



# Resting mononuclear cell *NR3C1* and *SKA2* expression levels predict blunted cortisol reactivity to combat training stress among elite army cadets exposed to childhood adversity

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## Abstract

Childhood adversity (CA) may alter reactivity to stress throughout life, increasing risk for psychiatric and medical morbidity, yet long-term correlates of milder CA levels among high functioning healthy adolescents are less studied. The current study examined the prevalence and impact of CA exposure among a cohort of healthy motivated elite parachute unit volunteers, prospectively assessed at rest and at the height of an intensive combat-simulation exposure. We found significantly reduced gene expression levels in resting mononuclear cell nuclear receptor, subfamily 3, member 1 (*NR3C1*), and its transactivator spindle and kinetochore-associated protein 2 (*SKA2*), that predict blunted cortisol reactivity to combat-simulation stress among CA exposed adolescents. Long-term alterations in endocrine immune indices, subjective distress, and executive functions persist among healthy high functioning adolescents following milder CA exposure, and may promote resilience or vulnerability to later real-life combat exposure.

## Introduction

Excessive or recurrent stress during a sensitive childhood time window may result in persisting aberrations in cortisol reactivity [1], with impaired suppression of inflammation and

structural brain changes affecting cognitive and executive functions, suggested to converge to play a role in increased propensity for medical and psychiatric morbidity [2]. Previous literature has predominantly focused upon adverse long-term sequel of CA in later life, documenting increased lifetime risk for cardiovascular morbidity, all cause-mortality [3], and a spectrum of psychopathology [4, 5]. Beyond the predominant focus on persisting health burden that follows severe CA levels [4], recent interest has extended to study the impact of milder CA levels [6], and the potential of CA levels that do not overwhelm coping abilities to induce post-traumatic growth and resilience [7–9]. Mechanisms through which CA may reprogram HPA axis and immune stress reactivity later in life [1], including altered methylation patterns [10] are only partially understood, and long-term studies of prevalent mild to moderate levels of CA among healthy high functioning individuals are needed. A large number of studies reported CA related blunted cortisol reactivity to laboratory stressors such as the Trier Social Stress Test (TSST), in later life, along with compromised coping, and psychopathology [11], although some reported increased CA related cortisol reactivity depending in part on age at adversity exposure [12]. Childhood adversity related alterations in function, expression, and methylation patterns of the nuclear receptor,

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subfamily 3, member 1 (*NR3C1*) gene coding the glucocorticoid receptor (GR), have been previously described in brain and blood [10] and associated with altered cortisol reactivity and neuropsychiatric/behavioral outcomes [13]. The spindle and kinetochore-associated protein 2 (*SKA2*) gene, plays a role in glucocorticoid receptor transactivation [14], and increased *SKA2* blood methylation and reduced gene expression levels have been associated with decreased cortisol stress reactivity, as well PTSD symptom severity [15, 16].

The aim of the current study was to assess the prevalence of CA and prospectively examine its potential long-term correlates on real-life coping among psychiatrically and medically healthy high functioning adolescents volunteering to an elite parachute combat unit. The current study focused on endocrine immune aspects as part of a larger study that further investigated psychometric (Kalla et al. [17]), and brain imaging indices among a smaller subgroup of subjects (Mirman et al. [18]). Subjects reporting elevated CA levels and matched NCA controls were prospectively followed at rest and at the height of a war simulation week with repeated morning salivary cortisol and psychometric assessments, as well as baseline mononuclear cell *NR3C1* and *SKA2* gene expression and methylation levels. In vivo cortisol stress reactivity was complemented by assessing in vitro mononuclear cell culture interleukin-6 (IL-6) inflammatory reactivity to LPS, following hydrocortisone pretreatment to assess mononuclear cell glucocorticoid sensitivity. Behavioral measurements included repeated anxiety, depression, and executive functions assessments. Our findings highlight persisting alterations in endocrine and immune indices, executive functions, and distress levels, following exposure to milder CA levels among healthy motivated high functioning adolescents. Lower mononuclear cell *NR3C1* and *SKA2* gene expression levels among CA exposed subjects at rest, predict blunted cortisol reactivity to combat-simulation stress.

## Materials and methods

### Participants

A cohort of 366 cadets, who had volunteered to undergo a 6-month military training course in the Israel Defense Forces (IDF) elite combat parachute unit, were screened for exposure to childhood adversity using the Childhood Trauma Questionnaire (CTQ) [19]. A prospective sample consisting of extremes case-control subgroups was approached for further prospective follow up. The NCA group consisted of subjects who obtained a score of 27, denoting a zero score of early childhood adversity. The CA group consisted of subjects who obtained CTQ scores

of  $\geq 37$ , (i.e., 10 or more points above the null score denoting mild to moderate adversity exposure was considered an elevated score). Both subgroups underwent comprehensive clinical assessments, including the Structured Clinical Diagnostic Interview (SCID-5) [20] administered by a trained psychiatrist to exclude psychiatric disorders. Exclusion criteria were substance use, medication treatment, current or past psychiatric disorder other than a history of ADHD, and chronic or intercurrent medical illness. All participants gave written informed consent and the study was approved by both the IDF and the Hadassah Medical Center Ethics committees and was conducted in accordance with the declaration of Helsinki.

### Diagnostic and Psychometric Instruments

The Structured Clinical Diagnostic Interview for DSM-5 (SCID-5) [20] was used to exclude major mental disorders. The Childhood Trauma Questionnaire (CTQ) [19] is a 28-item retrospective self-report questionnaire designed to assess 5 types of childhood maltreatment: sexual abuse, physical abuse, physical neglect, emotional abuse, and emotional neglect. The Beck Depression Inventory (BDI) [21] was used to rate depressive symptoms. The Behavior Rating Inventory of Executive Function—Adult Version (BRIEF-A) [22] was used to measure executive functions or self-regulation. The Spielberger State-Trait Anxiety Inventory (STAI) [23] was used to measure transient and enduring anxiety levels, respectively. A comprehensive report of the psychometric indices is in preparation for a separate publication (Kalla et al. [17]), and only a summary of the results in concise form relevant to the discussion of the endocrine immune investigation is presented here.

### Procedure

At the screening phase during the resting period at baseline, all 366 cadets filled out the CTQ. In order to compare baseline and stress reactivity between those with elevated CTQ scores and non-exposed null-scoring subjects, fifty-two CA and fifty-two matched NCA subjects were approached for the prospective follow-up part. Cadets in these extremes subgroups underwent a SCID-5 interview by a trained psychiatrist to exclude any subjects found to be suffering from major psychopathologies, and requested to continue for the prospective assessments that included blood drawn at the pre-training resting period and saliva sampled both at rest and at the height of the combat-simulation training week, taken in the morning within half-hour of awakening, as well as psychometric assessments (BDI, BRIEF-A, and STAI) administered at both time points. The clinical and lab evaluation and

analyses were performed with blinding as to the subject's group assignment.

### Salivary cortisol collection and assay

Free salivary cortisol was collected and assayed as previously described by us [24]. Briefly, saliva was sampled using dedicated tubes stored at 4 °C until centrifugation and long-term storage at −80 °C until assaying with duplicates using ELISA (Salimetrics LLC, Carlsbad CA). Intra and inter-assay variabilities were both below 10%.

### Blood mononuclear cell collection and separation

Venous blood was collected in EDTA, centrifuged, and peripheral blood mononuclear cells (PBMCs) separated using Histopack, and stored at −80 °C until processing as previously described [25].

### Gene Expression

Briefly, one microgram RNA from Monocytes was reverse transcribed using High Capacity cDNA RT kit (Applied Biosystems, Foster City, CA, USA) as per manufacturer instructions. Amplification reactions were performed in 20 µl volume: 10 µl TaqMan PCR master mix (Applied Biosystems), 2 µl cDNA, 1 µl Assays on-Demand™ (Applied Biosystems). The cDNA was quantified using real-time quantitative PCR performed in a GeneAmp 7500 Sequence Detection System (Perkin Elmer, USA), with Sequence Detection System software (Applied Biosystems, Foster City, CA, USA) using specific TaqMan™ primers and probe for the *NR3C1* and *SKA2* transcripts relative to 2 reference genes *TUBB* and *GUSB* with unchanged expression in mononuclear cells.

### Methylation analysis

Peripheral blood methylation levels of CpG sites were quantified by bisulfite pyrosequencing (EpigenDx Inc. Hopkinton, MA). We assayed the methylation status as previously described for the *NR3C1* CpG sites 35 through 38 immediately before exon 1F [13] and *SKA2* cg13989295 [15].

Bisulfite conversion was done using the EZ DNA methylation kit (ZymoResearch, Inc., CA). Pyrosequencing was done on the PSQ96 HS System (Pyrosequencing, Qiagen). Methylation level at each CpG site for each sample was calculated as the percentage of the methylated alleles over the sum of methylated and unmethylated alleles. Mean methylation level was calculated using methylation levels of all measured CpG sites within the targeted region. For

quality control, each experiment includes non-CpG cytosines as an internal control to verify completion of sodium bisulfite DNA conversion. Unmethylated and methylated DNAs were also included as controls in each run. Pyrosequencing assay was run in duplicates, with a high correlation ( $\geq 99.8\%$ ) of two runs for the same sample. Assays were validated by PCR preferential amplification, sensitivity, and reproducibility testing.

### In vitro LPS and hydrocortisone mononuclear cell culture assay

To assess in vitro inflammatory reactivity as well as glucocorticoid sensitivity, IL-6 secretion by mononuclear cell culture in response to bacterial LPS (Sigma Aldrich, USA) was determined followed by measuring the suppression of IL-6 response to LPS by preincubation with hydrocortisone (Sigma-Aldrich, USA) as previously described [26].

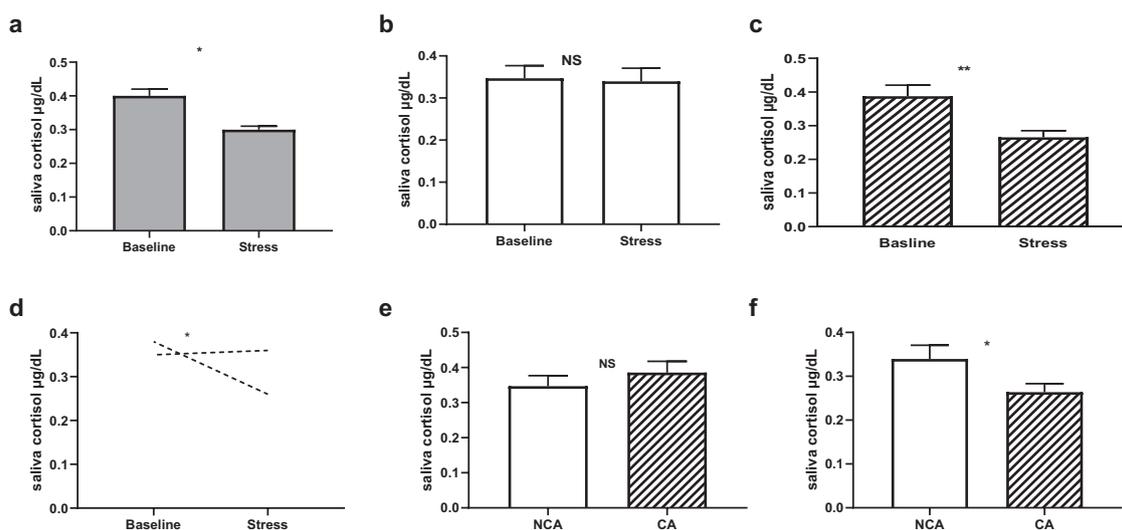
### Data analysis

To investigate the effects of childhood adversity, combat training, and their interaction, we conducted a two-way MANOVA with repeated measures comparing subgroups (CA vs. NCA), by time (baseline rest vs. height of combat training), for biological and psychometric indices, followed by 2-way ANOVA and *t*-tests to explore their effects and interactions. Independent *t*-test samples were used to examine differences between the CA vs. NCA subgroups with dependent *t*-tests applied for longitudinal analyses of findings at the rest and stress time points. Pearson correlations were calculated to examine the relationships between childhood maltreatment and the other variables in the study. Linear regression was applied to examine the ability of *NR3C1* and *SKA2* mRNA levels to predict cortisol dynamics.

## Results

### Childhood adversity scoring and grouping

During the initial ascertainment screening stage of the entire cohort of 366 parachute soldiers (age,  $19.1 \pm 0.1$  years, all male), using the CTQ, the mean CTQ score of all 366 subjects was  $32.11 \pm 7.295$ , with one third ( $n = 118$ ) reporting no early childhood adversity (NCA, CTQ score of 27), and 17% reporting adversity scores of 37 or more, with a mean score of  $44.58 \pm 8.66$ . Consenting cadets from the defined CA and matched cadets from the NCA groups were recruited for further prospective comparisons (CA  $n = 52$ , age  $19.1 \pm 0.7$  years; NCA  $n = 50$ , age  $19.1 \pm 1.1$  years;  $t = 0.85$   $df = 100$ ,  $p = 0.84$ ).



**Fig. 1 Salivary cortisol stress reactivity.** Comparison of free salivary cortisol levels between NCA and CA at rest and stress time points. **a** Cortisol levels decline between baseline rest before training, and stress time point among all soldiers participating in the prospective follow up (NCA and CA combined). **b** Cortisol levels do not change among NCA between resting and stress time points. **c** Cortisol levels significantly decline among CA between resting and stress time points. **d** ANOVA comparing cortisol levels between NCA and CA between

the resting and stress time points demonstrates a significant between groups interaction. **e** Cortisol levels are significantly different between NCA and CA at rest. **f** Cortisol is significantly lower among CA compared with NCA at the stress time point. Childhood Adversity group (CA, striped bar and dotted line); Non-Childhood Adversity group (NCA, white bar and continuous line), Combined CA and NCA groups (gray bar).

### Salivary free cortisol

Ninety-eight soldiers ascertained from the CA extremes completed the prospective follow-up with biological sampling at rest and at the height of the combat-simulation week (49 NCA and 49 CA). Free salivary cortisol levels showed a significant decline at the culmination of the war simulation week compared with baseline rest, among all soldiers combined NCA + CA ( $n = 98$ ) baseline cortisol  $0.37 \pm 0.2 \mu\text{g/dL}$ , stress cortisol  $0.3 \pm 0.1 \mu\text{g/dL}$ ,  $t = 2.4$ ,  $df = 97$ ,  $p = 0.019$ ; (Fig. 1a). However, separating the groups demonstrates that the NCA group does not show a significant difference between rest and stress conditions, NCA cortisol at baseline ( $n = 49$ )  $0.35 \pm 0.2 \mu\text{g/dL}$ , NCA cortisol at stress ( $n = 49$ )  $0.34 \pm 0.2 \mu\text{g/dL}$ ,  $t = 0.17$ ,  $df = 48$ ,  $p = 0.88$  (Fig. 1b), whereas only the CA group showed a significant decline between baseline and stress, CA cortisol at baseline ( $n = 49$ )  $0.39 \pm 0.2$ , CA cortisol at stress ( $n = 49$ )  $0.26 \pm 0.1$ ,  $t = 3.3$ ,  $df = 48$ ,  $p = 0.001$ ; (Fig. 1c) which appears to have contributed to the decline among the combined sample. Indeed, Analysis of variance demonstrated a significant main effect ( $F_{(1,96)} = 5.9$ ,  $p = 0.017$ ) with a significant between group interaction ( $F_{(1,96)} = 4.3$ ,  $p = 0.04$ ) in cortisol levels change from rest to the stress condition (Fig. 1d). Follow up analyses showed that while there was no significant difference in cortisol levels between CA and NCA at rest, NCA(49) baseline cortisol  $0.35 \pm 0.2 \mu\text{g/dL}$ , CA(49) baseline cortisol  $0.39 \pm 0.2 \mu\text{g/dL}$ ,

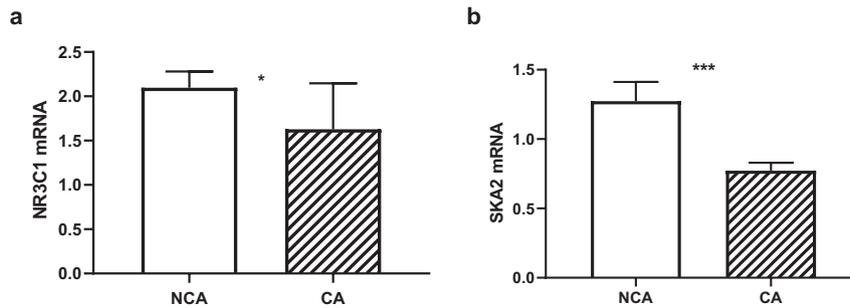
$t = -0.9$ ,  $df = 96$ ,  $p = 0.36$  (Fig. 1e), the CA group demonstrated significantly lower cortisol levels relative to NCA at the height of the combat-simulation week NCA ( $n = 49$ )  $0.34 \pm 0.2 \mu\text{g/dL}$ , CA( $n = 49$ )  $0.26 \pm 0.1 \mu\text{g/dL}$ ,  $t = -2.1$ ,  $df = 96$ ,  $p = 0.04$ ; (Fig. 1f).

### Mononuclear cell *NR3C1* and *SKA2* gene expression levels

We further examined blood mononuclear cell *NR3C1* mRNA levels, among 43 soldiers for whom PBMCs were available at baseline. We found PBMC *NR3C1* mRNA levels to be significantly reduced at the baseline resting condition among the CA as compared with NCA group NCA( $n = 21$ )  $2 \pm 0.8$ , CA( $n = 22$ )  $1.6 \pm 0.5$ ,  $t = 2.1$ ,  $df = 41$ ,  $p = 0.036$ ; (Fig. 2a). We further examined the *SKA2* gene mRNA and found a significantly lower level among CA compared with NCA at the baseline resting condition NCA( $n = 21$ )  $1.3 \pm 0.6$ , CA( $n = 22$ )  $0.7 \pm 0.2$ ,  $t = 3.3$ ,  $df = 41$ ,  $p = 0.003$  (Fig. 2b).

### Resting mononuclear *NR3C1* and *SKA2* gene expression levels predict cortisol dynamics

Gene expression levels of both genes among mononuclear cells sampled at baseline rest predicted later combat stress-induced cortisol decline between the rest and combat stress time points among the CA group. The model explained 40% of the variance in cortisol decline



**Fig. 2 Resting PBMC *NR3C1* and *SKA2* gene expression levels.** PBMC *NR3C1* (a) and *SKA2* (b) gene expression levels are lower at baseline among CA compared with NCA group. Peripheral blood mononuclear cell (PBMC); nuclear receptor subfamily 3, group C,

member 1 (*NR3C1*); *SKA2*- Spindle and kinetochore-associated protein 2(*SKA2*); childhood adversity (CA, striped bar) and non-childhood adversity (NCA, white bar) groups.

( $R^2 = 0.40$ ;  $F_{(3,20)} = 3.7$ ,  $p = 0.032$ ); *NR3C1* ( $\beta = -0.66$ ,  $p = 0.007$ ) and *SKA2* gene ( $\beta = 0.46$ ,  $p = 0.034$ ).

### Mononuclear cell *NR3C1* and *SKA2* site-specific methylation analyses

Mononuclear cell *SKA2* and *NR3C1* blood methylation levels at the sites examined were not significantly different between NCA and CA. Methylated percent *SKA2* cg13989295 methylation site (NCA group ( $n = 32$ ) and CA group ( $n = 28$ ) was  $96.5\% \pm 1.4\%$  and  $96.8\% \pm 1.5\%$ , respectively ( $t = -0.9$ ,  $df = 58$ ,  $p = .34$ ). For the *NR3C1* 1F site the methylated percent in NCA group ( $n = 33$ ) and CA group ( $n = 28$ ) was  $1.1\% \pm 0.9\%$  and  $0.9\% \pm 0.7\%$ , respectively ( $t = 1.1$ ,  $df = 58$ ,  $p = 0.26$ ).

### Mononuclear cell in vitro reactivity to LPS and hydrocortisone

We next examined the in vitro inflammatory reactivity of mononuclear cell culture by measuring the level of IL-6 secreted in response to LPS challenge, and cellular glucocorticoid sensitivity was assessed by measuring the suppression of the pro-inflammatory response following preincubation with hydrocortisone. Repeated measures ANOVA demonstrated a significant change from IL-6 levels at rest, following LPS challenge, and following preincubation with hydrocortisone in the combined sample ( $F_{(2,32)} = 3.85$ ,  $p = 0.032$ ). Paired  $t$ -test confirmed baseline IL-6 secretion ( $1.66 \pm 3.3$  pg/ml) was significantly elevated following LPS to ( $5.4 \pm 8.3$  pg/ml) ( $t = -2.49$ ,  $df = 17$ ,  $p = 0.023$ ), and this elevation was significantly suppressed following preincubation with hydrocortisone ( $2.8 \pm 4.5$  pg/ml) ( $t = -1.57$ ,  $df = 17$ ,  $p = 0.013$ ) in the combined sample. No significant differences were found comparing NCA with CA group, which in part may be due to the large standard deviation of the results and the smaller number of viable cell lines left for this experiment.

### Biological and behavioral measures

Under baseline resting conditions, compared to participants in the NCA subgroup, those with a history of CA had significantly higher state and trait anxiety and depression scores and reported more executive functions impairment on the BRIEF-A and all of its subsets. At the height of combat training, the CA group only showed an increase in state anxiety, whereas the NCA group significantly increased on all behavioral indices other than trait anxiety. Thus under stress, differences in state anxiety and executive function scores were no longer significant between groups (Kalla et al. [17]). The two groups showed significant separation on both the biological and the behavioral measures, and their combination. A MANOVA comprising resting salivary cortisol and mononuclear *NR3C1* and *SKA2* gene expression at rest showed a significant difference between the CA and NCA groups ( $F_{(3,38)} = 4.07$ ,  $p = 0.013$ ). Similarly a MANOVA comprising all psychological and cognitive variables over time showed a significant difference between groups ( $F_{(5,80)} = 5.75$ ,  $p < 0.001$ ), a significant effect of combat training ( $F_{(5,80)} = 20.68$ ,  $p < 0.001$ ) and a significant interaction ( $F_{(5,80)} = 2.47$ ,  $p = 0.039$ ). A MANOVA comprising resting biological and psychological variables increased the significance of the difference between groups ( $F_{(6,78)} = 5.07$ ,  $p < 0.001$ ), and a MANOVA considering psychological variables and cortisol available for both time points showed again a more significant difference between the CA and NCA groups ( $F_{(6,78)} = 17.48$ ,  $p < 0.001$ ), a significant effect of combat training ( $F_{(6,78)} = 17.48$ ,  $p < 0.001$ ) and a significant interaction ( $F_{(6,78)} = 2.55$ ,  $p = 0.026$ ).

### Discussion

Our results demonstrate CA related lowering of mononuclear *NR3C1* and *SKA2* gene expression predict curtailment of

cortisol stress reactivity persisting into young adulthood among healthy high functioning cadets. Psychometric evaluation revealed CA further associates with curtailed stress-induced exacerbation of distress and executive function impairment despite elevated depression anxiety and executive dysfunction at rest, detailed in Kalla et al. [17]. Notwithstanding large variability in the literature, a majority of studies found CA associates with blunted cortisol responses to stress incurred later in life, and less consistently also lower basal cortisol levels [27]. A meta-analysis of 4292 subjects assessed at different ages from adolescence to adulthood using the TSST supports CA-associated blunted cortisol response to social stress, suggesting long-term negative effects of early-life adversity increase with age [11]. In a cohort study of 860 children exposed to early adversity or prenatal substance exposure, decreased cortisol reactivity at 11 years of age was associated with maladaptive psychological outcomes [28]. Healthy adults exposed to CA show blunted basal diurnal cortisol slope [29], as well as blunted cortisol reactivity [30], with blunted stress reactivity predicting suicidality later in life [31, 32]. Results appear more consistent for adolescents and for males, with several studies showing inconsistent results, suggesting the effect of age [33] and gender [34] may add to outcome variability. Both *SKA2* and *NR3C1* transcripts were significantly lower in mononuclear cells at baseline resting conditions among the CA group in our sample, and while uncorrelated with salivary free cortisol at baseline, these levels predicted part of the variance of later free salivary cortisol response to stress among CA exposed subjects. Peripherally secreted pro-inflammatory cytokines such as IL-1beta increase cortisol secretion acting at the hypothalamus, pituitary, and adrenal levels [35], and altered glucocorticoid sensitivity of immune cells may thus result in altered cytokine response, altering immune feedback on stress-induced cortisol secretion. Our results are in accord with previous reports showing *SKA2* blood gene expression levels correlate with salivary cortisol suppression [15], consistent with its implicated role in glucocorticoid receptor transactivation, demonstrating *SKA2* knockdown prevents dexamethasone inhibition of cell proliferation [14]. Increased *SKA2* blood methylation has been associated with reduced *SKA2* transcript expression and decreased cortisol stress reactivity as well as suicidality and PTSD symptom severity [15, 16]. We did not find significant *NR3C1* or *SKA2* mononuclear cell methylation differences between CA and NCA in our cohort, nor a significant correlation with the respective gene expression levels in mononuclear cells, salivary cortisol reactivity, or other indices in the combined cohort. The low inter-individual variability in methylation levels in the *NR3C1* 1F and *SKA2* sites examined in our sample as in previous work [10] casts doubt as to the functional impact of these methylation sites in PBMCs for the majority of subjects. A larger sample is

required to assess the impact of CA on in vitro reactivity measures. Our study is unique in that it focused on the long-term correlates of milder forms of childhood adversity by prospectively following a psychiatrically screened, non-clinical, healthy cohort of adolescents through a real-life stressor. It cannot be surmised whether the CA-associated persisting endocrine and immune alterations observed here may impart susceptibility or resilience to later psychopathological reactions. A large body of research supports childhood adversity as a proximal risk factor for maladaptive stress reactivity, PTSD [36], and depression [37] in later life. Some previous research suggests, however, that early adversity may alternatively promote post-traumatic growth and resilience to stress incurred later in life [9]. The CA group's dampened cortisol recruitment at the face of the current manageable stress, could denote desensitized psychological interpretation of the intensive training situation as stressful enough to trigger a higher cortisol reaction, or a resetting of the HPA-reactivity set point. Although either could be taken to support the match/mismatch and stress inoculation theory proposing that manageable CA levels program the individual to actively seek in later life similar ("matching") adversity with which he is more adept to cope [1, 38], the cumulative evidence that blunted cortisol reactivity to subsequent stress may be detrimental [39], as well as the persistently elevated resting levels of distress and executive impairment in our sample (Kalla et al. [17]), may be taken to support the predominant view that repeated stressors incrementally escalate allostatic load associated wear and tear of physiological systems, thus progressively increasing risk for detrimental health consequences [40]. The CA-associated blunting of cortisol reactivity seen here among high functioning healthy adolescents can be seen to be generally in line with previous evidence for a timing effect in maladaptive HPA programming by early adversity among both humans and animal models whereby persisting hypoactive cortisol reactivity may follow adversity exposure in childhood, and may increase risk to later PTSD [1], whereas a persisting hyperactive cortisol response to subsequent stress may follow adverse perinatal exposure, increasing susceptibility for later depression [1]. In chronic PTSD, elevated corticotrophin-releasing hormone (CRH) secretion despite lower cortisol levels has been reported, with higher pituitary GR sensitivity resulting in greater cortisol suppression following low dose dexamethasone, as opposed to MDD, where higher cortisol and CRH and reduced GR reactivity are reported [41]. As lower cortisol reactivity has been linked to compromised regulation of sympathetic and inflammatory activity and maladaptive cognitive sequel of trauma [39], hydrocortisone has been tried as an acute preventive intervention immediately post trauma to reduce PTSD risk [42]. Previous prospective studies suggest that low Cortisol levels at the time of exposure to psychological

trauma may predict the development of PTSD, and that hypocortisolism might therefore constitute a risk factor for maladaptive stress reactivity predisposing to future PTSD [43, 44]. Taken together with previous evidence suggesting CA-associated blunted cortisol response to stress, may precede the blunted HPA activity reported in PTSD [45], our finding of a diminished CA related cortisol response to combat training may be taken to indicate a less stable CA-associated allostatic equilibrium, that marks an increased risk for incurring PTSD, should overwhelming stress be encountered later in life. Importantly, the pertinent question remains as to whether there is a differential ceiling effect to the adaptive ability of NCA vs. CA exposed subjects, when faced with overwhelming real-life stress such as real combat exposure. Further follow-up of larger cohorts is required to address whether the unique biological and behavioral profile among the CA subgroup observed at rest and under manageable life stress, can be used to predict later risk for PTSD upon exposure to real combat and other traumatic events.

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## Compliance with ethical standards

**Conflict of interest** The authors declare no competing interest.

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