



Autonomic nervous system activity predicts increasing serum cytokines in children

Nicholas V. Alen*, LillyBelle K. Deer, Camelia E. Hostinar*

Psychology Department Center for Mind and Brain, University of California, Davis, 267 Cousteau Place, Davis, CA 95618, United States

ARTICLE INFO

Keywords:

Inflammation
Cardiac autonomic balance
Autonomic nervous system
Individual differences
Cytokines

ABSTRACT

Systemic inflammation is associated with increased risk for prevalent and costly diseases, and animal models implicate the autonomic nervous system in the control of inflammatory processes. In humans, research on autonomic-immune connections has been much more limited, and has focused on single branch autonomic measures (i.e., either parasympathetic or sympathetic). The current study utilized cardiac autonomic balance (CAB), derived from dual-branch cardiac autonomic recordings, to test the relation between resting autonomic function and inflammatory reactivity to challenge in children.

Methods: Participants included 96 children (51 boys, 45 girls) ages 9–11 years (mean age = 9.93 years, $SD = 0.57$ years). CAB values were calculated from standardized measures of parasympathetic and sympathetic activity, namely resting respiratory sinus arrhythmia and pre-ejection period data, respectively. Children provided two blood samples, one before and one following exposure to an acute social stressor or control condition. Serum was assayed for four cytokines that orchestrate inflammation: interleukin-6 (IL6), interleukin-8 (IL8), interleukin-10 (IL10), and tumor necrosis factor-alpha (TNFa).

Results: We discovered large individual differences in inflammatory marker production across children, and no average main effect of stress condition. CAB significantly predicted these individual differences, such that children lower on CAB showed increasing serum cytokines from time 1 to time 2. In contrast, children with greater CAB tended to show declining inflammatory markers across the session.

Discussion: Low cardiac autonomic balance (i.e., the combination of low parasympathetic and high sympathetic activity) may be a useful marker of proinflammatory tendencies in children, suggesting novel paths for early risk detection and intervention.

1. Introduction

Extensive preclinical and clinical studies have implicated systemic inflammation in the pathophysiology of prevalent chronic diseases. These include coronary heart disease (Libby et al., 2018), obesity and metabolic disorders (Odegaard and Chawla, 2013), cancer (Crusz and Balkwill, 2015), and several psychiatric disorders including depression (A. H. Miller and Raison, 2016). Inflammation involves an acute response by innate immune cells to injury and infection, which eradicates invading pathogens, promotes tissue healing, and then terminates. Although beneficial in the short term, prolonged persistence of inflammation without a clear microbial target is a major contributor to highly prevalent and costly chronic diseases with inflammatory underpinnings (Libby, 2002; G. E. Miller et al., 2011). Thus, understanding risk factors for the development of elevated inflammation would pave the way for effective early intervention.

Motivated by the vision of early intervention, recent studies have begun assessing inflammatory markers in childhood. Importantly, childhood inflammatory markers can predict later health outcomes in a dose-response manner. For example, inflammatory markers in children under the age of 10 predict subsequent increases in body mass index (BMI) over baseline BMI (Bernard et al., 2019; Lourenco et al., 2014). Additionally, one large population-based study in England with 4500 children found that the inflammatory marker IL6 assessed in serum at age 9 predicted depressive symptomatology at age 18 in a dose-response manner (Khandaker et al., 2014). These patterns suggest the need to identify early risk factors that explain elevations in inflammatory markers during childhood, which could then become targets for early intervention.

Prevailing theory suggests that neuro-immune communication is an important driver of inflammation (Hostinar et al., 2017; Irwin and Cole, 2011). For example, connections between the autonomic nervous

* Corresponding authors.

E-mail addresses: nvalen@ucdavis.edu (N.V. Alen), cehostinar@ucdavis.edu (C.E. Hostinar).

system (ANS) and peripheral inflammation have been documented in both animal models and in humans. These studies have shown that sympathetic activation stimulates pro-inflammatory gene expression and cytokine production (Irwin and Cole, 2011; Nance and Sanders, 2007), whereas parasympathetic activity reduces the production of inflammatory markers by immune cells (Borovikova et al., 2000; Tracey, 2002). For instance, rodent studies have revealed a cholinergic anti-inflammatory pathway, such that electrical or pharmacological stimulation of the efferent vagus nerve inhibits systemic inflammation in response to endotoxin, reducing production of cytokines such as IL6 and TNFa (Borovikova et al., 2000; Tracey, 2002).

Consistent with this notion, human studies show that greater parasympathetic and less sympathetic modulation of cardiac activity at rest is associated with lower circulating levels of inflammatory markers such as IL6 (Cooper et al., 2015; Lockwood et al., 2017; Révész et al., 2014; Sloan et al., 2007), and reduced production of inflammatory cytokines in response to a bacterial product (Marsland et al., 2007a). However, to date these questions have not been examined in children, leaving open the question whether cardiac autonomic activity could serve as a risk marker for inflammation during development.

In addition, previous research on the relation between the ANS and immune processes has been largely limited to measures of a single branch of the ANS in isolation (i.e., either sympathetic or parasympathetic). The sympathetic and parasympathetic branches of the ANS do not always work in coordination (Berntson et al., 1991; Quigley and Moore, 2018). Considering evidence for both sympathetic and parasympathetic influences on the immune response, single branch ANS measures may therefore preclude a comprehensive understanding of neuro-immune connections.

In order to address these gaps in the literature, the current study tested the relation between autonomic functioning and inflammatory responses to challenge in children. We utilized calculations of cardiac autonomic balance, derived from sympathetic and parasympathetic autonomic recordings. We hypothesized that children exposed to an acute social stressor would exhibit greater increases in inflammatory markers, compared to controls. We also hypothesized that children who received social support from parents prior to acute stress exposure would exhibit changes in cytokine levels similar to controls, based on prior literature in adults suggesting that social support is associated with lower levels of serum inflammatory markers (Marsland et al., 2007b; Uchino et al., 2018). Lastly, we hypothesized that children with lower cardiac autonomic balance, indicating relatively lower parasympathetic and higher sympathetic modulation of cardiac activity at rest, would exhibit greater increases in inflammatory markers following challenge.

2. Methods

2.1. Participants

Participants included children, ages 9–11 years old, recruited from the Sacramento-Davis area through the University of California, Davis Participant Pool system and advertisements on Facebook. Exclusion criteria included: developmental disorder, chronic health condition, speech or language disorder, and currently taking psychotropic or steroid medication. Participants' parents were also asked if their child was, or had recently been, ill (e.g., the flu), and if so their lab visit was scheduled two weeks after symptoms abated. This study was part of a larger study looking at the social and emotional development of children; as such, sample size was determined for reasons not directly related to the current analysis. A total of 133 children were recruited for a laboratory visit. Of these children, 37 children did not participate in the blood draw procedure (21 participants declined to participate in one or both blood draws; the phlebotomist had a scheduling conflict for 13 visits; the phlebotomist was unable to draw sufficient blood from two participants; and one participant ended the session before the first

blood draw). This resulted in a sample of $N = 96$ children with data available for the current analysis. Participants in the current study included 51 boys and 45 girls (mean age = 9.93 years, $SD = 0.57$ years). Participants were 54.2 % non-Hispanic white, 33.3 % mixed race/ethnicity, 4.2 % Black/African American, 3.1 % Hispanic/Latino, 3.1 % Asian, and 2.1 % American Indian or Alaska Native. Participants' families had a mean yearly household income of \$127,194 ($SD = \$72,934$). The study was approved by the Institutional Review Boards of the University of California-Davis and the State of California Committee for the Protection of Human Subjects.

2.2. Procedure

Experiments were performed in late afternoon to account for diurnal variation in cytokine levels. Upon arrival at the laboratory, informed consent was obtained from the participant's parent or guardian, and informed assent was obtained from the participant. Participants were randomly assigned to one of three conditions, (1) the *alone* condition ($n = 29$), (2) the *parent* condition ($n = 33$), or (3) the *control* condition ($n = 34$), as described next.

2.2.1. Trier social stress test – modified (TSST-M)

The Trier Social Stress Test (TSST) is the most widely used laboratory social stressor for adults, and it involves a public speaking and an arithmetic challenge while being evaluated by judges and video-recorded for 10 min (Kirschbaum et al., 1993). The TSST-M is a modification of the TSST designed for children in our age range (Yim et al., 2010). The TSST-M is very similar to the adult TSST, with two differences: the prompt for the public speaking task was adapted to ask children to imagine introducing themselves to a new classroom and the mental arithmetic task was modified to be age-appropriate (see Yim et al., 2010 for additional details on conducting this protocol). In the current study, the procedure consisted of the following steps: participants were told that they had ten minutes to prepare a speech that would be evaluated by judges and recorded on camera to be later analyzed. In the *alone* condition, participants prepared alone in a room. In the *parent* condition, participants prepared with the help of their parent, who was instructed to support their child in any way they find natural. After the ten-minute preparation period, participants were taken to a novel room where there were two judges in white lab coats and a video camera. The participant then engaged in a five-minute speech followed by a five-minute arithmetic subtraction task. Judges refrained from showing facial affect or providing feedback to the child during the process.

Children randomly assigned to the *control* condition engaged in a placebo TSST-M that consisted of the following steps: participants were informed that they were part of a calm comparison group, and were asked to spend 10 min with their parent, thinking about their favorite book or movie, and writing down some ideas about the plot of the book or movie. Following this 10-minute period, participants were taken to a novel room where they engaged in five minutes of friendly conversation about the chosen book or movie with the experimenter, matching the speaking demands of the TSST-M but excluding the elements of social evaluation (i.e., judges and video camera). To match the mental arithmetic component of the TSST-M, the participants were then asked to play a Sudoku game (level: easy) for five min. Participants were told that their performance on the Sudoku was not important, and the experimenter spent time tidying up the room during this part of the task, to prevent the participant from feeling watched and evaluated on their performance.

2.2.2. Blood draw and serum processing procedure

The first blood draw occurred 30 min after arrival at the laboratory to allow children to acclimate to the new environment, before administration of the TSST-M or TSST-M placebo; the second blood draw occurred 100 min after the TSST-M, consistent with some evidence in

adults that cytokines such as IL6 peak in blood 90–120 min post-stress (Marsland et al., 2017; Steptoe et al., 2007). Blood draws were performed by trained and certified phlebotomists with previous experience drawing blood from pediatric samples. Blood draws were taken from the median cubital vein. Blood samples were inverted gently 5 times, and then left to clot in Serum Separator Tubes for 60 min. Following the 60-minute clotting time, samples were centrifuged at 2700 RPM for 10 min, after which sample serum was aliquoted into micro vials and stored at -80 degrees Celsius, until being shipped to Northwestern University for assaying.

2.3. Measures

Demographic data were collected from participants and parents through self-report or parent report questionnaires. This included: participant age, sex, and highest parental education level. Highest parental education level was the highest level of education among parents, either mother or father, that culminated in the attainment of a degree, and was coded as an ordinal variable: 0 = *less than high school* (1%); 1 = *high school diploma or GED* (11.5 %); 2 = *2-year or vocational degree* (12.5 %); 3 = *4-year degree* (31.3 %); 4 = *master's level degree* (32.2 %); 5 = *doctoral level degree* (11.5 %). Children's weight (kg) and height (m) were measured using a physician beam-scale and stadiometer, for the calculation of body mass index (BMI) using the following formula: $BMI = \text{weight}/(\text{height}^2)$.

2.3.1. Respiratory sinus arrhythmia

Respiratory Sinus Arrhythmia (RSA) was utilized as a marker of parasympathetic activity, where higher RSA reflects greater parasympathetic modulation of cardiac activity (Laborde et al., 2017). RSA was collected with a MindWare ambulatory electrocardiogram (ECG; MindWare, Westerville, OH), using three silver electrodes with a 7% chloride wet gel attached to the child's chest in Einthoven's triangle configuration. RSA data were collected during a resting 5-minute period, as part of a larger ECG data collection procedure. Current analysis focused on the resting period, during which children were in a seated position on a comfortable couch. All children were tested in the same room, on the same couch, and instructed to sit in the same seated position. Participants were instructed to not engage in any activity, to refrain from speaking to their parent, and to attempt to relax for the five-minute duration.

Interbeat Interval (IBI) data were calculated using an automated algorithm in the MindWare Biolab acquisition software. A high-frequency band pass filter set at .24–1.04 Hz was used to correspond to the average breathing rate of this age range (Quigley and Stifter, 2006). Sampling rate was set at 250 Hz. R-peaks were inspected and cleaned for artifacts by trained researchers using MindWare Heart Rate Variability software. Arrhythmias (e.g., ectopic beats, sinus pauses) were corrected using the MindWare mid-beat function, which averages the IBI interval and minimizes the influence of artifacts. RSA was calculated, using a Fast Fourier transformation algorithm, in 60-second epochs (Berntson et al., 1997). A 60-second epoch was considered usable when it met two criteria: (1) at least 30-seconds of clean, continuous data were available, and (2) less than 10 % of R-peaks were estimated. Manual inspection of respiration rate ensured that participant respiration rate did not fall outside of the high-frequency band pass filter range. RSA during the individual 60-second epochs were then averaged together, producing a mean resting RSA value. Three participants were missing resting RSA data for the following reasons: excessive, un-cleanable noise ($n = 2$), and ECG technical malfunction ($n = 1$).

2.3.2. Pre-ejection period

Pre-ejection period (PEP) was utilized as a marker of sympathetic activity, where longer PEP reflects less SNS modulation of cardiac activity (Berntson et al., 2004). PEP has been widely used as a non-

invasive index of sympathetic modulation of the heart (Bagley and El-Sheikh, 2014; Forouzanfar et al., 2018), and has been previously validated using pharmacological blockade (Berntson et al., 1994; Cacioppo et al., 1994; Mezzacappa et al., 1999; Schächinger et al., 2001; Winzer et al., 1999).

PEP was calculated from cardiac impedance data measured concurrently to ECG data collection using a MindWare ambulatory device (MindWare, Westerville, OH). Four silver electrodes with a 7% chloride wet gel were attached to the child's chest and back in standard configuration (Sherwood et al., 1990), including two on the chest (one at the top of the sternum and one at the xiphisternal junction) and two on the back (one over the C4 vertebrae and one over the thoracic spine). The impedance signal was used to derive dZ/dt , the first derivative of the change in thoracic impedance. PEP was defined as the amount of time in milliseconds between the Q-wave of the ECG signal and the B-notch of the dZ/dt signal (Berntson et al., 2004). For the identification of the B-notch we employed a two-stage approach recommended by Lozano and colleagues (2007). When impedance data provided a clear signal with a visible B-notch, an algorithm was utilized that identified the B-notch as the peak of the second derivative of the dZ/dt ; when impedance data did not provide a clear visible B-notch, the B-notch was estimated using a percentage of the R-peak to Z-peak interval (RZ interval) in milliseconds plus a constant, set at 4 milliseconds: $B\text{-notch} = 0.55 \times RZ \text{ interval} + 4$, (Lozano et al., 2007). Due to the small percentage of participants with impedance data that showed a clear visible B-notch (< 3%), all PEP values were calculated using the second estimation method for consistency. Current analysis focused on a 5-minute resting baseline measure of PEP, during which participants were in a seated position on a comfortable couch. All children were tested in the same room, on the same couch, and instructed to sit in the same seated position. Six participants were missing resting PEP data for the following reasons: excessive, un-cleanable noise ($n = 5$), and ECG technical malfunction ($n = 1$).

2.3.3. Inflammatory cytokines

Cytokine assays were performed at Northwestern University in the Foundations of Health Research Center using the following procedure: after the serum aliquots had been thawed, they were assayed in triplicate for the following cytokines: interleukin-6 (IL6), interleukin-8 (IL8), interleukin-10 (IL10), and tumor necrosis factor-alpha (TNF α). The assays were performed with a custom four-plex assay on the SimplePlex Platform (Protein Simple, San Jose, CA). This integrated system conducts automated fluorescence immunoassays using disposable microfluidic cartridges. It yields data with high levels of accuracy and reproducibility (Aldo et al., 2016.) In the current study, the mean inter-assay CVs for triplicate runs were: 4.89 % for IL6, 4.70 % for TNF α , 3.41 % for IL10, and 2.33 % for IL8.

2.4. Primary outcomes

2.4.1. Cardiac autonomic balance

Cardiac autonomic balance was calculated as the relative contribution of parasympathetic to sympathetic modulation of cardiovascular activity, using a formula developed by Berntson and colleagues (2008). RSA was used as an index of parasympathetic modulation of cardiovascular activity. PEP was utilized as an index of sympathetic modulation of cardiovascular activity. Due to differences in the scaling of these two indices, values were z-scored, producing: z-scored RSA (zRSA) and z-scored PEP (zPEP). RSA is positively related to parasympathetic activity, whereas PEP is negatively related to sympathetic activity, we therefore multiplied zPEP times (-1). CAB was calculated as: $CAB = zRSA - (-zPEP)$, as previously described (Berntson et al., 2008).

2.4.2. Cytokine change scores

Of the 96 participants who completed both blood draws, IL6 levels

for one participant were deemed abnormally high and indicative of illness by the laboratory that conducted the assaying procedure. This value was therefore excluded from analysis. From the remaining cytokine data, three IL6 values, two IL8 values, and two IL10 values, were outliers ($> 4 SD$ from the mean), these were therefore Winsorized to the highest value within $4 SD$ from the mean. Cytokine change scores were computed by subtracting Time 1 cytokine levels from Time 2 cytokine levels, such that higher change scores represent greater increases in circulating cytokine levels from Time 1 to Time 2.

2.5. Statistical analysis

Statistical analysis was conducted using SPSS version 25. To test the effects of condition (*alone*, *parent*, or *control*) we conducted four multiple linear regressions, regressing each cytokine change score (IL6, IL8, IL10, & TNFa) onto two dummy-coded variables representing the *alone* condition, and the *parent* condition, compared to controls. To test the relation between CAB and cytokine change, CAB values were included in the models, in addition to the following covariates: child age, child sex, child BMI, and highest parental education level. To control for inflated Type-I error due to multiple comparisons involving the four cytokines, estimated significance was assessed using the Benjamini-Hochberg False Discovery Rate (FDR) correction (Benjamini and Hochberg, 1995).

2.5.1. Missingness

Little's MCAR test was non-significant, $\chi^2(25) = 36.41, p = .07$, consistent with a missing completely at random (MCAR) pattern. In addition, individuals with and without cytokine change score data did not differ significantly in: resting CAB, age, sex, BMI, or highest parental education level.

3. Results

Sample descriptives for the main study variables and demographics are presented in Table 1. Complete bivariate correlational results are presented in Table 2.

3.1. Cardiac autonomic correlates

Consistent with its denotation as a spectrum from parasympathetic dominant to sympathetic dominant cardiac autonomic balance (CAB), CAB values were highly positively correlated with both RSA ($r = .71, p < .001$) and PEP ($r = .75, p < .001$; note: longer PEP indicates lower sympathetic activity). Unsurprisingly, CAB was significantly negatively correlated with heart rate (HR, $r = -0.62, p < .001$), consistent with previous research in adults (Berntson et al., 2008). CAB was not significantly correlated with any demographic variable, or with participant BMI. RSA was significantly correlated with participant sex ($r = -0.22, p = .03$), such that girls exhibited lower RSA. Consistent with lower parasympathetic modulation of cardiac activity (i.e., lower RSA) in girls, girls also exhibited higher HR compared to boys ($r = 0.23, p = .03$).

3.2. Cytokine change correlates

Change in IL6 was positively correlated with IL8 change ($r = 0.23, p = 0.03$), and TNFa change ($r = 0.29, p = 0.005$). IL8 change was also correlated with change in IL10 ($r = 0.42, p < 0.001$), and change in TNFa ($r = .29, p = 0.004$). IL10 change was positively correlated with TNFa change ($r = 0.24, p = 0.02$). IL8 change was higher in girls, compared to boys ($r = 0.23, p = 0.03$). No other significant relations between cytokine change and participant demographics were observed. Although inflammatory marker change scores were not associated with BMI, we found significant relations between baseline (i.e., Time 1) cytokine levels and BMI, as would be expected. Specifically, BMI was

Table 1
Sample Characteristics.

	M	SD	Range
CAB	-0.12	1.48	-3.81-3.43
RSA	6.52	1.20	3.83-9.33
PEP	79.31	11.07	42.67-105.20
HR	86.84	11.39	65.09-113.86
IL6 (pg/mL) Time 1	1.30	0.75	0.26-3.93
IL6 (pg/mL) Time 2	1.46	1.06	0.20-5.57
IL6 Change	0.16	0.77	-1.36-3.45
IL8 (pg/mL) Time 1	7.17	2.00	3.63-14.02
IL8 (pg/mL) Time 2	6.95	1.90	3.19-12.34
IL8 Change	-0.23	0.91	-3.13-1.49
IL10 (pg/mL) Time 1	2.45	1.14	1.13-7.99
IL10 (pg/mL) Time 2	2.40	1.04	0.90-6.10
IL10 Change	-0.05	0.45	-1.89-1.06
TNFa (pg/mL) Time 1	6.18	1.32	3.59-9.84
TNFa (pg/mL) Time 2	6.14	1.46	2.45-11.04
TNFa Change	-0.04	1.04	-3.38-2.28
Age	9.93	0.57	9.12-11.10
BMI	17.89	3.16	12.92-28.90
	Level	N	%
Sex	Male	51	53.1
	Female	45	46.9
Parental Education	Less than high school	1	1.0
	High school or GED	11	11.5
	2-year or vocational degree	12	12.5
	4-year degree	30	31.3
	Masters level degree	31	32.2
	Doctoral level degree	11	11.5

Note. CAB = cardiac autonomic balance. RSA = respiratory sinus arrhythmia. PEP = pre-ejection period. HR = heart rate. IL6 = interleukin-6. IL8 = interleukin-8. IL10 = interleukin-10. TNFa = tumor necrosis factor-alpha. BMI = body mass index. Parental education is the highest education level among parents.

positively correlated with baseline IL6 ($r = 0.28, p = .004$) and baseline IL10 ($r = 0.23, p = .02$).

3.3. Condition effects and cytokine change

Regression results revealed no significant differences in cytokine change by condition. Specifically, individuals in the *alone* condition did not differ from those in the *control* condition in: IL6 change ($B = -0.29, p = .13$), IL8 change ($B = 0.02, p = .94$), IL10 change ($B = -0.14, p = .21$), or TNFa change ($B = -0.32, p = .23$). In addition, individuals in the *parent* condition did not differ from controls in: IL6 change ($B = 0.15, p = .42$), IL8 change ($B = 0.12, p = .61$), IL10 change ($B = -0.03, p = .76$), or TNFa change ($B = -0.17, p = .51$). IL6 levels by condition are presented in Fig. 1 to illustrate both the non-significant main effects and large individual differences within each condition.

3.4. CAB and cytokine change

Across all four cytokine change scores, lower CAB was associated with greater increase in circulating cytokines over the course of the experiment. Specifically, at the bivariate level, CAB was negatively correlated with IL6 change ($r = -0.25, p = .02$), IL8 change ($r = -0.22, p = .04$), and IL10 change ($r = -0.30, p = .004$). CAB was also negatively correlated, at the trending level, with TNFa change ($r = -0.21, p = .05$).

Multiple linear regression results (Table 3) revealed that, after controlling for covariates, lower CAB remained significantly associated with greater increase in IL6 ($B = -0.14, SE = 0.06, \beta = -0.26, p = .015$), and IL10 ($B = -0.09, SE = 0.03, \beta = -0.30, p = .005$). Lower CAB was also marginally significantly associated with greater increase in IL8 ($B = -0.11, SE = 0.07, \beta = -0.19, p = .09$), and TNFa ($B = -0.14, SE = 0.08, \beta = -0.19, p = .09$). The associations between CAB, and change in IL6 and IL10, remained significant after controlling for

Table 2
Bivariate Correlation Matrix.

	2	3	4	5	6	7	8	9	10	11	12
1. CAB	.71***	.75***	-.62***	-.25*	-.22*	-.30**	-.21†	.00	-.20†	.03	.12
2. RSA	-	.07	-.79***	-.22*	-.18†	-.21*	-.19†	.02	-.22*	.05	-.13
3. PEP		-	-.16	-.13	-.10	-.24*	-.09	.02	-.07	-.01	.19†
4. HR			-	.30**	.13	.11	.14	.02	.23*	-.08	.15
5. IL6 Change				-	.23*	.08	.29**	-.12	.04	.03	.01
6. IL8 Change					-	.42***	.29**	-.03	.23*	-.06	.02
7. IL10 Change						-	.24*	.05	-.01	.12	-.09
8. TNFa Change							-	-.07	.08	.03	-.05
9. Age								-	.00	-.07	.07
10. Sex (female)									-	-.03	.10
11. Parental Education										-	-.11
12. BMI											-

Note. CAB = cardiac autonomic balance. RSA = respiratory sinus arrhythmia. PEP = pre-ejection period. HR = heart rate. IL6 = interleukin-6. IL8 = interleukin-8. IL10 = interleukin-10. TNFa = tumor necrosis factor-alpha. BMI = body mass index. Inflammatory change scores calculated by subtracting Time 1 from Time 2. Parental education is the highest education level among parents, coded as the following: less than highschool = 0, highschool or GED = 1, 2-year or vocational degree = 2, 4-year degree = 3, master's level degree = 4, doctoral level degree = 5.

† p < .10.
* p < .05.
** p < .01.
*** p < .001.

multiple comparisons. FDR calculations are presented in supplemental Table S1. The relations between CAB and cytokine change scores are presented in Fig. 2.

3.5. Post-hoc exploratory analysis

Post-hoc exploratory analysis was conducted to test associations between CAB and cytokine change within each condition. Within the TSST-M *alone* condition, CAB was correlated with change in IL8 ($r = -0.47, p = .02$), IL10 ($r = -0.42, p = .03$), and at the trending level with change in IL6 ($r = -0.34, p = .09$), but not with change in TNFa ($r = -0.23, p = .26$). Within the TSST-M *parent* condition, CAB was correlated with change in IL10 ($r = -0.47, p = .007$), TNFa ($r = -0.46, p = .009$), and at the trending level with change in IL6 ($r = -0.33, p = .07$), but not with change in IL8 ($r = -0.14, p = .45$). Within the non-stress *control* condition, CAB was not correlated with any cytokine change score: IL6 ($r = -0.14, p = .45$), IL8 ($r = -0.07, p = .68$), IL10 ($r = -0.01, p = .94$), and TNFa ($r = 0.07, p = .71$).

4. Discussion

This is the first study to test the relation between autonomic

function and changes in inflammatory markers in response to acute stress in children, to our knowledge. Increasing evidence suggests early risk detection may be crucial in implementing successful interventions in the etiology of inflammatory-based mental and physical health conditions (Chen et al., 2017). In addition, this is the first study to utilize measures of both sympathetic and parasympathetic modulation of cardiac activity, to test the association between cardiac autonomic balance and serum inflammatory markers. There is increasing understanding that single branch autonomic measures are unable to fully describe individual differences in autonomic functioning (Quigley and Moore, 2018).

Surprisingly, we did not find condition effects of acute stress exposure on changes in circulating cytokine levels. Previous meta-analytic results suggest that in adults acute stress exposure leads to average increases in circulating IL6 (Stephens et al., 2007), IL10, and TNFa (Marshall et al., 2017). However, several individual differences factors have been found to moderate stress-induced inflammatory responses in adults. For example, a greater inflammatory response to acute stress has been observed in individuals with depression (G. E. Miller et al., 2005), low self-esteem (O'Donnell et al., 2008), a history of childhood trauma (Carpenter et al., 2010), those of a lower social-economic status (Brydon et al., 2004; Stephens et al., 2002), and individuals with higher

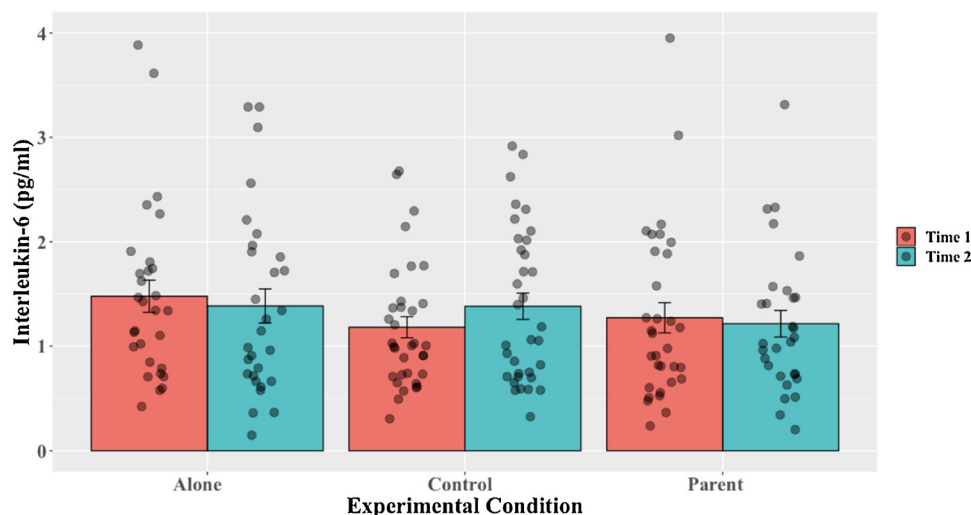


Fig. 1. Bar plot showing serum interleukin-6 (IL6) levels at Time 1 and Time 2, grouped by experimental condition. Error bars represent +/- 1 standard error.

Table 3
Linear Regression Results Predicting Cytokine Change.

Predictor	Model Dependent Variable											
	IL6 Change			IL8 Change			IL10 Change			TNFa Change		
	B	SE	p	B	SE	p	B	SE	p	B	SE	p
CAB	-.14 ^a	.06	.015	-.11	.07	.09	-.09 ^a	.03	.005	-.14	.08	.09
Age	-.09	.14	.52	-.02	.17	.90	.07	.08	.38	-.13	.20	.54
Sex	.01	.17	.94	.33	.20	.10	-.03	.10	.76	.18	.23	.44
Parental Education	.02	.07	.79	-.05	.08	.53	.05	.04	.18	.04	.10	.66
BMI	.02	.03	.49	.01	.03	.67	-.01	.02	.45	-.00	.04	.98
Alone Condition	-.31	.20	.12	.06	.24	.80	-.19	.12	.10	-.38	.28	.19
Parent Condition	.16	.19	.41	.20	.23	.40	-.05	.11	.65	-.16	.27	.56

Note. IL6 = interleukin-6. IL8 = interleukin-8. IL10 = interleukin-10. TNFa = tumor necrosis factor. CAB = cardiac autonomic balance. BMI = body mass index. Parental education is the highest education level among parents, coded as the following: less than highschool = 0, highschool or GED = 1, 2-year degree = 2, 4-year degree = 3, master's level degree = 4, doctoral level degree = 5.

^a estimate p-value significant after Benjamini-Hochberg correction for multiple comparisons (i.e., 4 models).

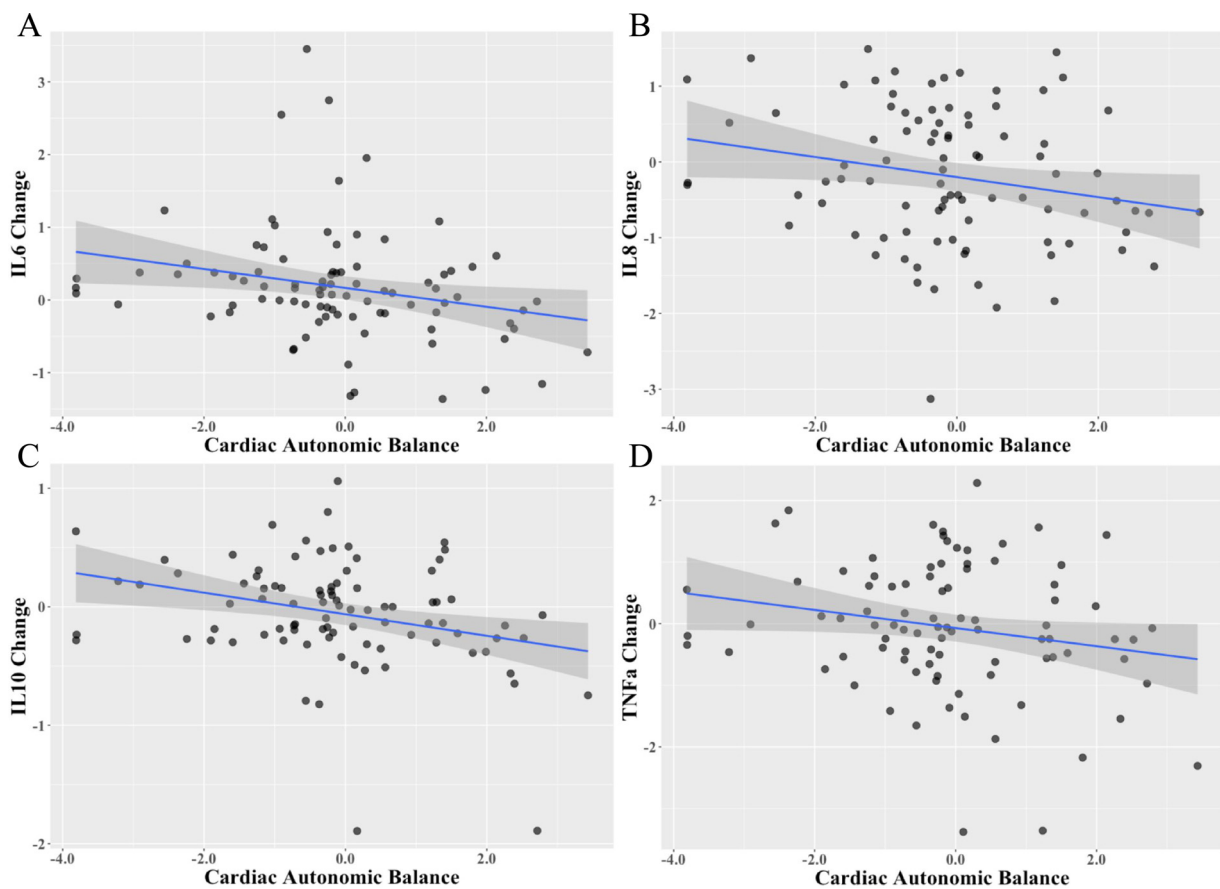


Fig. 2. Scatterplots of the relations between cardiac autonomic balance (CAB) and (A) interleukin-6 (IL6) change, (B) interleukin-8 (IL8) change, (C) interleukin-10 (IL10) change, and (D) tumor necrosis factor-alpha (TNFa) change. Change scores were calculated such that higher scores represent greater increases in cytokine levels from Time 1 to Time 2. Shaded area represents the 95 % confidence interval.

adiposity (Brydon et al., 2008). This suggests that individuals with poorer psychosocial functioning and physical health tend to exhibit greater increases in inflammatory markers in response to acute stress. Our non-clinical community sample consisted of healthy children with BMI in the normal range and middle-to-high SES, given the composition of the existing Participant Pool. This may explain why we did not find average main effects of acute stress exposure. Our future directions include the recruitment of lower-SES samples and children with mental and physical health diagnoses.

Alternatively, these results may suggest lower average stress-induced immune responses in children compared to adults. Acute stress effects on circulating IL6 levels have been previously documented in

adolescents, but only in those with higher BMI (Chiang et al., 2017), and in those who reported high peer-victimization and hopelessness (Giletta et al., 2018). There is very limited research on inflammatory responses to challenge in children, using salivary samples, and results are mixed. For example, no significant changes in salivary interleukin-1β following challenge have been observed in preschool children (Tyrka et al., 2015). A different study found stress-induced increases in salivary IL6 for girls ages 8–10 years, but decreases for boys (El-Sheikh et al., 2007). A third study, with preschoolers, found no significant changes in salivary IL6 following an emotionally challenging task, but found decreases in TNFa and IL8 (Riis et al., 2015). Future research in pediatric populations is needed to better understand if and when acute

stress exposure leads to average increases in circulating cytokine levels in children.

For our second hypothesis, we predicted that lower cardiac autonomic balance would be associated with greater increases in cytokine levels. Consistent with this hypothesis, we found that children with lower resting CAB (i.e., a combination of lower parasympathetic and higher sympathetic activity) exhibited greater increases in IL6 and IL10 across the experimental protocol. Importantly, these results remained significant after controlling for covariates and adjusting for multiple comparisons. These findings are consistent with previous research showing that higher baseline circulating cytokine levels are observed in adults with either low parasympathetic activity or high sympathetic activity (Révész et al., 2014; Williams et al., 2019). These findings are also consistent with prior studies in human and non-human animal models supporting modulation of inflammatory activity by the autonomic nervous system (Borovikova et al., 2000; Irwin and Cole, 2011; Nance and Sanders, 2007; Tracey, 2002).

Lower CAB was also associated with greater increases in IL8 and TNF α , though these relations were only trending and were not significant after controlling for multiple comparisons. This suggests that in children, CAB may be more strongly related to IL6 and IL10 increases during challenge compared to IL8 and TNF α increases. This could reflect differential associations between the autonomic nervous system and the four cytokines assayed in the current study. A recent meta-analysis found that lower parasympathetic modulation of cardiac activity (RSA) was consistently associated with higher circulating IL6 levels, but was not associated with TNF α levels (Williams et al., 2019). Too few studies have tested associations between the ANS and either IL10 or IL8 to facilitate meta-analysis, thus further research is needed on these cytokines. Post hoc analysis of achieved power suggested that we were underpowered to detect relations between CAB and both IL8 and TNF α (achieved power was only 0.71 and 0.67, respectively, which is below the recommended 0.8 power), largely due to missing data. Future research should therefore aim for a larger sample size, taking into account missing data problems associated with high rates of blood draw refusal in children. This will resolve the issue of whether non-significant links with TNF α and IL8 are true null effects or simply need larger samples to detect.

There are two possible interpretations for the increasing serum cytokine levels from Time 1 to Time 2. One interpretation is that these increases are stress-related, but only some children exhibited them so the group averages did not reflect these tendencies well, resulting in non-significant main effects of condition. As can be seen in Fig. 2, all cytokine change scores showed a broad spread with many children showing increases and many showing decreases in cytokine levels across conditions. Supporting the interpretation that cytokine increases may be stress-related in some children, our post-hoc exploratory analysis found stronger and significant associations between CAB and cytokine change within the two conditions experiencing the TSST-M stressor (alone or with prior parent support), but non-significant correlations in the non-stress *control* condition (section 3.5). This suggests that CAB may exhibit a stronger association with increases in inflammatory markers within contexts characterized by threat. These results should be interpreted with caution, given the exploratory methods utilized and the small sample sizes within each condition. The second possible interpretation is that our results reflect relations between children's CAB and differential diurnal trajectories of circulating cytokines. There is very limited literature on diurnal variation in serum cytokines, particularly in children. However, there is some evidence in adults that inflammatory cytokine levels display regular patterns throughout the day (Izawa et al., 2013), with cytokines increasing from afternoon to evening. Thus, our results may suggest that children with low cardiac autonomic balance show greater increases in cytokines across the afternoon, as evening approaches. Future research involving repeated sampling throughout the day could provide insight into how autonomic function may influence immune system diurnal patterns in

children, and how these underlying patterns can be differentiated from the effects of acute challenge.

Finally, a strength of our study is that it highlights the utility of cardiac autonomic balance measures in neuro-immune research. Our bivariate correlation matrix revealed several significant associations of cytokine change scores with individual parasympathetic (RSA) or sympathetic (PEP) measures, though notably all correlation coefficients were smaller between inflammatory markers and either RSA or PEP, compared to correlation coefficients between inflammatory markers and CAB. This suggests that the integrated relative contribution of parasympathetic to sympathetic activity may provide a stronger signal than single branch measures. Researchers interested in the association between autonomic and immune processes could benefit from utilizing valid indices of CAB, such as that derived from simultaneous recordings of RSA and PEP.

4.1. Limitations and future directions

This study contributes novel information towards a more comprehensive understanding of the connection between the autonomic and immune systems, extending past findings to include cardiac autonomic balance and inflammatory reactivity in children. Nevertheless, some limitations warrant attention.

First, our sample size was reduced due to missing serum data. Although our analysis of missingness did not reveal any systematic patterns, it is still possible that children who declined the blood draw were different from those who completed the blood draw in some unmeasured characteristic. Considering the challenges associated with pediatric research involving the collection of multiple biological samples, some missing data are expected. However, future research should consider the practical advice provided below in section 4.2, in order to help limit missing data in pediatric samples.

Second, it is important to note that CAB reflects the relative contribution of parasympathetic to sympathetic innervation of the heart, and therefore can only serve as a non-invasive proxy of autonomic activity in other bodily organs. Autonomic effects on immune activity are theorized to occur primarily through innervation of the spleen or liver, which are major sources of cytokine production (Tracey, 2007). Our results are also limited by the low-risk, healthy sample, which limits generalizability. We plan to examine these processes in more at-risk children in our future directions. Lastly, our findings are limited by the correlational design, which precludes causal inferences. Future research could benefit from creative methods of experimentally manipulating CAB, for example through random assignment to biobehavioral feedback training, in order to better test how CAB affects inflammatory processes. It is important to consider that the effects we detected may underestimate true effects due to the random error introduced by the inter-assay CV for serum cytokine assays. Nevertheless, our assay error was lower than most studies (average CV of 2.3–4.9%) and we assayed samples in triplicate to minimize such error.

4.2. Practical recommendations and theoretical implications

Pediatric research involving the collection of multi-system biological data has the potential to help further our understanding of how important biological systems, such as the immune system and the autonomic nervous system, interact across development. It is therefore critical to properly address the challenges to such a research endeavor, in order to mitigate reservations and promote the expansion of research in multi-system developmental psychobiology. With this in mind, we provide here several practical methodological strategies that we found to be helpful for the collection of autonomic and serum biomarkers in children. During ECG and impedance electrode placement, we found it necessary to refer to electrodes as *stickers* in order to avoid participant misconception, and resulting anxiety, that the ambulatory ECG would shock or electrocute them. We also found that expressing excitement

about the ECG device, comparing it to an astronaut's computer, and asking the participant to help identify the placement locations (e.g., *can you point to your lowest rib?*), helped make the participant feel more involved, less like a subject, and therefore more comfortable during the process.

During blood draws, we found that having several friendly and colorful images on the walls (e.g., smiling animals, jungle scenes) provided a distraction for the participant, and helped create a warm environment in an otherwise sterile phlebotomy room. The use of an oversized adult phlebotomy chair enabled us to allow parents to sit next to their child if the participant requested, which eased reservations in some participants. Phlebotomists were most successful when they provided a more concise description of the process before the blood draw; a lengthy and detailed introduction often led to increased participant anxiety and refusal to continue. Lastly, we provided participants with an optional topical anesthetic approved for pediatric use (lidocaine 4%) if requested; this numbing gel was offered when a participant expressed fear of the blood draw hurting. It is our goal that these recommendations can serve as a guide for researchers interested in developmental psychobiology.

In conclusion, this study found that children with low cardiac autonomic balance exhibited greater increases in circulating inflammatory cytokines. Importantly, we observed this association in healthy, low-risk children, under conditions of relatively mild challenge. This is a critical consideration, as it suggests that low CAB may represent a risk factor even in relatively favorable conditions. As such, it is possible that for children with poor mental and physical health, who may exhibit a greater inflammatory response to challenge, low CAB may represent an even greater risk factor. In addition, it is possible that CAB may be more strongly related to immune responses under conditions of greater environmental threat (e.g., abuse). If these contextual and individual risk factors co-occurred, we might find low CAB to be a crucial determinant of the development of inflammatory disease in at-risk populations. A comprehensive understanding of risk factors in the etiology of systemic elevated inflammation could pave the way for the development and application of targeted early risk identification and intervention.

Acknowledgments

This study was funded, in part, by the National Science Foundation grant 1327768. The first author's efforts were supported by the Dean's Distinguished Graduate Fellowship (DDGF) provided by the University of California, Davis. We thank Professor Gregory E. Miller and Mr. Adam K. Leigh from the Foundations of Health Research Center for their assistance with the cytokine assays.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.psypneu.2020.104745>.

References

- Aldo, P., Marusov, G., Svancara, D., David, J., Mor, G., 2016. Simple plexTM: a novel multi-analyte, automated microfluidic immunoassay platform for the detection of human and mouse cytokines and chemokines. *Am. J. Reprod. Immunol.* 75 (6), 678–693.
- Bagley, E.J., El-Sheikh, M., 2014. Relations between daytime pre-ejection period reactivity and sleep in late childhood. *J. Sleep Res.* 23 (3), 335–338. <https://doi.org/10.1111/jsr.12117>.
- Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. R. Stat. Soc. Ser. B* 57, 289–300.
- Bernard, K., Hostinar, C.E., Dozier, M., 2019. Longitudinal associations between attachment quality in infancy, C-reactive protein in early childhood, and BMI in middle childhood: preliminary evidence from a CPS-referred sample. *Attach. Hum. Dev.* 21 (1), 5–22. <https://doi.org/10.1080/14616734.2018.1541513>.
- Berntson, G.G., Cacioppo, J.T., Quigley, K.S., 1991. Autonomic determinism: The modes

- of autonomic control, the doctrine of autonomic space, and the laws of autonomic constraint. *Psychological Review* 98 (4), 459–487. <https://doi.org/10.1037/0033-295X.98.4.459>.
- Berntson, G.G., Cacioppo, J.T., Quigley, K.S., 1994. Autonomic cardiac control. I. Estimation and validation from pharmacological blockades. *Psychophysiology* 31, 572–585. <https://doi.org/10.1111/j.1469-8986.1994.tb02350.x>.
- Berntson, G.G., Bigger, J.T., Eckberg, D.L., Grossman, P., Kaufmann, P.G., Malik, M., van, d.M., 1997. Heart rate variability: origins, methods, and interpretive caveats. *Psychophysiology* 34 (6), 623–648.
- Berntson, G.G., Lozano, D.L., Chen, Y.J., Cacioppo, J.T., 2004. Where to Q in PEP. *Psychophysiology* 41 (2), 333–337. <https://doi.org/10.1111/j.1469-8986.2004.00156.x>.
- Berntson, G.G., Norman, G.J., Hawley, L.C., Cacioppo, J.T., 2008. Cardiac autonomic balance vs. cardiac regulatory capacity. *Psychophysiology* 45 (4), 1–7. <https://doi.org/10.1038/jid.2014.371>.
- Borovikova, L.V., Ivanova, S., Zhang, M., Yang, H., Botchkina, G.I., Watkins, L.R., Tracey, K.J., 2000. Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. *Nature* 405 (6785), 458–462. <https://doi.org/10.1038/35013070>.
- Brydon, L., Edwards, S., Mohamed-Ali, V., Steptoe, A., 2004. Socioeconomic status and stress induced increases in interleukin-6. *Brain Behav. Immun.* 18, 281–290.
- Brydon, L., Wright, C.E., O'Donnell, K., Zachary, I., Wardle, J., Steptoe, A., 2008. Stress-induced cytokine responses and central adiposity in young women. *Int. J. Obesity. (Lond.)* 32, 443–450.
- Cacioppo, J.T., Berntson, G.G., Binkley, P.F., Quigley, K.S., Uchino, B.N., Fieldstone, A., 1994. Autonomic cardiac control. II. Noninvasive indices and basal response as revealed by autonomic blockades. *Psychophysiology* 31, 586–598. <https://doi.org/10.1111/j.1469-8986.1994.tb02351.x>.
- Carpenter, L.L., Gawuga, C.E., Tyrka, A.R., Lee, J.K., Anderson, G.M., Price, L.H., 2010. Association between plasma IL-6 response to acute stress and early-life adversity in healthy adults. *Neuropsychopharmacology* 35, 2617–2623.
- Chen, E., Brody, G.H., Miller, G.E., 2017. Childhood close family relationships and health. *Am. Psychol.* 72 (6), 555–566. <https://doi.org/10.1037/amp0000067>.
- Chiang, J.J., Bower, J.E., Irwin, M.R., Taylor, S.E., Fuligni, A.J., 2017. Adiposity moderates links from early adversity and depressive symptoms to inflammatory reactivity to acute stress during late adolescence. *Brain Behav. Immun.* 66, 146–155. <https://doi.org/10.1016/j.physbeh.2017.03.040>.
- Cooper, T.M., McKinley, P.S., Seeman, T.E., Choo, T.H., Lee, S., Sloan, R.P., 2015. Heart rate variability predicts levels of inflammatory markers: evidence for the vagal anti-inflammatory pathway. *Brain Behav. Immun.* <https://doi.org/10.1016/j.bbi.2014.12.017>.
- Cruz, S.M., Balkwill, F.R., 2015. Inflammation and cancer: advances and new agents. *Nat. Rev. Clin. Oncol.* 12 (10), 584–596. <https://doi.org/10.1038/nrclinonc.2015.105>.
- El-Sheikh, M., Buckhalt, J.A., Granger, D.A., Erath, S.A., Acebo, C., 2007. The association between children's sleep disruption and salivary interleukin-6. *J. Sleep Res.* 16, 188–197. <https://doi.org/10.1111/j.1365-2869.2007.00593.x>.
- Forouzanfar, M., Baker, F.C., de Zambotti, M., McCall, C., Giovangrandi, L., Kovacs, G.T.A., 2018. Toward a better noninvasive assessment of prejection period: a novel automatic algorithm for B-point detection and correction on thoracic impedance cardiogram. *Psychophysiology* 55 (8), 1–12. <https://doi.org/10.1111/psyp.13072>.
- Gilletta, M., Slavich, G.M., Rudolph, K.D., Hastings, P.D., Nock, M.K., Prinstein, M.J., 2018. Peer victimization predicts heightened inflammatory reactivity to social stress in cognitively vulnerable adolescents. *J. Child Psychol. Psychiatry* 59 (2), 129–139. <https://doi.org/10.1111/jcpp.12804>.
- Hostinar, C.E., Nusslock, R., Miller, G.E., 2017. Future directions in the study of early-life stress and physical and emotional health: implications of the neuroimmune network hypothesis. *J. Clin. Child Adolesc. Psychol.* 1–15. <https://doi.org/10.1080/15374416.2016.1266647>.
- Irwin, M.R., Cole, S.W., 2011. Reciprocal regulation of the neural and innate immune systems. *Nat. Rev. Immunol.* 11 (9), 625–632. <https://doi.org/10.1038/nri3042>.
- Izawa, S., Miki, K., Liu, X., Ogawa, N., 2013. The diurnal patterns of salivary interleukin-6 and C-reactive protein in healthy young adults. *Brain Behav. Immun.* 27 (1), 38–41. <https://doi.org/10.1016/j.bbi.2012.07.001>.
- Khandaker, G.M., Pearson, R.M., Zammit, S., Lewis, G., Jones, P.B., 2014. Association of serum interleukin 6 and C-reactive protein in childhood with depression and psychosis in young adult life: a population-based longitudinal study. *JAMA Psychiatry* 71 (10), 1121–1128. <https://doi.org/10.1001/jamapsychiatry.2014.1332>.
- Kirschbaum, C., Pirke, K.-M., Hellhammer, D.H., 1993. The Trier Social Stress Test - A tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology* 28, 76–81.
- Laborde, S., Mosley, E., Thayer, J.F., 2017. Heart rate variability and cardiac vagal tone in psychophysiological research - Recommendations for experiment planning, data analysis, and data reporting. *Front. Psychol.* 8, 1–18. <https://doi.org/10.3389/fpsyg.2017.00213>. (FEB).
- Libby, P., 2002. Inflammation in atherosclerosis. *Nature* 420, 868. <https://doi.org/10.1038/nature01323>. Retrieved from.
- Libby, P., Loscalzo, J., Ridker, P.M., Farkouh, M.E., Hsue, P.Y., Fuster, V., Amar, S., 2018. Inflammation, immunity, and infection in atherothrombosis JACC review topic of the week. *J. Am. Coll. Cardiol.* 72 (17), 2071–2081. <https://doi.org/10.1016/j.jacc.2018.08.1043>.
- Lockwood, K.G., Jennings, J.R., Matthews, K.A., 2017. Psychophysiological correlates of systemic inflammation in black and white men. *Brain Behav. Immun.* 59, 93–102. <https://doi.org/10.1016/j.bbi.2016.08.013>.
- Lourenco, B.H., Cardoso, M.A., Team, A.S., 2014. C-reactive protein concentration predicts change in body mass index during childhood. *PLoS One* 9 (3), e90357. <https://doi.org/10.1371/journal.pone.0090357>.

- Lozano, D.L., Norman, G., Knox, D., Wood, B.L., Miller, B.D., Emery, C.F., Berntson, G.G., 2007. Where to B in dZ/dt. *Psychophysiology* 44 (1), 113–119. <https://doi.org/10.1111/j.1469-8986.2006.00468.x>.
- Marsland, A.L., Gianaros, P.J., Prather, A.A., Jennings, J.F., Neumann, S.A., Manuck, S.B., 2007a. Stimulated production of proinflammatory Cytokines covaries inversely with heart rate variability. *Psychosom. Med.* 69 (8), 709–716. <https://doi.org/10.1097/PSY.0b0136181576118>.
- Marsland, A.L., Sathanoori, R., Muldoon, M.F., Manuck, S.B., 2007b. Stimulated production of interleukin-8 covaries with psychosocial risk factors for inflammatory disease among middle-aged community volunteers. *Brain Behav. Immun.* 21 (2), 218–228. <https://doi.org/10.1016/j.bbi.2006.07.006>.
- Marsland, A.L., Walsh, C., Lockwood, K., John-henderson, N.A., 2017. The effects of acute psychological stress on circulating and stimulated inflammatory markers: a systematic review and meta-analysis. *Brain Behav. Immun.* (64), 208–219. <https://doi.org/10.1016/j.bbi.2017.01.011>.
- Mezzacappa, E.S., Kelsey, R.M., Katkin, E.S., 1999. The effects of epinephrine administration on impedance cardiographic measures of cardiovascular function. *Int. J. Psychophysiol.* 31, 189–196.
- Miller, A.H., Raison, C.L., 2016. The role of inflammation in depression: from evolutionary imperative to modern treatment target. *Nat. Rev. Immunol.* 16 (1), 22–34. <https://doi.org/10.1038/nri.2015.5>.
- Miller, G.E., Rohleder, N., Stetler, C., Kirschbaum, C., 2005. Clinical depression and regulation of the inflammatory response during acute stress. *Psychosom. Med.* 67 (5), 679–687. <https://doi.org/10.1097/01.psy.0000174172.82428.ce>.
- Miller, G.E., Chen, E., Parker, K.J., 2011. Psychological stress in childhood and susceptibility to the chronic diseases of aging: moving toward a model of behavioral and biological mechanisms. *Psychol. Bull.* 137 (6), 959–997. <https://doi.org/10.1037/a0024768>.
- Nance, D.M., Sanders, V.M., 2007. Autonomic innervation and regulation of the immune system (1987–2007). *Brain Behav. Immun.* 21 (6), 736–745. <https://doi.org/10.1016/j.bbi.2007.03.008>.
- O'Donnell, K., Brydon, L., Wright, C.E., Steptoe, A., 2008. Self-esteem levels and cardiovascular and inflammatory responses to acute stress. *Brain Behav. Immun.* 22, 1241–1247.
- Odegaard, J.I., Chawla, A., 2013. Pleiotropic actions of insulin resistance and inflammation in metabolic homeostasis. *Science* 339 (6116), 172–177. <https://doi.org/10.1126/science.1230721>.
- Quigley, K.M., Moore, G.A., 2018. Development of cardiac autonomic balance in infancy and early childhood: a possible pathway to mental and physical health outcomes. *Dev. Rev.* 49 (February), 41–61. <https://doi.org/10.1016/j.dr.2018.06.004>.
- Quigley, K.S., Stifter, C.A., 2006. A comparative validation of sympathetic reactivity in children and adults. *Psychophysiology* 43 (4), 357–365. <https://doi.org/10.1111/j.1469-8986.2006.00405.x>.
- Révész, D., Verhoeven, J.E., Milaneschi, Y., De Geus, E.J., Wolkowitz, O.M., Penninx, B.W., 2014. Dysregulated physiological stress systems and accelerated cellular aging. *Neurobiol. Aging* 35 (6), 1422–1430. <https://doi.org/10.1016/j.neurobiolaging.2013.12.027>.
- Riis, J.L., Granger, D.A., DiPietro, J.A., Bandeen-Roche, K., Johnson, S.B., 2015. Salivary cytokines as a minimally-invasive measure of immune functioning in young children: correlates of individual differences and sensitivity to laboratory stress. *Dev. Psychobiol.* 57 (2), 153–167. <https://doi.org/10.1002/dev.21271>. Retrieved from.
- Schächinger, H., Weinbacher, M., Kiss, A., Ritz, R., Langewitz, W., 2001. Cardiovascular indices of peripheral and central sympathetic activation. *Psychosom. Med.* 63, 788–796. <https://doi.org/10.1097/00006842-200109000-00012>.
- Sherwood, A., Allen, M.T., Fahrenberg, J., Kelsey, R.M., Lovallo, W.R., van Doornen, L.J.P., 1990. Methodological guidelines for impedance cardiography. *Psychophysiology* 27 (1), 1–23.
- Sloan, R.P., McCreath, H., Tracey, K.J., Sidney, S., Liu, K., Seeman, T., 2007. RR interval variability is inversely related to inflammatory markers: the CARDIA study. *Mol. Med.* 13 (3–4), 178–184. <https://doi.org/10.2119/2006-00112>.
- Steptoe, A., Owen, N., Kunz-Ebrecht, S., Mohamed-Ali, V., 2002. Inflammatory cytokines, socioeconomic status, and acute stress responsivity. *Brain Behav. Immun.* 16, 774–784.
- Steptoe, A., Hamer, M., Chida, Y., 2007. The effects of acute psychological stress on circulating inflammatory factors in humans: a review and meta-analysis. *Brain Behav. Immun.* 21 (7), 901–912. <https://doi.org/10.1016/j.bbi.2007.03.011>.
- Tracey, K.J., 2002. The inflammatory reflex. *Nature* 420 (6917), 853–859. <https://doi.org/10.1038/nature01321>.
- Tracey, K.J., 2007. Physiology and immunology of the cholinergic antiinflammatory pathway. *J. Clin. Invest.* <https://doi.org/10.1172/JCI30555>.
- Tyrka, A.R., Parade, S.H., Valentine, T.R., Eslinger, N.M., Seifer, R., 2015. Adversity in preschool-aged children: effects on salivary interleukin-1 β . *Dev. Psychopathol.* 27 (2), 567–576. <https://doi.org/10.1017/S0954579415000164>.
- Uchino, B.N., Trettervik, R., Kent de Grey, R.G., Cronan, S., Hogan, J., Baucom, B.R.W., 2018. Social support, social integration, and inflammatory cytokines: a meta-analysis. *Health Psychol.* 37 (5), 462–471. <https://doi.org/10.1037/hea0000594>.
- Williams, D.W.P., Koenig, J., Carnevali, L., Sgoifo, A., Jarczok, M.N., Sternberg, E.M., Thayer, J.F., 2019. Heart rate variability and inflammation: a meta-analysis of human studies. *Brain Behav. Immun.* 80, 219–226. <https://doi.org/10.1016/j.bbi.2019.03.009>.
- Winzer, A., Ring, C., Carroll, D., Willemsen, G., Drayson, M., Kendall, M., 1999. Secretory immunoglobulin A and cardiovascular reactions to mental arithmetic, cold pressor, and exercise: effects of beta-adrenergic blockade. *Psychophysiology* 36, 591–601. <https://doi.org/10.1111/1469-8986.3650591>.
- Yim, I.S., Quas, J.A., Cahill, L., Hayakawa, C.M., 2010. Children's and adults' salivary cortisol responses to an identical psychosocial laboratory stressor. *Psychoneuroendocrinology* 35 (2), 241–248. <https://doi.org/10.1016/j.psyneuen.2009.06.014>.