Supporting Information

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SI Materials and Methods

Biomarker Measurement. ***Resting systolic and diastolic blood pressure.*** Systolic and diastolic blood pressure were derived by averaging the second and third seated blood pressure readings taken after a 5-min rest period by using a sphygmomanometer (WA Baum).

Heart rate and heart rate variability. Heart rate (HR) and heart rate variability (HRV) data were obtained through a 10-min protocol previously described (1) and detailed methods for Coronary Artery Risk Development in Young Adults Study (CARDIA) substudy reported by Seeman et al. (2). Briefly, ECG electrodes were used to record heart rate over the seated resting period while the participant refrained from movement and talking. The ECG waveform was then converted to time series of RR intervals and examined by using spectral analysis on 5-min epochs. Data are presented as sum of low-frequency (0.04–0.15 Hz) and high-frequency (0.15–0.50 Hz) bands. Mean HR was also computed from these data.

Twelve-hour overnight urinary norepinephrine and epinephrine. Urine samples, preserved using sodium metabisulfite and frozen at –80 C, were analyzed using HPLC with electrochemical detection (3) and creatinine by Baranowski and Westenfelder (4). All inter- and intraassay coefficients of variation (CVs) were <7.1%. Epinephrine values below detection of the assay were assigned a zero ($n = 43$). Norepinephrine and epinephrine were adjusted by creatinine levels.

Cortisol. Cortisol morning rise (or AM rise), often referred to as the cortisol awakening response (CAR), and afternoon decline (diurnal slope) were selected as measures that reflect diurnal rhythm rather than total cortisol output (2). Diurnal salivary patterns were selected as elevated CAR and flattened diurnal slope represent aspects of dysregulated circadian rhythm and are related to poorer health outcomes (5–10). Salivary cortisol samples were taken using Salivettes (Sarstedt) at six time points by the participants during the day after their 15-y CARDIA visit. They were instructed to take a salivary sample upon awakening, 45 min after awakening, 2.5 h after awakening, 8 h after awakening, 12 h after awakening, and again before bed. Saliva was assayed by time-resolved immunoassay with fluorometric end point detection (11). Inter- and intraassay CVs were <12%. AM rise was determined by calculating the difference between waking and 45 min postwaking cortisol. Diurnal slope was estimated computing average hourly rate of decline from morning rise to bedtime logged cortisol values. Empirical Bayes best linear unbiased predictors of the subject-specific slope were used. Excluded data included individuals who woke up after noon ($n = 25$), individuals whose waking samples were collected more than 15 min after awakening ($n = 34$), those whose 45 min postawakening samples were collected less than 30 min or more than 60 min after the awakening sample ($n = 25$), and those individuals with cortisol samples equal or above 60 ($n = 54$), based on work suggesting values above this were potentially false values (12). Slopes were not used from individuals missing two of time 2, 3, or 4, missing both time 5 and 6 ($n = 6$), or who were awake less than 12 h or more than 20 h ($n = 12$).

Metabolic biomarkers. HDL cholesterol was measured by using precipitation with dextran sulfate/magnesium chloride (13). LDL cholesterol was calculated on samples using the Friedewald equation (14). Triglycerides were measured on an Abbott Spectrum using an enzymatic method (15). Glucose was determined with a Cobas Mira Plus chemistry analyzer using the hexokinase UV method (Roche Diagnostic Systems). Insulin was assessed by RIA using the overnight, equilibrium incubation format (16, 17), and calibrated with other sampling years. Waist circumference was taken by averaging two measurements at the minimum abdominal girth when participants were in an upright standing position.

Inflammatory biomarkers. C-reactive protein (CRP) and fibrinogen were measured by using a particle-enhanced immunonephelometric assay, with CVs within normal ranges, 2.1% to 5.7% (2). [A total of 53 subjects had CRP values greater than 10. We conducted sensitivity analyses, excluding these individuals, and found no difference in our primary results. In our allostatic load index, these individuals are given a score of 1 for being in the high risk group for CRP. Based on previous work (18), elevated CRP above 10 may reflect chronic inflammation rather than just acute infection.] IL-6 was measured by ultrasensitive ELISA (R&D Systems) for the detection of low levels of IL-6 in biological samples, with an average CV of 6.3%.

Lifestyle Risk Factor Measurement. Sleep quantity was assessed by asking participants to report average sleep duration per night for the preceding month. Values ranged from 3 to 15 h, with a majority of responses (90%) between 5 and 8 h. Physical activity was determined through administration of the physical activity history questionnaire used to calculate exercise units that reflect quantification of activity level during the previous 12 mo based on energy expenditure scores for common moderate and heavy intensity exercises (16) and self-reported frequency of engaging in these activities (ref. 19 includes further description). Physical activity data were not normally distributed, and logarithmic transformations failed to correct this. We created quartiles by using the following cutpoints: 1, 0–142; 2, 143–276; 3, 277–497; and 4, 498 and higher. Alcohol consumption was estimated by calculating typical daily alcohol consumption in milliliters (20). Log transformations were performed on alcohol consumption to approximate normal distribution. Smoking status was determined by self-report of smoking history, with two dummy-coded variables: Past and current smokers = 1, never smokers = 0; current smokers = 1, past and never smokers = 0.

SI Results

Sex Differences in Childhood Stress. Male and female subjects differed in mean childhood stress scores. Risky family scores were elevated in women (mean = 12.1, SD = 4.3) compared with men (mean = 11.4, SD = 3.7; $t = 2.3$, $P < 0.05$). Subscale scores were also different by sex. Mean parental warmth was higher in men (mean = 3.25, SD = 0.74) compared with women (mean = 3.11, SD = 0.87; $t = -2.40$, $P < 0.05$). Childhood abuse was significantly lower in men (mean = 1.4, SD = 0.6) compared with women (mean = 1.58, SD = 0.78; $t = 2.67$, $P < 0.01$).

Lifestyle Factors. All lifestyle risk factors were significantly associated with allostatic load, such that higher allostatic load was associated with greater prevalence of smoking, shorter sleep duration, less physical activity, and lower alcohol consumption (all $P < 0.05$). After adjustments for these lifestyle factors, risky family scores [B (SE) = 0.06 (0.03), $P < 0.05$] and the childhood abuse subscale score [B (SE) = 0.52 (0.16), $P = 0.001$] remained significant predictors of allostatic load. Parental warmth was modestly associated with allostatic load after adjustments by lifestyle factors [B (SE) = –0.24 (0.14), $P = 0.08$]. The interaction of abuse by parental warmth in the prediction of allostatic load after further adjustment by lifestyle factors was no longer

significant [B (SE) = −0.27 (0.18), $P = 0.13$], suggesting lifestyle factors may partially mediate this effect.

Individual Systems Risk Scores. Finally, we ran regression models testing for the unique association of childhood factors with individual biological system risk scores (Table S2). In analyses adjusting for age, sex, race, parental education, and oral contraceptive use (OCU), positive associations were present for childhood abuse with several of the system risk scores, including HRV ($P = 0.001$), blood pressure ($P < 0.05$) inflammation ($P = 0.01$), metabolic ($P = 0.003$) and cortisol risk scores ($P = 0.06$). Parental warmth was significantly inversely associated with metabolic and cortisol risk scores ($P < 0.05$), and showed a modest inverse association with HRV risk ($P = 0.08$). A significant interaction of abuse with parental warmth was also present for HRV and blood pressure ($P < 0.01$); however, this was not significant with any other individual system risk scores.

SI Discussion

Lifestyle Factors. Several established modifiable behavioral risk factors for diseases were also associated with elevated allostatic load in the present sample, including smoking, physical inactivity, and shorter sleep duration. This is of particular interest, as this provides evidence that these traditional behavioral risk factors for disease are also associated with elevated allostatic load, suggesting that poor health behaviors may impact multiple biological systems that then influence disease risk. Although these behavioral risk factors are often more prevalent among those who experience childhood stress, which is the case in the present study for smoking and sleep duration, further adjustments by these behavioral factors did not account for the effect of childhood abuse on allostatic load. However, the interaction term of abuse by parental warmth was reduced, suggesting that childhood toxic stress when accompanied by low parental warmth may be impacting adult allostatic load partially through influencing these lifestyle behaviors.

Multisystem vs. Individual System Scores. Our secondary aim was to examine the strength of the association between childhood stress with the multisystem biological risk score compared with individual system scores alone. Several of the individual system risk scores were associated with risky family environment and childhood abuse. The strength of these associations is smaller than the association with the multisystem risk score. It is possible that this reflects individual variability in the stress associated susceptibility of the various regulatory systems to imbalance, with some more likely to express the biological consequences of stress exposure in metabolic parameters whereas others show greater evidence in inflammatory markers. Indeed, in prior work, we have found that there is significant heterogeneity in the patterns of imbalance across systems (21). The strength of the multisystems perspective on biological consequences of childhood stress is that it allows us to capture the heterogeneous patterns of imbalance across multiple systems to provide a more macro-level assessment of the global impact of childhood stress on multiple major regulatory systems. As our results indicate, a multisystem biological risk scores is more strongly associated with childhood stress than with any one system alone.

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Table S1. Unstandardized regression coefficient of childhood factors predicting individual systems risk scores adjusted by age, sex, race, parental education, and OCU

	Risky family environment (range, 7–28)		Childhood abuse (range, 1–4)		Parental warmth (range, 1–4)		Abuse × warmth	
Variable	β (SE)	*P* value	β (SE)	*P* value	β (SE)	*P* value	β (SE)	*P* value
HRV risk (0–3)	0.02 (0.01)	0.01*	0.17 (0.05)	0.001*	−0.08 (0.04)	0.08	−0.15 (0.06)	0.007*
Blood pressure risk (0–2)	0.01 (0.01)	0.39	0.08 (0.04)	0.05	−0.04 (0.04)	0.30	−0.13 (0.05)	0.005*
Inflammation risk (0–3)	0.01 (0.01)	0.18	0.13 (0.05)	0.01*	−0.01 (0.04)	0.75	−0.02 (0.06)	0.71
Metabolic risk (0–6)	0.04 (0.01)	0.01*	0.23 (0.08)	0.003*	−0.15 (0.07)	0.02*	−0.10 (0.09)	0.28
SNS hormone risk (0–2)	0.01 (0.01)	0.33	0.02 (0.04)	0.57	−0.02 (0.03)	0.48	−0.02 (0.04)	0.63
Cortisol risk (0–2)	0.02 (0.01)	<0.001*	0.06 (0.03)	0.06	−0.09 (0.03)	0.001*	0.01 (0.04)	0.88

SNS, sympathetic nervous system.
*Significant at $P < .05$.

Table S2. Descriptive statistics and higher-risk cutpoint for each biomarker in the multisystem risk index

Biomarker	*N*	Mean	SD	Higher-risk cutpoints
Parasympathetic nervous system				
HR, beats/min	697	72.4	11.7	≥79.8
Low frequency HRV, ln ms^2	697	6.1	1.1	≤5.46
High frequency HRV, ln ms^2	697	5.9	1.3	≤5.12
Cardiovascular system				
SBP, mmHg	756	113.7	14.3	≥122
DBP, mmHg	756	75.4	10.7	≥82
Inflammatory system				
CRP, mg/L	756	3.4	5.1	≥4.22
Fibrinogen, mg/dL	689	403.7	90.9	≥455
IL-6, pg/mL	696	1.7	1.5	≥2.09
Metabolic system				
HDL, mg/dL	755	50.3	13.8	≤36 (men), ≤45 (women)
LDL, mg/dL	750	113.5	32.4	≥135
Triglycerides, mg/dL	755	105.1	116.4	≥120
Fasting glucose, mg/dL	755	85.6	18.2	≥89
Fasting insulin, μU/mL	753	14.6	10.9	≥12
Waist circumference, cm	754	89.8	15.6	≥100.75 (men), ≥96.38 (women)
Sympathetic nervous system				
Norepinephrine, μg/g creatinine	699	34.3	20.9	≥43.14
Epinephrine, μg/g creatinine	698	4.2	6.3	≥4.64
Hypothalamic–pituitary–adrenal				
AM rise (45-min postwaking nmol/L − waking sample), nmol/L	672	6.03	12.4	≤−1.7
Diurnal slope, ln (nmol/L)/h	718	−0.03	0.03	≥−0.01