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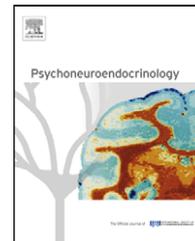**ScienceDirect**

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Steroid hormones in the saliva of adolescents after different exercise intensities and their influence on working memory in a school setting

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Summary   Little is known, about the influence of different exercise intensities on cognition, the concentration of steroid hormones (SHs), and their interaction in adolescents. Sixty high school students from the 9th grade were randomly assigned to two experimental (EG 1, EG 2) and a control group (CG). Saliva collection took place after a normal school lesson (t1) and after a 12-min resting control or exercise (t2) in a defined heart rate (HR) interval (EG 1: 50—65% HR max, $n = 18$; EG 2: 70—85% HR max, $n = 20$; CG: no intervention, $n = 21$). Saliva was analyzed for T and C. Cognitive performance was assessed using a working memory task (Letter Digit Span; LDS), which took place after t1 and t2. Repeated measure ANOVAs revealed a significant group by test interaction, indicating an increase of C and T level only for EG 2. Results for LDS showed a significant improvement due to exercise when groups were split into low and high performer at pre-test with a higher improvement of the low performers. In addition, post-test T levels negatively correlated with changes in LDS performance in EG 2. The results indicate that the concentrations of C and T are intensity dependent, and that exercise improves working memory in low performing adolescents. Only increased T, however, seems to be related to pre-to-post-test changes in working memory by having a detrimental effect on performance.
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1. Introduction

It is well known that acute bouts of physical activity in adults can influence the secretory processes of many endocrine tissues, releasing products such as testosterone (T) and cortisol (C). C, the main glucocorticoid form in humans, is a catabolic hormone secreted from the adrenal cortex in response to physical and psychological stress (Kirschbaum and Hellhammer, 1994). Given an adequate workload of >70% VO_{2max} in a short-term duration of 10–15 min, adult subjects showed increasing salivary C levels throughout the stress period (O'Connor and Corrigan, 1987). T is considered a key anabolic hormone with multiple physiological functions in the human body. With respect to exercise, T levels are often described as being associated with muscle mass and strength (Zitzmann and Nieschlag, 2001). Somewhat similar to C, T increases linearly in response to exercise once a specific intensity threshold of approximately 50% of VO_{2max} (Maresh et al., 1988) is reached, with peak concentrations occurring at the end of a 20-min exercise bout (Wilkerson et al., 1980).

Compared to adults, fewer data are available on hormone responses in children and adolescents to exercise-related stress (Boisseau and Delamarche, 2000). A study by Di Luigi et al. (2006) showed an increase in salivary C as well as T in adolescent boys 90 min after medium–high intensity exercise. Most other data on the influence of physical activity are extrapolated from information reported in studies with adults (Boisseau and Delamarche, 2000). Hence, more research on acute exercise-related endocrine system modifications in students appears to be essential.

This is of particular interest since glucocorticoids (see Lupien et al., 2005 for review) and T (see Hampson, 1995 for review) in young adults have been shown to influence cognitive performance. Thus, a beneficial relationship between acute physical activity and other measures of academic performance, such as academic achievement in the classroom, might be due to changes in SHs.

Moreover, studies with healthy young men and women mainly revealed a curvilinear (inverted U-shape) relationship between serum T levels and cognitive functions (Shute et al., 1983; Gouchie and Kimura, 1991; Moffat and Hampson, 1996; Muller et al., 2005). They found high and low levels of T being associated with low spatial ability scores whereas intermediate levels of T were associated with better spatial performance. Although other studies have reported linear (Silverman et al., 1999; Schattmann and Sherwin, 2007) relationships between serum levels of testosterone and cognitive function or failed to find any relationship (McKeever and Deyo, 1990; Gordon and Lee, 1986) an inverted nonlinear function might be best used to describe the relationship between circulating androgens and mean spatial cognition.

An inverted U-function shaped relation has also been reported between C and cognition (in a working memory task) for young subjects, and is consistent with current knowledge of the role of glucocorticoid hormones in brain function (Lupien et al., 2005). Regarding the acute effect of C a few human studies observed negative effects of high C-level or stress-treatment on working memory (Wolf et al., 2001; Elzinga and Roelofs, 2005; Oei et al., 2006; Schoofs et al., 2008), while mild stress is supposed to result in enhanced working memory performance (Schoofs et al., 2008). Animal models (see Roozendaal, 2000 for review) suggest that the level of adrenergic arousal induced by the training procedure is essential for the impairing effects of corticosterone on working memory. In rats, Okuda et al. (2004) showed that the relationship between corticosterone and object recognition depends on the level of training-induced emotional arousal. In humans, Elzinga and Roelofs (2005) found that working memory was significantly impaired among cortisol responders when they had to perform the memory task in a stressful context (i.e. in front of an audience), during which both cortisol levels and sympathetic activity were enhanced. Thus C might only be effective if a certain amount of testing induced arousal is present.

All studies that have shown an influence of T and C on cognitive performance, however, disregard the exercise factor in achieving a change in SHs. Research has shown that acute exercise of different intensities (see Tomporowski, 2003 for a review) and of different types (e.g. Budde et al., 2008) can positively influence cognition in adults and youth. Tomporowski (2003), for example, suggested an inverted U-function between exercise intensity and cognition. In addition, a recent study revealed that the impact of exercise on cognition is not uniform across all individuals (Sibley and Beilock, 2007), but rather that acute exercise predominantly helped those who performed sub-optimally in cognitive testing.

Less is known, however, about the link between exercise-related SHs changes and improved cognitive performance due to acute bouts of exercise. One could assume that the underlying mechanisms of a change in cognitive functions after acute exercise are due to an alteration in these SHs. The inverted U-function between exercise intensity and cognition might be explained by an inverted nonlinear function between C as well as T and cognitive functioning.

Data of schoolchildren provided by Aronen et al. (2005) showed that spatial working memory performance was associated with teacher-reported academic performance; keeping this in mind, we chose a working memory task to illustrate the academic demand of school.

Accordingly, the aims of our study were (1) to confirm exercise-related stress reactions in adolescents, as shown previously for adults; (2) to identify an exercise intensity for a short intervention that could potentially be implemented in a school setting (i.e. a school break), and that enhances working memory; and (3) to determine if exercise-related changes of C and T levels in adolescents are related to altered working memory.

As mentioned above, previous research with adults has shown that an increase in the plasma C concentration, above resting levels, requires an exercise intensity of >70% VO_{2max} (O'Connor and Corrigan, 1987). For testosterone, however, exercise intensities as low as 50% of VO_{2max} have been shown to elicit significant increases in plasma T (Maresh et al., 1988) in untrained subjects. Thus, we chose two exercise intensities that were assumed to optimally elevate C and T levels, respectively (i.e. 50–65% and 70–85% of the maximum heart rate [HR max]). To implement the intervention in a school setting (i.e. the exercise should be completed in a school break of usually 15 min) we chose a duration of 12 min. We expected an exercise-related increase of T and C in adolescents. Furthermore, we expected to find a relationship between working memory and exercise intensity that fits the U-shape curve reported in studies with young adults. More

specifically, we suggest that low performing individuals in the working memory task have a higher capacity to increase their working memory performance as compared to high performing individuals. The inverted U-function between exercise intensity and cognitive performance was anticipated to fit for the changes in T and C as well, and we expected to obtain exercise-related changes of C and T levels which altered working memory, with T and C being connected to the intensity of the exercise intervention.

2. Methods

2.1. Participants

Sixty healthy high school students from the 9th form of a Berlin (Germany) School aged 15–16 years participated in this study. The students were randomly assigned to two experimental (EG 1: 50–65% HR max; EG 2: 70–85% HR max) and a control group (CG). An experimenter who was not involved in the study generated the allocation sequence, enrolled participants, and assigned participants to their groups. The participants and their parents signed an informed consent waiver approved by the local board of the Humboldt Universität zu Berlin, Germany. Written informed consent was obtained before inclusion from all participants and their parents. Other inclusion criteria for study participation were: the absence of dyslexia (verified by teachers' statements), a maximum BMI of 25 and the absence of mental or physical impairments and no history of psychoactive substances (e.g. Ritalin). No participant had to be excluded due to these criteria. One participant was excluded from data analysis due to performing incongruently to instructions. The remaining sample (33 male and 26 female) had a mean age of 14.37 years (S.D. = 0.53, n = 59). The mean age in the CG was 14.50 years (S.D. = 0.51, n = 21, 13 male), in the EG 1 it was 14.38 years (S.D. = 0.50, n = 18, 9 male), and in the EG 2 it was 14.24 years (S.D. = 0.56, n = 20, 11 male).

2.2. Exercise testing

2.2.1. Maximum performance exercise
To determine the individual target HR for the exercise intervention, maximum performance was assessed using the Shuttle Run Test (Léger and Gadoury, 1989). The Shuttle Run Test ensured that exercise levels were related to the individual HR max. The Shuttle Run test was performed on a 20-m track. The exercise test started at a speed of 8.5 km/h. Every 1 min, running speed was enhanced by 0.5 km/h until exhaustion. On each speed level, participants received continuous acoustic signals in a given frequency as pacing signals. We calculated the maximum HR achieved at the end of the test.

2.2.2. Exercise interventions
The individual target HR was based on the results of the initial Shuttle Run Test (Léger and Gadoury, 1989). During the EG 1 and 2 conditions, the subjects ran on a 400-m track with their individual exercise intensity level, based on their individual HR max for 12 min. Each subject received an acoustic signal through the heart rate monitor when he or she was running too fast (fast beeping) or too slow (slow beeping). The control group condition consisted of being sedentary for 12 min. The conditions for the EG 1 were 12 min of low impact running at a level of 50–65% of the individual HR max (EG 2: 70–85% HR max) (cf. Table 1 for the HRs of the three groups during the intervention). HR was determined during exercise and during 5 min of the recovery phase afterwards. On the test days, the students refrained from any exercise prior to the investigation. The conditions for the CG, the EG 1, and EG 2 group differed only with regard to the intensity of physical activity.

2.2.3. Cardiovascular assessment
The heart rate was measured during exercise sessions in all groups, EG 1, EG 2 and CG, using a heart rate monitor (HRM RS400, Polar, Kempele, Finland). The HRM consists of an elasticized chest belt that detects and stores the subject's mean heart rate every 5 s. The heart rate data were downloaded to a computer using a Polar proprietary interface and commercial storage software.

2.3. Saliva sampling and analysis

On the day of the experiment, two saliva samples were collected with cotton rolls (Salivettes, Sarstedt, Nümbrecht, Germany). Samples of stimulated saliva (taken by chewing on a cotton roll for 1 min) were collected by the subjects after the fourth school lesson (t1: pre-test), after 12 min exercise intervention (EGs) in a defined HR interval relative to the individually identified HR max or at-rest (CG), respectively (t2: post-test). After chewing, the cotton rolls were placed in the plastic tube of the salivette, and collected. Although salivary C is known to maintain reliable values at room temperature (Kirschbaum and Hellhammer, 1994), samples were stored at −20 °C until analysis. Biochemical analyses were performed at the Dresden University of Technology. Salivary samples were prepared for biochemical analysis by centrifuging at 3000 rpm for 5 min to result in a clear supernatant of low viscosity. Free salivary cortisol and testosterone was analyzed with a commercially available chemi-luminescence-assay (IBL; Hamburg, Germany) with high sensitivity of 0.16 ng/ml (2.5 pg/ml for T). The samples were not measured in duplicates, but the intra- and interassay coefficients of variation were below 8% for C and 5% for T.

2.4. Cognitive testing

All groups received the Letter Digit Span (LDS) task (Gold et al., 1997). The LDS measures frontal lobe-mediated working memory. The task involves an auditory presentation of a mixed series of alternating numbers and letters. The presenting of the numbers/digits was recorded on CD before the test (one digit/number per second) to assure a similar timing and then presented by the examiner from a CD player. The subject was asked to respond by first writing the numbers in order from the smallest to the largest, followed by writing the letters in alphabetical order. For example, a subject heard "w7t4," the correct answer was "47tw." Following a series of practice trials, the test involved 4 trials at each string length, beginning with 2-item strings (such as "m3") and proceeding up to 7-item strings (such as "c7g4qls") for a total of 24 items. The total number of correct responses (of 24 possible) was used for the analyses. The test was terminated when a subject failed all four trials within one string length. To assess the internal

Table 1 Means (*M*) and standard errors (S.E.) for testosterone (T), cortisol (C), and working memory performance (LDS) at pre- and post-test as well as for pre-to-post-test changes in T (T-change), C (C-change), and LDS (LDS-change) for the two experimental groups (EG 1, EG 2) and the control group (CG). In addition, *M* and S.E. are shown for LDS performance at pre- and post-test as well as for pre-to-post-test changes for the three groups split into low (LP) and high performer (HP). Furthermore, mean heart rate during intervention (HR_exercise), mean heart rate during cognitive testing at post-test (HR_WM), frequency of male and female participants, body mass index (BMI), and physical activity levels (PA, in minutes per week) are shown. There were no significant group differences for BMI and PA, but for HR_exercise and HR_WM ($p < .05$).

Measure	EG 1		EG 2		CG	
	M	S.E.	*M*	S.E.	*M*	S.E.
Testosterone						
T-pre (pg/ml)	45.33	7.98	32.72	4.29	40.13	8.05
T-post (pg/ml)	40.77	8.76	42.67	5.69	25.55	3.62
T-change	−4.56	8.06	9.45	2.74	−14.58	8.02
Cortisol						
C-pre (mmol/l)	8.25	1.72	5.88	1.11	7.74	1.37
C-post (mmol/l)	5.28	0.64	8.40	1.25	5.75	0.56
C-change	2.97	1.57	2.52	1.21	−1.99	1.56
LDS						
LDS-pre	11.88	0.70	12.30	0.51	12.76	0.44
LDS-post	13.11	0.75	12.90	0.65	13.52	0.70
LDS-change	1.22	0.51	0.60	0.76	0.76	0.64
LDS_LP						
LDS-pre	9.90	0.60	10.40	0.45	11.18	0.35
LDS-post	11.70	0.93	12.60	0.98	12.82	1.09
LDS-change	1.80	0.55	2.20	1.10	1.64	1.03
LDS_HP						
LDS-pre	14.38	0.73	14.20	0.29	14.50	0.31
LDS-post	14.88	0.91	13.20	0.89	14.30	0.84
LDS-change	0.50	0.89	−1.00	0.79	−0.20	0.66
Covariates						
HR_exercise	123.93	2.47	160.21	3.32	86.31	2.04
HR_WM	87.45	9.63	98.25	9.64	87.72	9.45
Male/female	9/9		11/9		13/8	
BMI	19.61	0.55	19.82	0.38	21.64	0.93
PA	196.93	59.08	289.41	94.85	185.00	65.47

consistency of the LDS, Gold et al. (1997) calculated the Cronbach coefficient α, and obtained a score of .85; this suggests a high degree of consistency among test items. As described in the above study design, the LDS was completed two times (before and after the intervention). During the test, students were not allowed to talk to each other and they were asked to remain silent to prevent any possible interference by other students.

2.5. Study design

Each participant completed four normal academic school lessons (German, English or Latin). The first saliva collection took place at the end of the fourth lesson at 11:30 to minimize effects of time of day on SH levels. Students then received heart rate monitors and accomplished their first cognitive testing together in a quiet room. Groups were then separated into CG, EG 1, and EG 2, and the exercise intervention took place after walking 50 m to the 400-m track. The control group remained in the classroom, being sedentary for 12 min and not

allowed to talk to each other. Also in the experimental conditions, the subjects were neither talking nor discussing. After exercising or resting for 12 min, respectively the second saliva collection took place at 12:10, followed by the second cognitive testing (for all groups together) where the same version of the task was administered. The test ended with the return of the heart rate monitors at 12:30. The time lapse was the same for all participants.

2.6. Data analysis

A 2 × 3 mixed factor analysis of variance (ANOVA) was used to test for differences between pre-test and post-test (within), and for differences between the experimental (EG 1 and EG 2) and the control groups (CG) (between). Analyses were conducted separately for T, C, and working memory performance (LDS). Greenhouse Geyser adjustment was reported when the sphericity assumption was violated. Planned post-hoc comparisons (Bonferroni adjustment) were conducted to determine pre-to-post-test changes

Table 2 Means (*M*) and standard deviations (S.D.) for age and distribution of males and females for the two experimental groups (EG 1, EG 2) and the control group (CG) split in low (LP) and high performer (HP).

	EG 1		EG 2		CG	
	LP	HP	LP	HP	LP	HP
Age						
M	14.67	14.00	14.43	14.10	14.56	14.44
S.D.	0.50	0.00	0.79	0.32	0.53	0.53
Male/female	5/5	4/4	6/4	5/5	6/5	7/3

within the three groups (EG 1, EG 2, CG). Analyses have been controlled for sex, BMI, and leisure time sports activities. T level was significantly influenced by sex, while C level and LDS were influenced by none of the variables. Therefore, in the following, analyses for C and LDS were reported without any covariate, whereas analysis for T was conducted with sex as covariate. In ANOVAS effect sizes were calculated for significant results by partial eta-squared (η^2), expressing the amount of variance explained in the dependent variables by the respective effect. Effect sizes in paired samples (post-hoc comparisons) were reported as d and are measured relative to the standard deviation of the paired differences.

As described in the introduction we additionally divided the groups in low and high performers, i.e. participants with high and low working memory performance at pre-test (median split within each group). Table 2 shows the mean age and distribution of males and females within the groups. A 2 × 3 × 2 mixed factor ANOVA design was used to test for differences between pre-test and post-test (within), and for differences between the experimental (EG 1 and EG 2) and the control groups (CG) separated by low and high performers (between).

In a next step, we calculated partial correlation analyses to analyze the relationship between improvement in working memory performance and changes in C and T level, respectively. This was done separately for the exercise and control groups. Again, analyses were controlled for sex, BMI, and leisure time sports activities. Only the effect of sex was significant for testosterone. For all analyses, the significance level was α = .05. Multiple testing increases the risk for chance findings due to potential alpha error accumulation. Thus, in post-hoc comparisons and correlation analysis the nominal alpha level was adjusted for the three comparisons (EG 1, EG 2, CG) following Bonferroni ($\alpha = 1 - (1 - \alpha)^{1/m}$, with *m* = number of single comparisons and α = nominal α-level) (adjusted α = .017). Due to the explorative character of the study Bonferroni adjusted as well as unadjusted results are reported.

3. Results

3.1. Pre-to-post-test changes of cortisol, testosterone, and LDS

3.1.1. Testosterone
Repeated measure ANOVA revealed a main effect of the test ($F(1,39)$ = 4.42, *p* = .042, η^2 = .102), no significant group

effect ($F(2,39)$ = 0.05, *p* = .955), and a significant test by group interaction ($F(2,39)$ = 3.94, *p* = .028, η^2 = .168); this indicates a different pre-to-post-test trend for the three groups. Follow-up *t*-tests confirmed a significant increase of T level from t1 to t2 in the high intensity group (EG 2) ($t(18)$ = −3.45, *p* = .003, *d* = .039), but not in the low intensity group (EG 1) ($t(18)$ = 0.57, *p* = .579) and the control group ($t(19)$ = 1.82, *p* = .085) (cf. Fig. 1 and Table 1). Also after adjustment of the nominal α-level results for EG 2 remained significant.

3.1.2. Cortisol
C results also showed a significant test by group interaction ($F(2,55)$ = 4.10, *p* = .022, η^2 = .130). The test ($F(1,55)$ = 0.95, *p* = .335) and group effect ($F(2,55)$ = 0.58, *p* = .944), however, were not significant. C levels in the high intensity group increased significantly after training ($t(19)$ = −2.08, *p* = .050, *d* = .047), whereas the low intensity group ($t(17)$ = 1.89, *p* = .076) and the control group ($t(19)$ = 1.28, *p* = .218) showed no significant changes after exercise (cf. Fig. 1 and Table 1). However, after adjustment of the nominal α-level results did not reveal a significant change in C level due to the intervention.

3.1.3. LDS
For the working memory task, repeated measure ANOVA revealed a significant test effect ($F(1,56)$ = 5.20, *p* = .026, η^2 = .085) and no significant group effect ($F(2,56)$ = 0.43, *p* = .655), as well as no significant test by group interaction ($F(2,56)$ = 0.23, *p* = .793). Follow-up tests, however, indicated that only the low intensity exercise group significantly improved cognitive performance from pre- to post-test ($t(17)$ = −2.40, *p* = .028, *d* = .040; EG 2: ($t(19)$ = −0.79, *p* = .437; CG: ($t(20)$ = −1.18, *p* = .250) (cf. Fig. 1 and Table 1). This improvement was still marginally significant (adjusted α = .034) after correction for multiple comparisons.

3.2. Pre-to-post-test changes of LDS separated by high and low performer

To further analyze the effect of acute bouts of exercise with different intensities on working memory performance, the group was split into low and high performers (i.e. participants with high and low performance at pre-test, respectively, in the LDS task). Again, repeated measure ANOVA revealed not only a significant test effect ($F(1,55)$ = 5.19, *p* = .027, η^2 = .086), but also a significant effect of performance level ($F(1,55)$ = 30.26, *p* < .001, η^2 = .355) and a significant test by performance interaction ($F(1,55)$ = 9.17, *p* = .004, η^2 = .143), indicating a different pre-to-post-test development for the high and low performer. Group effect ($F(2,55)$ = 0.63, *p* = .539) and group by test interaction ($F(2,55)$ = 0.18, *p* = .837) were not significant (cf. Table 1 for descriptives).

Follow-up tests confirmed a significant performance improvement for the low performer of the low intensity group ($t(9)$ = −3.25, *p* = .010, *d* = .061), and a marginally significant improvement for the low performer of the high intensity group ($t(9)$ = −1.99, *p* = .077, *d* = .064; CG did not significantly change: $t(10)$ = −1.59, *p* = .143) (cf. Fig. 2). The performance improvement for the low performer of the low intensity group still remained marginally significant after Bonferroni adjustment (adjusted α = .034).
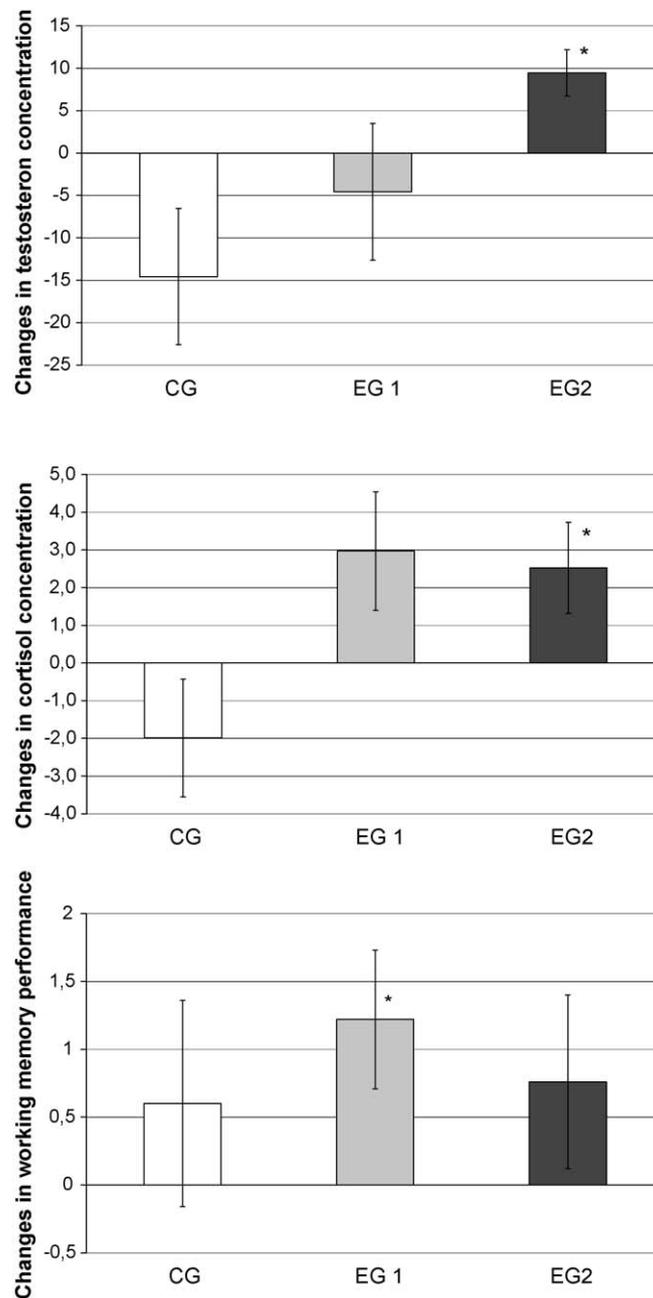
Figure 1 Changes in testosterone concentration, cortisol concentration and working memory performance for the control group (CG, white bars), experimental group 1 (EG 1, gray bars) and experimental group 2 (EG 2, black bars). *Note*. An asterisk marks significant pre-to-post-test changes within the groups (before Bonferroni adjustment). Error bars represent standard errors of the mean.

High performers revealed no performance improvement from pre- to post-test, neither in the CG nor in the low or high intensity group (p always > .10) (cf. Fig. 2). Thus, the exercise-related change in working memory performance seems to be driven by the low performer.

3.3. Relationship between physical and psychological variables

In a next step we calculated correlation analyses to analyze the relationship between improvement in working memory performance, and C as well as T level at post-test. This was done separately for the exercise and control groups. We did not find a significant correlation between C concentration and change in LDS performance, for none of the three groups (EG 1, EG 2, CG). This was also true for the relationship between improvement in working memory performance and T level at post-test (always p > .10, r < .30), with one exception: We found, however, a (marginally significant) correlation between post-test T level and changes in working memory performance ($r = -.40$, $p = .097$) in the high intensity group. After Bonferroni adjustment, however, this correlation was not significant. The elevated T level in the high intensity group seems to negatively influence pre-to-post-test changes in working memory performance; i.e. partici-
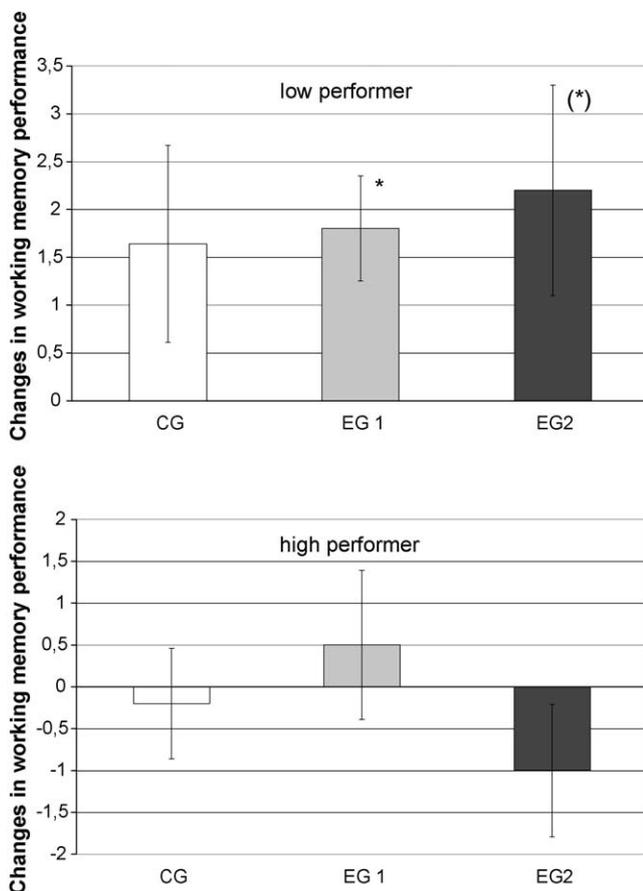
Figure 2 Changes in working memory performance for the low and high performers of the control group (CG, white bars), experimental group 1 (EG 1, gray bars) and experimental group 2 (EG 2, black bars). *Note*. An asterisk marks significant pre-to-post-test changes within the groups. An asterisk in brackets marks marginally significant pre-to-post-test changes groups (before Bonferroni adjustment). Error bars represent standard errors of the mean.

pants with a lower T level after high intensity exercise improved cognitive performance to a higher degree.

4. Discussion

The aims of our study were (1) to confirm exercise-related stress reactions in adolescents as previously shown for adults; (2) to identify an exercise intensity which enhances cognitive performance (i.e. working memory) in a school setting; and (3) to determine if the altered cortisol (C) and testosterone (T) levels in adolescents are related to changes in working memory performance.

The first main result was the intensity-dependent concentration of the SHs in adolescents. After high intensity exercise (EG 2), the levels of both SHs (C and T) were elevated. This was not true for the control and low intensity group.

The nearly consistent kinetics of T and C in the high intensity group are in line with earlier findings in adults and adolescents that indicate that T, similar to C, increases linearly in response to exercise once a specific intensity

threshold is reached (O'Connor and Corrigan, 1987; Maresh et al., 1988; Di Luigi et al., 2006).

Tharp and Barnes (1990) reported that swim training of different intensities only led to increased C levels after heavy intensity, but not moderate intensity training. Our results confirm that C level raises due to high exercise intensities (approx. 70% VO_{2max}; O'Connor and Corrigan, 1987). Fig. 1 reveals that also 12 min of low intensity exercise induced a similar increase in C levels. Due to the high standard error, these results were not significant. After correction for multiple comparisons none of the results for C remained significant. Thus, the results of the EG 1 have to be interpreted with adequate caution.

Rising T level needs less exercise intensity (approx. 50% VO_{2max}; Maresh et al., 1988) as compared to cortisol. In our study, however, T level remained stable in the low intensity group. This might be due to the relatively short exercise duration of 12 min. Tremblay et al. (2004) reported an increase in T while exercising with a moderate intensity of 50—55% of VO_{2max} for 40 min. It is assumed that the total load (product of intensity and duration) of exercise is an important determinant of the magnitude of the SHs response (Viru, 1985). Thus, low intensity exercise, if prolonged enough in duration, might result in significant elevations in T as shown by Tremblay et al. (2004) cf. also Galbo et al. (1977). To date, however, most research has suggested that exercise intensity plays a more important role than duration or mode in determining the magnitude of the endocrine response (Tremblay et al., 2004).

The assessment of salivary steroid concentrations represents an easy, non-invasive and stress-free technique of investigation. It is considered ideal for use in endocrine research, particularly in children and adolescents (Rosmond and Björntorp, 2001). The concentration of C in saliva closely correlates with the concentration of free C in the serum, and salivary C has been widely confirmed to be a valid and reliable indicator of the biological, active, and free fraction of serum C levels both at rest and after exercise (Kirschbaum and Hellhammer, 1994). A large variability in C levels both within and between subjects has frequently been reported (Kirschbaum and Hellhammer, 1994). This has been regarded as characteristic of an endocrine system that serves adaptive functions in a quickly changing environment (Kirschbaum et al., 1992). In the present study we observed the same instability of initial baseline C levels varying between the groups before the intervention (cf. Table 1). A similar instability of steroid levels in healthy adolescents has been reported for T (Couwenbergs et al., 1986). In these populations, however, it has been well documented that the salivary T concentration correlates well with serum T levels (Ohzeki et al., 1991). Our basal C and T data are consistent with what has been previously reported in the literature for salivary hormones (Matchock et al., 2007). The enhancement of the C and T concentration after exercise was comparable to results of other studies in adults (O'Connor and Corrigan, 1987; Maresh et al., 1988). Thus, exercise-related changes of T and C in adolescents are in accordance with results reported for adults.

Regarding our second aim, to identify an exercise intensity that enhances working memory in a school setting, our data partly support an inverted U-shaped relation between exercise intensity and cognition (see Tomporowski, 2003 for review). Although the interaction between group and pre-to-post-test changes was not significant, single group analysis

revealed that in the working memory task (LDS), only the low intensity group (EG 1) improved their performance significantly (and this effect still remained marginally significant after Bonferroni adjustment).

There was no such increase in either the control or in the high intensity group, except for the low performers in the high intensity group (see below).

By dividing the students into groups with good and poor results in the LDS at pre-test, however, our results revealed an improvement for low performers, whereas there was no significant change for high performers. Thus, students with a poorer working memory outcome benefited most from our intervention — regardless of exercise intensity. This supports the findings by Sibley and Beilock (2007) that acute exercise predominantly helps those who perform sub-optimally in cognitive testing. Low performers might have a stronger capacity for plastic reorganization and in turn an increase in cognitive performance. Acute exercise, which was reported to enhance the ability to concentrate (Budde et al., 2008), could be more beneficial for initial low performers in the working memory task. As no subject managed to complete the whole test, a ceiling effect of the high performers could not account for these results. One has to bear in mind that the sample sizes in the high and low performing groups are rather low and that the medium split procedure leads to a loss in statistical power. Nevertheless, we found that moderate exercise enhances working memory performance. However, the role of the exercise intensity needs to be further investigated, since the inverted U-shape function could not be replicated for the low performer. It appeared that they benefit from an exercise intervention regardless of the exercise intensity we chose.

Concerning the third aim, our data showed that moderate intensity seems to be mainly related to working memory performance (LDS) in the group of low performers (cf. Fig. 2), and does not have an effect on C or T concentrations. In contrast, high intensity exercise seems to enhance hormone concentrations and, again, only enhances working memory in the low performer group. C concentration at post-test, however, showed no significant relation to pre-to-post-test changes of LDS performance, in either the high or low performer groups. The lack of a significant correlation between C level and working memory performance might be ascribed to the fact that no clear-cut C responses of comparable magnitude had been induced by the low intensity interventions. The distinct enhancement of the C concentration in the EG 2 still had no effect on working memory. Thus, the working memory results cannot be explained by exercise-induced changes in C levels. These findings are in line with other studies failing to reveal an influence of Cs or psychosocial stress on WM (Monk and Nelson, 2002; Kuhlmann et al., 2005). But beside this psychosocial stress and hydrocortisone administration studies (e.g. Monk and Nelson, 2002), our study provides another form of stress, i.e. physical stress. Following induced mental stress Hoffman and al'Absi (2004) demonstrated significant increases in heart rate as well as elevated cortisol concentration, suggesting substantially increased adrenocortical reactivity. However, they did not find significant differences in any of the neuropsychological measures like Digit Span and Visual Memory Span compared to control. Intensive physical stress like in the EG 2 led to the same effects (an increased adrenocortical reactivity with an increase in heart rate and cortisol concentration but without significant differences in memory functions). To our knowledge, there is no other study comparing the influence of acute physical stress induction on working memory. This is important because our results are in contrast to studies observing WM impairments after psychosocial stress (Wolf et al., 2001; Elzinga and Roelofs, 2005; Oei et al., 2006).

Another explanation for the contradicting results compared with this previous studies might be the employment of different WM paradigms (e.g. n-back task vs. digit tasks) varying in the sensitivity, the involvement of distinguishable WM processes as well as in the demand they place on WM (Sliwinski et al., 2006; Unsworth and Engle, 2007). The absence of more than one performance criterion which is present in the LDS, might lead to a lack of sensitivity of this task for the detection of acute stress effects like it is said for the digit span (Schoofs et al., 2008).

It is possible that other mediators like BDNF, or catecholamines like dopamine and epinephrine, are more important for exercise-induced cognitive changes in a learning task (as shown by Winter et al., 2007). However, T post-test levels tends to be related to changes in the LDS performance in the high intensity group. Participants with a higher T level after high intensity exercise improved LDS performance to a lesser extent than those with lower T levels. This suggests that 12 min of intensive exercise enhanced the T concentration to an extent that was able to negatively influence working memory performance. This relationship between working memory performance and T level supports the outcomes of the study by Moffat and Hampson (1996), where moderate levels of androgens were associated with better spatial performance. In addition, Wolf and Kirschbaum (2002) reported an increased performance in cognitive tests in men with lower T concentrations. Also, Wolf et al. (2000) reported that the cognitive performance in a verbal fluency task was negatively affected by an acute high dose of T in elderly men. A negative association between T and the working memory task as used in the current study has not been reported previously. Working memory is mediated, in part, by the prefrontal cortex (Gold et al., 1997). Thus, our results suggest that T might modify the function of this region. However, a plausible biological mechanism has not yet been proposed to adequately explain this phenomenon (Beauchet, 2006). Many of Ts behavioural effects occur after it has been converted to its metabolically active derivatives, estradiol (E) or dihydrotestosterone (DHT) by means of the enzymes aromatase and 5-α reductase, respectively (Moffat, 2005). Thus, testosterone may interact not only with androgen receptors (Sternbach, 1998), but also with E receptors (Moffat, 2005). A possible mechanism for the effects of T on working memory is that T acts via aromatization to E on the striatal dopamine system with secondary effects on prefrontal functions (Janowsky et al., 2000). It cannot be determined from the present data whether T per se is the critical hormone or whether the observed relationships may depend on prior conversion to E and/or DHT.

Due to the pilot character of the current study, the size of the saliva sample time points was relatively small. Data about absolute levels of C secretion at different times during the day, such as elevated evening cortisol, and changes in

diurnal C rhythm would provide additional information about whether the transient endocrine changes after an acute bout of exercise have any lasting biological effects. Nevertheless, the data indicate for the first time that 12 min of exercise seem to cause improved working memory performance in high school students starting on a low cognitive level at pre-test. Surprisingly, our results fail to prove a relationship between C level and exercise-related changes in working memory performance. On the other hand, increased T levels due to high intensity exercise tend to be negatively related to working memory performance in adolescents.

We do not know, if a longer exercise duration would have changed the SH levels to an extent that would be able to significantly influence working memory. We did not expect this effect because most research suggests that exercise intensity plays a more important role than exercise duration in determining the magnitude of the endocrine response (e.g. Tremblay et al., 2004).

Our findings do not preclude the possibility of similar relationships between working memory and other steroids such as, e.g. estrogens. By sampling T and estrogens in the same male and female sample of subjects, it should be possible for future studies to more systematically investigate the possible effect of steroid hormones on working memory.

Taken together, our results support the request for more short bouts of exercise in schools via, for example, instructed exercise in school breaks. Even moderate intensity exercise is able to increase working memory in cognitively low performing children, and it does not harm the performance of higher cognitive performers. Acute bouts of exercise could simply consist of fast walking; this could be included very easily in a school setting because neither sports clothes nor sports facilities are necessary for this type of exercise.

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Conflict of interest

We declare that none of the authors has any financial or other relationships that might lead to a conflict of interests in relation to this study or the content of this manuscript.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.psyneuen. 2009.07.015.

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