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Randomized Trial Examines Effects of Equine Facilitated Learning on Adolescents' Basal Cortisol Levels

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Although equine facilitated programs have gained in popularity over the last decade, virtually nothing is known about the causal effects of equine facilitated interventions on human development and well-being. Researchers conducted a randomized trial to determine the effects of an 11-week equine facilitated learning program on the activity of the Hypothalamic Pituitary Adrenal (HPA) axis of fifth through eighth graders through salivary cortisol levels. Children (N = 131) referred by school counselors and recruited from the community were randomly assigned to either an experimental (N = 53) or waitlisted condition (N = 60). Six samples of salivary cortisol were collected in participants' own home over two consecutive days at pretest, and another set of six samples were collected at posttest in both groups of children. Children in the experimental group who participated in a series of once-weekly, 90-minute sessions of equine facilitated activities had lower afternoon cortisol levels ($F(1, 112) = 8.56$, $p = .017$; $d = .48$) and lower total cortisol concentration per waking hour ($F(1, 112) = 11.12$, $p = .017$, $d = .46$) at posttest, compared to waitlisted children. Multivariate regression analyses showed that program effects were independent from baseline levels of child cortisol, child gender, age, and referral status.

Keywords: randomized trial; adolescent; basal cortisol levels; equine facilitated intervention; HPA-axis

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Introduction

Since the practice of incorporating Human-Animal Interaction (HAI) into therapeutic and educational settings has grown in popularity (Nimer & Lundahl, 2007), there has been a call to conduct evidence-based research on its effects on child physical and mental health, well-being, and development (Esposito, McCune, Griffin, & Maholmes, 2011). Along with a call for clinical trials and examination of treatment effects in various populations, the importance of examining biobehavioral and physiological responses to HAI were emphasized (Esposito et al., 2011). Incorporating these themes, the present study reports on a randomized trial on the effects of an 11-week, equine facilitated learning (EFL)[1] program on activity of the Hypothalamic Pituitary Adrenal (HPA) axis of adolescents, measured through salivary cortisol. In particular, we focus on examining the effects of EFL on adolescents' basal cortisol levels, which in healthy adolescents are typically highest in the morning soon after waking, drop rapidly in the first few hours after waking, and then continue to drop more slowly, reaching a low point around midnight (Klimes-Dougan et al., 2001). The main goal was to examine whether engagement in an 11-week EFL program strengthens adaptive patterns of cortisol production of adolescents, with the goal of preventing development of physical and mental health problems associated with deviations in basal cortisol patterns, such as higher cortisol levels in the afternoon and a higher concentration of cortisol levels per waking hour (Ruttle et al., 2010; Zeiders, Doane, & Adam, 2011). This investigation thus responds to a recent call in the developmental literature to design and evaluate preventive interventions aimed at modifying basal activity of the HPA-axis (Cicchetti & Gunnar, 2008) and builds on the notion proposed in a recent HAI review that dampening of physiological stress parameters may represent a core mechanism in explaining positive effects of HAI (Beetz, Uvnäs-Moberg, Julius, & Kotrschal, 2012).

Rationale for Strengthening Basal Cortisol Patterns in Adolescents

Strengthening adolescents' basal cortisol levels - optimizing the development of adaptive daily patterns of production of cortisol - through participation in EFL was the main goal of this study for several reasons. First, the transition to adolescence is thought to represent a developmental period during which the brain might be especially sensitive to exposure to elevated levels of basal cortisol. Based on the observed links between the emergence of psychopathology and periods of heightened stress - and elevations in cortisol – heightened HPA activity is hypothesized to play a role in the development of stress-related disorders in adolescence (Lupien, McEwen, Gunnar, & Heim, 2009). Second, given research showing that increased HPA activity occurs as a result of puberty-associated neurobiological changes in systems underlying stress and emotional functioning (Stroud et al., 2009), the onset of puberty in and of itself may be a transition point that represents a period of increased risks in adolescence. This may be problematic given that this developmental period is already associated with psychosocial factors of vulnerability. Third, researchers have posited that lowered HPA activity (especially later in the day) in a relatively lower-risk, normally functioning sample of early adolescents may be considered more adaptive and thus, constitute a protective influence against the development of later psychopathology (Lupien, et al., 2009). Although associations between lower

[1] EFL combines mounted and unmounted, experiential learning, interaction with horses, ponies, miniature horses, donkeys, and mules, and counseling-based processing skills to increase adolescents' awareness and control of their emotions, cognitions, behaviors, and physiological arousal.

concentrations of cortisol levels and lower afternoon levels of cortisol and behavior and psychopathology are complex, evidence suggests that weaker diurnal patterns of cortisol are associated with various adjustment problems. For example, Tyrka et al. (2010) found that higher afternoon cortisol levels were linked to internalizing problems in a sample of 8-11-year-old boys. Furthermore, in a two-year follow-up, Tyrka et al. (2012) found that afternoon cortisol levels assessed at baseline were significant positive predictors of child depressive symptoms two years later. In addition, associations have also been found for average daily levels. Researchers (Scerbo & Kolko, 1994) found that elevated average child cortisol levels were positively related to child internalizing behavior, which has also been shown to relate to exclusion by peers at school (Peters, Riksen-Walraven, Cillessen, & de Weerth, 2011). In a sample of 9-14-year-olds, greater increases in average daily levels of cortisol measured across the span of a year predicted higher general and social anxiety in girls six months later at post-test (Schiefelbein & Susman, 2006). In sum, preventing or alleviating deviations from typical circadian patterns (e.g., high instead of low cortisol levels in the afternoon or evening, constant high or low levels of cortisol throughout the day) may have positive implications for various indices of adolescent physical and mental health.

Rationale for Reducing Basal Cortisol Levels Through Human Equine Interaction

Participation in an 11-week EFL program may strengthen adaptive patterns of adolescents' basal cortisol for several reasons. First, EFL incorporates several approaches (e.g., human-animal interaction; cognitive-behaviorally-based stress management; and physiological relaxation techniques) are thought to lower basal cortisol activity in adults. Although based on correlational designs, studies show promising within-person changes of cortisol levels in response to interactions with animals (Barker, Knisely, McCain, & Best, 2005; Barker et al., 2010; Odendaal & Meinjes, 2003), relaxation training (Pawlow & Jones, 2005), cognitive-behavioral stress management (Cruess, Antoni, Kumar, & Schneiderman, 2000; Gaab et al., 2003), yoga (Curtis, Osadchuk, & Katz, 2011), reiki and imagery (Bowden, Goddard, & Gruzelier, 2010), transcendental meditation (Kamei et al., 2000), and massage (Field et al., 2004). Second, although not specifically examined in the context of human-equine interaction, a recent review on the psychophysiological implications of human-animal interaction suggests that human-animal interaction, and possibly human-equine interaction, may modulate physiological stress parameters through their connection with the oxytocin system (Beetz et al., 2012). For example, several investigators (Odenaal, 2000; Odendaal & Meintjes, 2003; Handlin et al., 2011) have noted significant increases in oxytocin levels in human plasma levels after as little as three minutes of physically interacting (e.g., stroking) with a dog, with effects depending on the relationship quality between the human-dog pair. Similarly, physical interactions that 'tap into' caregiving and grooming behaviors have also been associated with increases in oxytocin in human to human contact (Carter, 2003; White-Traut, Schwertz, McFarlin, & Kogan, 2009). This is relevant, as the EFL program under study explicitly engages participants in physical interactions with the horse (i.e., grooming, stroking, petting, touching) for the purpose of down-regulating their physiological arousal. For example, in addition to the regular ten-minute grooming activity children engaged in during each session, children were at times instructed to use their hands to stroke (e.g., massage) the horse slowly and rhythmically for ten minutes, while mindfully engaging in relaxation behaviors. These behaviors included breathing slowly and rhythmically to match their breathing to the breathing of the horse, refraining from any verbal communication with others, and being mindful about the experience of touching the horse and feeling its warmth. Since increases in oxytocin are associated with decreases in cortisol levels in humans and non-human animals (Legros, Chiodera, & Geenen, 1988; Petersson, Lundeberg, & Uvnäs-Moberg, 1999; Neumann et al., 2000), engagement in this type of EFL activity - and the hypothesized increases in oxytocin - may thus lead to suppressed HPA activity as evidenced by reduced cortisol levels.

Last, given that cortisol levels are extremely sensitive to social stressors and supports, (Gunnar & Donzella, 2002), participation in EFL may effectively lower basal cortisol production through adaptations in participants' stress-relevant cognitive appraisals. Specifically, basal HPA activity is dependent not only on the nature and frequency of daily stressors encountered, but also on appraisal processes (Herman & Cullinan, 1997). These include both the individual's interpretation of stressors, as well as the assessment of the availability – and adequacy – of coping resources. One key component of EFL that may alter participants' stress-relevant cognitive appraisals is that participants are provided with countless opportunities to experience positive human-equine interactions in a supportive, non-judgmental environment. Combined with observed improvements in social competence (Pendry & Roeter, 2013), the development of a trust-based relationship with the horse, individual counselors, and other participants, EFL may thus effectively increase participants' performance accomplishment and perception of social support, which is likely to affect how stressors are perceived and, physiologically, responded to.

Research Questions and Hypothesis

Given that correlational studies have demonstrated promising alterations towards more adaptive basal cortisol patterns in adults (e.g., lower average cortisol, lower afternoon and evening levels), along with findings suggesting that HAI may increase oxytocin (associated with suppressing HPA activity), and improve stress-relevant cognitive appraisals, the following hypotheses guided our analyses. The first hypothesis predicted that children randomly assigned to participate in an 11-week equine facilitated learning program would on average have lower levels of average daily cortisol levels at post-test, compared to children assigned to a waitlisted condition. The second hypothesis predicted that children randomly assigned to participate in an 11-week equine facilitated learning program would have on average lower levels of afternoon and bedtime cortisol levels at post-test, compared to children assigned to a waitlisted condition.

Method

The Program

The equine facilitated learning program, PATH to Success, was conducted at a Premier Accredited Center of PATH, Int'l at a university setting. The stated goal of the program is to enhance child social competence and reduce stress through an 11-week session of once-weekly, 90-minute lessons of individual, team, and group-focused equine facilitated activities. Activities were based on principles of equitation science and natural horsemanship (McGreevy & McLean, 2010) featuring a combination of mounted and un-mounted activities and human-equine interactions, including observation of equine behavior, engagement in equine management (e.g., grooming), in-hand horsemanship, some riding, and personal and group reflection. The program was implemented by a team of certified instructors, professional counseling psychologists, graduate-level counseling students, and undergraduate students in child development, education, and animal science. Children in the experimental group were assigned to the same facilitation team for the entire 11-week session that included one equine, two child participants, an equine specialist, and a facilitator. Each day, a total of eight child participants - divided across four equine teams headed by four experienced facilitators - participated. The program was conducted four times a week, serving 32 children each week per 11-week session. The weekly lessons were conducted on weekday afternoons and included transportation of participants from school to the program site and back immediately following their regular school day. Children who were waitlisted participated after children in the experimental group completed the program. A description of weekly program objectives and a selection of sample activities are described in Table 1.

Procedures

The study was approved by Washington State's University Committee on Research Involving Human Subjects. Equine welfare of horses in the program was carefully monitored under the university's Institutional Animal Care and Use Committee (IACUC) regulations and campus veterinarian. Program participants were recruited through referral by school counselors and distribution of flyers and advertisements throughout schools and the community. Children referred by school counselors were those receiving counseling services for academic and/or behavioral adjustment issues or those whose parents had consulted with counseling staff about concerns over their child's exposure to school and/or home-based stress. Criteria for participation were 1) parents and children were proficient English speakers, 2) the child did not have physical or mental disabilities, and 3) children attended the fifth through eighth grade. Parents provided information about the adolescent's use of steroid-based medication,

Table 1
Outline of Lesson Objectives by Week

Week	Lesson Objective	Sample Activity
1	Basic safety: Meet horses and staff	Observing horse behavior and herd dynamics
2	Respect: Self, others and horses	Moving horses using 4 phases of direct or indirect pressure
3	Communication: Verbal and non-verbal	Leading horses, interpreting horse body language
4	Leadership: Assertive and aggressive cues	Driving activity using body language and phases
5	Trust: Coping with perceptions of stress	Riding and leading
6	Boundaries	Driving activity using indirect pressure
7	Overcoming challenges and building confidence	Desensitizing horses
8	Enhancing self-regulation and relaxation	Horse massage, riding
9	Prepare for parents/visitors day	Incorporating horsemanship skills for team challenge
10	Parents/visitors day	Participants 'teach' parents horsemanship skills
11	Program wrap up	Obstacle course, riding and reflection

health and behavior, and child's exposure to school and/or family based stress. Although this did not occur, children could have been excluded from participation if a history of animal abuse or suicidal behavior had been reported. Parents and children were instructed and consented/assented by the PI, and received materials and demonstrations on in-home salivary sampling procedures. Parents completed a standardized measure of child social competence (see measures) for which they were paid five dollars. Given that a limited number of program and study slots were available, children who were referred by school counselors, those with greater exposure to current or recent school or home-based stressors, and those with lower social competence were given priority during study selection - but not randomization - procedures.

Sample Recruitment, Screening, and Assignment

Program staff recruited a total of 131 interested children and their families from ten different schools in a rural university town in the Pacific Northwest area of the United States, of which 113 were selected (N_{boys} = 41; N_{girls} = 72; $N_{referred}$ = 16; M_{age} = 11.35 yrs; $M_{social\ competence}$ = 42.99) for study participation. Using a randomization schedule, study participants were randomly assigned to an experimental group (N = 53) or waitlisted control condition to start program participation 16 weeks later (N = 60). Participants were predominantly White (81.6%), and of non-Latino or Hispanic ethnicity (88.8%), with the remaining children reporting across racial categories that included more than one race (8%), Asian (3.2%), American Indian or Alaska Native (1.6%), or unknown race (5.6%). There were no significant differences between participants' age (p = .60), gender (p = .77), referral status (p = .18), grade level (p = .75), or pre-test levels of social competence (p =.31) by experimental condition. Sample enrollment, selection, randomization, follow up, and analyses counts are described in a flow diagram (Figure 1, page 86). Of participants originally assigned, 84% completed data collection at post-test (N_{boys} = 36; N_{girls} = 58; $N_{referred}$ = 13; M_{age} = 11.34 yrs). There were no significant group differences by age (p = .96), referral status (p = .91), grade level (p = .90), baseline social competence (p = .93), gender (p = .25), or amount of previous experience working with horses (p = .94) between participants who completed the study and those lost to attrition.

Measures

Child social competence

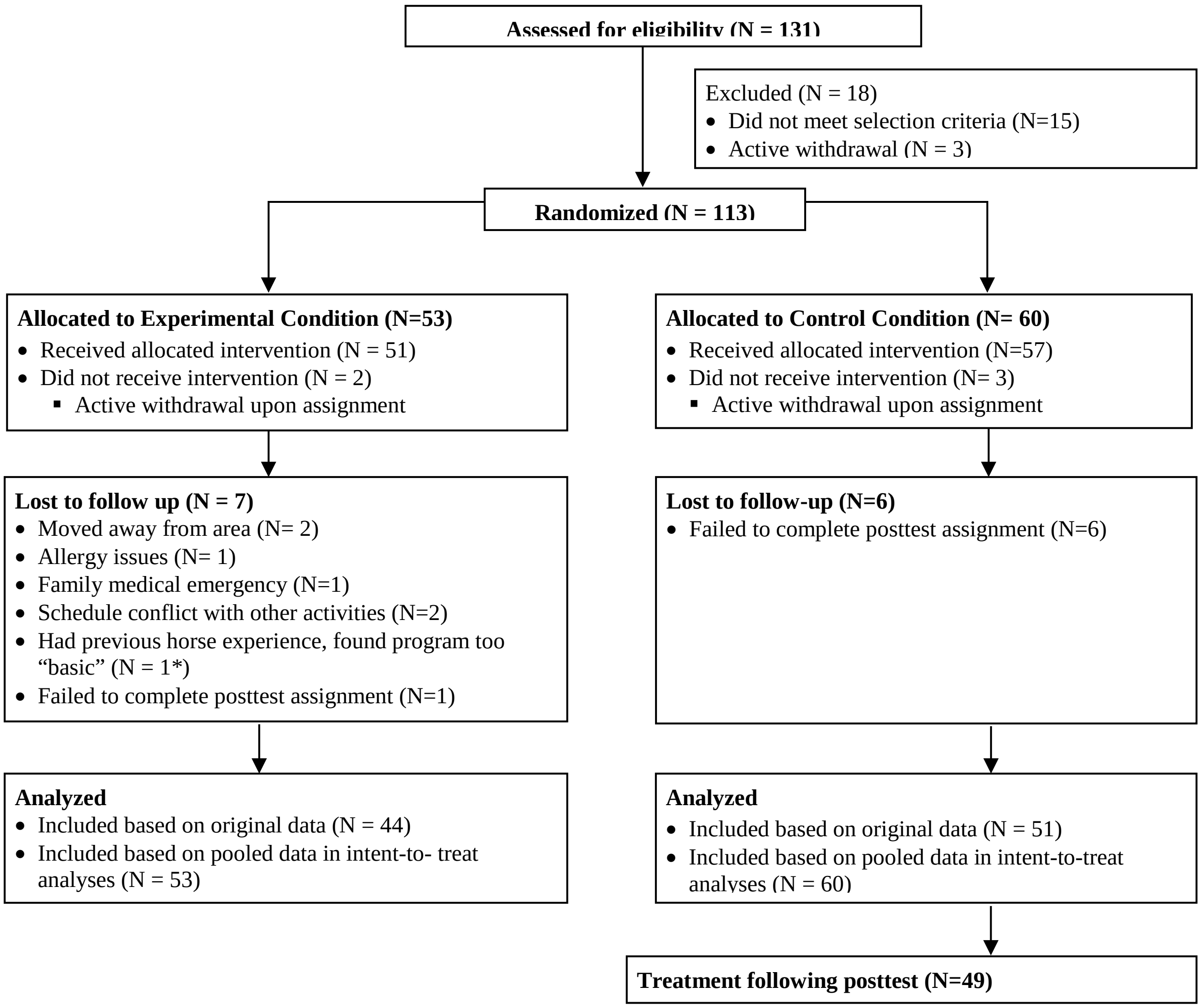
Parents completed the Devereux Student Strength Assessment (DESSA; LeBuffe, Shapiro, & Naglieri, 2009) which contains 72-items asking respondents to indicate how often various child behaviors occurred over the past four weeks based on a 5-point Likert scale ranging from 0 (*never*) to 4 (*very frequently*). The DESSA composite score (α_1 = .98) was derived from 8 subscales including Optimistic Thinking (α = .87), Self-Management (α = .86), Goal-Directed Behavior (α = .89), Self-Awareness (α = .82), Social-Awareness (α = .81), Personal Responsibility (α = .87), Decision Making (α = .91), and Relationship Skills (α = .93). Based on comparisons of mean levels of pre-test social competence in our sample to mean levels obtained in a 'normal' (M = 48.20; SD = 10.01) and 'emotionally disturbed' (M = 37.10; SD = 7.90) population sample (LeBuffe et al., 2009), it appears that the goal of recruiting children with relatively lower levels of social competence was met.

Salivary Cortisol Sampling

In-home cortisol sampling at pre-test was done by all participants over two consecutive

Figure 1

Flow Diagram describing sample enrollment, selection, randomization, follow up and analyses.



*Participant withdrew after 4 weeks of participation but completed post-test assessments.

days after participant consent/assent and selection, but before random assignment was communicated to participant, and before the start of the program. The in-home sampling paradigm conducted at pre-test was repeated at post-test i.e., after completion of the treatment period, twelve weeks later. During each sampling period, participants collected six salivary cortisol samples by spitting through a straw into a sterile 1.8 ml cryovial, three times a day, on each of two consecutive weekdays at prescribed events (immediately upon waking, immediately before bedtime) and at prescribed times (4:00 pm). Participants' recorded sampling time, use of steroid-based medication, timing of food/beverage intake, sleep and wake-times, as well as sampling confounds (e.g., sickness). Substantial efforts were made to impress upon participants the importance of compliance with the timing of sampling and reporting of sampling times. Payment of 5 dollars for each set of six samples was offered to each child upon completion at pre-test and again post-test. Compliance was high as 98% of children sampled provided all six samples as requested. Participants brought samples to the program site at the beginning and end of the program. Upon collection, samples were transferred by cooler to our laboratory-based freezer for storage at –20 degrees Celsius. Samples were analyzed by a laboratory specializing in the assaying of saliva by enzyme immunoassay. The test used for these assays had a range of sensitivity from .007 to 1.8 μg/dl, and average intra- and inter-assay coefficients of variation less than 3% and 7% respectively.

Data Reduction of Cortisol Values

Cortisol parameters (area under the curve (AUC), morning, afternoon, bedtime) for each child were calculated at pre-test and six at post-test. To limit the influence of extreme cortisol values, values were first winzorised to 3 standard deviations above and below the mean. Six cortisol values across two consecutive days were used to calculate each child's average daily cortisol level (AUC) using the polygon method, at pre-test and post-test. The polygon AUC is a valuable measure, especially in establishing a link between cortisol levels and psychological functioning (Stewart & Seeman, 2000). Next, based on averaging day one and day two cortisol values for each time of day (e.g., morning, afternoon, bedtime), we calculated an average cortisol value for each parameter (e.g., morning, afternoon, bedtime). A natural logarithmic transformation for each cortisol parameter at each time point was used to reduce positive skewness of the data. Descriptives on cortisol levels for each two-day period and their associations for each time period are presented in Table 2.

Table 2
Descriptives by Day and Correlations Between Days of Original Untransformed Child Cortisol Data

Variable	Day 1 M (SD)	Day 2 M (SD)	Correlation
Pre-test			
Morning Cortisol	.290 (.18)	.322 (.29)	.273**
Afternoon Cortisol	.085 (.12)	.116 (.23)	.149*
Bedtime Cortisol	.065 (.16)	.049 (.12)	.540**
Morning Time	7.32 (1.00)	7.41 (1.17)	.434**
Afternoon Time	16.40 (.88)	16.31 (.94)	.247*
Bedtime Time	21.12 (.90)	21.17 (.92)	.592**
Post-test			
Morning Cortisol	.289 (.16)	.301 (.16)	.255**
Afternoon Cortisol	.078 (.06)	.085 (.08)	.376**
Bedtime Cortisol	.057 (.09)	.050 (.08)	.500**
Morning Time	7.61 (1.11)	7.48 (1.10)	.565**
Afternoon Time	16.07 (1.25)	16.04 (1.13)	.591**
Bedtime Time	21.41 (1.02)	21.33 (1.11)	.473**

** Correlation is significant at the .01 level. * Correlation is significant at the .05 level.

Results

To determine whether participation in the program significantly strengthened child basal cortisol levels we conducted a series of intent-to-treat analyses (Fisher et al., 1990), which prevents overestimation of treatment effects by comparing participants in the groups to which they were originally randomly assigned, regardless of whether they subsequently withdrew or deviated from the protocol. Missing data on post-test assessments were imputed using five imputations according to multiple imputation procedures (Rubin, 1996). The post-test results thus reflect pooled estimates across five imputed datasets that were generated.

A series of one-way ANOVAs revealed no statistically significant differences in pre-test levels of child cortisol levels by experimental condition for any of the cortisol parameters. To test the first hypothesis - participation in the program would reduce average daily (AUC) levels of cortisol - researchers conducted a one-way ANOVA comparing average daily cortisol levels measured at post-test by experimental condition. Results support the first hypothesis indicating that children who participated in the intervention had significantly lower levels of cortisol per waking hour at post-test (F (1, 112) = 11.12, p = .017), compared to children who were waitlisted (see Figure 2). Based on calculations using pooled variances, the effect size of program participation on average daily cortisol level was .46, a moderate effect (Cohen, 1988).

Using a lagged regression approach, researchers next predicted adolescents' basal cortisol levels at post-test (Time -2), while including a pre-test (Time -1) covariate in the model. The aim of this approach was to examine program effects while considering omitted (unmeasured) time-invariant differences in children that may have been present at the beginning of the program. Important factors potentially related to adolescent basal cortisol levels at both time points (e.g., gender, age, referral status) were also included in the model as covariates. Results (Table 3, model 1)

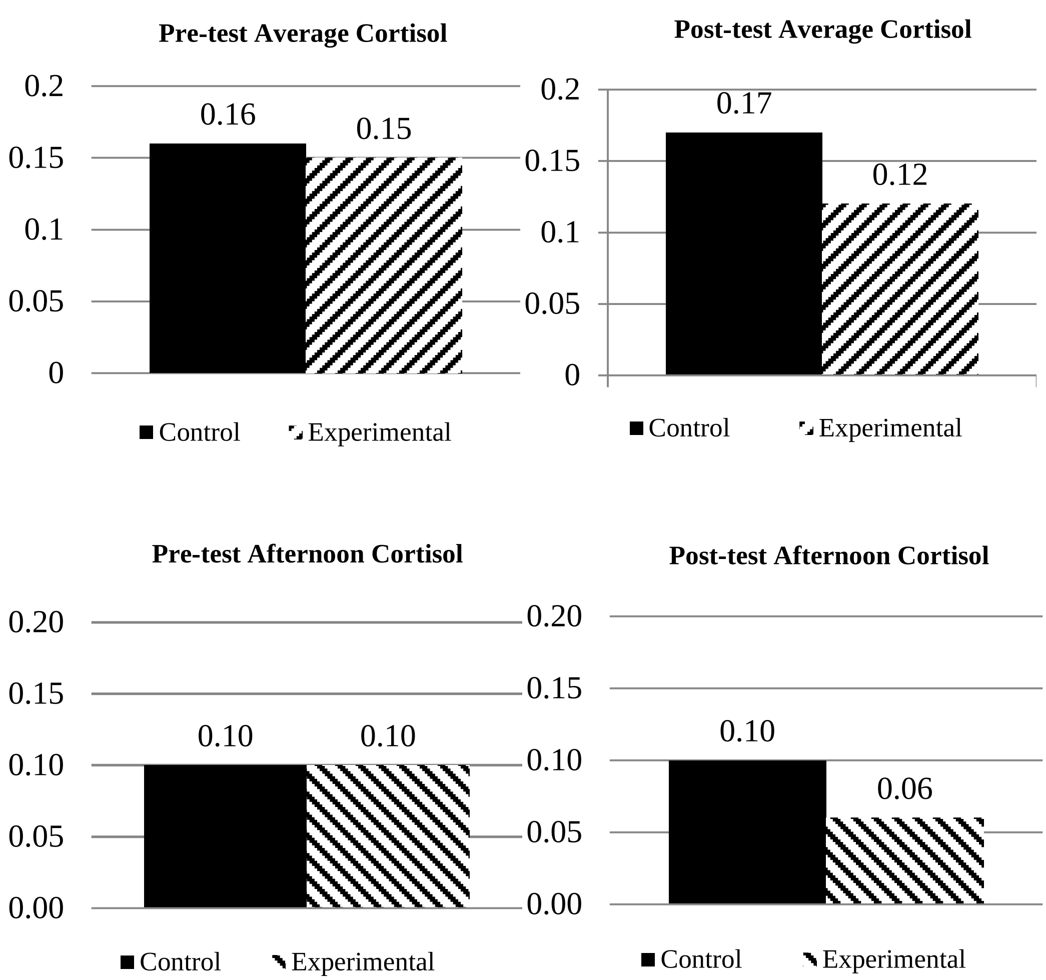
Table 3
Lagged Dependent Variable Models Predicting Posttest Basal Cortisol levels by Experimental Condition

Variable	Unstandardized B	SE	Beta	p-value
Average Daily (AUC) Cortisol				
Constant	-.639	.540		.242
Experimental Condition	**-.209**	**.087**	**-.223**	**.019**
Pre-test AUC Cortisol	.219	.078	.263	.006
Child Gender	-.143	.095	-.145	.137
Child Age	-.007	.004	-.159	.103
Whether Referred	-.054	.132	-.039	.684
Afternoon Cortisol				
Constant	-1.586	.784		.046
Experimental Condition	**.314**	**.119**	**-.278**	**.010**
Pre-test Afternoon Cortisol	.156	.086	.195	.074
Child Gender	-.158	1.33	-.131	.237
Child Age	-.004	.006	-.075	.486
Whether Referred	-.065	.176	-.040	.710

revealed a significant treatment effect on adolescents' average daily (AUC) cortisol levels (β = -.223; p = .019), which was independent of contributions of children's pre-test levels of average daily cortisol, which were positive and significant (β = .263; p < .006). There were no significant main effects of children's referral status, age, or gender, nor did we observe that treatment effects were moderated by these child characteristics.

Testing the second hypothesis - participation in the program would reduce afternoon levels of cortisol - researchers conducted a one-way ANOVA comparing afternoon cortisol levels measured at post-test by experimental condition. Results support the second hypothesis (Figure 2) by demonstrating a treatment effect of program participation as illustrated by significant group differences in afternoon cortisol levels, ($F(1, 112) = 8.56$, p = .017) by experimental condition. Based on calculations using pooled variances, program participation resulted in an effect size of d = .48, a moderate effect. Using a longitudinal lagged regression approach, researchers next predicted adolescents' afternoon basal cortisol levels at post-test (Time-2), while including a pre-test (Time-1) covariate in the model, as well as

Figure 2
Basal pattern of cortisol production demonstrating significant lower afternoon and daily average levels of cortisol at post-test for children assigned to experimental group.



factors potentially related to adolescent afternoon cortisol levels at both time points. Results (Table 3, model 2) revealed a significant treatment effect on adolescents' afternoon cortisol level (β = .278; p = .010), which was independent of contributions of children's pre-test levels of afternoon cortisol (β = .195; p < .074). There were no significant main effects of children's referral status, age, or gender, nor did we observe that treatment effects were moderated by these child characteristics. We found no evidence suggesting that morning or bedtime cortisol levels were significantly affected by program participation.

Discussion

The current study reports on the results of a randomized trial examining causal effects of participation in an 11-week equine facilitated learning program on basal HPA activity of adolescents assessed through in-home sampling of salivary cortisol at pre-test (before program assignment) and post-test (12 weeks later). Results showed a moderate lowering effect on average daily cortisol levels (AUC) (d = .48) and afternoon cortisol levels (d = .48) of adolescents who participated in an 11-week EFL program, compared to those who were waitlisted. Findings also showed that program effects on afternoon and daily average cortisol were independent from baseline levels of cortisol, child gender, age, and referral status. Although associations between lower average daily cortisol/afternoon levels of cortisol and developmental psychopathology and/or adolescent health are by no means clearly understood, several researchers have suggested that lower basal cortisol levels in a relatively normal adolescent sample may constitute a protective influence against the development of psychopathology and health problems in certain populations (Lupien et al., 2009). As such, this study suggests that EFL may be an effective approach to support positive development in adolescence. To our knowledge, this is the first randomized, controlled trial to demonstrate treatment effects of a prevention program on basal HPA activity in a sample of predominantly normal sample of adolescents. These results also represent the first causal evidence demonstrating significant effects of EFL on basal neuroendocrine functioning of adolescents. Our work complements findings based on recent randomized trials on EFL on social competence (Hauge et al., 2013; Pendry & Roeter, 2013) and responds to calls in the growing field of Human-Animal Interaction (HAI) and Animal Assisted Intervention (AAI) (Esposito et al., 2010) for evidence-based approaches examining effects on child development. This study also contributes to a relatively new and much needed body of literature of randomized trials on preventive interventions with adolescents, which have paid minimal attention to neurobiological and physiological systems in their evaluations of treatment efficacy (Cicchetti & Gunnar, 2008).

Human-Animal Interaction and Cortisol

The observed treatment effects of equine facilitated learning on young adolescents' cortisol levels represent a novel finding that complements a small body of research linking interaction between humans and dogs to reduced momentary cortisol activity. For example, Barker et al. (2010) compared cortisol reactivity and recovery of therapy dog owners to non-therapy dog owners in the context of a laboratory-based stress task. In both groups, cortisol levels dropped below baseline after one minute of interaction with the therapy dog, offsetting initial increases in cortisol levels in response to the stress task. Barker et al. (2005) also demonstrated that serum and salivary momentary cortisol levels of healthcare professionals were significantly lower after interacting with a therapy dog in a hospital setting. Lastly, interaction with a familiar dog in the home has been found to decrease human cortisol levels (Odendaal & Meinjes, 2003). While the use of therapy dogs has been shown to reduce cortisol levels in adults, interventions incorporating the use of therapy dogs with children have been less successful. For example, Kaminski, Pellino, and Wish (2002) compared salivary cortisol levels of participants participating in child-life intervention sessions - one group incorporating the use of therapy dogs and the other without – and found that reductions in salivary cortisol in either group were not significant.

Implications for adolescent development

The implications of these results for adolescent development need to be interpreted thoughtfully. First, extremely little is known about the extent to which cortisol activity of young adolescents - and changes therein - may precede adaptive changes on the behavioral level in the long term. As such, it is not clear whether the observed changes in cortisol levels constitute an influential protective factor that promotes future adaptation and resilience (Masten, 2001) in the context of risk factors. Second, it is important to consider both the nature of results (lower AUC and afternoon cortisol), as well as the population in which they were obtained (i.e. a relatively normal sample of young adolescents, rather than a clinical sample of children characterized by a history of severe maltreatment, extreme rearing conditions, and/or existing developmental psychopathology). Specifically, it is important to consider that basal cortisol levels in our sample at pre-test were characterized by what are thought of as relatively normal patterns of cortisol production, although a wide range of individual differences in steepness of these patterns were observed. Effects of lower AUC and afternoon cortisol levels observed in our study should thus not be equated with altering the presence of maladaptive patterns of HPA activity, such as hyper or hypocortisolism. In sum, the results of this study reflect moderate treatment effects of equine facilitated learning on average daily (AUC) and afternoon cortisol levels within the normal range of basal HPA activity.

Limitations and Implications

Future studies examining the effects of preventive equine facilitated learning programs on adolescent basal HPA activity will benefit from addressing the following limitations of this study. Since the program involved a significant amount of human interaction, the design of the study would have benefitted significantly from an additional control group to compare effects of equine facilitated activities to the effects of the intervention components that did not involve interactions with equines. Also, the use of additional control groups could have facilitated an examination of the role of particular types of equine interactions thought to be central to the intervention's success. For example, is it grooming horses, riding horses, or engaging in natural horsemanship activities that affect child HPA activity? What is the role of various other program features and activities possibly underlying the treatment effects (e.g. the role of cognitive behavioral approaches to affect cognitive appraisals about stress and coping versus relaxation activities aimed at reducing physiological arousal)? Second, although participation in the intervention was offered to all fifth through eighth grade students in the geographical area, students and parents had a choice to enroll their child in the program. As such, the study cannot address whether this equine facilitated intervention would be equally effective for children who may have been less willing or comfortable to interact with horses.

Our findings have several implications for practice. First, the experimental nature of these findings gives greater credence to the claim of therapeutic horsemanship professionals, participants, and parents who have reported significant positive effects of equine facilitated activities firsthand. Faced with skepticism about the efficacy of equine facilitated programs by potential funders and third party payers, therapeutic professionals and clients can now point to causal effects on physiological outcomes associated with current and future physical and mental health. Second, this study suggests that equine facilitated learning programs may serve as viable alternatives to after school programs focusing on athletic or academic achievement, providing after school opportunities to children with different interests and needs. Third, while this is only the first step in more fully understanding our ability to alter HPA activity of adolescents through the use of preventive interventions, the effect of a relatively short-term EFL program on diurnal HPA activity may serve as the impetus to conduct research on effects of EFL in other populations, including children with a history of stress exposure or those with diagnoses of stress-related adjustment issues, as well as closer examination of potential mediating variables, underlying program effects.

References

Barker, S.B., Knisely, J.S., McCain, N.L., & Best, A.M. (2005). Measuring stress and immune response in healthcare professionals following interaction with a therapy dog: A pilot study. *Psychological Reports*, *96*, 713-729. doi: 10.2466/pr0.96.3.713-729

Barker, S.B., Knisely, J.S., McCain, N.L., Schubert, C.M., & Pandurangi, A.K. (2010). Exploratory study of stress-buffering response patterns from interaction with a therapy dog. *Anthrozoös*, *23*, 79-91. doi: 10.2752/175303710X12627079939341

Beetz, A., Uvnäs-Moberg, K., Julius, H., & Kotrschal, K. (2012). Psychosocial and psychophysiological effects of human-animal interactions: The possible role of oxytocin. *Frontiers in Psychology*, *3*, 1-15. doi: 10.3389/fpsyg.2012.00234

Bowden, D., Goddard, L., & Gruzelier, J. (2010). A randomised controlled single-blind trial of the effects of Reiki and positive imagery on well-being and salivary cortisol. *Brain Research Bulletin, 81*, 66–72. doi:10.1016/j.brainresbull.2009.10.002

Carter, S.C. (2003). Developmental consequences of oxytocin. *Physiology & Behavior, 79*, 383– 397. doi:10.1016/S0031-9384(03)00151-3

Cicchetti, D., & Gunnar, M. R. (2008). Integrating biological processes into the design and evaluation of preventive interventions. *Development and Psychopathology*, *20*, 737–743. doi:10.1017/S0954579408000357

Cohen, J. (1988). *Statistical power analysis for the behavioral sciences* (2nd ed.). Hillsdale, NJ: Erlbaum.

Cruess, D. G., Antoni, M. H., Kumar, M., & Schneiderman, N. (2000). Reductions in salivary cortisol are associated with mood improvement during relaxation training among HIV seropositive men. *Journal of Behavioral Medicine*, *23*, 107–122. doi:10.1023/A:1005419917023

Curtis, K., Osadchuk, A., & Katz, J. (2011). An eight-week yoga intervention is associated with improvements in pain, psychological functioning and mindfulness, and changes in cortisol levels in women with fibromyalgia. *Journal of Pain Research*, *4*, 189-201. doi:10.2147/JPR.S22761

Esposito, L. E., McCardle, P., Maholmes, V., McCune, S., & Griffin, J. A. (2010). Introduction. In P.McCardle, M.McCune, J. A.Griffin, L.Esposito, & L.Freund *(Eds.). Animals in our lives: Human-animal interaction in family, community, & therapeutic settings (pp.* 1-5*).* Baltimore: Brookes.

Esposito, L., McCune, S., Griffin, J. A., & Maholmes, V. (2011). Directions in human-animal interaction research: Child development, health, and therapeutic interventions. *Child Development Perspectives, 5*(3), 205-211. doi 10.1111/j.1750-8606.2011.00175.x

Field, T., Hernandez-Reif, M., Diego, M., Feijo, L., Vera, Y., & Gil, K. (2004). Massage therapy by parents improves early growth and development. *Infant Behavior & Development*, *27*, 435-442. doi:10.1016/j.infbeh.2004.03.004

Fisher, L.D., Dixon, D.O., Herson, J., Frankowski, R.K., Hearon, M.S., & Pearce, K.E. (1990). Intention to treat in clinical trials. In K.E. Pearce (Ed.), *Statistical issues in drug research and development* pp. 331–350. New York: Marcel Dekker.

Gaab, J., Blättler, N., Menzi, T., Pabst, B., Stoyer, S., & Ehlert, U. (2003). Randomized controlled evaluation of the effects of cognitive-behavioral stress management on cortisol responses to acute stress in healthy subjects. *Psychoneuroendocrinology*, *28*, 767–779. doi:10.1016/S0306-4530(02)00069-0

Gunnar M. R., & Donzella, B. (2002). Social regulation of the cortisol levels in early human development. *Psychoneuroendocrinology*, *27*, 199–220. doi:10.1016/S0306-4530(01)00045-2

Handlin, L., Hydbring-Sandberg, E., Nilsson, A., Ejdgeback, M., Jansson, A., & Unvas-Moberg, K. (2011). Short-term interaction between dogs and their owners—effects on oxytocin, cortisol, insulin and heart rate—an exploratory study. *Anthrozoös, 24*, 301-316.

Hauge, H., Kvalem, I. L, Berget, B., Enders-Slegers, M., & Braastad, B. O. (2013). Equine-assisted activities and the impact on perceived social support, self-esteem and self-efficacy among adolescents—an intervention study. *International Journal of Adolescence and Youth.* http://dx.doi.org/10.1080/02673843.2013.779587

Herman, J., & Cullinan, W. (1997) Neurocircuitry of stress: Central control of the hypothalamo-pituitary-adrenocortical axis. *Trends in Neuroscience*, *20*, 78-84. doi:10.1016/S0166-2236(96)10069-2

Kamei, T., Torui, Y., Kimura, H., Ohno, S., Kumano, H., & Kimura, K. (2000). Decrease in serum cortisol during yoga exercise is correlated with alpha wave activation. *Perceptual and Motor Skills*, *90*, 1027–1032. doi:10.2466/PMS.90.3.1027-1032

Kaminski, M., Pellino, T., & Wish, J. (2002). Play and pets: The physical and emotional impact of child-life and pet therapy on hospitalized children. *Children's Health Care*, *31*(4), 321-335.

Klimes-Dougan, B., Hastings, P. D., Granger, D. A., Usher, B.A., & Zahn-Waxler, C.

(2001). Adrenocortical activity in at-risk and normally developing adolescents: Individual differences in salivary cortisol basal levels, diurnal variation, and responses to social challenges. *Developmental Psychopathology*, *13*, 695–719. doi:10.1017/S0954579401003157

LeBuffe, P.A., Shapiro, V.B., & Naglieri, J.A. (2009). *The Devereux Student Strengths Assessment (DESSA).* Lewisville, NC: Kaplan Press.

Legros, J. J., Chiodera, P., & Geenen, V. (1988). Inhibitory action of exogenous oxytocin on plasma cortisol in normal human subjects: Evidence of action at the adrenal gland. *Neuroendocrinology, 48*, 204-206.

Lupien, S. J., McEwen, B. S., Gunnar, M. G., & Heim, C. (2009). Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nature Reviews Neuroscience, 10*, 434-445. doi:10.1038/nrn2639

Masten, A. S. (2001). Ordinary magic: Resilience processes in development. *American Psychologist*, *56*, 227-238. doi:10.1037/0003-066X.56.3.227

McGreevy, P. D., & McLean, A. N. (2010). *Equestrian Science*. United Kingdom: Blackwell Publishing.

Neumann, I. D., Wigger, A., Torner, L., Holsboer, F., & Landgraf, R. (2000). Brain oxytocin inhibits basal and stress-induced activity of the hypothalamo-pituitary-adrenal axis in male and female rats: Partial action within the paraventricular nucleus. *Journal of Neuroendocrinology, 12*, 235-243.

Nimer, J., & Lundahl, B. (2007). Animal-assisted therapy: A meta-analysis. *Anthrozoös, 20*(3), 225-238. doi: 10.2752/089279307X224773

Odendaal, J. S. (2000). Animal assisted therapy—magic or medicine? *Journal of Psychosomatic Research*, *49,* 275-280.

Odendaal, J. S. J., & Meinjes, R. (2003). Neurophysiological correlates of affiliative behavior between humans and dogs. *Veterinary Journal*, *165*, 296-301. doi: 10.1016/S1090-0233(02)00237-X

Pawlow, L. A., & Jones, G. E. (2005). The impact of abbreviated progressive muscle relaxation on salivary cortisol and salivary immunoglobulin a (sIga). *Applied Psychophysiology and Biofeedback*, *30*, 375–387. doi:10.1007/s10484-005-8423-2

Pendry, P., & Roeter, S. (2013). Experimental trial demonstrates positive effects of equine facilitated learning on child social competence. *Human-Animal Interaction Bulletin, 1*, 1-19.

Peters, E., Riksen-Walraven, J. M., Cillessen, A. H., & de Weerth, C. (2011). Peer rejection and HPA activity in middle childhood: Friendship makes a difference. *Child Development*, *82*(6), 1906-1920. doi: 10.1111/j.1467-8624.2011.01647.x

Petersson, J., Lundeberg, T., & Unvas-Moberg. (1999). Short-term increase and long-term decrease of blood pressure in response to oxytocin-potentiating effect of female steroid hormones. *Journal of Cardiovascular Pharmacology, 33*, 102-108.

Rubin, D.B. (1996). Multiple imputation after 18+ year. *Journal of the American Statistical Association*, *91*, 473-489. doi:10.2307/2291635

Ruttle, P. L., Shirtcliff, E. A., Serbin, L. A., Fisher, D. B., Stack, D. M., & Schwartzman, A. E. (2010). Disentangling psychobiological mechanisms underlying internalizing and externalizing behaviors in youth: Longitudinal and concurrent associations with cortisol. *Hormones and Behavior*, *59*, 123-132. doi:10.1016/j.yhbeh.2010.10.015

Scerbo, A. S., & Kolko, D. J. (1994). Salivary testosterone and cortisol in disruptive children: Relationship to aggressive, hyperactive, and internalizing behaviors. *Journal of the American Academy of Child & Adolescent Psychiatry*, *33*(8), 1174-1184.

Schiefelbein, V. L., & Susman, E. J. (2006). Cortisol levels and longitudinal cortisol change as predictors of anxiety in adolescents. *The Journal of Early Adolescence, 26*(4), 397-413. doi:10.1177/0272431606291943

Stewart, J., & Seeman, T. (2000). Salivary Cortisol Measurement. Retrieved from http://www.macses.ucsf.edu/research/allostatic/salivarycort.php

Stroud, L. R., Foster, E., Papandonatos, G. D., Handwerger, K., Granger, D. A., Kivlighan, K. T., & Niaura, R. (2009). Stress response and the adolescent transition: Performance versus peer rejection stressors. *Development and Psychopathology*, *21*, 47-68. doi:10.1017/S0954579409000042

Tyrka, A. R., Kelly, M. M., Graber, J. A., DeRose, L., Lee, J. K., Warren, M. P., & Brooks-Gunn, J. (2010). Behavioral adjustment in a community sample of boys: Links with basal and stress-induced salivary cortisol concentrations. *Psychoneuroendocrinology, 35*, 1167-1177. doi:10.1016/j.psyneuen.2010.02.002

Tyrka, A. R., Lee, J. K., Graber, J. A., Clement, A. M., Kelly, M. M., DeRose, L., & Brooks-Gunn, J. (2012). Neuroendocrine predictors of emotional and behavioral adjustment in boys: Longitudinal follow-up of a community sample. *Psychoneuroendocrinology, 37*, 2042-2046. doi:10.1016/j.psyneuen.2012.04.004

White-Traut, R. C., Schwertz, D., McFarlin, B., & Kogan, J. (2009). Salivary cortisol and behavioral state responses of healthy newborn infants to tactile-only and multisensory interventions. *Journal of Obstetric, Gynecologic, & Neonatal Nursing: Clinical Scholarship for the Care of Women, Childbearing Families, & Newborns*, *38*, 22-34. doi:10.1111/j.1552-6909.2008.00307.x

Zeiders, K. H., Doane, L. D., & Adam, E. K. (2011). Reciprocal relations between objectively measured sleep patterns and diurnal cortisol rhythms in late adolescence. *Journal of Adolescent Health, 48*, 566-571. doi:10.1016/j.jadohealth.2010.08.012