Supplementary Statistical Analyses

Maternal EPC cortisol and children’s HPAA habituation to a new school year, a non-experimental stressor

We examined the associations between average maternal FMU cortisol 1) during each of the 8 EPC weeks or 2) across the entire eight-week EPC period and whether children were able to habituate to the start of school (i.e., whether a child’s FMU cortisol returned to basal levels after increasing in response to the start of school). Fixed effects were habituation status and children’s sex. Random effects were maternal week of pregnancy and child identification number. We also tested the interaction between sex and habituation status. We analysed significant effects using post-hoc comparisons.

Principal component analysis of DNA methylation data

We performed PCA on normalized, tissue-corrected methylation data using Python to determine associations between the mothers’ and children’s global DNA methylation signals and the mother’s and children’s cortisol variables of interest. PCA was used to decompose the methylation patterns measured into a set of linearly independent principal components (PCs). These PCs represent the mean ways through which DNA methylation varies across samples, and their contribution is quantified by the amount of total variance they capture. We then used Spearman correlations to associate the observed main patterns of variation in the data (PCs) with the known traits of the participants (i.e., the mother’s and children’s cortisol variables of
interest). This procedure quantifies to which extent these traits are reflected in genome-wide DNA methylation patterns.

Supplementary Results

Maternal EPC cortisol and children’s HPAA habituation to a new school year

Average maternal cortisol levels during specific EPC weeks were not related to whether their children habituated to the start of school, as determined by whether the children’s FMU cortisol levels after the start of school returned to or below levels observed prior to the start of school (all $p > 0.05$). In contrast, the relationship between overall average maternal cortisol across the eight-week EPC period and their children’s habituation status was dependent on the sex of the children (sex by habituation status interaction: $F_{1,18.18} = 4.10, p = 0.05$). Post-hoc analyses indicated that sons who did not habituate to the start of school were exposed to lower maternal cortisol levels across the entire EPC period compared to daughters that did not habituation and children of both sexes that did habituate (all three $p$-values $< 0.02$; Supplementary Figure S4). All other groups did not differ from each other in maternal cortisol levels across the eight-week EPC period (all $p$-values $> 0.68$; Supplementary Figure S4).

Principal component analysis of DNA methylation data

Children’s global DNA methylation signals were more strongly associated with weekly maternal EPC cortisol than with either children’s or mothers’ cortisol levels at the time of children’s DNA methylation assessment in 2013. Specifically, PC2 of children’s DNA methylation signals, which accounted for 9.1% of the total variance, was positively associated with maternal $\log_{10}$ FMU
cortisol in EPC weeks 1 (R = 0.444, p = 0.039), 2 (R = 0.476, p = 0.022), 3 (R = 0.458, p = 0.024), 4 (R = 0.425, p = 0.038), 5 (R = 0.504, p = 0.017), and 8 (R = 0.499, p = 0.021) (Supplementary Figure S5 a-f). Similarly, PC3 accounted for 4.6% of the total variance and was positively associated with maternal log₁₀ FMU cortisol in EPC weeks 1 (R = 0.428, p = 0.047) and 4 (R = 0.552, p = 0.005) (Supplementary Figure S5 g and h). In contrast, PC1 of children’s DNA methylation signals, which accounted for 15.4% of the total variance in the data, was only marginally negatively associated with children’s average log₁₀ FMU cortisol prior to (R = -0.361, p = 0.076) and following (R = -0.376, p = 0.064) the start of a new school year (Supplementary Figure S5 i and j), while PC3 was positively associated with mothers’ average log₁₀ FMU cortisol in 2013 (R = 0.583, p = 0.036) (Supplementary Figure S5 k). Mother’s DNA methylation signals were not significantly associated with maternal EPC cortisol levels (all p > 0.05).
Supplementary Figure S1. Tissue correction of blood-contaminated buccal samples. A joint dataset was created, containing the DNA methylation data from the buccal samples of the mothers and children, together with 16 whole blood samples and 10 buccal samples from two independent studies. Principal Components Analysis (PCA) was run on the joint dataset, and principal component (PC) 1 was identified as a carrier of tissue-specific variation. Each tick on the x-axis represents the PC 1 score of one individual. This PC was then subtracted from the data, correcting for this tissue contamination while leaving the rest of the variance intact.
Supplementary Figure S2. Children’s cortisol before and after the start of a new school term

Children’s log_{10}-transformed FMU cortisol (mean ± standard error of the mean) before and after the start of a new school term (Day 0), a non-experimental stressor.
Supplementary Figure S3. Children’s cortisol response to the TSST-C. Children’s log_{10}-transformed salivary cortisol profile before and in response to the TSST-C, an experimental stressor.
Supplementary Figure S4. Average maternal cortisol across the entire eight-week early post-conception (EPC) period and children’s habituation status in response to the start of a new school term (i.e., whether the children’s $\log_{10}$ FMU cortisol after the start of school returned to or below levels seen prior to the start of school). Sons that did not habituate to the start of school were exposed to lower maternal $\log_{10}$ FMU cortisol levels across the entire eight-week EPC period compared to daughters that did not habituate and sons and daughters that did habituate (all three $p$-values < 0.02). Different letters indicate significant differences ($p < 0.05$).
Supplementary Figure S5. Relationships between Principal Components 1, 2 and 3 (PC1, PC2 and PC3) of children’s DNA methylation signals and maternal cortisol during the early post-conception period (EPC) and children’s and mothers’ current cortisol levels. PC2 was positively associated with average maternal log₁₀ FMU cortisol during EPC weeks a) 1, b) 2, c) 3, d) 4, e) 5, and f) 8 (all \( p < 0.05 \)). PC3 was positively associated with average maternal log₁₀ FMU cortisol during EPC weeks g) 1 (\( p = 0.047 \)) and h) 4 (\( p = 0.005 \)). PC1 was marginally negatively associated with children’s average log₁₀ FMU cortisol i) in the week prior to the start of a new school term (\( p = 0.076 \)) and j) in the two weeks following the start of a new school term (\( p = 0.064 \)). k) PC3 was positively correlated with average maternal log₁₀ FMU cortisol at the time of children’s DNA methylation assessment in 2013 (\( p = 0.036 \)).