children for 4 months after the STP (follow-up STP). A recent consensus regarding the guidelines for assessing the CAR requires the collection of 3 post-awakening samples [18]. However, according to the study by Bäumler et al.[19], we instructed participants to collect saliva on just two occasions

during each day: immediately upon awakening and 30 minutes after. We required 2 samples per day instead of 3 to reduce the burden of participation in the study and limit the effect of the study on the daily routines of the families and children. Researchers explained the protocol and demonstrated the saliva sampling procedure to the participants and their parents. Participants received training for regarding the saliva collection protocol prior to beginning the study. They were asked not to collect saliva within 30 minutes after eating, drinking, walking, or brushing their teeth. Parents were asked to gently wake up their child. We used a well-established technique for salivary sampling, which is a common method of cortisol measurement in adults and children. Saliva samples were collected using Salisoft® (Sarstedt, Inc., Germany). A cotton swab was chewed for 2 min without moving the jaw or stimulating saliva production in any way. Then the swab was inserted into a double-chamber plastic test tube. Saliva samples were centrifuged at 4°C and frozen at −80°C until assayed.

Measurement of cortisol

Salivary cortisol concentrations were measured via an enzyme immunoassay (high sensitivity salivary cortisol enzyme immunoassay kit; Salimetrics LLC, USA). The limit of detection of this assay in our laboratory was 0.19 nmol/liter. Intra- and inter-assay coefficients were 5.43% and 6.41%, respectively. The assay plates were read at 450 nm

for cortisol, using a Multiskan Ascent microplate photometer (Thermo Labsystems Oy, Helsinki, Finland).

Summer Treatment Program (STP)

The STP procedures include a variety of behavioral components that have been extensively described and documented elsewhere [16]. There are 3 major components of treatment in the STP: behavioral therapy (e.g., point system, time out, daily report card, parent training), social skills training, and sports skills training. Parents participated in two sessions before the beginning of the STP, in which they learned general behavioral principles, such as reinforcement systems, appropriate commands, and time outs. They were also taught to implement home-based rewards for their child's good performance during STP days, as measured by the daily report card. There were 37 staff members: 7 clinical psychologists, 20 student counselors, 3 pediatricians, 1 nurse, and 6 special education teachers. The children attended the STP at an elementary school from 9:30 AM until 4:20 PM each weekday. They were placed into 2 age-matched groups of 12 children that stayed together throughout the day. Each group spent 1.5 h daily in classroom sessions conducted by special education teachers, during which the children completed individualized paper and pencil activities, computer-assisted learning, and peer tutoring in small groups. The remainder of each day was spent engaged in

192 recreationally based group activities supervised by 7 clinical psychologists. The
193 intervention was implemented in both classroom and recreational settings while the
194 children were engaged in classroom tasks and group-based recreation.

195 Using a systemic reward/response cost program, children earned points for
196 appropriate behavior (e.g., helping, compliance, good sportsmanship) and lost points for
197 inappropriate behavior (e.g., teasing, noncompliance, rule violations) throughout the
198 day. Counselors recorded the points taken from and awarded to each child throughout
199 the day. Children were disciplined for prohibited behaviors (i.e., intentional aggression,
200 intentional destruction of property, repeated non-compliance), with discipline in the
201 form of loss of privileges or time out from the ongoing activities.

202 Questionnaires

203 Parents completed a questionnaire that rated the symptoms of ADHD, oppositional
204 defiant disorder, and conduct disorder, based on DSM-IV-TR criteria, for behavior
205 observed at the time of saliva collection. Simultaneously, the children completed the
206 Kid-KINDL^{R} questionnaire (Questionnaire for measuring health-related quality of life
207 in children [20]). The Kid-KINDL^{R} questionnaire comprises six domains, (1) physical
208 health, (2) emotional well-being, (3) self-esteem, (4) family, (5) friends, and (6) school,

209 with four items for each domain. The Kid-KINDL^R has been validated as a reliable and
210 practical instrument for assessing the QOL of Japanese elementary school children [21].
211 Scores were transformed such that the range of possible values for the total score
212 extended from 0 (most negative state) to 100 (most positive state).

213 ## Statistical Analyses

214 We analyzed the data using SPSS (Statistical Package for the Social Sciences)
215 Version 20.0J. Specifically, we analyzed the levels of cortisol and CAR (increase in
216 cortisol levels between awakening and 30 min after awakening) using repeated-
217 measures analyses of variance (ANOVA), in which the measurement day was set as an
218 independent variable (i.e., pre-STP, post-STP, and 4-month follow up) and sampling
219 time (i.e., awakening and 30 min after awakening) was set as a within-subject repeated
220 measure. We determined the correlations between CAR and symptom scores, and
221 between CAR and QOL via Pearson's correlation coefficient. Post hoc tests were
222 conducted using Tukey's least significant difference test. The data are presented as the
223 means ± standard deviation (SD).

224 # Results

225 Forty-eight children with ADHD completed the STP in 2009 and 2010. The
226 complete set of saliva samples and questionnaires were obtained from 37 children aged
227 7–12 years (mean 9.4 years, SD 1.5 year) and their 37 mothers. Table 1 provides
228 descriptive information about the participants and control children. One of the children
229 had hyperactive/impulsive type ADHD, 11 had inattentive type, and the others had
230 combined type (Table 1). In the STP group, 21 children were on ADHD medication: 17
231 children were receiving methylphenidate, one was receiving atomoxetine, one was
232 receiving both, one was receiving methylphenidate and risperidone, and one was
233 receiving methylphenidate and clonidine. The other 16 children were not taking
234 medication. In the STP group, 23 of the children had comorbidities; eight had ASD,
235 seven had LD, four had ASD and LD, two had ASD and ODD, and two had LD and
236 ODD (Table 1). Fourteen of the children had no comorbidities. None of the participants
237 had CD or AxD (Table 1).

238 The mean awakening time was not significantly different between the STP group
239 and the control group (6:51 ± 0:29 STP group vs. 6:45 ± 0:14 control group).

240 **Cortisol levels and CAR**

241 The mean cortisol levels for all sample points and the CAR (increase of cortisol
242 levels between awakening and 30 min after awakening) are presented in Table 2. In
243 both the STP and control groups, the levels of cortisol upon awakening were not
244 significantly different among the pre-STP, post-STP, and follow-up measurements. In
245 the STP group, the level of cortisol 30 min after awakening was not significantly
246 different between the pre- and post-STP conditions, but it was significantly higher at the
247 follow-up compared with the pre-STP measurement ($p = 0.004$) (Fig. 2). There was a
248 significant increase in cortisol levels between awakening and 30 min after awakening
249 post-STP and follow-up, but not pre-STP. In addition, the CAR in the STP group was
250 lower pre-STP compared with the measures in the control group, and increased to the
251 control range 4 months after STP (Table 2, Fig. 3).

252 We observed no significant differences in cortisol and CAR levels between
253 children with ADHD alone and children with ADHD plus comorbidities at any of the
254 pre-STP, post-STP, or follow-up measurement points (Table 3).

255 We found no significant difference in the CAR between non- medicated (N =
256 16) and medicated (N = 21) participants at any of the pre- STP (-0.15 ± 4.74 vs 0.93 ±
257 4.05; $p = 0.46$), post- STP (3.83 ± 4.71 vs 5.62 ± 6.70; $p = 0.37$), or follow-up STP (3.68
258 ± 7.45 vs 10.31 ± 9.63; $p = 0.13$) measurement points.

Changes in symptom scores and CAR

Changes in symptom scores are presented in Table 4. Inattention scores tended to differ among the pre-STP, post-STP, and follow-up measurements ($F(2, 68) = 3.05$, $p = 0.054$) and post-STP scores tended to be lower than pre-STP scores ($p = 0.100$). Hyperactivity/impulsivity scores were significantly different among the pre-STP, post-STP, and follow-up measurements ($F(2, 68) = 6.77$, $p = 0.002$), and follow-up hyperactivity/impulsivity scores were lower than pre-STP ($p = 0.018$) and post-STP scores ($p = 0.013$). Oppositional defiant scores were not significantly different among the pre-STP, post-STP, and follow-up measurements. Additionally, improvements in inattention subscale scores were weakly correlated with the levels of CAR after completing the STP ($r = -0.29$, $p = 0.091$). Hyperactivity/impulsivity subscale scores were not correlated with CAR levels.

Inattention scores in children with ADHD and LDs tended to be higher than those in children with ADHD and no LD ($p = 0.095$). Additionally, inattention scores in children with ADHD on medication tended to be higher than those in unmedicated children ($p = 0.073$).

Changes in QOL scores and CAR

Changes in QOL scores are presented in Table 4. For the Kid-KINDL^R, QOL scores were not significantly different among the pre-STP, post-STP, and follow-up measurements. The changes in CAR between the pre-STP and STP follow-up measurement points tended to be correlated with improved QOL scores in the school (r = 0.41, p = 0.079) and physical health (r = 0.424, p = 0.070) categories of the Kid-KINDL^R questionnaire. The hyperactivity/impulsivity score at the pre-STP measurement was negatively correlated with the QOL score in the emotional well-being (r = −0.55, p = 0.001), family (r = −0.59, p = 0.000), and friends (r = −0.40, p = 0.02) categories, and the oppositional defiant score at the pre-STP measurement was negatively correlated with the QOL emotional well-being score (r = −0.42, p = 0.013) in the Kid-KINDL^R questionnaire. The inattention score at the follow-up measurement was negatively correlated with QOL scores in the physical health (r = −0.30, p = 0.081) and friends (r = −0.38, p = 0.026) categories, and the oppositional defiant score at the follow-up measurement was negatively correlated with QOL scores in the physical health (r = −0.32, p = 0.063), emotional well-being (r = −0.40, p = 0.019), family (r = −0.33, p = 0.060), and school (r = −0.31, p = 0.077) categories in the Kid-KINDL^R questionnaire.

Changes in the hyperactivity/impulsivity score between the pre-STP and follow-up measurements points tended to be correlated with an improved QOL self-esteem score ($r = -0.30$, $p = 0.082$) in the Kid-KINDL^R questionnaire. Changes in the ODD score between the post and follow-up measurements were correlated with an improved QOL friends score ($r = -0.38$, $p = 0.029$) in the Kid-KINDL^R questionnaire. Comorbidities (ASD and LD) did not affect the QOL score.

Cortisol levels and CAR in mothers of children in the STP group

Changes in cortisol levels and the CAR of mothers of children in the STP group are presented in Table 2 and Figure 4. We found a gradual increase in CAR through the pre-STP, post-STP, and follow-up measurements, even though cortisol levels were not significantly different ($F(2, 88) = 1.543$, $p = 0.219$).

Discussion

The HPA axis plays an important role in regulating neurotransmitters and behaviors associated with the central nervous system, such as attention, emotion, learning, memory, and movement [22, 23]. Previous studies have reported that the HPA axis might function abnormally in children with ADHD. Indeed, this dysfunction may

310 be directly tied to symptoms of ADHD, such as attention deficit, hyperactivity, and
311 impulsive behavior [24].

312 In this study, we investigated levels of salivary cortisol in children with
313 ADHD, their mothers, and control children, along with ADHD symptom scores and
314 QOL scores at pre-STP, post-STP, and follow-up timepoints after STP. To our
315 knowledge, this is the first study to demonstrate that low CAR levels in children with
316 ADHD increased following completion of a summer treatment program with intensive
317 behavioral therapy, social skills training, and sports skills training.

318 Our finding that children with ADHD had a lower CAR was consistent with
319 previous studies [9], and supports the notion of dysregulation of the HPA-axis in
320 children with ADHD. Freitag et al. (2009) compared CAR levels in children with
321 ADHD + comorbid disorders with those with ADHD alone and found that diminished
322 CAR levels appeared to be specific to children with ADHD + ODD. Similarly, another
323 study found no evidence of diminished CAR in children with ADHD without comorbid
324 disorders, and also found no decrease in CAR levels in children with ADHD + CD or
325 ADHD + AxD [25]. Although our participants were free of CD and AxD, they still
326 showed decreased CAR levels. This could indicate that HPA axis dysfunction also
327 exists in children with ADHD and no CD or AxD.

328 Several researchers have reported an association between HPA axis
329 dysfunction and hyperactivity [9, 26], general ADHD symptoms [8], or genetic factors
330 [27-29]. In our study, improvements in inattention and hyperactivity/ impulsivity
331 subscale scores were correlated with changes in CAR. ADHD has been associated with
332 behavioral disinhibition [30]. Disinhibition appears to be related to dysfunction of the
333 HPA axis, whereas behavioral inhibition has been associated with activity in the HPA
334 axis [31]. To our knowledge, no previous studies have investigated the therapeutic
335 effect of STP and associated changes in CAR levels in children with ADHD. Our results
336 indicate that intensive behavioral STP treatment led to improved behavioral inhibition
337 and QOL. Such changes might lead to improved HPA axis function in children with
338 ADHD, as reflected by the observed increase in CAR levels after STP.

339 Recently, a specific haplotype (G:A:G:G; ER22/23EK- N363S- *Bcl*I- A3669G)
340 of the glucocorticoid receptor (GR) gene was found to be significantly associated with
341 behaviors related to ADHD symptoms, comorbidity with ODD, and executive function
342 domains [32]. Furthermore, the GR variants have been associated with changes in HPA
343 axis and stress-related disorders, and contribute to individual differences in resilience
344 and vulnerability to stressors [33, 34]. Taking these findings together with ours, ADHD
345 symptoms appear to be associated with ADHD and HPA axis dysregulation.

346 The opportunities uncovered via epigenetic modification of the GR gene
347 indicate that it is possible to establish an epigenetic state of a gene through behavioral
348 programming, and that this process has the potential to be reversible [35]. In addition,
349 Robert et al. found suggestive evidence for an association between changes in the
350 methylation of *FKBP5,* which is a functional negative regulator of GR, and
351 psychological therapy response [36, 37]. These mechanisms might lead to the observed
352 increase in CAR levels following intensive behavioral STP treatment. Moreover, the
353 effect was sustained until the follow-up at 4 months after the STP, indicating long-
354 lasting effects.

355 In the present study, we found that CAR levels in mothers of children with
356 ADHD also tended to increase following the STP. Improvements in mother–child
357 interactions have been found to modify cortisol responses to stress [38], and our
358 findings that low maternal CAR also increased after the STP support this notion.

359 Our results suggest that intensive STP behavioral treatment results in improved
360 behavior inhibition and QOL in children with ADHD. These improvements might be
361 associated with epigenetic changes to the GR gene or its associated genes, which could
362 consequently improve HPA axis function, as reflected by the increased CAR observed
363 after the STP.

The findings of this study should be interpreted within the context of certain limitations. The principal limitation of our study was the small sample size. Second, our sample contained many more boys than girls, reflecting the fact that ADHD is more commonly observed in boys. Third, ADHD medication presents a potential bias. Previous studies about methylphenidate or atomoxetine have demonstrated the increase and normalization of cortisol levels by noradrenergic potentiation [39-41]. Another study showed that the cortisol levels of ADHD patients increased significantly after 1 month of MPH treatment, but were found not to change significantly during a 6-month treatment period [42]. In this study, most of the medicated children with ADHD had been taking oral MPH or ATX for more than 6 months, and thus we would not observe any significant differences in cortisol levels between the medicated and unmedicated group. Further research with a larger sample is required to identify differential association based on gender or medication. Additionally, to fully examine the precise effect of the STP on CAR levels in children with ADHD, it is necessary to also consider changes in CAR levels in children with ADHD who do not participate in the STP. Finally, children with ADHD unexpectedly provide extremely positive reports of their own competence in comparison to other criteria reflecting actual competence, in a phenomenon known as positive illusory bias (PIB). Hypothesized explanations for the PIB include cognitive

382 immaturity, neuropsychological deficits leading to ignorance of incompetence, and self-
383 protection [43, 44]. Thus, the PIB may have affected the accuracy of the QOL
384 questionnaire, which was completed by the participants. Further studies thus could
385 include evaluations of QOL, completed not only by the participants but also by their
386 mothers or teachers.

387 Conclusions

388 The STP improved behavior in children with ADHD and may improve HPA
389 axis function, as reflected by an increase in CAR levels after STP. Furthermore, we
390 demonstrated that improvements in mother–child interactions could modify cortisol
391 responses to stress. Further studies are needed to confirm the influence of mother–child
392 interactions on HPA axis activity.

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Figure legends

Figure 1. Study protocol

Figure 2. Comparison of cortisol levels in children with ADHD at pre-STP, post-STP, and at a STP follow-up measurement.
In the STP group, cortisol levels 30 min after awakening were significantly higher 4 months after STP (Follow-up STP) compared with those in the Pre-STP measurement.
# $p < 0.01$ vs. Pre-STP

Figure 3. Cortisol awakening response (CAR) in children with ADHD pre-STP, post-STP, and at a STP follow-up measurement.
*****$p < 0.05$ vs. control group
Pre-STP CAR in the STP group was significantly lower than that in the control group.

Figure 4. Comparison of cortisol levels in mothers of children with ADHD at pre-STP, post-STP, and at STP follow-up measurement points.
Cortisol levels were not significantly different.

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571

572 Table 1. Participant demographic characteristics

		STP group	Control group
		N=37	N=16
Age [mean (SD)]		9.4 (1.5)	9.3 (1.4)
Sex [N (%)]			
	Male	31(83.8)	15 (93.7)
	Female	6 (16.2)	1 (6.3)
Time of awakening [mean (SD)]		6:51 (00:29)	6:45 (00:14)
ADHD subtype [N (%)]			
	Hyperactive/ Impulsive type	1 (2.7)	
	Inattentive type	11 (29.7)	
	Combined type	25 (67.6)	
Medication [N (%)]			
	Methylphenidate	17(45.9)	
	Atomoxetine	1 (2.7)	
	Methylphenidate + Atomoxetine	1 (2.7)	
	Methylphenidate + Risperidone	1 (2.7)	
	Methylphenidate + Clonidine	1 (2.7)	
	None	16 (43.2)	
Comorbidities [N (%)]			
	ASD	8 (21.6)	
	LD	7 (18.9)	
	ASD + LD	4 (10.8)	
	ASD + ODD	2 (5.4)	
	LD + ODD	2 (5.4)	
	None	14 (37.8)	
WISC-III [mean (SD)]			
	VIQ	100.2 (11.3)	
	PIQ	92.1 (12.6)	
	FIQ	96.2 (10.5)	

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574 ASD; Autism spectrum disorder, LD; Learning disorder, ODD; Oppositional defiant

disorder

Table 2. Changes in salivary cortisol concentration

		Pre-STP	Post-STP	Follow-up STP
STP group		N=37	N=37	N=20
	on awakening	11.56 (6.32)	9.87 (3.49)	11.23 (7.18)
	30 min after awakening	12.02 (5.31)	14.71 (7.10)[c]	18.56 (9.72)[ce]
	CAR	0.46 (4.33)	4.84 (5.91)[d]	7.33 (9.15)[e]
Control group		N=16	N=16	N=16
	on awakening	7.91 (2.04)	9.78 (4.24)	8.23 (3.62)
	30 min after awakening	14.89 (8.21)[c]	17.31 (8.04)[c]	17.03 (8.71)[c]
	CAR	6.98 (7.73)[g]	7.53 (6.45)[h]	8.80 (6.08)[i]
Mothers of STP group		N=37	N=37	N=20
	on awakening	11.10 (6.75)	10.80 (5.53)	13.63 (10.45)
	30 min after awakening	13.69 (8.81)[a]	14.65 (10.11)[b]	18.56 (11.28)[b]
	CAR	2.59 (8.06)	3.84 (7.68)	4.92 (4.84)

[a] $p \leq 0.05$ vs. on awakening; [b] $p \leq 0.01$ vs. on awakening ; [c] $p \leq 0.001$ vs. on awakening
[d] $p \leq 0.05$ vs. Pre-STP; [e] $p \leq 0.01$ vs. Pre-STP ; [f] $p \leq 0.001$ vs. Pre-STP;
[g] $p \leq 0.05$ vs. Pre-STP of STP group; [h] $p \leq 0.01$ vs. Pre-STP of STP group; [i] $p \leq 0.001$ vs. Pre-STP of STP group
CAR cortisol awakening response (increase in cortisol levels between awakening and 30 min after awakening)

Table 3. Changes in salivary cortisol concentration in children with ADHD alone vs. children with ADHD with comorbidities

		pre STP	**post STP**	**follow-up STP**
ADHD alone		N=14	N=14	N=7
	on awakening (*nmol/l*)	6.74 (3.32)	5.88 (2.21)	8.63 (5.76)
	30 min after awakening (*nmol/l*)	6.86 (3.25)	9.80 (4.11)	11.14 (5.75)
	CAR	0.12 (1.93)	3.92 (3.77)	2.51 (4.32)
ADHD + comorbidities		N=23	N=23	N=13
	on awakening (*nmol/l*)	6.23 (3.68)	5.24 (1.77)	4.95 (1.86)
	30 min after awakening (*nmol/l*)	6.57 (2.82)	7.18 (3.57)	9.86 (5.39)
	CAR	0.34 (2.69)	1.94 (2.77)	4.91 (5.42)

Table 4. Changes in psychometric profiles of children with ADHD

		Pre-STP	**Post-STP**	**Follow-up STP**
		N=37	N=37	N=37
Symptom scores [mean (SD)]				
	hyperactivity-impulsivity	11.47 (5.86)	10.50 (5.36)	8.84 (4.86)[ab]
	inattention	18.25 (4.56)	16.56 (5.22)	16.54 (5.05)[a]
	oppositional defiance	8.97 (6.22)	8.39 (5.47)	7.92 (5.49)
The Kid-KINDL^R [mean (SD)]				
	physical health	80.88 (19.64)	88.60 (15.35)	83.27 (19.99)
	emotional well being	80.70 (27.50)	85.48 (17.99)	84.19 (20.08)
	self-esteem	54.04 (28.24)	59.19 (28.67)	62.68 (31.81)
	family	67.65 (28.35)	73.16 (20.90)	70.96 (21.37)
	friends	70.4 (18.48)	72.24 (22.57)	74.82 (22.90)
	school	64.15 (24.68)	63.24 (28.03)	56.80 (21.78)
	total QOL	70.24 (16.66)	74.05 (14.68)	72.65 (17.98)

[a] $p \leq 0.05$ vs Pre-STP; [b] $p \leq 0.05$ vs Post-STP

Symptom scores: ADHD questionnaire, oppositional defiant disorder, and conduct disorder rating scales based on DSM-IV criteria.

Figure 1. Protocol

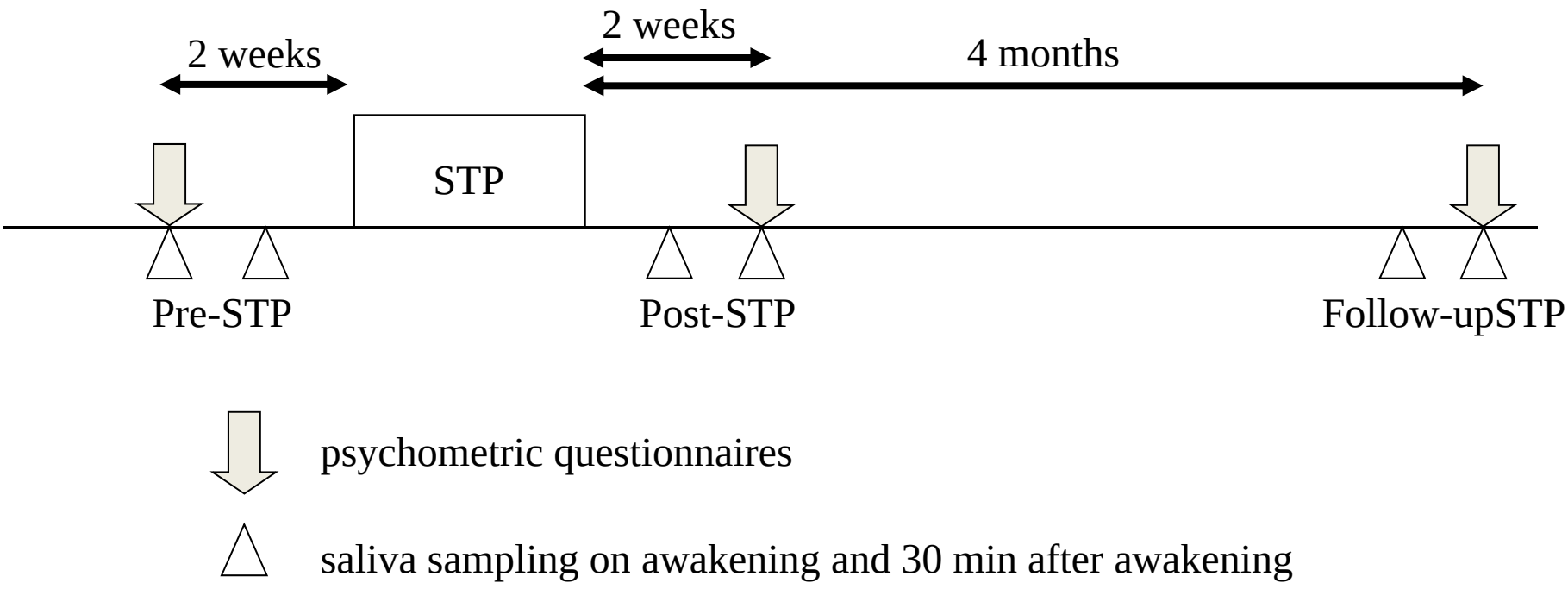
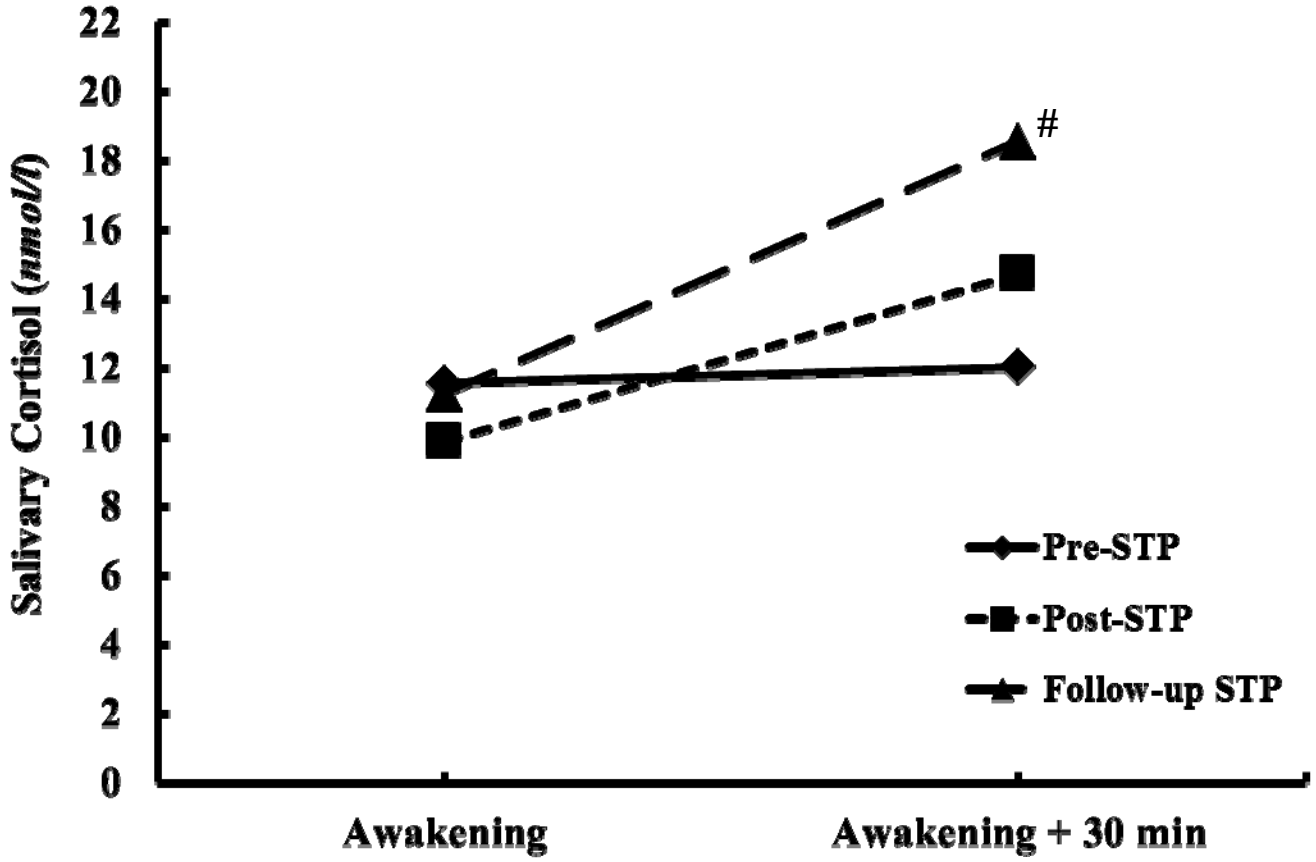


Figure 2.

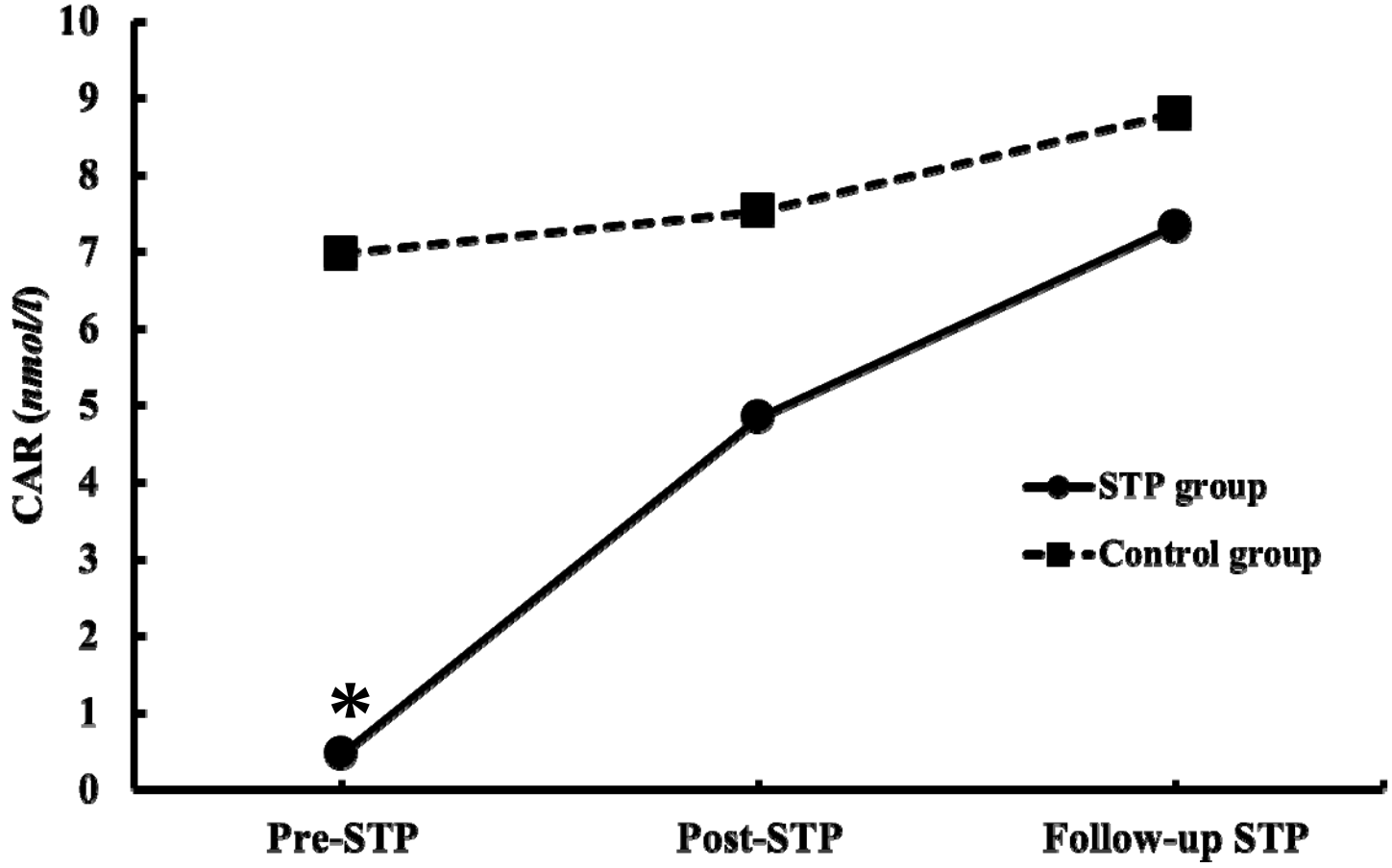
Comparison of the cortisol levels in children with ADHD
at pre- and post-STP, and at a follow-up measurement after STP.



In the STP group, the level of cortisol 30 min after awakening was significantly higher 4 months after STP (Follow-up STP) compared with that in the Pre-STP measurement.

# $p < 0.01$ vs. Pre-STP

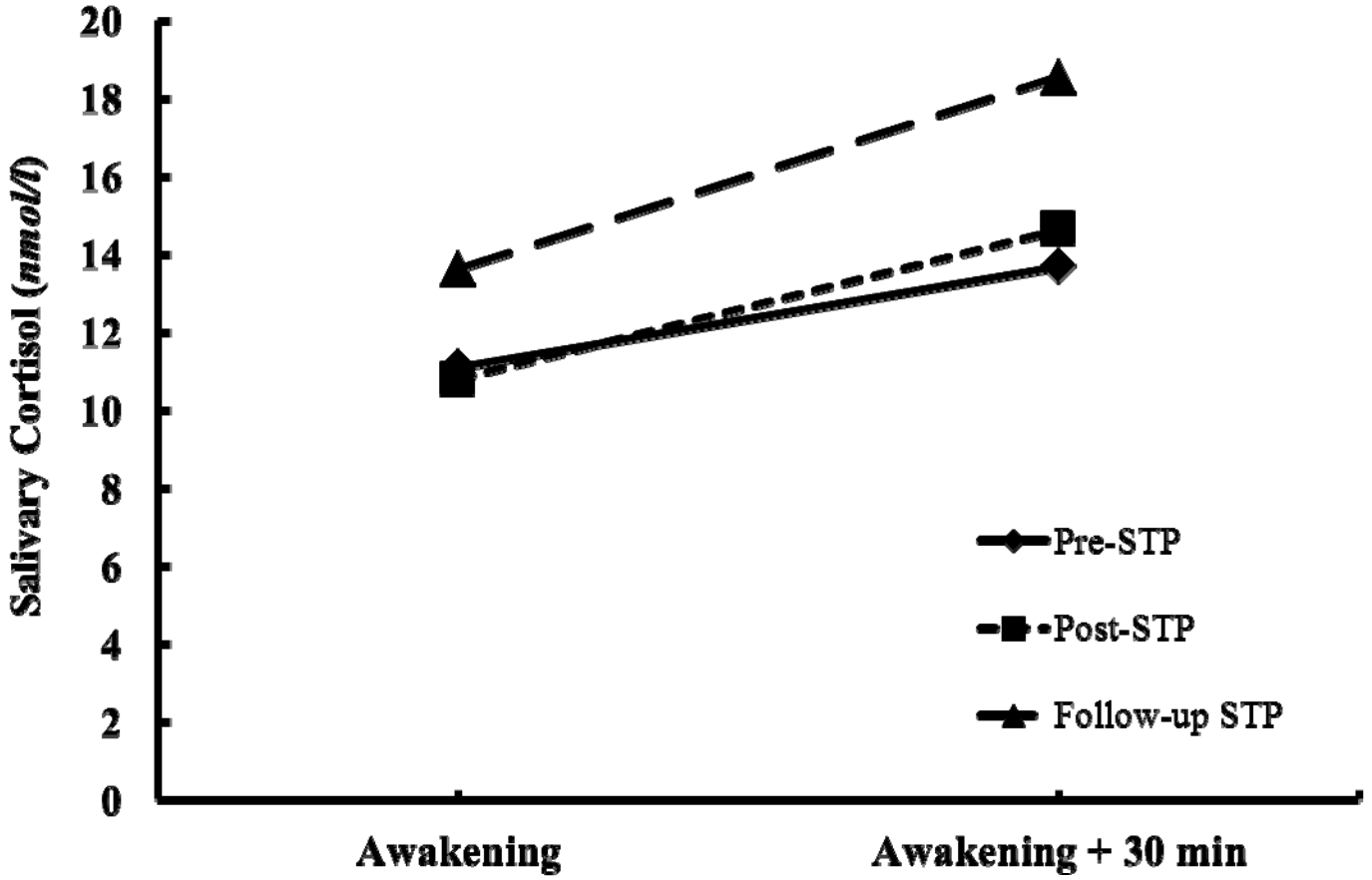
Figure 3. Cortisol awakening response (CAR) in children with ADHD pre- and post-STP and at a follow-up measurement after STP.



*$p < 0.05$ vs. control group

Pre-STP CAR in the STP group was significantly lower than that in the control group.

Figure 4 Comparison of the cortisol levels in mothers of children with ADHD at pre, post, and STP follow-up measurement points.



Cortisol levels were not significantly different.