Actigraphic sleep and cortisol in middle childhood: A multivariate behavioral genetics model

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ABSTRACT

To date, behavioral genetic studies investigated either sleep or cortisol levels in middle childhood, but not both simultaneously. Therefore, a pertinent question is the degree to which genetic factors and environmental factor contribute to the correlation between sleep and cortisol levels. To address this question, we employed the classical twin design. We measured sleep in 6-9-year-old twins (N = 436 twin pairs, "Together Unique" study) over four consecutive nights using actigraphy, and we measured morning cortisol on two consecutive days. Sleep duration, sleep efficiency, and wake episodes were used as indicators of sleep. Morning cortisol level was used as cortisol indicator. A structural equation model was fitted to estimate the contribution of additive genetic effects (A), shared (common) environmental effects, (C) and unique environmental effects (E) to phenotypic variances and covariances. Age, cohort, and sex were included as covariates. The heritability of sleep duration, sleep efficiency, and wake episodes were 52%, 45%, and 55%, respectively. Common environmental factors played no significant role. High genetic correlations between sleep duration and sleep efficiency and high genetic correlations between sleep efficiency and wake episodes were found. Shared environmental (29%) and unique environmental factors (53%) explained the variance in morning cortisol levels. Because the sleep and cortisol measures were found to be uncorrelated, we did not consider genetic and environmental contributions to the association between the sleep and cortisol measures. Our findings indicate that sleep duration, sleep efficiency, and wake episodes in children are mostly impacted by genetic factors and by unique environmental factors (including measurement error).

1. Introduction

Sleep and cortisol both have an impact on the psychological and physiological functioning of children [1–3]. Sleep is controlled by two systems. There is the suprachiasmatic nucleus, which is the biological clock indicating that it is time to sleep when it is dark and time to be awake when it is light [4], and there is the sleep homeostat which keeps track of how much sleep we had and when we must refill the sleep reserve [5]. The production of steroid hormone cortisol is regulated by the hypothalamus-pituitary-adrenal (HPA) axis. Cortisol has several functions, including regulation of the metabolism, the immune system,

and the response to stress [6]. Its release from the HPA axis follows the circadian rhythm: It rises after midnight and rapidly at wake-up, peaking around half an hour after wake-up. Cortisol levels gradually decline during the day, with the lowest point at around midnight [7].

Previous studies have suggested that sleep and cortisol are associated in children. Using a one-night measurement of polysomnography in 6–12-year-old children, Fernandez-Mendoza et al. [8] found that children with short sleep duration and parent-reported sleep problems had increased evening and morning cortisol levels. Again using data based on one night of sleep, Lemola et al. [9] also found a negative association between morning cortisol and sleep duration in 6–10-year-old children.

Two studies have employed actigraphy to measure sleep in middle childhood. In a group of 282 8-year-old children, children with short sleep duration, compared to children with average sleep duration, had a higher cortisol awakening response, while children with low sleep efficiency, compared to children with average or high sleep efficiency had higher diurnal cortisol secretion [10]. El-Sheikh and colleagues [11] found that higher levels of cortisol were associated with shorter sleep duration and poor sleep quality in a group of 64 children with a mean age of 8.75 years. Overall, shorter sleep duration has been related to higher cortisol secretion. However, most studies did not report correlations or effect sizes which is why we have no information about the strength of the association.

An open question concerns the extent to which genetic and environmental factors are involved in the association of sleep and cortisol. The physiological processes of sleep and cortisol are likely to be related. Cortisol-releasing-hormones (CRH) have been suggested to be associated with sleep and wakefulness [12]. High CRH has been associated with decreased slow wave sleep, and increased light sleep and wakefulness [13]. Suppression of CRH depends on inhibitory feedback by cortisol [14]. Glucocorticoid receptor (GR) activation at the level of the paraventricular nucleus decreases release of CRH, whereas GR activation on the amygdala increases release of CRH [15]. Studies administering corticosterone have also reported either increased or decreased wakefulness or slow wave sleep [12,13]. Specifically, low doses seem to decrease wakefulness and increase slow wave sleep whereas high doses seem to do the opposite [13]. A possible explanation lies in the binding of cortisol to receptors. At low levels, cortisol binds to the MR, at higher levels cortisol binds to the GR and at very high levels, it binds to the amygdala GR [12]. Binding to the amygdala GR is associated with an increase of CRH (and of subsequent cortisol) [15]. Environmental factors can lead to high cortisol levels and/or sleep deprivation [6,16]. Besides environmental factors explaining associations between sleep and cortisol, genetic factors may (partly) explain this association. Alexander et al. [17] found a link between the brain-derived neurotrophic factor gene polymorphism (BDNF Val66Met) and stress reactivity: carriers of the Met allele had significantly less activation of the HPA axis in response to a psychosocial stressor. This might indicate that carriers of this polymorphism do not display high cortisol levels in which cortisol is binding to the amygdala GR impacting sleep.

To date, most twin studies investigating sleep have been conducted in adults, and a majority of these used self-reports of sleep duration and/or quality. Heritability coefficients of different sleep measures ranged from 30% to 60% [18–21]. Behavioral genetic studies of sleep in children are scarce. Heritability coefficients for self- or parent-reported sleep quality or sleep duration range between 36% and 41% [22,23]. A recent study investigated the heritability of some objective sleep measures, and the covariance between these measures in a sample of 8-year-old children [24]. Genetic factors accounted for 81% of the variance in sleep duration, and for 79% of the variance in sleep efficiency. Common environmental factors played no role. This study also investigated the contributions of genetic and environmental factors to the covariance between sleep duration and sleep efficiency: A common genetic factor (genetic correlation of .85) underlies sleep duration and sleep efficiency. However, not all sleep measures were subject to this common genetic factor: e.g. sleep duration and sleep midpoint time variability did not share a common genetic factor. In this study, not all sleep measures were investigated together in a multivariate ACE model, which leaves the question open if different aspects of sleep, such as wake episodes may be subject to the same genetic and environmental factors. Different processes may be at play: pulsatile release of cortisol occurs when a person wakes up during the night. However, it is not clear whether these pulses build up to higher cortisol during the day [12]. Given the low sample sizes in most actigraphy studies, a modest contribution of shared environmental factors may have gone undetected due to low statistical power.

Another important consideration is whether the extent to which genetic and/or environmental factors explain variation in sleep depends on the structure of the day of the children. A recent study in 11-to 14-year-olds indicated that common environmental factors do play a role on several sleep measures on school days, but not on free days, while genetic factors impacted sleep measures on the free days [25]. It is not yet clear whether this pattern also presents in middle childhood samples, as adolescence is especially linked to changes in sleep, and no studies so far have investigated younger children using actigraphy.

Twin studies on cortisol levels have included samples ranging from 9-year old children to adults. In a study on 14-year-olds, heritability of the cortisol level after morning awakening was 28% [26]. In a study on 9 to 16-year old children, genetic factors accounted for 28% of the variation in cortisol levels directly after morning awakening, 60% 30 min after morning awakening and 8% in the evening [27].

Twin studies to date have considered either sleep or cortisol levels, but not both together. Therefore, little is known about the contributions of genetic and environmental factors to the covariance of sleep and cortisol. In the current study, we employed a multivariate model based on the classical twin design to investigate the contribution of genetic and environmental factors to the phenotypic variance in actigraphy, cortisol levels, and to their phenotypic covariance. This study has three aims: 1) to investigate the contributions of genetic, shared environmental, and non-shared environmental factors to the variance in sleep duration, sleep efficiency, and wake episodes; 2) to investigate the contributions of genetic, shared environmental, and non-shared environmental factors to the variance in morning cortisol levels and the total cortisol production during the day; and 3) to investigate the contributions of genetic, shared environmental, and non-shared environmental factors to the covariances of sleep and cortisol.

Based on previous research in adults [19,20], we expected genetic influences on sleep as well as cortisol. Sleep studies in adults did not report shared environmental influences of sleep measures. In our middle-childhood-aged sample, we expected shared environmental influences as we anticipated potential influences of school hours and parental rules about bedtime, which are shared by the twins, to affect sleep. Regarding cortisol, we expected genetic influences on the cortisol measures, but not shared environmental influences [28–30]. Lastly, we examined common genetic influences to explain the covariance between the cortisol and sleep measures, as previous research has found genetic influences on both cortisol and sleep [31,32]. In exploratory analyses, we investigated whether the contribution of genetic and environmental factors differed depending on the day, testing for differences between weekdays and weekend days.

2. Methods

2.1. Participants

The "Together Unique" project is a longitudinal twin study with an experimental cohort-sequential design (see website: http://www.samen-uniek.com/) which includes two cohorts: an early childhood cohort ($N = 239$ families) and a middle childhood cohort ($N = 257$ families). Through municipal records, twin families from the western region of the Netherlands were invited to participate. Families were contacted if the twins had the same gender, their parents were Dutch speaking, and both parents and grandparents were of European descent. Exclusion criteria were the presence of congenital disability, psychological disorder, chronic illness, hereditary disease, visual/hearing impairment, or an IQ of <70. For a detailed description of the recruitment, see Euser et al. [33] for the early childhood cohort, and van der Meulen et al. [34] for the middle childhood cohort. The study was approved by the central committee on research involving human subjects in the Netherlands (CCMO; Early childhood cohort NL49069.000.14; Middle childhood cohort NL50277.058.14).

The current study was pre-registered (https://osf.io/karqx/) and utilized data from the first measurement wave of the middle childhood

cohort (collected in 2015/2016) ($N = 256$) and the fifth measurement wave of the early childhood cohort (collected in 2019) ($N = 180$), resulting in a total of 436 twin pairs ($N = 872$ participants) of the same age range (6–9 years old). Sixty families dropped out of the study before the fifth current wave of the early childhood cohort. The mean age of the total group was 7.5 ($SD = 0.59$; early childhood cohort: $M = 7.53$, $SD = 0.59$, middle childhood cohort: $M = 7.48$, $SD = 0.58$), 48% were male, and 58% of the twins were monozygotic. Descriptives, separately for monozygotic (MZ) and dizygotic (DZ) twins, of both samples are shown in Table 1.

2.2. Procedure

All families received a set of actigraphs for their children via the mail. Actigraphs are watch-like devices which register sleep and wake states by recording movement. The children wore these devices on four consecutive nights, first two weeknights and then two weekend nights. All actigraphs were color-coded to reliably match them to each twin. During the research visit the parents were asked to download an app which served as an e-diary. Additionally, parents received daily reminders to use the actigraph. Parents were asked to put the actigraphs on their children's non-dominant hand wrist each night before the children went to sleep and to remove the actigraphs in the morning after the children woke up. Furthermore, parents received a paper logbook to report on any possible occurring problems regarding the app or actigraphs. Over the course of the two weekdays (first two days of data collection), parents collected saliva samples from their children at three time-points (after awakening, between 16:00 and 18:00 and 30 min after dinner). Via the app, they were reminded to collect saliva and to report on whether the children had consumed any food or drinks, had engaged in physical activity, or had experienced any stress during the 30 min before saliva collection. The saliva was collected by requiring the children to spit into a small tube (passive drooling). Parents also reported in the paper logbook whether there were any issues during the saliva collection. Parental report on sleep times was used to check the data manually against errors in the measurement and/or analysis (see measures). This information was used to enhance the accuracy of the actigraphy and e-diary data.

2.3. Measures

2.3.1. Sleep

Sleep was assessed for four subsequent nights, using wrist-actigraphy. Actigraphy is a well-validated and non-intrusive way to estimate sleep and wakefulness in the home [35]. Previous research indicated that four nights are sufficient to reliably estimate the sleep measures included in this study [36]. Actigraphy has concordance rates of more than 90% in comparison with polysomnography, the gold standard for measuring sleep [37]. MicroMini-Motionlogger actigraphs from Ambulatory Monitoring Inc. (Ardsley, NY) were used to collect data within fixed 1-min time frames using Zero Crossing (ZC) mode. The

sleep data was analyzed using Action-W software (Version 2.7.2305). First, we manually checked the actigraph data according to the Action-W user guidelines (Version 2.7.1). Bedtimes and rise times of the parental report were manually compared to the times of the actigraphs to detect possible inconsistencies in the actigraph data. Cases in which the parental report deviated 30 minutes or more from the actigraph data were reassessed independently by two raters (JR and MBK). In cases of rater disagreement, a third rater (MO) made the final decision. Subsequently, the following, commonly used, sleep variables were computed automatically using the validated Sadeh algorithm for children older than 12 months [38]: sleep duration, sleep efficiency, and wake episodes. *Sleep duration* is the total number of minutes asleep while being in bed. *Sleep efficiency* is the percentage of time being asleep between sleep onset and morning awakening. *Wake episodes* are the number of occasions of adjacent 1-min wake epochs while being in bed. We computed the means of sleep efficiency, sleep duration, and wake episodes over the four days, as a recent study did not find substantial differences in sleep measures on weekend or on weekdays in this age group [24]. Nevertheless, we also computed the means for two weekdays and two weekend days separately to test this in our sample.

2.3.2. Cortisol

Saliva samples were taken three times per day over two consecutive days. Assessing cortisol via saliva has been widely used and proven as a reliable proxy for unbound cortisol in blood [7]. The non-invasiveness makes it easy to use in studies with children. By collecting saliva on two days, we obtained a more robust measure than with a single day, and we can account for non-compliance or mistakes during collection on one occasion. The primary parent performed the saliva collection directly after the awakening of the children. Parents received the instructions orally from the researcher beforehand as well as written in a paper logbook. Furthermore, parents received reminders via the app before each moment of collection ensuring that the time of saliva collection was adhered to. The primary parent performed the saliva collection 1) directly after the awakening of the children, 2) between 16.00 and 18.00 o'clock, and 3) 30 minutes after dinner. Due to the number of missing values on the second and third time point, and a high correlation between the morning cortisol and the daily cortisol production, we decided to deviate from our pre-registered plan and only use the morning cortisol samples in subsequent analyses. Parents were asked to store the saliva in the freezer. Once collected, saliva samples were stored at $-20°$ Celsius at the university until they were sent to the laboratory of the University Trier for cortisol analyses. To determine the cortisol concentration in the saliva sample, a time-resolved fluorescence immunoassay was used. On each batch, the same three saliva control samples (low, medium and high) were run. If control samples were out of a $2SD$ range, the whole batch was reanalyzed. The intra-assay coefficient of variation was between 4.0% and 6.7%, and the corresponding inter-assay coefficients of variation were between 7.1% and 9.0% indicating that the variation between batches was low [39]. Inadmissible data values, as indicated by the lab, were treated as missing: These data values (in total six measurements or 0.1%) most likely did not reflect cortisol levels, but high glucose intake just before saliva sampling, which can distort the measurement accuracy of cortisol values. The mean morning cortisol levels of the two measurement days were used for the analyses. We expected the effect to be the strongest for the morning cortisol as cortisol builds up during the night [7].

2.3.3. Zygosity

To determine the zygosity of the twins, DNA samples of the twins were taken by buccal swabs. For three twin-pairs in the early childhood cohort and one twin-pair in the middle childhood cohort, no DNA data was available. For these, zygosity was determined with a questionnaire filled in by the primary parent with eight items about physical resemblance and the confusion of the parents in distinguishing the twins [40]. The questionnaire predicted zygosity in 93% of the cases compared to

Table 1
Means and Standard deviations of the Outcome Variables.

	Total *M* (*SD*)	MZ *M* (*SD*)	DZ *M* (*SD*)
N twin pairs	436	251	185
Age	7.50 (0.59)	7.54 (0.61)	7.45 (0.55)
Sex % female	52	52	52
Bedroom % shared	55	59	51
Sleep duration in hours	8.54 (0.73)	8.51 (0.73)	8.56 (0.73)
Sleep efficiency in %	86.09 (6.19)	86.28 (6.16)	85.83 (6.23)
Wake episodes	24.10 (6.42)	23.78 (6.29)	24.55 (6.59)
MCL	9.35 (3.70)	9.40 (3.52)	9.28 (3.94)

Note. MCL = morning cortisol level in nmol/liter; means and standard deviations only of the oldest twin displayed for readability.

our own DNA analyses. Furthermore, two samples were re-analyzed as parents indicated their doubts concerning the zygosity determination. Most likely these two samples had been switched in the first analysis, as re-analysis showed that one twin pair was monozygotic instead of dizygotic and the other twin pair was dizygotic instead of monozygotic. These results were checked again and confirmed.

2.3.4. Covariates

To examine potential confounding factors influencing cortisol or sleep data, information on age, sex and whether the twins shared a bedroom were collected using questionnaires, completed by the primary caregiver. Sleep duration and cortisol levels change during the development of children, therefore we controlled for age effects. Sharing a bedroom might also have an impact on sleeping patterns of the children. The data collection of the two cohorts was in different years, which also might have an impact on the data, so we investigated whether cohort was significantly associated with the phenotypes. Furthermore, in exploratory analyses, we checked whether sleep assessments took place in the vacation or during school times, in winter or summer time or during the change between winter and summer time.

Also, the following potential confounders of cortisol measures were included: body-mass-index, medication use, food or beverage intake 30 minutes prior to sampling, physical activity 30 minutes prior to sampling, or experience of stress 30 minutes prior to sampling (see Table S1 for frequencies of the dichotomous confounders). Studies indicated that obesity is associated with lower cortisol levels [41]. Medications can have an influence on salivary cortisol, such as corticosteroids, which act on the release of CRH or ADHD medication, where a dry mouth is a common side effect [42]. Consumption of food or drinks, as well as physical activity, and (psychological) stress have been found to increase unbound cortisol in saliva [7,41].

2.4. Statistical analyses

Data points deviating >3.29 *SD* from the mean were winsorized in line with previous cortisol and sleep studies (e.g. [11]). Before conducting the main analyses, we computed descriptive statistics of the sleep and cortisol variables per cohort and for MZ and DZ twins separately. We also computed correlations and within-twin correlations for each variable (sleep efficiency, sleep duration, wake episodes, and morning cortisol levels). ANOVAs and regression analyses were conducted with the potential confounding variables as predictors of the sleep and cortisol variables. Confounders with significant effects ($p <$ .05) on the phenotypes were subsequently included in the main analyses. This resulted in the inclusion of only cohort.

We employed genetic covariance structure modeling as implemented in the OpenMx library in R [43] to estimate contributions of genetic and environmental factors to the phenotypic variances of, and covariances among, the sleep variables and cortisol levels using maximum likelihood estimation. The genetic covariance structure model included the contributions of additive genetic factors (A), shared environmental factors (C), and unshared environmental factors (E). The E factors may contribute to the phenotypic covariance among the four phenotypes (3 sleep phenotypes and cortisol), but do not contribute to the covariance between the twins (hence "unshared"). To estimate these contributions, we exploit the fact that MZ twins share 100% of their alleles (i.e., are genetically identical), while DZ twin on average share 50% of their alleles [44]. Consequently, MZ twins will show greater resemblance than DZ twin with respect to phenotypes that are subject to genetic influences. In covariance structure modeling, the MZ and DZ covariance matrices are modeled as follows:

$$
\begin{array}{ccc}
 & \text{twin1} & \text{twin2} \\
S_{MZ} = \begin{array}{c}\text{twin1} \\ \text{twin2}\end{array} & \begin{array}{c}S_A + S_C + S_E \\ S_A + S_C\end{array} & \begin{array}{c}S_A + S_C \\ S_A + S_C + S_E\end{array} \\
S_{DZ} = \begin{array}{c}\text{twin1} \\ \text{twin2}\end{array} & \begin{array}{c}S_A + S_C + S_E \\ .5^* S_A + S_C\end{array} & \begin{array}{c}.5^* S_A + S_C \\ S_A + S_C + S_E,\end{array}
\end{array}
$$

where S_{MZ} and S_{DZ} are the 8x8 phenotypic covariance matrices. S_A, S_C, and S_E are the 4x4 additive genetic (A), shared environmental (C), and unshared environmental (E) covariance matrices, respectively. The expected 4x4 phenotypic matrix equals $S_A+S_C+S_E$, the 4x4 twin 1-2 covariance matrices are S_A+S_C (MZs) and $0.5*S_A+S_C$ (DZs). We first fitted the saturated model, in which we estimated the (unconstrained) MZ and DZ 8x8 covariance matrices. This saturated model served as a baseline model. Subsequently, we fitted the ACE model, where we parameterized the A, C, and E covariance matrices using the Cholesky or lower triangular decompositions. For example, in the case of S_A, we parameterized S_A as $D_A D_A^t$, where D_A is a lower triangular 4x4 matrix (s1 to s3 are the sleep phenotypes), superscript t denotes matrix transposition:

$$
D_A = \begin{array}{c|cccc}
 & s1 & s2 & s3 & \text{cortisol} \\
\hline
s1 & a_{11} & 0 & 0 & 0 \\
s2 & a_{21} & a_{22} & 0 & 0 \\
s3 & a_{31} & a_{32} & a_{33} & 0 \\
\text{cortisol} & a_{41} & a_{42} & a_{43} & a_{44}
\end{array}
$$

The shared environmental and unshared environmental covariance matrices (S_C and S_E) were estimated in the same way: $S_C = D_C D_C^t$ and $S_E = D_E D_E^t$. In the ACE model, we tested the additive genetic, shared environmental, and unshared environmental correlations between the sleep phenotypes and cortisol. In terms of the Cholesky parameterization, this test involves fixing parameter a_{41}, a_{42}, and a_{43} to zero in the S_A matrix, or analogous parameters in the Cholesky matrices of S_C (i.e., parameters c_{41}, c_{42}, and c_{43} in D_C) and S_E (i.e., parameters e_{41}, e_{42}, and e_{43} in D_E).

We conducted the statistical tests using the log-Likelihood Ratio Test (LRT) statistic. In this procedure, we fitted two models, models M0 and M1, where M1 is nested under M0, that is, M1 can be derived from M0 by the imposition of parameter constraints. The test statistic used to evaluate the constraints is the minus twice the difference in the log-likelihood values of the two models. If the constraints are tenable, this (log-likelihood ratio) test statistic should follow a χ^2 distribution with the number of degrees of freedom equal to the difference in the number of parameters of model M0 and model M1. If the LRT statistic is larger than the critical value, associated with the given choice of alpha, the constraints are rejected. For instance, suppose model M0 includes S_A based on the 10 parameters a_{11} to a_{44} (in D_A), and model M1 includes S_A but with the parameters a_{41}, a_{42}, and a_{43} fixed to zero. If these constraints are correct, then the LRT test statistic follows a chi-square ($df =$ 3) distribution, in which 3 is the difference in the number of freely estimated parameters in M0 and M1. Given $\alpha = .05$, the critical value equals 7.81. That is, if the LRT test statistical is greater than 7.81, given $df = 3$, we reject the constraints $a_{41} = a_{42} = a_{43} = 0$.

We adopted an alpha of .05 in carrying out the LRTs. In follow-up analyses, we assessed whether the contribution of genetic factors differed depending on the environmental structure of the day (weekdays versus weekend days).

3. Results

3.1. Preliminary analyses

The children slept on average 8.5 hours per night and had a sleep

efficiency of 86%. The average number of 1-min adjacent wake episodes was 24. The mean morning cortisol level was 9.35 nmol/l. All variables were approximately normally distributed (both skewness and kurtosis between −1 and 1). Variables containing outliers (deviating >3.29 SD from the mean) were winsorized: the 5% of the smallest and largest values were replaced by the lowest/highest retained values (see Table S2 for a list of frequencies of outliers per variable). There were missing data in all outcome variables. Of all children, 78% of the oldest twins provided the full four nights of sleep measurement, 14% provided three nights, 5% provided two nights, 1.5% provided one night of measurement and another 1.5% did not provide any sleep data. For the youngest twin, the numbers were 80% for four nights, 13% for three nights, 4% for two nights, 1% for one night and 2% for no sleep data. The means and standard deviations of all mean sleep variables did not differ when we included only cases with complete sleep data or all cases with at least one night of sleep data (see Table S3). Therefore, there were 33 and 34 missing cases on each sleep variable for the oldest and the youngest twin respectively (8%). Missingness of the sleep variables is comparable to other child samples (9.8%) [24]. With regard to morning cortisol, 80% of the oldest (81% of the youngest) twin provided both morning measurements, 4% (5%) provided one morning measurement and 16% (14%) provided no morning cortisol data. There were 70 and 63 missing cases on the morning cortisol level for the oldest and the youngest twin, respectively (16% and 15%). We conducted Little's MCAR test to assess the missingness across the variables. Little's MCAR test was not significant ($X(33) = 33.63$, $p = .44$) indicating that missingness occurred completely at random. For our main analyses, the sample size was 402 twin pairs (235 MZ twin pairs and 167 dizygotic twin pairs). Sleep duration was positively correlated with sleep efficiency and negatively with wake episodes ($p < .01$). Sleep efficiency and wake episodes were also negatively correlated ($p < .01$). However, no sleep variables were significantly correlated to morning cortisol levels (MCL) ($p > .05$) (see Table 2). Therefore, we decided to exclude morning cortisol from the multivariate behavioral genetics model, as common genetic and environmental factors are most likely absent for unrelated variables. We report the results of the univariate twin model for morning cortisol.

We tested associations between possible covariates and the sleep and cortisol variables. 55% of twin pairs (59% of MZ twins and 51% of DZ twins) shared a room, however, room sharing was not associated with the sleep variables. For all three sleep variables, a main effect of cohort was found: sleep duration ($F(3, 846) = 13.17$, $p < .001$, adjusted $R^2 = 4.1\%$), sleep efficiency ($F(3, 846) = 13.05$, $p < .001$, adjusted $R^2 = 4.1\%$), and wake episodes ($F(3, 846) = 13.08$, $p < .001$, adjusted $R^2 = 4.1\%$). All other variables (age, sex, BMI, medication use, drinking/eating, physical activity or stress 30 min prior to saliva sampling) did not have a significant association with the sleep or cortisol variables. Therefore, only cohort was included in the twin analyses.

We computed the cross-twin within-trait correlations. The suitability of the ACE model can be evaluated by comparing the MZ and DZ correlations, where $2*r_{DZ} > r_{MZ}$ suggests that the ACE model is suitable. The correlations of the MZ twin pairs did not exceed twice the correlation of the DZ twin pairs for sleep duration and MCL (Fig. 1).

3.2. Multivariate ACE models

We fitted the full ACE model with the phenotypes sleep duration, sleep efficiency, and wake episodes. The model fitted the data in comparison to the saturated model (ΔX^2 [24] = 34.42, $p = .078$; AIC = 5849.17 vs AIC = 5862.75). Table 3 shows the model fit statistics and Table 4 shows all parameter estimates including confidence intervals. Fig. 2 displays the proportion of phenotypic variance and covariance accounted for by A, C, and E.

As can be seen in Fig. 2, the heritabilities of sleep duration, sleep efficiency, and wake episodes were 46%, 42%, and 55%, respectively. Unique environmental factors explained 47%, 55%, and 45% of the variance in sleep duration, sleep efficiency, and wake episodes, respectively. Common environmental factors played no significant role. A high genetic correlation was found between both sleep duration and sleep efficiency ($r = .91$) and sleep efficiency and wake episodes ($r = .71$).

3.3. Univariate ACE model for morning cortisol

We fitted the full ACE model for MCL (ΔX^2 [3] = 3.53, $p = .316$, AIC = 3956.83 vs AIC = 3959.29). The model fitted the data in comparison to the saturated model. Tables 3 and 4 show the model indices and heritability indices: 18% of the variance in MCL was attributed to genetic factors (note that the confidence interval included zero), 29% was explained by shared environmental factors and 53% was explained by unique environmental factors.

3.4. Exploratory analyses

As pre-registered exploratory analyses, we investigated the sleep variables on weekdays and weekend days separately. Because the sleep and the cortisol variables were unrelated, MCL was not included. The mean sleep duration, sleep efficiency and wake episodes did not differ much between weekdays and weekend days. Sleep duration was shorter on weekend days compared to weekdays (Table 5). Cross-twin-within-trait correlations also tended to be similar, with only DZ correlations on sleep duration and sleep efficiency being lower on weekdays compared to weekend days.

We ran the ACE model consisting of the three sleep variables for weekdays and weekend days separately. The ACE model for the weekdays showed adequate fit in comparison to the saturated model (ΔX^2 [30] = 35.23, $p = .234$; AIC = 19287.29 vs AIC = 19312.05). We tested an AE model but constraining the C parameters led to significant worsening of the model fit; therefore, the ACE model was retained. There was no common shared environmental factor explaining variance in sleep duration, sleep efficiency and wake episodes. A high genetic correlation was found for sleep duration and sleep efficiency and a high genetic correlation was found for sleep efficiency and wake episodes. Heritability indices ranged between 33% and 48%, the unique environment accounted for between 52% and 66% of the variance in the phenotypes (Fig. S2).

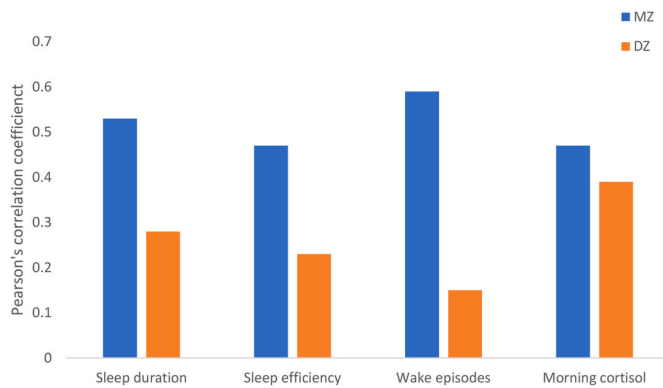We also fitted an ACE model for the weekend days. This model fit

Table 2
Phenotypic correlations of the Outcome Variables.

	1	2	3	4
1 Sleep duration in hours		.76* [.71, .79]	.31* [.22, .39]	.09 [-.01, .20]
2 Sleep efficiency in %	.80* [.76, .83]		.56* [.49, .63]	.08 [-.03, .18]
3 Wake episodes	.25* [.15, .34]	.49* [.41, .56]		-.03 [-.14, .07]
4 MCL	.06 [-.04, .17]	.04 [-.07, .14]	.01 [-.09, .12]	

Note. MCL = morning cortisol level in nmol/liter; *$p < .01$, Correlations for the oldest twin are shown above the diagonal, for the youngest twin below the diagonal; 95% confidence intervals of the correlations are shown in brackets.

Fig. 1. Cross-twin within-trait correlations.
Note. MZ = monozygotic twin pairs, DZ = dizygotic twin pairs.

poorly in comparison to the saturated model (ΔX^2 [30] = 130.36, $p <$ .001; AIC = 19662.21 vs AIC = 19591.85). However, the explained variance and covariance in the phenotypes were similar to the weekday model. There was no common shared environmental factor explaining variance in sleep duration, sleep efficiency and wake episodes. A high genetic correlation was found for sleep duration and sleep efficiency and a high genetic correlation was found for sleep efficiency and wake episodes. Heritability indices ranged between 36% and 46%, the unique environment accounted for between 52% and 64% of the variance in the phenotypes (Fig. S3). As measurement during holidays, winter-, or summertime and during the switch between these two were not associated with any of the sleep variables ($p >$ .05), no further exploratory model was run with these variables as covariates.

4. Discussion

We performed a multivariate behavioral genetic study on sleep in school-aged children. We found that sleep duration, sleep efficiency, and wake episodes were moderately heritable. A high genetic correlation was found between sleep duration and sleep efficiency, and also between sleep efficiency and wake episodes. Shared and unique environmental factors played a role in the variance of morning cortisol levels. We did not investigate common genetic, shared environmental, or unique environmental factors between actigraphic sleep and morning cortisol levels because there was no phenotypic correlation between actigraphic sleep and cortisol.

4.1. Heritability of sleep

Our findings with regard to the heritability of actigraphic sleep do not deviate much from the previous actigraphic sleep study in 12-year-old children [32]. Sleep duration was highly heritable in that study with 65%, which was comparable in our sample (46%). Sleep efficiency was also quite heritable with 52% in that study and 42% in the current study. Wake episodes had a heritability of 57% in the previous study and 55%

in our study. The results of the small Sletten et al. (2013) study, including only 25 MZ twins and 41 DZ twins were thus replicated in our larger sample (251 MZ twins and 185 DZ twins). However, our heritability indices are somewhat lower in comparison to a more recent study [24]. Sleep duration was highly heritable with 81% in that study as well as sleep efficiency with 79%.

We also investigated whether the heritability of actigraphic sleep would be different on weekdays or weekend days. Contrary to Inderkum and Tarokh [25], we found no differences. An explanation could be that our sample is much younger (6–9-year-olds compared to 11–14-year-olds). In adolescence, the need for sleep increases and adolescents have more freedom in deciding when to go to bed [25]. In our sample, children probably go to bed at the same time every night independent of a week or weekend day which is also reflected in the reported waking-/sleeping times. Moreover, in the Netherlands, sports competitions for child teams tend to be early in the morning on weekends, creating a much similar situation on a weekend day as compared to a weekday.

4.2. Genetic correlations in sleep variables

In the current study we found high genetic correlations between sleep duration and sleep efficiency and high genetic correlations between sleep efficiency and wake episodes, but low genetic correlations between sleep duration and wake episodes. Genome-wide association studies have predominantly focused on finding loci for sleep duration, and the PAX8 locus was consistently found to be associated with sleep duration [45,46]. In a recent study, Dashti et al. [45] detected 78 loci for self-reported sleep duration. These loci have also been found to associate with actigraphy derived sleep duration and sleep efficiency, indicating a common genetic background of both as identified in our study. Unfortunately, wake episodes were not included as a variable in these analyses. A GWAS on insomnia symptoms, in which participants were asked whether they would wake up often in the middle of the night, has identified several loci associated with insomnia symptoms (near MEIS1, TMEM132E, and CYCL1), but not with sleep duration in adults [47]. This indicates that distinct genetic factors might be implicated in sleep duration compared to waking episodes. At the same time these genetic factors might as well be correlated, some of the loci associated with sleep duration and sleep efficiency might also be contributing to wake episodes. Therefore, more twin studies and more genome-sequencing research are needed to shed light on the contribution of different genes to the variation in sleep duration, sleep efficiency and wake episodes.

4.3. Heritability of cortisol

In this study, we found no significant heritability of morning cortisol levels. The literature shows a large variability in heritability estimates, depending on age, time of sampling, and type of measurement (urine, blood, or saliva). For example, Bartels et al. [31] found in a sample of 12-year-old children that heritability of morning cortisol was 22% – 24% when measured at 7.30am, but 56 – 59% when measured at 8.30am. In a meta-analysis the combined heritability for basal cortisol

Table 3

Full and Best-fitting Cholesky Decomposition Fit Statistics for the Multivariate Sleep Model and for the Univariate Morning Cortisol Levels Model.

Model	Test	-2LL	df	AIC	Δ df	Δ χ^2	p
Sleep Model							
0. Saturated Model		5766.75	2367	5862.75			
1. ACE-ACE-ACE	1 vs 0	5801.17	2391	5849.17	24	34.42	.078
MCL							
0. Saturated Model		3943.29	731	3959.29			
1. ACE	1 vs 0	3946.83	734	3956.83	3	3.53	.316

Note. -2LL = −2 log-likelihood ratio test statistic; AIC = Akaike's information criterion; Δ df = change in degrees of freedom when model parameters were dropped; Δ χ^2 = change in -2LL when model parameters were dropped; p = p-value of significance of the chi-square test; Cohort was included as a covariate.

Table 4
Full and Best fitting Cholesky Decomposition Parameter Estimates.

	A	C	E
Multivariate Sleep model			
ACE-ACE-ACE			
Sleep duration	.46 [.20 - .58]	.07 [.00 - .29]	.47 [.39 - .57]
Sleep efficiency	.42 [.29 - .52]	.03 [.00 - .13]	.55 [.46–65]
Wake episodes	.55 [.20 - .63]	.00 [.00 - .14]	.45 [.37 - .54]
	*r*A	*r*C	*r*E
Sleep duration x Sleep efficiency	.91 [.89 - .97]	-.99 [-1.00 – 1.00]	.84 [.81 - .87]
Sleep duration x Wake episodes	.36 [.06 - .48]	-.99 [-1.00 – 1.00]	.24 [.12 - .35]
Sleep efficiency x Wake episodes	.71 [.69 - .87]	1.00 [-1.00 – 1.00]	.34 [.23 - .44]
Univariate MCL model			
ACE	.18 [.00-.51]	.29 [.01 - .50]	.53 [.43 - .64]

Note. *r*A is the genetic correlation between two variables, *r*C is the shared environmental correlation between two variables, *r*E = unique environmental correlation between two variables; Cohort was included as a covariate.

levels was 62%, but values ranged between 0 and 88% [28]. However, the review also indicated that power was insufficient for every singular study included in the review [28].

4.4. Association between sleep and cortisol

We were surprised that there was no correlation between sleep and cortisol variables in our study. Previous research found significant associations between sleep and cortisol in middle childhood. However, there are indications that the association holds only for sleep problems, and not for the normal range of healthy sleep patterns in children: Fernandez-Mendoza et al. [8] found that only the combination of reported insomnia symptoms with a shorter sleep duration was significantly related to morning and evening cortisol levels. Moreover, Pesonen et al. [48] found that sleep problems were related to lower diurnal cortisol. In samples without reported sleep problems, sleep duration and sleep efficiency were related to afternoon cortisol levels in a healthy middle childhood sample [11]. However, the mean sleep duration in that study was 6.58 h, which deviates from the recommendation of 9 to 12 hours for children of that age [49]. This is in line with

Table 5
Means, Standard deviations and Pearson Correlations for Weekdays and Weekend Days separately.

Statistic	Phenotype	Weekday		Weekend day	
		MZ	DZ	MZ	DZ
Mean (*SD*)	Sleep duration	8.66 (0.77)	8.66 (0.74)	8.36 (0.83)	8.46 (0.89)
	Sleep efficiency	86.37 (6.42)	85.95 (6.57)	86.07 (6.94)	85.65 (7.18)
	Wake episodes	46.87 (18.33)	45.05 (17.43)	46.56 (18.49)	46.41 (24.17)
Correlation	Sleep duration	.50	.20	.51	.35
	Sleep efficiency	.45	.19	.42	.27
	Wake episodes	.49	.09	.49	.07

Note. MZ = Monozygotic, DZ = Dizygotic; means and standard deviations only of the oldest twin displayed for readability; Cross-twin-within-trait Pearson's correlations.
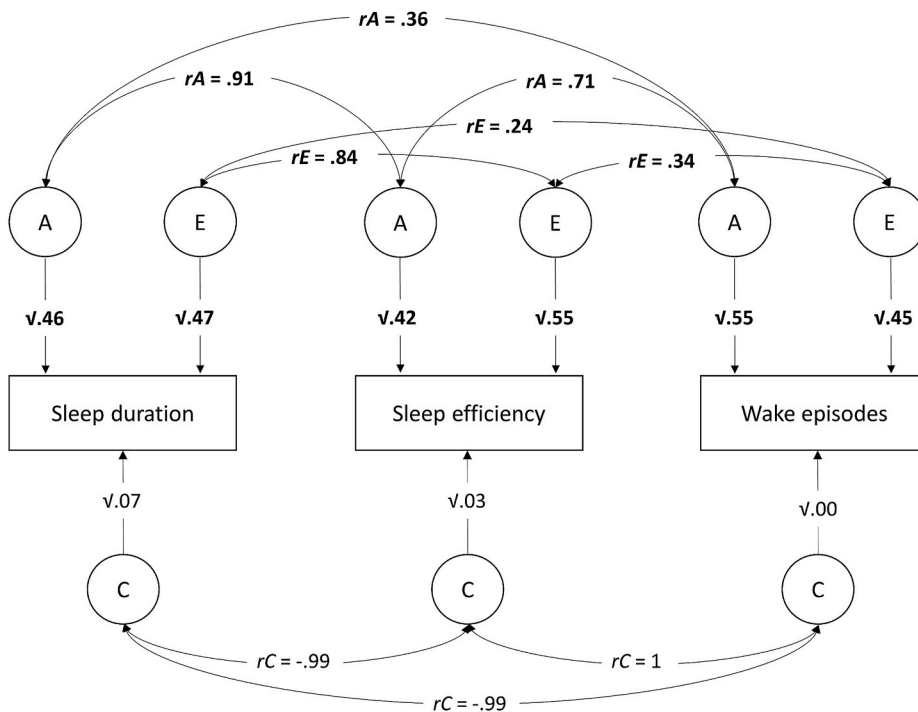

Fig. 2. Final multivariate model for sleep in children.
Note. The model with genetic and unique environmental effects for all variables and shared environmental effects for sleep duration; Variance components (i.e., squared standardized parameter estimates) shown for A, C and E of all phenotypes; *r*A is the genetic correlation between two variables, *r*C is the shared environmental correlation between two variables, *r*E = unique environmental correlation between two variables; Estimates with confidence intervals not including zero are shown in bold; Note that the C parameter of wake episodes is zero, which implies that the *rC* correlations of the other two sleep phenotypes with episodes are not identified, and uninterpretable"; Note that the other C parameters are also very low which renders the relevant *r*C highly unreliable and therefore effectively uninterpretable; Confidence intervals omitted for readability (see Table 4); Cohort was included as a covariate.

another study that compared children with a normal sleep duration and children with a low sleep duration (less than 7.7 h), and only found an association with cortisol for the short sleepers [10]. In line with our results, a recent study analyzing the association between sleep and hair cortisol in a sample of non-clinical children also found no association between sleep and cortisol [50]. Also, Marceau and colleagues [51] investigated parent-reported sleep duration and morning cortisol in children longitudinally and did not find an association at any time point between 4.5 and 9 years.

Therefore, it might be that sleep and cortisol are not linearly related in the normal range of variation in sleep, but only in individuals with reported sleep problems or deviating sleep patterns.

Another possibility is that any association between sleep and cortisol may have gone undetected by looking at average sleep and cortisol parameters over several days. Several studies found that hours of sleep were associated with cortisol levels of the following day and cortisol levels in turn predicted the sleep hours of the following night [52,53]. This might indicate that sleep and cortisol may be associated in normal ranges of variation in sleep, but more subtle and subject to day-to-day fluctuations.

4.5. Strengths and limitations

To our knowledge, this is the first study combining sleep and cortisol in a behaviour genetics analysis using actigraphic sleep measurements over four days in the home environment and using cortisol measurements over two days. Furthermore, we are among the first to study heritability of cortisol and sleep in middle childhood. However, some limitations also must be noted. Although we tried to standardize the measurements as much as possible, some measurement error may be present. Having the parents put on the actigraphs is a great advantage when it comes to investigating sleep in a natural environment, but it also leads to less control over the measurements. The actigraphs might have been put on the dominant hand or put on the wrist too loose, affecting the measurements. The same is true for cortisol measurement, having the parents oversee the saliva collection in the home environment ensures that no external factors (nervousness in a laboratory setting for example) influence the measurement. At the same time, there is less control about whether the parents use the correct tubes and whether timing is in congruence with the instructions. Cortisol rises quickly in the morning after awakening, therefore timing of saliva collection is an important issue. In addition to instructing participants, we used an app to record the times that children woke up and that saliva was collected. However, parental reports of waking times were not convergent with actigraph data, and both measures may be biased in different directions: Children's early awakening may escape their parents' awareness, while actigraphy can be overly sensitive to waking and movement. Also, by studying cortisol and sleep in a children sample in their home environment, we could not prevent that some parents skipped some measurements, leading to missingness. Furthermore, some parents skipped the questions regarding possible covariates of morning cortisol (e.g. food intake before saliva collection). Although unlikely in the early morning, such unnoticed covariates might have affected the cortisol estimates. Thus, although the amount of missing data was similar to other studies [51], future research should try to decrease these and other possible measurement errors by controlling the time of data collection more closely. An interesting avenue for the field of cortisol measurement are wearable devices that continuously measure cortisol. A first study by Parlak [54] has tested a device that detects cortisol in sweat. However, more research and development are needed until such devices are suitable as an ambulatory assessment method in child research. Another

point concerning the reliability of the cortisol data are possible batch effects, which were not taken into account in our analyses. However, quality control processes were employed in the laboratory. We recommend that future studies use a double control standard by also including the batch numbers in their analyses, which is already done in many other research fields.

The fact that the correlations between the sleep variables and MCL were found to be zero also raises the issue of power. Therefore, we conducted a power analysis to assess whether our sample size would have been sufficient to detect any the additive genetic, shared environmental and unshared environmental correlations between the sleep phenotypes and cortisol. Based on previous research, we expected that the phenotypic correlation between the sleep phenotypes and morning cortisol in children would be about .25. We explored the power to detect additive genetic correlations and unique environmental correlations of differing sizes. In the case of a genetic correlation of 0.2 and a unique environmental correlation of 0.4, the power to reject $a_{41} = a_{42} = a_{43} = 0$ (no contribution of A to the phenotypic correlations) was 0.38. Given genetic correlations of 0.6 and unique environmental correlations of 0.1, the power to reject $e_{41} = e_{42} = e_{43} = 0$ (no contribution of E to the phenotypic correlations) was 0.44, given an alpha of .05. In all other scenarios (genetic correlation of 0.8, 0.4 and 0), power was sufficient. We also did not find significant heritability estimates for the univariate twin model of MCL. Assuming a small A of 0.18, the power to detect A (given a C of 0.29 and E of 0.53) was 0.34.

Although we collected data from more than 400 twin pairs, resulting in more than 800 children engaged in four nights of ambulatory assessments, this sample size was still too small to have sufficient power to detect small additive genetic correlations or unique environmental correlations. Future studies should replicate our study with even larger samples to have a higher probability to detect possible correlations of A, C and E.

5. Conclusion

In this study, we investigated the genetic factors implicated in the variation in actigraphic measured sleep and cortisol in children. Sleep is moderately heritable whereas cortisol levels are mostly explained by the shared and unique environment. High genetic correlations between sleep duration and efficiency were found as well as high genetic correlations between sleep efficiency and wake episodes. Sleep and cortisol were not related in our non-clinical low-risk sample. Future research should focus on disentangling the genetic contributions at play in aspects of sleep as well as investigating under which circumstances sleep and cortisol are correlated.

Declaration of cometing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cpnec.2021.100094.

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