Dynamics of the human stress system in depression

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Markers of stress and inflammation as potential mediators of the relationship between exercise and depressive symptoms: The TRAILS Study

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ABSTRACT

The hypothalamic-pituitary-adrenal axis, autonomic nervous system, and immune system have been proposed to underlie the antidepressant effect of exercise. Using a population sample of 715 adolescents, we examined whether pathways from exercise to affective and somatic symptoms of depression were mediated by these putative mechanisms. Exercise (hr/week) and depressive symptoms were assessed at age 13.5 (±0.5) and 16.1 (±0.6). Cortisol and heart rate responses to a standardized social stress test and C-reactive protein levels were measured at age 16. Exercise was prospectively and inversely related to affective (B=-0.16, 95%CI=-0.30 – -0.03) but not somatic symptoms (B=-0.04, 95%CI=-0.21 – 0.13). Heart rate during social stress partially mediated this relationship (B=-0.03, 95%CI=-0.07 – -0.01). No other mediating effects were found. Hence, the autonomic stress system may play a role in the relationship between exercise and depressive symptoms.

INTRODUCTION

Exercise may be a highly valuable treatment for individuals suffering from mood problems, and serve as a prevention strategy for those at risk. However, effects reported in the literature are inconsistent and the operating mechanisms not well known (Rimer et al., 2012). To maximize the effectiveness of exercise programs against depressive symptoms, discerning physiological mechanisms that drive the antidepressant effects may be helpful.

Two candidate mechanisms through which physical exercise exerts its influence are the major components of the body's stress system: the hypothalamic-pituitary-adrenal (HPA) axis and the autonomic nervous system (ANS). Physical exercise triggers heart rate and HPA axis responses. Physically fit individuals show reduced cardiovascular and neuroendocrine responses, not only to exercise but also to psychosocial stress, compared to unfit individuals (Forcier, Stroud, & Papandonatos, 2006). Depression is a stress-related disorder, and has often been reported to be associated with increased reactivity of the stress system (e.g. Carney, Freedland, & Veith, 2005; Stetler & Miller, 2011). Also reduced reactivity has been related to (risk of) depression (e.g. (Miller, Chen, & Zhou, 2007; Phillips, Hunt, Der, & Carroll, 2011): hypoactivity of the HPA axis seems to be associated with severe and chronic forms of depression (Burke, Davis, Otte, & Mohr, 2005; Miller et al., 2007; Booij, Bouma, de Jonge, Ormel, & Oldehinkel, 2013). This hypoactivity has been proposed to result from chronically elevated cortisol levels, necessitating a downregulation of the stress system (Fries, Hesse, Hellhammer, & Hellhammer, 2005). Taken together, these findings suggest that training stress systems by physical exercise may protect against the depressogenic effects of stress.

Another candidate mechanism is the immune system. Continued activation of the immune system could lead to sickness behavior and subsequently to depressive symptoms in vulnerable individuals (Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008). Increased levels of inflammatory proteins such as C-reactive protein (CRP) have indeed been found in individuals with depression (e.g. Dantzer et al., 2008), although not in all studies (e.g. Piletz et al., 2009). Regular exercise can reduce inflammation levels (Mattusch, Dufaux, Heine, Mertens, & Rost, 2000; Lopresti, Hood, & Drummond, 2013), thereby possibly preventing or mitigating sickness behavior and depressive symptoms.

We studied whether heart rate and cortisol responses to psychosocial stress, as well as CRP, mediated the prospective relationship between exercise and depressive symptoms in an adolescent population cohort. Because the first-incidence of depression rises dramatically in adolescence (Kessler et al., 2005), this is a relevant age group to target in terms of prevention and early intervention. Also, there are probably relatively few individuals with chronic problems (see also Booij et al., 2013), and hence, chronicity-related hyporeactivity of the stress system. An additional advantage is

that the prevalence of potentially confounding somatic disorders is relatively low at this age. A previous study in the same sample found that physical activity was more strongly related to affective than to somatic symptoms (Stavrakakis, de Jonge, Ormel, & Oldehinkel, 2012). Somatic and affective symptoms have been differentially related to HPA axis and ANS function (e.g. Bosch et al., 2009) and to CRP levels (Duivis, Vogelzangs, Kupper, de Jonge, & Penninx, 2013). Thus, pathways from exercise to depression may be symptom-dependent and were therefore explored separately for affective and somatic symptoms.

METHOD

Participants

The study was conducted in a focus sample of TRAILS (Tracking Adolescents' Individual Lives Survey), a large prospective cohort study of Dutch adolescents with bi- or triennial measurements from age 11 to at least 21. The data came from the second (T2; mean age 13.5 years, SD = 0.5) and third (T3; mean age 16.1, SD = 0.6) assessment wave. The focus sample consisted of 715 adolescents (50.9% girls) who were submitted to more extensive measurements at T3, including a social stress test. For more information about this sample and the selection procedure, see elsewhere (Bouma, Riese, Ormel, Verhulst, & Oldehinkel, 2009). The participants were treated in accordance with the Declaration of Helsinki, and informed consent was obtained from both the participants and their parents.

Measures

Exercise

At both T2 and T3, adolescents completed an exercise questionnaire. Participants could specify up to four different types of exercise that they regularly performed, and indicate for every exercise how many days and hours per week they spent on those activities. The total number of hours per week spent on these exercises was calculated as an overall measure for exercise. Because prior findings (Hoffman et al., 2011) suggest that the exercise-depression relationship is non-linear and weakens with increasing levels of exercise, we calculated the natural logarithm (ln) of exercise in addition to the untransformed score.

Cortisol and heart rate responses to stress

At T3, cortisol and heart rate responses to psychosocial stress were induced by means of the Groningen Social Stress Test (GSST). The GSST is a standardized protocol, inspired by the Trier Social Stress Test, for the induction of moderate performance-related social stress (Kirschbaum, Pirke, & Hellhammer, 1993). The GSST has been found to elicit significant changes in heart rate and the HPA system (Bouma et al., 2009; Bouma et al., 2011). It entails the elements necessary to induce a significant cortisol response, namely uncontrollability and social-evaluative aspects (Dickerson & Kemeny, 2004). In short, the participants were, on the spot, instructed to prepare

and deliver a six-minute speech about themselves and their lives, and to perform a difficult mental arithmetic task in front of a camera, while being videotaped. The videotape was said to be judged by peers on content of speech and use of voice and posture. Participants were debriefed directly after the task.

HPA axis responses towards the GSST were assessed by four salivary samples of cortisol, collected before as well as immediately after, 20 min after, and 40 min after the end of the GSST. Free cortisol levels in saliva reflect HPA axis activity about 20 min earlier, as there is a time window between the production of cortisol by the adrenal glands upon stress and the presence of cortisol in saliva (Aardal-Eriksson, Karlberg, & Holm, 1998). Salivary cortisol samples were collected using Salivettes, which are small cotton swabs in plastic tubes (Sarstedt, Numbrecht, Germany). After the experimental session, the samples were placed in a refrigerator at 4 °C, and within three to four days brought to the laboratory of the University Medical Center Groningen, and stored at -20 °C until analysis. All samples were analyzed with the same reagent, and all experimental samples from a participant were assayed in the same batch. Missing experimental samples (C1, n = 9; C2, n = 4; C3, n = 8; C4, n = 7) were due to detection failures in the lab (54%) or insufficient saliva in the tubes (46%). Missing values were imputed on the basis of a combination of the group mean and corresponding standard deviation of the missing cortisol sample and the mean of the participant's cortisol samples that were valid. Stress-induced HPA axis functioning was operationalized as cortisol secretion during the GSST, calculated as the area under the curve with respect to the ground (AUCgCort), and cortisol reactivity to the GSST, calculated as the area under the curve with respect to increase (AUCiCort) (see, Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003).

Cardiac autonomic function was assessed at the start of the experimental session as well as during and after the GSST, in seven blocks: pretest, speech preparation, speech, silent interlude after speech, mental arithmetic, silent interlude after mental arithmetic, and posttest. A three-lead electrocardiogram was registered using 3M/RedDot Ag/AgCl electrodes (type 2255, 3M Health Care, Neuss, Germany), while the participant was sitting and breathing spontaneously. With a BIOPAC Amplifier-System (MP100, Goleta, CA), the signals were amplified and filtered before digitization at 250 samples/second. Dedicated software (PreCARSPAN, previously used in, e.g., Dietrich et al., 2007) was used to check signal stationarity, to correct for artifacts, to detect R-peaks, and to calculate the interbeat-interval (IBI) between two heartbeats. Blocks were considered invalid if they contained artefacts with a duration of more than 5 s, if the total artefact duration was more than 10% of the registration, or if the block length was less than 100 s (invalid blocks pretest: n=15, preparation: n=28, speech: n=27, interlude after speech: n=35, mental arithmetic: n=29, interlude after mental arithmetic: n=31, posttest: n=32). HR is inversely related to IBI by the equation HR=60000/IBI. HR was defined as the number of beats per minute (bpm). Comparable to the cortisol measures, heart rate during social stress (AUCgHR) and heart rate reactivity (AUCiHR) were computed, based on heart rates during speech

preparation, speech, mental arithmetic and posttest.

C-Reactive Protein

At T3, fasting blood samples (39.5 ml) of participants were drawn, and transported to the laboratory within 4h. High-sensitivity C-reactive Protein (hsCRP) was determined using an immunonephelometric method, BN2, CardioPhase® hsCRP, Siemens with a lower detection limit of 0.175 mg/L. Intra-assay coefficients of variance ranged from 2.1 to 4.4, and inter-assay coefficients of variance ranged from 1.1 to 4.0.

Depressive symptoms

At T2 and T3, depressive symptoms were assessed with the Affective Problems Scale (APS) of the Youth Self-Report (Achenbach, Dumenci, & Rescorla, 2003). The APS contains 13 items covering depressed mood, anhedonia, loss of energy, feelings of worthlessness and guilt, suicidal ideation, sleep problems, and eating problems; which could be rated as 0 = not true, 1 = somewhat or sometimes true, or 2 = very or often true in the past six months. These items reflect symptoms of a Major Depressive Episode according to the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV) (Achenbach et al., 2003). Removal of the item 'I sleep more than most kids' increased the internal consistency of the scale; therefore this item was excluded (see also Bosch et al., 2009). Of the remaining items (Cronbach's α T2 = 0.78, T3 = 0.79), a somatic symptoms subscale and an affective symptoms subscale were constructed. The somatic symptoms subscale contained the following items: lack of appetite, overtired, reduced sleep, trouble sleeping, and lack of energy. The affective symptoms subscale contained the following items: loss of pleasure, crying, self-harm, suicidal ideation, feelings of worthlessness, feeling guilty and sadness.

Covariates

Gender, social economic status (SES) (based on parental professional occupation, educational attainment and household income at T1), T2 Body Mass Index (BMI) and total affective or somatic symptom scores at T2 (corresponding with the outcome measure), were included as potential confounders in all of the analyses. In addition, mediation effects were corrected for smoking at T3, GSST start time, and oral contraceptive use, because these potentially confound the relationship between heart rate and cortisol and depressive symptoms (Bouma et al., 2009). Smoking was assessed as part of the regular T3 questionnaire, which was filled out at school, on average 3.07 months (SD=5.12) before the experimental session. We distinguished between non-smokers and habitual smokers (i.e. at least one cigarette a day). Oral contraceptive (OC) use was assessed by means of a checklist on current medication use administered at the start of the experimental session. In addition, OC use as assessed in the T3 regular questionnaire was used if checklist data from the experimental session was missing, and the time between filling out the questionnaire and the experimental session was less than six months.

Statistical Analysis

Missing values were imputed by means of Expectation-Maximization imputation, with all variables used in the analysis and similar variables collected at adjacent waves as predictor variables. The variables with the most missing values were hsCRP (n=126, 17.6%) and AUCgHR / AUCiHR (n=32, 4.5%).

The prospective relationship between exercise (T2) and affective and somatic symptoms (T3) was examined using linear regression models. We first compared the effects of the untransformed and transformed (ln) measure of exercise to examine whether the relationship was linear or rather asymptotic. When the main effect of (ln) exercise was significant ($p<.05$), we examined whether stress responses and CRP mediated the relationship between exercise and depressive symptoms, using the indirect method (Preacher & Hayes, 2008). This method estimates total, direct and indirect (bootstrapped) unstandardized effects of the independent variable on the outcome through the mediator variables. All putative mediators were included simultaneously, thus individual mediation effects were adjusted for all other mediators. For comparison, the analysis was repeated in the non-imputed dataset. Because of our hypothesis that exercise reduces reactivity of the stress system, and our previous finding that the cortisol response to psychosocial stress, as well as depressive symptoms, was reduced instead of increased in individuals with chronic depressive problems (Booij et al., 2013), we repeated the analysis without the participants with chronic depressive problems (n=653) as well. Chronicity of depressive problems was defined as previously described by Booij et al. (2013). Furthermore, because of the assumed causal direction of the associations under study, we favored a prospective design, in which the assessment of physical exercise preceded that of the mediators and outcomes. However, the two-year interval between the waves was quite large, and exercise patterns may have changed in between the waves. Therefore, we also examined (putative mediation of) the cross-sectional relationship between exercise and depressive symptoms at T3. The same potential confounders were included in these analyses, except for affective/somatic symptoms at T2. Further, BMI at T2 was replaced by BMI at T3.

RESULTS

Descriptive statistics

Characteristics of the study sample are displayed in Table 1. The participants reported exercising regularly for 4.1h per week at T2 and 4.3h per week at T3. The affective symptom scores ranged from 0 to 13 and the somatic symptom scores from 0 to 10. On average, affective symptoms scores were relatively lower and less variable than somatic symptom scores.

Table 1. Characteristics of the study sample (n=715)	
Variable	**Mean (SD)**
Demographics	
Age T3	16.1 (0.6)
Gender (% female)	50.9
Lifestyle factors	
Exercise T2 (h/week)	4.1 (4.1)
Exercise T3 (h/week)	4.3 (4.3)
BMI T2 (kg/m2)	18.9 (3.1)
BMI T3 (kg/m2)	21.3 (3.3)
Smoking status T3 (% smoking)	27.8
Oral contraception T3 (% using girls)	63.9
Depressive symptoms	
Affective symptoms T2 (0-14)	1.4 (1.9)
Affective symptoms T3 (0-14)	1.3 (1.8)
Somatic symptoms T2 (0-10)	2.1 (2.0)
Somatic symptoms T3 (0-10)	2.3 2.1)
Stress / inflammation markers	
AUCg Cortisol (nmol/l)	275.5 (174.8)
AUCi Cortisol (nmol/l)	37.9 (117.4)
AUCg Heart rate (bpm)	849.1 (120.5)
AUCi Heart rate (bpm)	9.9 (47.0)
HsCRP (mg/l)	1.4 (5.1)

Note: AUCgCort = Area under the curve with respect to the ground of the cortisol response; AUCiCort = Area under the curve with respect to baseline of the cortisol response; AUCgHR = Area under the curve with respect to the ground of the heart rate response; AUCiHR = Area under the curve with respect to baseline of the heart rate response; HsCRP = High-sensitive C-reactive protein.

Bivariate correlations are displayed in Table 2. Exercise was more strongly related to affective symptoms than somatic symptoms in both waves. Exercise (both T2 and T3) was negatively correlated to the AUCgHR, while affective symptoms at T3 were positively correlated to the AUCgHR. Finally, affective symptoms at T2 were negatively correlated to CRP at T3.

Table 2. Bivariate correlations

	ExT2	ExT3	AST2	AST3	SST2	SST3	AgH	AiH	AgC	AiC	CRP
ExT2											
ExT3	.46**										
AST2	-.11**	-.10**									
AST3	-.16**	-.17**	.58**								
SST2	-.08*	-.05	.47**	.33**							
SST3	-.05	-.11**	.34**	.48**	.50**						
AgH	-.18**	-.23**	.04	.14**	-.02	.04					
AiH	.05	.03	-.01	-.02	.03	.05	.25**				
AgC	.02	.00	-.04	-.01	.00	-.03	.11**	.05			
AiC	-.07	.03	-.04	-.05	-.04	-.10*	.19**	.13	.45**		
CRP	-.00	.03	-.10*	.02	.07	.01	-.00	-.03	.00	-.07	

Note: ExT2 = Exercise at T2; ExT3 = Exercise at T3; AST2 = Affective symptoms at T2; AST3 = Affective symptoms at T3; SST2 = Somatic symtoms at T2; SST3 = Somatic symptoms at T3; AgH = Area under the curve with respect to the ground for the heart rate response; AiH = Area under the curve with respect to baseline for the heart rate response; AgC = Area under the curve with respect to the ground for the cortisol response; AiC = Area under the curve with respect to baseline for the cortisol response; CRP = C-reactive protein.
* p<.05
** p<.01

Prospective relationship between exercise and depressive symptoms
The association between ln exercise (T2) and affective symptoms (T3) was slightly stronger (R2 change=0.5%, B=-0.16, p=.02) than the association with the untransformed measure of exercise (R2 change=0.4%, B=-0.03, p=.03), suggesting a nonlinear negative association. The association between exercise (T2) and somatic symptoms (T3) was non-significant (R2 change=0.0%, B=0.01, p=.70), as was the association between ln exercise and somatic symptoms (R2 change=0.0%, B=-0.04, p=.66). The mediation analysis was therefore limited to affective symptoms.

Mediation analysis
Total, direct and indirect effects of ln exercise (T2) on affective symptoms (T3) are displayed in Table 3. There was a significant indirect pathway from exercise to affective symptoms through AUCgHR. As a group, the mediators accounted for 19% of the association between exercise and affective symptoms, but this entirely amounted to the AUCgHR. The analysis in which individuals with chronic depressive symptoms were excluded yielded similar results (Table 3). A mediation analysis on complete cases also suggested a significant indirect pathway through the AUCgHR (B=-0.03, 95%CI= -0.08 – -0.00).

Table 3. Mediation model for the prospective association between exercise (T2) and affective symptoms (T3)*

	Total group (n=715)			Without chronic depressive problems (n=653)		
Variable	**B**	**T**	**P**	**B**	**T**	**P**
Total effect†						
Exercise (ln)	-0.16	-2.27	.02	-0.21	-3.35	.00
Direct effect†						
Exercise (ln)	-0.13	-1.83	.07	-0.18	-2.80	.01
	B	**CI lower‡**	**CI upper‡**	**B**	**CI lower‡**	**CI upper‡**
Indirect effect of exercise (ln) through mediators (bootstrapped results) †						
Total	-0.03	-0.07	0.01	-0.03	-0.07	0.00
AUCgCort	-0.00	-0.02	0.00	-0.00	-0.02	0.00
AUCiCort	0.01	-0.00	0.04	0.00	-0.02	0.02
AUCgHR	-0.03	-0.07	-0.01	-0.03	-0.06	-0.00
AUCiHR	-0.00	-0.02	0.00	-0.00	-0.02	0.00
HsCRP	0.00	-0.02	0.02	0.00	-0.01	0.00

Note: Ln = natural logarithm; AUCgCort = Area under the curve with respect to the ground of the cortisol response; AUCiCort = Area under the curve with respect to baseline of the cortisol response; AUCgHR = Area under the curve with respect to the ground of the heart rate response; AUCiHR = Area under the curve with respect to baseline of the heart rate response.
* Adjusted for gender, social economic status, affective symptoms at T2, body mass index, oral contraceptive use, smoking, time of the GSST.
† The direct effect is the effect of the predictor variable on the outcome variable, when taking the mediator variables into account. The indirect effect is the effect of the predictor variable on the outcome variable through the mediator variables. The total effect is the sum of the direct and indirect effect.
‡ 95% confidence interval.

Individual associations between predictor and mediator variables, and between mediator variables and outcome are displayed in Table 4. Exercise (T2) was significantly related to the AUCiCort and the AUCgHR. The AUCgHR was also significantly related to affective symptoms (T3). There was a trend towards a relationship between HsCRP and affective symptoms.

Table 4. Associations between exercise and mediators, and between mediators and affective symptoms (n=715)*

Variable	B	T	P
Exercise (ln) to mediators			
AUCgCort	-7.33	-0.89	.37
AUCiCort	-16.74	-3.03	.00
AUCgHR	-25.92	-4.71	.00
AUCiHR	2.86	1.26	.21
HsCRP	-0.45	-0.18	.86
Mediators to affective symptoms			
AUCgCort	0.001	1.51	.13
AUCiCort	-0.001	-1.16	.25
AUCgHR	0.001	2.45	.01
AUCiHR	-0.001	-0.95	.34
HsCRP	-0.020	-1.89	.06

Note: Ln = natural logarithm; AUCgCort = Area under the curve with respect to the ground of the cortisol response; AUCiCort = Area under the curve with respect to baseline of the cortisol response; AUCgHR = Area under the curve with respect to the ground of the heart rate response; AUCiHR = Area under the curve with respect to baseline of the heart rate response.
* Adjusted for gender, social economic status, affective symptoms at T2, body mass index, oral contraceptive use, smoking, time of the GSST.

Cross-sectional mediation analysis

The results of the mediation analysis on the cross-sectional data (i.e., all variables assessed at T3) are displayed in Table 5. Again, there was a significant indirect pathway from exercise to affective symptoms through AUCgHR.

Table 5. Mediation model for the cross-sectional association between exercise (T3) and affective symptoms (T3) (n=715)*

Variable	B	T	P
Total effect†			
Exercise (ln)	-0.27	-3.38	.00
Direct effect†			
Exercise (ln)	-0.22	-2.77	.01
	B	**CI lower‡**	**CI upper‡**
Indirect effect of exercise (ln) through mediators (bootstrapped results) †			
Total	-0.04	-0.10	-0.00
AUCgCort	-0.00	-0.02	0.00
AUCiCort	0.00	-0.00	0.02
AUCgHR	-0.04	-0.09	-0.00
AUCiHR	-0.00	-0.01	0.01
HsCRP	-0.00	-0.01	0.01

Note: Ln = natural logarithm; AUCgCort = Area under the curve with respect to the ground of the cortisol response; AUCiCort = Area under the curve with respect to baseline of the cortisol response; AUCgHR = Area under the curve with respect to the ground of the heart rate response; AUCiHR = Area under the curve with respect to baseline of the heart rate response.
* Adjusted for gender, social economic status, body mass index, oral contraceptive use, smoking, time of the GSST.
† The direct effect is the effect of the predictor variable on the outcome variable, when taking the mediator variables into account. The indirect effect is the effect of the predictor variable on the outcome variable through the mediator variables. The total effect is the sum of the direct and indirect effect.
‡ 95% confidence interval.

DISCUSSION

The results suggest that exercise influences affective symptoms, but not somatic symptoms of depression. A similar conclusion was drawn from another TRAILS study in a larger sample, using general physical activity instead of exercise as a predictor (Stavrakakis et al., 2012). In the present study, markers of stress-induced HPA axis and ANS function and inflammation were tested as mediators of the prospective relationship between exercise and affective symptoms. As a group, these variables partially mediated the association. This indirect effect was mainly accounted for by heart rate (AUCgHR) during social stress. Because we found no mediating effect of heart rate reactivity (AUCiHR), we speculate that regular exercise reduces overall heart rate in various conditions (Forcier et al., 2006) and that this protects against the depressogenic effects of stress.

The natural logarithm of exercise appeared to be a better predictor of affective symptoms than the untransformed measure, indicating that the relative benefit of moderate amounts of exercise compared to no exercise was larger than the relative benefit of large amounts of exercise compared to moderate amounts. Similar non-linear effects of physical activity on health have been reported earlier for depression (Hoffman et al., 2011). This information may motivate inactive depressed individuals to start exercising: it is not necessary to exercise very intensively in order to achieve benefit.

Prior research has revealed both positive and negative associations between depression and cardiovascular responses to stress (e.g. Carney et al., 2005; Light, Kothandapani, & Allen, 1998; de Rooij, Schene, Phillips, & Roseboom, 2010; Phillips et al., 2011). We found evidence for neither hyper- nor hyporeactivity of the cardiovascular system, but did find an association between depression and overall heart rate during stress. Possibly, it is not the increase during stress, but rather a general imbalance of the ANS in depression. Another explanation for the discrepancies in the literature may be that, comparable to HPA axis reactivity (e.g. Miller et al., 2007), both hyper- and hyporeactivity of the ANS reflect an unbalanced system, and hence may confer a risk for stress-related disorders. In line with this, Barton et al. (2007) found that sympathetic activity in patients with Major Depressive Disorder followed a bimodal distribution, with one group displaying extremely high and the other very low sympathetic activity. Similar subgroups may exist with regard to cardiovascular reactivity. Future studies in clinically depressed individuals may examine this possibility.

It is not known how heart rate during stress may mediate the relationship between exercise and depressive symptoms, but several studies provide clues for possible explanations. Chronic exercise has been found to enhance executive function and inhibitory capacities, which require prefrontal control (Padilla et al., 2014). Long-term cardiovascular fitness has also been related to volumetric and functional improvements in the prefrontal cortex (PFC) (Hillman, Erickson, & Kramer, 2008). The PFC plays an important role in regulating parasympathetic activity and its activation is associated with successful suppression of affective responses to negative emotional stimuli as well (Hänsel & von Känel, 2008). One of its main targets is the central nucleus of the amygdala, which is the major efferent source of modulation of autononomic responses, and an important component of the fight-flight network (Thayer & Lane, 2009). By increasing top-down control (i.e. modulating parasympathetic activity), the fight-flight response may be more easily overruled, resulting in more adaptive response patterns in the context of typical daily life. Therefore, physical exercise may decrease risk of depression through increased prefrontal control, and this goes together with a reduced heart rate during stress (Thayer & Lane, 2009). Alternatively, heart rate itself may increase risk for depression. The body is a dynamic system and the heart and brain have strong reciprocal connections. Heightened response patterns to stressful situations in daily life may cause wear and tear of the body, and so increase risk for stress-related disease (Juster, McEwen, & Lupien, 2010). Finally, increased heart

rate may be interpreted by the limbic system as evidence of imminent threat, and so trigger additional stress reactions and negative emotions (Kinser, Goehler, & Taylor, 2012), which may promote development of depressive symptoms. In short, the heart and the brain are reciprocally connected and form complex feedback loops, which together may influence (risk of) depression. Exercise may modulate this feedback loop at the level of the heart as well as the brain.

The indirect pathways through hsCRP and HPA axis activity were not significant, probably because of lacking associations between exercise and these variables, or between these variables and depressive symptoms. Affective symptoms were predicted by hsCRP levels, but hsCRP levels were not predicted by exercise. In contrast, exercise predicted cortisol reactivity to social stress, but cortisol reactivity did not predict affective symptoms. The lack of association between exercise and hsCRP levels may relate to the nature of our sample; adolescents from the general population. On average, older and clinically depressed individuals experience more somatic problems, likely accompanied by inflammation (Dantzer et al., 2008). Possibly, exercise only reduces hsCRP levels when the initial level of inflammation is (pathologically) high. Associations between exercise and cortisol reactivity to social stress were previously reported in adults (Rimmele et al., 2007) and children (Martikainen et al., 2013), and now also in adolescents.

The lack of associations between stress-induced cortisol levels and affective symptoms contradicted our expectations, but illustrates the many inconsistencies regarding this relationship reported in the literature (Stetler & Miller, 2011). Depression can present differently in different individuals (van Loo, de Jonge, Romeijn, Kessler, & Schoevers, 2012). Moreover, in a previous study, we found that the relationship between cortisol responses to social stress and depressive problems depended on chronicity of these problems (Booij et al., 2013). In the current study, however, a sensitivity analysis without individuals with chronic depressive problems yielded similar results. This suggests that chronicity was not responsible for the lack of associations. Because of our hypothesis of a general downregulation of the stress system by exercise, we used stress-reactivity measures. However, others studies have found that lack of recovery after stress may be more strongly related to depression than increased reactivity (Burke et al., 2005). Hence, whether or not exercise improves HPA axis functioning and subsequently depressive symptoms may depend on the measures used and be moderated by the nature of the depressive symptoms.

The present study has strengths and limitations. In the current study, we measured DSM-oriented depressive symptoms, which is not necessarily the same as depression. Nevertheless, it is increasingly recognized that depression is a dimensional, not categorical, construct (e.g. Hankin, Fraley, Lahey, & Waldman, 2005; Judd et al., 1998). This is further supported by the fact that subthreshold depressive symptoms are strong predictors of depression (e.g. Cuijpers & Smit, 2004). By measuring DSM-oriented symptoms, we measured the full range of the spectrum instead of both ends, which

increased precision of our outcome estimates. A drawback is that we had less power to detect significant differences compared to studies selecting more individuals from the high end of the spectrum (i.e. clinically depressed individuals). Furthermore, potential mediators and outcome were measured simultaneously, so we cannot conclude on the direction of the relationship between them. Also, there was a two-year interval between the waves, and exercise patterns may have changed in that period. However, a cross-sectional analysis led to comparable results, i.e. a stronger association of exercise with affective symptoms than with somatic symptoms, and a single significant pathway through the AUCg of heart rate.

Taken together, our findings support the view that exercise may prevent or reduce depressive symptoms, and that the stress system plays a role in this relationship. However, they also suggest that pathways from exercise to depression are symptom-dependent and have limited explanatory power at the group level, possibly because of inter- and intraindividual variability. Future studies may adopt a more person-centered approach by examining intra-individual processes in subjects' natural environments to enhance knowledge about subject-specific mechanisms.

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