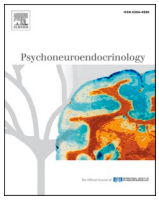
Psychoneuroendocrinology 129 (2021) 105252

Contents lists available at ScienceDirect

Psychoneuroendocrinology

journal homepage: www.elsevier.com/locate/psyneuen



Blunted circadian cortisol in children is associated with poor cardiovascular health and may reflect circadian misalignment

Weiying Dai [a], Swanand A. Wagh [a], Steffi Chettiar [a], Grace D. Zhou [b], Runia Roy [b,c], Xingye Qiao [d], Paul S. Visich [e], Eric P. Hoffman [b,*]

[a] Department of Computer Science, Binghamton University – SUNY, Binghamton, NY, USA
[b] Department of Pharmaceutical Sciences, Binghamton University -SUNY, Binghamton, NY, USA
[c] Department of Biomedical Engineering, Binghamton University -SUNY, Binghamton, NY, USA
[d] Department of Mathematical Sciences, Binghamton University -SUNY, Binghamton, NY, USA
[e] Exercise and Sport Performance Department, University of New England, Biddeford, ME, USA

ARTICLE INFO

Keywords:
Circadian rhythm
Blood pressure
Metabolic syndrome
Cortisol

ABSTRACT

Objectives: Circadian cues in children (sunlight, exercise, diet patterns) may be associated with health outcomes. The primary objective was to assess associations of daily cortisol fluctuations (morning, night) with cardiovascular health outcomes. A secondary objective was to determine if 1-year longitudinal changes in circadian cortisol levels are associated with longitudinal changes in health outcomes.

Study design: The Cardiovascular Health Intervention Program (CHIP) was a cross-sectional and longitudinal study of cardiovascular risk profiles in public elementary school children in Southern Maine. Participants were 689 students in 4th grade (baseline; age = 9.20 ± 0.41 years), and 647 students in 5th grade (age = 10.53 ± 0.52 years). Longitudinal data (4th and 5th grade) was available for 347 participants. Clinical outcomes were blood pressure, hip/waist ratios, body mass index, percent fat. Laboratory measures were fasting glucose, lipids, and salivary cortisol measures (morning and evening).

Results: Lower first-in-morning diurnal cortisol levels were associated with increased blood pressure (β −0.23 ± 0.05; $p < 0.001$), increased body fat (β −0.22 ± 0.05; $p < 0.001$), and poor lipid profiles (β −0.15 ± 0.07; $p < 0.05$). Inclusion of night cortisol in the model (stress-related) improved associations of the model with bodyfat composition (morning β −0.27 ± 0.05; $p < 0.001$; night β +0.16 ± 0.06; $p < 0.01$). Adjustments for potential confounding variables improved associations of morning cortisol with lipids (β −0.19 ± 0.07; $p < 0.01$). Longitudinal analysis showed that lower morning diurnal cortisol in 4th grade was associated with increases in blood pressure a year later (β −0.18 ± 0.08; $p = 0.017$) after adjusting for confounding variables.

Conclusion: Data presented suggest adding circadian misalignment (lower amplitude of first-in-morning cortisol) to existing models of metabolic syndrome in children. Further, circadian misalignment may be a factor contributing to high blood pressure.

1. Introduction

Increased prevalence of chronic inflammatory and allergic conditions is associated with modern lifestyles (Haahtela et al., 2019). While prevalence for chronic inflammatory disease (allergy/asthma, inflammatory bowel disease, Type II diabetes) in both adults and children has plateaued in more developed countries, it is rapidly increasing in less developed countries (NCD Risk Factor Collaboration NCD-RisC, 2016; McAloon et al., 2016; Lundbäck et al., 2016; Ng et al., 2018; Benchimol et al., 2014; Serebrisky and Wiznia, 2019). The chronic

Abbreviations: BMI, Body mass index; BP, Blood pressure; CFA, Confirmatory factor analysis; CFI, Comparative fit index; CHIP, Cardiovascular Health Intervention Program; dL, deciliter; FIML, Full Information Maximum Likelihood; HDL, High density lipoprotein; LDL, Low density lipoprotein; LTC, Latent trait cortisol; SEM, Structural Equation Modeling; RMSEA, Root Mean Square Error of Approximation; SES, Socioeconomic status; SRMR, Standardized Root Mean Square Residual; TG, Triglycerides.

* Correspondence to: School of Pharmacy and Pharmaceutical Sciences, Binghamton University – SUNY, PO Box 6000, Binghamton, NY 13902, USA.
E-mail address: ehoffman@binghamton.edu (E.P. Hoffman).

https://doi.org/10.1016/j.psyneuen.2021.105252
Received 31 January 2021; Received in revised form 7 April 2021; Accepted 30 April 2021
Available online 11 May 2021

inflammatory state and immune dysregulation for each disease shows associations with obesity (Saltiel and Olefsky, 2017), and physical inactivity (Sallam and Laher, 2016). Both obesity and inactivity influence the gut microbiome as well (Ticinesi et al., 2019; Lee et al., 2020).

We hypothesized that the removal of circadian cues leads to a form of circadian misalignment that may be detected by blunting of first-in-morning diurnal cortisol rhythms. This circadian misalignment may in turn lead to a pro-inflammatory state and increases in cardiovascular risks, as has been shown in mice (Inokawa et al., 2020). It is well-known that sunlight exposure, exercise, regular diet, and stress all serve to entrain the circadian clock (Tahara et al., 2017). A central aspect of the built environment is less sunlight and exercise, with the expected disruption of circadian entrainment cues. Indeed, there is increasing pre-clinical and clinical evidence of associations between improving circadian clocks and decreasing pro-inflammatory disease states, including asthma (Ehlers et al., 2018), cardiovascular risk factors (Morris et al., 2016; Alibhai et al., 2017), inflammatory bowel disease (Liu et al., 2017; Gombert et al., 2019), and diabetes (Jakubowicz et al., 2019; Bosma-den Boer et al., 2012).

Circadian rhythms are regulated by clock genes and encoded proteins (CLOCK, BMAL1), and these in turn drive expression of cortisol as the primary hormone driving circadian rhythms, where it is the ligand for the glucocorticoid receptor expressed in all tissues and cells (Trott and Menet, 2018). Circadian rhythms are typically measured by serum cortisol levels, where levels peak pre-awakening, and trough by Noon. First-in-morning cortisol levels approach the maximum (peak) serum concentrations during daily circadian cycles. Cortisol, and its pharmacological derivatives (corticosteroid drugs; prednisone and others), show potent anti-inflammatory activities (Barnes, 2006), and it could be assumed that diurnal variations in cortisol have immunomodulatory roles.

To study circadian health and relationship to cardiovascular risk factors, we hypothesized that a population-based study of children might provide the best approach. Elementary school children within a single grade level within a school have relatively uniform diurnal sleep, diet, and activity patterns. We worked with two public elementary schools in Southern Maine to develop a research and education program with a focus on healthy dietary choices and physical activity. This included assessments of each child for anthropomorphic data (blood lipid and glucose measures) at a neighboring university, and working with parents to collect 9 salivary samples from each child (two upon wakening with 30 min apart, one before bed) over 3 consecutive days for measures of cortisol. The study performed a cross-sectional analysis to assess the data at 9 years old and a longitudinal analysis to assess the children's data at both 9 and 10 years of age. Our preliminary cross-sectional study with 474 children provided initial evidence for the association of morning cortisol levels and cardiovascular risk factors (lipids, blood pressure, body composition) (Yeung et al., 2016). Here, we report relationships between diurnal cortisol fluctuations in 1336 child assessments, with 587 children assessed at 9 years old and 347 children assessed at both 9 and 10 years of age.

2. Materials and methods

2.1. Participants

The Cardiovascular Health Intervention Program (CHIP) is a study of circadian rhythms and cardiovascular risk factors in children in Southern Maine, and participants and procedures have been described previously (Yeung et al., 2016; White et al., 2017). Children studies were 689 students at baseline (4th grade) (age: 9.20 ± 0.41 years; 53% female; 89% Caucasian) and 647 students at follow-up (5th grade) (age: 10.53 ± 0.52 years; 55% female; 82% Caucasian). All students in the entire school in each grade were invited to participate, and participation rate was 78%. Parents provided informed consent for their children, and ethics review and approval was by the University of New England (UNE) Institutional Review Board.

2.2. Procedures

Procedures have been previously described, including participant recruitment and assessments of cardiovascular risk factors and are provided in an appendix (Methods A.1) (Yeung et al., 2016). Methods for salivary cortisol collection and analyses have been previously described in detail (Yeung et al., 2016).

2.3. Data analytic strategy

Full Information Maximum Likelihood (FIML) estimation in Mplus version 8.4 was used as previously described (Yeung et al., 2016). We used a 4-stage analytic strategy using structural equation modeling (SEM) framework: (1) estimates of cortisol latent state-trait models (LTC) defined by the baseline (4th grade) cortisol data; (2) confirmatory factor analysis (CFA) to define multi-factor structure reflective of cardiovascular risk factors (baseline; 4th grade); (3) path analysis from steps 1 and 2 (hypothesis testing); (4) adjustments for potential confounding effects of sex, age, race, socioeconomic, and medication use. The four steps were applied to 4th grade data.

For longitudinal analyses, path analysis tested whether the baseline LTC (from the 4th graders) predicted the cardiovascular risk factors at follow-up (from the 5th graders). In the longitudinal model, the baseline LTC from the latent state-trait model (using the best baseline model structure) and baseline Lipids (baseline Blood Pressure or baseline Body Composition, using the multi-factor model structure) were specified to predict the follow-up Lipids (follow-up Blood Pressure or follow-up Body Composition, using the multi-factor model structure). The longitudinal model was adjusted for sex, age, race, socioeconomic, and medication use.

Both baseline and longitudinal models were tested for fit using FIML using four global fit indices: 1. Chi-square test (p-value >0.05); Comparative Fit Index (CFI ≥0.95); 2. Standardized Root Mean Square Residual (SRMR; <0.05 [good], 0.05–0.08 [fair], >0.08 [poor]); 3. Root Mean Square Error of Approximation (RMSEA; < 0.05 [good], 0.05–0.08 [fair], >0.08 [poor]) (Hu and Bentler, 1999).

Table 1
Participant demographics.

	4th Grade (n = 689)	**5th Grade** (n = 647)
Age	9.20 ± 0.41 years	10.53 ± 0.52 years
Female n (%)	365 (53%)	356 (55%)
Male n (%)	324 (47%)	291 (45%)
Caucasian n (%)	613 (89%)	530 (82%)
Female Overweight n (%) (BMI)	77/362 (21%) (19.2–22.0 kg/m^2)	70/356 (20%) (20.4–23.3 kg/m^2)
Female Obese n (%) (BMI)	83/362 (23%) (>22.0 kg/m^2)	99/356 (28%) (>23.3 kg/m^2)
Male Overweight n (%) (BMI)	51/313 (16%) (18.8–21.0 kg/m^2)	65/284 (23%) (19.8–22.6 kg/m^2)
Male Obese n (%) (BMI)	84/313 (27%) (>21.0 kg/m^2)	62/284 (22%) (>22.6 kg/m^2)

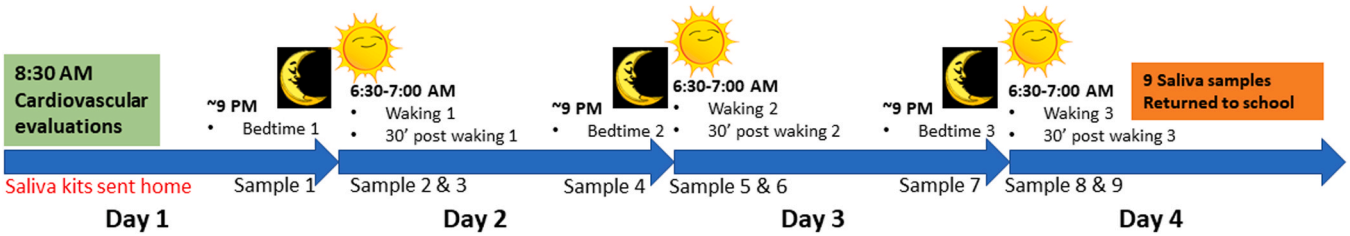


Fig. 1. Study design. Shown is a schematic of the overall study design. Participating children (4th and 5th grades; 9–10 years) were sent home with an informed consent and 9 saliva collection kits with instructions for sublingual saliva collection at bedtime, at first awakening (Waking), and 30 min post awakening (3′ post waking) on 3 consecutive days (9 samples total). Samples were stored in the home freezer until Day 4. On Day 4, children were instructed to fast (skip breakfast) and once arriving at school, bused to the University of New England where phenotyping for cardiovascular risk factors were carried out. Saliva samples were collected at that time and stored at − 80 °C until analysis. Cortisol measures were carried out on all samples.

Table 2
Salivary cortisol measures in 4th and 5th grade school children.

	PRE (4th grade) N = 689 9.2±0.4 years		POST (5th grade) N = 647 10.5±0.5 years		Longitudinal paired *t*-test N = 347	Cross-sectional change (p value)
	Mean *μg/dl*	SD	Mean *μg/dl*	SD		
Day 1: Bedtime	0.07	0.12	0.09	0.13	0.038	+ 0.02 (0.007)
Day 1: Waking	0.28	0.18	0.31	0.19	ns	+ 0.03 (0.045)
Day 1: 30-min post-waking	0.33	0.20	0.39	0.22	0.004	+ 0.07 (0.00005)
Day 2: Bedtime	0.07	0.11	0.10	0.15	0.015	+ 0.03 (0.0005)
Day 2: Waking	0.28	0.17	0.32	0.19	0.007	+ 0.04 (0.002)
Day 2: 30-min post-waking	0.33	0.20	0.37	0.21	0.003	+ 0.04 (0.005)
Day 3: Bedtime	0.09	0.16	0.09	0.14	0.018	0 (ns)
Day 3: Waking	0.28	0.17	0.31	0.18	ns	+ 0.03 (0.015)
Day 3: 30-min post-waking	0.33	0.19	0.37	0.21	0.018	+ 0.04 (0.0021)
Mean Days 1–3: Bedtime	0.08	0.10	0.09	0.11	0.007	+ 0.01 (0.027)
Mean Days 1–3: Waking	0.28	0.13	0.31	0.14	0.019	+ 0.03 (0.0016)
Mean Days 1–3: 30-min post-waking	0.33	0.15	0.38	0.16	0.0006	+ 0.05 (0.0002)

3. Results

3.1. Participant demographics

All children attending public middle school (4th and 5th grades) were invited to participate, with consent from parents, with some children participating during both 4th and 5th grade. There was a 78% participation rate of all children, suggesting a population-based cohort for study. Mean age in 4th grade was 9.20 ± 0.41 years, and in 5th grade 10.53 ± 0.52 years, with the majority of Caucasian race (89% 4th grade; 82% 5th grade) (Table 1). Using CDC growth charts and cut-offs, about 20% of participants were overweight (85th-95th percentile), and 25% obese (>95th percentile) (Table 1).

3.2. Cortisol measures at baseline (4th grade children) and follow-up (5th grade children)

The study design for collection of salivary samples is shown in Fig. 1. Salivary cortisol data, including first awakened (waking), 30 min later (30-min post-waking), and at bedtime, for 3 consecutive days (9 samples per child), both during 4th and 5th grades, are summarized in Table 2. For both 4th grade and 5th grade students, mean morning levels of cortisol were substantially higher than evening levels (waking ~0.3 μg/dL; bedtime ~0.09 μg/dL). The cortisol levels of 5th grade students increased by 10–11% compared to those of 4th grade students, and increases were significant for both cross-sectional analyses and intra-subject longitudinal analyses (Table 2). Across sampling days, intra-subject correlations for repeated measures of the same time point ranged from 0.30 to 0.59 for each measure. The medium level cortisol correlations were expected as the cortisol levels can be affected by many factors, motivating us to use the latent state trait model to approximate the trait cortisol (with variance common across sampling days).

3.3. Cardiovascular risk factor measures at baseline (4th grade children) and follow-up (5th grade children)

Cardiovascular risk indices, including TG, HDL-C, BP (systolic; diastolic), BMI, waist-to-hip ratio, and percent fat, both during 4th and 5th grades are summarized in Table 3. Cardiovascular risk factors increased from 4th grade to 5th grade for both cross-sectional and longitudinal (intra-subject) measures. HDL, BP, and BMI each increased about 6% over the year, whereas TG increased 11%. Changes in Waist-to-hip ratio and percent fat were not significant.

The intercorrelations observed between cardiovascular risk factors (Table A.1) suggested that the cardiovascular indices may reflect the variations of some underlying latent (unobserved) factors, which could be constructed from confirmatory factor analysis. High correlation values were observed between TG and HDL-C (r = −0.40), between systolic BP and diastolic BP (r = 0.69), between BMI, waist-to-hip ratio, and percent fat (r = 0.46, 0.58, and 0.75 respectively).

3.4. Cortisol latent state-trait models

Three Single-Trait Multistate (STMS) models were tested, and one single-trait model estimated (waking, 30 min post-waking and bedtime salivary cortisol levels), as previously described (Yeung et al., 2016). Model 1: Waking and 30 min post-waking samples from 3 sequential days (6 measures/child) were combined into a single subject LTC. Fit indices were χ^2 (11) = 74.035 ($p < 0.001$); CFI = 0.950; SRMR = 0.049; RMSEA = 0.098 (90% CI: 0.078–0.120); SRMR and CFI (good fit). Model 2: Night (bedtime) samples from the 3 sequential days (3 measures/-child) were combined into single subject LTC. The fit indices for Model 2 were χ^2 (29) = 248.796 ($p < 0.001$); CFI = 0.770; SRMR = 0.094; RMSEA = 0.113 (90% CI: 0.100–0.126); CFI, SRMR and CFI (poor fit). Model 3: Each of the 3 times of day cortisol assessments were considered

Table 3
Cardiovascular risk factors in children 4th and 5th grade children.

	PRE (4th grader) N = 689 9.2±0.4 years		POST (5th grader) N = 647 10.5±0.5 years		Longitudinal paired *t*-test N = 347	Cross-sectional change (%) (p value)
	Mean	SD	Mean	SD		
Triglycerides (mg/dl)	67.25	42.93	75.64	38.95	0.0002	+ 8.39 (+11%) (0.0008)
High-density lipoprotein (mg/dl)	51.35	12.64	54.52	14.24	< 0.0001 (2.36×10^{-8})	+ 3.17 (+6%) (0.0001)
Systolic blood pressure (mmHg)	105.94	11.12	112.11	9.72	< 0.0001 (2.59×10^{-25})	+ 6.17 (5.5%) (<0.0001) (9.79×10^{-26})
Diastolic blood pressure (mmHg)	68.41	9.47	72.59	8.74	< 0.0001 (3.37×10^{-14})	+ 4.18 (5.7%) (<0.0001) (1.95×10^{-16})
Body mass index (kg/m^2)	19.35	4.03	20.59	4.50	< 0.0001 (4.75×10^{-58})	+ 1.24 (6%) (<0.0001) (1.95×10^{-7})
Waist-to-hip ratio	0.87	0.06	0.86	0.07	ns	− 0.01 (ns)
Percent fat (%)	28.49	10.28	29.52	9.70	0.004	+ 1.03 (ns)

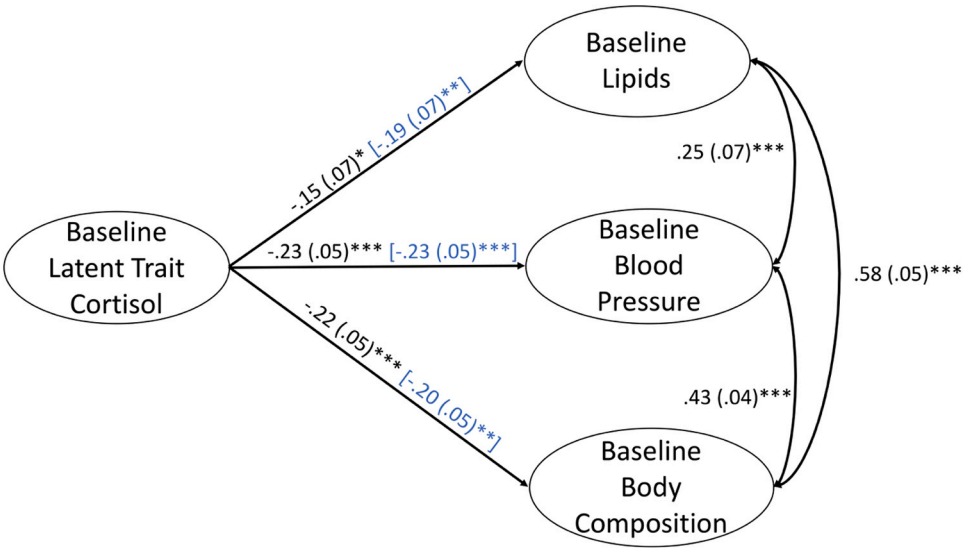


Fig. 2. Model fitting for 4th grade students (n = 587 subjects; age 9 years). Parameter estimations (standard errors) are shown between latent factors (cortisol and cardiovascular risk factors). CFI and SRMR suggest a good fit of the data, while RMSEA suggested a fair fit (χ^2[61] = 167.684 [p < 0.001]; CFI = 0.950; SRMR = 0.041; and RMSEA = 0.055 [90% CI: 0.045–0.065]). *p < 0.05, **p < 0.01, and ***p < 0.001. Black = before adjusting for covariates. Blue = after adjusting for covariates. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

separate traits (awakening, 30-minute post-awakening, bedtime) (9 measures/child). The 3 latent state factors were then loaded onto a single latent trait factor. Model 3 did not converge. Model 4: Six morning samples collected across days were loaded onto one morning latent trait factor. The fit indices resulting from Model 4 were χ^2 (9) = 75.682 (p < 0.001); CFI = 0.910 (poor); SRMR = 0.047 (good); RMSEA = 0.112 (90% CI: 0.089–0.136) (poor). Similar to our preliminary study (Yeung et al., 2016), Model 1 was shown as the best fit and therefore was retained to calculate LTC values at both Baseline (4th grade) and Follow up (5th grade) (Fig. A.1). The LTC accounted for 33.5–52.2% of the variance in six morning cortisol values. On average, the LTC explained the 43.3% of variance in cortisol measures both within a single day, and across 3 sequential days.

3.6. Salivary biomarkers at baseline and cardiovascular risk factors at baseline

STMS model LTC at Baseline (4th grade) was defined using SEM to test latent factors for Lipids, Blood Pressure, and Body Composition (Fig. 2). This analysis showed fit indices of: χ^2(61) = 167.684 (p < 0.001); CFI = 0.950 (good); SRMR = 0.041 (good); and RMSEA = 0.055 (90% CI: 0.045–0.065) (fair). LTC showed significant association with cardiovascular traits, including Blood Pressure (LTC explained 4.9%), Body Composition (LTC 4.9%), and Lipids (LTC 2.2%), with lower LTC associated with poorer cardiovascular risk factors.

In the above SEM model, only morning cortisol values were involved in the calculation of LTC. As a separate interest, we explored the effect of night cortisol on cardiovascular risk factors. Night LTC factor was formed from night samples across the three sampling days. The LTC calculated using the morning cortisol values is referred to as morning LTC (to be in contrast with night LTC). We investigated the effects of (1) night LTC alone and (2) both morning LTC and night LTC, on cardiovascular risk factors. For the night LTC alone model, the night LTC at baseline was tested to predict the Lipids, Blood Pressure, and Body Composition latent factors at Baseline (4th grade). The fit indices were: χ^2(30) = 62.212 (p < 0.001); CFI = 0.979 (good); SRMR = 0.035 (good); and RMSEA = 0.043 (90% CI: 0.028–0.058) (good). However, night LTC alone was not significantly associated with Lipids, Blood Pressure, and Body Composition (Model 1; Table A.2). The parameter estimates for the association of morning LTC were also listed here for comparison purpose (Model 2; Table A.2). Strong standardized parameter estimates were seen for Blood Pressure, and Body composition, and less for Lipids. When both morning LTC and night LTC were tested as a single LTC, and then modeled with Lipids, Blood Pressure, and Body Composition latent factors at Baseline (4th grade), fit indices were: χ^2(95) = 216.591 (p < 0.001); CFI = 0.950 (good); SRMR = 0.040 (good); and RMSEA = 0.047 (90% CI: 0.038–0.055) (good). When morning LTC and night LTC were analyzed collectively, the parameter estimations of both LTCs are increased with cardiovascular risk factors, but in opposite directions (Model 3; Table A.2). Lower morning LTC was associated with higher levels of Lipids, Blood Pressure, and Body Composition, whereas higher night LTC was associated with higher

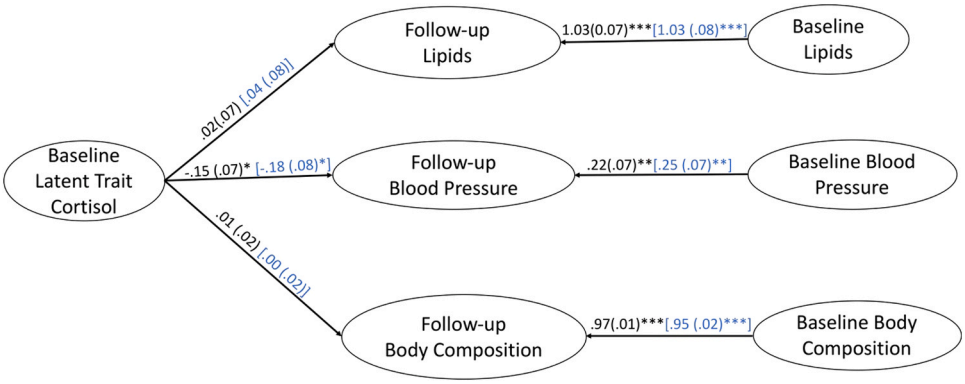


Fig. 3. Predictability of baseline (4th grade) salivary latent trait cortisol to follow-up (5th grade) cardiovascular risk factors from 347 subjects. Parameter estimations (standard errors) are shown between latent factors (cortisol and cardiovascular risk factors). SRMR and RMSEA suggested a fair fit of data to the model (χ^2[284] = 607.334 [p = 0.0000], CFI = 0.905, SRMR = 0.064, RMSEA = 0.057 [90% CI = 0.051 0.064]). *p < 0.05, **p < 0.01, and ***p < 0.001. Black = before adjusting for covariates. Blue = after adjusting for covariates. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

weight (Body Composition). This finding is consistent with higher morning diurnal cortisol (diurnal health) and lower evening stress related cortisol associated with improvements in cardiovascular risk factors.

3.7. Baseline (4th grade) LTC and Blood Pressure together predict follow-up (5th grade) Blood Pressure

Longitudinal analyses with a SEM model was carried out, with the LTC from the STMS model at Baseline (4th grade) and the Lipids, Blood Pressure, and Body Composition latent factors at Baseline (4th grade) modeled to predict the same latent factors at Follow-up (5th grade) (Fig. 3). The fit indices were: $\chi^2(284) = 607.334$ (p = 0.0000), CFI = 0.905 (fair), SRMR = 0.064 (fair), RMSEA = 0.057 (90% CI = [0.051 0.064]) (fair). Lower Lipids, Blood Pressure, and Body Composition latent factors in 4th grade were associated with the same latent factors in 5th grade. More interestingly, lower LTC at baseline was associated with higher level of Blood Pressure at follow-up ($\beta = -0.15 \pm 0.07$, p = 0.030) (Fig. 3). LTC at baseline and blood pressure at baseline explained 9.9% of the variation in blood pressure at follow-up. Adjustment for covariates (sex, age, race, socioeconomic, and medication use) did not weaken the effects of Baseline (4th grade) LTC on Follow-up (5th grade) blood pressure ($\beta = -0.18 \pm 0.08$, p = 0.017).

We also investigated the feasibility of using both morning LTC and night LTC to predict cardiovascular risk latent factors at Follow-up (5th grade). Night LTC factor was formed from 3 consecutive sampling days. The fit indices for the prediction model were: $\chi^2(433) = 786.389$ (p < 0.001); CFI = 0.907; SRMR = 0.062; and RMSEA = 0.048 (90% CI: 0.043–0.054); all suggesting a fair fit. Lower morning LTC at Baseline (4th grade) was associated with higher Blood Pressure at Follow-up (5th grade) ($\beta = -0.19 \pm 0.08$, p = 0.017), while night LTC was not associated with any cardiovascular risk factors (p > 0.05).

4. Discussion

We explored relationships of circadian health (diurnal fluctuations in cortisol) and cardiovascular risk factors in a population-based study of young children (9–10 years; n = 1336 assessments; 689 in 4th grade; 647 in 5th grade). Key advances in this study compared to previous reports are the population-based longitudinal design, the study of children during school days where sleep and activity patterns may be most uniform, and the testing of 9 cortisol measures per child per assessment (6 morning, 3 evening). A previous study of cortisol in school children collected samples during school hours, but this would not be expected to have captured circadian cortisol at first awakening (measured stress-related cortisol and not diurnal cortisol) (Dimolareva et al., 2018). In a study of hourly blood cortisol measures in children 6–12 years, peak cortisol was between 5 a.m. and 9 a.m., with nadir between 7 p.m. and 3 a.m. (Feder et al., 2004), emphasizing the importance of first-in-morning cortisol measures, as done in this current report. While there are numerous studies of cortisol levels in children and adults that accumulate in hair, nail clippings, or 24-h urine, these are all felt to be reflective of stress-related cortisol elevations (persistent over days or months), and not diurnal cortisol, as morning cortisol fluctuations are relatively short-lived (~4 h).

The LTC values in the study were modeled from multiple saliva cortisol samples (9 per child) collected within (morning, night) and across three sequential days. Consistent with our preliminary work (Yeung et al., 2016) and other prior work (Doane et al., 2015; Stroud et al., 2016), the waking and 30 min post-waking samples contributed to a LTC factor. Our data provide evidence that the same LTC factor structure can be extended from early and late adolescence (Doane et al., 2015; Stroud et al., 2016) to middle childhood. Our finding that LTC explained 43% of the variance in cortisol levels in 9–10 year old children is similar to findings of the cortisol awaking response in adults using a similar state trait model (46% on average; Hellhammer et al., 2007), and also consistent with a study of 39 children (47% on average; Fries et al., 2008).

In mice, circadian misalignment leads to blunted diurnal cortisol, and poor health (increased inflammation and early death) (Inokawa et al., 2020). We hypothesized that blunted first-in-morning cortisol might reflect circadian misalignment in children, and likewise be associated with poor health outcomes. Specifically, the hypothesis tested was that high morning (diurnal) cortisol would be associated with improved cardiovascular risk factors (improved circadian and cardiovascular health) at baseline (with 4th grader only). The application of structural equation modeling (SEM) framework supported our hypothesis of improved circadian health measured by increased morning diurnal cortisol showing strong associations with cardiovascular risk factors in a population-based study of children (9–10 years). The baseline analysis with a larger cohort (587 subjects) confirmed our preliminary report (474 subjects) (Yeung et al., 2016), showing that lower morning LTC was associated with higher levels of lipids, higher blood pressure, and higher body fat composition, explaining 2.2%, 5.4%, and 4.9% of the variation of each (Fig. 2). Our longitudinal analysis demonstrated that the baseline (4th grade) latent trait cortisol was predictive of 5th grade blood pressure, where lower morning LTC at 4th grade predicted higher blood pressure in 5th grade, consistent with the circadian health (circadian misalignment) model.

Although the variance explained by the morning LTC were relatively small, their associations were significant. Our findings may underestimate the associations of morning LTC associations due to limitations of our study design, including the home collection of salivary samples, inconsistency in following the saliva collection and storage protocol by children and their families, and likely challenges in protocol adherence regarding fasting instructions for lipids collection.

In contrast to the present results, prior studies have linked higher cortisol with higher diastolic or systolic blood pressure (Walker et al., 2000; Filipovský et al., 1996; Ward et al., 2003; Reynolds et al., 2003; Soriano-Rodriguez et al., 2010; Rice et al. 2018). This discrepancy may

be because these prior studies typically measure stress-related cortisol, and not first-in-morning diurnal cortisol, and thus have not studied circadian alignment. For example, in a children cortisol study, higher morning serum cortisol was associated with increased systolic blood pressure in 6.8 years old school children (n = 223), but samples were collected at a clinic between 8 and 9 a.m. presumably after the children had been awake for some hours (Soriano-Rodríguez et al., 2010). Another pilot children study of associations of morning (9–10 a.m.) and afternoon (1–2 p.m.) cortisol measures with blood pressure showed little difference in cortisol levels between these two time points, and some positive association of increased afternoon cortisol levels with increased blood pressure; this study more likely measured stress-related cortisol and not circadian alignment (n = 56; 3–5 year old children) (Rice et al., 2018). In addition, exposure to chronic stress can lead to adrenal suppression and reduced cortisol levels, such as reported with early adversity, family conflicts in adolescents, maternal depression, and social deprivation in 9–14 years old (Stroud et al., 2016; Apter-Levi et al., 2016; Hostinar et al., 2015; Kushner et al., 2016; Zhang et al., 2016). Two of the studies used first-in-morning home-based cortisol measures, and both found increased stress to be associated with reduced first-in-morning cortisol, possibly consistent with stress leading to circadian misalignment and reduced diurnal cycling (Stroud et al., 2016; Zhang et al., 2016). A study of 450 obese children (age 4–18 years) showed that higher cortisol and ACTH serum levels were associated with increased cardiovascular risk factors (blood pressure, triglycerides, insulin resistance) (Prodam et al., 2013). A similar study of 205 overweight Latino youth (age 8–13 years) also showed higher cortisol associated with increased metabolic syndrome feature, with the highest correlation with increased systolic blood pressure ($r = 0.34$; $P < 0.001$) (Weigensberg et al., 2008). Both of these studies showed higher cortisol associated with higher blood pressure, while in our current study we found lower cortisol associated with higher blood pressure. However, there are marked differences in the populations studied (obese or overweight vs. our population-based cohort), the ages studied (4–18 years vs. our 9–10 years cohort), the type of sample tested for cortisol (blood vs. our salivary samples), and the time of day of cortisol sampling (travel to clinics with sampling at 8 a.m., vs. our first-in-morning home-based sampling).

In embarking on this study, our hypothetical model was that the intra-subject difference between first-in-morning cortisol and late evening cortisol would define the "amplitude" of circadian cortisol rhythms. However, models testing first-in-morning cortisol alone vs. the difference between first-in-morning and late evening cortisol showed that first-in-morning cortisol alone leads to better fitting of models (addition of evening cortisol weakened data fit). In further consideration of our models, we believe that evening cortisol does not reflect the 'nadir' of circadian cortisol amplitude, but instead reflects stress-related cortisol. Thus, using evening cortisol as a nadir may mix 'apples and oranges', where first-in-morning cortisol reflects the peak amplitude of circadian cortisol rhythms, but evening cortisol (stress-related) may not be an accurate indicator of the circadian nadir. Future studies will need to clearly delineate measures of circadian alignment (first-in-morning upon awakening cortisol) vs. stress-related cortisol.

Our longitudinal data extend our cross-sectional results, indicating that morning trait cortisol is a causal factor for the elevated blood pressure a year later in middle childhood. Therefore, morning trait cortisol could be a vascular risk factor in children. It is well-documented that childhood cardiovascular risk factors predict adult cardiovascular disease (Berenson, 2002). If this longitudinal result can be replicated in a larger study in children or adult population, strategies targeting at lower cortisol action should be evaluated for their effects on cardiovascular diseases.

When evening stress-related cortisol levels were tested for association with cardiovascular risk factors alone, we found no significant association with cardiovascular risk factors. However, when both morning and evening cortisol levels were included in the model, significant but inverted associations of cortisol with body composition were observed; lower morning cortisol and elevated evening cortisol were both associated with increased body fat (Table A.2). This data suggests that increases in evening stress-related cortisol is associated with poorer cardiovascular outcomes in children, as has been observed by many other authors (Veldhorst et al., 2014; Papafotiou et al., 2017; Ricotti et al., 2020). Our data also suggests that the effect of increased stress-related cortisol on poor cardiovascular health is much weaker than the effect of decreased diurnal cortisol (poor circadian health). However, we did not find the predictability of the stress-related cortisol at 4th grade on any cardiovascular risk.

To our knowledge, this is the first longitudinal study of first-in-morning cortisol in young children during typical school days. We found support for a circadian health model, where strong diurnal cycling reflected by increased morning cortisol was associated with improvements in cardiovascular risk factors. We suggest that the built environment has removed circadian cues, and in turn blunted the diurnal cycling of morning cortisol. This may increase cardiovascular risk factors through poor cyclical regulation of inflammation and other physiological processes driven by cortisol. A prediction of this model is that efforts to improve circadian health (increased exercise, increased sunlight, altered diet patterns) may increase diurnal morning cortisol and decrease risk of poor cardiovascular risk factors. A limitation to our study is that the STMS and LTC modeling utilized in our study do not define thresholds of first-in-morning cortisol levels that could be used to define 'at-risk' children. Also, the methods utilized to collect blood pressure in this population-based study were suboptimal, and additional time points for cortisol measures throughout the day would better define true circadian cortisol cycling. Finally, our data may be consistent with our hypothetical model of circadian misalignment as a factor contributing to poor child health, but our study does not identify or study specific risk factors contributing to circadian misalignment. Future studies could refine methods and improve on the models presented here.

Funding source

Funding provided by the Clark Charitable Foundation, Bethesda, MD, USA. The Foundation had no role in study design, collection, analysis or interpretation of data, writing of the report ordecision to submit the paper for publication.

Conflict of interest

The authors have no conflicts of interest relevant to this article to disclose. The authors have no financial disclosures relevant to this article to report.

Acknowledgements

We appreciate the opportunity and commitment of the Biddeford and BuxtonElementary Schools, parents and children to let us carry out the Cardiovascular Health Intervention Program. G.D. Zhou conducted her research in the Department of Pharmaceutical Sciences at Binghamton University in the summer of 2019 when she was a visiting research assistant in E.P. Hoffman's research group.

Appendix A

Methods A.1

Procedures:

Procedures and testing protocols for the pediatric Cardiovascular Health Intervention Program (CHIP) have been described in detail (Yeung et al., 2016; White et al., 2017; Downing et al., 2021). Briefly, all assessments were carried out by faculty, staff and students of the University of New England. Participants were recruited from two elementary schools, with the entire 4th and 5th grade classes participating. Clinical assessments were done on site at University of New England, where participants had measures of blood lipids and glucose, resting blood pressure (BP), and anthropometric assessments. For cortisol measures, participants were provided 9 saliva collection kits to bring home, with instructions for the parents/guardians and child to collect sublingual samples at awakening, 30 min later, and at bedtime, on 3 consecutive days. Samples were stored in the home freezer, then brought by the participants to school and collected by study staff (Table A.1, Table A.2, Fig. A.1 and Fig. A.2).

Laboratory analyses included a skin prick blood sample tested on a lipids/glucose panel (Cholestech LDX), and measured total cholesterol, triglycerides, high density lipoprotein-cholesterol, and blood glucose. For the reported longitudinal data analyses, the missing data rate for all three

Table A.1
Correlations among cardiovascular risk indices.

Metabolic Index	Triglycerides	HDL	Systolic BP	Diastolic BP	BMI	Waist-to-hip ratio	% fat
Triglycerides	1						
HDL	-0.40	1					
Systolic blood pressure	.20	-0.12	1				
Diastolic blood pressure	.20	-0.08	.69	1			
BMI	.32	-0.32	.41	.31	1		
Waist-to-hip ratio	.35	-0.27	.22	.16	.58	1	
% fat	.32	-0.28	.40	.32	.75	.46	1

Table A.2
The effects of morning latent trait cortisol (LTC) and night LTC on cardiovascular risk factors at baseline (4th grade).

Model	Latent Factors	Lipids	Blood Pressure	Body Composition
1	Night LTC	.07±.09	-0.07±.11	.14±.11
2	Morning LTC	-0.15±.07*	-0.23±.05***	-0.22±.05***
3	Morning LTC	-0.18±.07*	-0.25±.06***	-0.27±.05***
	Night LTC	.11±.08	.04±.06	.16±.06**

Standardized parameter estimations ± standard errors are shown.
* $p < 0.05$.
** $p < 0.01$
*** $p < 0.001$.

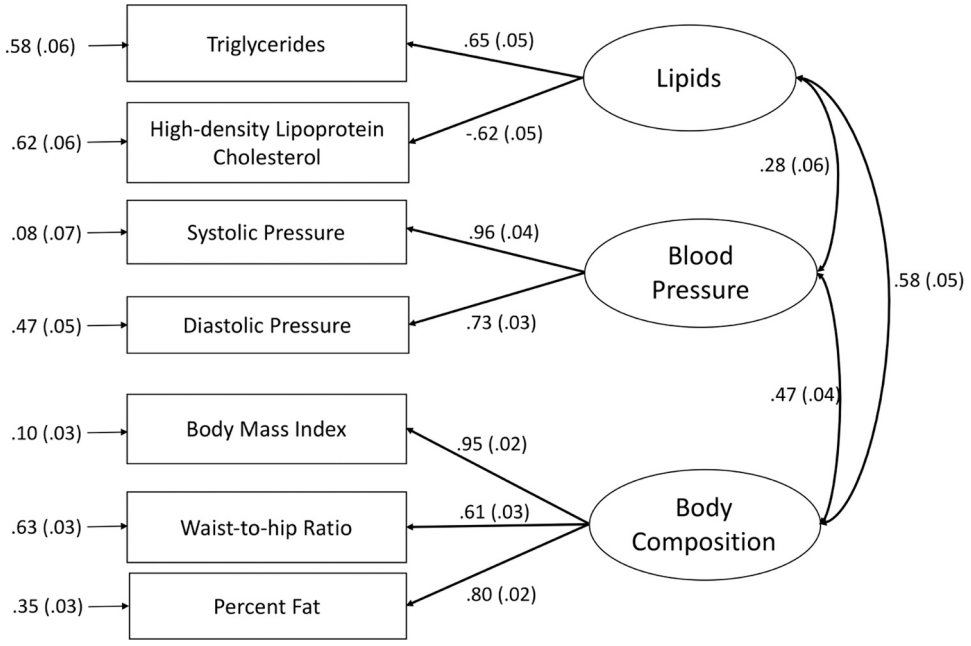


Fig. A.1. Confirmatory factor analysis of 4th grade children (684 subjects). All loadings were p-value < 0.001 (standard errors).

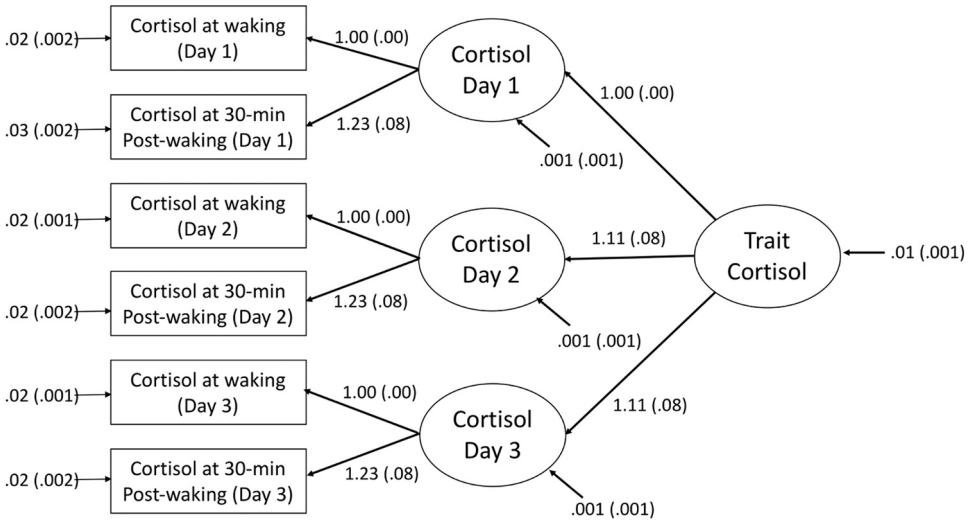


Fig. A.2. Single trait-multistate (STMS) model showing associations of cortisol measures over consecutive days (3 days; 592 children). All loadings p-value < 0.001 (standard errors). /.

biomarkers extracted from blood samples was 17.4% at Baseline (4th grade) and 18.0% at Follow-up (5th grade). Two blood pressure measurements from the right arm of each participant were taken two minutes apart and averaged. The missing data rate for blood pressure was 0.2% at Baseline and 0.5% at Follow-up. In addition to height and weight, FitnessGram software calculated percent fat, integrating participant's right tricep, calf and sex in determinations (Meredith and Welk, 2010). The missing data rates were BMI (4th grade 2.0%; 5th grade 0.9%), waist-to-hip ratio (4th grade 1.0%; 5th grade 1.9%), and percent fat (4th grade 0.3%; 5th grade 1.4%).

Each saliva sample was assayed for cortisol immunoassay in duplicate (Salimetrics, State College, PA). The immunoassay used 25 μl saliva, with standard curve from 0.01 to 3 μg/dl. At baseline (4th grade), missingness in the cortisol data was from 97 children who did not provide any samples on any day, and 5 children that provided cortisol samples but did not have cardiovascular phenotyping assessments. In terms of laboratory (cortisol) data, missingness was defined as insufficient volume for assay, cortisol values below the lower limit of sensitivity, cortisol values that were biologically implausible (>4.0 μg/dl), or greater than 4 standard deviations above the sample mean; 13.72% had missing cortisol data by these definitions.

At follow-up (5th grade), missingness in the cortisol data was from 194 children that did not provide samples, and 2 children that did not participate in phenotyping. These 196 participants were not significantly different from the 451 children who provided at least one cortisol sample, and had lipids, blood pressure, body composition and confounding factors measures. Missingness of cortisol measures was 9.01%.

347 participants were used the longitudinal analysis. 240 participants at baseline (out of 587 participants) did not have the follow-up data (with at least one cortisol sample, and data on lipids, blood pressure, body composition and confounding factors). 104 participants at follow-up (out of 451 participants) did not have the baseline data. For the 347 participants, there was totally 11.73% missing cortisol data (10.63% missing samples, 0.90% outliers) at baseline and 8.71% missing cortisol data (7.01% missing samples, 1.70% outliers) at follow-up.

Socioeconomic status was assessed using the child's free lunch status (0 = low income [free or subsidized lunch], or 1 = medium/high income [no free or subsidized lunch]). The low income percentage was 49.63% at Baseline (4th grade) and 31.26% at Follow-up (5th grade). The Medication use percentage is 25.40% in 4th grade and 17.62% in 5th grade. The missing data rates for socioeconomic status were 0.58% at Baseline and 3.09% at Follow-up. Missing data rates for Medication use were 0% at Baseline and at Follow-up.

No transformation was applied to any variable even if their skewness (e.g., cortisol, Triglycerides, and BMI) is greater than 1 (either at baseline or follow-up) because Mplus version 8.4 is robust to non-normality of the input data.

References for Supplemental Methods:

Downing R 2nd, Michael T, Place R, Hoffman E, Visich P, The Influence of Metabolic Syndrome Risk Factors on Carotid Intima Media Thickness in Children. Glob. Pediatr. Health 2021 Jan 11;8:2333794X20987453. doi: 10.1177/2333794X20987453. PMID: 33490309; PMCID: PMC7804354.

White D, Place R, Michael T, Hoffman E, Gordon PM, Visich P. The Relationship between Coronary Artery Disease Risk Factors and Carotid Intima-Media Thickness in Children. J. Pediatr. 2017 Nov;190:38–42. doi: 10.1016/j.jpeds.2017.07.034. Epub 2017 Sep 11. PMID: 28912051.

Yeung EW, Place R, Gordish-Dressman H, Visich P, Hoffman E, Walker SO, Granger DA. Salivary latent trait cortisol (LTC): Relation to lipids, blood pressure, and body composition in middle childhood. Psychoneuroendocrinology. 2016 Sep;71:110–8. doi: 10.1016/j.psyneuen.2016.05.013. Epub 2016 May 18. PMID: 27262343.

References

Alibhai, F.J., LaMarre, J., Reitz, C.J., Tsimakouridze, E.V., Kroetsch, J.T., Bolz, S.S., Shulman, A., Steinberg, S., Burris, T.P., Oudit, G.Y., Martino, T.A., 2017. Disrupting the key circadian regulator CLOCK leads to age-dependent cardiovascular disease. J. Mol. Cell Cardiol. 105, 24–37.

Apter-Levi, Y., Pratt, M., Vakart, A., Feldman, M., Zagoory-Sharon, O., Feldman, R., 2016. Maternal depression across the first years of life compromises child psychosocial adjustment; relations to child HPA-axis functioning. Psychoneuroendocrinology 64, 47–56.

Barnes, P.J., 2006. How corticosteroids control inflammation: Quintiles Prize Lecture 2005. Br. J. Pharmacol. 148, 245–254.

Benchimol, E.I., Manuel, D.G., Guttmann, A., Nguyen, G.C., Mojaverian, N., Quach, P., Mack, D.R., 2014. Changing age demographics of inflammatory bowel disease in Ontario, Canada: a population-based cohort study of epidemiology trends. Inflamm. Bowel Dis. 20, 1761–1769.

Berenson, G.S., 2002. Childhood risk factors predict adult risk associated with subclinical cardiovascular disease. The Bogalusa Heart Study. Am. J. Cardiol. 90, 3L–7L.

Bosma-den Boer, M.M., van Wetten, M.L., Pruimboom, L., 2012. Chronic inflammatory diseases are stimulated by current lifestyle: how diet, stress levels and medication prevent our body from recovering. Nutr. Metab. 9, 32.

Dimolareva, M., Gee, N.R., Pfeffer, K., Maréchal, L., Pennington, K., Meints, K., 2018. Measuring cortisol in the classroom with school-aged children-a systematic review and recommendations. Int. J. Environ. Res. Public Health 15, 15.

Doane, L.D., Chen, F.R., Sladek, M.R., Van Lenten, S.A., Granger, D.A., 2015. Latent trait cortisol (LTC) levels: reliability, validity, and stability. Psychoneuroendocrinology 55, 21–35.
Ehlers, A., Xie, W., Agapov, E., Brown, S., Steinberg, D., Tidwell, R., Sajol, G., Schutz, R., Weaver, R., Yu, H., Castro, M., Bacharier, L.B., Wang, X., Holtzman, M.J., Haspel, J. A., 2018. BMAL1 links the circadian clock to viral airway pathology and asthma phenotypes. Mucosal Immunol. 11, 97–111.
Feder, A., Coplan, J.D., Goetz, R.R., Mathew, S.J., Pine, D.S., Dahl, R.E., Ryan, N.D., Greenwald†, S., Weissman, M.M., 2004. Twenty-four-hour cortisol secretion patterns in prepubertal children with anxiety or depressive disorders. Biol. Psychiatry 56, 198–204.
Filipovský, J., Ducimetiére, P., Eschwége, E., Richard, J.L., Rosselin, G., Claude, J.R., 1996. The relationship of blood pressure with glucose, insulin, heart rate, free fatty acids and plasma cortisol levels according to degree of obesity in middle-aged men. J. Hypertens. 14, 229–235.
Fries, A.B.W., Shirtcliff, E.A., Pollak, S.D., 2008. Neuroendocrine dysregulation following early social deprivation in children. Dev. Psychobiol. 50, 588–599.
Gombert, M., Carrasco-Luna, J., Pin-Arboledas, G., Codoñer-Franch, P., 2019. The connection of circadian rhythm to inflammatory bowel disease. Transl. Res. 206, 107–118.
Haahtela, T., von Hertzen, L., Anto, J.M., Bai, C., Baigenzhin, A., Bateman, E.D., Behera, D., Bennoor, K., Camargos, P., Chavannes, N., de Sousa, J.C., Cruz, A., Do Céu Teixeira, M., Erhola, M., Furman, E., Gemicioğlu, B., Gonzalez Diaz, S., Hellings, P.W., Jousilahti, P., Khaltaev, N., Kolek, V., Kuna, P., La Grutta, S., Lan, L., Maglakelidze, T., Masjedi, M.R., Mihaltan, F., Mohammad, Y., Nunes, E., Nyberg, A., Quel, J., Rosado-Pinto, J., Sagara, H., Samolinski, B., Schraufnagel, D., Sooronbaev, T., Tag Eldin, M., To, T., Valiulis, A., Varghese, C., Vasankari, T., Viegi, G., Winders, T., Yañez, A., Yorgancioğlu, A., Yusuf, O., Bousquet, J., Billo, N. E., 2019. Helsinki by nature: the nature step to respiratory health. Clin. Transl. Allergy 9, 57.
Hellhammer, J., Fries, E., Schweisthal, O.W., Schlotz, W., Stone, A.A., Hagemann, D., 2007. Several daily measurements are nec- essary to reliably assess the cortisol rise after awakening: state- and trait components. Psychoneuroendocrinology 32, 80–86.
Hostinar, C.E., Johnson, A.E., Gunnar, M.R., 2015. Early social deprivation and the social buffering of cortisol stress responses in late childhood: an experimental study. Dev. Psychol. 51, 1597–1608.
Hu, L.T., Bentler, P.M., 1999. Cutoff criteria for fit indexes in covariance structure analysis: conventional criteria versus new alternatives. Struct. Equ. Model. Multidiscip. J. 6, 1–55.
Inokawa, H., Umemura, Y., Shimba, A., Kawakami, E., Koike, N., Tsuchiya, Y., Ohashi, M., Minami, Y., Cui, G., Asahi, T., Ono, R., Sasawaki, Y., Konishi, E., Yoo, S. H., Chen, Z., Teramukai, S., Ikuta, K., Yagita, K., 2020. Chronic circadian misalignment accelerates immune senescence and abbreviates lifespan in mice. Sci. Rep. 10, 2569.
Jakubowicz, D., Landau, Z., Tsameret, S., Wainstein, J., Raz, I., Ahren, B., Chapnik, N., Barnea, M., Ganz, T., Menaged, M., Mor, N., Bar-Dayan, Y., Froy, O., 2019. Reduction in glycated hemoglobin and daily insulin dose alongside circadian clock upregulation in patients with type 2 diabetes consuming a three-meal diet: a randomized clinical trial. Diabetes Care 42, 2171–2180.
Kushner, M.R., Barrios, C., Smith, V.C., Dougherty, L.R., 2016. Physiological and behavioral vulnerability markers increase risk to early life stress in preschool-aged children. J. Abnorm. Child Psychol. 44, 859–870.
Lee, C.J., Sears, C.L., Maruthur, N., 2020. Gut microbiome and its role in obesity and insulin resistance. Ann. N. Y. Acad. Sci. 1461, 37–52.
Liu, X., Yu, R., Zhu, L., Hou, X., Zou, K., 2017. Bidirectional regulation of circadian disturbance and inflammation in inflammatory bowel disease. Inflamm. Bowel Dis. 23, 1741–1751.
Lundbäck, B., Backman, H., Lötvall, J., Rönmark, E., 2016. Is asthma prevalence still increasing? Expert Rev. Respir. Med. 10, 39–51.
McAloon, C.J., Boylan, L.M., Hamborg, T., Stallard, N., Osman, F., Lim, P.B., Hayat, S.A., 2016. The changing face of cardiovascular disease 2000-2012: an analysis of the world health organisation global health estimates data. Int. J. Cardiol. 224, 256–264.
Morris, C.J., Purvis, T.E., Hu, K., Scheer, F.A., 2016. Circadian misalignment increases cardiovascular disease risk factors in humans. Proc. Natl. Acad. Sci. USA 113, E1402–E1411.
NCD Risk Factor Collaboration (NCD-RisC), 2016. Worldwide trends in diabetes since 1980: a pooled analysis of 751 population-based studies with 4.4 million participants. Lancet 387, 1513–1530.
Ng, S.C., Shi, H.Y., Hamidi, N., Underwood, F.E., Tang, W., Benchimol, E.I., Panaccione, R., Ghosh, S., Wu, J.C.Y., Chan, F.K.L., Sung, J.J.Y., Kaplan, G.G., 2018. Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. Lancet 390, 2769–2778.
Papafotiou, C., Christaki, E., van den Akker, E.L., Wester, V.L., Apostolakou, F., Papassotiriou, I., Chrousos, G.P., Pervanidou, P., 2017. Hair cortisol concentrations exhibit a positive association with salivary cortisol profiles and are increased in obese prepubertal girls. Stress 20, 217–222.
Prodam, F., Ricotti, R., Agarla, V., Parlamento, S., Genoni, G., Balossini, C., Walker, G.E., Aimaretti, G., Bona, G., Bellone, S., 2013. High-end normal adrenocorticotropic hormone and cortisol levels are associated with specific cardiovascular risk factors in pediatric obesity: a cross-sectional study. BMC Med. 11, 44.
Reynolds, R.M., Syddall, H.E., Walker, B.R., Wood, P.J., Phillips, D.I., 2003. Predicting cardiovascular risk factors from plasma cortisol measured during oral glucose tolerance tests. Metabolism 52, 524–527.
Rice, M., Turner-Henson, A., Hage, F.G., Azuero, A., Joiner, C., Affuso, O., Ejem, D., Davis, S.L., Soistmann, H., 2018. Factors that influence blood pressure in 3- to 5-year-old children: a pilot study. Biol. Res. Nurs. 20, 25–31.
Ricotti, R., Solito, A., Mariotti Zani, E., Caputo, M., Genoni, G., Barone-Adesi, F., Mancioppi, V., Agosti, E., Aimaretti, G., Bellone, S., Prodam, F., 2020. The relationship between cortisol and IGF-I influences metabolic alteration in pediatric overweight and obesity. Eur. J. Endocrinol. 182, 255–264.
Sallam, N., Laher, I., 2016. Exercise modulates oxidative stress and inflammation in aging and cardiovascular diseases. Oxid. Med. Cell. Longev. 2016, 7239639.
Saltiel, A.R., Olefsky, J.M., 2017. Inflammatory mechanisms linking obesity and metabolic disease. J. Clin. Investig. 127, 1–4.
Serebrisky, D., Wiznia, A., 2019. Pediatric asthma: a global epidemic. Ann. Glob. Health 85 (6), 1–6.
Soriano-Rodríguez, P., Osiniri, I., Grau-Cabrera, P., Riera-Pérez, E., Prats-Puig, A., Carbonell-Alferez, M., Schneider, S., Mora-Maruny, C., De Zegher, F., Ibánez, L., Bassols, J., López-Bermejo, A., 2010. Physiological concentrations of serum cortisol are related to vascular risk markers in prepubertal children. Pedia Res. 68, 452–455.
Stroud, C.B., Chen, F.R., Doane, L.D., Granger, D.A., 2016. Individual differences in early adolescents' latent trait cortisol (LTC): relation to early adversity. Dev. Psychobiol. 58, 700–713.
Tahara, Y., Aoyama, S., Shibata, S., 2017. The mammalian circadian clock and its entrainment by stress and exercise. J. Physiol. Sci. 67, 1–10.
Ticinesi, A., Lauretani, F., Tana, C., Nouvenne, A., Ridolo, E., Meschi, T., 2019. Exercise and immune system as modulators of intestinal microbiome: implications for the gut-muscle axis hypothesis. Exerc. Immunol. Rev. 25, 84–95.
Trott, A.J., Menet, J.S., 2018. Regulation of circadian clock transcriptional output by CLOCK:BMAL1. PLoS Genet. 14, 1007156.
Veldhorst, M.A., Noppe, G., Jongejan, M.H., Kok, C.B., Mekic, S., Koper, J.W., van Rossum, E.F., van den Akker, E.L., 2014. Increased scalp hair cortisol concentrations in obese children. J. Clin. Endocrinol. Metab. 99, 285–290.
Walker, B.R., Soderberg, S., Lindahl, B., Olsson, T., 2000. Independent effects of obesity and cortisol in predicting cardiovascular risk factors in men and women. J. Intern. Med. 247, 198–204.
Ward, A.M., Fall, C.H., Stein, C.E., Kumaran, K., Veena, S.R., Wood, P.J., Syddall, H.E., Phillips, D.I., 2003. Cortisol and the metabolic syndrome in South Asians. Clin. Endocrinol. 58, 500–505.
Weigensberg, M.J., Toledo-Corral, C.M., Goran, M.I., 2008. Association between the metabolic syndrome and serum cortisol in overweight Latino youth. J. Clin. Endocrinol. Metab. 93, 1372–1378.
White, D., Place, R., Michael, T., Hoffman, E., Gordon, P.M., Visich, P., 2017. The relationship between coronary artery disease risk factors and carotid intima-media thickness in children. J. Pediatr. 190, 38–42.
Yeung, E.W., Place, R., Gordish-Dressman, H., Visich, P., Hoffman, E., Walker, S.O., Granger, D.A., 2016. Salivary latent trait cortisol (LTC): relation to lipids, blood pressure, and body composition in middle childhood. Psychoneuroendocrinology 71, 110–118.
Zhang, J., Lam, S.P., Kong, A.P., Ma, R.C., Li, S.X., Chan, J.W., Yu, M.W., Zhou, J., Chan, M.H., Ho, C.S., Li, A.M., Tang, X., Wing, Y.K., 2016. Family conflict and lower morning cortisol in adolescents and adults: modulation of puberty. Sci. Rep. 6, 22531.