Systematic review and meta-analysis of the effect of adverse childhood experiences (ACEs) on brain-derived neurotrophic factor (BDNF) levels

ABSTRACT

There is continued interest in identifying dysregulated biomarkers that mediate associations between adverse childhood experiences (ACEs) and negative long-term health outcomes. However, little is known regarding how ACE exposure modulates neural biomarkers to influence poorer health outcomes in ACE-exposed children. To address this, we performed a systematic review and meta-analysis of the impact of ACE exposure on Brain Derived Neurotrophic Factor (BDNF) levels - a neural biomarker involved in childhood and adult neurogenesis and long-term memory formation. Twenty-two studies were selected for inclusion within the systematic review, ten of which were included in meta-analysis. Most included studies retrospectively assessed impacts of childhood maltreatment in clinical populations. Sample size, BDNF protein levels in ACE-exposed and unexposed subjects, and standard deviations were extracted from ten publications to estimate the BDNF ratio of means (ROM) across exposure categories. Overall, no significant difference was found in BDNF protein levels between ACE-exposed and unexposed groups (ROM: 1.08; 95% CI: 0.93-1.26). Age at sampling, analyte type (*e.g.*, sera, plasma, blood), and categories of ACE exposure contributed to high between-study heterogeneity, some of which was minimized in subset-based analyses. These results support continued investigation into the impact of ACE exposure on neural biomarkers and highlight the potential importance of analyte type and timing of sample collection on study results.

1. INTRODUCTION

The term adverse childhood experiences (ACEs) refers to potentially traumatic events occurring during childhood (*e.g.*, physical abuse, emotional abuse, sexual abuse) (Felitti et al., 1998). In 2019, a study by the Centers for Disease Control and Prevention (CDC) reported 61% of individuals within the United States had been exposed to at least one traumatic incident during childhood (Merrick et al., 2019). Exposure to ACEs has been found to confer enduring negative impacts on the physical and mental health of ACE exposed children (Hughes et al., 2017; Kalmakis & Chandler, 2015). Due to the prevalence of ACE exposure and its impact on a diverse set of physiological systems, a field of research has emerged that focuses on identifying quantifiable biomarkers of ACE exposures. Such biomarkers could elucidate the biological pathways through which ACEs negatively impact health and could be further leveraged to investigate mechanisms for enhancing physical and psychological resilience. Prior research has focused primarily on dysregulation of the endocrine system and the hypothalamic-pituitary-adrenal (HPA) axis, primarily by investigating the effect of ACEs on cortisol levels (K. S. Dempster, O'Leary, MacNeil, Hodges, & Wade, 2021) and inflammatory biomarkers (Baumeister, Akhtar, Ciufolini, Pariante, & Mondelli, 2016; Kuhlman, Horn, Chiang, & Bower, 2020). Substantially less is known regarding the effect of ACEs on the developing nervous system, despite suggestive evidence of ACE-driven, detrimental impacts on the long-term mental health of ACE exposed children during adulthood (Schauss et al., 2019).

ACEs are a major contributing factor to early mortality, and exposure to even a single ACE has been found to increase risk of chronic diseases, such as heart, lung, and liver disease, in addition to cancer, type 2 diabetes, obesity, and psychiatric disorders (Deighton, Neville, Pusch, & Dobson, 2018; Godoy et al., 2021; Grummitt et al., 2021), but how ACE exposure directly perturbs diverse physiological systems remains poorly defined (Deighton et al., 2018; Kalmakis & Chandler, 2015; Petruccelli, Davis, & Berman, 2019). ACE-associated increases in inflammatory markers, including C-reactive protein (CRP), Tumor Necrosis Factor- α (TNF- α), and Interleukin-6 (IL-6), have been observed to partially mediate subsequent development of obesity and other chronic conditions (Kylie S Dempster, O'Leary, MacNeil,

Hodges, & Wade, 2020). ACE-mediated alterations in the HPA axis have also been associated with dysregulated cortisol levels in children, which can subsequently affect biomarkers and functioning of other biological systems (Deighton et al., 2018). Increased exposure to ACEs is associated with detrimental impacts on cardiometabolic health, such as increases in blood pressure, body mass index (BMI), waist circumference, and triglyceride levels, all of which elevate cardiovascular disease risk (Godoy et al., 2021; Pretty, D O'Leary, Cairney, & Wade, 2013). Thus, it is evident that exposure to ACEs affects physical health in a manner that spans several physiological systems.

Beyond the multifaceted effects of ACEs on one's physical health, ACE exposure also has strong associations with psychiatric diagnoses later in life, such as depression, PTSD, anxiety, and substance abuse disorders (Deighton et al., 2018; Kalmakis & Chandler, 2015). Alterations in neural development due to ACE exposure may underlie this link, but the mechanisms mediating such a relationship remain poorly defined (Schauss et al., 2019; Wilson & Perez Vallejos, 2021). Childhood, especially early childhood, is a time of extensive neural development, as children begin to accumulate memories and strengthen the neuronal connections that underlie memory formation (Buss et al., 2007). Exposure to ACEs at this stage of life may impair proper memory consolidation and increase a child's likelihood for developing phobias, anxiety disorders, or other trauma-related disorders (Schauss et al., 2019). Additionally, childhood is also a time of extensive brain growth, and traumatic experiences during childhood have been observed to impair growth of specific brain regions. For instance, Navalta, McGee, and Underwood (2018) found that childhood exposure to traumatic experiences was associated with decreased volumes of the hippocampus, amygdala, medial prefrontal cortex, and limbic structures in adulthood. Because these structures are each essential for regulation of emotional arousal, developmental perturbation of any of these structures could increase the likelihood of developing anxiety disorders or mood dysregulation (Navalta et al., 2018). Thus, the potential impact of ACEs on the structure of the nervous system may explain an increased propensity toward mood disorders.

One biomarker hypothesized to mediate ACE-driven impacts on nervous system function is brain derived neurotrophic factor (BDNF). A member of the neurotrophin family of growth factor proteins,

BDNF binds to Tropomyosin receptor kinase B (TrkB) receptors on neurons of the central and peripheral nervous systems where it supports neuronal survival and encourages neurogenesis (Binder & Scharfman, 2004). BDNF has predominantly been treated as a biomarker of synaptic plasticity (Benedetti et al., 2017; Kim, Watt, Ceballos, & Sharma, 2019), and due to its secretion by activated lymphocytes and the ability of IL-6 and TNF- α to augment BDNF release from the nervous system (Jin, Sun, Yang, Cui, & Xu, 2019; Kraneveld et al., 2014), BDNF activity resides at the intersection of neural development, memory formation, and neuroimmunology (Bekinschtein, Cammarota, Izquierdo, & Medina, 2007; Calabrese et al., 2014; Kim et al., 2019). In addition to BDNF's modulation of the immune axis, it has also been implicated in instigating dysregulation of the HPA axis, with elevated BDNF levels contributing to hyperactivity of the HPA axis – a common molecular change seen in ACE-exposed children (K. S. Dempster et al., 2021; Naert, Ixart, Maurice, Tapia-Arancibia, & Givalois, 2011). Furthermore, because BDNF can be non-invasively measured in saliva and plasma, it is frequently investigated as a potential biomarker of ACE exposure – one that is hypothesized to mediate the contributions of ACEs to nervous system dysfunction and the development of neuropsychiatric illnesses. BDNF levels have been implicated in the development of mood disorders which have been associated with ACE exposure, such as bipolar disorder, depression, and schizophrenia (Bocchio-Chiavetto et al., 2010; Gaglia, 2021; Kauer-Sant'Anna et al., 2007).

While BDNF has been frequently investigated, findings have predominantly explored the impact of ACE exposure on methylation of the *BDNF* gene promoter regions, and subsequent effects on the expression of the BDNF gene, both in blood and in neural tissues (Jiang, Postovit, Cattaneo, Binder, & Aitchison, 2019; Neves, Dinis-Oliveira, & Magalhães, 2019; Pilkay, Combs-Orme, Tylavsky, Bush, & Smith, 2020). However, findings have remained largely inconclusive due to the key drawback of using methylation-based studies of BDNF, as DNA methylation varies in association with age, sex, and ethnicity of subjects, as well as the tissue type from which DNA is extracted (Tsai, 2018). While methylation-based studies can help reveal the functional genetic mechanisms underlying variability in BDNF gene expression, directly measuring BDNF protein level and its association with prior ACE

exposure can substantially simplify interpretation of results, particularly when BDNF protein is measured from a single, consistent type of biospecimen.

Thus, we sought to clarify the relationship regarding the impact of ACE exposure on BDNF levels via specifically analyzing protein levels, which can be more reliably quantified and thus will enable generalizability of results. We conducted a systematic literature review and meta-analysis to identify and integrate data from all published studies investigating the impact of ACE exposure on BDNF protein levels. We further performed diagnostics to evaluate the quality and consistency of the published literature, and subgroup analyses to explore potential heterogeneity due to differences in study designs and the specific types of ACEs evaluated. By rigorously amalgamating data across studies, our results can help to clarify the relationship between ACE exposure and BDNF levels, reveal current knowledge gaps, and inform future mechanistic research into how ACEs may impact long-term neurological health and disease.

2. MATERIALS AND METHODS

2.1 Definition of ACEs

ACEs were first defined in the 1998 CDC-Kaiser Study (Felitti et al., 1998), which delineated specific traumatic exposures during childhood that were found to have long-term, detrimental impacts on the child's physiological and mental health. We employed this definition of ACEs, including ten distinct exposures: physical abuse, emotional abuse, sexual abuse, physical and emotional neglect (including extreme poverty/deprivation), presence of parent with mental illness, incarcerated family member, exposure to domestic abuse or intimate partner violence, presence of parent with history of substance abuse, and family separation (parental death, foster care, or parental divorce).

2.2 Search Strategy

A medical librarian (SK) with expertise in the design and conduct of systematic literature reviews assisted with development of a search strategy that utilized a mix of keywords and subject headings representing

the concepts of ACEs (*e.g.*, child abuse, domestic violence, family separation, poverty), biomarkers (*e.g.*, endocrine, immune, cardiovascular, and nervous), stress reaction, and children. The search underwent internal peer-review by another medical librarian with similar expertise. A full accounting of search terms and strategies appears in **Supplementary Table 1**. The search study was applied to four databases: MEDLINE via Ovid Wolters Kluwer, Embase via Elsevier, Scopus via Elsevier, and APA PsycINFO via EBSCO and was conducted on April 27, 2020. When possible, conference abstracts and non-human studies were removed. Results were compiled into EndNote, imported into Covidence for screening, and duplicate studies were removed.

2.3 Study Screening and Selection

Title and abstract screening were completed by all authors. Abstract screening was conducted by a minimum of two authors, of which one author must have been a senior team lead (EG, KW, LR, and JW). Criteria for inclusion of abstracts required mention of biomarkers, ACEs, or exposure to childhood trauma within the sample. Screening discordances were resolved through discussion with the full team. Full-text manuscripts of selected abstracts were then uploaded to REDCap for full-text screening. A sample REDCap screening tool can be found in **Supplementary Figure 1**.

Selected studies were required to 1) report both ACE exposure and measurement of relevant biomarkers, and 2) quantify the impact of ACEs on selected biomarker(s). Studies were excluded if they: 1) were conducted in animal models, 2) were not original research (reviews, editorials, or commentaries), 3) were reports of single patients (*i.e.*, case-reports), and 4) were unavailable in English. Following identification of all studies that passed the full-text inclusion/exclusion criteria, all studies that were annotated as quantifying neurotransmitter levels were individually evaluated to retain only those studies which specifically measured BDNF protein levels. The citations list of all included studies was also screened using the original inclusion/exclusion criteria to identify any additional relevant studies not captured by the search strategy. For studies that quantified BDNF protein levels but did not report specific

numeric levels in the manuscript, we contacted study authors in order to obtain these data (*n=13*). If no response was received, the study was excluded from the meta-analysis (*n=11*).

2.4 Data Extraction + Synthesis

All publications retained, following full-text screening, were uploaded into REDCap for preliminary data extraction. Data extracted from publications included: sample description, sample size, specific ACE exposure and time of exposure, specific biomarker and time of measurement, study method, and summary of findings. Exclusion reason was also annotated for full-text publications that were reviewed but did not meet inclusion criteria. Following extraction of these preliminary data, all studies with mention of neurotransmitters were isolated and studies analyzing BDNF protein levels were selected for inclusion in the systematic review. All studies measuring the relationship between ACEs and BDNF levels were reviewed, and the following data were extracted: sample size and study population, type of ACE measured and timing of measurement (*e.g.*, during childhood, retrospectively in adulthood), BDNF measurement methodology and timing, and general impact of ACE exposures on BDNF levels.

From these studies, a small subset of studies was isolated for inclusion in the meta-analysis, all of which explicitly quantified mean BDNF protein levels and standard deviations in an ACE-exposed sample and a comparator population. Data extracted from studies included within the meta-analysis were: ACE exposure(s), BDNF measurement methodology, sample characteristics, mean BDNF levels (in ACE-exposed and unexposed), and standard deviation of BDNF measures (in ACE-exposed and unexposed). Mean BDNF levels and standard deviations were used to calculate the ratio of means (ROM) for ACE-exposed versus ACE-unexposed subjects, including 95% confidence intervals. Fixed and random-effects meta-analysis of the ROM across studies was conducted in R-Studio using the meta, metafor, and dmetar packages to calculate a summary ROM with its 95% confidence interval, study weight (based on sample size), between-study heterogeneity via the I^2 statistic, to assess the presence of publication bias using the Egger's regression asymmetry test, and to generate forest plots. Mixed-effects

meta-analysis was also performed in R-Studio to explore heterogeneity in the summary-level ROM across strata of study design.

3. RESULTS

3.1 Search Strategy Results

Following application of the search strategy to databases, 11,845 abstracts were uploaded onto COVIDENCE. 3,710 of these entries were excluded as they were duplicates, and an additional 7,009 records were excluded following abstract and title review. 1,126 articles were selected for full-text screening, of which 145 were excluded due to lack of adherence with inclusion criteria. Of the 981 studies**,** thirty-nine full-text articles were recorded as measuring neurotransmitters as a biomarker of ACE exposure and were rigorously evaluated for potential inclusion in our meta-analyses. Twenty studies were excluded because they did not measure BDNF protein levels (9 measured different neurotransmitters, 9 measured DNA methylation levels at the *BDNF* gene locus, and 2 evaluated BDNF as a mediator without reporting the direct effect of ACE exposure on BDNF levels). Three additional studies were screened in via referencing the citations of included studies. Twenty-two studies were thus included in our systematic review, of which ten quantified and reported mean BDNF levels and standard deviations in ACE-exposed and unexposed subjects (or study authors provided these data via personal communication) and were included in the meta-analysis. A detailed overview of the flow of studies through the screening process is presented in a PRISMA diagram (**Figure 1**).

3.2 Key Findings from Systematic Literature Review

Of the twenty-two studies included within the systematic review, eighteen studies explored the impact of ACEs in clinical populations, and four studies had a sample representative of the general population. The majority of studies (*n=17*) investigated one or more forms of childhood maltreatment as the ACE exposure. Specifically, sexual abuse (n=*16*), physical abuse (*n=13*), neglect (*n=12*), and emotional abuse (*n=11*) were all evaluated within this subset of studies. Other ACE exposures measured include: parental

mental health (*n=3)*, parental substance abuse (*n=2)*, parental incarceration (*n=1*), parental death (*n=2*), intimate partner violence (*n=1*), socioeconomic disadvantage (*n=1),* and overall childhood trauma (*n=1*). ACE exposure was predominantly measured retrospectively in adulthood (*n=14*) rather than during childhood while exposure was ongoing (*n=8*).

BDNF protein levels were primarily measured from serum (*n= 16)*, plasma (*n= 5)*, and whole blood (*n=1)*. Levels were measured predominantly with an ELISA assay (*n=21*); one study utilized a Luminex assay, but was not included in the meta-analysis due to omission of necessary data elements. BDNF measurements were conducted both retrospectively in adulthood (*n=14)* and during childhood while the ACE was ongoing (*n=8)*. Qualitatively, eight studies reported elevated BDNF level in the ACE-exposed group versus the ACE-unexposed group, while eleven studies reported lower BDNF level in the ACE-exposed group, and three studies reported no detectable difference in BDNF levels between exposure groups. Of the four studies measuring ACE exposure and BDNF levels in a representative sample drawn from the general population, two studies reported higher BDNF level in the ACE-exposed group, and two studies reported lower BDNF level in the ACE-exposed group. For studies investigating clinical populations, six studies reported higher BDNF level in the ACE-exposed group, nine studies reported lower BDNF level in the ACE-exposed group, and three studies found no detectable difference in BDNF levels across exposure groups. Detailed study characteristics appear in **Supplementary Table 2**.

3.3 Key Findings from Meta-Analyses

Within the subset of ten studies included in the meta-analysis, six studies investigated the impact of ACEs on BDNF levels within clinical patients and four studies investigated subjects recruited from the general population. Childhood maltreatment was the most common ACE exposure (*n=7)* reported by studies. Within these studies, exposure to sexual abuse (*n=7)*, physical abuse (*n=6)*, emotional abuse (*n=5*), and neglect (*n=5)* were specifically reported. ACE exposure was predominantly measured retrospectively in adulthood (*n=6).* BDNF levels were most commonly measured in serum (*n=6)*, plasma (*n=3)*, and whole

blood (*n=1)*, and all these studies used an ELISA assay. Five studies observed lower BDNF level in the ACE-exposed group versus the unexposed group, and five studies observed elevated BDNF level in the ACE-exposed group. Among studies looking at the general population, two studies reported lower BDNF level in the ACE-exposed group, and two studies reported higher BDNF level in the ACE-exposed group. Three studies with clinic-based participants reported lower BDNF level in the ACE-exposed group, and three studies reported higher BDNF level in the ACE-exposed group. Detailed characteristics of studies included in the meta-analysis appear in **Table 1**.

Aggregate analysis was performed on ten studies from which mean BDNF levels and standard deviations for both the ACE-exposed and an unexposed comparator group could be ascertained. A random-effects meta-analysis of the ROM in ACE-exposed versus unexposed subjects was not significantly different from 1.0 (ROM = 1.08; 95% CI: 0.93-1.26) (**Figure 2**). While the meta-analytic ROM estimate was not significant, four studies reported significant differences in BDNF protein levels between the ACE-exposed and unexposed groups. Do Prado et al. reported significantly lower BDNF levels in the exposed group than the unexposed group (ROM = 0.57, 95% CI: 0.43-0.78). Hauck et al. (ROM = 1.56, 95% CI: 1.17-2.09), Bortoluzzi et al. (ROM = 1.43, 95% CI: 1.11-1.83), and Trajkovska et al. (ROM: 1.15, 95% CI: (1.01, 1.31) reported significantly higher BDNF levels in the exposed group than the unexposed group. High between-study heterogeneity was observed ($I^2 = 0.76$), and Cochran's Q value also revealed statistically significant heterogeneity ($p<0.01$). Egger's regression test indicated that no single study unduly influenced the results of the meta-analysis ($p=0.41$). Additionally, use of the Duval and Tweedie's trim-and-fill procedure revealed that removal of possible outlier studies did not substantially impact results of the meta-analysis (**Supplementary Figure 2).** Taken together, these diagnostic procedures indicate that no single study was driving results, that publication biases were minimal, and that all ten studies should be included within the meta-analysis despite substantial between-study heterogeneity.

Studies were further analyzed by investigating the following sub-groups of interest: timing of BDNF measurement (childhood versus adulthood), childhood maltreatment as a specific subtype of ACE,

and analyte in which BDNF was measured. To explore the impact of BDNF measurement timing, studies were grouped into two groups: BDNF measurement before the age of 20 and after the age of 20. Twenty years was identified as a cut-off based on 1) the specific demographics of samples from the included studies and 2) an interest in understanding the relationship of biomarker measurement during childhood and adolescence vs adulthood. In the subset of studies (*n=4*) measuring BDNF levels in subjects under the age of 20, BDNF levels were not significantly different in ACE-exposed versus ACE-unexposed individuals (ROM: 1.08, 95% CI: 0.61, 1.91) (**Figure 3**). Non-significant differences between the ACE-exposed and unexposed groups were also observed in the subset of studies (*n=6*) in which BDNF levels were measured after age 20 (ROM: 1.10, 95% CI: 0.97, 1.25) (**Figure 3**). No substantial heterogeneity in ROM estimates across the two age strata was observed in mixed-effects analysis (p=0.95).

Limiting meta-analysis to the subgroup of studies specifically exploring childhood maltreatment as the ACE exposure (*n=5*), BDNF levels were non-significantly elevated in the ACE-exposed group in the random-effects models (ROM: 1.06; 95% CI: 0.71, 1.58) (**Figure 4**). Between-study heterogeneity was particularly high in this subset of studies (I^2 = 83%). In addition to type of ACE and timing of measurements, the specific analyte assayed may also influence meta-analysis findings. Within the subset of studies quantifying serum levels of BDNF (*n=6*), BDNF levels were significantly elevated in the ACE-exposed subjects in a random-effects model (ROM 1.19, 95% CI: 1.03, 1.38) (**Figure 5**). Subset analysis of other analytes, such as plasma or blood, could not be performed due to the limited number of studies, and further investigation into tissue-specific alterations in BDNF signaling is needed to increase standardization and facilitate cross-study comparisons.

4. DISCUSSION

This is the first systematic review and meta-analysis exploring the impact of ACE exposure on protein levels of BDNF, a neural biomarker involved in childhood and adult neurogenesis and long-term memory formation. Results from the systematic review of twenty-two published studies indicate substantial heterogeneity in results, which may be related to differences in study design. Our systematic review

revealed that studies in the field are largely restricted to clinical populations, primarily adults with various psychopathologies. The predominant study methodology remains measuring ACE exposure retrospectively during adulthood and assaying BDNF levels in these adult subjects, whereas a relative paucity of studies measured BDNF levels cross-sectionally during childhood and none have investigated this biomarker longitudinally. Studies also varied substantially in how ACE-exposed and ACE-unexposed participants were defined, which further contributes to a lack of consensus on the value of BDNF as a potential biomarker of ACE exposure. Findings from our random-effects meta-analysis of ten studies identified no statistically significant differences in BDNF protein levels between ACE-exposed and ACE-unexposed groups, although BDNF levels were generally elevated in ACE-exposed subjects in studies specifically investigating childhood maltreatment as the ACE exposure, and the meta-analysis observed higher BDNF levels in ACE-exposed individuals when limited to studies measuring sera. Meta-analysis revealed substantial between-study heterogeneity, which likely contributed to the inconsistency of results across studies.

Within the ten studies undergoing formal synthesis through meta-analysis, clinical populations were predominant, being explored by six studies. However, all studies investigating clinical populations cannot be treated the same. Clinical populations encompassed by our meta-analysis included patients with a diagnosis of PTSD, first-episode psychosis, and major depressive disorder (MDD) (Bocchio-Chiavetto et al., 2010; Green, Corsi-Travali, & Neumeister, 2013; Pillai et al., 2010; Zhang et al., 2014). Both MDD and first-episode psychosis have been associated with lowered BDNF levels, while PTSD patients have been shown to display elevated levels of BDNF (Hsieh, Lin, Lee, & Huang, 2019; Mojtabavi, Saghazadeh, van den Heuvel, Bucker, & Rezaei, 2020; Pillai et al., 2010). Additionally, as shown in Supplementary Table 2, these publications varied in both the racial/ethnic composition and the sex distribution of the study samples, another source of between-study heterogeneity that complicates drawing firm conclusions about the effects of ACE exposure within subjects previously diagnosed with a psychopathology.

ACE exposure in our study was defined as exposure to *any* early-life stress exposure meeting 1998 CDC-Kaiser Study criteria, although others have alternatively defined it as having experienced more than

three stressful life events (do Prado, Grassi-Oliveira, Daruy-Filho, Wieck, & Bauer, 2017; Trajkovska, Vinberg, Aznar, Knudsen, & Kessing, 2008). Similarly, definitions of the ACE-unexposed group in our study sample ranged from healthy controls to clinical patients with a psychiatric diagnosis who experienced fewer than three stressful life events. Utilization of varied ACE-exposure definitions may cloud results and is expected to limit precision of our meta-analytic point estimates. Future studies that adopt a more formally standardized ACE exposure definition would improve the field's ability to better establish the relationship between ACE exposure and BDNF levels.

A previous meta-analysis that explored BDNF protein levels in patients with anxiety disorder also observed substantial between-study heterogeneity (Suliman, Hemmings, & Seedat, 2013). This may be partially attributable to the relatively few studies that specifically examined BDNF protein levels, the relatively small sample sizes within these studies, and the variation in study design that our own meta-analysis also encountered. Despite a strong rationale for the involvement of BDNF in mental health disorders, the possible link between ACE exposure and BDNF activity remains unclear, particularly in comparison to HPA biomarkers (*e.g.*, cortisol) and inflammatory biomarkers (Kim et al., 2019).

Although outside the scope of our systematic review and meta-analysis of the effect of ACEs on BDNF protein levels, several studies have investigated ACE-associated changes in DNA methylation of the *BDNF* gene promoter region. Gene promoter methylation is a known epigenetic mechanism of regulating gene expression, and such an approach to understanding the relationship between ACEs and BDNF has both advantages and disadvantages compared with direct protein measurement, such as by ELISA. A major advantage to measuring ACE-associated changes in the dynamic methylation state of the *BDNF* gene promoter is that it includes a clear mechanism through which ACE exposure may modify BDNF activity. However, DNA methylation is strongly associated with differences in racial/ethnic ancestry and with age, so such studies need to explicitly account for such potential confounding factors in their analyses (Horvath, 2013; Kazmi et al., 2020) Furthermore, directly assaying BDNF protein levels would be expected to enhance cross-study comparisons and directly assesses what is likely a more physiologically-

relevant endpoint. Indeed, while BDNF protein levels are perhaps more distal to the ACE exposure than methylation changes, they are more proximate to the clinical outcomes of greatest interest.

While our meta-analysis on all ten studies did not identify a significant association between ACE exposure and BDNF protein levels, subset analyses of more homogenous study designs revealed suggestive patterns. Subset analysis of studies exclusively investigating the impact of childhood maltreatment indicated a trend towards elevated BDNF levels in the ACE-exposed group, results which contrast with a prior study detailing decreases in BDNF protein levels and mRNA expression due to stressors and activation of the HPA axis (Bath, Schilit, & Lee, 2013). However, when looking at the specific studies in the subset analysis, heterogeneity persisted. Do Prado et al., the only study displaying a significant decrease in BDNF levels in the ACE-exposed group, measured only the impact of early-life stress. In contrast, the studies reporting significantly elevated levels of BDNF in ACE-exposed subjects had unique clinical samples with participants reporting foster care exposure and anxiety disorder onset. Thus, it may be that these additional factors modify the effects of ACE exposure on BDNF regulation in a different manner than is traditionally acknowledged within the field.

Two hypotheses have been investigated in relation to elevated BDNF levels in the face of stress exposure. One hypothesis attributes increased BDNF protein levels to compensatory effect, wherein stress exposure leads to increased BDNF levels in order to accommodate for stress-related decreases in neurogenesis (Greisen, Altar, Bolwig, Whitehead, & Wörtwein, 2005). A second hypothesis considers the form of BDNF that is being upregulated or downregulated. BDNF can be present as proBDNF or mature-BDNF, and these forms have opposing functions. Pro-BDNF has been found to induce apoptosis via interaction with the inflammatory system, while mature-BDNF contributes to synaptogenesis and memory consolidation (Mojtabavi et al., 2020). An increase in pro-BDNF levels specifically could be detrimental due to its contribution to neuronal death. Commercially available ELISA assays for BDNF typically measure mature-BDNF unless explicitly specified to measure pro-BDNF, however, it remains possible that a subset of studies included in our meta-analysis measured pro-BDNF without clearly stating so.

Subgroup analysis of BDNF levels in groups under and over the age of 20 also indicates possible time dependency in changes of BDNF protein levels. While there was no significant difference between the protein levels of BDNF between the two groups in our mixed-effects model, there was a trend towards higher BDNF protein levels measured in the ACE-exposed group prior to the age of 20. The highest levels of BDNF mRNA and protein levels are found during the adolescent/young adult period, after which levels are found to decrease during middle to late adulthood (Katoh-Semba et al., 2007). Our results may reflect developmental changes in BDNF protein levels, or a waning effect of ACE-exposure on BDNF at more removed timepoints. BDNF levels may also be elevated following ACE exposure due to the previously mentioned compensatory mechanism, but such changes may not persist longitudinally. Exposure to a chronic stress-state has been found to be associated with lowered BDNF levels in murine models (Algamal et al., 2018). ACE exposure can result in persistent experiences of chronic stressors due to the longitudinal impacts of ACEs, as children exposed to parental substance abuse or sexual abuse have been found to engage in riskier future behaviors such as engaging in abusive relationships or pursuing risky sexual behaviors, but teasing out these effects across diverse studies designs with varied ACE exposures remains difficult (Lalor & McElvaney, 2010; Senn, Carey, & Vanable, 2008).

If ACE exposure increases the likelihood of chronic stress exposure and these jointly lead to BDNF blunting in adulthood, such a mechanism has particular relevance given the increased susceptibility of children with ACE exposure to the development of mental disorders during adulthood. Decreased BDNF levels have been associated with MDD, anxiety, and schizophrenia, all diagnoses seen at higher rates in ACE exposed adults (Hsieh et al., 2019). Changes in BDNF levels could thus link ACE exposure to poorer mental health outcomes. Future studies should focus on both further investigating developmental changes in BDNF levels and also on measuring lifelong stress in addition to ACE exposure in order to evaluate the impact of chronic stress on BDNF blunting.

Several limitations must be acknowledged within this study. First, several publications did not report the true mean BDNF values within their samples, instead displaying the data graphically or reporting relevant statistical analysis results. Authors were contacted, and studies were excluded if data

were not provided. Inclusion of these studies would have been beneficial given the small sample size of the overall meta-analysis and the sub-group meta-analyses. It would be beneficial for future studies to provide raw biomarker data in order to facilitate cross-study comparisons and meta-analyses. Provision of all relevant data would be in-line with the general shift towards data transparency in health research and the increased willingness of journals to permit the inclusion of online-only supplemental data files and tables. Second, the small sample size (<10) of the subset analyses prevented evaluation of publication bias via the Egger's test, and results may also be unduly influenced by a single study. Additionally, the small sample size of studies included within the meta-analysis prevented analysis of all possible sub-groups, such as exploring the impact of ACEs other than childhood maltreatment or differential impact of other analytes (*e.g.,* whole blood or plasma) on BDNF protein levels. Relationships between these variables and BDNF should not be disregarded and instead be further investigated in order to determine mechanistic details of putative ACE-mediated dysregulation of BDNF. Increased data reporting along with further investigation into the ACE-BDNF relationship would attenuate these limitations and help to better elucidate the impact of ACEs on BDNF protein levels specifically. Future investigations could also consider expanding ACE exposures analyzed in relation to BDNF levels, moving beyond exploring the impact of traditional ACEs, such as parental mental health or parental substance abuse, and towards acknowledging an expanded list of ACE exposures, such as experiences of discrimination or exposure to violent environments (Afifi, 2020; Cronholm et al., 2015). Diversification of the ACE exposures considered in addition to explicit sub-analysis of the traditional ACEs and BDNF protein levels will ensure a more thorough understanding of the ACE-BDNF relationship that reflects unique stressors present in the environment today.

In summary, while individual studies provide evidence of ACE-dependent changes in BDNF protein levels, limited sample sizes and heterogeneous study designs limit the ability to reach a consensus on the strength and breadth of this possible relationship. Differential regulation of BDNF levels based on the type of ACE exposure, the acute versus chronic nature of the ACE exposure, and the timing of biomarker measurement seem likely. Our meta-analysis was purposefully broad in nature to begin the process of

consolidating the state of the field and to investigate the overarching relationship of ACE exposure and BDNF protein levels. While it was limited by heterogeneity and the small sample sizes used by individual studies, it can serve as a starting point to inform the design of future studies. Understanding the complex ACE-BDNF relationship will enable greater insight into how ACE exposure may predispose ACE-exposed children towards poorer mental health outcomes, and thus may help nominate treatment approaches to mitigate mental health risks in ACE-exposed children.

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Conceptualization, Data Curation; Methodology, Project Administration; Resources, Supervision, Writing – Review & Editing; **Kyle M. Walsh:** Conceptualization, Methodology, Resources; Supervision, Writing – Review & Editing

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TABLES

Table 1. Characteristics of Studies Included in Meta-Analysis

Study	ACEs Measured	BDNF Measurement	ACE+ Sample (N)	Mean (SD) BDNF in ACE+	ACE- Sample (N)	Mean (SD) BDNF in ACE-
Bortoluzzi et al. (2014)	Childhood abuse (sexual, physical, and/or emotional), neglect (physical and/or emotional)	Serum BDNF levels by sandwich ELISA	Adolescents with anxiety and exposure to high levels of trauma *(n=10)*	54.05 ng/mL (20.21)	Adolescents with anxiety and exposure to low levels of trauma *(n=50)*	37.87 ng/mL (13.08)
Bücker et al. (2015)	Childhood abuse (sexual, physical, and/or emotional) and neglect (physical and/or emotional)	Plasma BDNF levels by Sandwich ELISA	Children in foster care /protective services exposed to measured ACEs between the age of 3-12 (*n= 36)*	1.42 ng/mL (log transformed) (0.25)	Healthy controls (*n=26)*	0.89 ng/mL (log transformed) (0.57)
do Prado et al. (2017)	Childhood Abuse (sexual, physical, and/or emotional abuse) and neglect (emotional and/or physical)	Plasma BDNF levels by ELISA	Adolescents with history of childhood maltreatment (*n=30)*	200.07 pg/mL (97.48)	Healthy Controls (*n=27)*	347.96 pg/mL (224.11)
Hauck et al. (2010)	Remote Trauma (not explicitly defined)	Serum BDNF levels by sandwich ELISA	PTSD patients with remote exposure to trauma (*n=13*)	0.39 pg/ug (0.16)	Healthy Controls (*n=34)*	0.25 pg/ug (0.14)
Sharma, Graham, Rohde, and Ceballos (2017)	Parental substance abuse	Serum BDNF levels by ELISA	Healthy social drinkers with positive family history of alcohol abuse (*n=46)*	423.99 pg/mL (260.18)	Healthy social drinkers with negative family history of alcohol abuse (*n=22)*	424.96 pg/mL (324.89)

Simsek, Uysal, Kaplan, Yuksel, and Aktas (2015)	Sexual abuse	Serum BDNF levels by ELISA	Children with exposure to sexual abuse with PTSD (*n=27)*	2300 pg/mL (2200)	Children with exposure to sexual abuse without PTSD (*n=28)*	2400 pg/mL (2300)
Sordi et al. (2019)	Childhood abuse (sexual, physical, and/or emotional) and neglect (physical and/or emotional)	Serum BDNF levels by ELISA	Crack-cocaine users defined to have high childhood trauma exposure (*n=11)*	30.77 pg/mL (8.13)	Crack-cocaine users defined to have low childhood trauma exposure (*n=11)*	29.28 pg/mL (11.62)
Theleritis et al. (2014)	Severe childhood abuse (sexual and/or physical), parental death, and parental separation	Plasma BDNF levels by ELISA	First Episode Psychosis Patients having been exposed to physical and/or sexual abuse (*n=47*)	24.7541 ng/mL (6.3805)	Healthy controls without exposure to physical and/or sexual abuse (*n=75)*	25.485 ng/mL (6.4408)
Trajkovska et al. (2008)	Familial mental health parental death, and additional recent stressors (including abuse, marital problems, illness, etc.)	Whole blood BDNF levels by ELISA	Females at high risk for MDD diagnosis with 3+ stressful life events (*n=26*)	18500 pg/mL (4100)	Females at high risk for MDD without stressful life events (*n=35)*	21600 pg/mL (7000)
Watt, Ceballos, Kim, Pan, and Sharma (2020)	Childhood abuse (sexual, physical, and/or emotional) and neglect (physical and/or emotional),parental mental health, parental substance abuse, parental incarceration, and exposure to intimate partner violence	Serum BDNF levels by ELISA	ACE score greater than or equal to 4 (*n= 30)*	421.08 pg/mL (132.07)	ACE score less than or equal to 3 (*n= 63)*	377.34 pg/mL (153.58)

Abbreviations: MDD (major depressive disorder), SD (standard deviation), ELISA (enzyme-linked immunosorbent assay), BDNF (Brain Derived Neurotrophic Factor), ACE (Adverse childhood experience), PTSD (post-traumatic stress disorder)

FIGURE LEGENDS

FIGURE 1. PRISMA flowchart depicting study selection strategy and reasons for exclusion.

FIGURE 2. Forest plot evaluating ratio of mean BDNF protein levels between ACE-exposed and ACE-unexposed samples across all ten studies included within the meta-analysis. ROM: ratio of means ($\frac{ACEexposed}{ACEunexposed}$). CI: Confidence Interval.

FIGURE 3. Forest plot evaluating ratio of mean BDNF protein levels between ACE-exposed and unexposed groups within subgroups investigating samples over and under the age of twenty. ROM: ratio of means ($\frac{ACEexposed}{ACEunexposed}$). CI: Confidence Interval.

FIGURE 4. Forest plot evaluating ratio of mean BDNF protein levels between ACE-exposed and unexposed groups within studies investigating childhood maltreatment as the ACE exposure. ROM: ratio of means ($\frac{ACEexposed}{ACEunexposed}$). CI: Confidence Interval.

FIGURE 5. Forest plot of ratio evaluating mean BDNF protein levels between ACE-exposed and unexposed groups within studies measuring BDNF from the serum specifically. ROM: ratio of means ($\frac{ACEexposed}{ACEunexposed}$). CI: Confidence Interval.

SUPPLEMENTARY FILE 1

SUPPLEMENTARY TABLE 1. Search Strategy Report

Topic: How does experiencing adverse childhood experience changes children's biologic reaction to stress and other biomarkers of health.

Searcher: SJK

Date: 4.27.2020

Database (including vendor/platform): Ovid/Medline

Net	Set		Results
ACEs	1	exp "Adult Survivors of Child Adverse Events"/ or exp Adverse Childhood Experiences/	3333
	2	exp Child Welfare/ OR exp "Child of Impaired Parents"/ OR exp Child Abuse/ or exp "Adult Survivors of Child Abuse"/ OR exp Child Abuse, Sexual/ OR exp Physical Abuse/ OR exp Substance-Related Disorders/ OR exp Domestic Violence/ or exp Divorce/ OR exp Family Separation/ OR exp Poverty/ OR exp Racism/ or exp Suicide/ or exp Intimate Partner Violence/ or exp Depressive Disorder	540707
	3	(ACEs or (toxic adj1 stress) or (childhood adj1 adversity) or (childhood trauma) or (adverse adj1 childhood) or (childhood adj1 adversities) or ((Adverse or adversity or adversities) adj3 (child or childhood or children) adj3 (exposure or exposures or exposed or event or events or experience or experiences or experienced or experiencing))).ti,ab.	7888
	4	1 OR 2 OR 3	545608
Biomarkers	5	exp Biomarkers/	735410

	6	(Biomarker or biomarkers or epigenomics OR epigenetic OR epigenetics OR interleukins OR interleukin OR TNF OR interferon OR interferons OR fibrinogen OR leukocyte OR leukocytes OR cytokine OR cytokines OR chemokines OR chemokine OR insulin OR telomere OR cortisol OR telomeres OR (Tumor adj1 Necrosis adj1 Factor adj1 alpha) OR (c adj1 reactive adj1 protein) OR CRP OR telomeres OR telomere or ((Biological OR bio OR immune OR serum OR plasma OR blood OR saliva OR urine OR laboratory OR viral OR clinical OR surrogate OR immunologic OR biochemical or molecular or genetic or inflammatory OR inflammation OR physiological OR cardiometabolic OR cardiovascular OR proinflammatory) adj2 (Marker OR markers OR endpoint OR levels OR level))).ti,ab	2085320
	7	5 or 6	2537119
	8	4 and 7	26947
Stress reaction	9	exp Stress, Psychological/ or exp Stress, Physiological/ OR exp Stress Disorders, Post-Traumatic/ or exp Stress Disorders, Traumatic/ or exp Adaptation, Biological/ or exp Adaptation, Psychological/ or exp Adaptation, Physiological/ or exp Cognitive Dysfunction/ or exp Life Change Events/ OR exp Allostasis/	627568
	10	(reaction or reactions or reacted or reactive or reacting or stress or stresses or stressor or stressors or stressed or adaptation or adaption or adapt or adapted or adapts or adapting or development or developing or developed or develop or develops or developmental or maladaptation or maladaption or maladapted or outcome or outcomes or impact or impacts or influence or influences or function or functions or functioning or functioned OR allostasis OR (allostatic adj1 load)).ti,ab	10147768
	11	9 or 10	10336323

	12	8 and 11	14520
Children	13	exp child/ or exp child, preschool/ or exp infant/ or exp Adolescent/	3524617
	14	(child or children or childhood or pediatric or pediatrics or paediatric or paediatrics or kid or kids or boy or girl or boys or girls or infant or infants or infancy or neonate or neonates or baby or babies or juvenile or juveniles or adolescent or adolescents or adolescence or teen or teens or teenager or teenagers or boyhood or girlhood or newborn or newborns).ti,ab	2189417
	15	13 or 14	4132033
	16	12 and 15	3940
	17	16 not (case reports or editorial or letter or comment).pt.	3809
	18	17 not (Animals/ not (Animals/ and Humans/))	3686
		(25783196 OR 30605796 OR 27647050 OR 27275737 OR 25496803 OR 30067291).ui	Catches 3

Topic: How does experiencing adverse childhood experience changes children's biologic reaction to stress and other biomarkers of health.

Searcher: SJK

Date: 4.28.2020

Database (including vendor/platform): Embase

Set		Results

1	'child welfare'/exp OR 'child of impaired parents'/exp OR 'child abuse'/exp OR 'childhood trauma survivor'/exp OR 'child sexual abuse'/exp OR 'physical abuse'/exp OR 'drug dependence'/exp OR 'domestic violence'/exp OR 'divorce'/exp OR 'family separation'/exp OR 'poverty'/exp OR 'racism'/exp OR 'suicide'/exp OR 'partner violence'/exp OR 'depression'/exp	888108
2	(ACEs OR (toxic NEAR/1 stress) OR (childhood NEAR/1 adversity) OR ("childhood trauma") OR (adverse NEAR/1 childhood) OR (childhood NEAR/1 adversities) OR ((Adverse OR adversity OR adversities) NEAR/3 (child OR childhood OR children) NEAR/3 (exposure OR exposures OR exposed OR event OR events OR experience OR experiences OR experienced OR experiencing))):ti,ab	11124
3	1 OR 2	893335
4	'biological marker'/exp	298625
5	(Biomarker OR biomarkers OR epigenomics OR epigenetic OR epigenetics OR interleukins OR interleukin OR TNF OR interferon OR interferons OR fibrinogen OR leukocyte OR leukocytes OR cytokine OR cytokines OR chemokines OR chemokine OR insulin OR telomere OR cortisol OR telomeres OR (Tumor NEAR/1 Necrosis NEAR/1 Factor NEAR/1 alpha) OR (c NEAR/1 reactive NEAR/1 protein) OR CRP OR telomeres OR telomere OR ((Biological OR bio OR immune OR serum OR plasma OR blood OR saliva OR urine OR laboratory OR viral OR clinical OR surrogate OR immunologic OR biochemical OR molecular OR genetic OR inflammatory OR inflammation OR physiological OR cardiometabolic OR cardiovascular OR proinflammatory) NEAR/2 (Marker OR markers OR endpoint OR levels OR level))):ti,ab	2907043
6	4 or 5	2970217

7	3 and 6	57001
8	'mental stress'/exp OR 'physiological stress'/exp OR 'posttraumatic stress disorder'/exp OR 'adaptation'/exp OR 'cognitive defect'/exp OR 'life event'/exp OR 'allostasis'/exp	835833
9	(reaction OR reactions OR reacted OR reactive OR reacting OR stress OR stresses OR stressor OR stressors OR stressed OR adaptation OR adaption OR adapt OR adapted OR adapts OR adapting OR development OR developing OR developed OR develop OR develops OR developmental OR maladaptation OR maladaption OR maladapted OR outcome OR outcomes OR impact OR impacts OR influence OR influences OR function OR functions OR functioning OR functioned OR allostasis OR (allostatic NEAR/1 load)):ti,ab	13451136
10	8 or 9	13774791
11	10 and 7	36799
12	'pediatrics'/exp OR 'infant'/exp OR 'child'/exp OR 'adolescent'/exp OR 'minor (person)'/exp OR 'puberty'/exp OR pediatric:ab,ti OR pediatrics:ab,ti OR paediatric:ab,ti OR paediatrics:ab,ti OR infant:ab,ti OR infants:ab,ti OR infantile:ab,ti OR baby:ab,ti OR babies:ab,ti OR preterm:ab,ti OR prematurity:ab,ti OR child:ab,ti OR children:ab,ti OR childhood:ab,ti OR toddler:ab,ti OR toddlers:ab,ti OR boy:ab,ti OR boys:ab,ti OR boyhood:ab,ti OR girl:ab,ti OR girls:ab,ti OR girlhood:ab,ti OR schoolchild:ab,ti OR schoolgirl:ab,ti OR schoolgirls:ab,ti OR schoolboy:ab,ti OR schoolboys:ab,ti OR 'school age':ab,ti OR preadolescent:ab,ti OR preadolescents:ab,ti OR preadolescence:ab,ti OR adolescent:ab,ti OR adolescents:ab,ti OR adolescence:ab,ti OR juvenile:ab,ti OR juveniles:ab,ti OR youth:ab,ti OR youths:ab,ti OR teenager:ab,ti OR teenagers:ab,ti OR teenaged:ab,ti OR teen:ab,ti OR teens:ab,ti OR pubescent:ab,ti OR puberty:ab,ti OR	4606004

	pubescence:ab,ti OR prepubescent:ab,ti OR prepubescence:ab,ti OR minors:ab,ti	
13	12 and 11	6481
14	13 NOT 'conference abstract'/it	4428
15	17 AND [humans]/lim	4106

Topic: How does experiencing adverse childhood experience changes children's biologic reaction to stress and other biomarkers of health.

Searcher: SJK

Date: 4.27.2020

Database (including vendor/platform): Scopus

Set		Results
1	TITLE-ABS-KEY("toxic stress" OR ACEs OR ((child OR childhood OR children) W/3 (adverse or adversity or adversities)))	69755

2	TITLE-ABS-KEY (Biomarker or biomarkers or epigenomics OR epigenetic OR epigenetics OR interleukins OR interleukin OR TNF OR interferon OR interferons OR fibrinogen OR leukocyte OR leukocytes OR cytokine OR cytokines OR chemokines OR chemokine OR insulin OR telomere OR cortisol OR telomeres OR (Tumor W/1 Necrosis W/1 Factor W/1 alpha) OR (c W/1 reactive W/1 protein) OR CRP OR telomeres OR telomere or ((Biological OR bio OR immune OR serum OR plasma OR blood OR saliva OR urine OR laboratory OR viral OR clinical OR surrogate OR immunologic OR biochemical or molecular or genetic or inflammatory OR inflammation OR physiological OR cardiometabolic OR cardiovascular OR proinflammatory) W/2 (Marker OR markers OR endpoint OR levels OR level)))	4308860
3	TITLE-ABS-KEY (reaction or reactions or reacted or reactive or reacting or stress or stresses or stressor or stressors or stressed or adaptation or adaption or adapt or adapted or adapts or adapting or development or developing or developed or develop or develops or developmental or maladaptation or maladaption or maladapted or outcome or outcomes or impact or impacts or influence or influences or function or functions or functioning or functioned OR allostasis OR (allostatic W/1 load))	29958682
4	TITLE-ABS-KEY (child or children or childhood or pediatric or pediatrics or paediatric or paediatrics or kid or kids or boy or girl or boys or girls or infant or infants or infancy or neonate or neonates or baby or babies or juvenile or juveniles or adolescent or adolescents or adolescence or teen or teens or teenager or teenagers or boyhood or girlhood or newborn or newborns)	5404959
5	1 AND 2 AND 3 AND 4	1983

Searcher: SJK

Date: 4.28.2020

Database (including vendor/platform): PsycINFO

Set		Results
1	DE "Childhood Adversity" OR DE "Child Abuse" OR DE "Child Neglect" OR DE "Child Welfare" OR DE "Domestic Violence" OR DE "Emotional Abuse" OR DE "Physical Abuse" OR DE "Sexual Abuse" OR DE "Verbal Abuse" OR DE "Alcoholism" OR DE "Alcohol Abuse" OR DE "Suicide" OR DE "Divorce" OR DE "Racism" OR DE "Parental Absence" OR DE "Poverty" OR DE "Homeless" OR DE "Intimate Partner Violence" OR DE "Major Depression"	305648
2	(TI ACEs OR AB ACEs OR ((TI toxic OR AB toxic) N1 (TI stress OR AB stress)) OR ((TI childhood OR AB childhood) N1 (TI adversity OR AB adversity)) OR (TI "childhood trauma" OR AB "childhood trauma") OR ((TI adverse OR AB adverse) N1 (TI childhood OR AB childhood)) OR ((TI childhood OR AB childhood) N1 (TI adversities OR AB adversities)) OR ((TI Adverse OR AB Adverse OR TI adversity OR AB adversity OR TI adversities OR AB adversities) N3 (TI child OR AB child OR TI childhood OR AB childhood OR TI children OR AB children) N3 (TI exposure OR AB exposure OR TI exposures OR AB exposures OR TI exposed OR AB exposed OR TI event OR AB event OR TI events OR AB events OR TI experience OR AB experience OR TI experiences OR AB experiences OR TI experienced OR AB experienced OR TI experiencing OR AB experiencing)))	9664
3	1 OR 2	310863
4	DE "Biological Markers"	12701

5	(TI Biomarker OR AB Biomarker OR TI biomarkers OR AB biomarkers OR TI epigenomics OR AB epigenomics OR TI epigenetic OR AB epigenetic OR TI epigenetics OR AB epigenetics OR TI interleukins OR AB interleukins OR TI interleukin OR AB interleukin OR TI TNF OR AB TNF OR TI interferon OR AB interferon OR TI interferons OR AB interferons OR TI fibrinogen OR AB fibrinogen OR TI leukocyte OR AB leukocyte OR TI leukocytes OR AB leukocytes OR TI cytokine OR AB cytokine OR TI cytokines OR AB cytokines OR TI chemokines OR AB chemokines OR TI chemokine OR AB chemokine OR TI insulin OR AB insulin OR TI telomere OR AB telomere OR TI cortisol OR AB cortisol OR TI telomeres OR AB telomeres OR (TI Tumor OR AB Tumor N1 TI Necrosis OR AB Necrosis N1 TI Factor OR AB Factor N1 TI alpha OR AB alpha) OR (TI c OR AB c N1 TI reactive OR AB reactive N1 TI protein OR AB protein) OR TI CRP OR AB CRP OR TI telomeres OR AB telomeres OR TI telomere OR AB telomere OR ((TI Biological OR AB Biological OR TI bio OR AB bio OR TI immune OR AB immune OR TI serum OR AB serum OR TI plasma OR AB plasma OR TI blood OR AB blood OR TI saliva OR AB saliva OR TI urine OR AB urine OR TI laboratory OR AB laboratory OR TI viral OR AB viral OR TI clinical OR AB clinical OR TI surrogate OR AB surrogate OR TI immunologic OR AB immunologic OR TI biochemical OR AB biochemical OR TI molecular OR AB molecular OR TI genetic OR AB genetic OR TI inflammatory OR AB inflammatory OR TI inflammation OR AB inflammation OR TI physiological OR AB physiological OR TI cardiometabolic OR AB cardiometabolic OR TI cardiovascular OR AB cardiovascular OR TI proinflammatory OR AB proinflammatory) N2 (TI Marker OR AB Marker OR TI markers OR AB markers OR TI endpoint OR AB endpoint OR TI levels OR AB levels OR TI level OR AB level)))	203021
6	4 or 5	206051
7	3 and 6	16179

8	DE "Physiological Stress" OR DE "Post-Traumatic Stress" OR DE "Psychological Stress" OR DE "Stress Reactions" OR DE "Stress and Trauma Related Disorders" OR DE "Acute Stress Disorder" OR DE "Posttraumatic Stress Disorder"	53237
9	(TI reaction OR AB reaction OR TI reactions OR AB reactions OR TI reacted OR AB reacted OR TI reactive OR AB reactive OR TI reacting OR AB reacting OR TI stress OR AB stress OR TI stresses OR AB stresses OR TI stressor OR AB stressor OR TI stressors OR AB stressors OR TI stressed OR AB stressed OR TI adaptation OR AB adaptation OR TI adaption OR AB adaption OR TI adapt OR AB adapt OR TI adapted OR AB adapted OR TI adapts OR AB adapts OR TI adapting OR AB adapting OR TI development OR AB development OR TI developing OR AB developing OR TI developed OR AB developed OR TI develop OR AB develop OR TI develops OR AB develops OR TI developmental OR AB developmental OR TI maladaptation OR AB maladaptation OR TI maladaption OR AB maladaption OR TI maladapted OR AB maladapted OR TI outcome OR AB outcome OR TI outcomes OR AB outcomes OR TI impact OR AB impact OR TI impacts OR AB impacts OR TI influence OR AB influence OR TI influences OR AB influences OR TI function OR AB function OR TI functions OR AB functions OR TI functioning OR AB functioning OR TI functioned OR AB functioned OR TI allostasis OR AB allostasis OR (TI allostatic OR AB allostatic N1 TI load OR AB load))	2,326,627
10	8 or 9	2,330,372
11	7 and 10	9556

12	MH "Adolescence+" OR MH "Child+" OR MH "Pediatrics+" OR MH "Minors (Legal)" OR MH "Puberty+" OR TI(Infant OR infants OR infancy OR newborn OR newborns OR neonatal OR neonate OR neonates OR baby OR babies OR preterm OR prematurity OR toddler OR toddlers OR boy OR boys OR boyhood OR girl OR girls OR girlhood OR kid OR kids OR child OR childhood OR children OR schoolchild OR schoolgirl OR schoolgirls OR schoolboy OR schoolboys OR "school age" OR "school aged" OR preadolescent OR preadolescents OR preadolescence OR adolescent OR adolescents OR adolescence OR juvenile OR juveniles OR youth OR youths OR teen OR teens OR teenager OR teenagers OR teenaged OR puberty OR pubescent OR pubescence OR prepubescent OR prepubescence OR pediatric OR pediatrics OR paediatric OR paediatrics OR minors) OR AB(Infant OR infants OR infancy OR newborn OR newborns OR neonatal OR neonate OR neonates OR baby OR babies OR preterm OR prematurity OR toddler OR toddlers OR boy OR boys OR boyhood OR girl OR girls OR girlhood OR kid OR kids OR child OR childhood OR children OR schoolchild OR schoolgirl OR schoolgirls OR schoolboy OR schoolboys OR "school age" OR "school aged" OR preadolescent OR preadolescents OR preadolescence OR adolescent OR adolescents OR adolescence OR juvenile OR juveniles OR youth OR youths OR teen OR teens OR teenager OR teenagers OR teenaged OR puberty OR pubescent OR pubescence OR prepubescent OR prepubescence OR pediatric OR pediatrics OR paediatric OR paediatrics OR minors)	988388
13	11 AND 12	2213
	Limit to academic journals	1910

SUPPLEMENTARY TABLE 2. Characteristics of Studies Included within the Systematic Review

Author (year)	Study Population (Sample Size), Sample Characteristics (sex makeup and racial/ethnic composition)	ACEs Measured, ACE Timing	BDNF Measurement Methodology, Measurement Timing	Impact of ACE exposure on BDNF levels
Aksu, Unlu, Kardesler, Cakaloz, and Aybek (2018)	9 comparator group; 22 ACE+ (mean age of each group not provided, overall age range is 8-18) Sample Characteristics: Turkish children and adolescents recruited from the community (controls) and hospital (PTSD patients) (100% female, race breakdown not provided)	Childhood sexual abuse, during childhood following ACE exposure	ELISA on serum, during childhood post ACE	Lower in ACE+
Benedetti et al. (2017)	5HTTLPR l/l (15) (mean age: 44.40), 5-HTTLPR l/s (18) (mean age: 46.33), 5-HTTLPR-s/s (7) (mean age: 51.14), Sample Characteristics: Italian MDD inpatients with Type 1 Bipolar Disorder (67.5% female, race breakdown not provided)	Childhood abuse (sexual, physical, and/or emotional), neglect (physical and/or emotional), retrospectively in adulthood	ELISA on serum, retrospectively during adulthood	Lower in ACE+
Bortoluzzi et al. (2014)	50 comparator group; 10 ACE+ (mean age of overall sample: 13.56) Sample Characteristics: Brazilian adolescents with and without anxiety disorders	Childhood abuse (sexual, physical, and/or emotional), neglect (physical and/or emotional), during childhood following ACE exposure	ELISA on serum, during childhood	Higher in ACE+

	(65.6% female, 72.2% Caucasian)			
Bücker et al. (2015)	26 comparator group (mean age: 8.96); 36 ACE+ (mean age: 9.44) Sample Characteristics: Brazilian children recruited from child protection program and foster care home and healthy controls recruited from the community (40.3% female, race breakdown not provided)	Childhood abuse (sexual, physical, and/or emotional) and neglect (physical and/or emotional), during childhood both during ACE exposure and following ACE exposure	ELISA on plasma, during childhood	Higher in ACE+
Counotte et al. (2019)	32 comparator; 7 ACE+ (mean age of each group not provided, age range was 21-26) Sample Characteristics: Dutch study participants with liability to psychosis and healthy controls (38.5% female, race breakdown not provided)	Childhood abuse (sexual, physical, and/or emotional), neglect (physical and/or emotional), retrospectively during adulthood	Luminex assay on serum, retrospectively in adulthood	No significant difference in levels of BDNF
do Prado, Grassi-Oliveira, Daruy-Filho, Wieck, and Bauer (2017)	27 comparator group (mean age: 14.19); 30 ACE+ (mean age: 16.47) Sample Characteristics: Brazilian adolescents (57.9% female, race breakdown not provided)	Childhood Abuse (sexual, physical, and/or emotional abuse) and neglect (emotional and/or physical), during childhood following ACE exposure	ELISA on plasma, during childhood following ACE exposure	Lower in ACE+
Druzhkova et al. (2019)	ACE+, comparator breakdown not reported despite analyses on trauma scores and biomarkers (age range: 18-45)	Childhood abuse (sexual, physical, and/or emotional), neglect (physical and/or emotional), retrospectively in adulthood	ELISA on serum, retrospectively in adulthood	No significant correlation found between trauma scores and BDNF levels

	Sample Characteristics: Russian patients with borderline mental disorders (76.3% female, race breakdown not provided)			
Grassi-Oliveira, Stein, Lopes, Teixeira, and Bauer (2008)	15 comparator group (mean age: 36.47); 17 ACE+ (mean age: 39.35) Sample Characteristics: Brazilian female, MDD outpatients and healthy controls (100% female, race breakdown not provided)	Childhood neglect (physical), retrospectively in adulthood	ELISA on plasma, retrospectively during adulthood	Lower in ACE+
Hauck et al. (2010)	34 comparator group (mean age: 36.2); 13 ACE+ (mean age: 39.5) Sample Characteristics: Brazilian adults with PTSD (caused by remote vs. recent trauma) and healthy controls (92% female ACE+, 79% female comparator, race breakdown not provided)	Not explicitly reported (defined as remote trauma), retrospectively in adulthood	ELISA on serum, retrospectively in adulthood	Higher in ACE+
Kauer-Sant'Anna et al. (2007)	85 comparator group (mean age: 43.01); 78 ACE+ (mean age: 42.13) Sample Characteristics: Brazilian bipolar patients with and without childhood trauma (overall female percentage not noted, race	Childhood abuse (sexual and physical), retrospectively in adulthood	ELISA on serum, retrospectively in adulthood	Lower in ACE+

	distribution not provided)			
Kavurma et al. (2017)	35 comparator group; 70 ACE+ Sample Characteristics: Turkish adolescents with non-suicidal self-harm and healthy controls (73.3% female, race distribution not provided)	Childhood abuse (sexual, physical, and/or emotional), neglect (physical and/or emotional), during childhood following ACE exposure	ELISA on serum, during childhood	Lower in ACE+
Mansur et al. (2016)	28 comparator group; 467 ACE+ (overall mean age of sample: 10.06) Sample Characteristics: Brazilian children at high risk for psychiatric disorders (45.1% female, 58% Caucasian)	Socioeconomic disadvantage, during childhood while ACE exposure ongoing	ELISA on serum, during childhood	Higher in the ACE+
Palmos et al. (2019)	256 comparator group (mean age: 48.50); 108 ACE+ (mean age: 47.38) Sample Characteristics: British residents recruited from MDD clinical trial and from the local community (controls) (54.8%, ethnicities encompassed include Black, White, and Other)	Childhood abuse (sexual, physical, and/or emotional) and neglect (physical and/or emotional), retrospectively during adulthood	ELISA on serum, retrospectively in adulthood	Childhood maltreatment did not affect BDNF in comparator vs. ACE+ group
Sharma, Graham, Rohde, and Ceballos (2017)	22 comparator group; 46 ACE+ (mean age of each group not provided, overall age range is 18-29) Sample Characteristics:	Family history of substance abuse (alcohol use disorder), retrospectively in adulthood	ELISA on serum, retrospectively in adulthood	Lower in ACE+

	Participants recruited from Texas State University (70.6% female, race distribution not given for this sample but both White and Hispanic participants were reported)			
Simsek, Uysal, Kaplan, Yuksel, and Aktas (2015)	28 comparator group (mean age: 13.9); 27 ACE+ (mean age: 14.9) Sample Characteristics: Turkish children admitted to outpatient clinic for PTSD assessment and Turkish children without PTSD (69.1% female, race distribution not provided)	Childhood sexual abuse, during childhood following ACE exposure	ELISA on serum, during childhood	Lower in ACE+
Snijders et al. (2017)	50 comparator group (mean age: 15.0); 96 ACE+ (mean age: 16.4) Sample Characteristics: healthy Dutch children and Dutch children recruited from Dutch Bipolar offspring study (43.8% female, race distribution not provided)	Exposure to parent with bipolar disorder, during childhood while ACE exposure ongoing	ELISA on serum, during childhood	Lower in ACE+
Sordi et al. (2019)	11 comparator group (mean age: 29.64); 11 ACE+ (mean age: 25.45) Sample Characteristics: All male, Brazilian participants recruited from addiction unit (crack cocaine users) (100% male, race	Childhood abuse (sexual, physical, and/or emotional) and neglect (physical and/or emotional), retrospectively in adulthood	ELISA on serum, retrospectively in adulthood	Higher in ACE+ group

	distribution not provided)			
Theleritis et al. (2014)	75 comparator group (mean age: 32.1); 47 ACE+ (mean age: 30.6) Sample Characteristics: UK patients with first episode psychosis and residents (45.2% females, ethnicities encompassed were Black/ African-Caribbean and White	Childhood abuse (sexual and/or physical) and parental death, retrospectively in adulthood	ELISA on plasma, retrospectively in adulthood	Lower in ACE+
Trajkovska, Vinberg, Aznar, Knudsen, and Kessing (2008)	35 comparator group; 26 ACE+ (overall age range of sample 22-70) Sample Characteristics: Danish Participants at high and low risk for affective disorders (risk is defined as having twin with affective disorder) (59.2% female, racial distribution not provided)	Familial mental health parental death, and additional recent stressors (including abuse, marital problems, illness, etc.), retrospectively in adulthood	ELISA on whole blood, retrospectively in adulthood	Higher in ACE+
van der Meij, Comijs, Dols, Janzing, and Voshaar (2014)	231 comparator group; 108 ACE+ (mean age of specific groups not provided, mean age of overall sample: 70.6) Sample Characteristics: Dutch participants recruited from Netherlands Study of Depression in Older People (64.4% female, race distribution not provided)	Childhood abuse (sexual, physical, and/or emotional) and neglect (physical and/or emotional),, retrospectively in adulthood	ELISA on serum, retrospectively in adulthood	Higher in ACE+

Viola et al. (2014)	20 comparator group (mean age: 29.5); 22 ACE+ (mean age: 31.8) Sample Characteristics: Crack cocaine users recruited from detox facility (100% female, race distribution not provided)	Childhood sexual abuse, retrospectively in adulthood	ELISA on plasma, retrospectively in adulthood	Higher in ACE+
Watt, Ceballos, Kim, Pan, and Sharma (2020)	63 comparator group; 30 ACE+ (overall sample mean age: 21.05) Sample Characteristics: Texas University students (73% females, 40% non-Hispanic White, 60% Non-White)	Childhood abuse (sexual, physical, and/or emotional) and neglect (physical and/or emotional),parental mental health, parental substance abuse, parental incarceration, and exposure to intimate partner violence, retrospectively in adulthood	ELISA on serum, retrospectively in adulthood	Higher in ACE+

SUPPLEMENTARY FIGURE 1. Sample REDCap Data Collection Tool

ACES and Biomarkers

Record Number

Article Information

Title

Authors

Type

Year

Journal

Volume

Pages

Abstract

Population

◯ General population
◯ Mother/child dyad
◯ Specific clinical sample (specify)
◯ Other (specify)

Describe specific clinical sample

Describe other population

Sample size

_____

ACE [check all that apply]

	Yes
Child maltreatment	☐
sexual abuse	☐
physical abuse	☐
neglect	☐
emotional	☐
unspecified	☐
Parental mental health (depression, anxiety, bipolar, PTSD, other)	☐
maternal	☐
paternal	☐
household member	☐
Parental substance use	☐
maternal	☐
paternal	☐
household member	☐
Parental death	☐
Suicide	☐
Foster care/institutionalization	☐
Parental Incarceration/criminal involvement	☐
Family separation (other than foster care or parental incarceration)	☐
Intimate partner/Domestic violence	☐
Racism	☐
Poverty	☐
Peer victimization/bullying	☐
Natural disaster	☐
Other (specify)	☐

Specify other ACE

_____

Developmental Timing of ACE [check all that apply]

- ☐ In utero
- ☐ Before age 6
- ☐ After age 6
- ☐ Chronic
- ☐ Ever during childhood
- ☐ Other (specify)

Specify other developmental timing of ACE

_____

Measurement Timing of ACE [check all that apply]

- ☐ During childhood
- ☐ Retrospectively in adulthood

Biomarker [check all that apply]

	Yes
Cortisol	☐
C-Reactive protein	☐
Inflammatory biomarkers, other than CRP (specify, e.g. "STAT3")	☐
Telomere length	☐
Methylation	☐
Neuro-imaging parameters	☐
Neurotransmitters/Neurotrophic factors (specify, e.g. "BDNF")	☐
Cardiopulmonary parameters (e.g. FEV1, heart rate)	☐
Hormones, other than cortisol (specify, e.g. "insulin")	☐
Standard clinical values (e.g. specify, BMI, LDL, HBA1C)	☐
Other (specify)	☐

Specify inflammatory biomarkers, other than CRP

_____

Specify neurotransmitters/neurotrophic factors

_____

Specify hormones, other than cortisol

_____

Specify standard clinical values

Specify other biomarker

Developmental Timing of biomarker: [check all that apply]

- ☐ During childhood while ACE is ongoing
- ☐ During childhood, post-ACE
- ☐ Young Adulthood 19-24
- ☐ Middle Adulthood 25-40
- ☐ Older Adulthood 40+

Method [check all that apply]

- ☐ Cross-sectional,
- ☐ Longitudinal,
- ☐ Retrospective,
- ☐ Prospective,
- ☐ Other (specify)

Specify other method

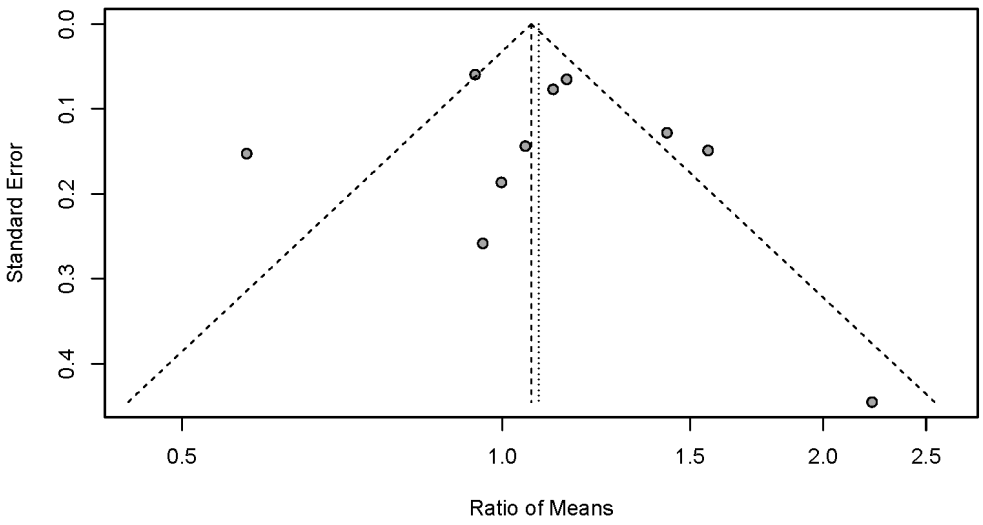
Findings

Exclusion reason (if excluded we need to provide a reason) [check all that apply]

- ☐ Not in English
- ☐ No full text available
- ☐ Did not assess the effect of an ACE on Biomarker
- ☐ Retracted
- ☐ Other, specify

Other reason excluded

SUPPLEMENTARY FIGURE 2. Duval and Tweedie's Funnel Plot



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