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Multidimensional Sleep Characteristics and Cardiometabolic Health in Adolescents and Young Adults

Dissertation
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I hereby declare, that the submitted thesis entitled:

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Table of content

List of abbreviations

BMI	Body mass index
BP	Blood pressure
CI	Confidence interval
DSPD	Delayed sleep-wake phase disorder
FMI	Fat mass index
GINIplus	German Infant Study on the influence of Nutrition Intervention PLUS environmental and genetic influences on allergy development
HDL	High-density lipoprotein cholesterol
HOMA-IR	Homeostatic model assessment of insulin resistance
hs-CRP	High-sensitivity C-reactive protein
LISA	Influence of Lifestyle factors on the development of the Immune System and Allergies in East and West Germany
OR	Odds ratio
PRS	Polygenic risk scores
SNPs	Single nucleotide polymorphisms
15y	15-year follow-up of birth cohorts
20y	20-year follow-up of birth cohorts

List of publications in this thesis

Publication I

Wang M, Flexeder C, Kilanowski A, Kress S, Herberth G, Schikowski T, Peters A, Standl M. Changes in sleep duration and sleep difficulties from adolescence to young adulthood and the risk of obesity: Bidirectional evidence in the GINIplus and LISA studies. *Sleep Medicine*. 2023;101:401-410. https://doi.org/10.1016/j.sleep.2022.11.031.

Publication II

Wang M, Flexeder C, Harris CP, Thiering E, Koletzko S, Bauer CP, Schulte-Körne G, von Berg A, Berdel D, Heinrich J, Schulz H, Schikowski T, Peters A, Standl M. Accelerometry-assessed sleep clusters and cardiometabolic risk factors in adolescents. *Obesity (Silver Spring)*. 2024;32(1):200-213. https://doi.org/10.1002/oby.23918.

Publication III

Wang M, Flexeder C, Harris CP, Kress S, Schikowski T, Peters A, Standl M. Accelerometry-assessed sleep clusters and obesity in adolescents and young adults: a longitudinal analysis in GINIplus/LISA birth cohorts. *World Journal of Pediatrics*. Published online January 4, 2025. https://doi.org/10.1007/s12519-024-00872-5.

Manuscripts: Overview and contributions

Publication I

The first publication, entitled “Changes in sleep duration and sleep difficulties from adolescence to young adulthood and the risk of obesity: Bidirectional evidence in the GINIplus and LISA studies” explored bidirectional relationships of reported sleep duration and sleep difficulties with overweight or obesity within two German birth cohorts. We observed that persistent sleep difficulties from adolescence to young adulthood was linked to young adult overweight or obesity, and vice versa. However, insufficient sleep only showed a cross-sectional relationship with overweight or obesity in young adulthood, while overweight or obesity presented a long-term association with insufficient sleep from adolescence to young adulthood.

Contribution to Publication I

As the first author of the first publication, I conducted extensive literature research and contributed to study design. Then I performed the statistical analyses, drafted the initial manuscript, incorporated co-authors’ and reviewers’ comments, finalized the manuscript, and led the submission process.

Publication II

The second publication, entitled “Accelerometry-assessed sleep clusters and cardiometabolic risk factors in adolescents” investigated the associations between objectively assessed multidimensional sleep characteristics and multiple cardiometabolic markers by using cluster analysis. We identified five distinctive sleep patterns, named “good sleep”, “delayed sleep phase”, “sleep irregularity and variability”, “fragmented sleep”, and “prolonged sleep latency”. Of note, the “prolonged sleep latency” pattern demonstrated a link with elevated fat mass index, and males with the “sleep irregularity and variability” pattern exhibited higher odds of having elevated triglyceride levels.

Contribution to Publication II

As the first author of the second publication, I began with a literature review and contributed to the research question development. I further performed all statistical analyses and results visualization, wrote the original draft, engaged in reviewing and editing, as well as was responsible for the submission process.

Publication III

The third publication, entitled “Accelerometry-assessed sleep clusters and obesity in adolescents and young adults: a longitudinal analysis in GINIplus/LISA birth cohorts”

extended cluster analysis in young adulthood, and assessed longitudinal relationships of sleep clusters with overweight or obesity. We discovered that the five sleep patterns were not only evident in adolescents but also in young adults. Furthermore, the “prolonged sleep latency” pattern was observed to be associated with higher body mass index and higher odds of having overweight or obesity.

Contribution to Publication III

As the first author of the third publication, I contributed to study design, conducted the statistical analyses, visualized the results, drafted the initial manuscript, integrated comments from co-authors and reviewers, and led the paper for submission.

Introductory summary

1. Background

1.1 Understanding sleep: Importance, disorders, and assessments

Sleep importance

Sleep occupies almost one-third of our human lives [1]. Sleep plays an essential role on restoration of neurological, skeletal, and muscular systems, modulation of endocrine and immune responses, cognitive performance, and maintenance of mental and physical health [2-4]. Accordingly, the American Sleep Association has recommended that children (ages 6-12 years) sleep 9-12 hours each 24 hours, adolescents (ages 13-18 years) sleep 8-10 hours each 24 hours, and healthy adults sleep at least 7 hours each night to promote optimal health [5,6].

Sleep duration in children, adolescents and adults has declined rapidly in recent decades, leading to a growing concern of sleep deprivation as a critical public health issue [7,8]. Notably, children aged 5-18 years experienced a significant reduction of over one hour in sleep duration from 1905 to 2008 [7]. A study with a representative sample reported that one in eight children aged 12-17 years in Germany had chronic sleep reduction [9]. Insufficient sleep is associated with cognitive problems (e.g., memory and performance), psychosocial issues (e.g., stress and depression), and increased risks of adverse cardiometabolic outcomes (e.g., obesity, cardiovascular disease, and diabetes) [10-14].

Sleep disorders

A variety of sleep disorders may disrupt quantity and quality of sleep. The most prevalent sleep disorders include insomnia disorders, circadian rhythm sleep-wake disorders, sleep apnea, restless legs syndrome, and narcolepsy [15,16]. Insomnia is a prevalent sleep disorder, marked by difficulty initiating sleep, difficulty maintaining sleep, or early waking, causing daytime impairment [17]. The prevalence of reported insomnia symptoms is 10%-30% in childhood and adolescence [18,19], and is 30%-50% in general adult population, with around 10% of being insomnia disorder [20-22]. Notably, delayed sleep-wake phase disorder (DSPD), a kind of circadian rhythm sleep-wake disorders, is more common among adolescents and young adults with the prevalence as 7%-16% [18,23], compared to adults aged 30-59 years (0.03%-2.75%) [24]. DSPD is characterized by difficulty initiating sleep at a socially acceptable bedtime, leading to delayed sleep onset and challenges in waking up at conventional morning times, such as for school [23]. However, individuals with DSPD have normal sleep quantity and quality when given unrestricted sleep opportunities, like on weekends.

Sleep assessment methods

The assessments of sleep can be divided into subjective and objective measurements. Subjective measures are widely applied in large epidemiological studies, including simple generic questions (e.g., “on average, how many hours do you sleep each night?”), standardized developed questionnaires (e.g., Pittsburgh Sleep Quality Index [25]), sleep diaries [26] and logs [27]. These self-administered methods are low-cost and convenient, but their accuracy is lower compared to polysomnography [28]. Overnight polysomnography in a laboratory setting is the gold standard for measuring sleep characteristics. However, it is not feasible in large-scale sleep research due to high costs and potential disruption of sleep routine [29]. Besides polysomnography, wearable devices provide a feasible and cost-effective means to objectively measure most sleep characteristics in population studies [28]. Accelerometers are the primary wearable devices utilized for measuring both sleep and physical activities, with wrist-worn actigraphy specifically designed and validated for sleep measurements [30]. Actigraphy can collect sleep data over consecutive nights with less disruption to sleep routines, providing information on variations in sleep patterns related to insomnia or circadian sleep disorders [29,30]. However, it tends to overestimate total sleep time, due to its limited ability to detect wakefulness after sleep onset [30].

1.2 Cardiometabolic risk factors

Cardiometabolic risk factors can emerge in childhood, and tend to persist into adulthood, raising long-term risks of cardiovascular events, diabetes, and mortality [31-33]. Cardiometabolic risk factors describe a cluster of conditions and physiological markers that include overweight or obesity, elevated blood pressure (BP), dyslipidemia, and dysglycemia [34]. Inflammatory markers are also recognized as key cardiometabolic risk factors, as they are linked to heightened risks of developing cardiovascular disease and diabetes [35,36].

From 1990 to 2022, the prevalence of overweight and obesity in children and adolescents (ages 5-19 years) has increased by 12% globally (from 8% to 20%), impacting over 390 million individuals [37]. Additionally, childhood hypertension prevalence has risen from 1994 to 2018, reaching around 4% globally in individuals aged 19 years and younger [38]. Other associated conditions like dyslipidemia and dysglycemia are increasingly detected in childhood [39,40]. Thus, it is vital to prevent cardiometabolic risk factors in children by addressing modifiable factors, with potential benefits in extending lifespan and reducing health costs.

1.3 Sleep characteristics and cardiometabolic health in adolescents and young adults

Adolescence and young adulthood are critical developmental periods when individuals experience significant physiological, psychological, and environmental changes, like the onset of puberty, physical maturity, increased social activities, and more digital media use [41,42]. Consequently, these changes may sometimes lead to challenges such as sleep disturbances and negative impacts on cardiometabolic health [43-45]. The overview of research progress on relationships of sleep characteristics with cardiometabolic health among adolescents and young adults is displayed in **Figure 1**.

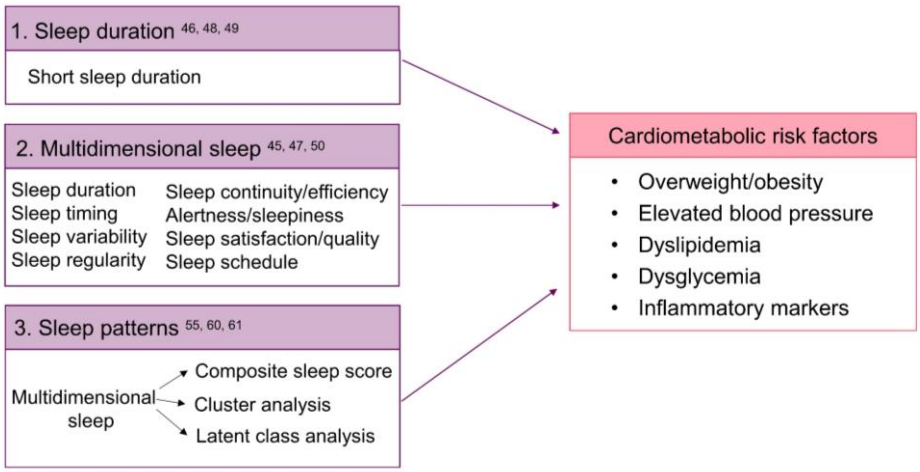

Figure 1. From single sleep duration to multidimensional sleep characteristics: Integrative methods to study their associations with cardiometabolic risk factors among adolescents and young adults. The reference numbers in this figure are formatted as superscripts.

Sleep duration

Sleep, a modifiable factor, may provide a unique opportunity for improving cardiometabolic health in early stages of life. Cumulative epidemiological studies have recognized short sleep duration as a risk factor contributing to adverse cardiometabolic health among children and adults [46-48]. For instance, in a systematic review, my colleagues and I, as a co-first author, found strong evidence linking short sleep duration to adiposity and high BP among children and adolescents [46]. Consequently, sleep duration was incorporated as the eighth component to the construct of cardiovascular health known as “Life’s Essential 8”, by the American Heart Association for the first time in June 2022 [49].

Sleep as a multidimensional construct

Most studies primarily dedicated to examining sleep duration, which can be easily assessed through questionnaires. However, it is important to acknowledge that sleep is a complex concept that includes multiple dimensions. A novel concept defining sleep health based on several dimensions was proposed by Daniel J. Buysse in 2014, including sleep duration,

satisfaction/quality, alertness/sleepiness, timing, and continuity/efficiency [50]. Sleep variability, regularity, and schedule have also been increasingly recognized as crucial components of sleep over the past decade [45,47,51].

Recent studies have investigated relationships of multiple sleep dimensions with cardiometabolic health among children and adolescents, with a few employing objective sleep measures, but discovered inconsistent findings [47,52,53]. For example, self-reported long sleep latency (the amount of time from going to bed to falling asleep) was linked to an increased z-score of body mass index (BMI) among 624 UK adolescents aged 11-18 years [54]. In contrast, no significant relationship was observed between objective sleep latency and BMI z-score in 559 children aged 9-16 years in Europe [55]. Objectively assessed higher sleep efficiency (the proportion of actual total sleep time relative to total time spent in bed) was inversely associated with adiposity and systolic BP in adolescents aged 11.9-16.6 years [56]. Emerging research focuses on day-to-day variations of sleep duration (variability) and sleep timing (irregularity) during adolescence, revealing significant links to adiposity and adverse cardiometabolic health [45]. Furthermore, LeMay-Russell et al.[57] reported that objectively assessed earlier sleep midpoint timing (the halfway between sleep onset and wake up timing) was linked to fat mass gain over one year in youth with mean age of 12.5 years. However, Jansen et al.[58] suggested the relationship of objectively assessed later sleep midpoint timing with higher likelihood of developing insulin resistance during a two-year period among 362 adolescents with mean age of 14.4 years. Moreover, time awake after sleep onset showed no significant association with adolescent adiposity in most previous studies [47].

Sleep patterns

The sleep characteristics were commonly evaluated independently, yet they occur simultaneously within an individual in context of one another [50,51]. A few papers have investigated the relationships of multidimensional sleep with cardiometabolic health among adolescents and young adults from a holistic perspective, such as using a composite sleep score [59,60], cluster analysis [61], and latent class analysis [55]. For instance, the Sleep Health Composite score was summed up by six reported dichotomized sleep dimensions, and showed that higher score, indicating greater sleep health, was inversely associated with adolescent obesity [60]. However, this score allocated equal weights to each sleep dimension and did not consider their correlations. Cluster analysis accounts for correlations between multiple sleep characteristics, and groups similar individuals into clusters based on certain similarity criteria [51]. In 2021, Matricciani et al.[61] conducted the first study using cluster analysis to assess the relationships of sleep patterns with some cardiometabolic risk factors in 1043 children aged 11-12 years and 1337 adults with a mean age of 43 years in Australia, across four objective sleep characteristics. They identified four sleep clusters, named “long

sleepers", "short sleepers", "overall good sleepers", and "late to bed", with the "overall good sleepers" cluster linking to more favorable cardiometabolic health. Additionally, Thumann et al.[55] identified four sleep patterns in 559 European children and adolescents aged 9-16 years using latent class analysis across five subjective and objective sleep characteristics. These patterns included "poor sleep quality", "optimal sleep", "short sleep duration", and "early birds", with no significant link observed to BMI.

Sex differences

Sleep problems and cardiometabolic risk factors vary by sex in adolescence, with females sleeping longer but experiencing more insomnia symptoms [62,63], while males exhibiting a higher prevalence of obesity and high BP [64,65]. However, evidence on sex differences in associations between sleep and cardiometabolic health during adolescence and young adulthood remains inconclusive. Several reviews have reported stronger associations of short sleep duration with overweight or obesity and elevated BP in males than females [46]. An evolutionary perspective suggests females may be more tolerant to environmental stressors and less affected by sleep deprivation than males [66]. Additionally, variations in sex hormone may contribute to different impacts between sexes [67]. However, a nationally representative US study observed a cross-sectional link of short sleep with obesity only among adolescent boys, yet found a longitudinal link among both males and females in young adulthood [68].

1.4 Current research gaps

Currently, there are still several research gaps in adolescents and young adults that need to be urgently addressed. First, most studies employed a cross-sectional design, lacking longitudinal evidence to assess causality and directionality between sleep patterns and cardiometabolic health [46,47]. Second, the measurements of sleep duration relied on subjective reporting, either by the youths themselves or their parents in most previous studies, which may inaccurately reflect sleep quantity and quality [46]. Third, only a few previous studies considered sleep as a multidimensional construct and examined their relationships with some cardiometabolic risk factors [51]. However, the relationships of sleep with other cardiometabolic markers, like fat mass, insulin resistance, and C-reactive protein, have not yet been explored. Fourth, the role of genetic predisposition for BMI in the relationship of sleep with obesity among adolescents and young adults is unclear [69]. Yet, a study based on UK Biobank reported no interaction effect of genetic risk with nighttime sleep behaviors on adult BMI [70].

2. Aims

The overarching aim of this dissertation is to explore the associations of multiple sleep characteristics with cardiometabolic risk factors considering genetic risk variants among adolescents and young adults, using prospectively collected information from two German birth cohorts. The three main objectives were to investigate:

(1) bidirectional relationships of subjective sleep duration and sleep difficulties with overweight or obesity among adolescents and young adults, and to test the role of genetic risk for BMI.
(2) sleep clusters identified by objectively multidimensional sleep characteristics, and to assess their cross-sectional relationships with cardiometabolic risk factors in adolescents.
(3) longitudinal relationships of objective sleep clusters with overweight or obesity among adolescents and young adults, and to evaluate if they are modified with time or by genetic risk.

3. Methods

3.1 Study population

This thesis utilizes data from the 15-year (15y) and 20-year (20y) follow-ups of two German birth cohorts, GINIplus (German Infant Study on the influence of Nutrition Intervention PLUS environmental and genetic influences on allergy development) and LISA (Influence of Lifestyle factors on the development of the Immune System and Allergies in East and West Germany). Briefly, during 1995-1998, the GINIplus cohort enrolled 5991 healthy neonates in Munich and Wesel. The GINIplus cohort included an intervention arm targeting infants with a family allergy history to investigate different hydrolyzed formulas' allergy prevention effects, and an observation arm for infants without such history and those whose parents did not want to take part in the intervention arm [71]. During 1997-1999, the LISA cohort enrolled 3094 healthy newborns in Munich, Wesel, Leipzig, and Bad Honnef [71]. Both cohorts completed questionnaires and accelerometry measurements at 15y and 20y, with physical examinations at 15y. Both studies received approvals from local ethics committees and obtained written consents from participants and their families. **Figure 2** provides an illustration of the study design and the data utilized.

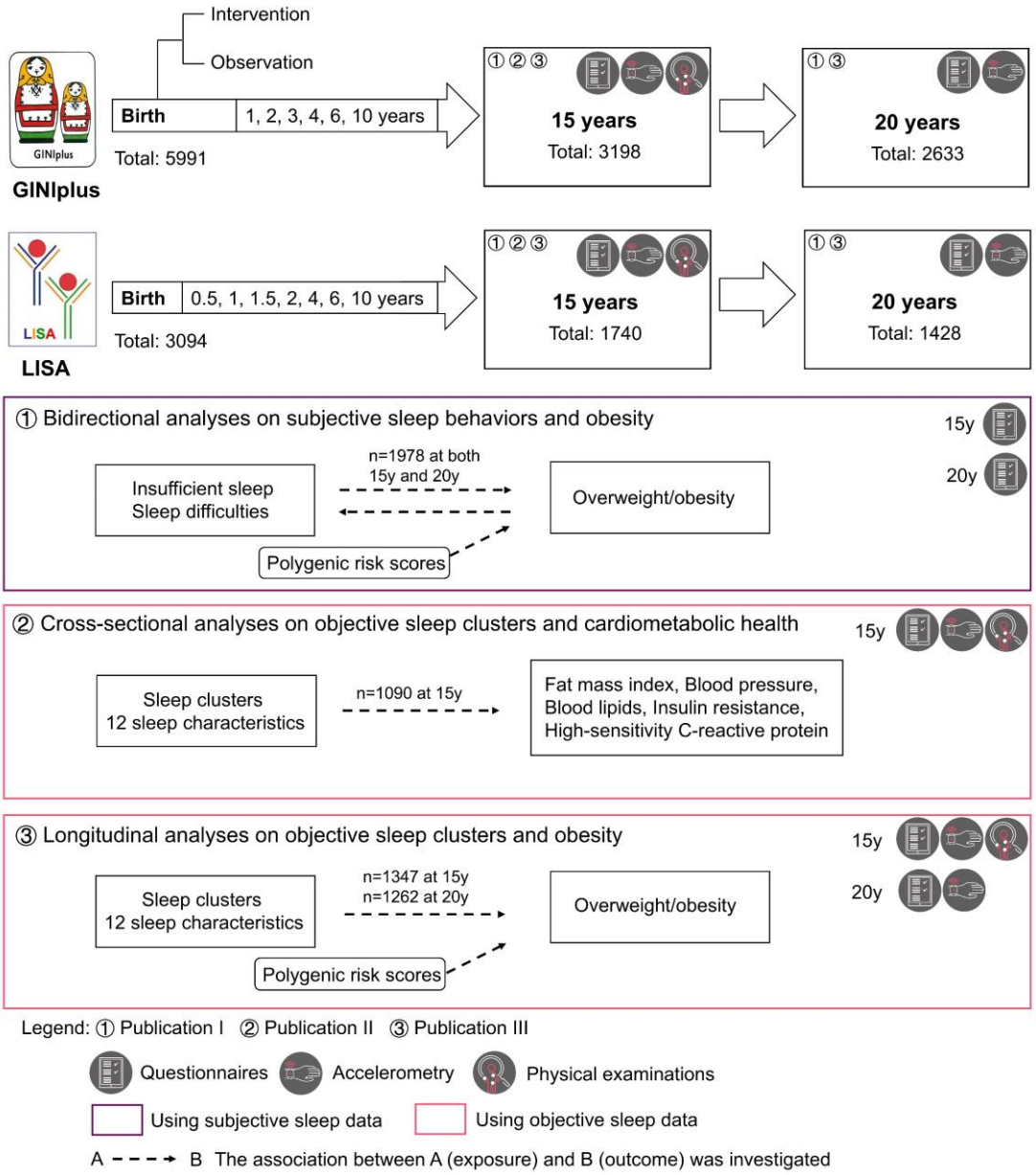

Figure 2. The study design and the data utilized in this cumulative dissertation. Abbreviations: GINIplus, German Infant Study on the influence of Nutrition Intervention PLUS environmental and genetic influences on allergy development; LISA, Influence of Lifestyle factors on the development of the Immune System and Allergies in East and West Germany; 15y, 15-year follow-up of birth cohorts; 20y, 20-year follow-up of birth cohorts. Note: the data from GINIplus and LISA were merged for analysis in this cumulative dissertation.

3.2 Variable assessments

Exposures

Subjective sleep duration (in hours) and sleep difficulties (“yes” or “no”) were reported by parents at 15y and by participants at 20y using questionnaires. Sleep duration was categorized into either sufficient sleep or insufficient sleep, with insufficient sleep defined as less than 8 hours among adolescents [5] and 7 hours among young adults [6]. To explore changes of sleep duration from 15y to 20y, participants were divided into four mutual exclusive groups based on the status (“yes” or “no”) of insufficient sleep at two follow-ups.

Participants were also grouped into four categories based on the status (“yes” or “no”) of sleep difficulties at two follow-ups. Please see the detailed definitions in Publication I [72].

Objective sleep characteristics were assessed by accelerometry at 15y and 20y. Study participants wore triaxial accelerometers (ActiGraph GT3X, Pensacola, Florida) for a continuous period of seven days and nights during a regular week [73,74]. Participants wore accelerometers on the dominant hip during the day and on the non-dominant wrist at night. Additionally, participants also completed daily sleep diaries to document the bedtime and getting up times. After quality control and data management, sleep and wake periods during each night were identified applying the Sadeh algorithm [30,75].

Figure 3 displays the overview of sleep in one night and objective sleep characteristics. Twelve objective sleep characteristics were derived based on accelerometry and sleep diaries, and included for cluster analysis. They were daily average (mean) and day-to-day variability (standard deviation) of six sleep characteristics as shown in **Figure 3**, respectively [76]. Publication II provides the detailed definitions of 12 sleep characteristics [76].

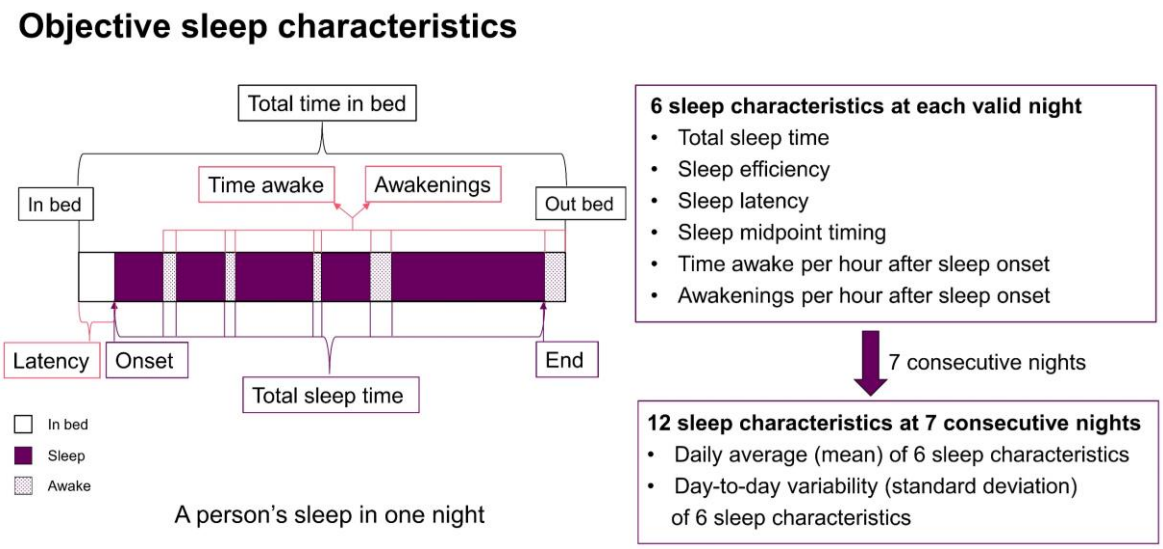

Figure 3. Overview of sleep in one night and objective sleep characteristics. The objective sleep data measured by accelerometry was used for Publication II and Publication III.

Outcomes

Height and weight were reported by parents at 15y and by participants at 20y using questionnaires. During the physical examination at 15y, height, weight, systolic BP, diastolic BP, and fat free mass were measured, and blood samples were collected. Blood lipids were measured, including triglycerides, total cholesterol, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol (HDL), as well as high-sensitivity C-reactive protein (hs-CRP) was assessed. Fasting glucose and insulin were measured in a sub-sample at 15y.

The outcomes of interest were continuous variables including BMI, fat mass index (FMI), systolic BP, diastolic BP, triglycerides, total cholesterol, low-density lipoprotein cholesterol, HDL, Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), as well as dichotomous variables including overweight or obesity (overweight/obesity), weight status changes, high FMI, high triglycerides, high hs-CRP, high BP, high HOMA-IR and low HDL. BMI was categorized as either normal weight or overweight/obesity. Overweight/obesity was defined as BMI z-scores exceeding 1 among adolescents [77] and BMI equal to or exceeding 25 kg/m^2 among young adults [37], respectively. Weight status changes were categorized into four groups according to the status ("yes" or "no") of overweight/obesity at two follow-ups. Please see other detailed definitions in Publication I [72] and II [76].

Polygenic risk scores for BMI

Genetic data was obtained from sub-samples of GINIplus and LISA cohorts in Munich and Wesel. Genotyping was conducted using Affymetrix Chip 5.0 and 6.0 (Thermo Fisher, USA) for Munich datasets and Infinium Global Screening Array GSA v2 MD (Illumina, USA) for Wesel datasets. Following quality control and genotype imputation [78], a total of 1511 participants from Munich and 792 participants from Wesel had accessible genetic information. Polygenic risk scores (PRS) related to adult BMI were calculated according to 97 significant genome-wide single nucleotide polymorphisms (SNPs) [79]. Out of the 97 SNPs, the Munich datasets utilized 95 available SNPs for PRS calculation, while the Wesel datasets employed 96 available SNPs for PRS calculation. The PRS were standardized to z-scores for final analyses, with higher values indicating increased risk for high BMI. The details and SNPs list can be found in Publication I [72].

3.3 Statistical analyses

Bidirectional analyses on subjective sleep behaviors and obesity

The relationships of unfavorable sleep behaviors changes (insufficient sleep and sleep difficulties, respectively) from 15y to 20y, with overweight or obesity at 20y were examined by logistic regression models. The confounders adjusted for were age, sex, education/occupation types, parental education, screen time, physical activity, traffic noise, study, study center at 20y, as well as pubertal stage and overweight or obesity at 15y. Additionally, the role of PRS for BMI was examined in a subset of the sample.

In parallel, the relationships of weight status changes from 15y to 20y with unfavorable sleep behaviors at 20y were explored by logistic regression models. The variables controlled for were age, sex, education/occupation types, parental education, screen time, physical activity, traffic noise, insufficient sleep (for sleep difficulties) or sleep difficulties (for insufficient sleep), study, study center at 20y, as well as pubertal stage, insufficient sleep, and sleep difficulties at 15y.

Cross-sectional analyses on objective sleep clusters and cardiometabolic health

The identification of sleep clusters at 15y was performed by K-means cluster analysis with k set to five, using 12 accelerometry-assessed sleep characteristics. The relationships of identified sleep clusters with continuous and dichotomous cardiometabolic risk factors were assessed by linear and logistic regression analyses, respectively. The adjusted variables were age, sex, season of sleep measurements, pubertal stage, parental education, sedentary behavior, moderate-to-vigorous physical activity, depression, total energy intake, carbohydrate intake, study, study center, fasting condition (except for HOMA-IR), and FMI (except for FMI). Moreover, the Bonferroni correction with Nyholt method was applied for multiple testing correction [80].

Longitudinal analyses on objective sleep clusters and obesity

K-means cluster analysis was employed utilizing 12 accelerometry-assessed sleep characteristics to identify five sleep clusters at 15y as before, and was extended to 20y. The longitudinal associations of sleep clusters with BMI and overweight or obesity were evaluated by linear and logistic regression with generalized estimating equations modeling. The adjustments were the time of follow-ups, sex, age, study, study center, season of sleep measurements, BMI measurement methods, parental education, sedentary behavior, moderate-to-vigorous physical activity, and total energy intake. Furthermore, the interaction effects with PRS and time of follow-ups were examined, respectively.

4. Results

4.1 Subjective sleep behaviors and obesity: Bidirectional findings

The first publication addressed the first specific aim of this thesis: To explore bidirectional relationships of subjective sleep duration and sleep difficulties with overweight or obesity among adolescents and young adults, and to test the role of genetic risk for BMI.

Among 1978 participants with repeatedly assessed data, in comparison to participants with persistent sufficient sleep between both 15y and 20y, those with insufficient sleep only at 20y had higher odds of having overweight or obesity at 20y (odds ratio (OR) =1.85, 95% confidence interval (CI) = [1.27, 2.69], **Figure 4**). Compared to participants without sleep difficulties at two follow-ups, only those with persistent sleep difficulties had higher odds of having overweight or obesity at 20y (OR=2.15, 95%CI= [1.22, 3.77], **Figure 4**). In a sub-sample (n=918), the PRS for BMI was linked to higher odds of overweight or obesity at 20y (OR=1.41, 95%CI= [1.17, 1.70]), yet without observed significant gene-sleep interaction effect (p-interaction > 0.05). Reversely, when compared to participants with persistent normal weight at two follow-ups, only those with persistent overweight or obesity had higher

odds of insufficient sleep (OR=1.81, 95%CI= [1.21, 2.70]), and sleep difficulties (OR=1.77, 95%CI= [1.18, 2.66], **Figure 4**) at 20y, respectively.

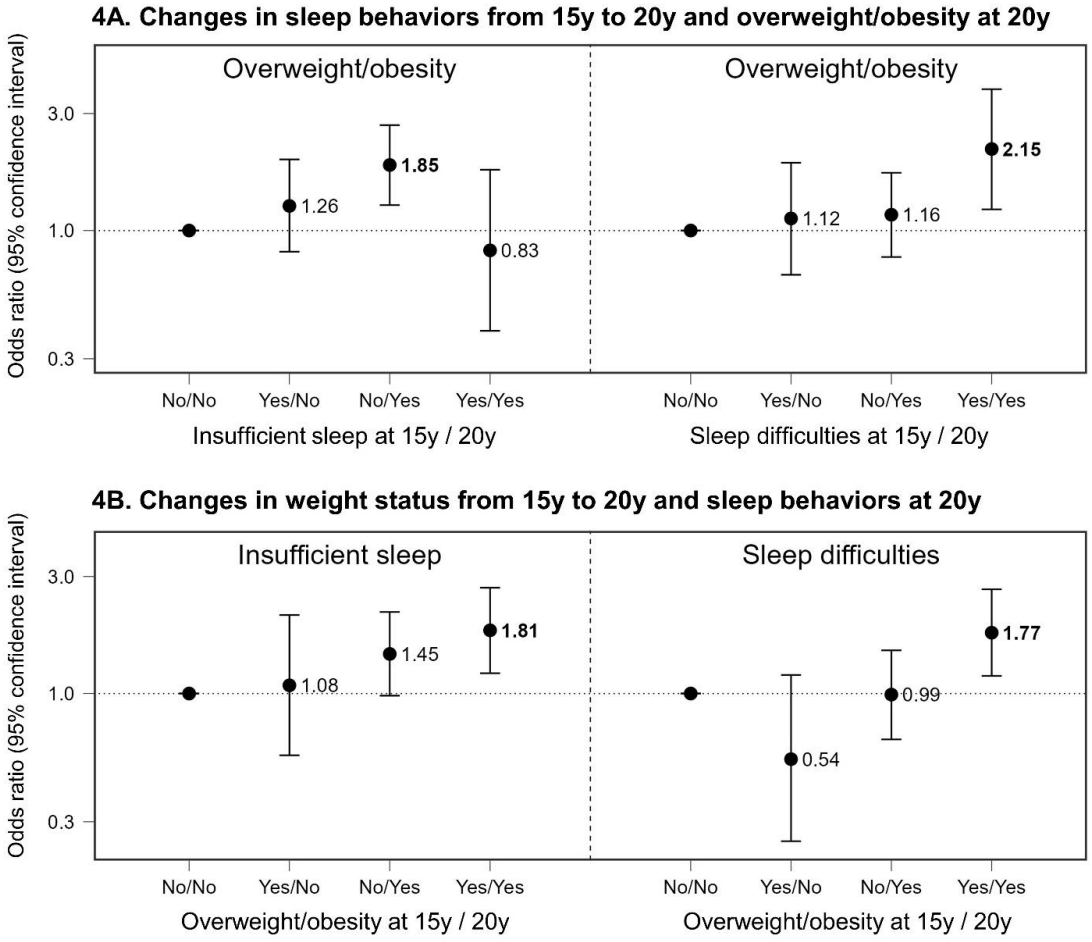

Figure 4. Bidirectional relationships of two sleep behaviors with overweight or obesity (overweight/obesity) from 15y to 20y. The significant odds ratios are highlighted in bold. Abbreviations: 15y, 15-year follow-up of birth cohorts; 20y, 20-year follow-up of birth cohorts.

4.2 Objective sleep clusters and cardiometabolic health: Cross-sectional findings

The second publication assessed the second specific aim of this thesis: To explore sleep clusters identified by objectively multidimensional sleep characteristics, and to assess their cross-sectional relationships with cardiometabolic risk factors in adolescents.

K-means cluster analysis classified five sleep clusters based on 12 accelerometry-derived sleep characteristics among 1090 German adolescents aged 14.3-16.4 years. They were named by their unique features, including “good sleep” (30.9% of the sample), “delayed sleep phase” (22.4%), “sleep irregularity and variability” (9.9%), “fragmented sleep” (28.7%) and “prolonged sleep latency” (8.1%). In comparison to adolescents within “good sleep” cluster, those within “prolonged sleep latency” cluster had elevated FMI scaled by sex (β=0.39, 95% CI = [0.15, 0.62], **Figure 5**). Only male adolescents with “sleep irregularity

and variability" cluster exhibited elevated odds of high triglycerides (OR =9.50, 95%CI= [3.22, 28.07], **Figure 5**), yet the association did not replicate in the linear analysis.

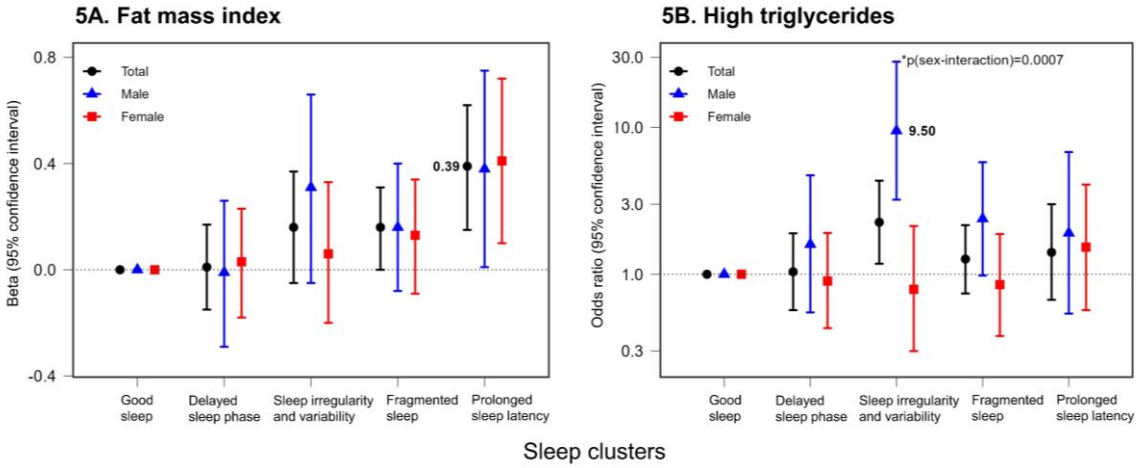

Figure 5. Cross-sectional associations between five sleep clusters with fat mass index (kg/m^2) and high triglycerides (≥1.7 mmol/L) among adolescents. The significant estimates (beta or odds ratio) are displayed in bold after Bonferroni correction with Nyholt method. *p means significant interaction effects of sex with sleep clusters on outcomes.

4.3 Objective sleep clusters and obesity: Longitudinal findings

The third publication addressed the third specific aim of this thesis: To explore longitudinal relationships of objective sleep clusters with overweight or obesity among adolescents and young adults, and to evaluate if they are modified with time or by genetic risk.

Although sleep characteristics changed from 15y to 20y, such as shorter total sleep time (mean 7.2 to 6.6 hours), the previously identified five sleep clusters in adolescents (n=1347) were also identified in young adults (n=1262). Among 636 participants having repeated data at both follow-ups, adolescents within the "good sleep", "fragmented sleep", and "delayed sleep phase" clusters showed more stability into the same cluster during the transition (consistency rate ≥ 40%), while those with the "sleep irregularity and variability", and "prolonged sleep latency" clusters showed less stability (consistency rate < 10%). In generalized estimating equations models, compared to the "good sleep" cluster, the "prolonged sleep latency" cluster exhibited increased BMI (β=0.56, 95% CI = [0.06, 1.05]) and increased odds of having overweight or obesity (OR=1.55, 95%CI= [1.02, 2.34], **Figure 6**). PRS was linked to increased BMI (β=0.65, 95%CI= [0.39, 0.91]) and elevated odds of having overweight or obesity (OR=1.52, 95%CI= [1.15, 2.00]), yet without significant gene-sleep cluster interaction effect (p-interaction > 0.05). Only among males, significant interaction effects with time of follow-ups on odds of having overweight or obesity were observed in the "fragmented sleep", "sleep irregularity and variability", and "delayed sleep phase" clusters (p-interaction < 0.05), showing more adverse impacts with increasing age.

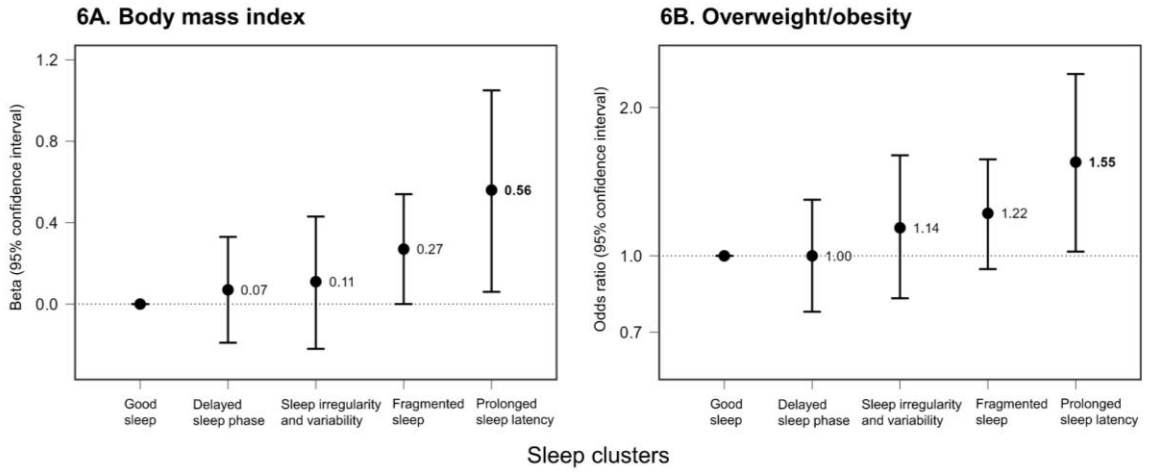

Figure 6. Longitudinal associations of five sleep clusters with body mass index (kg/m^2) and overweight or obesity (overweight/obesity) between 15-year and 20-year follow-ups of birth cohorts. The significant estimates (beta or odds ratio) are highlighted in bold.

5. Discussion

5.1 Summary of key findings

This dissertation aimed to investigate the associations of multiple sleep characteristics with cardiometabolic risk factors considering genetic risk variants among adolescents and young adults, using prospectively collected information from two German birth cohorts. This thesis first utilized subjective sleep and BMI data among both adolescents and young adults, and observed bidirectional relationships of sleep behaviors with overweight or obesity (Publication I). For example, persistent sleep difficulties from adolescence through young adulthood were linked to having overweight or obesity among young adults. Conversely, consistent overweight or obesity was linked to young adult sleep difficulties. Further, this dissertation identified five distinctive sleep clusters among adolescents by cluster analysis utilizing 12 objective sleep characteristics. Individuals with “prolonged sleep latency” cluster had higher FMI, and males with “sleep irregularity and variability” cluster seemed to have elevated odds of high triglycerides (Publication II). Furthermore, this thesis extended the cluster analysis to young adults by using objective sleep data, and demonstrated that five sleep clusters can be identified consistently in both adolescence and young adulthood. The “prolonged sleep latency” cluster had longitudinal links with higher BMI and overweight or obesity, independent of genetic risk. Only among males, the clusters of “fragmented sleep”, “sleep irregularity and variability”, and “delayed sleep phase” exhibited increasingly adverse impacts on overweight or obesity as age advanced (Publication III).

Relevance between findings

In Publication I, subjective sleep difficulties (symptoms of insomnia) had a long-term association with obesity (indicated by BMI) from adolescence through young adulthood. In Publication II and the Publication III, the objective "prolonged sleep latency" pattern was linked to adiposity (indicated by FMI or BMI), which precisely corroborating a part of the first finding. As the "prolonged sleep latency" pattern serves as the objective phenotype of "difficulty falling asleep", reflecting one component of reported "sleep difficulties" and insomnia symptoms. Taken together, this thesis supported the significant association between prolonged sleep latency and obesity, indicating that prolonged sleep latency may elucidate the established link between reported short sleep and obesity. Prolonged sleep latency may lead to increased calorie intake due to stress or anxiety from difficulty falling asleep [81,82], hormonal imbalances affecting energy intake and expenditure [81], and reduced physical activity from fatigue caused by less deep sleep [83].

In addition, the findings from both Publication II and Publication III suggested that males during adolescence and young adulthood with unfavorable sleep patterns are more susceptible to have adverse cardiovascular risk factors. Furthermore, the results in both Publication I and Publication III highlighted again the significant association between genetic risk and obesity, indicating that the link between poor sleep and obesity is independent from the genetic prediction.

5.2 Implications of sleep clusters

Five sleep clusters were classified consistently among both adolescents and young adults, according to 12 accelerometry-measured sleep characteristics indicating sleep quality, quantity, variability, regularity, and schedule. The "good sleep" cluster accounts for approximately one-third of overall study population, which has greater total sleep time and efficiency, with lower values in other sleep characteristics. The "delayed sleep phase" cluster, which has later sleep midpoint timing yet higher sleep efficiency, clearly depicts objective features associated with DSPD [23]. As described above, DSPD prevalence among adolescents and young adults is 7%-16%. In our study, over 18% of individuals were allocated to the "delayed sleep phase" cluster, despite we did not ask this DSPD question or diagnose them with DSPD. Nevertheless, this finding highlighted prevalent sleep issues frequently associated with DSPD among adolescents and young adults, deserving of greater attention. The "sleep irregularity and variability" cluster, marked by higher day-to-day variability in total sleep time, and other sleep characteristics, accounted for around 10% of adolescents and young adults. Male adolescents with "sleep irregularity and variability" cluster had elevated odds of high triglycerides, consistent with recent findings linking sleep variability/irregularity to adverse cardiometabolic outcomes [45]. This may involve the potential biological mechanisms in males such as increased lipid absorption,

synthesis of triglycerides in the liver [84], hormonal changes associated with earlier puberty [85], and irregular breakfast habits [45,86]. The “fragmented sleep” cluster, characterized by more time awake and higher number of awakenings, and “prolonged sleep latency” cluster, characterized by longer sleep latency, both were symptoms of insomnia, like “difficulty staying asleep”, and “difficulty falling asleep”, respectively. Furthermore, as adolescents transitioned into young adulthood, the “good sleep”, “fragmented sleep”, and “delayed sleep phase” clusters demonstrated greater stability, while the “sleep irregularity and variability”, and “prolonged sleep latency” clusters displayed lower stability. These findings supported the utility of accelerometry and cluster analysis in identifying various sleep patterns in early stages of life, such as variability, insomnia, and circadian sleep disorders [29,30].

5.3 Strengths and limitations

A detailed discussion is included in the respective publication. Here, some major strengths and limitations are briefly summarized.

This dissertation utilizes data from two follow-ups of two well-established German birth cohorts, facilitating longitudinal analyses among a large sample of adolescents and young adults to explore their developmental changes. Furthermore, the availability of multidimensional sleep characteristics with different measurements (questionnaires, accelerometry, and sleep diaries), along with multiple cardiometabolic risk factors, and genetic data, provides a comprehensive opportunity for thorough investigation. Additionally, the application of cluster analysis considering correlations between sleep characteristics, enable us to understand unique sleep patterns and their associations with cardiometabolic health from a holistic perspective. Moreover, the inclusion of genetic data provides the opportunity to identify populations at risk and those who would specifically benefit from a targeted intervention. Besides, to mitigate the risk of type-1 error from examining multiple cardiometabolic outcomes, I applied Bonferroni correction with Nyholt method, considering their correlations, as detailed in Publication II [76].

On the other hand, some limitations in this thesis should be acknowledged. First, although bidirectional analyses (Publication I) and a longitudinal design (Publication III) were applied, all three observational studies in the dissertation inherently limited causal inferences. Second, accelerometers may overestimate total sleep time compared to polysomnography [30], although they are widely adopted as practical tools in epidemiological studies [87]. Third, the absence of daytime sleep data hindered examining their associations with cardiometabolic health in adolescents and young adults. A recent review found no clear link between short daytime napping (<30 minutes per day) and cardiometabolic risk factors in young and middle-aged adults [88]. Fourth, all three papers analyzed data from a limited age range (14-16 years and 19-22 years) within the German population, restricting the

generalizability to other age groups or cultures. Fifth, the 7-day accelerometry measurement protocol may influence sleep behaviors, as participants might tend to exhibit good sleep hygiene.

6. Conclusion and Outlook

In conclusion, this thesis found bidirectional relationships of subjective insufficient sleep and sleep difficulties with having overweight or obesity between adolescence and young adulthood. Further, this dissertation demonstrated that adolescents and young adults shared five consistent sleep patterns based on objective sleep characteristics. Most importantly, prolonged sleep latency was observed to be linked to obesity in adolescence and young adulthood. Notably, males during these developmental periods with poor sleep patterns are more prone to developing adverse cardiometabolic risk factors.

These findings contributed to the adverse impacts of multidimensional sleep on cardiometabolic health among adolescents and young adults. They provided additional insights through the application of cluster analysis on objective sleep characteristics and longitudinal analysis on two time-points data. This thesis suggests prioritizing preventive measures to reduce sleep latency and target males during the transition from adolescence through young adulthood. As adolescents and young adults become more independent and take control of their sleep habits, implementing educational programs and intervention strategies during this crucial period can yield a substantial impact on both sleep and subsequent overall health.

I acknowledge that this thesis used the observational data from a limited age range within the German population, potentially limiting the causality and generalizability of our findings. Therefore, more observational, and interventional studies involving other age groups, ethnicities and cultures are necessary to confirm our results and explore the causal relationship. In addition, I suggest using polysomnography to measure sleep for consecutive nights in relatively small sample sizes of adolescents and young adults to confirm our findings, given the difference between accelerometry and the gold standard. Moreover, future research including more objective sleep characteristics, such as daytime sleep information, may provide a more comprehensive understanding of sleep patterns. Besides, while accelerometry can measure sleep over a 1- or 2-week period but may alter sleep behaviors, validated digital tool monitoring sleep in everyday life may provide additional important information on regular sleep patterns. Furthermore, the molecular studies, like investigating biomarkers of subclinical inflammation, are essential to deepen our understanding of the potential mechanism linking sleep and cardiometabolic health.

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Publication I

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Changes in sleep duration and sleep difficulties from adolescence to young adulthood and the risk of obesity: Bidirectional evidence in the GINIplus and LISA studies

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ABSTRACT

Objective: This study aimed to assess the association of changes in sleep behaviors from adolescence to young adulthood with the risk of overweight/obesity, and the reverse relationship.
Methods: Data of 1978 participants was obtained from the 15- and 20-year follow-ups of the GINIplus and LISA birth cohorts. Insufficient sleep was defined as reported sleep duration <8 h for adolescents, <7 h for adults, and sleep difficulties as reported having sleeping difficulties. Logistic regression models were used to assess bidirectional associations of changes in insufficient sleep and sleep difficulties with overweight/obesity. The polygenic risk scores (PRS) for body mass index (BMI) was tested in a sub-sample (n = 918).
Results: Compared with sufficient sleep in both adolescence and young adulthood, insufficient sleep only in young adulthood was associated with an increased risk of overweight/obesity (odds ratio = 1.85, 95% confidence interval = [1.27–2.69]). Compared with no sleep difficulties at both time-points, only persistent sleep difficulties was associated with a higher risk of overweight/obesity (2.15 [1.22–3.77]). The PRS for BMI was associated with overweight/obesity (1.41 [1.17–1.70]), but no significant gene-sleep interaction effect was observed. Reversely, only persistent overweight/obesity was associated with increased risks of insufficient sleep (1.81 [1.21–2.70]), and sleep difficulties (1.77 [1.18–2.66]), respectively.
Conclusions: Insufficient sleep only presented a cross-sectional association with overweight/obesity in young adulthood, while long-term sleep difficulties from adolescence to young adulthood was associated with young adult overweight/obesity. Reversely, long-term overweight/obesity from adolescence to young adulthood was associated with insufficient sleep and sleep difficulties in young adulthood.

1. Introduction

The prevalence of pediatric insufficient sleep and insomnia has greatly increased over the past decades [1,2]. Insufficient sleep and insomnia have detrimental effects on health and well-being among children and adolescents [3], especially on overweight/obesity in adulthood [4–6]. However, the cause and direction of the

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association between sleep behaviors and overweight/obesity remain unclear, and few longitudinal studies have investigated the relationships between changes in sleep behaviors (insufficient sleep or sleep difficulties) from adolescence transitioning to young adulthood and the risk of young adult overweight/obesity [5,7].

The transition from adolescence to young adulthood is an important developmental period when individuals undergo substantial physiological and psychological changes, which might lead to changes in sleep behavior, in turn impacts health [8,9]. Previous prospective studies analyzed a different number of follow-up time-points and sleep assessment methods in childhood and adolescence [4,10–12] to predict subsequent obesity or fat mass, but these had not considered the changes in sleep duration at different time-points. Additionally, only a few studies investigated changes in sleep duration and obesity in children and adolescents [7,13]. One study found that girls with insufficient sleep at 11 years but sufficient sleep at 18 years had an increase in body mass index (BMI) z-scores at 18 years compared to those with sufficient sleep at both time-points [7], while another study observed that change in total sleep duration was not significantly associated with changes in BMI over 2 years in adolescence [13].

Insufficient sleep has been identified as an important risk factor for the development of obesity [3,4,6,14], but as a reverse direction of association cannot be ruled out, the association may be bidirectional [15–17]. It might be plausible that obesity predisposes people to poor sleep quality, such as sleep apnea disrupting sleep [18]. Only a few studies examined the potential bidirectional longitudinal associations between sleep duration and obesity in children and adolescents with inconsistent findings [12,19,20]. Some studies discovered that higher BMI was associated with subsequent shorter sleep during adolescence to adulthood [19], or in infancy and early childhood [20], but not vice versa. However, another study revealed a bidirectional association between sleep duration and adiposity among South Asian children [12].

The application of polygenic risk scores (PRS), as an estimate of a participant's genetic liability to a trait or disease, represents a possibility to study gene-environment interaction effects on obesity with increased power [21]. A few gene-environment interaction studies have investigated the interactions of sleep duration with PRS on obesity in adults, which indicated that short sleep duration accentuated the effect of PRS on obesity [22,23]. However, the research on gene-sleep interactions in children and adolescents is rare, and previous PRS only focused on several common adult BMI single nucleotide polymorphisms (SNPs) [24,25] and were not based on the latest comprehensive loci for BMI [26].

In the present study, we assessed the associations of changes in sleep duration and sleep difficulties from adolescence to young adulthood, with overweight/obesity in young adulthood, considering genetic risk variants, using data from two prospective German birth cohorts. In parallel, we examined the relationships of the changes in overweight/obesity status with insufficient sleep and sleep difficulties from adolescence to young adulthood.

2. Methods

2.1. Study participants

Data in the present study was obtained from the 15- and 20-year follow-up examinations of two ongoing German birth cohort studies, GINIplus (German Infant Study on the influence of Nutrition Intervention PLUS environmental and genetic influences on allergy development) and LISA (Influence of Lifestyle factors on the development of the Immune System and Allergies in East and West Germany). In brief, a total of 5991 mothers and their newborns were recruited into the GINIplus study between 1995 and 1998 in Munich and Wesel, which consisted of the intervention arm (n = 2252), and the observation arm (n = 3739). The intervention study arm was a double-blind, randomized, intervention trial using three hydrolyzed formula nutrition and cow's milk formula, and was carried out among newborns with at least one allergic parent and/or sibling during the first 4 months, while breastfeeding was not wished or feasible. Newborns without a family history of allergic disease, and those with a family history whose parents refused to participate in the trial were followed-up in the observation arm. For the LISA study, a total of 3094 healthy, full term neonates were recruited between 1997 and 1999 in Munich, Leipzig, Wesel and Bad Honnef and surrounding areas. Four study centers were selected to represent different living areas across Germany, with Munich and Leipzig being considered more urban, and Wesel and Bad Honnef more rural areas. The study designs and recruitments have been described in more detail elsewhere [27–29].

Finally, a total of 1978 participants with complete information on variables of interest at both 15- and 20-year follow-ups were included for the main analysis, and a sub-sample of 918 participants with genotype data available in Munich and Wesel study centers were included for the genetic analysis (Fig. 1). Both studies were approved by the respective local ethics committees (Bavarian Board of Physicians, University of Leipzig, Board of Physicians of North-Rhine-Westphalia), and written informed consents were given from participants and their families.

2.2. Unfavorable sleep behaviors

Two unfavorable sleep behaviors, insufficient sleep duration and sleep difficulties, were assessed in the questionnaires at the 15- and 20-year follow-up examinations of both cohorts. In the 15-year follow-up, participants' parents were asked to answer the sleep duration related question "How many hours in total during the day and night does the child sleep on average?" and the sleep difficulties related question "Does the child have sleeping difficulties? (answer: yes; no)". In the 20-year follow-up, participants responded to the sleep duration related questions "How many hours do you sleep on average on school/working days?" and "How many hours do you sleep on average on days off from school/work?". Information on sleep difficulties was collected by the question "Do you have sleeping difficulties? (answer: yes; no)". Average sleep duration in the 20-year follow-up was calculated as [(sleep duration on school/working days * 5 + sleep duration on days off from school/work * 2)/7]. Insufficient sleep was defined as sleep duration of <8 h for adolescents (15-year follow-up) [30] and < 7 h for young adults (20-year follow-up) [31]. Sleep difficulties were defined as parent-reported or self-reported sleeping difficulties.

To investigate the changes in insufficient sleep between adolescence and young adulthood, the participants were categorized into four groups according to insufficient sleep at both follow-ups, describing presence/absence at both time-points or at either time-point:

1) persistent sufficient sleep at both time-points (No/No, n = 1372)
2) insufficient sleep in adolescence but sufficient sleep in young adulthood (Yes/No, n = 259)
3) sufficient sleep in adolescence but insufficient sleep in young adulthood (No/Yes, n = 267)
4) persistent insufficient sleep at both time-points (Yes/Yes, n = 80).

For the changes in sleep difficulties over time, the participants were also divided into four groups:

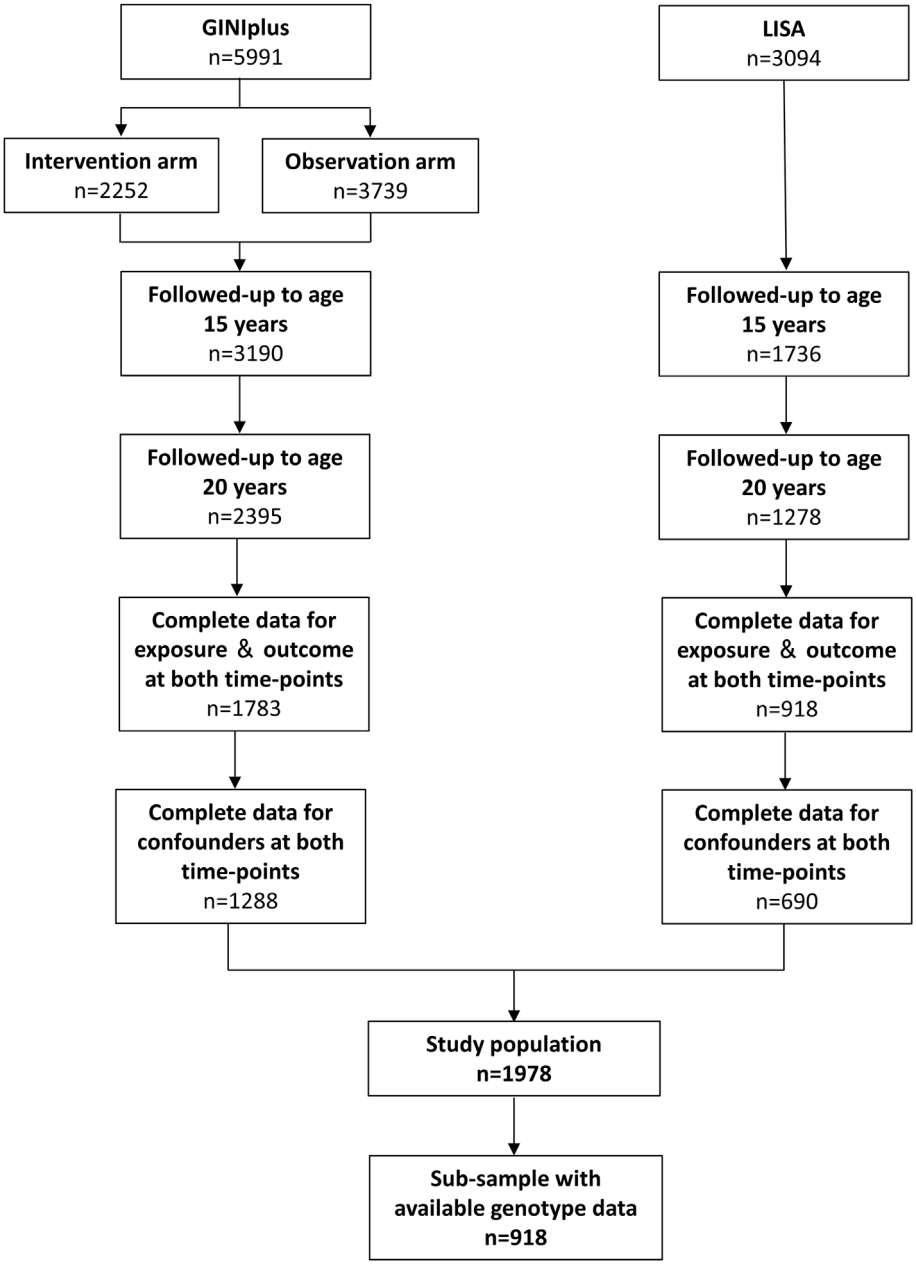

Fig. 1. Flow chart of participants.

1) persistent no sleep difficulties at both time-points (No/No, n = 1412)
2) sleep difficulties in adolescence but no sleep difficulties in young adulthood (Yes/No, n = 162)
3) no sleep difficulties in adolescence but sleep difficulties in young adulthood (No/Yes, n = 293)
4) persistent sleep difficulties at both time-points (Yes/Yes, n = 111).

2.3. Overweight/obesity

The weight and height of participants were reported by the parents at the 15-year follow-up, and by the participants at the 20-year follow-up. BMI was calculated as weight (kg)/height's square (m^2). For the 15-year follow-up, BMI z-scores were calculated based on the World Health Organization (WHO) growth reference for school-aged children and adolescents [32]. BMI was categorized into overweight/obesity (BMI z-scores $\geq$1 for adolescents [32] and BMI $\geq$25 kg/m^2 for young adults [33]) and normal weight (BMI z-scores <1 for adolescents and BMI <25 kg/m^2 for young adults).

To elucidate the changes in weight status from adolescence transitioning to young adulthood, participants were categorized into four groups, describing the presence/absence of overweight/obesity at both time-points or at either time-point:

1) persistent normal weight at both time-points (No/No, n = 1581)
2) overweight/obesity in adolescence but normal weight in young adulthood (Yes/No, n = 72)
3) normal weight in adolescence but overweight/obesity in young adulthood (No/Yes, n = 172)

4) persistent overweight/obesity at both time-points (Yes/Yes, n = 153).

2.4. Calculation of PRS

Genotyping for GINIplus and LISA was performed in 1511 samples using the Affymetrix Chip 5.0 and 6.0 (Thermo Fisher, USA) in the Munich study center and 883 samples using the Infinium Global Screening Array GSA v2 MD (Illumina, USA) in the Wesel study center. After quality control and genotype imputation, genome-wide data available for 918 participants in Munich and Wesel study centers with complete information on variables of interest was included in the present study. The quality control and genotype imputation have been described in detail previously [34,35].

PRS for adult BMI, as genetic determinants, were calculated based on previously published genome-wide association studies (GWAS) results on 97 significant BMI-associated loci ($P < 5 \times 10^{-8}$) [26]. The individual number of effect alleles of the selected SNPs were extracted for each participant and were weighted with the determined GWAS effect size [26]. For the Munich study center, of the 97 SNPs [26], only 96 SNPs were available, where 2 more were excluded due to low imputation quality ($R^2 < 0.4$) but one proxy with $R^2 > 0.7$ was added to replace one of the missing SNPs, and 95 SNPs in total were finally included in the calculation of the PRS. For the Wesel study center, 96 SNPs were available to be calculated into a PRS, but there was no proxy available for the missing variant. All PRS were normalized for the final analysis. The lists of SNPs for Munich and Wesel datasets can be found in the Supplemental Table S1.

2.5. Potential confounders

Potential confounders were sex, study (GINI observation arm, GINI intervention arm, and LISA study), study center (Munich, Leipzig, Bad Honnef, and Wesel), a parental highest education level (low: <10th grades; medium: 10th grades; and high: >10th grades), puberty stage (at 15-year follow-up) as well as exact age, physical activity, screen time, traffic noise and education/occupation types at the 20-year follow-up. Puberty stage was obtained from a self-rated scale for pubertal development: 1) prepubertal, 2) early pubertal, 3) midpubertal, 4) late pubertal, 5) postpubertal [36], and then was combined into 3 groups for the final analysis: 1) pre-/early/mid-pubertal, 2) late pubertal, and 3) postpubertal. Participants were asked how many hours per week (h/week) they spent in moderate physical activity (slight sweating, slightly increased breathing e.g. cycling, swimming, skating) in summer and winter, respectively; and how many hours they spent in vigorous physical activity (a lot of sweating, rapid breathing, e.g. ball games, training) in summer and winter, respectively. Then the average number of hours in moderate physical activity (summer and winter), and in vigorous physical activity (summer and winter), in h/week were calculated, respectively; followed by the sum of average moderate activity and average vigorous activity in h/week, which was defined as moderate to vigorous physical activity (MVPA). Physical activity was classified as low (MVPA <7 h/week), medium (7 h/week ≤ MVPA <10.5 h/week; MVPA ≥10.5 h/week but vigorous physical activity <3.5 h/week), high (MVPA ≥10.5 h/week and vigorous physical activity ≥3.5 h/week), according to Janssen (2007) [37]. Participants were asked how many hours they spent in front of a screen (television, computer, video games) per working/school day in summer and winter, respectively: 1) < 1 h, 2) 1–2 h, 3) 3–4 h, 4) 5–6 h, 5) 7–8 h, 6) 9–10 h, 7) > 10 h. Screen time was categorized into ≤2 h in summer and winter, and >2 h in summer or winter. Traffic noise was the self-reported degree to which the participant was disturbed by traffic noise at home when the window is open, from 0 (does not disturb) to 10 (unbearable), and was defined as no (0) and yes (>0). Participants were asked about the activity they currently mainly carried out: 1) school, 2) job training (a dual system accompanied with school attendance), 3) voluntary social/environmental year, 4) university, 5) employed, 6) unemployed, 7) housewife/househusband, 8) other. Then, education/occupation types were categorized into five types: 1) university, 2) job training, 3) school, 4) employed, and 5) other activities (voluntary social/environmental year, unemployed, housewife/household, and other).

2.6. Statistical analysis

The characteristics of the study participants were described in the total population and by sex, using mean value and standard deviation (SD) for continuous variables, and frequency (n) and percentage (%) for categorical variables. T-test for continuous variables and Chi-square test for categorical variables were used to explore differences between males and females.

In order to investigate the effects of changes in sleep behaviors on overweight/obesity, unfavorable sleep behaviors changes from adolescence to young adulthood, insufficient sleep and sleep difficulties, respectively, were modelled as exposures, and overweight/obesity in young adulthood as the outcome in multivariable logistic regression analyses. Three models were developed to adjust for potential confounding variables and PRS: Model 1 with adjustment for age, sex, study, study center, parental education, puberty (in adolescence), physical activity, screen time, traffic noise and education/occupation types in young adulthood; Model 2 with adjustment for covariates in Model 1 plus overweight/obesity in adolescence to test the effect of pre-existing overweight/obesity; and Model 3 with adjustment for covariates in Model 1 plus the PRS for BMI, followed by Model 3a that further added the interaction term with PRS. The insufficient sleep changes and sleep difficulties changes were mutually adjusted for in the same models.

In parallel, to determine the impact of the changes in overweight/obesity on unfavorable sleep behaviors, the exposures and outcomes were reversed. In the multivariable logistic regression models, the overweight/obesity status changes from adolescence to young adulthood were included as independent variables, and unfavorable sleep behaviors in young adulthood, insufficient sleep and sleep difficulties, respectively, as dependent variables. Two models were conducted to assess the effects of potential confounders. Model 1 included age, sex, study, study center, parental education, puberty (in adolescence), physical activity, screen time, traffic noise, education/occupation types, and sleep difficulties (for insufficient sleep) or insufficient sleep (for sleep difficulties) in young adulthood. Model 2 additionally included insufficient sleep and sleep difficulties in adolescence to examine the impact of pre-existing unfavorable sleep behaviors.

Furthermore, the interaction effects with sex were tested, and if the interaction term reached nominal significance, followed by sex-stratified analyses. Three sensitivity analyses were used to examine the robustness of our findings: first, excluding participants with overweight/obesity in adolescence; second, excluding those who had insufficient sleep or sleep difficulties in adolescence; third, using insufficient sleep defined only on school/working days in young adulthood.

3. Results

A total of 1978 participants with available data in adolescence and young adulthood were included in the final analyses. Table 1 presents the characteristics of the participants in the total

Table 1
Characteristics of participants in adolescence and young adulthood.

Variable	Total (n = 1978)	Male (n = 864)	Female (n = 1114)	*P*-value
Study, %				0.081
GINI observation	715 (36.1)	289 (33.4)	426 (38.2)	
GINI intervention	573 (29.0)	257 (29.7)	316 (28.4)	
LISA	690 (34.9)	318 (36.8)	372 (33.4)	
Study center, %				0.176
Munich	1080 (54.6)	495 (57.3)	585 (52.5)	
Leipzig	152 (7.7)	66 (7.6)	86 (7.7)	
Bad Honnef	69 (3.5)	29 (3.4)	40 (3.6)	
Wesel	677 (34.2)	274 (31.7)	403 (36.2)	
Parental education, %				0.219
Low	79 (4.0)	40 (4.6)	39 (3.5)	
Medium	454 (23.0)	186 (21.5)	268 (24.1)	
High	1445 (73.1)	638 (73.8)	807 (72.4)	
Adolescence (15-year follow-up)				
Age, years	15.0 ± 0.3	15.0 ± 0.2	15.1 ± 0.3	0.180
BMI, kg/m^2	20.1 ± 2.8	20.1 ± 2.9	20.1 ± 2.6	0.982
BMI z-score	−0.1 ± 1.0	0.0 ± 1.0	−0.2 ± 0.9	0.011
Sleep duration, hours	8.2 ± 0.8	8.3 ± 0.7	8.1 ± 0.8	<0.001
Overweight/obesity[a], %				<0.001
No	1753 (88.6)	735 (85.1)	1018 (91.4)	
Yes	225 (11.4)	129 (14.9)	96 (8.6)	
Insufficient sleep[b], %				<0.001
No	1639 (82.9)	752 (87.0)	887 (79.6)	
Yes	339 (17.1)	112 (13.0)	227 (20.4)	
Sleep difficulties, %				0.009
No	1705 (86.2)	765 (88.5)	940 (84.4)	
Yes	273 (13.8)	99 (11.5)	174 (15.6)	
Puberty, %				<0.001
Pre-/early/mid-pubertal	406 (20.5)	359 (41.6)	47 (4.2)	
Late pubertal	1376 (69.6)	499 (57.8)	877 (78.7)	
Postpubertal	196 (9.9)	6 (0.7)	190 (17.1)	
Young adulthood (20-year follow-up)				
Age, years	20.3 ± 0.4	20.3 ± 0.4	20.2 ± 0.4	<0.001
BMI, kg/m^2	22.4 ± 3.4	22.9 ± 3.4	22.1 ± 3.4	<0.001
Sleep duration, hours	7.6 ± 0.8	7.6 ± 0.8	7.7 ± 0.8	0.005
Overweight/obesity[a], %				0.032
No	1653 (83.6)	704 (81.5)	949 (85.2)	
Yes	325 (16.4)	160 (18.5)	165 (14.8)	
Insufficient sleep[b], %				0.409
No	1631 (82.5)	705 (81.6)	926 (83.1)	
Yes	347 (17.5)	159 (18.4)	188 (16.9)	
Sleep difficulties, %				0.001
No	1574 (79.6)	716 (82.9)	858 (77.0)	
Yes	404 (20.4)	148 (17.1)	256 (23.0)	
Physical activity, %				<0.001
Low	872 (44.1)	322 (37.3)	550 (49.4)	
Medium	589 (29.8)	263 (30.4)	326 (29.3)	
High	517 (26.1)	279 (32.3)	238 (21.4)	
Screen time, %				0.380
≤ 2 h	322 (16.3)	133 (15.4)	189 (17.0)	
> 2 h	1656 (83.7)	731 (84.6)	925 (83.0)	
Traffic noise, %				0.039
No	788 (39.8)	367 (42.5)	421 (37.8)	
Yes	1190 (60.2)	497 (57.5)	693 (62.2)	
Education/occupation types, %				0.096
University	1119 (56.6)	480 (55.6)	639 (57.4)	
Job training	436 (22.0)	184 (21.3)	252 (22.6)	
School	80 (4.0)	42 (4.9)	38 (3.4)	
Employed	191 (9.7)	97 (11.2)	94 (8.4)	
Other activities	152 (7.7)	61 (7.1)	91 (8.2)	

[a] Overweight/obesity: BMI z-score ≥1 according to WHO for adolescents; BMI ≥25 kg/m^2 for adults.
[b] Insufficient sleep: sleep duration <8 h for adolescents; <7 h for adults.

population and by sex in adolescence and young adulthood. Overall, the prevalence of insufficient sleep and sleep difficulties in adolescence was 17.1% and 13.8%, respectively, and the corresponding prevalence in young adulthood was 17.5% and 20.4%, respectively. The range of sleep duration in adolescence and young adulthood was 5.0–12.0 and 4.6–12.1 h, and the number of participants sleeping more than 10 h was 7 (0.3%) and 6 (0.3%), respectively. In addition, the prevalence of insufficient sleep on school/working days and on days off from school/work in young adults was 22.2% and 1.6%, respectively. The prevalence of sleep difficulties in participants with insufficient sleep was significantly higher than that in those with sufficient sleep in adolescence (25.4% vs 11.4%), and in young adulthood (32.6% vs 17.8%), respectively. However, no significant interaction effect between sleep difficulties and insufficient sleep on overweight/obesity was observed (data not shown).

In adolescence, 225 (11.4%) participants were overweight or obese, and in young adulthood, 325 (16.4%) were overweight or obese. Regarding the difference between sexes, female adolescents had a significantly higher prevalence of insufficient sleep and sleep difficulties than males, while the prevalence of overweight/obesity in females was lower than in males. In young adulthood, a similar pattern was found, although the difference in the prevalence of insufficient sleep between males and females was non-significant.

3.1. Associations between unfavorable sleep behaviors changes and overweight/obesity

Table 2 shows the associations from multivariable logistic regression models analysing changes in unfavorable sleep behaviors from adolescence to young adulthood with overweight/obesity in young adulthood. In Model 1, sufficient sleep in adolescence but insufficient sleep in young adulthood, and persistent sleep difficulties at both time-points were associated with increased risks of young adult overweight/obesity (odds ratio (OR) = 1.80, 95%confidence interval (CI) = [1.29–2.51] and 1.76 [1.05–2.94]), respectively. In Model 2, the effects were consistent with further adjustment for overweight/obesity in adolescence, and corresponding OR [95%CI] were 1.85 [1.27–2.69] and 2.15 [1.22–3.77], respectively. In contrast, participants with insufficient sleep or sleep difficulties in adolescence transitioning to be favorable in young adulthood, had no increased risk of young adult overweight/obesity (1.26 [0.82–1.95] and 1.12 [0.66–1.89]; respectively, Model 2). In Model 3, additionally including the PRS for BMI, the PRS was independently associated with the risk of young adult overweight/obesity (1.41 [1.17–1.70]), but the effect of persistent sleep difficulties on overweight/obesity attenuated to statistical non-significance (2.05 [0.95–4.42]). However, there was no significant interaction effect between PRS and the changes in insufficient sleep or sleep difficulties (Model 3a).

For the interaction analyses with sex, there was a significant interaction effect between sex and the group with sufficient sleep in adolescence but insufficient sleep in young adulthood (*P*-value interaction = 0.029). In the sex-stratified analyses, insufficient sleep only in young adulthood was associated with an increased risk of young adult overweight/obesity among males (2.62 [1.58–4.35]), but no such association was found in females (Table S2). However, females with persistent sleep difficulties at both time-points had a higher risk of young adult overweight/obesity (2.29 [1.09–4.81], Table S2). In the sensitivity analyses excluding those who were overweight/obese in adolescence, the overall findings did not change (Table S3).

3.2. Associations between overweight/obesity status changes and unfavorable sleep behaviors

Participants with persistent overweight/obesity at both time-points had higher risks of insufficient sleep (1.81 [1.21–2.70]), and sleep difficulties (1.77 [1.18–2.66]), respectively, compared to those who had normal weight at both time-points, including adjustment for insufficient sleep and sleep difficulties in adolescence (Table 3, Model 2). In contrast, the risk of young adult insufficient sleep or sleep difficulties did not seem to increase, when overweight/obesity in adolescence transitioned to normal weight in young adulthood (1.08 [0.56–2.09] and 0.54 [0.25–1.19]; respectively).

No significant interaction effect between sex and weight status changes was observed. In females, persistent overweight/obesity in both periods was associated with an increased risk of sleep difficulties (2.47 [1.42–4.27]) in young adulthood, but there was no association between weight status changes and insufficient sleep (Table S4). However, among males, no significant association between weight status changes and sleep difficulties was found, but overweight/obesity only in young adulthood was associated with a higher risk of insufficient sleep (2.06 [1.17–3.60]) (Table S4). In additional sensitivity analyses, after exclusion of those who had insufficient sleep or sleep difficulties in adolescence, the risk of sleep difficulties in the group with persistent overweight/obesity at both time-points was attenuated and not significant (1.59 [0.96–2.64], Table S5).

In further sensitivity analyses, the overall findings remained robust and unchanged, when using the definition of insufficient sleep restricted on school/working days only (Table S6).

4. Discussion

In the present study, we assessed the changes in unfavorable sleep behaviors and the risk of young adult overweight/obesity, and

Table 2
Associations between unfavorable sleep behaviors changes from adolescence to young adulthood and young adult overweight/obesity.

Outcome	Exposure		Model 1 (N = 1978)			Model 2 (N = 1978)		Model 3 (N = 918)			Model 3a
	Adolescence	Young adulthood	n (%)	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	n (%)	OR (95% CI)	*P*-value	*P-value interaction*
Overweight/obesity	**Insufficient sleep**										
	No	No	201 (14.7)	1.00		1.00		92 (14.5)	1.00		
	Yes	No	42 (16.2)	1.10 (0.75–1.61)	0.639	1.26 (0.82–1.95)	0.289	20 (15.7)	0.99 (0.55–1.75)	0.960	0.767
	No	Yes	65 (24.3)	1.80 (1.29–2.51)	0.001	1.85 (1.27–2.69)	0.001	34 (27.4)	2.18 (1.34–3.56)	0.002	0.844
	Yes	Yes	17 (21.2)	1.22 (0.66–2.24)	0.520	0.83 (0.39–1.77)	0.634	8 (23.5)	1.36 (0.55–3.37)	0.503	0.090
	Sleep difficulties										
	No	No	216 (15.3)	1.00		1.00		101 (15.3)	1.00		
	Yes	No	27 (16.7)	1.18 (0.74–1.87)	0.488	1.12 (0.66–1.89)	0.678	10 (14.7)	0.97 (0.46–2.06)	0.945	0.705
	No	Yes	57 (19.5)	1.32 (0.93–1.86)	0.117	1.16 (0.78–1.72)	0.476	31 (22.0)	1.37 (0.84–2.25)	0.212	0.930
	Yes	Yes	25 (22.5)	1.76 (1.05–2.94)	0.030	2.15 (1.22–3.77)	0.008	12 (25.5)	2.05 (0.95–4.42)	0.066	0.575
	PRS								1.41 (1.17–1.70)	<0.001	

Model 1: Adjusted for age, sex, study, study center, parental education, puberty (in adolescence), physical activity, screen time, traffic noise, and education/occupation types in young adulthood.
Model 2: Model 1 + overweight/obesity in adolescence.
Model 3: Model 1 + PRS for BMI.
Model 3a: Model 3 + interaction term between unfavorable sleep behaviors and PRS.
n (%): number of cases (prevalence). OR: odds ratio; 95%CI: 95% confidence interval. PRS: polygenic risk scores. The insufficient sleep changes and sleep difficulties changes were mutually adjusted for in the same models.

Table 3
Associations between overweight/obesity status changes from adolescence to young adulthood and unfavorable sleep behaviors in young adulthood.

Outcome	Exposure			Model 1 (N = 1978)		Model 2(N = 1978)	
	Adolescence	Young adulthood	n (%)	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Insufficient sleep	**Overweight/obesity**						
	No	No	253 (16.0)	1.00		1.00	
	Yes	No	12 (16.7)	1.07 (0.56–2.06)	0.838	1.08 (0.56–2.09)	0.813
	No	Yes	39 (22.7)	1.46 (0.98–2.16)	0.060	1.45 (0.98–2.15)	0.067
	Yes	Yes	43 (28.1)	1.81 (1.21–2.70)	0.004	1.81 (1.21–2.70)	0.004
Sleep difficulties	**Overweight/obesity**						
	No	No	314 (19.9)	1.00		1.00	
	Yes	No	8 (11.1)	0.55 (0.26–1.19)	0.129	0.54 (0.25–1.19)	0.126
	No	Yes	35 (20.3)	1.06 (0.71–1.59)	0.784	0.99 (0.65–1.50)	0.959
	Yes	Yes	47 (30.7)	1.74 (1.17–2.58)	0.006	1.77 (1.18–2.66)	0.006

Model 1: Adjusted for age, sex, study, study center, parental education, puberty (in adolescence), physical activity, screen time, traffic noise, education/occupation types, and sleep difficulties (for insufficient sleep) or insufficient sleep (for sleep difficulties) in young adulthood.
Model 2: Model 1 + insufficient sleep and sleep difficulties in adolescence.
n (%): number of cases (prevalence). OR: odds ratio; 95%CI: 95% confidence interval.

the reverse association of changes in overweight/obesity with the risk of young adult unfavorable sleep behaviors, using data from the 15- and 20-year follow-ups of the GINIplus and LISA cohorts. We found that insufficient sleep only had a cross-sectional association with overweight/obesity in young adulthood, while persistent sleep difficulties from adolescence to young adulthood was associated with overweight/obesity. Reversely, persistent overweight/obesity had impacts on young adult insufficient sleep and sleep difficulties.

The risk of overweight/obesity in young adulthood was associated with current insufficient sleep, independent of insufficient sleep in adolescence. Participants who favourably altered their insufficient sleep between adolescence and young adulthood had a similar risk of overweight/obesity to those with persistent sufficient sleep at both time points. To our knowledge, only one other study assessed the relationships between combinations of the presence or absence of insufficient sleep at two time-points and BMI or BMI z-scores from childhood to young adulthood among 3974 Brazilian participants [7]. In contrast to the findings of the present study, compared to those with adequate sleep duration at both time-points, girls who altered the inadequate sleep duration at 11 years to adequate sleep duration at 18 years, had an increase in BMI z-scores ($\beta = 0.39$, 95%CI = 0.13–0.65) and fat mass index (FMI) z-scores ($\beta = 0.30$, 95%CI = 0.07–0.53) [7]. In addition, a study reported that the change in total sleep duration was not associated with changes in BMI or percent body fat (PBF) over 2 years in 723 US adolescents [13]. On the contrary, another prospective study comprising 14800 US participants found that cumulative exposure to short sleep from adolescence to young adulthood had a dose-response association with the odds of obesity [4].

Unlike the cross-sectional association between insufficient sleep and overweight/obesity in young adulthood, sleep difficulties exhibit a long-term relationship with overweight/obesity throughout adolescence and young adulthood. While the cross-sectional association of BMI with sleep duration is well known, the association of sleep difficulties with overweight/obesity is less clear. A meta-analysis mostly comprising cross-sectional studies including 25,082 children, adolescents, and young adults found that poor sleep quality (subjectively reported) was significantly associated with a higher odds of overweight/obesity (1.46 [1.24–1.72]), independent of sleep duration [38]. In contrast, 233 German patients with a confirmed diagnosis of insomnia (mean age 52 years old) showed a lower BMI (23.8 kg/m^2 versus 27.1 kg/m^2; $P < 0.05$), compared to the representative population matched by age and sex [39]. Varying findings between previous studies might be due to different study designs, age groups, and sample sizes.

In addition to previous studies, we also considered genetic risk variants and found that PRS for BMI was independently associated with overweight/obesity in young adulthood, while there was no interaction effect with unfavorable sleep behaviors. This is different from the previous studies related to the interaction effects between genetic risk scores (GRS) with sleep duration in children and adolescents. For example, in a Chinese cohort study of 3211 children and adolescents aged 6–18 years, Fu et al. [25] revealed that a GRS consisting of six leptin-related SNPs had an interaction with sleep duration, where GRS was robustly associated with a higher BMI and overweight/obesity among short sleepers (<8 h/day). Similarly, Prats-Puig et al. [24] reported that a GRS of three common SNPs in the obesity genes (*FTO*, *TMEM*18, and *NRXN*3) had a greater effect on the negative association between short sleep duration and BMI in 297 Caucasian children aged 5–9 years. Unlike the previous few studies of gene-sleep interactions in children and adolescents [22], our study utilized the most comprehensive SNPs (96 SNPs in Munich center and 95 SNPs in Wesel center) for BMI to analyse the gene-sleep interaction on the risk of overweight/obesity in young adults. The discrepancy between the above studies may be due to the different study approaches, where we evaluated the genetic risk using GWAS-selected SNPs, but other studies focused on specific pathways, as well as different sample sizes and ethnicities [22].

Our study also found that associations vary between sexes, where females with persistent sleep difficulties from adolescence to young adulthood had a higher risk of overweight/obesity, yet males with insufficient sleep only in young adulthood was associated with an increased risk of overweight/obesity. This finding related to short sleep among males was consistent with the majority of previous research. Several studies have reported a stronger association between short sleep and overweight/obesity in boys than in girls among adolescence [40,41]. For example, a US study composed of a nationally representative sample found that short sleep presented a cross-sectional association with obesity in adolescent boys but not in girls, while exhibiting a longitudinal association with obesity in both young adult males and females [41]. In contrast, Lytle et al. [13] did not observe a significant association between change in sleep duration and change in BMI or PBF over 2 years during adolescence in either girls or boys. The evidence about the sex discrepancy in association between sleep difficulties and overweight/obesity is rare. Our findings suggested that females with sleep difficulties between adolescence and young adulthood are more susceptible to develop overweight/obesity than males, which might be due to different physiologic mechanisms and sex hormones between sexes during adolescence [41,42].

While exploring the direction of unfavorable sleep behaviors with overweight/obesity, we observed that overweight/obesity had long-term impacts on insufficient sleep and sleep difficulties from adolescence to young adulthood. Our finding regarding the bidirectional associations of sleep duration with overweight/obesity was similar to the results in children observed by Collings et al. [12], where sleep duration was inversely associated with total and abdominal adiposity, and higher adiposity was also associated with shorter sleep duration among South Asian children, using data at 4 time-points from 12 to 36 months of age. However, Sokol et al. [19] found that higher BMI was associated with subsequent shorter sleep during adolescence to adulthood, but sleep duration was not associated with subsequent BMI. Until now, longitudinal studies on obesity and sleep difficulties, as well as their bidirectional associations were very rare in children and adolescents. A meta-analysis conducted in adults based on three prospective studies found that the odds of developing future insomnia symptoms among participants with obesity at baseline were not significantly higher than among those with normal-weight at baseline (1.07 [0.91, 1.26]) [43].

Regarding the bidirectional relationships between insufficient sleep, sleep difficulties, and overweight/obesity, it is hard to distinguish what comes first [44,45], and it might be that there are also shared risk factors. In our sensitivity analyses, after exclusion of adolescents who were overweight/obese at baseline, persistent sleep difficulties was still associated with the risk of developing new-onset young adult overweight/obesity (2.04 [1.11–3.73]). However, persistent overweight/obesity was no longer statistically related to the risk of developing new-onset young adult sleep difficulties (1.59 [0.96–2.64]) after the exclusion of participants with insufficient sleep/sleep difficulties at baseline, although the p-value (0.071) was borderline significant. The mechanism of sleep restriction leading to obesity may be that sleep deprivation influences physiological, autonomic nervous system, hormonal system, and food preferences, further promoting the increased dietary intake, decreased physical activity, and weight gain [44–46]. On the other side, the relationship that obesity could cause sleep loss or sleep disorders should also be worth noting. The biological mechanisms regarding this association might involve the changes in pro-inflammatory cytokines levels, diet food components, and vitamin D deficiency [44,47,48].

Our study has several strengths. We investigated the relationships between the changes in sleep difficulties from adolescence transitioning to young adulthood and the risk of overweight/obesity for the first time. Our data also allowed us to consider the role of genetic variants for BMI on the associations. In addition, we explored the bidirectional associations between unfavorable sleep behaviors and overweight/obesity in adolescence and young adulthood. The limitations of the present study should also be noted. Firstly, the information on sleep behaviors, including sleep duration and sleep difficulties, were obtained by parent-reported questionnaire at 15-year follow-up and self-reported questionnaire at 20-year follow-up. Although there is a potential bias between subjective sleep and objective sleep, some evidence showed that parent-reported and self-reported sleep data were moderately correlated with objective sleep data [49,50]. However, self-reported sleep duration has been used by several previous studies, but applied different methodology with regards to number and time-points of sleep assessment as well as age and follow-up period which limits comparability [4,10–12]. Secondly, the weight and height of participants were reported by parents at the 15-year follow-up, and by participants at the 20-year follow-up. Despite males tend to overestimate their heights and females tend to underestimate their weights, self-reported height and weight has been confirmed to be statistically associated with actual measures and can be calculated for BMI and weight categories in young adults [51]. Thirdly, we could not account for other sleep characteristics, such as sleep timing, sleep efficiency, sleep onset latency, and day-to-day variability in sleep duration, which could also have impacts on weight status in adolescents [52,53]. Fourthly, our study used the average sleep duration in the 15-year follow-up and average sleep duration [(sleep duration on school/working days * 5 + sleep duration on days off from school/work * 2)/7] in the 20-year follow-up to represent participants' average and habitual sleep duration, and was unable to consider the weekend catch-up sleep. Our results showed that the prevalence of insufficient sleep in a week and on school/working days were similar, but the prevalence of insufficient sleep was much lower on days off from school/work. However, the sensitivity analyses restricting the definition of insufficient sleep only on school/working days showed robust results. Fifthly, we were not able to distinguish sufficient sleep and hypersomnia, due to missing information about the daytime sleepiness in the study. Sixthly, we could not differentiate sleep difficulties from other sleep disorders, especially insomnia and delayed sleep phase syndrome, which are common sleep disorders in adolescents [54].

5. Conclusions

Long-term sleep difficulties between adolescence and young adulthood were associated with young adult overweight/obesity, and vice versa, indicating a bidirectional association. Insufficient sleep only showed a cross-sectional relationship with young adult overweight/obesity, while overweight/obesity had a longitudinal association with young adult insufficient sleep. In contrast, the risk of young adult overweight/obesity did not seem to increase when unfavorable sleep behaviors in adolescence transitioned to be favorable in young adulthood. Our study highlighted the impacts of long-term sleep difficulties on young adult obesity, and emphasized the importance of maintaining a healthy sleep from adolescence to young adulthood through future public health interventions to prevent obesity later in life.

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Wesel, Pediatric Practice, Bad Honnef, IUF – Leibniz-Research Institute for Environmental Medicine at the University of Düsseldorf) and in addition by a grant from the Federal Ministry for Environment (IUF Düsseldorf, FKZ 20462296). Further, the 15-year follow-up examination of the LISA study was supported by the Commission of the European Communities, the 7th Framework Program: MeDALL project.

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Declaration of competing interest

None

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sleep.2022.11.031.

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Supplementary Material

Changes in sleep duration and sleep difficulties from adolescence to young adulthood and the risk of obesity: Bidirectional evidence in the GINIplus and LISA studies

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List of captions for supplementary tables and figures

Table S1. List of included SNPs for PRS calculation

Table S2. Associations between unfavorable sleep behaviors changes from adolescence to young adulthood and young adult overweight/obesity stratified by sex

Table S3. Associations between unfavorable sleep behaviors changes from adolescence to young adulthood and young adult overweight/obesity after exclusion of those who were overweight/obese in adolescence (N=1753)

Table S4. Associations between overweight/obesity status changes from adolescence to young adulthood and unfavorable sleep behaviors in young adulthood stratified by sex

Table S5. Associations between overweight/obesity status changes from adolescence to young adulthood and unfavorable sleep behaviors in young adulthood after exclusion of those who had insufficient sleep or sleep difficulties in adolescence (N=1452)

Table S6. Associations between sleep behaviors and overweight/obesity, with insufficient sleep defined only on school/working days

Table S1. List of included SNPs for PRS calculation

Legend: SNP = single nucleotide polymorphism, Beta = beta coefficient from genome-wide association study, Switched effect allele (0=no, 1=yes), PRS = polygenic risk scores.

	Munich study center					Wesel study center				
SNP	**Proxy**	**R^2**	**Beta**	**Effect allele**	**Switched effect allele**	**Proxy**	**R^2**	**Beta**	**Effect allele**	**Switched effect allele**
rs1558902	NA	NA	0.0818	A	0	NA	NA	0.0818	A	0
rs6567160	NA	NA	0.0556	C	0	NA	NA	0.0556	C	0
rs13021737	NA	NA	0.0601	G	0	NA	NA	0.0601	G	0
rs10938397	NA	NA	0.0402	G	0	NA	NA	0.0402	G	0
rs543874	NA	NA	0.0482	G	0	NA	NA	0.0482	G	0
rs2207139	NA	NA	0.0447	G	0	NA	NA	0.0447	G	0
rs11030104	NA	NA	0.0414	A	1	NA	NA	0.0414	A	1
rs3101336	NA	NA	0.0334	C	0	NA	NA	0.0334	C	0
rs7138803	NA	NA	0.0315	A	0	NA	NA	0.0315	A	0
rs10182181	NA	NA	0.0307	G	0	NA	NA	0.0307	G	0
rs3888190	NA	NA	0.0309	A	0	NA	NA	0.0309	A	0
rs1516725	NA	NA	0.0451	C	0	NA	NA	0.0451	C	0
rs12446632	NA	NA	0.0403	G	1	NA	NA	0.0403	G	1
rs16951275	NA	NA	0.0311	T	1	NA	NA	0.0311	T	1
rs3817334	NA	NA	0.0262	T	0	NA	NA	0.0262	T	0
rs2112347	NA	NA	0.0261	T	1	NA	NA	0.0261	T	1
rs12566985	NA	NA	0.0242	G	1	NA	NA	0.0242	G	1
rs3810291	NA	NA	0.0283	A	0	NA	NA	0.0283	A	0
rs7141420	NA	NA	0.0235	T	0	NA	NA	0.0235	T	0
rs13078960	NA	NA	0.0297	G	0	NA	NA	0.0297	G	0
rs10968576	NA	NA	0.0249	G	0	NA	NA	0.0249	G	0
rs17024393	NA	NA	0.0658	C	0	NA	NA	0.0658	C	0
rs657452	NA	NA	0.0227	A	1	NA	NA	0.0227	A	1
rs12429545	NA	NA	0.0334	A	0	NA	NA	0.0334	A	0
rs12286929	NA	NA	0.0217	G	0	NA	NA	0.0217	G	0
rs13107325	NA	NA	0.0477	T	0	NA	NA	0.0477	T	0
rs11165643	NA	NA	0.0218	T	0	NA	NA	0.0218	T	0
rs7903146	NA	NA	0.0234	C	1	NA	NA	0.0234	C	1
rs10132280	NA	NA	0.0230	C	1	NA	NA	0.0230	C	1
rs17405819	NA	NA	0.0224	T	1	NA	NA	0.0224	T	1
rs6091540	NA	NA	0.0188	C	1	NA	NA	0.0188	C	1
rs1016287	NA	NA	0.0229	T	1	NA	NA	0.0229	T	1
rs4256980	NA	NA	0.0209	G	0	NA	NA	0.0209	G	0
rs17094222	NA	NA	0.0249	C	0	NA	NA	0.0249	C	0
rs12401738	NA	NA	0.0211	A	0	NA	NA	0.0211	A	0
rs7599312	NA	NA	0.0220	G	1	NA	NA	0.0220	G	1
rs2365389	NA	NA	0.0200	C	1	NA	NA	0.0200	C	1
rs205262	NA	NA	0.0221	G	0	NA	NA	0.0221	G	0
rs2820292	NA	NA	0.0195	C	0	NA	NA	0.0195	C	0
rs12885454	NA	NA	0.0207	C	1	NA	NA	0.0207	C	1
rs9641123	NA	NA	0.0191	C	0	NA	NA	0.0191	C	0
rs16851483	NA	NA	0.0483	T	0	NA	NA	0.0483	T	0

rs1167827	NA	NA	0.0202	G	0	NA	NA	0.0202	G	0
rs758747	NA	NA	0.0225	T	0	NA	NA	0.0225	T	0
rs1928295	NA	NA	0.0188	T	1	NA	NA	0.0188	T	1
rs9925964	NA	NA	0.0192	A	1	NA	NA	0.0192	A	1
rs11126666	NA	NA	0.0207	A	0	NA	NA	0.0207	A	0
rs2650492	NA	NA	0.0207	A	0	NA	NA	0.0207	A	0
rs6804842	NA	NA	0.0185	G	0	NA	NA	0.0185	G	0
rs12940622	NA	NA	0.0182	G	1	NA	NA	0.0182	G	1
rs7164727	NA	NA	0.0180	T	0	NA	NA	0.0180	T	0
rs11847697	NA	NA	0.0492	T	0	NA	NA	0.0492	T	0
rs4740619	NA	NA	0.0179	T	1	NA	NA	0.0179	T	1
rs492400	NA	NA	0.0158	C	1	NA	NA	0.0158	C	1
rs13191362	NA	NA	0.0277	A	1	NA	NA	0.0277	A	1
rs3736485	NA	NA	0.0176	A	1	NA	NA	0.0176	A	1
rs17001654	NA	NA	0.0306	G	0	NA	NA	0.0306	G	0
rs11191560	NA	NA	0.0308	C	0	NA	NA	0.0308	C	0
rs2080454	NA	NA	0.0168	C	1	NA	NA	0.0168	C	1
rs7715256	NA	NA	0.0163	G	1	NA	NA	0.0163	G	1
rs2176040	NA	NA	0.0141	A	1	NA	NA	0.0141	A	1
rs1528435	NA	NA	0.0178	T	0	NA	NA	0.0178	T	0
rs2075650	NA	NA	0.0258	A	1	NA	NA	0.0258	A	1
rs1000940	NA	NA	0.0192	G	0	NA	NA	0.0192	G	0
rs2033529	NA	NA	0.0190	G	0	NA	NA	0.0190	G	0
rs11583200	NA	NA	0.0177	C	1	NA	NA	0.0177	C	1
rs7239883	NA	NA	0.0164	G	1	NA	NA	0.0164	G	1
rs2836754	NA	NA	0.0164	C	0	NA	NA	0.0164	C	0
rs9400239	NA	NA	0.0188	C	0	NA	NA	0.0188	C	0
rs10733682	NA	NA	0.0174	A	1	NA	NA	0.0174	A	1
rs11688816	NA	NA	0.0172	G	1	NA	NA	0.0172	G	1
rs11057405	NA	NA	0.0307	G	1	NA	NA	0.0307	G	1
rs9914578	NA	NA	0.0201	G	0	NA	NA	0.0201	G	0
rs977747	NA	NA	0.0167	T	1	NA	NA	0.0167	T	1
rs2121279	NA	NA	0.0245	T	0	NA	NA	0.0245	T	0
rs29941	NA	NA	0.0182	G	0	NA	NA	0.0182	G	0
rs11727676	NA	NA	0.0358	T	1	NA	NA	0.0358	T	1
rs3849570	NA	NA	0.0188	A	0	NA	NA	0.0188	A	0
rs9374842	NA	NA	0.0187	T	0	NA	NA	0.0187	T	0
rs6477694	NA	NA	0.0174	C	1	NA	NA	0.0174	C	1
rs4787491	NA	NA	0.0159	G	0	NA	NA	0.0159	G	0
rs1441264	NA	NA	0.0175	A	0	NA	NA	0.0175	A	0
rs7899106	NA	NA	0.0395	G	0	NA	NA	0.0395	G	0
rs2176598	NA	NA	0.0198	T	1	NA	NA	0.0198	T	1
rs2245368	NA	NA	0.0317	C	1	NA	NA	0.0317	C	1
rs17203016	NA	NA	0.0210	G	0	NA	NA	0.0210	G	0
rs7243357	NA	NA	0.0217	T	1	NA	NA	0.0217	T	1
rs16907751	NA	NA	0.0350	C	1	NA	NA	0.0350	C	1
rs1808579	NA	NA	0.0167	C	1	NA	NA	0.0167	C	1
rs13201877	NA	NA	0.0233	G	0	NA	NA	0.0233	G	0

rs2033732	NA	NA	0.0192	C	0	NA	NA	0.0192	C	0
rs9540493	NA	NA	0.0172	A	1	NA	NA	0.0172	A	1
rs1460676	NA	NA	0.0197	C	0	NA	NA	0.0197	C	0
rs6465468	NA	NA	0.0166	T	0	NA	NA	0.0166	T	0
rs12016871	rs76790205	0.9503	0.0298	T	0					
rs2287019						NA	NA	0.0360	C	1
rs17724992						NA	NA	0.0194	A	1

Table S2. Associations between unfavorable sleep behaviors changes from adolescence to young adulthood and young adult overweight/obesity stratified by sex

Outcome	Exposure		n(%)	OR (95% CI)	*P*-value
	Adolescence	Young adulthood			
Males(n=864)					
Overweight/ obesity	**Insufficient sleep**				
	No	No	100(16.2)	1.00	
	Yes	No	17(19.3)	1.44 (0.73-2.83)	0.287
	No	Yes	43(31.9)	2.62 (1.58-4.35)	<0.001
	Yes	Yes	0(0.0)	Inf	0.983
	Sleep difficulties				
	No	No	119(18.2)	1.00	
	Yes	No	12(19.0)	1.26 (0.58-2.75)	0.553
	No	Yes	23(20.5)	1.10 (0.60-2.04)	0.752
	Yes	Yes	6(16.7)	1.71 (0.63-4.66)	0.295
Females(n=1114)					
Overweight/ obesity	**Insufficient sleep**				
	No	No	101(13.4)	1.00	
	Yes	No	25(14.6)	1.13 (0.62-2.05)	0.683
	No	Yes	22(16.7)	1.15 (0.63-2.09)	0.659
	Yes	Yes	17(30.4)	1.51 (0.62-3.66)	0.359
	Sleep difficulties				
	No	No	97(12.8)	1.00	
	Yes	No	15(15.2)	0.99 (0.47-2.11)	0.984
	No	Yes	34(18.8)	1.14 (0.65-1.99)	0.647
	Yes	Yes	19(25.3)	2.29 (1.09-4.81)	0.029

Adjusted for age, study, study center, parental education, puberty (two levels in adolescence; pre-/early/mid-pubertal vs. late/post-pubertal for males; pre-/early/mid-/late pubertal vs. postpubertal for females), physical activity, screen time, traffic noise, and education/occupation types in young adulthood, as well as overweight/obesity (in adolescence);
n (%): number of cases (prevalence). OR: odds ratio; 95%CI: 95% confidence interval. Inf: no case in this group. The insufficient sleep changes and sleep difficulties changes were mutually adjusted for in the same models.

Table S3. Associations between unfavorable sleep behaviors changes from adolescence to young adulthood and young adult overweight/obesity after exclusion of those who were overweight/obese in adolescence (N=1753)

Outcome	Exposure		n(%)	OR (95% CI)	*P*-value
	Adolescence	Young adulthood			
Overweight /obesity	**Insufficient sleep**				
	No	No	107(8.7)	1.00	
	Yes	No	26(10.9)	1.24 (0.77-2.00)	0.376
	No	Yes	35(15.4)	1.81 (1.19-2.78)	0.006
	Yes	Yes	4(6.2)	0.64 (0.22-1.84)	0.405
	Sleep difficulties				
	No	No	122(9.7)	1.00	
	Yes	No	15(10.5)	1.16 (0.64-2.09)	0.628
	No	Yes	19(7.6)	0.77 (0.46-1.29)	0.316
	Yes	Yes	16(16.0)	2.04 (1.11-3.73)	0.021

Adjusted for age, sex, study, study center, parental education, puberty (in adolescence), physical activity, screen time, traffic noise, and education/occupation types in young adulthood;
n (%): number of cases (prevalence). OR: odds ratio; 95%CI: 95% confidence interval. The insufficient sleep changes and sleep difficulties changes were mutually adjusted for in the same models.

Table S4. Associations between overweight/obesity status changes from adolescence to young adulthood and unfavorable sleep behaviors in young adulthood stratified by sex

Outcome	Exposure		n(%)	OR (95% CI)	*P*-value
	Adolescence	Young adulthood			
Male(n=864)					
Insufficient sleep	**Overweight/obesity**				
	No	No	108(16.5)	1.00	
	Yes	No	8(16.3)	1.05 (0.47-2.37)	0.900
	No	Yes	22(27.5)	2.06 (1.17-3.60)	0.012
	Yes	Yes	21(26.2)	1.73 (0.98-3.08)	0.061
Sleep difficulties	**Overweight/obesity**				
	No	No	114(17.4)	1.00	
	Yes	No	5(10.2)	0.54 (0.20-1.48)	0.229
	No	Yes	14(17.5)	0.90 (0.46-1.76)	0.765
	Yes	Yes	15(18.8)	1.24 (0.65-2.39)	0.514
Female(n=1114)					
Insufficient sleep	**Overweight/obesity**				
	No	No	145(15.7)	1.00	
	Yes	No	4(17.4)	1.31 (0.42-4.07)	0.644
	No	Yes	17(18.5)	1.11 (0.62-2.00)	0.729
	Yes	Yes	22(30.1)	1.78 (0.99-3.20)	0.056
Sleep difficulties	**Overweight/obesity**				
	No	No	200(21.6)	1.00	
	Yes	No	3(13.0)	0.44 (0.12-1.60)	0.214
	No	Yes	21(22.8)	1.00 (0.58-1.74)	0.992
	Yes	Yes	32(43.8)	2.47 (1.42-4.27)	0.001

Adjusted for age, study, study center, parental education, puberty (two levels in adolescence; pre-/early/mid-pubertal vs. late/post-pubertal for males; pre-/early/mid-/late pubertal vs. postpubertal for females), physical activity, screen time, traffic noise, education/occupation types, sleep difficulties (for insufficient sleep) or insufficient sleep (for sleep difficulties) in young adulthood, as well as insufficient sleep and sleep difficulties (in adolescence);

n (%): number of cases (prevalence). OR: odds ratio; 95%CI: 95% confidence interval.

Table S5. Associations between overweight/obesity status changes from adolescence to young adulthood and unfavorable sleep behaviors in young adulthood after exclusion of those who had insufficient sleep or sleep difficulties in adolescence (N=1452)

Outcome	Exposure		n(%)	OR (95% CI)	*P-value*
	Adolescence	Young adulthood			
Insufficient sleep	**Overweight/obesity**				
	No	No	166(14.3)	1.00	
	Yes	No	9(15.8)	1.12 (0.53-2.36)	0.772
	No	Yes	30(24.4)	1.87 (1.18-2.96)	0.007
	Yes	Yes	28(25.0)	1.73 (1.07-2.81)	0.026
Sleep difficulties	**Overweight/obesity**				
	No	No	188(16.2)	1.00	
	Yes	No	5(8.8)	0.60 (0.23-1.54)	0.287
	No	Yes	16(13.0)	0.78 (0.44-1.37)	0.383
	Yes	Yes	25(22.3)	1.59 (0.96-2.64)	0.071

Adjusted for age, sex, study, study center, parental education, puberty (in adolescence), physical activity, screen time, traffic noise, education/occupation types, and sleep difficulties (for insufficient sleep) or insufficient (for sleep difficulties) in young adulthood;
n (%): number of cases (prevalence). OR: odds ratio; 95%CI: 95% confidence interval.

Table S6. Associations between sleep behaviors and overweight/obesity, with insufficient sleep defined only on school/working days

Outcome	Exposure		n(%)	OR (95% CI)	*P-value*
	Adolescence	Young adulthood			
Overweight/ obesity	**Insufficient sleep**				
	No	No	192(14.7)	1.00	
	Yes	No	34(14.7)	1.16 (0.73-1.85)	0.528
	No	Yes	74(22.3)	1.62 (1.13-2.30)	0.008
	Yes	Yes	25(23.1)	1.10 (0.58-2.06)	0.777
	Sleep difficulties				
	No	No	216(15.3)	1.00	
	Yes	No	27(16.7)	1.11 (0.66-1.89)	0.685
	No	Yes	57(19.5)	1.15 (0.77-1.71)	0.506
	Yes	Yes	25(22.5)	2.08 (1.18-3.65)	0.011
Adjusted for age, sex, study, study center, parental education, puberty (in adolescence), physical activity, screen time, traffic noise, education/occupation types, and overweight/obesity in young adulthood. The insufficient sleep changes and sleep difficulties changes were mutually adjusted for in the same models.					
Insufficient sleep	**Overweight/obesity**				
	No	No	328(20.7)	1.00	
	Yes	No	13(18.1)	0.83 (0.44-1.58)	0.574
	No	Yes	47(27.3)	1.32 (0.91-1.91)	0.150
	Yes	Yes	52(34.0)	1.61 (1.10-2.36)	0.014
Sleep difficulties	**Overweight/obesity**				
	No	No	314(19.9)	1.00	
	Yes	No	8(11.1)	0.56 (0.26-1.23)	0.147
	No	Yes	35(20.3)	1.00 (0.66-1.51)	0.985
	Yes	Yes	47(30.7)	1.79 (1.19-2.68)	0.005
Adjusted for age, sex, study, study center, parental education, puberty (in adolescence), physical activity, screen time, traffic noise, education/occupation types, and sleep difficulties (for insufficient sleep) or insufficient sleep (for sleep difficulties) in young adulthood, as well as insufficient sleep and sleep difficulties in adolescence.					
n (%): number of cases (prevalence). OR: odds ratio; 95%CI: 95% confidence interval.					

Publication II

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Accelerometry-assessed sleep clusters and cardiometabolic risk factors in adolescents

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Abstract

Objective: This study aimed to identify sleep clusters based on objective multidimensional sleep characteristics and test their associations with adolescent cardiometabolic health.

Methods: The authors included 1090 participants aged 14.3 to 16.4 years (mean = 15.2 years) who wore 7-day accelerometers during the 15-year follow-up of the German Infant Study on the influence of Nutrition Intervention PLUS environmental and genetic influences on allergy development (GINIplus) and the Influence of Lifestyle factors on the development of the Immune System and Allergies in East and West Germany (LISA) birth cohorts. K-means cluster analysis was performed across 12 sleep characteristics reflecting sleep quantity, quality, schedule, variability, and regularity. Cardiometabolic risk factors included fat mass index (FMI), blood pressure, triglycerides, high-density lipoprotein cholesterol, high-sensitivity C-reactive protein, and insulin resistance ($n = 505$). Linear and logistic regression models were examined.

Results: Five sleep clusters were identified: good sleep ($n = 337$); delayed sleep phase ($n = 244$); sleep irregularity and variability ($n = 108$); fragmented sleep ($n = 313$); and

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prolonged sleep latency ($n = 88$). The "prolonged sleep latency" cluster was associated with increased sex-scaled FMI ($\beta = 0.39$, 95% CI: 0.15–0.62) compared with the "good sleep" cluster. The "sleep irregularity and variability" cluster was associated with increased odds of high triglycerides only in male individuals (odds ratio: 9.50, 95% CI: 3.22–28.07), but this finding was not confirmed in linear models.

Conclusions: The prolonged sleep latency cluster was associated with higher FMI in adolescents, whereas the sleep irregularity and variability cluster was specifically linked to elevated triglycerides (≥1.7 mmol/L) in male individuals.

INTRODUCTION

Cardiometabolic risk factors may appear as early as childhood and track into adulthood, increasing cardiovascular disease risk [1]. Accumulating evidence has linked short sleep to increased cardiometabolic risk in children and adolescents [2, 3]. Recently, the American Heart Association added sleep duration as the eighth metric to cardiovascular health's definition (Life's Essential 8) [4]. Besides sleep duration, other sleep characteristics, including sleep efficiency, timing, variability, regularity, and wake time, have also been associated with adolescent cardiometabolic health [5–8]. These sleep characteristics within an individual were mainly assessed independently; however, they tend to be correlated with each other [9].

Cluster analysis provides the ability to consider multidimensional sleep characteristics from a holistic perspective [9]. To date, only one study has applied this approach to identify sleep patterns and explored their relationships with cardiometabolic health in children and adults using accelerometry-measured sleep data [10]. Four patterns were identified, and the "overall good sleepers" pattern was associated with more favorable body mass index (BMI) and metabolic syndrome severity score. However, associations among sleep patterns and other cardiometabolic risk factors such as fat mass index (FMI), high-sensitivity C-reactive protein (hs-CRP), or insulin resistance have not been investigated [2, 3]. For instance, several studies have linked short sleep to higher CRP in children and adolescents [2]. Additionally, earlier objective sleep midpoint timing was associated with increased 1-year fat mass in youth [5]. However, adolescents with late objective sleep midpoint timing had increased odds of developing insulin resistance within 2 years [6].

Therefore, we applied cluster analysis to identify sleep clusters across 12 accelerometry-assessed sleep characteristics in 1090 adolescents and investigated their associations with cardiometabolic risk factors, including FMI, blood pressure (BP), lipids, hs-CRP, and Homeostatic Model Assessment of Insulin Resistance (HOMA-IR).

Study Importance

What is already known?

- Multiple objective sleep characteristics, including sleep duration, efficiency, timing, variability, regularity, and wake time, are associated with cardiometabolic risk in children and adolescents.
- These sleep characteristics within an individual are often assessed independently, although they tend to be correlated with each other.

What does this study add?

- Five sleep clusters were identified in adolescents by cluster analysis across 12 accelerometry-derived sleep characteristics, i.e., "good sleep," "delayed sleep phase," "sleep irregularity and variability," "fragmented sleep," and "prolonged sleep latency."
- The prolonged sleep latency cluster was associated with increased fat mass index, and male individuals within the sleep irregularity and variability cluster had higher odds of having high triglycerides.

How might these results change the direction of research or the focus of clinical practice?

- Considering the relationships among multidimensional sleep characteristics and health from a holistic perspective deserves further investigation.
- Our results suggest that improvements in sleep latency, variability, and regularity may enrich existing sleep-targeted intervention strategies for cardiometabolic health that mainly focus on improving adequate sleep.

METHODS

Study participants

We used data from the 15-year follow-up of two ongoing German birth cohorts, the German Infant Study on the influence of Nutrition Intervention PLUS environmental and genetic influences on allergy development (GINIplus) and the Influence of Lifestyle factors on the development of the Immune System and Allergies in East and West Germany (LISA). More details of both studies have been published [11]. Briefly, the GINIplus study recruited 5991 healthy

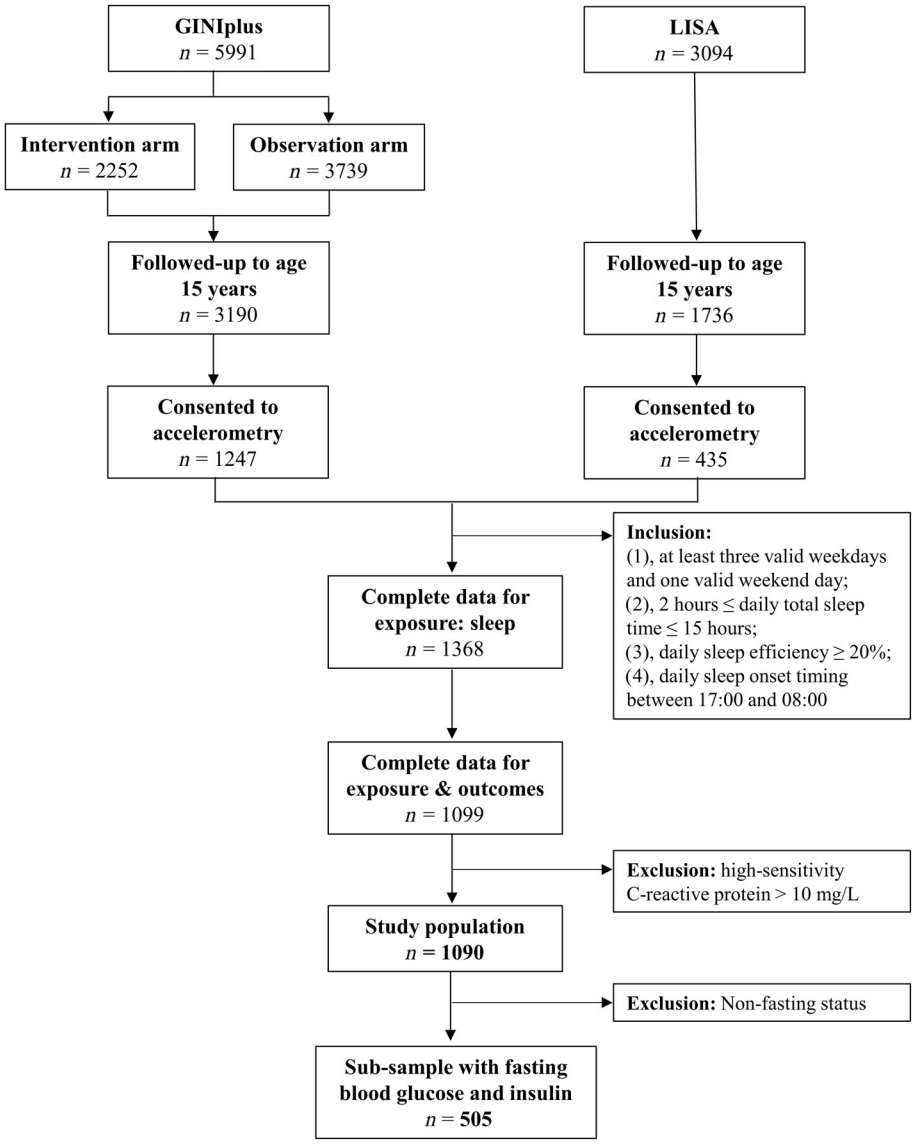

FIGURE 1 Flowchart of participants. GINIplus, German Infant Study on the influence of Nutrition Intervention PLUS environmental and genetic influences on allergy development; LISA, Influence of Lifestyle factors on the development of the Immune System and Allergies in East and West Germany.

newborns in Munich and Wesel from 1995 to 1998, comprising an intervention arm, which aimed to investigate the hydrolyzed formulae effects for allergy prevention in infants with a family history of allergy, and an observation arm, including newborns without a family history of allergy and those whose parents declined to participate in the intervention. The LISA study recruited 3094 healthy neonates in Munich, Wesel, Leipzig, and Bad Honnef between 1997 and 1999. At the 15-year follow-up between May 2011 and July 2014, a subset of participants (1247 in GINIplus and 435 in LISA) consented to wear accelerometers to measure sleep and physical activity (PA) in Munich and Wesel. Finally, a total of 1090 participants with valid accelerometry-measured sleep data and complete information on cardiometabolic outcomes (except for HOMA-IR in a subsample, $n = 505$) were included in the analyses. Participants were included only with at least 3 weekdays and 1 weekend day of valid accelerometry recording for ≥10 h/day [12]. More details are described in Figure 1. Both studies were approved by the respective local ethics committees, and written consents were provided by all participants and their families.

Sleep assessment and characteristics

Accelerometry

Nighttime sleep and daytime PA were measured by a triaxial accelerometer (ActiGraph GT3X, Pensacola, Florida) during a regular school week, the validity of which has been demonstrated in adolescents [13]. Participants wore accelerometers for 7 consecutive days and nights, with

accelerometers worn on the nondominant wrists at night, and kept sleep diaries. Accelerometry protocol details have been provided elsewhere [12].

Sleep characteristics

Accelerometry-measured sleep data were analyzed with the ActiLife software (version 5.5.5, firmware 4.4.0) using the Sadeh algorithm [14]. The sampling rate was set to 30 Hz, and measured accelerations were stored at 1 Hz after conversion into proprietary "activity counts," which were summed over 60-s epochs. The "probability of sleep" was computed as a score centered around zero for each minute participants indicated as time-in-bed in their diary (time between going to bed and getting up). The minute was identified as "asleep" if the score was equal to or greater than zero, and the minute was identified as "awake" if the score was less than zero [14]. The following six sleep characteristics were derived for each valid night:

1. Total sleep time (hours): the total number of minutes scored as asleep by the algorithm, divided by 60;
2. Sleep efficiency (percentage): the ratio of algorithm-scored asleep minutes to the total diary-recorded minutes in bed;
3. Sleep midpoint timing (24-h clock): the first minute algorithm-scored as asleep, adding half of the total sleep time, then converted to 24-h clock;
4. Sleep latency (minutes): the total number of minutes between diary-recorded time of going to bed and the first minute algorithm-scored as asleep;
5. Time awake per hour after sleep onset (minutes per hour): the total number of algorithm-scored awake minutes after sleep onset (WASO), divided by the hours in bed after sleep onset (total sleep time + WASO/60);
6. Awakenings per hour after sleep onset: the number of algorithm-scored different awakening episodes after sleep onset, divided by the hours in bed after sleep onset.

For each of six daily sleep characteristics, the daily average was calculated as mean value across all valid days, and the day-to-day variability was calculated as standard deviation (SD) across all valid days. In total, 12 sleep characteristics reflecting sleep quantity (total sleep time), quality (sleep efficiency), schedule (sleep midpoint timing, sleep latency, time awake per hour after sleep onset, and awakenings per hour after sleep onset), variability (SD in total sleep time, SD in sleep efficiency, SD in sleep latency, SD in time awake per hour after sleep onset, and SD in awakenings per hour after sleep onset), and regularity (SD in sleep midpoint timing) were used in subsequent cluster analysis.

Cardiometabolic risk factors

Participants' body weight (kilograms), height (meters), systolic BP (SBP, millimeters of mercury), and diastolic BP (DBP, millimeters of mercury) were measured. Fat-free mass (kilograms) was assessed by means of phase sensitive bioelectrical impedance (NutriBox, Data Input GmbH, Pöcking, Germany), and fat mass (kilograms) was calculated by subtracting fat-free mass from body weight. FMI was calculated as fat mass (kilograms) per height squared (meters squared). Serum total cholesterol (TC, millimoles per liter), triglycerides (TG, millimoles per liter), high-density lipoprotein cholesterol (HDL, millimoles per liter), low-density lipoprotein cholesterol (LDL, millimoles per liter), and hs-CRP (milligrams per liter) were measured. Fasting glucose (millimoles per liter) and fasting insulin (picomoles per liter) were measured, and HOMA-IR was calculated as follows: (glucose $\times$ insulin)/(22.5 $\times$ 6.945) [15]. Details on the measurements are provided in online Supporting Information Methods.

Cardiometabolic risk factors were dichotomized based on established cutoffs or sex-specific percentiles. According to three components of metabolic syndrome definitions in children and adolescents by the International Diabetes Federation (IDF) [16], high BP was defined as SBP ≥ 130 mm Hg or DBP ≥ 85 mm Hg; high TG was defined as TG ≥ 1.7 mmol/L; and low HDL was defined as HDL < 1.03 mmol/L at ages 10 to 16 years and, at ages ≥16 years, <1.03 mmol/L in male individuals and <1.29 mmol/L in female individuals. High hs-CRP was defined as hs-CRP ≥ 75% sex-specific percentile of the current population with hs-CRP ≥ 0.2 mg/L (0.91 mg/L in male and 0.87 mg/L in female individuals) [17]. High FMI was defined as FMI ≥ 75% sex-specific percentile (5.01 kg/m^2 in male and 6.68 kg/m^2 in female individuals), and high HOMA-IR was defined as HOMA-IR ≥ 75% sex-specific percentile (2.59 in male and 2.74 in female individuals).

Confounders

Sex, age at blood sampling, study (GINIplus observation arm, GINIplus intervention arm, and LISA study), study center (Munich and Wesel), season of sleep measurement (spring, summer, autumn, and winter), parental highest education (low/medium: ≤10th grade; high: >10th grade), and fasting status at blood sampling (yes, no) were collected by questionnaires. Pubertal stage was categorized into two groups: pre-, early, or midpubertal and late or postpubertal stage based on a self-rated questionnaire [18]. Accelerometry-measured PA was classified into sedentary, light, moderate, and vigorous PA according to published triaxial cutoffs by Aguilar-Farías [19] (for sedentary) and Romanzini [20], and then moderate and vigorous PA were merged into moderate-to-vigorous PA (MVPA) [12]. Average sedentary (hours) and MVPA (minutes) across all valid days were included. Depressive symptoms were evaluated by the Depression Screener for Teenagers and defined as a score ≥ 12 [21]. Dietary information was assessed by a self-administered food frequency questionnaire [22]. Total energy intake (EI, kilocalories) was calculated [23], and carbohydrate intake was expressed as its percentage in total EI (%EI).

Statistical analysis

All statistical analyses were performed in R (version 4.1.2, R Center for Statistical Computing, Vienna, Austria). A total of 12 sleep

TABLE 1 Participant characteristics in total population and stratified by sex

	Total	Male	Female	*p* value
n	1090	489	601	
Age (y)	15.2 ± 0.3	15.2 ± 0.3	15.2 ± 0.3	0.230
Weight (kg)	61.2 ± 11.0	64.1 ± 11.7	58.8 ± 9.9	**<0.001**
Height (cm)	171.4 ± 8.0	176.2 ± 7.4	167.5 ± 6.1	**<0.001**
FMI (kg/m^2)	5.1 ± 2.1	4.1 ± 1.9	5.9 ± 1.9	**<0.001**
High FMI, *n* (%)	272 (25.0)	122 (24.9)	150 (25.0)	1.000
SBP (mm Hg)	118.5 ± 11.7	121.2 ± 12.3	116.3 ± 10.8	**<0.001**
DBP (mm Hg)	69.4 ± 8.9	68.7 ± 8.9	70.1 ± 9.0	**0.010**
High BP, *n* (%)	206 (18.9)	124 (25.4)	82 (13.6)	**<0.001**
TC (mmol/L)	4.3 (3.8, 4.8)	4.1 (3.6, 4.6)	4.4 (3.9, 5.0)	**<0.001**
TG (mmol/L)	1.0 (0.7, 1.3)	1.0 (0.7, 1.4)	1.0 (0.7, 1.3)	0.561
High TG, *n* (%)	130 (11.9)	70 (14.3)	60 (10.0)	**0.036**
HDL (mmol/L)	1.5 ± 0.4	1.4 ± 0.4	1.6 ± 0.4	**<0.001**
Low HDL, *n* (%)	84 (7.7)	56 (11.5)	28 (4.7)	**<0.001**
LDL (mmol/L)	2.3 (1.9, 2.7)	2.2 (1.8, 2.6)	2.4 (2.0, 2.8)	**<0.001**
hs-CRP (mg/L)	0.4 (0.2, 0.7)	0.4 (0.2, 0.7)	0.4 (0.2, 0.7)	0.305
High hs-CRP, *n* (%)	218 (20.0)	96 (19.6)	122 (20.3)	0.843
HOMA-IR	2.1 (1.5, 2.6)	2.0 (1.4, 2.6)	2.1 (1.6, 2.7)	0.095
High HOMA-IR, *n* (%)	128 (25.3)	61 (25.4)	67 (25.3)	1.000
Total EI (kcal/day)	2093.9 ± 645.2	2406.4 ± 643.4	1868.0 ± 544.9	**<0.001**
Carbohydrate intake (%EI)	53.0 ± 7.3	52.5 ± 7.2	53.4 ± 7.4	0.095
Sedentary (h)	8.2 ± 1.4	8.0 ± 1.5	8.4 ± 1.3	**<0.001**
MVPA (min)	50.4 ± 26.5	57.3 ± 25.4	44.7 ± 26.2	**<0.001**
Depression, *n* (%)	141 (13.9)	48 (10.6)	93 (16.5)	**0.010**
Fasting status (yes), *n* (%)	511 (46.9)	241 (49.3)	270 (44.9)	0.170
Sleep clusters, *n* (%)				**<0.001**
Good sleep	337 (30.9)	109 (22.3)	228 (37.9)	
Delayed sleep phase	244 (22.4)	103 (21.1)	141 (23.5)	
Sleep irregularity and variability	108 (9.9)	43 (8.8)	65 (10.8)	
Fragmented sleep	313 (28.7)	193 (39.5)	120 (20.0)	
Prolonged sleep latency	88 (8.1)	41 (8.4)	47 (7.8)	
Study, *n* (%)				0.278
GINIplus observation	414 (38.0)	179 (36.6)	235 (39.1)	
GINIplus intervention	437 (40.1)	192 (39.3)	245 (40.8)	
LISA	239 (21.9)	118 (24.1)	121 (20.1)	
Study center, *n* (%)				0.136
Munich	625 (57.3)	293 (59.9)	332 (55.2)	
Wesel	465 (42.7)	196 (40.1)	269 (44.8)	
Season, *n* (%)				0.357
Spring	281 (25.8)	133 (27.2)	148 (24.6)	
Summer	167 (15.3)	65 (13.3)	102 (17.0)	
Autumn	353 (32.4)	158 (32.3)	195 (32.4)	
Winter	289 (26.5)	133 (27.2)	156 (26.0)	
Parental highest education, *n* (%)				0.824
Low/medium	337 (30.9)	149 (30.5)	188 (31.3)	
High	753 (69.1)	340 (69.5)	413 (68.7)	

TABLE 1 (Continued)

	Total	Male	Female	*p* value
Pubertal stage, *n* (%)				**<0.001**
Pre-, early, or midpubertal	202 (21.7)	178 (43.8)	24 (4.6)	
Late or postpubertal	729 (78.3)	228 (56.2)	501 (95.4)	

Note: The results are presented as mean ± SD, median (first quartile, third quartile), or *n* (percentage). The number of participants with available information was as follows: HOMA-IR (505); total EI (865); carbohydrate intake (865); sedentary (1082); MVPA (1082); depression (1017); and pubertal stage (931). $P < 0.05$ are highlighted in bold.

Abbreviations: BP, blood pressure; carbohydrate intake (%EI), carbohydrate as percentage of total energy intake; DBP, diastolic blood pressure; EI, energy intake; FMI, fat mass index; GINIplus, German Infant Study on the influence of Nutrition Intervention PLUS environmental and genetic influences on allergy development; HDL, high-density lipoprotein cholesterol; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; hs-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein cholesterol; LISA, Influence of Lifestyle factors on the development of the Immune System and Allergies in East and West Germany; MVPA, moderate-to-vigorous physical activity; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides.

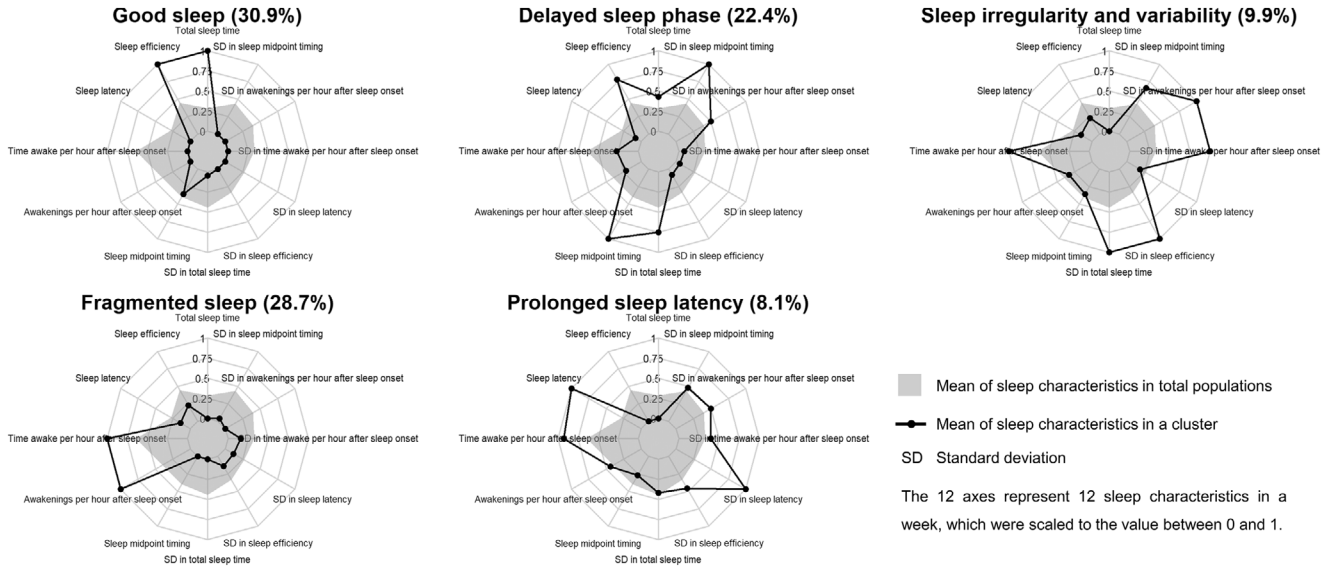

FIGURE 2 Distributions of sleep characteristics in a week in five sleep clusters.

characteristics were standardized, and their Spearman correlation was examined (Figure S1). Hierarchical cluster analysis was conducted using Euclidean distance and Ward's linkage (Ward.D2). K-means cluster analysis was applied testing three, four, five, and six clusters. The final number of sleep clusters was set to five, considering the combination of the following methods: 1) interpretability of k-means results; 2) hierarchical cluster dendrogram (Figure S2); 3) results of principal component analysis (Table S1; 5 components account for 80% cumulative percentage of variance); and 4) sum of squares method (Figure S3, by minimizing the within-cluster sum of squares and maximizing the between-cluster sum of squares). More details are provided in online Supporting Information Methods. In final k-means cluster analysis, the number of clusters was specified as five, with 50 random initial centroids. One-way ANOVA and Kruskal–Wallis rank sum test for continuous variables and χ^2 test for categorical variables were used to explore differences among sexes and sleep clusters, followed by Bonferroni-adjusted post hoc tests.

Linear regression models were conducted to evaluate associations among sleep clusters and continuous cardiometabolic markers, which were examined for normality and naturally log-transformed as appropriate. Outliers were detected visually using box plots (median ± 3 interquartile range, outliers were not excluded). Three models were performed: Model 1 was adjusted for sex, age, study, study center, and parental highest education; Model 2 was additionally adjusted for season, pubertal stage, sedentary, MVPA, depression, fasting status (except for HOMA-IR), total EI, and carbohydrate intake; and Model 3 was Model 2 plus adjustment for FMI. For comparability, FMI (sex-specific), SBP, DBP, and HDL (inverse) were scaled, and results were described as β with 95% confidence intervals (CI). TC, TG, LDL, and HOMA-IR were log-transformed, and the β estimate of linear models were then back-transformed to means ratio (MR = exp[β]) with 95% CI. MR represents the ratio of the mean of the outcome variable in one group versus the reference group. Considering the correlation among outcomes, the number of independent tests was calculated as seven according to Nyholt [24] using the R package "poolr" [25], yielding a Bonferroni-

TABLE 2 Associations among sleep clusters and continuous cardiometabolic risk factors in adolescents

Total (1090)	Good sleep (337)	Delayed sleep phase (244)		Sleep irregularity and variability (108)		Fragmented sleep (313)		Prolonged sleep latency (88)	
Outcomes	**β (95% CI)**	**β (95% CI)**	***p***	**β (95% CI)**	***p***	**β (95% CI)**	***p***	**β (95% CI)**	***p***
FMI sex-scaled									
Model 1	Ref.	0.06 (−0.11 to 0.22)	0.500	0.14 (−0.07 to 0.36)	0.195	0.11 (−0.05 to 0.27)	0.167	0.28 (0.04 to 0.51)	0.020
Model 2	Ref.	0.01 (−0.15 to 0.17)	0.888	0.16 (−0.05 to 0.37)	0.139	0.16 (0.00 to 0.31)	0.050	0.39 (0.15 to 0.62)	**0.001**
SBP scaled									
Model 1	Ref.	−0.01 (−0.17 to 0.15)	0.885	0.04 (−0.17 to 0.25)	0.727	0.00 (−0.16 to 0.15)	0.960	0.11 (−0.13 to 0.34)	0.372
Model 2	Ref.	0.01 (−0.15 to 0.16)	0.950	0.03 (−0.18 to 0.24)	0.779	0.02 (−0.14 to 0.17)	0.843	0.12 (−0.12 to 0.35)	0.327
Model 3	Ref	0.00 (−0.15 to 0.16)	0.974	0.00 (−0.21 to 0.20)	0.965	−0.02 (−0.17 to 0.13)	0.814	0.03 (−0.19 to 0.26)	0.781
DBP scaled									
Model 1	Ref.	0.04 (−0.12 to 0.21)	0.605	0.19 (−0.03 to 0.40)	0.087	−0.04 (−0.20 to 0.12)	0.616	0.00 (−0.24 to 0.23)	0.987
Model 2	Ref.	0.06 (−0.11 to 0.22)	0.500	0.21 (−0.01 to 0.42)	0.058	0.00 (−0.16 to 0.15)	0.971	−0.01 (−0.25 to 0.22)	0.905
Model 3	Ref.	0.05 (−0.11 to 0.22)	0.509	0.18 (−0.03 to 0.40)	0.093	−0.03 (−0.18 to 0.13)	0.729	−0.08 (−0.31 to 0.16)	0.528
HDL inversely scaled									
Model 1	Ref.	−0.05 (−0.21 to 0.11)	0.561	−0.16 (−0.37 to 0.06)	0.147	0.08 (−0.08 to 0.23)	0.336	0.01 (−0.22 to 0.24)	0.913
Model 2	Ref.	−0.05 (−0.21 to 0.11)	0.549	−0.16 (−0.37 to 0.06)	0.150	0.10 (−0.06 to 0.26)	0.205	0.04 (−0.20 to 0.27)	0.763
Model 3	Ref.	−0.05 (−0.21 to 0.11)	0.523	−0.19 (−0.40 to 0.02)	0.080	0.07 (−0.08 to 0.22)	0.366	−0.04 (−0.27 to 0.19)	0.743
	MR (95% CI)	**MR (95% CI)**	***p***	**MR (95% CI)**	***p***	**MR (95% CI)**	***p***	**MR (95% CI)**	***p***
TC									
Model 1	Ref.	1.01 (0.98 to 1.04)	0.500	1.02 (0.98 to 1.06)	0.269	1.01 (0.98 to 1.04)	0.674	1.02 (0.97 to 1.06)	0.453
Model 2	Ref.	1.01 (0.98 to 1.04)	0.431	1.02 (0.98 to 1.07)	0.241	1.01 (0.98 to 1.04)	0.677	1.01 (0.97 to 1.06)	0.591
Model 3	Ref.	1.01 (0.98 to 1.04)	0.438	1.02 (0.98 to 1.06)	0.313	1.00 (0.97 to 1.03)	0.847	1.00 (0.96 to 1.05)	0.868
TG									
Model 1	Ref.	1.00 (0.93 to 1.08)	0.951	1.03 (0.93 to 1.13)	0.551	1.05 (0.98 to 1.13)	0.178	1.00 (0.90 to 1.11)	0.982
Model 2	Ref.	1.01 (0.94 to 1.09)	0.726	1.02 (0.93 to 1.12)	0.659	1.05 (0.98 to 1.13)	0.137	1.01 (0.92 to 1.12)	0.775
Model 3	Ref.	1.01 (0.94 to 1.08)	0.743	1.01 (0.92 to 1.10)	0.893	1.04 (0.97 to 1.11)	0.272	0.98 (0.89 to 1.08)	0.690
LDL									
Model 1	Ref.	1.01 (0.97 to 1.06)	0.595	1.02 (0.95 to 1.08)	0.616	1.03 (0.98 to 1.08)	0.253	1.04 (0.97 to 1.12)	0.271
Model 2	Ref.	1.02 (0.97 to 1.07)	0.525	1.02 (0.96 to 1.09)	0.533	1.03 (0.98 to 1.08)	0.221	1.03 (0.96 to 1.11)	0.411
Model 3	Ref.	1.02 (0.97 to 1.07)	0.535	1.01 (0.95 to 1.08)	0.691	1.02 (0.98 to 1.07)	0.354	1.01 (0.94 to 1.09)	0.749

TABLE 2 (Continued)

	MR (95% CI)	MR (95% CI)	*p*	MR (95% CI)	*p*	MR (95% CI)	*p*	MR (95% CI)	*p*
HOMA-IR[a]									
Model 1	Ref.	1.04 (0.93 to 1.17)	0.466	1.18 (1.01 to 1.39)	0.041	1.05 (0.93 to 1.17)	0.442	1.09 (0.92 to 1.29)	0.307
Model 2	Ref.	1.03 (0.92 to 1.15)	0.604	1.19 (1.01 to 1.40)	0.039	1.07 (0.95 to 1.20)	0.249	1.15 (0.97 to 1.36)	0.120
Model 3	Ref.	1.04 (0.94 to 1.16)	0.472	1.14 (0.98 to 1.33)	0.084	1.04 (0.93 to 1.15)	0.505	1.08 (0.92 to 1.27)	0.367

Note: Model 1: Adjusted for sex, age, study, study center, and parental highest education; Model 2: Model 1 + season, pubertal stage, sedentary, MVPA, depression, fasting status (except for HOMA-IR), total EI, and carbohydrate intake; and Model 3: Model 2 + FMI. $P < 0.007$ are highlighted in bold, which remained significant after Bonferroni correction using the Nyholt method.

Abbreviations: DBP, diastolic blood pressure; EI, energy intake; FMI, fat mass index; HDL, high-density lipoprotein cholesterol; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; LDL, low-density lipoprotein cholesterol; MR, means ratio; MVPA, moderate-to-vigorous physical activity; Ref., reference; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides.

[a]HOMA-IR available for 505 participants.

corrected, two-sided α level of 0.007 (0.05/7; online Supporting Information Methods). Given the highly skewed distribution of hs-CRP, it was not tested in linear models.

To explore the vulnerable/extreme subgroups of cardiometabolic outcomes, logistic regression models were used to assess associations among sleep clusters and dichotomous cardiometabolic markers (high FMI, high BP, high TG, low HDL, high hs-CRP, and high HOMA-IR), with the same adjustment levels as in linear models. Results were described as odds ratios (OR) with 95% CI. Bonferroni correction was applied with the Nyholt method [24], yielding a two-sided α level of 0.010 (0.05/5).

Additionally, the interaction effects among sleep clusters and sex were tested, followed by sex-stratified analyses. The following two sensitivity analyses were performed: 1) restricted to only fasting participants; and 2) sleep clusters were identified by sleep characteristics only on weekdays. Multiple imputation by sex was applied to some confounders with missing values (pubertal stage, sedentary, MVPA, depression, total EI, and carbohydrate intake) using the R package "mice" [26].

RESULTS

The overall prevalence of high BP, high TG, low HDL, and high hs-CRP was 18.9%, 11.9%, 7.7%, and 20.0%, respectively (Table 1). Male individuals had a higher prevalence of high BP, high TG, and low HDL than female individuals, but female individuals had higher FMI, TC, and LDL than male individuals ($p < 0.05$).

Five sleep clusters were identified and named by their characteristics: "good sleep" ($n = 337$; average total sleep time = 7.6 h); "delayed sleep phase" ($n = 244$; 7.2 h); "sleep irregularity and variability" ($n = 108$; 6.9 h); "fragmented sleep" ($n = 313$; 6.9 h); and "prolonged sleep latency" ($n = 88$; 6.9 h; Table 1, Table S2). Figure 2 displays the distributions of sleep characteristics in a week in each sleep cluster. The good sleep cluster was characterized by higher total sleep time and sleep efficiency. The delayed sleep phase cluster was characterized by higher sleep midpoint timing, SD in sleep midpoint timing, and sleep efficiency. The sleep irregularity and variability cluster exhibited higher SD in most sleep characteristics such as total sleep time, sleep midpoint timing, and higher time awake per hour after sleep onset. Furthermore, the fragmented sleep cluster had higher time awake and awakenings per hour after sleep onset, whereas the prolonged sleep latency cluster had higher sleep latency, SD in sleep latency, and time awake per hour after sleep onset. Figure S4 demonstrates the stability and robustness of the identified sleep clusters in the present study by demonstrating that the distributions of sleep characteristics only during weekdays were similar to those of the entire week (Figure 2). Table S3 shows the participant characteristics in five sleep clusters.

In linear analyses, compared with the good sleep cluster, the prolonged sleep latency cluster was significantly associated with increased sex-scaled FMI (β = 0.39, 95% CI: 0.15–0.62; Model 2, Table 2). The sleep irregularity and variability cluster was associated

TABLE 3 Associations among sleep clusters and dichotomous cardiometabolic risk factors in adolescents

Total (1090)	Good sleep (337)	Delayed sleep phase (244)		Sleep irregularity and variability (108)		Fragmented sleep (313)		Prolonged sleep latency (88)	
Outcomes	**OR (95% CI)**	**OR (95% CI)**	***p***	**OR (95% CI)**	***p***	**OR (95% CI)**	***p***	**OR (95% CI)**	***p***
High FMI									
No. of cases (%)	72 (21.4)	63 (25.8)		27 (25.0)		82 (26.2)		28 (31.8)	
Model 1	Ref.	1.22 (0.83–1.81)	0.312	1.13 (0.68–1.89)	0.644	1.32 (0.90–1.92)	0.153	1.60 (0.95–2.72)	0.080
Model 2	Ref.	1.13 (0.75–1.68)	0.565	1.22 (0.72–2.07)	0.468	1.46 (0.99–2.15)	0.059	1.98 (1.14–3.45)	0.016
High BP									
No. of cases (%)	59 (17.5)	48 (19.7)		19 (17.6)		65 (20.8)		15 (17.0)	
Model 1	Ref.	1.03 (0.67–1.59)	0.877	0.89 (0.50–1.59)	0.686	0.97 (0.65–1.47)	0.903	0.79 (0.42–1.50)	0.469
Model 2	Ref.	1.09 (0.70–1.69)	0.708	0.86 (0.47–1.56)	0.616	1.00 (0.66–1.53)	0.999	0.77 (0.40–1.49)	0.440
Model 3	Ref.	1.09 (0.70–1.71)	0.699	0.79 (0.43–1.46)	0.453	0.92 (0.60–1.42)	0.716	0.64 (0.33–1.25)	0.192
High TG									
No. of cases (%)	31 (9.2)	23 (9.4)		21 (19.4)		42 (13.4)		13 (14.8)	
Model 1	Ref.	0.98 (0.55–1.73)	0.932	2.29 (1.24–4.22)	**0.008**	1.32 (0.79–2.20)	0.282	1.59 (0.79–3.24)	0.197
Model 2	Ref.	1.07 (0.59–1.93)	0.827	2.35 (1.24–4.48)	**0.009**	1.35 (0.79–2.29)	0.271	1.61 (0.76–3.39)	0.212
Model 3	Ref.	1.04 (0.57–1.90)	0.890	2.26 (1.18–4.34)	0.014	1.27 (0.74–2.16)	0.386	1.41 (0.67–3.00)	0.368
Low HDL									
No. of cases (%)	22 (6.5)	18 (7.4)		8 (7.4)		29 (9.3)		7 (8.0)	
Model 1	Ref.	0.96 (0.50–1.85)	0.897	0.99 (0.42–2.33)	0.986	1.07 (0.59–1.95)	0.828	1.03 (0.41–2.55)	0.954
Model 2	Ref.	0.98 (0.50–1.92)	0.950	1.00 (0.42–2.40)	0.999	1.12 (0.60–2.09)	0.711	1.00 (0.39–2.55)	0.996
Model 3	Ref.	0.97 (0.49–1.94)	0.939	0.88 (0.36–2.16)	0.783	1.01 (0.54–1.90)	0.978	0.79 (0.30–2.06)	0.627
High hs-CRP									
No. of cases (%)	62 (18.4)	53 (21.7)		27 (25.0)		63 (20.1)		13 (14.8)	
Model 1	Ref.	1.19 (0.78–1.80)	0.420	1.42 (0.84–2.40)	0.187	1.16 (0.77–1.73)	0.482	0.74 (0.38–1.43)	0.367
Model 2	Ref.	1.18 (0.77–1.80)	0.445	1.39 (0.82–2.38)	0.225	1.15 (0.76–1.74)	0.520	0.71 (0.36–1.40)	0.321
Model 3	Ref.	1.17 (0.76–1.82)	0.471	1.29 (0.74–2.25)	0.367	1.03 (0.67–1.59)	0.883	0.53 (0.26–1.08)	0.080
High HOMA-IR[a]									
No. of cases (%)	37 (23.3)	28 (22.4)		13 (29.5)		37 (27.2)		13 (31.7)	
Model 1	Ref.	0.99 (0.56–1.75)	0.966	1.41 (0.66–3.01)	0.373	1.23 (0.71–2.12)	0.457	1.64 (0.76–3.55)	0.212
Model 2	Ref.	0.90 (0.50–1.62)	0.721	1.59 (0.72–3.48)	0.251	1.48 (0.83–2.61)	0.182	2.70 (1.17–6.25)	0.021
Model 3	Ref.	0.92 (0.49–1.72)	0.801	1.45 (0.64–3.28)	0.367	1.34 (0.73–2.46)	0.339	2.32 (0.96–5.63)	0.064

Note: Model 1: Adjusted for sex, age, study, study center, and parental highest education; Model 2: Model 1 + season, pubertal stage, sedentary, MVPA, depression, fasting status (except for HOMA-IR), total EI, and carbohydrate intake; and Model 3: Model 2 + FMI. $P < 0.01$ are highlighted in bold, which remained significant after Bonferroni correction using the Nyholt method.

Abbreviations: BP, blood pressure; EI, energy intake; FMI, fat mass index; HDL, high-density lipoprotein cholesterol; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; hs-CRP, high-sensitivity C-reactive protein; MVPA, moderate-to-vigorous physical activity; OR, odds ratio; Ref., reference; TG, triglycerides.

[a]High HOMA-IR in 505 participants.

with higher HOMA-IR (MR = 1.19, 95% CI: 1.01–1.40, Model 2); however, it was nonsignificant after adjustment for FMI. No significant interaction with sex was found. Male and female individuals within the prolonged sleep latency cluster had higher FMI (β = 0.38, 95% CI: 0.01–0.75; β = 0.41, 95% CI: 0.10–0.72), respectively, but these associations were not significant after multiple testing correction (Table S4).

In logistic analyses, the sleep irregularity and variability cluster was associated with increased odds of high TG (OR = 2.35, 95% CI: 1.24–4.48, Model 2); however, it was not significant after adjustment for FMI and multiple testing correction (Table 3). The association of the prolonged sleep latency cluster with high FMI (OR = 1.98, 95% CI: 1.14–3.45; Model 2, Table 3) was detected, but it did not reach the significance threshold after multiple testing correction. Additionally, the prolonged sleep latency cluster was associated with high HOMA-IR (OR = 2.70, 95% CI = 1.17–6.25; Model 2, Table 3), but it was nonsignificant after adjustment for FMI. A significant interaction effect between sex and the sleep irregularity and variability cluster on high TG was observed ($p = 0.002$), restricting this association only to male individuals (OR = 9.50, 95% CI: 3.22–28.07; Figure 3, Table S5). In female individuals, the association between the prolonged sleep latency cluster and high FMI (OR = 2.23, 95% CI: 1.05–4.72), as well as the association between sleep irregularity and variability cluster and high hs-CRP (OR = 2.05, 95% CI: 1.02–4.14), were observed, but they were nonsignificant after multiple testing correction (Figure 3, Table S5).

In sensitivity analyses, the overall findings did not change substantially when considering only fasting adolescents (Tables S6 and S7) and when sleep clusters were limited to being defined based on sleep characteristics on weekdays only (Tables S8 and S9).

DISCUSSION

Based on 1090 adolescents, five sleep clusters, i.e., "good sleep," "delayed sleep phase," "sleep irregularity and variability," "fragmented sleep," and "prolonged sleep latency," were identified by applying cluster analysis across 12 accelerometry-derived sleep characteristics. The prolonged sleep latency cluster was associated with increased FMI. Furthermore, the sleep irregularity and variability cluster was associated with high TG (≥1.7 mmol/L) only in male individuals, but this finding was not replicated in linear models.

We identified five sleep clusters using 12 sleep characteristics reflecting sleep quantity, quality, schedule, variability, and regularity. Several studies have identified sleep patterns by comprehensively considering multiple objectively assessed sleep characteristics, including cluster analysis [10, 27], latent class analysis [28, 29], and composite sleep scores considering self-reported sleep behaviors [30], but only a few studies have been conducted in children and adolescents. Matricciani et al. used cluster analysis to identify four sleep clusters (overall good sleepers, short sleepers, late to bed, and long sleepers) in

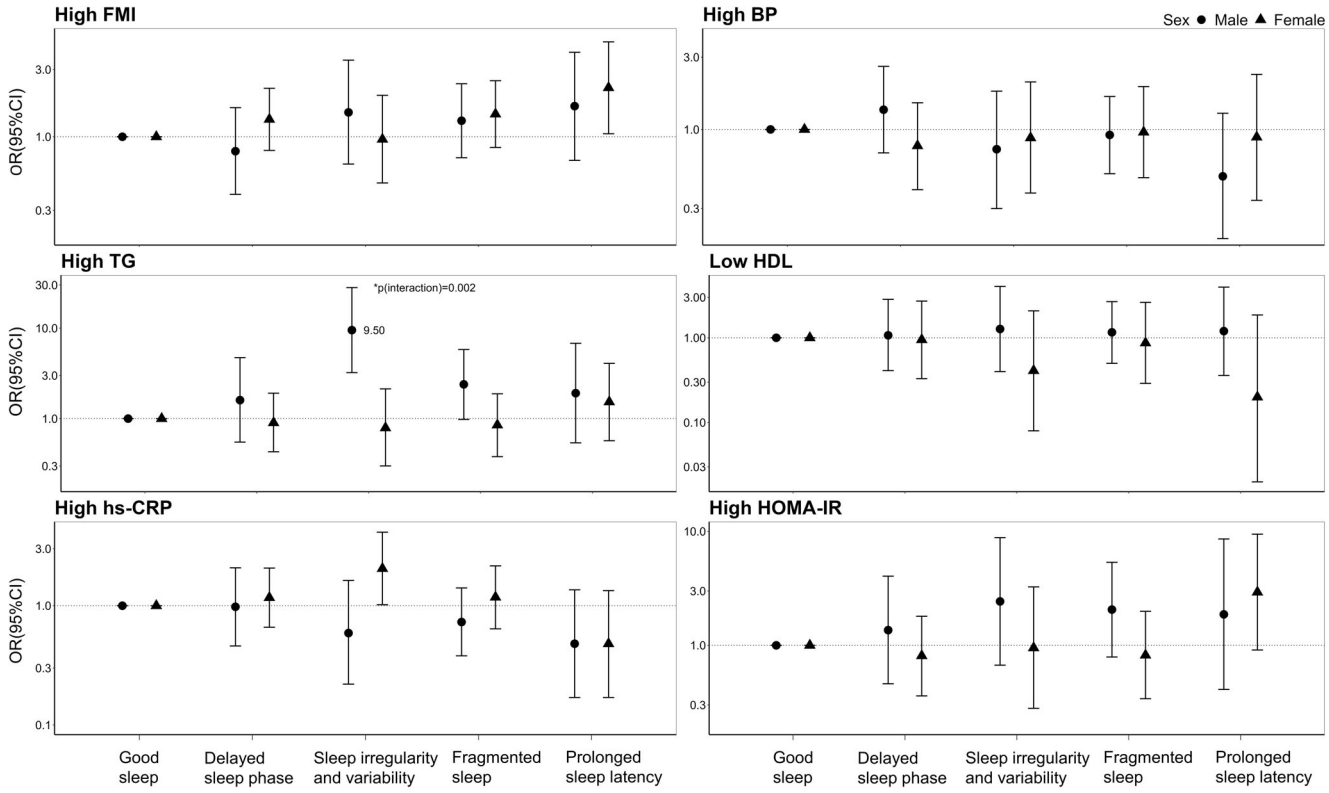

FIGURE 3 Associations among sleep clusters and dichotomous cardiometabolic risk factors in adolescents by sex. BP, blood pressure; FMI, fat mass index; HDL, high-density lipoprotein cholesterol; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; hs-CRP, high-sensitivity C-reactive protein; OR, odds ratio; TG, triglycerides.

1043 Australian children aged 11 to 12 years, using four accelerometry-measured sleep characteristics (period, efficiency, midpoint timing, and sleep period variability) [10]. Furthermore, Thumann et al. applied latent class analysis to classify four sleep subtypes (optimal sleep, early birds, short sleep duration, and poor sleep quality) among 559 European children aged 9 to 16 years, using five sleep characteristics (duration, efficiency, latency, reported wake-up time, and reported lights-off time) [29]. Other studies have detected various sleep patterns in adults, but they all identified a cluster or group named good sleep or healthy sleep [28, 30]. Our findings provided further insights into more diverse sleep patterns by additionally including time awake, awakenings, sleep latency, and their variabilities because we were able to classify sleep profiles labeled fragmented sleep, prolonged sleep latency, and sleep irregularity and variability. Notably, the identified delayed sleep phase cluster clearly presented the common phenotype of delayed sleep phase disorder in adolescents [31]. Furthermore, to enhance comparability among participants with varying total sleep time and effectively capture distinct sleep patterns, we used the time awake per hour after sleep onset (ratio of WASO to (total sleep time + WASO/60)) [12] rather than relying solely on WASO. This standardized assessment approach was also applied to the awakenings per hour after sleep onset.

The prolonged sleep latency cluster was significantly associated with increased FMI in linear models, which was also observed in logistic models but was nonsignificant after multiple testing correction, possibly due to lower power. Previous studies have demonstrated an association between short sleep and childhood obesity, but only a few studies have examined the impact of objective sleep latency, an aspect leading to sleep loss, on obesity [8]. One study showed no association of accelerometry-measured long sleep latency with BMI *z* scores among 107 Swedish children aged 2 to 6 years [32]. Moreover, Thumann et al. reported that accelerometry-measured sleep latency was not associated with BMI *z* scores among 559 European children aged 9 to 16 years [29]. However, in our study, the prolonged sleep latency cluster ($n = 88$) was characterized by extremely high sleep latency (46.4 min; Table S2) compared with the other four sleep clusters (13.8–18.9 min). Possible mechanisms for this association may include the following: 1) prolonged sleep latency can cause frustration, anxiety, or stress, leading to emotional eating, preference for energy-dense foods, and increased calorie intake [3, 33]; 2) prolonged sleep latency may delay the onset of the first sleep stage and reduce time spent in deep sleep (third sleep stage), impacting physical restoration and leading to fatigue and decreased motivation for PA [34]; and 3) disruption of hormonal balance such as cortisol due to prolonged sleep latency can impact EI and expenditure [3].

Male individuals within the sleep irregularity and variability pattern were at increased odds of high TG. Similarly, Spruyt et al. found that accelerometry-determined sleep duration variability during school days was correlated with TG among 47 children with obesity aged 4 to 10 years [35]. Duan et al. also observed a relationship between short sleep and high TG (≥1.24 mmol/L) only in adolescent boys [36]. Furthermore, a recent review supported associations of greater sleep variability and irregularity with obesity and adverse cardiometabolic health in adolescence [7]. The underlying mechanism may involve sociocultural and biological influences. Adolescents within the sleep irregularity and variability cluster may have irregular breakfast behaviors, which were associated with higher TG [7, 37]. Additionally, it may be explained by increased absorption of dietary lipids with increased *de novo* synthesis of TG in the liver or decreased ability to catabolize absorbed dietary fat in male individuals with sleep deprivation [38]. Moreover, in our study, male individuals with a sleep irregularity and variability pattern were more likely to be in late or postpuberty (70.6% compared with 55.9% in the good sleep cluster), whereas no difference in female individuals was found. This suggests that, in male individuals with a sleep irregularity and variability pattern, puberty may start earlier, leading to increased testosterone and decreased sex hormone-binding globulin, and may potentially affect TG level [39]. However, the causality needs to be verified to determine whether pubertal hormonal changes drive sleep behaviors changes [40].

Although we found a significant association of sleep irregularity and variability cluster with high TG (≥1.7 mmol/L) in male individuals, this was not confirmed in linear analyses. We further explored potential reasons. The median TG values were similar across five sleep clusters, but the prevalence of high TG in the sleep irregularity and variability cluster (19.4%; Table S3) was higher than in other sleep clusters (9.2%–14.8%). Additionally, this finding was only found in male individuals and was consistent in fasting male individuals (Figure S5). Regarding the cutoff for TG, the IDF recommended that elevated TG (≥1.7 mmol/L) was most commonly observed in adults with metabolic syndrome, and using adult levels was a wise, easy-to-apply definition to identify children and adolescents at increased risk [16]. Because linear models can only discover differences in the mean TG, logistic models suggested a higher prevalence of extreme values of TG, which could point toward vulnerable subgroups at risk.

Notably, the prolonged sleep latency cluster and the sleep irregularity and variability cluster seemed to be associated with higher insulin resistance, possibly due to increased adiposity, because associations were nonsignificant after adjustment for FMI. The relationship among sleep, adiposity, and insulin resistance may be bidirectional and potentially causal [41]. Sleep disturbance affects metabolic pathways, increasing insulin resistance, potentially reducing energy expenditure, and boosting appetite. Conversely, psychological and endocrine abnormalities in individuals with obesity and/or diabetes disrupt sleep, creating a harmful cycle.

This study investigated associations among sleep patterns identified by cluster analysis and cardiometabolic health in a large adolescent population, with accelerometry-measured sleep data and a comprehensive assessment of cardiometabolic risk factors. However, some limitations should be noted. First, our cross-sectional, observational study was unable to infer causality. Notably, our previous study found a bidirectional association between reported sleep difficulty and overweight/obesity from adolescence to young adulthood [42]. Second, we used sex-specific upper quartiles to dichotomize FMI, HOMA-IR, and hs-CRP to improve comparability across outcomes because no standard thresholds are available in adolescents, which

may limit comparability with other studies. Third, although the accelerometer is a practical approach to measure sleep in epidemiological research [43], it differs from polysomnography (gold standard) [44]. Fourth, daytime sleep data were unavailable. Fifth, we assumed that 1-week sleep measurements estimated habitual sleep patterns over a longer period [43], although the measurements of cardiometabolic risk factors preceded sleep assessments in our study, with a mean age difference of 0.36 years. Sixth, caution should be exercised when generalizing our findings to other age groups or cultures because our participants are German adolescents aged 14 to 16 years.

CONCLUSION

We identified five distinctive sleep patterns by cluster analysis and found that the cluster describing "prolonged sleep latency" pattern was associated with higher fat mass in adolescents. Additionally, the cluster describing "sleep irregularity and variability" pattern seemed to be associated with high TG in male individuals. Our results suggest that improvements in sleep latency, variability, and regularity may enrich existing sleep-targeted intervention strategies for cardiometabolic health that mainly focus on improving adequate sleep.O

AUTHOR CONTRIBUTIONS

Mingming Wang conceptualized and designed the study, conducted the statistical analyses, drafted the initial manuscript, and revised and finalized the manuscript. Claudia Flexeder conceptualized and designed the study, contributed to data acquisition and interpretation, supervised the statistical analyses, and critically reviewed and revised the manuscript. Carla P Harris contributed to data interpretation, supervised the statistical analyses, and critically reviewed and revised the manuscript. Elisabeth Thiering, Sibylle Koletzko, Carl-Peter Bauer, Gerd Schulte-Körne, Andrea von Berg, Dietrich Berdel, Joachim Heinrich, Holger Schulz, and Tamara Schikowski contributed to data acquisition and interpretation and critically reviewed and revised the manuscript. Annette Peters contributed to study design and data interpretation and critically reviewed and revised the manuscript. Marie Standl conceptualized and designed the study, contributed to data acquisition and interpretation, supervised the statistical analyses and the manuscript process, and critically reviewed and revised the manuscript. All authors approved the final manuscript as submitted and agreed to be accountable for all aspects of the work.

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CONFLICT OF INTEREST STATEMENT

The authors declared no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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Online supporting information

Accelerometry-assessed sleep clusters and cardiometabolic risk factors in adolescents

Supplementary methods

Supplementary results

Method S1. Details on blood cardiometabolic markers measurements

Serum total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL), and low-density lipoprotein cholesterol (LDL) (mmol/L) were measured using homogenous enzymatic colorimetric methods on a Modular Analytics System from Roche Diagnostics GmbH Mannheim. Serum hs-CRP (mg/L) was measured using Roche (Mannheim, Germany) Tina-quant CRP (latex) high-sensitive assay. Fasting glucose (mmol/L) was measured by standard laboratory methods by the two individual hospitals, and fasting insulin (pmol/L) was performed centrally by a fully mechanized system, LIAISON (DiaSorin, Saluggia, Italy).

Method S2. Consideration of final number of sleep clusters

We used a combination of four methods to derive a common number of five groups, which we determined to be the most robust and interpretable solution based on a thorough evaluation of the clustering results.

K-means clustering partitions data points into k groups based on their similarity, assigning each point to the cluster whose centroid is closest. The number of clusters K must be predefined, and the initial centroid positions significantly impact the clustering outcome [1].

Hierarchical cluster analysis groups data points into a hierarchy of clusters based on feature similarity, forming a tree-like structure (called dendrogram), where similar data points are grouped together at different levels of the tree. The resulting clusters are influenced by the choice of distance metric, which measures dissimilarity between data points, and the linkage method, which calculates distances between clusters based on their constituent data points [1].

Principal component analysis is a dimensionality reduction technique that transforms correlated variables into uncorrelated principal components, capturing the most variance in the data. It calculates the covariance matrix to assess linear dependence, decomposes it into eigenvectors and eigenvalues, which represent the direction and magnitude of maximum variation [2]. Typically, enough principal components are retained to account for 80% of the total variance.

The sum of squares method is a technique used to determine the ideal number of clusters by minimizing the within-cluster sum of squares, which indicates the level of compactness of each cluster, and maximizing the between-cluster sum of squares, which indicates the level of separation among the clusters [3].

Each of these four methods provided valuable insights and different perspectives on the structure of the data. Regarding the interpretation of five clusters (**Figure 2**), for instance, the “delayed sleep phase”, characterized by higher sleep midpoint timing, higher SD in sleep midpoint timing, and higher sleep efficiency, clearly identifies objectively what is known about the delayed sleep phase phenotype, which is common in adolescents and young adults [4]. **Figure S2** also shows that all participants could be divided into five different groups (represented by five colors). In addition, in **Table S1**, we observed that five components account for 80% cumulative percentage of variance, which could give us a hint about five. Furthermore, In **Figure S4**, when the number is five, the within-cluster sum of squares is relatively lower, and the between-cluster sum of squares is relatively higher. Therefore, considering all this information, we decided to define the number of clusters to five.

Method S3. Bonferroni correction for multiple testing using the Nyholt method

To minimize the risk of type I errors (false-positive findings) and ensure more accurate results, we applied the Bonferroni correction using the Nyholt method [5] for multiple testing, thereby avoiding excessively conservative results. This method estimates the effective number (n) of independent tests

based on the correlation matrix between outcomes. Subsequently, it applies the Bonferroni correction to establish a stringent significance threshold (0.05/n).

Table S1. Results of principal component analysis

Component	Eigenvalue	Percentage of variance (%)	Cumulative percentage of variance (%)
1	3.84	32.00	32.00
2	2.18	18.20	50.20
3	1.44	11.98	62.18
4	1.20	10.01	72.19
5	0.91	7.56	79.75
6	0.87	7.27	87.02
7	0.62	5.15	92.17
8	0.38	3.14	95.31
9	0.29	2.43	97.74
10	0.19	1.60	99.33
11	0.08	0.65	99.98
12	0.00	0.02	100.00

Table S2. Sleep characteristics in sleep clusters

Sleep parameters	Good sleep		Delayed sleep phase		Sleep irregularity and variability		Fragmented sleep		Prolonged sleep latency		*p**
	337		244		108		313		88		
	Mean	Median [Q_1, Q_3]	Mean	Median [Q_1, Q_3]	Mean	Median [Q_1, Q_3]	Mean	Median [Q_1, Q_3]	Mean	Median [Q_1, Q_3]	
Daily averages											
Total sleep time, hours	7.6	7.5 [7.2, 7.9]^{a}	7.2	7.2 [6.8, 7.7]^{b}	6.9	6.9 [6.5, 7.4]^{c}	6.9	6.9 [6.5, 7.2]^{c}	6.9	7.0 [6.5, 7.4]^{c}	<0.001
Sleep efficiency, %	84.7	84.5 [82.6, 86.7]^{a}	82.0	81.8 [79.6, 84.4]^{b}	75.1	76.5 [72.0, 78.6]^{c}	75.2	75.7 [72.6, 78.2]^{c}	72.3	72.8 [69.3, 76.0]^{c}	<0.001
Sleep latency, mins	13.8	12.2 [8.3, 17.4]^{a}	16.3	14.7 [9.7, 22.2]^{b}	18.9	16.3 [11.4, 24.6]^{b}	18.5	17.5 [12.5, 23.5]^{b}	46.4	43.3 [37.6, 53.6]^{c}	<0.001
Time awake per hour after sleep onset, mins/h	7.7	7.9 [6.6, 9.0]^{a}	9.2	9.3 [7.9, 10.3]^{b}	13.2	12.4 [10.9, 14.5]^{c}	13.2	12.8 [11.4, 14.9]^{c}	12.8	12.5 [10.6, 14.9]^{c}	<0.001
Awakenings per hour after sleep onset	2.5	2.6 [2.2, 2.9]^{a}	2.7	2.7 [2.3, 3.1]^{b}	2.8	2.8 [2.5, 3.1]^{b}	3.4	3.4 [3.1, 3.7]^{c}	2.9	2.8 [2.5, 3.2]^{b}	<0.001
Sleep midpoint timing, 24-hour clock	02:30	02:29 [02:09, 02:49]^{a}	03:12	03:11 [02:46, 03:32]^{b}	02:30	02:32 [02:07, 02:56]^{a}	02:06	02:08 [01:47, 02:29]^{c}	02:24	02:33 [01:59, 02:52]^{a}	<0.001
Day-to-day variability											
SD in total sleep time, mins	50.8	49.1 [34.0, 63.9]^{a}	79.5	77.3 [60.5, 98.4]^{b}	89.9	86.0 [69.0, 110.1]^{b}	48.8	47.4 [33.9, 60.1]^{a}	66.2	60.2 [44.7, 82.0]^{c}	<0.001
SD in sleep efficiency, %	4.2	3.9 [3.0, 5.1]^{a}	4.8	4.7 [3.7, 6.0]^{b}	11.3	10.8 [9.1, 13.2]^{c}	5.2	5.1 [3.9, 6.4]^{b}	7.5	7.2 [5.7, 8.4]^{d}	<0.001
SD in sleep latency, mins	10.1	8.5 [5.2, 13.2]^{a}	11.8	10.7 [6.3, 15.4]^{b}	16.2	14.6 [8.9, 21.8]^{c}	13.9	12.6 [8.5, 18.7]^{c}	42.8	38.6 [30.8, 49.7]^{d}	<0.001
SD in time awake per hour after sleep onset, mins/h	2.5	2.3 [1.7, 3.0]^{a}	2.8	2.8 [2.0, 3.5]^{b}	6.8	6.4 [5.5, 7.6]^{c}	3.2	3.1 [2.5, 4.0]^{a}	4.2	4.1 [3.2, 5.2]^{e}	<0.001
SD in awakenings per hour after sleep onset	0.5	0.5 [0.4, 0.6]^{a}	0.6	0.6 [0.5, 0.8]^{b}	0.7	0.7 [0.5, 0.9]^{b}	0.5	0.5 [0.4, 0.6]^{a}	0.6	0.6 [0.4, 0.7]^{ab}	<0.001
SD in sleep midpoint timing, mins	54.1	53.6 [40.4, 67.3]^{a}	91.3	89.6 [73.1, 105.6]^{b}	78.5	77.1 [53.4, 96.8]^{c}	55.6	53.6 [39.2, 70.6]^{a}	72.1	68.7 [50.1, 90.4]^{c}	<0.001

*: Kruskal-Wallis test with Dunn post hoc tests and Bonferroni adjustment. Different letters (a b c d e) are considered significantly different (adjusted *P*-values < 0.05). SD: standard deviation. Q_1: first quartile. Q_3: third quartile

Table S3. Participant characteristics in sleep clusters

	Good sleep	Delayed sleep phase	Sleep irregularity and variability	Fragmented sleep	Prolonged sleep latency	*P**
n (%)	337 (30.9)	244 (22.4)	108 (9.9)	313 (28.7)	88 (8.1)	
Male, n(%)	109 (32.3)[a]	103 (42.2)[a]	43 (39.8)[a]	193 (61.7)[b]	41 (46.6)[ab]	**<0.001**
Age, year	15.2 ± 0.3	15.2 ± 0.3	15.2 ± 0.3	15.2 ± 0.3	15.2 ± 0.3	0.440
Weight, kg	59.7 ± 10.2[a]	60.7 ± 11.0[ab]	61.9 ± 11.7[ab]	62.5 ± 10.8[b]	62.7 ± 13.1[ab]	**0.008**
Height, cm	170.2 ± 8.1[a]	171.2 ± 7.8[a]	171.6 ± 7.9[ab]	173.1 ± 7.6[b]	170.6 ± 8.4[ab]	**<0.001**
FMI, kg/m^2	5.1 ± 1.9	5.1 ± 2.1	5.3 ± 2.0	4.8 ± 2.1	5.5 ± 2.1	**0.023**
High FMI, n(%)	72 (21.4)	63 (25.8)	27 (25.0)	82 (26.2)	28 (31.8)	0.299
SBP, mmHg	117.6 ± 11.9	118.3 ± 11.8	119.0 ± 10.1	119.1 ± 12.1	120.1 ± 11.6	0.334
DBP, mmHg	69.5 ± 8.9	69.5 ± 8.9	70.8 ± 9.1	68.9 ± 9.0	69.3 ± 8.4	0.401
High BP, n(%)	59 (17.5)	48 (19.7)	19 (17.6)	65 (20.8)	15 (17.0)	0.818
TC, mmol/L	4.3 [3.8, 4.9]	4.2 [3.9, 4.8]	4.4 [3.8, 4.9]	4.2 [3.7, 4.7]	4.3 [3.8, 4.8]	0.427
TG, mmol/L	1.0 [0.7, 1.3]	1.0 [0.7, 1.3]	0.9 [0.7, 1.4]	1.0 [0.8, 1.3]	1.0 [0.7, 1.3]	0.823
High TG, n(%)	31 (9.2)	23 (9.4)	21 (19.4)	42 (13.4)	13 (14.8)	**0.027**
HDL, mmol/L	1.5 ± 0.4[ab]	1.5 ± 0.3[a]	1.5 ± 0.4[ab]	1.4 ± 0.3[b]	1.5 ± 0.4[ab]	**0.015**
Low HDL, n(%)	22 (6.5)	18 (7.4)	8 (7.4)	29 (9.3)	7 (8.0)	0.775
LDL, mmol/L	2.3 [1.9, 2.7]	2.3 [1.9, 2.7]	2.3 [1.9, 2.7]	2.3 [1.9, 2.7]	2.4 [1.9, 2.9]	0.918
Hs-CRP, mg/L	0.4 [0.2, 0.7]	0.3 [0.2, 0.7]	0.5 [0.2, 0.9]	0.4 [0.2, 0.8]	0.3 [0.2, 0.7]	0.581
High hs-CRP, n(%)	62 (18.4)	53 (21.7)	27 (25.0)	63 (20.1)	13 (14.8)	0.381
HOMA-IR	2.0 [1.5, 2.6]	2.1 [1.4, 2.6]	2.3 [1.7, 2.7]	2.1 [1.4, 2.7]	2.2 [1.5, 2.9]	0.344
High HOMA-IR, n(%)	37 (23.3)	28 (22.4)	13 (29.5)	37 (27.2)	13 (31.7)	0.650
Total EI, kcal/day	1989.3 ± 618.3[a]	2059.5 ± 634.5[ab]	2187.2 ± 709.1[ab]	2213.3 ± 649.3[b]	2094.2 ± 625.0[ab]	**0.001**
Carbohydrate intake (%EI)	53.8 ± 7.2	52.5 ± 7.8	52.1 ± 8.2	52.4 ± 6.7	54.1 ± 7.5	0.066
Sedentary, hour	8.4 ± 1.3[a]	8.7 ± 1.4[a]	8.1 ± 1.1[ab]	8.0 ± 1.4[b]	7.5 ± 1.3[b]	**<0.001**
MVPA, mins	48.4 ± 30.4	48.7 ± 25.9	50.0 ± 24.4	54.2 ± 24.0	49.9 ± 22.4	0.053
Depression, n(%)	43 (13.8)	35 (15.0)	13 (12.7)	34 (11.8)	16 (19.3)	0.500
Fasting status (yes), n(%)	162 (48.1)	125 (51.2)	45 (41.7)	137 (43.8)	42 (47.7)	0.347
Study, n(%)						0.094
GINI observation	136 (40.4)	101 (41.4)	35 (32.4)	109 (34.8)	33 (37.5)	

GINI intervention	141 (41.8)	96 (39.3)	47 (43.5)	116 (37.1)	37 (42.0)	
LISA	60 (17.8)	47 (19.3)	26 (24.1)	88 (28.1)	18 (20.5)	
Study center, n(%)						**0.012**
Munich	205 (60.8)	126 (51.6)	53 (49.1)	196 (62.6)	45 (51.1)	
Wesel	132 (39.2)	118 (48.4)	55 (50.9)	117 (37.4)	43 (48.9)	
Season, n(%)						0.079
Spring	87 (25.8)	67 (27.5)	30 (27.8)	76 (24.3)	21 (23.9)	
Summer	38 (11.3)	35 (14.3)	20 (18.5)	57 (18.2)	17 (19.3)	
Autumn	107 (31.8)	77 (31.6)	32 (29.6)	115 (36.7)	22 (25.0)	
Winter	105 (31.2)	65 (26.6)	26 (24.1)	65 (20.8)	28 (31.8)	
Parental highest education, n(%)						**<0.001**
Low/medium	93 (27.6)	72 (29.5)	39 (36.1)	88 (28.1)	45 (51.1)	
High	244 (72.4)[a]	172 (70.5)[a]	69 (63.9)[ab]	225 (71.9)[a]	43 (48.9)[b]	
Pubertal stage, n(%)						**0.005**
Pre-/early-/mid-pubertal	53 (18.5)	47 (22.1)	12 (13.2)	76 (29.1)	14 (17.7)	
Late-/post-pubertal	234 (81.5)[a]	166 (77.9)[ab]	79 (86.8)[a]	185 (70.9)[b]	65 (82.3)[ab]	

The results are presented as mean ± standard deviation, median [first quartile, third quartile], n(%) (numbers(percentage)). Abbreviations: FMI, fat mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; BP, blood pressure; TC, total cholesterol; TG, triglycerides; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; hs-CRP, high-sensitivity C-reactive protein; HOMA-IR, homeostatic model assessment of insulin resistance; EI, energy intake; carbohydrate intake (%EI), carbohydrate as percentage of total energy intake; MVPA, moderate-to-vigorous physical activity.

The number of participants with available information: HOMA-IR (505), total EI (865), carbohydrate intake (865), sedentary (1082), MVPA (1082), depression (1017), and pubertal stage (931).

*: Post hoc tests after one-way ANOVA test or Kruskal-Wallis test or Chi-square test, with Bonferroni adjustment. Different letters (a b c) are considered significantly different. *P*-values < 0.05 are highlighted in bold.

Table S4. Sex-stratified associations between sleep clusters and continuous cardiometabolic risk factors in adolescents

	Good sleep	Delayed sleep phase		Sleep irregularity and variability		Fragmented sleep		Prolonged sleep latency	
Outcomes	**β (95%CI)**	**β (95%CI)**	***P***	**β (95%CI)**	***P***	**β (95%CI)**	***P***	**β (95%CI)**	***P***
Males (489)									
FMI scaled	Ref	-0.01 (-0.29-0.26)	0.931	0.31 (-0.05-0.66)	0.089	0.16 (-0.08-0.40)	0.184	0.38 (0.01-0.75)	0.043
SBP scaled	Ref	0.08 (-0.19-0.34)	0.566	-0.26 (-0.61-0.08)	0.136	-0.15 (-0.38-0.08)	0.199	-0.09 (-0.45-0.26)	0.609
DBP scaled	Ref	0.26 (-0.01-0.53)	0.057	0.10 (-0.25-0.45)	0.566	-0.07 (-0.30-0.17)	0.580	-0.15 (-0.52-0.21)	0.403
HDL inversely scaled	Ref	-0.06 (-0.33-0.21)	0.647	-0.11 (-0.47-0.24)	0.537	0.06 (-0.17-0.30)	0.597	0.02 (-0.34-0.39)	0.901
	MR (95%CI)	**MR (95%CI)**	***P***	**MR (95%CI)**	***P***	**MR (95%CI)**	***P***	**MR (95%CI)**	***P***
TC	Ref	1.03 (0.98-1.08)	0.242	1.05 (0.98-1.12)	0.145	1.03 (0.98-1.07)	0.238	1.00 (0.94-1.08)	0.889
TG	Ref	1.04 (0.92-1.17)	0.521	1.10 (0.94-1.28)	0.221	1.06 (0.96-1.18)	0.250	0.94 (0.80-1.11)	0.464
LDL	Ref	1.03 (0.95-1.12)	0.456	1.06 (0.95-1.18)	0.296	1.07 (0.99-1.15)	0.079	1.03 (0.92-1.15)	0.648
HOMA-IR[a]	Ref	1.11 (0.93-1.31)	0.243	1.16 (0.92-1.46)	0.209	1.03 (0.89-1.21)	0.667	0.96 (0.74-1.24)	0.742
Females (601)									
FMI scaled	Ref	0.03 (-0.18-0.23)	0.809	0.06 (-0.20-0.33)	0.636	0.13 (-0.09-0.34)	0.243	0.41 (0.10-0.72)	0.010
SBP scaled	Ref	-0.07 (-0.27-0.13)	0.500	0.16 (-0.11-0.43)	0.244	0.14 (-0.08-0.35)	0.218	0.10 (-0.21-0.41)	0.510
DBP scaled	Ref	-0.11 (-0.31-0.10)	0.312	0.25 (-0.02-0.53)	0.069	0.05 (-0.17-0.27)	0.630	0.01 (-0.31-0.32)	0.970
HDL inversely scaled	Ref	-0.07 (-0.28-0.14)	0.536	-0.26 (-0.54-0.02)	0.065	0.09 (-0.14-0.31)	0.449	-0.15 (-0.47-0.18)	0.377
	MR (95%CI)	**MR (95%CI)**	***P***	**MR (95%CI)**	***P***	**MR (95%CI)**	***P***	**MR (95%CI)**	***P***
TC	Ref	1.01 (0.97-1.05)	0.745	1.00 (0.95-1.06)	0.913	0.98 (0.94-1.02)	0.392	1.01 (0.96-1.08)	0.638
TG	Ref	1.00 (0.92-1.09)	0.974	0.95 (0.84-1.06)	0.342	1.02 (0.93-1.11)	0.709	1.02 (0.89-1.16)	0.795
LDL	Ref	1.01 (0.95-1.08)	0.665	0.99 (0.91-1.07)	0.721	0.98 (0.92-1.05)	0.570	1.01 (0.92-1.11)	0.800
HOMA-IR[a]	Ref	1.00 (0.87-1.15)	0.987	1.12 (0.91-1.38)	0.294	1.02 (0.87-1.19)	0.797	1.15 (0.92-1.43)	0.210

Abbreviations: FMI, fat mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides; LDL, low-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance; MR, means ratio. HOMA-IR[a]: 240 males and 265 females.

Adjusted for age, study, study center, parental highest education, season, pubertal stage, sedentary, MVPA, depression, fasting status (except for HOMA-IR), total EI, carbohydrate intake, and FMI (except for FMI).

Table S5. Sex-stratified associations between sleep clusters and dichotomous cardiometabolic risk factors in adolescents

	Good sleep		Delayed sleep phase			Sleep irregularity and variability			Fragmented sleep			Prolonged sleep latency		
Outcomes	**%**	**OR (95%CI)**	**%**	**OR (95%CI)**	***P***	**%**	**OR (95%CI)**	***P***	**%**	**OR (95%CI)**	***P***	**%**	**OR (95%CI)**	***P***
Males (489)														
n		109		103			43			193			41	
High FMI	21.1	Ref	22.3	0.79 (0.39-1.61)	0.521	30.2	1.49 (0.64-3.50)	0.355	25.9	1.30 (0.71-2.38)	0.389	31.7	1.65 (0.68-3.98)	0.266
High BP	24.8	Ref	29.1	1.35 (0.70-2.61)	0.373	23.3	0.74 (0.30-1.79)	0.502	25.4	0.92 (0.51-1.65)	0.770	19.5	0.49 (0.19-1.28)	0.146
High TG	7.3	Ref	9.7	1.60 (0.55-4.72)	0.392	34.9	9.50 (3.22-28.07)	**<0.001**	16.1	2.39 (0.98-5.80)	0.056	14.6	1.91 (0.54-6.80)	0.316
Low HDL	9.2	Ref	10.7	1.07 (0.41-2.84)	0.888	14.0	1.27 (0.40-4.04)	0.686	11.9	1.16 (0.50-2.67)	0.729	14.6	1.20 (0.36-3.96)	0.764
High hs-CRP	19.3	Ref	21.4	0.98 (0.46-2.08)	0.952	18.6	0.59 (0.22-1.63)	0.310	19.7	0.73 (0.38-1.41)	0.350	17.1	0.48 (0.17-1.36)	0.168
High HOMA-IR[a]	21.4	Ref	20.3	1.36 (0.46-4.05)	0.580	38.1	2.43 (0.67-8.82)	0.178	27.9	2.06 (0.79-5.36)	0.139	27.8	1.87 (0.41-8.59)	0.423
Female (601)														
n		228		141			65			120			47	
High FMI	21.5	Ref	28.4	1.33 (0.80-2.21)	0.279	21.5	0.96 (0.47-1.97)	0.917	26.7	1.45 (0.84-2.50)	0.182	31.9	2.23 (1.05-4.72)	0.037
High BP	14.0	Ref	12.8	0.78 (0.40-1.50)	0.455	13.8	0.88 (0.38-2.06)	0.766	13.3	0.96 (0.48-1.92)	0.915	14.9	0.89 (0.34-2.31)	0.814
High TG	10.1	Ref	9.2	0.90 (0.43-1.91)	0.792	9.2	0.79 (0.30-2.13)	0.648	9.2	0.85 (0.38-1.88)	0.690	14.9	1.53 (0.57-4.08)	0.399
Low HDL	5.3	Ref	5.0	0.95 (0.33-2.71)	0.918	3.1	0.41 (0.08-2.08)	0.281	5.0	0.87 (0.29-2.62)	0.801	2.1	0.20 (0.02-1.86)	0.159
High hs-CRP	18.0	Ref	22.0	1.17 (0.66-2.07)	0.581	29.2	2.05 (1.02-4.14)	0.045	20.8	1.18 (0.64-2.16)	0.603	12.8	0.48 (0.17-1.34)	0.164
High HOMA-IR[a]	24.3	Ref	24.2	0.81 (0.36-1.80)	0.601	21.7	0.95 (0.28-3.26)	0.940	26.0	0.82 (0.34-1.99)	0.661	34.8	2.93 (0.91-9.46)	0.074

Abbreviations: FMI, fat mass index; BP, blood pressure; TG, triglycerides; HDL, high-density lipoprotein cholesterol; hs-CRP, high-sensitivity C-reactive protein; HOMA-IR, homeostatic model assessment of insulin resistance. High HOMA-IR[a]: 240 males and 265 females.

Adjusted for age, study, study center, parental highest education, season, pubertal stage, sedentary, MVPA, depression, fasting status (except for high HOMA-IR), total EI, carbohydrate intake, and FMI (except for high FMI).

P-values < 0.01 are highlighted in bold, which remained significant after Bonferroni correction using the Nyholt method.

Table S6. Associations between sleep clusters and continuous cardiometabolic risk factors in adolescents only with fasting status

	Good sleep	Delayed sleep phase		Sleep irregularity and variability		Fragmented sleep		Prolonged sleep latency	
Outcomes	**β (95%CI)**	**β (95%CI)**	***P***	**β (95%CI)**	***P***	**β (95%CI)**	***P***	**β (95%CI)**	***P***
Total (511)									
FMI scaled	Ref	-0.05 (-0.28-0.18)	0.680	0.25 (-0.08-0.58)	0.138	0.18 (-0.05-0.41)	0.132	0.35 (0.00-0.70)	**0.048**
SBP scaled	Ref	-0.07 (-0.30-0.16)	0.557	-0.02 (-0.35-0.32)	0.919	0.01 (-0.22-0.25)	0.912	0.05 (-0.30-0.41)	0.766
DBP scaled	Ref	-0.04 (-0.27-0.19)	0.740	0.19 (-0.14-0.52)	0.252	-0.04 (-0.28-0.19)	0.707	-0.17 (-0.53-0.18)	0.337
HDL inversely scaled	Ref	-0.07 (-0.29-0.16)	0.570	-0.13 (-0.45-0.20)	0.450	0.18 (-0.05-0.41)	0.129	0.06 (-0.29-0.40)	0.750
	MR (95%CI)	**MR (95%CI)**	***P***	**MR (95%CI)**	***P***	**MR (95%CI)**	***P***	**MR (95%CI)**	***P***
TC	Ref	1.00 (0.96-1.05)	0.996	1.00 (0.94-1.07)	0.944	1.00 (0.96-1.05)	0.995	0.98 (0.91-1.05)	0.490
TG	Ref	1.03 (0.94-1.13)	0.487	1.09 (0.96-1.24)	0.183	1.08 (0.99-1.18)	0.086	1.01 (0.88-1.15)	0.938
LDL	Ref	1.01 (0.94-1.08)	0.867	1.00 (0.90-1.10)	0.962	1.04 (0.96-1.11)	0.336	0.99 (0.89-1.10)	0.835
Males (241)									
FMI scaled	Ref	-0.15 (-0.52-0.22)	0.429	0.61 (0.09-1.12)	**0.021**	0.18 (-0.16-0.53)	0.302	0.52 (-0.03-1.06)	0.066
SBP scaled	Ref	0.06 (-0.32-0.44)	0.750	-0.18 (-0.72-0.36)	0.515	-0.23 (-0.59-0.12)	0.202	0.09 (-0.48-0.66)	0.764
DBP scaled	Ref	0.12 (-0.26-0.49)	0.551	0.06 (-0.48-0.59)	0.834	-0.21 (-0.56-0.14)	0.247	-0.34 (-0.91-0.23)	0.242
HDL inversely scaled	Ref	-0.12 (-0.47-0.24)	0.517	-0.16 (-0.66-0.34)	0.526	0.17 (-0.16-0.51)	0.304	0.01 (-0.52-0.53)	0.985
	MR (95%CI)	**MR (95%CI)**	***P***	**MR (95%CI)**	***P***	**MR (95%CI)**	***P***	**MR (95%CI)**	***P***
TC	Ref	1.00 (0.93-1.07)	0.952	1.02 (0.92-1.13)	0.672	1.00 (0.94-1.07)	0.984	0.97 (0.87-1.08)	0.583
TG	Ref	1.02 (0.88-1.18)	0.759	1.04 (0.85-1.28)	0.699	1.05 (0.92-1.20)	0.470	0.88 (0.71-1.09)	0.253
LDL	Ref	1.00 (0.89-1.13)	0.969	1.04 (0.89-1.23)	0.612	1.05 (0.94-1.17)	0.397	1.01 (0.85-1.20)	0.938
Females (270)									
FMI scaled	Ref	0.03 (-0.27-0.33)	0.832	-0.02 (-0.46-0.42)	0.919	0.15 (-0.19-0.48)	0.397	0.19 (-0.28-0.66)	0.422
SBP scaled	Ref	-0.21 (-0.51-0.10)	0.182	0.05 (-0.39-0.50)	0.812	0.33 (-0.01-0.67)	0.062	-0.02 (-0.49-0.46)	0.951
DBP scaled	Ref	-0.21 (-0.50-0.09)	0.174	0.31 (-0.13-0.74)	0.169	0.16 (-0.17-0.49)	0.343	-0.05 (-0.52-0.41)	0.825
HDL inversely scaled	Ref	-0.07 (-0.38-0.23)	0.641	-0.19 (-0.64-0.27)	0.421	0.13 (-0.21-0.48)	0.457	-0.02 (-0.50-0.46)	0.943
	MR (95%CI)	**MR (95%CI)**	***P***	**MR (95%CI)**	***P***	**MR (95%CI)**	***P***	**MR (95%CI)**	***P***
TC	Ref	1.01 (0.95-1.07)	0.774	0.98 (0.90-1.07)	0.655	1.00 (0.93-1.06)	0.936	0.99 (0.90-1.08)	0.771
TG	Ref	1.04 (0.93-1.16)	0.508	1.08 (0.91-1.28)	0.360	1.07 (0.94-1.22)	0.308	1.05 (0.88-1.26)	0.569
LDL	Ref	1.02 (0.93-1.11)	0.707	0.95 (0.84-1.09)	0.487	1.02 (0.92-1.13)	0.744	0.98 (0.85-1.13)	0.810

Abbreviations: FMI, fat mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides; LDL, low-density lipoprotein cholesterol; MR, means ratio.

Adjusted for age, sex (except for males and females), study, study center, parental highest education, season, pubertal stage, sedentary, MVPA, depression, total EI, carbohydrate intake, and FMI (except for FMI).

P-values < 0.05 are highlighted in bold (No correction for multiple testing was performed because of the reduced sample size).

Table S7. Associations between sleep clusters and dichotomous cardiometabolic risk factors in adolescents only with fasting status

	Good sleep		Delayed sleep phase			Sleep irregularity and variability			Fragmented sleep			Prolonged sleep latency		
Outcomes	**%**	**OR (95%CI)**	**%**	**OR (95%CI)**	***P***	**%**	**OR (95%CI)**	***P***	**%**	**OR (95%CI)**	***P***	**%**	**OR (95%CI)**	***P***
Total (511)														
n		162		125			45			137			42	
High FMI	20.4	Ref	24.0	1.07 (0.59-1.93)	0.822	28.9	1.53 (0.69-3.41)	0.297	26.3	1.48 (0.82-2.64)	0.191	31.0	1.95 (0.84-4.51)	0.118
High BP	14.2	Ref	14.4	1.04 (0.51-2.12)	0.916	15.6	0.78 (0.29-2.12)	0.631	16.1	1.06 (0.52-2.15)	0.876	19.0	1.56 (0.56-4.31)	0.396
High TG	3.1	Ref	2.4	0.61 (0.13-2.97)	0.543	15.6	5.97 (1.46-24.45)	**0.013**	5.1	1.76 (0.46-6.73)	0.411	2.4	0.80 (0.07-9.06)	0.856
Low HDL	5.6	Ref	4.8	0.53 (0.16-1.79)	0.306	8.9	0.65 (0.14-3.06)	0.590	7.3	0.87 (0.28-2.69)	0.811	4.8	0.62 (0.10-3.84)	0.609
High hs-CRP	19.1	Ref	21.6	1.29 (0.68-2.43)	0.431	26.7	1.16 (0.50-2.70)	0.725	19.0	0.97 (0.50-1.88)	0.921	16.7	0.49 (0.17-1.38)	0.177
Male (241)														
n		56		59			21			86			19	
High FMI	23.2	Ref	18.6	0.58 (0.21-1.57)	0.286	42.9	2.28 (0.68-7.64)	0.181	24.4	1.11 (0.46-2.67)	0.815	42.1	2.56 (0.71-9.25)	0.153
High BP	16.1	Ref	20.3	1.43 (0.50-4.09)	0.509	19.0	0.67 (0.16-2.83)	0.588	18.6	1.04 (0.37-2.93)	0.937	21.1	1.20 (0.26-5.55)	0.820
High TG	3.6	Ref	1.7	0.23 (0.01-4.20)	0.319	23.8	10.23 (0.69-152.3)	0.093	5.8	1.92 (0.23-16.26)	0.550	0.0	Inf	0.993
Low HDL	8.9	Ref	6.8	0.42 (0.08-2.19)	0.302	14.3	0.37 (0.04-3.16)	0.368	11.6	1.22 (0.28-5.30)	0.790	10.5	0.70 (0.08-6.25)	0.753
High hs-CRP	12.5	Ref	18.6	1.53 (0.46-5.09)	0.485	19.0	0.47 (0.09-2.37)	0.359	16.3	0.82 (0.25-2.62)	0.734	15.8	0.29 (0.04-1.85)	0.190
Female (270)														
n		106		66			24			51			23	
High FMI	18.9	Ref	28.8	1.63 (0.76-3.51)	0.212	16.7	0.81 (0.23-2.85)	0.747	29.4	1.66 (0.71-3.91)	0.246	21.7	1.11 (0.32-3.86)	0.866
High BP	13.2	Ref	9.1	0.58 (0.19-1.80)	0.349	12.5	1.06 (0.25-4.57)	0.935	11.8	1.09 (0.34-3.54)	0.881	17.4	2.12 (0.47-9.64)	0.333
High TG	2.8	Ref	3.0	1.40 (0.18-10.67)	0.744	8.3	5.61 (0.57-55.66)	0.142	3.9	1.39 (0.15-13.3)	0.774	4.3	0.89 (0.04-18.91)	0.941
Low HDL	3.8	Ref	3.0	0.51 (0.06-4.70)	0.554	4.2	2.18 (0.11-42.02)	0.606	0.0	Inf	0.995	0.0	Inf	0.997
High hs-CRP	22.6	Ref	24.2	1.06 (0.48-2.34)	0.877	33.3	1.91 (0.66-5.55)	0.234	23.5	1.03 (0.43-2.51)	0.943	17.4	0.53 (0.13-2.13)	0.375

Abbreviations: FMI, fat mass index; BP, blood pressure; TG, triglycerides; HDL, high-density lipoprotein cholesterol; hs-CRP, high-sensitivity C-reactive protein.

Adjusted for age, sex (except for males and females), study, study center, parental highest education, season, pubertal stage, sedentary, MVPA, depression, total EI, carbohydrate intake, and FMI (except for high FMI).

P-values < 0.05 are highlighted in bold (No correction for multiple testing was performed because of the reduced sample size).

Table S8. Associations between sleep clusters and continuous cardiometabolic risk factors in adolescents only on weekdays

	Good sleep	Delayed sleep phase		Sleep irregularity and variability		Fragmented sleep		Prolonged sleep latency	
Outcomes	**β (95%CI)**	**β (95%CI)**	***P***	**β (95%CI)**	***P***	**β (95%CI)**	***P***	**β (95%CI)**	***P***
Total (1090)									
FMI sex-scaled	Ref	-0.04 (-0.20-0.12)	0.630	0.17 (-0.05-0.40)	0.130	0.15 (0.00-0.30)	0.044	0.39 (0.14-0.64)	**0.002**
SBP scaled	Ref	-0.04 (-0.20-0.12)	0.610	-0.03 (-0.25-0.19)	0.781	-0.02 (-0.16-0.13)	0.818	0.09 (-0.16-0.33)	0.484
DBP scaled	Ref	0.11 (-0.06-0.27)	0.205	0.03 (-0.20-0.25)	0.821	0.03 (-0.12-0.18)	0.676	-0.03 (-0.28-0.22)	0.803
HDL inversely scaled	Ref	-0.08 (-0.24-0.08)	0.299	-0.08 (-0.30-0.15)	0.505	0.17 (0.02-0.31)	0.026	0.05 (-0.20-0.29)	0.708
	MR (95%CI)	**MR (95%CI)**	***P***	**MR (95%CI)**	***P***	**MR (95%CI)**	***P***	**MR (95%CI)**	***P***
TC	Ref	1.02 (0.99-1.05)	0.318	1.02 (0.97-1.06)	0.441	0.99 (0.96-1.02)	0.560	1.02 (0.98-1.07)	0.322
TG	Ref	0.99 (0.93-1.07)	0.874	1.02 (0.93-1.12)	0.680	1.02 (0.96-1.09)	0.519	0.96 (0.87-1.07)	0.496
LDL	Ref	1.02 (0.97-1.07)	0.508	1.00 (0.94-1.07)	0.907	1.01 (0.97-1.06)	0.602	1.07 (0.99-1.15)	0.083
HOMA-IR[a]	Ref	0.99 (0.89-1.10)	0.782	1.17 (0.99-1.37)	0.062	1.01 (0.91-1.12)	0.795	1.04 (0.88-1.23)	0.638
Males (489)									
FMI scaled	Ref	0.02 (-0.25-0.29)	0.881	0.43 (0.09-0.78)	0.014	0.26 (0.04-0.47)	0.022	0.31 (-0.06-0.68)	0.098
SBP scaled	Ref	0.10 (-0.17-0.36)	0.476	-0.03 (-0.38-0.31)	0.847	-0.09 (-0.30-0.13)	0.434	0.05 (-0.32-0.41)	0.801
DBP scaled	Ref	0.34 (0.07-0.60)	0.013	0.14 (-0.20-0.48)	0.420	-0.02 (-0.23-0.20)	0.881	-0.08 (-0.45-0.28)	0.659
HDL inversely scaled	Ref	-0.02 (-0.29-0.25)	0.877	-0.28 (-0.63-0.07)	0.114	0.08 (-0.14-0.30)	0.462	0.09 (-0.28-0.46)	0.624
	MR (95%CI)	**MR (95%CI)**	***P***	**MR (95%CI)**	***P***	**MR (95%CI)**	***P***	**MR (95%CI)**	***P***
TC	Ref	1.01 (0.96-1.06)	0.844	1.03 (0.96-1.10)	0.434	1.00 (0.96-1.04)	0.891	0.97 (0.91-1.04)	0.391
TG	Ref	0.99 (0.89-1.12)	0.931	0.94 (0.81-1.09)	0.420	0.98 (0.90-1.08)	0.750	0.88 (0.75-1.04)	0.127
LDL	Ref	1.00 (0.92-1.09)	0.942	1.01 (0.91-1.12)	0.878	1.04 (0.97-1.11)	0.302	0.99 (0.88-1.10)	0.813
HOMA-IR[a]	Ref	1.02 (0.86-1.20)	0.826	1.14 (0.90-1.45)	0.283	0.95 (0.82-1.10)	0.509	0.91 (0.70-1.17)	0.444
Females (601)									
FMI scaled	Ref	-0.11 (-0.31-0.10)	0.303	-0.06 (-0.37-0.24)	0.676	0.03 (-0.18-0.23)	0.807	0.52 (0.18-0.86)	**0.003**
SBP scaled	Ref	-0.14 (-0.35-0.06)	0.171	-0.03 (-0.32-0.27)	0.867	0.09 (-0.12-0.30)	0.397	0.08 (-0.27-0.42)	0.663
DBP scaled	Ref	-0.07 (-0.28-0.14)	0.524	-0.04 (-0.34-0.26)	0.798	0.14 (-0.07-0.35)	0.204	0.02 (-0.34-0.37)	0.931
HDL inversely scaled	Ref	-0.14 (-0.35-0.07)	0.189	0.07 (-0.24-0.37)	0.676	0.23 (0.02-0.45)	0.032	-0.07 (-0.42-0.29)	0.714
	MR (95%CI)	**MR (95%CI)**	***P***	**MR (95%CI)**	***P***	**MR (95%CI)**	***P***	**MR (95%CI)**	***P***
TC	Ref	1.03 (0.99-1.07)	0.204	1.00 (0.95-1.06)	0.894	0.97 (0.94-1.01)	0.188	1.09 (1.02-1.16)	0.013
TG	Ref	0.99 (0.91-1.08)	0.851	1.06 (0.94-1.21)	0.330	1.03 (0.94-1.12)	0.502	1.03 (0.89-1.20)	0.648
LDL	Ref	1.03 (0.97-1.10)	0.347	1.00 (0.91-1.09)	0.965	0.98 (0.92-1.04)	0.469	1.17 (1.05-1.29)	**0.004**

HOMA-IR[a]	Ref	0.96 (0.83-1.11)	0.612	1.18 (0.94-1.48)	0.147	1.07 (0.92-1.24)	0.406	1.15 (0.92-1.44)	0.220

Abbreviations: FMI, fat mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides; LDL, low-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance; MR, means ratio. HOMA-IR[a]: 240 males and 265 females.

Adjusted for age, sex (except for males and females), study, study center, parental highest education, season, pubertal stage, sedentary, MVPA, depression, fasting status (except for HOMA-IR), total EI, carbohydrate intake, and FMI (except for FMI).

P-values < 0.007 are highlighted in bold, which remained significant after Bonferroni correction using the Nyholt method.

Table S9. Associations between sleep clusters and dichotomous cardiometabolic risk factors in adolescents only on weekdays

	Good sleep		Delayed sleep phase			Sleep irregularity and variability			Fragmented sleep			Prolonged sleep latency		
Outcomes	**%**	**OR (95%CI)**	**%**	**OR (95%CI)**	***P***	**%**	**OR (95%CI)**	***P***	**%**	**OR (95%CI)**	***P***	**%**	**OR (95%CI)**	***P***
Total (1090)														
n		407		210			89			311			73	
High FMI	22.4	Ref	23.3	0.94 (0.62-1.42)	0.775	29.2	1.54 (0.89-2.65)	0.119	27.0	1.45 (1.004-2.10)	0.048	30.1	1.75 (0.97-3.18)	0.064
High BP	18.9	Ref	15.2	0.78 (0.49-1.25)	0.305	20.2	0.85 (0.46-1.57)	0.597	20.6	0.87 (0.58-1.30)	0.499	20.5	0.81 (0.41-1.59)	0.543
High TG	9.6	Ref	10.5	1.23 (0.69-2.20)	0.487	21.3	2.42 (1.25-4.65)	**0.008**	12.9	1.22 (0.73-2.02)	0.449	13.7	1.43 (0.64-3.23)	0.383
Low HDL	6.4	Ref	6.7	1.08 (0.53-2.18)	0.840	9.0	1.11 (0.46-2.70)	0.818	9.3	1.15 (0.63-2.08)	0.656	9.6	1.04 (0.40-2.72)	0.928
High hs-CRP	19.7	Ref	22.4	1.17 (0.76-1.80)	0.479	20.2	0.89 (0.49-1.65)	0.721	19.0	0.85 (0.56-1.28)	0.444	19.2	0.66 (0.33-1.32)	0.239
High HOMA-IR[a]	20.8	Ref	23.6	1.19 (0.64-2.25)	0.581	37.1	2.25 (0.97-5.25)	0.060	27.6	1.45 (0.80-2.62)	0.216	34.2	3.06 (1.24-7.57)	0.016
Male (489)														
n		148		80			41			183			37	
High FMI	20.3	Ref	23.8	1.24 (0.62-2.48)	0.547	31.7	2.14 (0.92-4.96)	0.077	27.9	1.78 (1.01-3.11)	0.045	24.3	1.42 (0.55-3.62)	0.469
High BP	26.4	Ref	21.2	0.79 (0.40-1.56)	0.491	34.1	1.06 (0.47-2.37)	0.891	25.1	0.80 (0.47-1.37)	0.424	21.6	0.58 (0.23-1.49)	0.259
High TG	11.5	Ref	12.5	1.42 (0.56-3.59)	0.455	26.8	2.33 (0.87-6.23)	0.092	15.3	1.24 (0.60-2.53)	0.563	10.8	0.89 (0.25-3.20)	0.853
Low HDL	9.5	Ref	12.5	1.57 (0.62-3.97)	0.345	7.3	0.54 (0.13-2.21)	0.396	12.6	1.17 (0.54-2.49)	0.693	16.2	1.41 (0.44-4.49)	0.560
High hs-CRP	20.9	Ref	18.8	0.74 (0.35-1.59)	0.444	14.6	0.35 (0.12-1.03)	0.058	19.7	0.62 (0.34-1.13)	0.120	21.6	0.58 (0.21-1.61)	0.300
High HOMA-IR[a]	22.4	Ref	21.3	0.93 (0.32-2.71)	0.889	41.2	2.89 (0.77-10.88)	0.119	26.5	1.24 (0.50-3.08)	0.635	29.4	2.03 (0.45-9.08)	0.356
Female (601)														
n		259		130			48			128			36	
High FMI	23.6	Ref	23.1	0.76 (0.45-1.29)	0.308	27.1	1.11 (0.52-2.37)	0.788	25.8	1.17 (0.69-1.97)	0.562	36.1	2.43 (1.08-5.49)	0.033
High BP	14.7	Ref	11.5	0.67 (0.34-1.33)	0.248	8.3	0.56 (0.19-1.72)	0.315	14.1	1.01 (0.52-1.93)	0.986	19.4	1.16 (0.43-3.10)	0.769
High TG	8.5	Ref	9.2	1.10 (0.50-2.41)	0.812	16.7	2.16 (0.85-5.47)	0.105	9.4	1.09 (0.50-2.36)	0.832	16.7	2.62 (0.90-7.63)	0.079
Low HDL	4.6	Ref	3.1	0.63 (0.18-2.19)	0.467	10.4	2.55 (0.77-8.49)	0.126	4.7	0.97 (0.33-2.88)	0.953	2.8	0.40 (0.04-3.63)	0.414
High hs-CRP	18.9	Ref	24.6	1.40 (0.80-2.45)	0.234	25.0	1.69 (0.76-3.73)	0.199	18.0	0.96 (0.53-1.75)	0.896	16.7	0.53 (0.19-1.53)	0.242
High HOMA-IR[a]	19.8	Ref	25.4	1.34 (0.57-3.10)	0.501	33.3	1.84 (0.55-6.22)	0.324	29.4	1.58 (0.67-3.70)	0.296	38.1	4.23 (1.24-14.44)	0.022

Abbreviations: FMI, fat mass index; BP, blood pressure; TG, triglycerides; HDL, high-density lipoprotein cholesterol; hs-CRP, high-sensitivity C-reactive protein; HOMA-IR, homeostatic model assessment of insulin resistance. High HOMA-IR[a]: 240 males and 265 females.

Adjusted for age, sex (except for males and females), study, study center, parental highest education, season, pubertal stage, sedentary, MVPA, depression, fasting status (except for high HOMA-IR), total EI, carbohydrate intake, and FMI (except for high FMI).

P-values < 0.01 are highlighted in bold, which remained significant after Bonferroni adjustment using the Nyholt method.

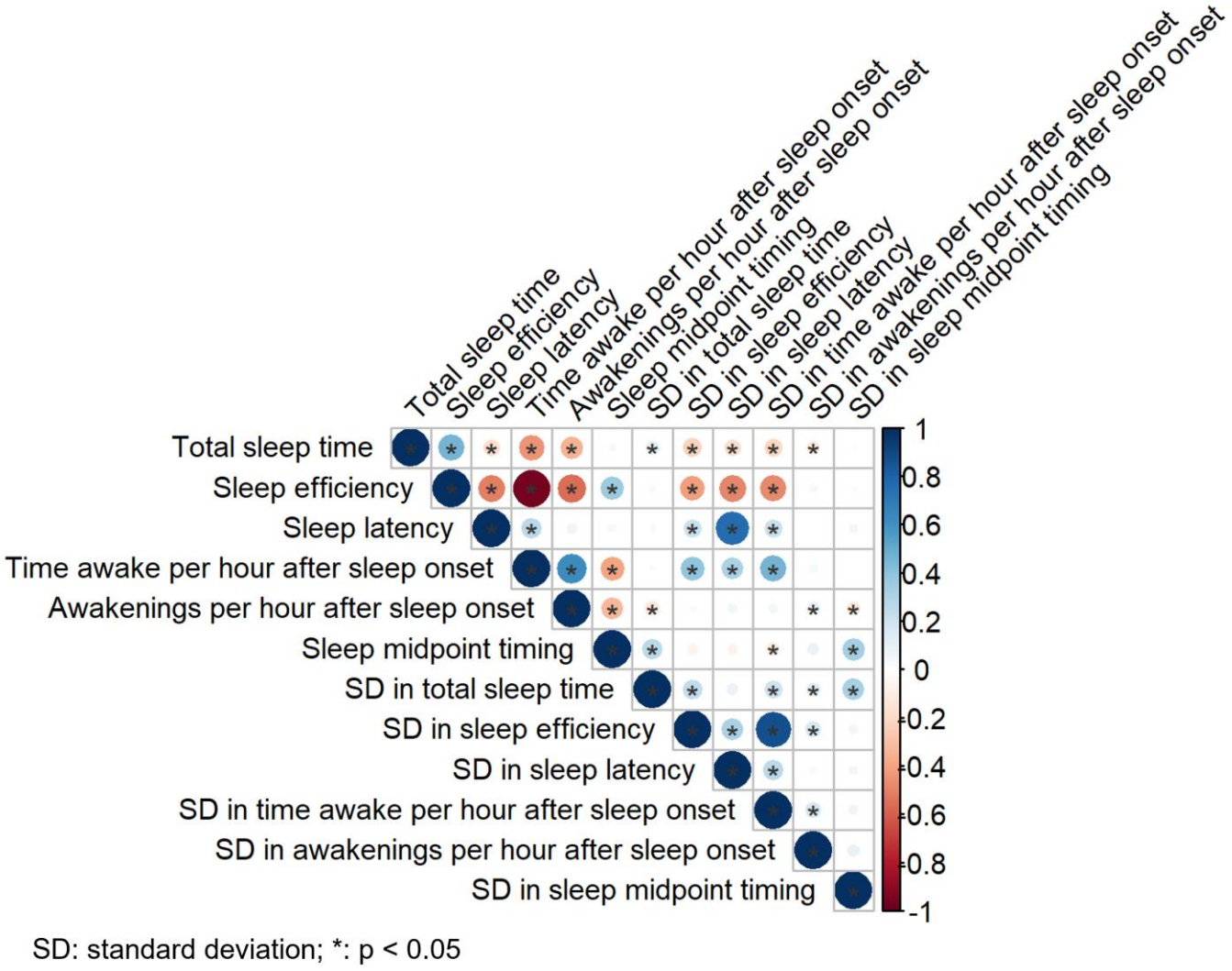

Figure S1. Correlation plot between 12 sleep characteristics

Figure S1 shows the Spearman correlations between 12 standardized sleep characteristics. Dark blue color represents a strong positive correlation and dark red color shows a strong negative correlation. Significant correlation coefficients are indicated by an asterisk. The strong correlations are observed between total sleep time and sleep efficiency (positive), sleep efficiency and time awake per hour after sleep onset (negative), sleep latency and SD in sleep latency (positive), as well as SD in sleep efficiency and SD in time awake per hour after sleep onset (positive).

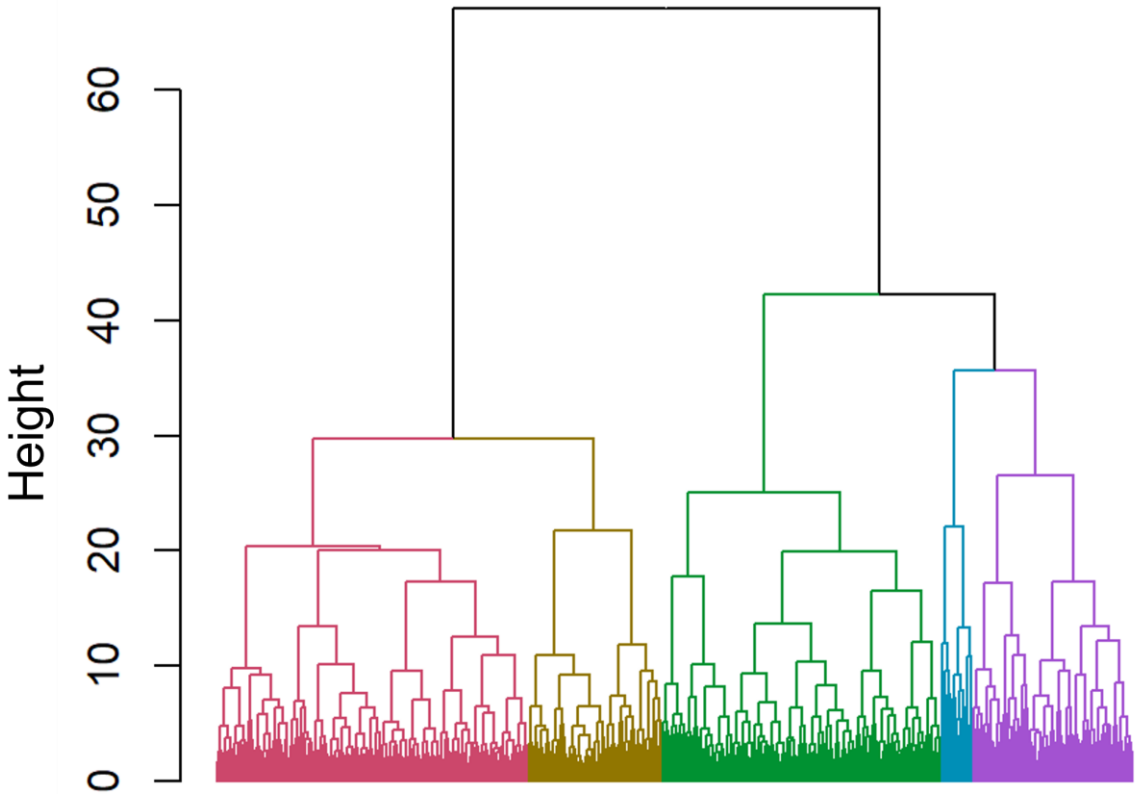

Figure S2. Hierarchical clustering dendrogram based on 12 sleep characteristics

Figure S2 shows the dendrogram of the hierarchical clustering, where Euclidean distance and Ward's linkage (accounting for the total within cluster variance) were applied on 12 standardized sleep characteristics. The distance used to cluster the objects ("height") is shown on the y-axis of the dendrogram. From bottom to top, each observation is initially considered as a cluster of its own, then similar objects are clustered together at an early stage (agglomerative approach). The height increases with the decreased number of clusters, indicating greater heterogeneity among clusters. Five different colors were used to represent the finally determined five clusters.

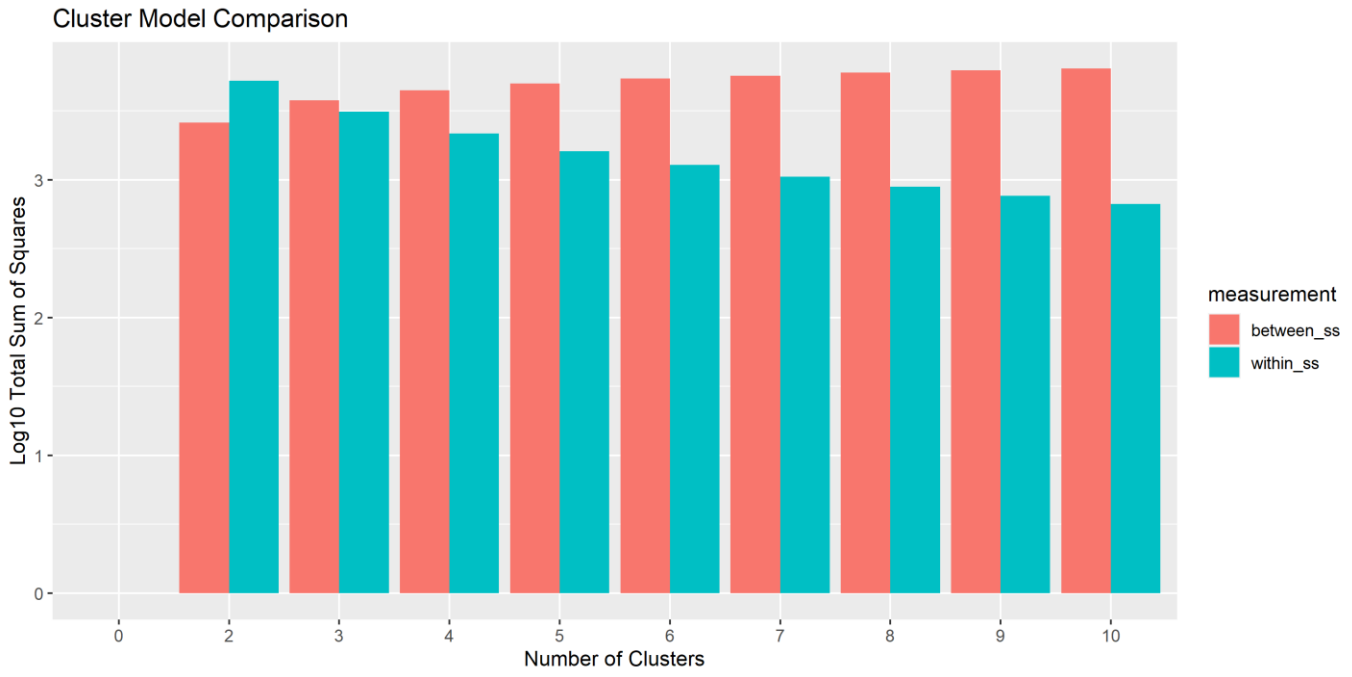

Figure S3. Sum of squares method for k-means cluster model comparison

Figure S3 visualizes results of the sum of squares method, which is a technique used to determine the ideal number of clusters. This method accounts for the within-cluster sum of squares that indicates the level of compactness of each cluster (blue color, the smaller the better), and the between-cluster sum of squares that indicates the level of separation among the clusters (red color, the larger the better). From this figure, it appears that five clusters would be the appropriate choice.

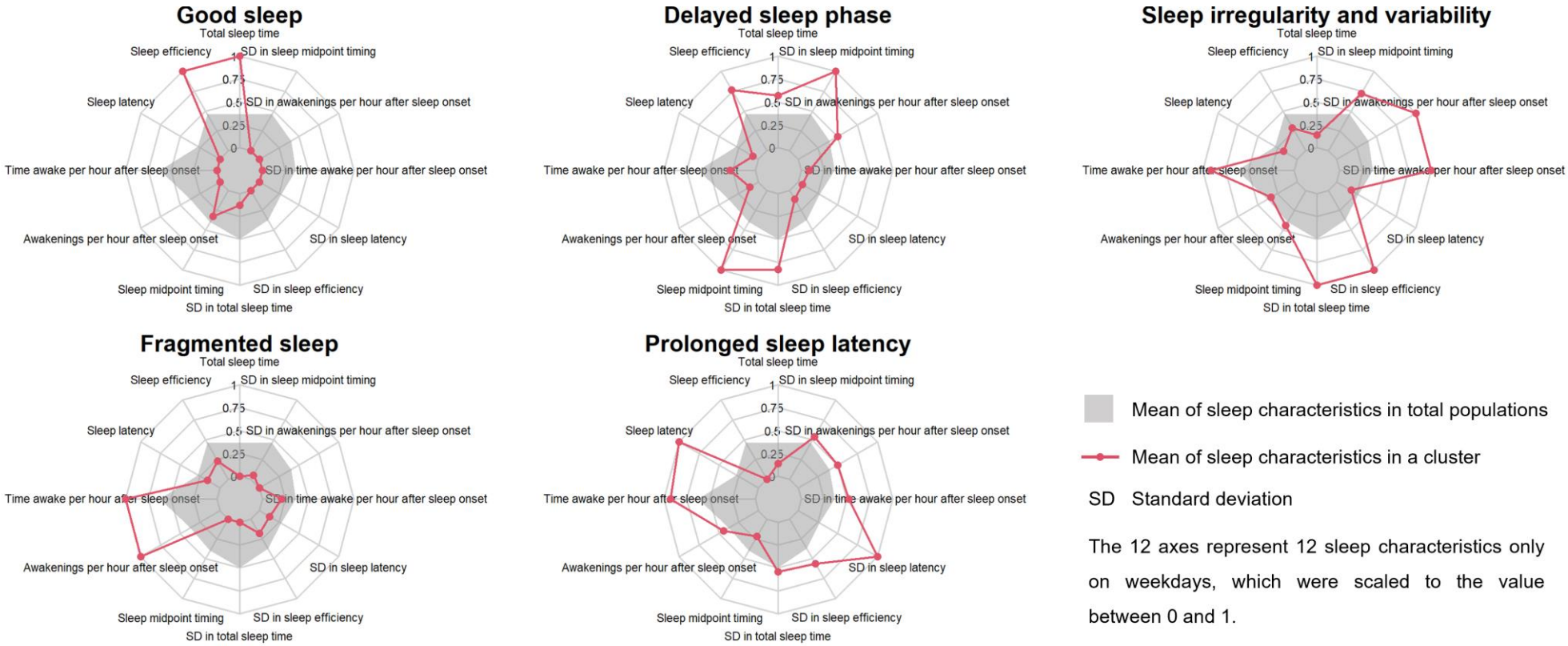

Figure S4. Details of only weekday sleep characteristics in the distribution of the five sleep clusters identified in Figure 2

Figure S4 shows the distributions of only weekday sleep characteristics in the identified five sleep clusters based on sleep characteristics on weekdays and weekend days, whereas **Figure 2** shows the distributions of sleep characteristics across the entire week (including weekdays and weekend) in the identified five sleep clusters based on sleep characteristics on weekdays and weekend days.

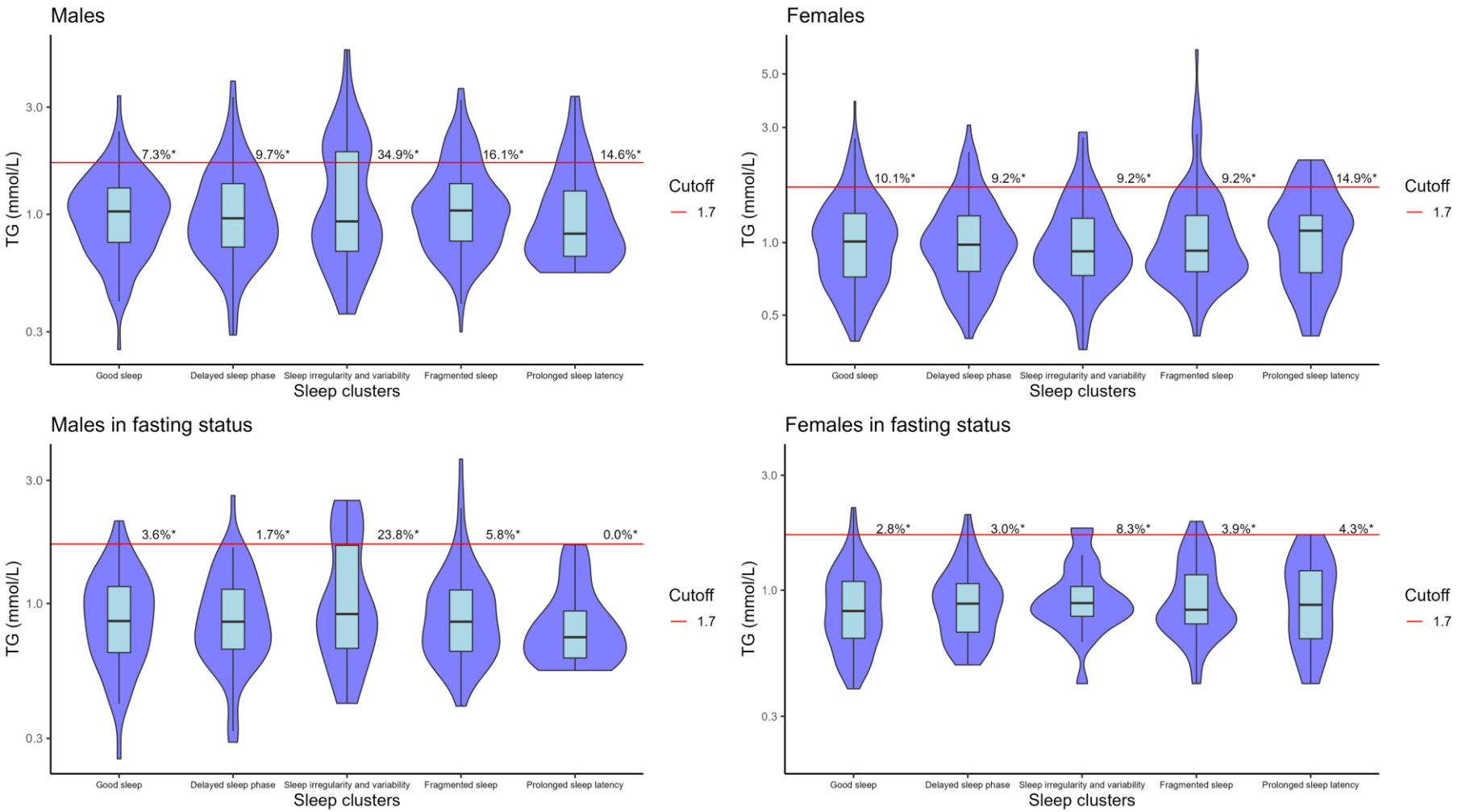

Figure S5. Distributions of triglycerides (TG) in five sleep clusters, stratified by sex and fasting status

Figure S5 illustrates the distributions of triglycerides (TG) in five sleep clusters, stratified by sex and fasting status. The top two violin plots are the distributions of TG in all males, and females, regardless of their fasting status. The bottom two violin plots are the distributions of TG in males, and females, only in fasting status. This figure confirms that the prevalence of high TG in the "sleep irregularity and variability" cluster was significantly higher than in the other sleep clusters, both in overall males and in fasting males.

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Publication III

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ORIGINAL ARTICLE

Accelerometry-assessed sleep clusters and obesity in adolescents and young adults: a longitudinal analysis in GINIplus/LISA birth cohorts

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Abstract

Background Some studies have revealed various sleep patterns in adolescents and adults using multidimensional objective sleep parameters. However, it remains unknown whether these patterns are consistent from adolescence to young adulthood and how they relate to long-term obesity.

Methods Seven-day accelerometry was conducted in German Infant Study on the influence of Nutrition Intervention PLUS environmental and genetic influences on allergy development (GINIplus) and Influence of Lifestyle factors on the development of the Immune System and Allergies in East and West Germany (LISA) birth cohorts during the 15-year and 20-year follow-ups, respectively. Five sleep clusters were identified by k-means cluster analysis using 12 sleep characteristics at each follow-up. Adjusted linear and logistic regression models using generalized estimating equations were examined. Further, the interaction effects with time of follow-ups and polygenic risk scores (PRS) for body mass index (BMI) were tested.

Results Five sleep clusters were classified consistently in both adolescence ($n = 1347$, aged 14.3–16.4 years) and young adulthood ($n = 1262$, aged 19.5–22.4 years). Adolescents in the "good sleep", "delayed sleep phase", and "fragmented sleep" clusters displayed greater stability transitioning into young adulthood, while those in the "sleep irregularity and variability", and "prolonged sleep latency" clusters showed lower stability ($n = 636$). Compared to the "good sleep" cluster, the "prolonged sleep latency" cluster exhibited associations with higher BMI [$\beta = 0.56$, 95% confidence interval (CI) = (0.06, 1.05)] and increased odds of overweight/obesity [Odds ratio = 1.55, 95% CI = (1.02, 2.34)]. No significant PRS-sleep cluster interaction was found for BMI or overweight/obesity. Among males only, the "delayed sleep phase", "sleep irregularity and variability" and "fragmented sleep" clusters showed stronger associations with overweight/obesity as age increased.

Conclusion Adolescents and young adults shared five consistent sleep patterns, with the "prolonged sleep latency" pattern linked to higher BMI and overweight/obesity.

Keywords Accelerometry · Adolescents · Obesity · Sleep clusters · Young adults

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Introduction

Insufficient sleep is associated with increased risks of obesity and cardiovascular diseases [1, 2], prompting its inclusion as an eighth essential factor for cardiovascular health by the American Heart Association in 2022 [3]. Apart from sleep duration [4], other sleep dimensions are recognized as obesity risk factors, such as sleep efficiency [5], variability [6], and timing [7]. These sleep characteristics are interrelated within an individual, making sleep health a multidimensional construct with overlapping components [8].

Some studies have identified diverse sleep patterns in children, adolescents, and adults, using comprehensive approaches by considering multiple objective sleep characteristics [9–13]. Cluster analysis effectively groups similar individuals by accounting for the correlations among various sleep characteristics [8]. Recently, we identified five sleep clusters in German adolescents by K-means cluster analysis across 12 accelerometry-assessed sleep characteristics, and observed an association between "prolonged sleep latency" cluster and higher fat mass index [14]. Another recent study identified three sleep clusters for males and females among Brazil young adults using K-means cluster analysis on seven sleep characteristics measured mainly by accelerometry, with "healthy sleepers" cluster showing lower prevalence of overweight [13]. However, it is poorly understood whether objective sleep patterns change during the transition from adolescence into young adulthood due to substantial physiological, psychological, and environmental shifts [15, 16]. To our knowledge, no study has comprehensively identified sleep patterns with multiple objective sleep characteristics, across both adolescence and young adulthood.

In addition, it is unclear whether objective sleep patterns interact with obesity-related genetic variants [17]. One study reported uncorrected interactions between objective sleep duration and three gene loci affecting body mass index (BMI) in 643 New Zealand children [18]. Furthermore, evidence remains scarce on examining sex differences in relationships between objective sleep characteristics and obesity during the transition from adolescence into young adulthood. A study from USA revealed that subjective short sleep was linked to obesity only in adolescent males, but was linked to incident obesity in both sexes during young adulthood [19].

Therefore, we aimed to investigate longitudinal associations of clustering-identified sleep patterns, using multidimensional accelerometry-assessed sleep characteristics, with BMI and overweight/obesity in adolescence and young adulthood, and to explore sleep interaction effects with time of follow-ups and genetic risk.

Methods

Study population

Data were obtained from two ongoing German birth cohorts, German Infant Study on the influence of Nutrition Intervention PLUS environmental and genetic influences on allergy development (GINIplus) and Influence of Lifestyle factors on the development of the Immune System and Allergies in East and West Germany (LISA). More details are available elsewhere [20–22]. For two cohorts, 1682 participants from Munich and Wesel at the 15-year follow-up (15 y) between 2011 and 2014, and 1595 participants from Munich, Wesel and Bad Honnef at the 20-year follow-up (20 y) between 2016 and 2020, consented to and completed accelerometry measurements.

For the main analyses, a total of 1973 participants at 15 y ($n=1347$) and/or 20 y ($n=1262$) in Munich or Wesel were included, with 636 participants having repeated data at both follow-ups. A subset of 1087 participants ($n=775$ at 15 y; $n=701$ at 20 y) with genetic data were used for genetic analysis, including 389 with repeated exposures and outcomes. Participants were included if they had at least three weekdays and one weekend day of valid accelerometry data for ≥ 10 hours/day. Figure 1 offers detailed inclusion criteria. Local ethics committees approved both cohorts, and all participants and their parents wrote informed consents.

Sleep assessment, characteristics, and clusters

Accelerometry

A validated triaxial accelerometer (ActiGraph GT3X, Pensacola, Florida) was applied at both follow-ups [23, 24]. Participants wore accelerometers on non-dominant hand-side wrists at night for assessing sleep, and on dominant hips during the day for measuring physical activity (PA), for seven consecutive days during a regular school/work week. Participants also kept sleep diaries to record the times they went to bed and got up, corresponding with the transition of the accelerometer from hip to wrist in the evening and back in the morning. Accelerometry measurements details are available elsewhere [25–27].

Objective sleep characteristics

Accelerometry-assessed sleep data were analyzed with ActiLife software (firmware 4.4.0; version 5.5.5 at 15 y; version 6.11.2 at 20 y) based on the Sadeh algorithm [28]. Accelerations were sampled at 30 Hz, converted to proprietary "activity count units", and stored at 1 Hz, aggregated over one-second intervals. The "probability of sleep" was

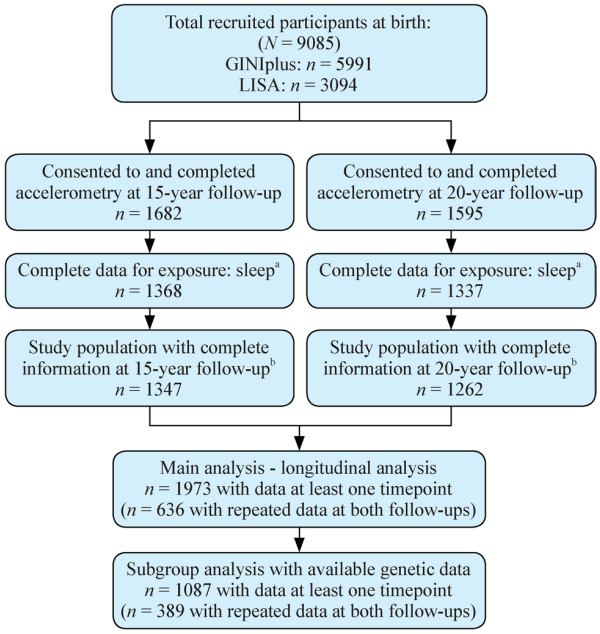


Fig. 1 Flow chart of participants. *GINIplus* German Infant Study on the influence of Nutrition Intervention PLUS environmental and genetic influences on allergy development, *LISA* Influence of Lifestyle factors on the development of the Immune System and Allergies in East and West Germany. **a**: Inclusion for sleep data: (1) at least three weekdays and one weekend day of valid accelerometry recording for ≥ 10 hours/day; (2) 2 hours ≤ daily total sleep time ≤ 15 hours; (3) daily sleep efficiency ≥ 20%; (4) daily sleep onset timing between 17:00 and 08:00. **b**: Inclusion for participants: (1) participants in Munich and Wesel study centers; (2) complete outcomes; (3) complete confounders, except for total energy intake

calculated as a score centered around zero for each minute of recorded time-in-bed. A score was classified as "asleep" if it was zero or positive, and "awake" if negative. Six sleep characteristics were generated per valid night: (1) total sleep time (hours), algorithm-scored total "asleep" minutes divided by 60; (2) sleep efficiency (%), ratio of algorithm-scored "asleep" minutes to diary-recorded total time in bed; (3) sleep midpoint timing (24-hour clock), first algorithm-scored minute of "asleep" plus half of total sleep time, converted to 24-hour clock; (4) sleep latency (minutes), total number of minutes between diary-recorded timing of going to bed and the first algorithm-scored minute of "asleep"; (5) time awake per hour after sleep onset (minutes/hour), total algorithm-scored awake minutes after sleep onset, divided by hours spent in bed after sleep onset; and (6) awakenings per hour after sleep onset (numbers/hour), total algorithm-scored different awakening episodes after sleep onset, divided by hours spent in bed after sleep onset. Six average sleep characteristics were computed as mean value across all valid days, and their day-to-day variability was computed as standard deviation (SD). Twelve sleep characteristics were included for cluster analysis: (averaged) total sleep time (TST), sleep efficiency (SE), sleep latency (SL), time awake per hour after sleep onset (WASO/h), awakenings per hour after sleep onset (awakenings/h), sleep midpoint timing (SMT), SD in total sleep time (SD in TST), SD in sleep efficiency (SD in SE), SD in sleep latency (SD in SL), SD in time awake per hour after sleep onset (SD in WASO/h), and SD in awakenings per hour after sleep onset (SD in awakenings/h), SD in sleep midpoint timing (SD in SMT).

Sleep clusters

Five sleep clusters at 15 y were identified previously: good sleep, delayed sleep phase, sleep irregularity and variability, fragmented sleep, and prolonged sleep latency [14]. Sleep cluster at 20 y were identified in the current study.

Subjective sleep characteristics included reported time in bed, sleep quality, sleep difficulties, difficulty falling asleep, difficulty staying asleep. More detailed definitions are presented in Supplementary Method S1.

Body mass index measurements

At 15 y, participants' body weight (kg) and height (m) were measured objectively by physical examinations ($n = 1197$) and parent-reported by questionnaires ($n = 150$). At 20 y, participants self-reported weight and height by questionnaires. BMI was calculated as weight divided by height squared (kg/m^2). The objective BMI strongly correlated with subjective BMI (Pearson coefficient = 0.95) at 15 y, and BMI z-scores were computed following World Health Organization references [29]. BMI were categorized into overweight/obesity and non-overweight/obesity, defined as BMI z-scores > 1 and ≤ 1 for adolescents [29], and BMI ≥ 25 and < 25 kg/m^2 for adults [30], respectively.

Confounders

Potential confounders included time of follow-ups (15 y and 20 y), the time-independent variables: sex, study (GINI observation arm, GINI intervention arm, and LISA study), study center (Munich and Wesel), parental highest education level (low/medium: ≤ 10th grade; high: > 10th grade); and the time-dependent variables: age at BMI measurements, BMI measurement methods (examination vs. questionnaire at 15 y; questionnaire at 20 y), season of sleep measurements (spring, summer, autumn, and winter), accelerometry-measured sedentary behavior, moderate-to-vigorous physical activity (MVPA), and total energy intake (kcal) at two follow-ups, respectively. In both follow-ups, sedentary, MVPA [25] and total energy intake were obtained using the same protocol. Accelerometry-measured PA was categorized into sedentary (by Aguilar-Farías [31]), light, moderate, and

vigorous PA using triaxial cutoffs by Romanzini [32], with moderate and vigorous PA combined into MVPA. This study used the averaged sedentary (hours) and MVPA (minutes) from all valid days. Total energy intake was computed from a self-administered food frequency questionnaire [33, 34], with missing values ($n = 284$ at 15 y; $n = 243$ at 20 y) imputed using a linear regression model based on sex, age, study, study center, and parental highest education [35].

Polygenic risk score for BMI

Genotyping for GINIplus and LISA was conducted using Affymetrix Chip 5.0 and 6.0 (Thermo Fisher, USA) in Munich and Infinium Global Screening Array GSA v2 MD (Illumina, USA) in Wesel. Quality control and genotype imputation details were previously published [36, 37]. PRS for BMI were computed according to 97 genome-wide significant single nucleotide polymorphisms (SNPs) [38]. Of the 97 SNPs, 95 SNPs available in Munich, and 96 SNPs available in Wesel were included for polygenic risk score (PRS) calculation, respectively [39]. Standardized PRS were used, with higher values indicating increased risk for high BMI. SNPs lists can be found previously [39].

Statistical analysis

At both follow-ups, 12 sleep characteristics were first standardized, then tested for their Spearman correlation, followed by hierarchical cluster analysis using Euclidean distance and Ward's linkage (Ward.D2), and k-means cluster analysis. Details on sleep cluster number selection at 15 y has been described previously [14]. The final number of sleep clusters at 20 y was determined to be five after evaluating: (1) interpretation of k-means results; (2) results of principal component analysis (Supplementary Table S1, where five components balance the criteria of eigenvalues > 1 and a cumulative variance > 80%); (3) dendrogram of hierarchical clustering (Supplementary Fig. S1); and (4) visualized results from sum of squares method (Supplementary Fig. S2). The principal component analysis plots (Supplementary Fig. S3) visually validated similar clustering patterns at 15 y and 20 y, according to a systematic framework [40]. In the final K-means cluster analysis at each follow-up, five clusters were designated, using 50 random initial centroids. In addition, the K-means cluster analyses were limited to participants with data available at both time points ($n = 636$) to test the agreement of sleep pattern classification between the full dataset and the subsample. Differences in characteristics by sex and by sleep clusters were assessed using one-way analysis of variance and Kruskal–Wallis rank sum test for continuous variables, and Chi-square test for categorical variables, followed by Bonferroni-adjusted post-hoc tests.

Linear and logistic regression models using generalized estimating equations (GEE) were used to evaluate longitudinal associations of sleep clusters with BMI and overweight/obesity. GEE models can estimate population-averaged effects across repeated measurements and provide robust estimates, even with only one time-point data for some participants. Boxplot inspection identified BMI outliers, none of which were excluded. Three models were examined: Model 1 was adjusted for time of follow-ups, sex, age, study, study center, parental highest education, and BMI measurement methods; Model 2 was further adjusted for season, sedentary, MVPA, and total energy intake; Model 3 was Model 2 plus PRS interaction term with sleep clusters. The results were presented as β with 95% confidence interval (CI), and odds ratio (OR) with 95%CI, respectively. $P < 0.05$ was considered statistically significant.

Furthermore, the interaction effects of sleep clusters with sex and time of follow-ups were examined, followed by sex- and time-stratified analyses (cross-sectional analyses at two time-points). Four sensitivity analyses were conducted: (1) including participants with repeated sleep and BMI data at both follow-ups ($n = 636$); (2) excluding participants with missing total energy intake; (3) excluding participants with parent-reported BMI at 15 y; (4) determining sleep clusters by sleep characteristics excluding weekends (the nights from Friday to Saturday and Saturday to Sunday). All statistical analyses were conducted in *R* (version 4.3.1) [41].

Results

Table 1 presents participants characteristics overall and by sex, in adolescence and young adulthood. In adolescence, males had higher prevalence of having overweight/obesity than females, yet no significant difference between sexes was observed in young adulthood. From adolescence to young adulthood, averaged TST decreased (7.2–6.6 hours), SE increased (79.3%–84.4%), SMT was one hour later (2:36–3:36) and SL was shortened (18.7–6.8 minutes, Table 2). Day-to-day variability in six sleep characteristics exhibited minimal changes, except for a decrease of SD in SL (14.9–6.7 minutes). In addition, females had higher TST than males (7.3 vs. 7.0 hours in adolescence; 6.8 vs. 6.5 hours in young adulthood). More details can be found in Supplementary Table S2.

Five sleep clusters in young adulthood were identified and consistent with those during adolescence [14], and named by their unique parameters: (1) "good sleep", marked by higher TST and SE; (2) "delayed sleep phase", distinguished by later SMT, higher SE, and SD in SMT; (3) "sleep irregularity and variability", characterized by higher SD in most sleep characteristics and higher WASO/h; (4) "fragmented sleep", demonstrating longer WASO/h and more frequent

Table 1 Participants characteristics in adolescence and young adulthood

Characteristics	Adolescence				Young adulthood			
	Total	Male	Female	*P* value	Total	Male	Female	*P* value
N	1347	611	736		1262	491	771	
Age, y	15.2 ± 0.3	15.2 ± 0.3	15.2 ± 0.3	0.397	20.2 ± 0.4	20.3 ± 0.4	20.2 ± 0.4	**< 0.001**
Study, *n* (%)				0.238				**0.036**
GINIplus observation	497 (36.9)	217 (35.5)	280 (38.0)		488 (38.7)	174 (35.4)	314 (40.7)	
GINIplus intervention	509 (37.8)	226 (37.0)	283 (38.5)		480 (38.0)	185 (37.7)	295 (38.3)	
LISA	341 (25.3)	168 (27.5)	173 (23.5)		294 (23.3)	132 (26.9)	162 (21.0)	
Study center, *n* (%)				**0.016**				**0.003**
Munich	818 (60.7)	393 (64.3)	425 (57.7)		762 (60.4)	322 (65.6)	440 (57.1)	
Wesel	529 (39.3)	218 (35.7)	311 (42.3)		500 (39.6)	169 (34.4)	331 (42.9)	
Weight, kg	61.1 ± 11.0	63.9 ± 11.8	58.7 ± 9.7	**< 0.001**	68.7 ± 12.6	76.6 ± 10.9	63.8 ± 11.0	**< 0.001**
Height, cm	171.4 ± 8.1	176.2 ± 7.4	167.4 ± 6.3	**< 0.001**	174.5 ± 9.6	183.2 ± 6.8	169.0 ± 6.4	**< 0.001**
BMI, kg/m^2	20.7 ± 3.0	20.5 ± 3.1	20.9 ± 3.0	**0.022**	22.5 ± 3.3	22.8 ± 2.9	22.3 ± 3.5	**0.009**
BMI z-score	0.1 ± 1.0	0.0 ± 1.1	0.1 ± 0.9	0.661				
Overweight/obesity, *n* (%)				**0.004**				0.635
No	1120 (83.1)	488 (79.9)	632 (85.9)		1040 (82.4)	401 (81.7)	639 (82.9)	
Yes	227 (16.9)	123 (20.1)	104 (14.1)		222 (17.6)	90 (18.3)	132 (17.1)	
BMI measurement methods, *n* (%)				0.342				NA
Examination	1197 (88.9)	537 (87.9)	660 (89.7)					
Questionnaire	150 (11.1)	74 (12.1)	76 (10.3)		1262 (100.0)	491 (100.0)	771 (100.0)	
Sleep clusters, *n* (%)				**< 0.001**				**< 0.001**
Good sleep	440 (32.7)	154 (25.2)	286 (38.8)		389 (30.8)	103 (21.0)	286 (37.1)	
Delayed sleep phase	245 (18.2)	101 (16.5)	144 (19.6)		330 (26.2)	133 (27.1)	197 (25.6)	
Sleep irregularity and variability	130 (9.6)	54 (8.8)	76 (10.3)		132 (10.5)	60 (12.2)	72 (9.3)	
Fragmented sleep	424 (31.5)	249 (40.8)	175 (23.8)		340 (26.9)	158 (32.2)	182 (23.6)	
Prolonged sleep latency	108 (8.0)	53 (8.7)	55 (7.5)		71 (5.6)	37 (7.5)	34 (4.4)	
Season, *n* (%)				0.326				0.454
Spring	355 (26.4)	168 (27.5)	187 (25.4)		349 (27.7)	129 (26.3)	220 (28.5)	
Summer	198 (14.7)	79 (12.9)	119 (16.2)		342 (27.1)	129 (26.3)	213 (27.6)	
Autumn	437 (32.4)	205 (33.6)	232 (31.5)		297 (23.5)	127 (25.9)	170 (22.1)	
Winter	357 (26.5)	159 (26.0)	198 (26.9)		274 (21.7)	106 (21.6)	168 (21.8)	
Total energy intake, kcal/day	2076.4 ± 647.6	2374.5 ± 645.0	1851.5 ± 552.1	**< 0.001**	1776.3 ± 661.1	2146.2 ± 690.7	1583.6 ± 555.3	**< 0.001**
Sedentary behavior, hours	8.3 ± 1.4	8.0 ± 1.5	8.5 ± 1.3	**< 0.001**	8.4 ± 1.5	8.3 ± 1.7	8.4 ± 1.5	0.762
MVPA, minutes	50.8 ± 27.1	57.7 ± 27.3	45.2 ± 25.7	**< 0.001**	46.2 ± 23.7	48.9 ± 23.6	44.6 ± 23.6	**0.002**
Parental highest education, *n* (%)				0.748				**0.017**
Low/medium	395 (29.3)	176 (28.8)	219 (29.8)		355 (28.1)	119 (24.2)	236 (30.6)	
High	952 (70.7)	435 (71.2)	517 (70.2)		907 (71.9)	372 (75.8)	535 (69.4)	

The results are presented as mean ± standard deviation or *n* (%). *BMI* body mass index, *MVPA* moderate-to-vigorous physical activity, *GINIplus* German Infant Study on the influence of Nutrition Intervention PLUS environmental and genetic influences on allergy development; *LISA* Influence of Lifestyle factors on the development of the Immune System and Allergies in East and West Germany

Overweight/obesity: BMI z-score > 1 for adolescents; BMI ≥ 25 kg/m^2 for adults according to World Health Organization

The number of participants with available total energy intake: $n = 1063$ in adolescence; $n = 1019$ in young adulthood. *P* value: one-way analysis of variance was used for continuous variables, and Chi-square test was used for categorical variables. *P* values < 0.05 were highlighted in bold

Table 2 Sleep characteristics in the total population and in five sleep clusters during adolescence and young adulthood

Sleep characteristics	Total		Sleep clusters										
			Good sleep		Delayed sleep phase		Sleep irregularity and variability		Fragmented sleep		Prolonged sleep latency		*p value
	Mean	Median (IQR)	Mean	Median (IQR)	Mean	Median (IQR)	Mean	Median (IQR)	Mean	Median (IQR)	Mean	Median (IQR)	
Adolescence (14–16 y), *n* (%)	**1347**		**440 (32.7)**		**245 (18.2)**		**130 (9.6)**		**424 (31.5)**		**108 (8.0)**		
Averages across all valid days													
TST, h	7.2	7.2 (0.9)	7.5	7.5 (0.7)[a]	7.2	7.2 (0.9)[b]	6.9	6.8 (0.8)[c]	6.9	6.9 (0.7)[c]	7.0	7.0 (0.9)[c]	<0.001
SE, %	79.3	79.8 (8.0)	84.4	84.2 (4.2)[a]	82.4	82.3 (5.3)[b]	74.8	75.7 (7.0)[cd]	75.5	76.1 (5.3)[c]	72.6	73.2 (6.9)[d]	<0.001
SL, min	18.7	15.9 (13.4)	14.1	12.3 (9.9)[a]	16.5	14.7 (11.8)[b]	18.9	17.6 (11.3)[b]	17.8	16.8 (10.8)[b]	45.4	42.4 (17.2)[c]	<0.001
WASO/h, min/h	10.6	10.2 (4.5)	7.9	8.0 (2.5)[a]	8.9	9.0 (2.5)[b]	13.4	12.9 (4.1)[c]	13.1	12.8 (3.1)[c]	12.6	12.2 (4.0)[c]	<0.001
Awakenings/h, numbers/h	2.9	2.9 (0.8)	2.5	2.6 (0.7)[a]	2.6	2.6 (0.7)[a]	2.8	2.8 (0.6)[b]	3.4	3.3 (0.6)[c]	2.9	2.9 (0.7)[b]	<0.001
SMT, 24-h clock	2:36	2:36 (54 min)	2:36	2:30 (42 min)[a]	3:18	3:18 (48 min)[b]	2:30	2:30 (48 min)[a]	2:12	2:12 (42 min)[c]	2:30	2:36 (54 min)[a]	<0.001
Day-to-day variability across all valid days													
SD in TST, min	61.4	57.6 (38.1)	51.0	50.1 (28.8)[a]	84.5	81.3 (38.8)[b]	86.8	84.9 (39.8)[b]	49.6	48.2 (27.6)[a]	66.9	59.7 (38.3)[c]	<0.001
SD in SE, %	5.6	5.0 (3.0)	4.2	4.0 (2.1)[a]	5.1	4.9 (2.4)[b]	11.1	10.7 (3.8)[c]	5.1	5.0 (2.4)[b]	7.5	7.2 (2.9)[d]	<0.001
SD in SL, min	14.9	11.7 (12.2)	10.3	8.5 (7.9)[a]	12.5	10.8 (10.1)[b]	16.5	15.1 (13.7)[c]	13.5	12.3 (9.9)[bc]	42.7	38.7 (18.8)[d]	<0.001
SD in WASO/h, min/h	3.3	3.0 (1.9)	2.5	2.4 (1.3)[a]	3.0	2.9 (1.6)[b]	6.8	6.4 (2.1)[c]	3.1	3.0 (1.5)[b]	4.3	4.1 (2.1)[d]	<0.001
SD in Awakenings/h, numbers/h	0.6	0.5 (0.3)	0.5	0.5 (0.2)[a]	0.6	0.6 (0.3)[b]	0.7	0.6 (0.4)[b]	0.5	0.5 (0.2)[ac]	0.6	0.6 (0.3)[bc]	<0.001
SD in SMT, min	67.5	64.4 (35.8)	56.3	55.4 (26.3)[a]	96.1	92.9 (37.3)[b]	75.0	73.9 (39.3)[c]	59.3	59.3 (31.1)[a]	71.3	67.3 (37.3)[c]	<0.001
Young adulthood (19–22 y), *n* (%)	**1262**		**389 (30.8)**		**330 (26.2)**		**132 (10.5)**		**340 (26.9)**		**71 (5.6)**		
Averages across all valid days													
TST, h	6.6	6.6 (1.0)	7.1	7.1 (0.8)[a]	6.7	6.7 (0.9)[b]	6.3	6.3 (0.8)[c]	6.4	6.4 (0.9)[c]	6.3	6.2 (1.0)[c]	<0.001
SE, %	84.4	85.2 (7.5)	88.4	88.3 (4.1)[a]	87.7	87.7 (4.3)[a]	80.0	80.5 (6.1)[b]	79.8	80.4 (4.7)[b]	76.8	78.6 (7.7)[b]	<0.001
SL, min	6.8	5.2 (5.8)	5.0	4.2 (4.3)[a]	5.4	4.6 (4.5)[a]	7.6	7.6 (6.2)[b]	6.6	6.0 (5.6)[b]	22.4	21.1 (8.5)[c]	<0.001
WASO/h, min/h	8.6	8.2 (4.3)	6.4	6.4 (2.5)[a]	6.8	6.9 (2.5)[a]	11.2	10.9 (3.3)[b]	11.5	11.1 (2.8)[b]	11.6	10.8 (5.4)[b]	<0.001
Awakenings/h, numbers/h	2.9	2.9 (1.0)	2.5	2.6 (0.7)[a]	2.5	2.5 (0.8)[a]	2.9	2.9 (0.8)[b]	3.6	3.5 (0.6)[c]	3.1	3.1 (0.9)[b]	<0.001
SMT, 24-h clock	3:36	3:30 (84 min)	3:24	3:18 (78 min)[a]	4:06	4:00 (84 min)[b]	3:42	3:36 (78 min)[c]	3:18	3:12 (66 min)[a]	3:42	3:30 (72 min)[ac]	<0.001
Day-to-day variability across all valid days													
SD in TST, min	64.8	61.1 (36.5)	50.2	48.2 (27.0)[a]	81.6	79.6 (35.1)[b]	84.3	81.4 (38.4)[b]	56.3	53.4 (28.9)[c]	70.9	62.3 (32.6)[d]	<0.001
SD in SE, %	4.9	4.4 (2.7)	3.4	3.1 (1.7)[a]	4.3	4.1 (1.9)[b]	9.9	9.0 (2.9)[c]	5.0	4.9 (2.0)[d]	6.7	6.0 (3.4)[e]	<0.001
SD in SL, min	6.7	5.2 (5.0)	4.7	4.0 (3.3)[a]	5.3	4.6 (4.3)[a]	8.8	8.1 (8.2)[b]	6.3	5.7 (4.3)[c]	22.8	19.9 (7.7)[d]	<0.001
SD in WASO/h, min/h	2.9	2.5 (1.5)	2.0	1.9 (1.0)[a]	2.5	2.4 (1.1)[b]	5.8	5.3 (1.7)[c]	3.0	3.0 (1.3)[d]	3.3	3.1 (1.7)[d]	<0.001

Table 2 (continued)

Sleep characteristics	Total		Sleep clusters									
			Good sleep		Delayed sleep phase		Sleep irregularity and variability		Fragmented sleep		Prolonged sleep latency	*p value
	Mean	Median (IQR)	Mean	Median (IQR)	Mean	Median (IQR)	Mean	Median (IQR)	Mean	Median (IQR)	Mean	Median (IQR)	
SD in Awakenings/h, numbers/h	0.6	0.6 (0.3)	0.5	0.5 (0.2)[a]	0.7	0.6 (0.3)[b]	0.9	0.9 (0.4)[c]	0.6	0.6 (0.3)[b]	0.7	0.6 (0.3)[b]	< 0.001
SD in SMT, min	70.4	65.2 (46.0)	49.9	47.9 (29.2)[a]	94.5	90.8 (41.8)[b]	93.0	88.3 (53.0)[b]	61.9	57.7 (37.4)[c]	69.5	65.1 (42.9)[c]	< 0.001

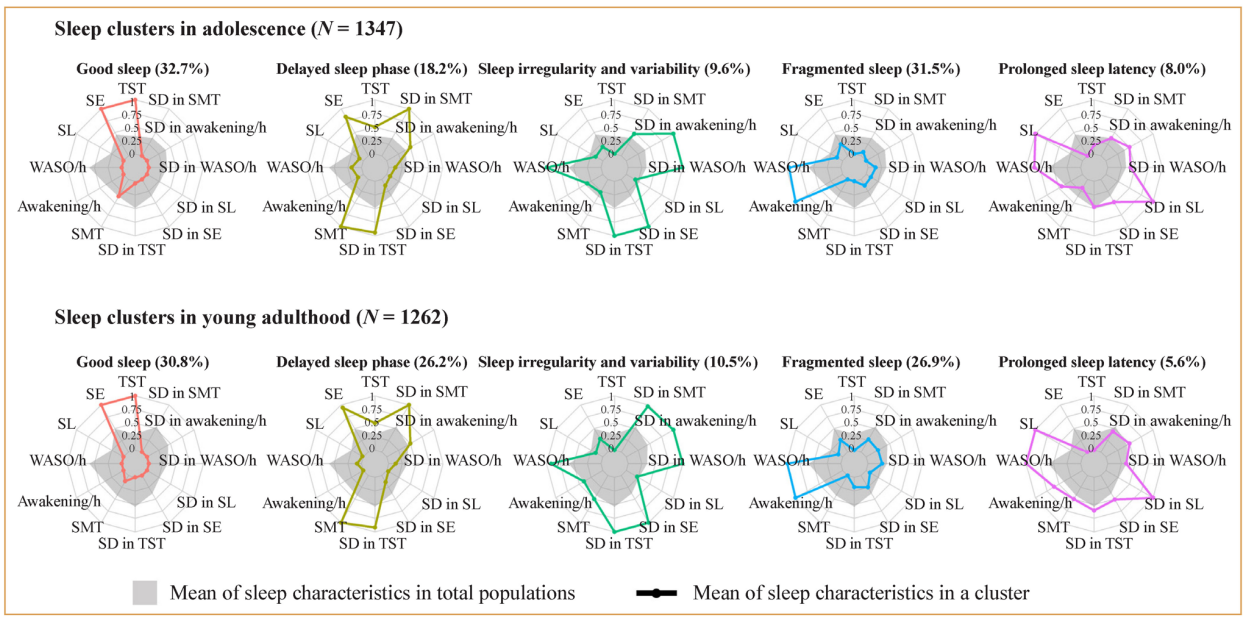
The results are presented as mean and median (IQR) of sleep characteristics, given that most of them did not follow a normal distribution. *P* value: Kruskal–Wallis test with Dunn post hoc tests and Bonferroni adjustment. Sharing the same letter (a b c d e) are considered not significantly different (adjusted *P* values < 0.05). *Awakenings/h* awakenings per hour after sleep onset, *IQR* interquartile range, *SD* standard deviation, *SE* sleep efficiency, *SL* sleep latency, *SMT* sleep midpoint timing, *TST* total sleep time, *WASO/h* time awake per hour after sleep onset

awakenings/h; and (5) "prolonged sleep latency", exhibiting higher SL, SD in SL, and WASO/h (Table 2 and Fig. 2a). Supplementary Table S3 demonstrates that, in adolescence, the "good sleep" and "delayed sleep phase" clusters subjectively reported shorter time in bed with higher sleep quality, while the "prolonged sleep latency" cluster had the opposite. In young adulthood, the "delayed sleep phase" cluster reported the lowest time in bed. Supplementary Table S2 and Supplementary Table S4 display sex-stratified sleep characteristics and participants characteristics in five sleep clusters. Figure 2b and Supplementary Table S5 illustrate sleep clusters transition from adolescence to young adulthood among participants with repeated data ($n = 636$). Adolescents within the "good sleep", "delayed sleep phase", and "fragmented sleep" clusters were more stable into the same cluster during the transition (consistency rate ≥ 40%). Conversely, adolescents within the "sleep irregularity and variability", and "prolonged sleep latency" clusters showed lower stability (consistency rate < 10%). Similar transition patterns were found in both sexes. Furthermore, the re-identified sleep clusters specifically among participants with repeated data showed strong agreement with the original analysis (Cohen's kappa = 0.87 in adolescence, 0.78 in young adulthood), supporting the robustness and representativeness of the sleep pattern classifications (Supplementary Table S6).

In GEE regression models, compared to the "good sleep" cluster, the "prolonged sleep latency" cluster was associated with higher BMI [$\beta = 0.56$, 95% CI = (0.06, 1.05)] and increased odds of having overweight/obesity [OR = 1.55, 95% CI = (1.02, 2.34)], after adjustment for confounders (Model 2, Table 3). In addition, PRS was associated with higher BMI [$\beta = 0.65$, 95% CI = (0.39, 0.91)] and overweight/obesity [OR = 1.52, 95% CI = (1.15, 2.00)], yet had no significant gene-sleep cluster interaction (Model 3, Table 3). No interaction between sleep clusters and sex was detected (P-interaction > 0.05, Supplementary Table S7).

No significant interaction of time of follow-ups with sleep clusters was found in the total population and in females (Supplementary Table S8). However, among males, significant interactions were observed between time of follow-ups and the "delayed sleep phase" cluster on BMI, as well as time of follow-ups with "delayed sleep phase", "sleep irregularity and variability" and "fragmented sleep" clusters on overweight/obesity (P-interaction < 0.05). Figure 3 shows the visualizations of marginal means for BMI and prevalence of overweight/obesity in five sleep clusters among males and females. These visualizations also incorporate interaction terms between sleep clusters and time of follow-ups from GEE models. In males only, marginal BMI means in the "delayed sleep phase" cluster, and overweight/obesity prevalence in "delayed sleep phase", "sleep irregularity and variability" and "fragmented sleep" clusters display higher values

a Sleep characteristics in each of five clusters in adolescence and young adulthood



b Transitions of sleep clusters from adolescence to young adulthood (*N* = 636)

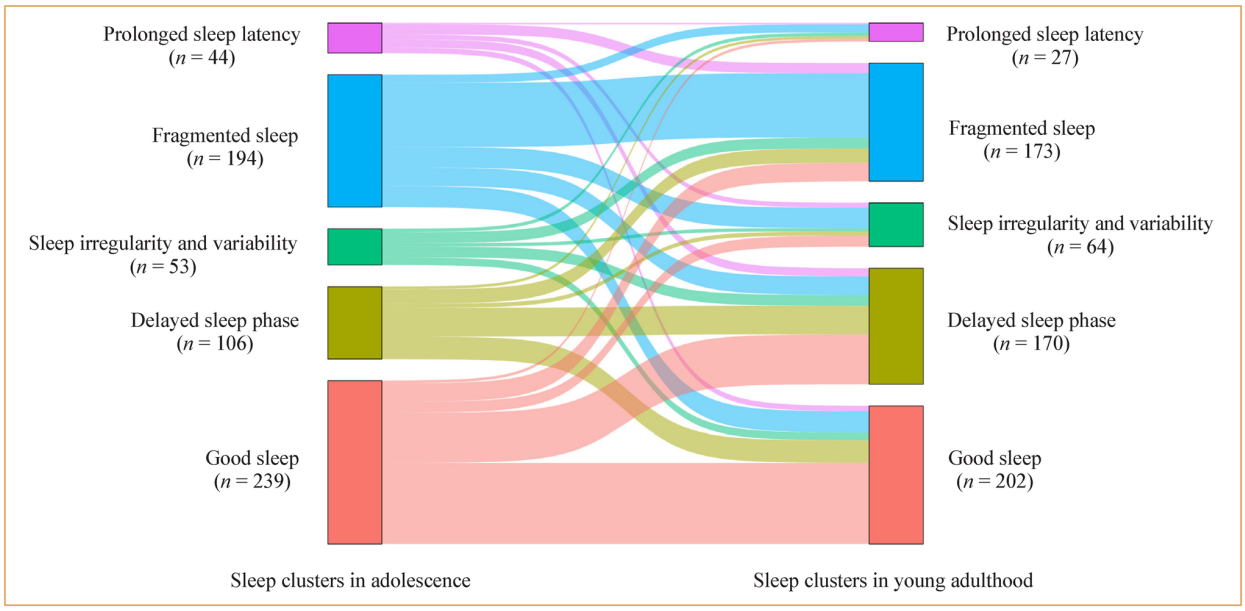


Fig. 2 Five sleep clusters and their transitions in adolescence and young adulthood. **a**, Sleep characteristics in each of five clusters in adolescence and young adulthood. The 12 axes represent 12 sleep characteristics, which were scaled to the value between 0 and 1. Five colors represent five sleep clusters (same as b). *Awakenings/h* awakenings per hour after sleep onset, *SD* standard deviation, *SE* sleep efficiency, *SL* sleep latency, *SMT* sleep midpoint timing, *TST* total sleep time, *WASO/h* time awake per hour after sleep onset. **b**, Transitions of sleep clusters from adolescence to young adulthood (*N* = 636), during 5-year follow-up. The line thickness between adolescence and young adulthood represents the proportions of each sleep cluster in adolescence that remained in the same cluster or transitioned to a different cluster in young adulthood. The thicker the line, the higher the proportion. Modified from Wang M, et al., Obesity (Silver Spring), 2024, under CC BY-NC-ND 4.0

Table 3 Associations of sleep clusters and genetic risk with BMI and overweight/obesity

Sleep clusters		Model 1 (2609 observations)		Model 2 (2609 observations)		Model 3 (1476 observations)		
BMI	Observations	β (95%CI)	*P* value	β (95% CI)	*P* value	β (95%CI)	*P* value	*P* value interaction
Good sleep	829	Ref		Ref				
Delayed sleep phase	575	0.07 (− 0.18, 0.33)	0.574	0.07 (−0.19, 0.33)	0.587	0.01 (− 0.35, 0.36)	0.969	0.358
Sleep irregularity and variability	262	0.05 (− 0.27, 0.38)	0.752	0.11 (−0.22, 0.43)	0.528	0.13 (−0.31, 0.57)	0.551	0.606
Fragmented sleep	764	0.22 (− 0.05, 0.48)	0.109	0.27 (−0.00, 0.54)	0.053	0.19 (−0.16, 0.54)	0.283	0.722
Prolonged sleep latency	179	0.47 (− 0.02, 0.97)	0.062	0.56 (0.06, 1.05)	**0.028**	0.78 (0.03, 1.54)	**0.042**	0.228
PRS						0.65 (0.39, 0.91)	**< 0.001**	
Overweight/obesity	Cases/observations	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value	*P* value interaction
Good sleep	15.6%	Ref		Ref				
Delayed sleep phase	16.3%	1.00 (0.77, 1.29)	0.990	1.00 (0.77, 1.30)	0.993	0.98 (0.68, 1.40)	0.904	0.942
Sleep irregularity and variability	19.5%	1.08 (0.78, 1.50)	0.641	1.14 (0.82, 1.60)	0.443	1.43 (0.94, 2.16)	0.095	0.327
Fragmented sleep	17.8%	1.15 (0.89, 1.48)	0.278	1.22 (0.94, 1.57)	0.137	1.40 (0.99, 1.98)	0.055	0.825
Prolonged sleep latency	21.8%	1.41 (0.95, 2.10)	0.087	1.55 (1.02, 2.34)	**0.039**	1.81 (1.01, 3.25)	**0.047**	0.182
PRS						1.52 (1.15, 2.00)	**0.003**	

Model 1: Adjusted for time of follow-ups, sex, age, study, study center, parental highest education, BMI measurement methods;

Model 2: Model 1 + season, sedentary behavior, moderate-to-vigorous physical activity, and total energy intake;

Model 3: Model 2 + PRS interaction term with sleep clusters. *P* values < 0.05 were highlighted in bold

BMI body mass index, *CI* confidence interval, *OR* Odds ratio, *PRS* polygenic risk score

in young adulthood compared to adolescence. In two cross-sectional analyses, results in adolescence aligned with GEE findings, but in young adulthood, only males exhibited associations of the "sleep irregularity and variability" and "fragmented sleep" clusters with overweight/obesity [OR = 3.84, 95% CI = (1.53, 9.64); OR = 2.62, 95% CI = (1.17, 5.85)] (Supplementary Table S9).

Main results remained largely consistent across sensitivity analyses. Results were comparable, with similar estimates but larger standard deviations due to the smaller sample sizes, when analyzing participants with repeated data (Supplementary Table S10) and excluding those with missing total energy intake (Supplementary Table S11). Supplementary Table S12 and Supplementary Table S13 confirmed the main findings when excluding participants with parent-reported BMI at 15 y and determining sleep clusters by sleep characteristics excluding weekends.

Discussion

This study identified five consistent sleep clusters in both 1347 adolescents and 1262 young adults, including "good sleep", "delayed sleep phase", "sleep irregularity and variability", "fragmented sleep", and "prolonged sleep latency", using K-means cluster analysis involving 12 accelerometry-assessed sleep characteristics. Adolescents within the "good sleep", "delayed sleep phase", and "fragmented sleep" clusters were more stable into the same cluster during the transition into young adulthood. Notably, the "prolonged sleep latency" pattern exhibited longitudinal associations with higher BMI and overweight/obesity, independent of genetic risk. Among males, the "delayed sleep phase", "sleep irregularity and variability" and "fragmented sleep" clusters were more strongly associated with overweight/obesity as age increased.

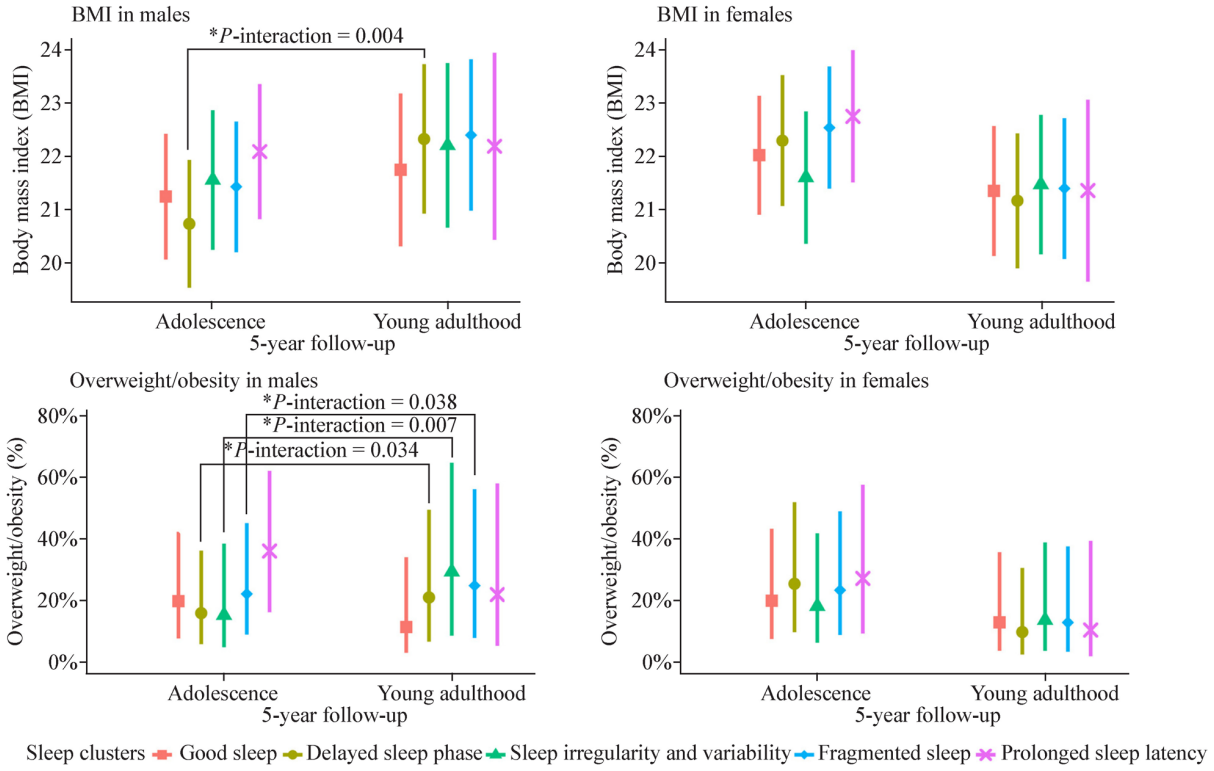


Fig. 3 Generalized estimating equation marginal means for BMI and prevalence of overweight/obesity in males and females, with interaction terms between time of follow-ups and sleep clusters. *There is a significant interaction effect between this sleep cluster and time on the health outcomes. *BMI* body mass index

Although TST decreased and SE increased from adolescence to young adulthood, we identified five consistent sleep patterns in both periods. Notably, we previously identified five sleep patterns in adolescence, and found a significant link between "prolonged sleep latency" cluster and higher fat mass index [14]. Now, we extend this analysis to young adults, aiming to bridge the gap in understanding objective sleep patterns during the transition from adolescence to young adulthood. This period involves significant physiological, psychological, and environmental changes, such as minimal adult supervision, independent living, more digital media use, and irregular schedule [15]. A recent study identified three sleep patterns among 2738 Brazil young adults aged 21.9–23.5 years for males (healthy sleepers, late and variant sleepers, and shorter and poorer sleepers) and for females (healthy sleepers, late and poor-quality sleepers, and shorter, variant, and inefficient sleepers), using k-means cluster analysis across seven sleep characteristics (accelerometry-measured sleep onset, offset, efficiency, TST, TST variability, the Epworth Sleep Scale and the Pittsburgh Sleep Quality Index) [13]. The "late and variant sleepers" and "late and poor-quality sleepers" clusters showed delayed sleep onset and higher SE, which was similar with our "delayed sleep phase" cluster, indicating the delayed sleep phase disorder phenotype [42]. Our current study recognized five sleep clusters in both adolescence and young adulthood, indicating consistent sleep patterns shared between these age groups. Similarly, previous studies have reported that issues like insufficient sleep, irregular sleep–wake patterns and delayed sleep phase disorder persisted from adolescence into young adulthood [16, 43]. Furthermore, the differences in subjective sleep characteristics across five sleep clusters supported our identified objective sleep clusters from a subjective perspective (Supplementary Table S3). For example, the "good sleep" cluster seemed to have higher subjective sleep quality and lower prevalence of sleep difficulties in both adolescence and young adulthood. The shorter self-reported time in bed for the "good sleep" cluster may be due to the lower SL and time awake, as the time in bed is the sum of TST, SL and time awake after sleep onset.

To our knowledge, no study has identified sleep patterns across both adolescence and young adulthood, comprehensively considering multiple objective sleep characteristics. Previous studies have either examined objective sleep patterns at one time-point [10, 11, 13], or identified subjective sleep patterns at two time-points from adolescence to young

adulthood [44]. Chang et al. identified sleep categories in both adolescence and young adulthood, three for males (good sleepers, some sleep problems, poor sleepers) and two for females (good sleepers, and poor sleepers) by latent class analysis across five reported sleep problem indicators, and "good sleepers" were more stable over time [44]. Similarly, we observed that the "good sleep", "delayed sleep phase", and "fragmented sleep" clusters demonstrated greater stability over time. Other studies also classified consistent sleep patterns in childhood and adolescence using subjective sleep characteristics [45, 46].

The "prolonged sleep latency" cluster was longitudinally associated with higher BMI and overweight/obesity in adolescence and young adulthood, aligning with our cross-sectional findings of a link to higher adolescent fat mass [14]. A few studies examining objective sleep latency in relation to obesity had relatively small sample sizes of children and adolescents (< 600) and reported no significance [11, 47]. However, Wirth et al. reported higher BMI in individuals with sleep latency of ≥ 12 minutes (close to the median) compared to those with < 12 minutes among 430 young adults aged 21–35 years [48]. Moreover, our current research using 1347 adolescents and 1262 young adults, identified the "prolonged sleep latency" cluster, distinguished by notably higher sleep latency than other sleep clusters (mean = 45.4 vs.14.1–18.9 minutes in adolescence; 22.4 vs. 5.0–7.6 minutes in young adulthood, Table 2). As discussed in more details in our previous work [14], the association may be attributed to various mechanisms including emotional eating and more calorie intake due to anxiety, or stress from trouble falling asleep; fatigue and reduced motivation for PA caused by delayed sleep stages; hormonal imbalances like cortisol disruption affecting energy intake and expenditure [49–51].

Furthermore, among males only, relationships of the "delayed sleep phase", "sleep irregularity and variability" and "fragmented sleep" clusters with overweight/obesity changed over time. Previous research poorly explored the evolving associations between objective sleep characteristics and obesity over time. Asarnow et al. found that reported later workday bedtime was longitudinally associated with increased BMI from adolescence to adulthood, with no age interaction [52]. However, we discovered a borderline association between the "delayed sleep phase" cluster and overweight/obesity [OR = 2.24, 95% CI = (0.98, 5.12), Supplementary Table S9] among young adult males, indicating a stronger association with BMI compared to adolescence (mean SMT = 4:24 vs. 3:24, Supplementary Table S2). Although slight changes among males from adolescence to young adulthood in "sleep irregularity and variability" and "fragmented sleep" clusters (e.g., SD in TST: 83.2 vs. 78.0 minutes; WASO/h:13.4 vs. 11.7 minutes/hour, respectively), young adult males within these clusters exhibited higher odds of having overweight/obesity. In line with an evolutionary perspective suggesting females' higher ability to tolerate environmental stress in early life [53], our findings indicated that during the transition into young adulthood, young males may exhibit higher vulnerability to unfavorable sleep patterns and increased odds of having overweight/obesity.

In addition, PRS was independently associated with higher BMI and overweight/obesity, without sleep clusters interaction. Consistently with our prior research, PRS showed increased odds of having overweight/obesity from adolescence to young adulthood, without interaction with reported sleep duration or difficulties [39]. Similarly, in the largest study (362,496 Caucasian adults from the UK Biobank), only daytime napping, but not sleep duration or other reported sleep characteristics showed significance in interaction with PRS on BMI [54]. These results collectively suggest that independent of genetic susceptibility, nighttime sleep disturbance may be linked to increased risk of obesity.

Despite strengths like repeated measures of multiple accelerometry-assessed sleep characteristics in adolescence and young adulthood, clustering-identification of five distinct sleep clusters, availability of BMI-related genetic variants, and a large sample size, our study has several limitations. First, BMI data from a subset ($n = 150$) were parent-reported at 15 y, and were self-reported at 20 y. Including subjective BMI increased the sample size and statistical power, given a strong correlation (coefficient = 0.95) between measured and parent-reported BMI at 15 y. Olfert et al. also supported the use of self-reported anthropometric data in young adults for BMI classification [55]. Moreover, the analysis accounted for potential differences in BMI measurement methods. Second, accelerometers may tend to overestimate TST in comparison to the gold standard, polysomnography. Yet they have been validated and widely utilized as practical tools in epidemiologic studies [24, 56, 57]. Third, we assumed that one-week sleep measurements represented long-term sleep patterns, despite BMI measurements preceded sleep assessments (15 y: mean age difference = 0.38 years; 20 y: 0.28 years). Fourth, we acknowledged that some objective sleep characteristics were partly derived from subjective measures (sleep diaries). Fifth, information on daytime or evening naps, and other subjective aspects of sleep was lacking. While social jetlag and catch-up sleep were not included, we used SD in SMT and SD in TST as proxy measurements. Although we tested differences in some subjective sleep characteristics across five sleep clusters, further research could benefit from incorporating more subjective and objective sleep characteristics in a single study. Sixth, despite the longitudinal design, observational studies inherently constrain causal inference. Seventh, caution is warranted when comparing these results to others as our study involved only German adolescents aged 14–16 years and young adults aged 19–22 years.

Eighth, some environmental factors, like artificial light at night, known for affecting circadian rhythms and obesity [58, 59], were unavailable for adjustment in our study. Ninth, we acknowledged that the missing of total energy intake might not be at random, despite using linear regression for imputation [35]. The sensitivity analysis excluding participants with missing data showed comparable results (Supplementary Table S11).

In conclusion, adolescents and young adults shared five consistent distinct sleep patterns, and the "prolonged sleep latency" pattern was linked to increased BMI and overweight/obesity, independent of genetic predisposition. Compared to individuals with the "good sleep" cluster, those with the "prolonged sleep latency" cluster have 1.55 times higher odds of having overweight/obesity. Young male adults with "delayed sleep phase", "sleep irregularity and variability" and "fragmented sleep" patterns appeared to have stronger associations with overweight/obesity, compared to male adolescents. Our findings suggest that improvements on sleep latency, timing, irregularity, variability, and awakenings, may help address obesity from adolescence onward.

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Author contributions WM: conceptualization, formal analysis, funding acquisition, investigation, methodology, software, validation, visualization, writing—original draft, and writing—review and editing. FC: conceptualization, data acquisition, data interpretation, supervision, and writing—review and editing. HCP, KS, and ST: data acquisition, data interpretation, and writing—review and editing. PA: conceptualization, data interpretation, supervision, and writing—review and editing. SM: conceptualization, funding acquisition, project administration, resources, data acquisition, data interpretation, supervision, and writing—review and editing. All authors approved the final manuscript as submitted and agreed to be accountable for all aspects of the work.

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Data Availability Due to data protection reasons, the datasets generated and/or analyzed during the current study cannot be made publicly available. The datasets are available to interested researchers from the corresponding author on reasonable request (e.g. reproducibility), provided the release is consistent with the consent given by the GINIplus and LISA study participants. Ethical approval might be obtained for the release and a data transfer agreement from the legal department of Helmholtz Munich must be accepted.

Declarations

Conflict of interest No financial or non-financial benefits have been received or will be received from any party related directly or indirectly to the subject of this article.

Ethical approval The GINIplus and LISA studies were approved by the local ethics committees, and all participants and their parents gave written informed consents.

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Online supporting information

Accelerometry-assessed sleep clusters and obesity in adolescents and young adults: a longitudinal analysis in GINIplus/LISA birth cohorts

Supplementary methods

Method S1. Definitions of subjective sleep characteristics.

Supplementary results

Table S1. Results of principal component analysis from the 20-year data.

Table S2. Sex-stratified sleep characteristics in total population and in five sleep clusters during adolescence and young adulthood.

Table S3. Subjective sleep characteristics in five sleep clusters during adolescence and young adulthood.

Table S4. Participants characteristics in each of five sleep clusters in adolescence and young adulthood.

Table S5. Number of participants with repeated data in sleep clusters in adolescence and young adulthood.

Table S6. Comparisons of identified sleep clusters by two methods in participants with repeated data in adolescence and young adulthood (*N*=636).

Table S7. Sex-interaction and sex-stratified associations of sleep clusters with BMI and overweight/obesity.

Table S8. The interactions of sleep clusters with time of follow-ups on BMI and overweight/obesity in total population and by sex.

Table S9. Cross-sectional associations of sleep clusters with BMI and overweight/obesity in adolescence and young adulthood, respectively.

Table S10. Associations of sleep clusters with BMI and overweight/obesity in participants with repeated two time-points data.

Table S11. Associations of sleep clusters with BMI and overweight/obesity, excluding participants with missing total energy intake information.

Table S12. Associations of sleep clusters with BMI and overweight/obesity, excluding participants with parent-reported BMI in adolescence.

Table S13. Associations of BMI and overweight/obesity with sleep clusters identified by sleep characteristics, excluding the Friday night and Saturday night.

Fig. S1. Hierarchical clustering dendrogram based on 12 sleep characteristics from the 20-year data.

Fig. S2. Sum of squares method for k-means cluster model comparison from the 20-year data.

Fig. S3. Principal component analysis (PCA) plots for the validation of clustering results from 15-year data on 20-year data.

Method S1. Definitions of subjective sleep characteristics

Self-reported time in bed and self-reported sleep quality were assessed using the same sleep diary over seven consecutive days during accelerometry measurements in both adolescence and young adulthood. Time in bed was defined as the average total number of minutes participants spent in bed according to the diary, calculated as the difference between the in-bed and out-of-bed time, across all valid days. Sleep quality was defined as the average sleeping quality of the night (1-6; 1=worst, 6=best) according to diary, across all valid days.

Information on sleep difficulties, difficulty falling asleep, and difficulty staying asleep was collected using questionnaires filled out separately from the sleep diary. Note, the time of questionnaires completion was not at exactly the same time as the sleep diary, although both were done during the same follow-ups. In adolescence, parents reported these difficulties (yes or no), while participants provided the information in young adulthood.

Table S1. Results of principal component analysis from the 20-year data

Component	Eigenvalue	Percentage of variance (%)	Cumulative percentage of variance (%)
1	3.99	33.26	33.26
2	1.89	15.78	49.04
3	1.50	12.51	61.55
4	1.13	9.44	70.99
5	0.94	7.83	78.82
6	0.80	6.65	85.47
7	0.72	6.00	91.47
8	0.60	4.98	96.45
9	0.20	1.70	98.15
10	0.19	1.59	99.74
11	0.03	0.25	99.99
12	0.00	0.01	100.00

Note, the results of principal component analysis from the 15-year data can be found in previous publication [1].

Table S2. Sex-stratified sleep characteristics in total population and in five sleep clusters during adolescence and young adulthood

Sleep characteristics	Total		Sleep clusters										
			Good sleep		Delayed sleep phase		Sleep irregularity and variability		Fragmented sleep		Prolonged sleep latency		*P-value
	Mean	Median [IQR]	Mean	Median [IQR]	Mean	Median [IQR]	Mean	Median [IQR]	Mean	Median [IQR]	Mean	Median [IQR]	
Males in adolescence (14-16 years), n (%)	**611**		**154 (25.2)**		**101 (16.5)**		**54 (8.8)**		**249 (40.8)**		**53 (8.7)**		
Averages across all valid days													
TST, hours	7.0	7.0 [0.8]	7.4	7.4 [0.6] [a]	7.1	7.0 [0.9] [b]	6.7	6.6 [0.8] [c]	6.8	6.8 [0.7] [c]	6.7	6.8 [0.7] [c]	<0.001
SE, %	78.1	78.5 [8.9]	84.3	84.2 [3.7] [a]	82.4	82.5 [4.5] [a]	73.7	74.3 [6.9] [bc]	74.8	75.2 [5.6] [b]	71.5	72.2 [7.1] [c]	<0.001
SL, minutes	18.4	15.4 [13.4]	12.7	11.1 [9.7] [a]	15.2	13.6 [9.0] [ab]	17.2	15.9 [9.7] [bc]	17.9	16.6 [11.2] [c]	44.1	41.6 [16.6] [d]	<0.001
WASO/h, minutes/hour	11.4	11.0 [4.8]	8.0	8.1 [2.4] [a]	9.0	9.0 [1.9] [a]	14.2	14.0 [4.4] [b]	13.4	13.1 [3.5] [b]	13.3	12.7 [4.6] [b]	<0.001
Awakenings/h, numbers/hour	3.0	3.0 [0.8]	2.6	2.6 [0.6] [a]	2.6	2.7 [0.7] [a]	2.9	2.9 [0.6] [ab]	3.4	3.4 [0.6] [c]	2.9	2.9 [0.6] [b]	<0.001
SMT, 24-hour clock	2:30	2:30 [54 mins]	2:36	2:36 [42 mins] [a]	3:24	3:24 [48 mins] [b]	2:24	2:24 [42 mins] [ac]	2:12	2:12 [48 mins] [c]	2:30	2:36 [48 mins] [a]	<0.001
Day-to-day variability across all valid days													
SD in TST, minutes	58.0	54.6 [37.0]	48.0	43.8 [26.8] [a]	81.4	78.3 [39.3] [b]	83.2	80.7 [32.1] [b]	47.8	46.6 [26.3] [a]	65.2	59.9 [38.2] [c]	<0.001
SD in SE, %	5.7	5.2 [3.2]	4.2	4.0 [1.9] [a]	5.4	5.3 [2.6] [b]	11.5	10.8 [4.2] [c]	5.2	5.0 [2.5] [b]	7.6	7.5 [3.3] [d]	<0.001
SD in SL, minutes	15.2	12.1 [12.8]	9.8	8.3 [7.3] [a]	12.4	10.3 [8.3] [ab]	16.4	15.6 [15.1] [c]	14.1	13.2 [10.3] [bc]	40.3	36.5 [18.1] [d]	<0.001
SD in WASO/h, minutes/hour	3.4	3.1 [1.9]	2.5	2.4 [1.2] [a]	3.1	3.1 [1.5] [b]	6.9	6.7 [2.2] [c]	3.2	3.1 [1.7] [b]	4.6	4.5 [1.7] [d]	<0.001
SD in Awakenings/h, numbers/hour	0.6	0.5 [0.3]	0.5	0.5 [0.3] [a]	0.7	0.7 [0.3] [b]	0.7	0.7 [0.5] [bc]	0.5	0.5 [0.2] [a]	0.6	0.5 [0.3] [ac]	<0.001
SD in SMT, minutes	67.0	64.8 [36.1]	54.5	54.0 [27.2] [a]	99.1	93.5 [37.3] [b]	76.7	73.0 [41.1] [c]	59.1	59.8 [31.0] [ad]	69.6	66.0 [35.9] [cd]	<0.001
Females in adolescence (14-16 years), n (%)	**736**		**286 (38.8)**		**144 (19.6)**		**76 (10.3)**		**175 (23.8)**		**55 (7.5)**		
Averages across all valid days													
TST, hours	7.3	7.3 [0.9]	7.6	7.4 [0.6] [a]	7.3	7.0 [0.9] [b]	7.0	6.6 [0.8] [cd]	7.0	6.8 [0.7] [c]	7.2	6.8 [0.7] [bd]	<0.001
SE, %	80.4	80.7 [7.2]	84.4	84.2 [3.7] [a]	82.3	82.5 [4.5] [b]	75.5	74.3 [6.9] [c]	76.4	75.2 [5.6] [c]	73.6	72.2 [7.1] [c]	<0.001
SL, minutes	18.9	16.0 [13.3]	14.9	11.1 [9.7] [a]	17.4	13.6 [9.0] [b]	20.1	15.9 [9.7] [b]	17.6	16.6 [11.2] [b]	46.5	41.6 [16.6] [c]	<0.001
WASO/h, minutes/hour	10.0	9.7 [4.0]	7.8	8.1 [2.4] [a]	8.8	9.0 [1.9] [b]	12.8	14.0 [4.4] [c]	12.5	13.1 [3.5] [c]	12.0	12.7 [4.6] [c]	<0.001
Awakenings/h, numbers/hour	2.7	2.7 [0.8]	2.5	2.6 [0.6] [a]	2.6	2.7 [0.7] [ab]	2.7	2.9 [0.6] [b]	3.3	3.4 [0.6] [c]	2.8	2.9 [0.6] [b]	<0.001

SMT, 24-hour clock	2:36	2:30 [54 mins]	2:30	2:36 [42 mins] [a]	3:12	3:24 [48 mins] [b]	2:30	2:24 [42 mins] [a]	2:06	2:12 [48 mins] [c]	2:24	2:24 [48 mins] [a]	<0.001
Day-to-day variability across all valid days													
SD in TST, minutes	64.1	60.2 [37.7]	52.6	43.8 [26.8] [a]	86.6	78.3 [39.3] [b]	89.5	80.7 [32.1] [b]	52.1	46.6 [26.3] [a]	68.5	59.9 [38.2] [c]	<0.001
SD in SE, %	5.5	4.9 [2.9]	4.2	4.0 [1.9] [a]	4.9	5.3 [2.6] [b]	10.9	10.8 [4.2] [c]	5.0	5.0 [2.5] [b]	7.4	7.5 [3.3] [d]	<0.001
SD in SL, minutes	14.6	11.3 [11.7]	10.6	8.3 [7.3] [a]	12.5	10.3 [8.3] [ab]	16.6	15.6 [15.1] [c]	12.5	13.2 [10.3] [bc]	45.0	36.5 [18.1] [d]	<0.001
SD in WASO/h, minutes/hour	3.2	2.9 [1.8]	2.5	2.4 [1.2] [a]	2.9	3.1 [1.5] [b]	6.6	6.7 [2.2] [c]	3.0	3.1 [1.7] [b]	4.0	4.5 [1.7] [d]	<0.001
SD in Awakenings/h, numbers/hour	0.5	0.5 [0.2]	0.5	0.5 [0.3] [a]	0.6	0.7 [0.3] [b]	0.6	0.7 [0.5] [b]	0.5	0.5 [0.2] [a]	0.6	0.5 [0.3] [b]	<0.001
SD in SMT, minutes	67.9	64.4 [34.9]	57.3	54.0 [27.2] [a]	94.0	93.5 [37.3] [b]	73.9	73.0 [41.1] [c]	59.6	59.8 [31.0] [a]	73.0	66.0 [35.9] [c]	<0.001
Males in young adulthood (19-22 years), n (%)		**491**		**103 (21.0)**		**133 (27.1)**		**60 (12.2)**		**158 (32.2)**		**37 (7.5)**	
Averages across all valid days													
TST, hours	6.5	6.4 [0.9]	6.9	6.9 [0.8] [a]	6.6	6.7 [0.8] [b]	6.1	6.0 [0.9] [c]	6.2	6.3 [0.9] [c]	6.3	6.2 [1.0] [bc]	<0.001
SE, %	83.0	83.7 [8.3]	88.2	87.7 [4.4] [a]	87.5	87.7 [4.3] [a]	78.6	78.8 [6.8] [b]	79.3	79.7 [5.0] [b]	75.7	77.8 [9.2] [b]	<0.001
SL, minutes	7.5	5.9 [5.9]	5.7	5.3 [3.7] [a]	5.5	4.6 [4.1] [a]	8.0	7.7 [6.1] [b]	6.6	6.0 [5.5] [ab]	22.5	21.3[10.4] [c]	<0.001
WASO/h, minutes/hour	9.4	9.0 [4.8]	6.4	6.5 [2.7] [a]	6.9	6.9 [2.5] [a]	12.0	11.8 [4.1] [b]	11.7	11.4 [2.7] [b]	12.5	11.4 [6.7] [b]	<0.001
Awakenings/h, numbers/hour	3.0	3.0 [1.0]	2.5	2.5 [0.9] [a]	2.5	2.5 [0.8] [a]	3.1	3.1 [0.8] [b]	3.6	3.5 [0.7] [c]	3.3	3.2 [0.7] [bc]	<0.001
SMT, 24-hour clock	3:54	3:42 [84 mins]	3:42	3:42 [84 mins] [ab]	4:24	4:00 [108 mins] [c]	4:06	4:00 [72 mins] [ac]	3:30	3:24 [72 mins] [b]	3:48	3:42 [72 mins] [abc]	<0.001
Day-to-day variability across all valid days													
SD in TST, minutes	63.5	61.0 [36.8]	46.7	45.0 [27.0] [a]	78.2	78.2 [38.5] [b]	78.0	76.6 [31.7] [b]	55.2	51.3 [29.9] [ac]	69.6	62.5 [35.8] [bc]	<0.001
SD in SE, %	5.2	4.6 [3.0]	3.5	3.4 [2.0] [a]	4.2	4.1 [1.9] [b]	10.0	9.0 [3.2] [c]	5.0	4.9 [2.1] [d]	7.0	6.4 [3.6] [e]	<0.001
SD in SL, minutes	7.5	5.8 [5.8]	5.3	4.2 [3.7] [a]	5.4	4.5 [4.6] [a]	8.8	8.1 [8.4] [b]	6.3	6.1 [4.1] [ab]	23.3	20.6 [9.0] [c]	<0.001
SD in WASO/h, minutes/hour	3.0	2.7 [1.8]	2.0	1.9 [1.1] [a]	2.4	2.3 [1.2] [b]	5.8	5.4 [1.7] [c]	3.0	2.9 [1.5] [d]	3.6	3.3 [1.8] [d]	<0.001
SD in Awakenings/h, numbers/hour	0.7	0.6 [0.3]	0.5	0.5 [0.3] [a]	0.7	0.6 [0.4] [b]	0.9	0.9 [0.5] [c]	0.6	0.6 [0.3] [b]	0.7	0.6 [0.3] [b]	<0.001
SD in SMT, minutes	73.4	68.1 [50.8]	46.3	43.6 [28.1] [a]	97.9	93.7 [43.5] [b]	99.2	94.9 [55.5] [b]	61.0	56.8 [36.7] [c]	71.8	65.1 [50.5] [c]	<0.001
Females in young adulthood (19-22 years), n (%)		**771**		**286 (37.1)**		**197 (25.6)**		**72 (9.3)**		**182 (23.6)**		**34 (4.4)**	
Averages across all valid days													
TST, hours	6.8	6.7 [1.0]	7.1	7.1 [0.9] [a]	6.7	6.7 [0.9] [b]	6.4	6.4 [0.7] [bc]	6.5	6.5 [0.9] [c]	6.2	6.2 [0.8] [c]	<0.001
SE, %	85.2	85.9 [6.6]	88.5	88.5 [4.0] [a]	87.8	87.6 [4.3] [a]	81.1	81.4 [5.1] [b]	80.2	81.1 [4.5] [b]	78.1	79.2 [5.7] [b]	<0.001

SL, minutes	6.3	5.0 [5.6]	4.7	3.8 [3.9] [a]	5.3	4.4 [4.5] [a]	7.4	7.4 [6.2] [b]	6.6	6.1 [5.6] [b]	22.3	20.8 [6.4] [c]	<0.001
WASO/h, minutes/hour	8.2	7.8 [3.8]	6.4	6.4 [2.5] [a]	6.7	6.8 [2.4] [a]	10.5	10.5 [3.6] [b]	11.2	10.8 [2.7] [b]	10.7	9.8 [4.2] [b]	<0.001
Awakenings/h, numbers/hour	2.8	2.8 [0.9]	2.5	2.6 [0.7] [a]	2.5	2.5 [0.7] [a]	2.8	2.8 [0.7] [b]	3.5	3.5 [0.6] [c]	2.9	2.9 [0.7] [b]	<0.001
SMT, 24-hour clock	3:24	3:18 [78 mins]	3:18	3:12 [78 mins] [a]	4:00	4:00 [78 mins] [b]	3:24	3:18 [78 mins] [a]	3:06	3:06 [60 mins] [a]	3:36	3:24 [66 mins] [ab]	<0.001
Day-to-day variability across all valid days													
SD in TST, minutes	65.6	61.1 [36.8]	51.5	49.3 [26.9] [a]	83.8	82.2 [32.1] [b]	89.6	88.7 [48.4] [b]	57.2	54.9 [27.1] [ac]	72.3	62 [27.2] [bc]	<0.001
SD in SE, %	4.7	4.2 [2.6]	3.3	3.1 [1.6] [a]	4.3	4.1 [1.9] [b]	9.8	9.0 [2.6] [c]	5.0	5.0 [1.8] [d]	6.4	5.6 [3.1] [d]	<0.001
SD in SL, minutes	6.3	4.9 [4.7]	4.5	3.8 [3.2] [a]	5.3	4.7 [4.1] [ab]	8.9	8.3 [7.9] [c]	6.2	5.6 [4.3] [bc]	22.2	19.2 [4.8] [d]	<0.001
SD in WASO/h, minutes/hour	2.8	2.5 [1.4]	2.0	1.9 [0.9] [a]	2.5	2.4 [1.1] [b]	5.8	5.1 [1.8] [c]	3.0	3.0 [1.1] [d]	3.1	3.0 [1.3] [bd]	<0.001
SD in Awakenings/h, numbers/hour	0.6	0.6 [0.3]	0.5	0.5 [0.2] [a]	0.7	0.6 [0.3] [b]	0.8	0.8 [0.4] [c]	0.6	0.6 [0.3] [b]	0.6	0.6 [0.2] [ab]	<0.001
SD in SMT, minutes	68.5	63.6 [42.4]	51.2	48.9 [29.9] [a]	92.3	87.7 [41.1] [b]	87.8	85.2 [47] [bc]	62.8	58.5 [38.3] [d]	66.9	64.5 [41.5] [cd]	<0.001

The results are presented as mean and median [IQR] of sleep characteristics, given that most of them did not follow a normal distribution. *: Kruskal-Wallis test with Dunn post hoc tests and Bonferroni adjustment. Sharing the same letter (a b c d e) are considered not significantly different (adjusted *P*-values < 0.05). Abbreviations: Awakenings/h, awakenings per hour after sleep onset; IQR, interquartile range; SD, standard deviation; SE, sleep efficiency; SL, sleep latency; SMT, sleep midpoint timing; TST, total sleep time; WASO/h, time awake per hour after sleep onset.

Table S3. Subjective sleep characteristics in five sleep clusters during adolescence and young adulthood

Sleep characteristics	Sleep clusters						
	Good sleep	Delayed sleep phase	Sleep irregularity and variability	Fragmented sleep	Prolonged sleep latency	*_P_-value	Number of participants
Adolescence (14-16 years)							
Self-reported time in bed, hours	8.95 ± 0.70 [a]	8.80 ± 0.86 [a]	9.24 ± 0.84 [b]	9.15 ± 0.77 [b]	9.68 ± 1.00 [c]	**<0.001**	1347
Self-reported sleep quality	4.67 ± 0.72 [a]	4.56 ± 0.68 [ab]	4.46 ± 0.75 [b]	4.59 ± 0.67 [ab]	4.46 ± 0.66 [b]	**0.004**	1347
Parent-reported sleep difficulties, yes	57 (13.2)	25 (10.4)	23 (18.9)	55 (13.2)	18 (17.3)	0.180	1313
Parent-reported difficulty falling asleep, yes	52 (12.1)	24 (10.0)	19 (15.8)	49 (11.8)	17 (16.3)	0.370	1311
Parent-reported difficulty staying asleep, yes	16 (3.7)	4 (1.7)	5 (4.2)	10 (2.4)	2 (1.9)	0.437	1311
Young adulthood (19-22 years)							
Self-reported time in bed, hours	8.01 ± 0.76 [a]	7.61 ± 0.75 [b]	7.85 ± 0.95 [a]	7.97 ± 0.80 [a]	8.17 ± 0.90 [a]	**<0.001**	1262
Self-reported sleep quality	4.43 ± 0.82	4.35 ± 0.83	4.26 ± 0.83	4.33 ± 0.75	4.21 ± 0.83	0.137	1262
Self-reported sleep difficulties, yes	74 (19.8)	68 (21.8)	36 (29.8)	63 (19.6)	19 (28.4)	0.095	1194
Self-reported difficulty falling asleep, yes	53 (14.2)	58 (18.6)	29 (24.0)	45 (14.0)	16 (23.9)	**0.025**	1193
Self-reported difficulty staying asleep, yes	35 (9.4)	29 (9.3)	14 (11.6)	35 (10.9)	9 (13.4)	0.788	1193

The results are presented as mean ± SD or number (%) of sleep characteristics. *: Post hoc tests after one-way analysis of variance or Chi-square test, with Bonferroni adjustment. Sharing the same letter (a b c) are considered not significantly different (adjusted *P*-values < 0.05). *P*-values < 0.05 were highlighted in bold. Self-reported sleep quality was presented on a scale of 1 (worst) to 6 (best) according to the sleep diary. Self-reported time in bed and sleep quality represented averages assessed from the sleep diary across valid days. Sleep difficulties, difficulty falling asleep, and difficulty staying asleep were assessed by questionnaires.

Table S4. Participants characteristics in each of five sleep clusters in adolescence and young adulthood

	Good sleep	Delayed sleep phase	Sleep irregularity and variability	Fragmented sleep	Prolonged sleep latency	*P-value
Adolescence	440 (32.7)	245 (18.2)	130 (9.6)	424 (31.5)	108 (8.0)	
Age, year	15.2 ± 0.3	15.2 ± 0.3	15.2 ± 0.3	15.2 ± 0.3	15.2 ± 0.3	0.538
Study, n(%)						**0.018**
GINIplus observation	173 (39.3)	103 (42.0)	42 (32.3)	140 (33.0)	39 (36.1)	
GINIplus intervention	171 (38.9)	93 (38.0)	53 (40.8)	149 (35.1)	43 (39.8)	
LISA	96 (21.8)	49 (20.0)	35 (26.9)	135 (31.8)	26 (24.1)	
Study center, n(%)						**0.003**
Munich	278 (63.2)	130 (53.1)	70 (53.8)	280 (66.0)	60 (55.6)	
Wesel	162 (36.8) [ab]	115 (46.9) [a]	60 (46.2) [ab]	144 (34.0) [b]	48 (44.4) [ab]	
Weight, kg	59.9 ± 10.8 [a]	60.7 ± 10.8 [ab]	61.8 ± 11.5 [ab]	61.8 ± 10.7 [ab]	63.2 ± 13.0 [b]	**0.019**
Height, cm	170.4 ± 8.3 [a]	171.5 ± 7.9 [ab]	171.3 ± 7.8 [ab]	172.4 ± 7.8 [b]	171.5 ± 8.7 [ab]	**0.007**
BMI, kg/m^2	20.6 ± 2.9	20.6 ± 3.1	21.0 ± 3.2	20.7 ± 3.0	21.4 ± 3.4	0.089
BMI z-score	0.0 ± 1.0	0.0 ± 1.0	0.1 ± 0.9	0.1 ± 1.0	0.3 ± 1.0	0.056
Overweight/obesity, n(%)						0.169
No	374 (85.0)	204 (83.3)	110 (84.6)	351 (82.8)	81 (75.0)	
Yes	66 (15.0)	41 (16.7)	20 (15.4)	73 (17.2)	27 (25.0)	
BMI measurement methods, n(%)						0.191
Examination	397 (90.2)	219 (89.4)	119 (91.5)	364 (85.8)	98 (90.7)	
Questionnaire	43 (9.8)	26 (10.6)	11 (8.5)	60 (14.2)	10 (9.3)	
Season, n(%)						**0.032**
Spring	110 (25.0)	78 (31.8)	34 (26.2)	109 (25.7)	24 (22.2)	
Summer	50 (11.4)	33 (13.5)	24 (18.5)	69 (16.3)	22 (20.4)	
Autumn	146 (33.2)	72 (29.4)	39 (30.0)	152 (35.8)	28 (25.9)	
Winter	134 (30.5)	62 (25.3)	33 (25.4)	94 (22.2)	34 (31.5)	
Total energy intake, kcal/day	1998.7 ± 628.7 [a]	2020.4 ± 625.8 [ab]	2129.4 ± 663.2 [ab]	2173.5 ± 673.3 [b]	2088.9 ± 610.6 [ab]	**0.005**
Sedentary behavior, hours	8.5 ± 1.3 [ab]	8.6 ± 1.5 [a]	8.2 ± 1.2 [bc]	8.0 ± 1.4 [c]	7.5 ± 1.3 [d]	**<0.001**
MVPA, mins	48.3 ± 26.1 [a]	49.8 ± 34.3 [ab]	49.3 ± 23.6 [ab]	54.3 ± 25.0 [b]	52.2 ± 23.5 [ab]	**0.019**
Parental highest education, n(%)						**0.001**
Low/medium	111 (25.2)	72 (29.4)	47 (36.2)	117 (27.6)	48 (44.4)	
High	329 (74.8) [ab]	173 (70.6) [ab]	83 (63.8) [ab]	307 (72.4) [a]	60 (55.6) [b]	
Young adulthood	389 (30.8)	330 (26.2)	132 (10.5)	340 (26.9)	71 (5.6)	

Age, year	20.2 ± 0.4 [a]	20.2 ± 0.4 [ab]	20.3 ± 0.5 [b]	20.2 ± 0.4 [ab]	20.2 ± 0.3 [ab]	**0.003**
Study, n(%)						0.419
GINIplus observation	166 (42.7)	121 (36.7)	55 (41.7)	120 (35.3)	26 (36.6)	
GINIplus intervention	136 (35.0)	139 (42.1)	46 (34.8)	131 (38.5)	28 (39.4)	
LISA	87 (22.4)	70 (21.2)	31 (23.5)	89 (26.2)	17 (23.9)	
Study center, n(%)						0.114
Munich	244 (62.7)	201 (60.9)	66 (50.0)	205 (60.3)	46 (64.8)	
Wesel	145 (37.3)	129 (39.1)	66 (50.0)	135 (39.7)	25 (35.2)	
Weight, kg	66.5 ± 11.1 [a]	68.7 ± 13.2 [ab]	72.0 ± 13.8 [b]	69.9 ± 12.6 [b]	70.0 ± 13.9 [ab]	**<0.001**
Height, cm	172.9 ± 9.3 [a]	174.2 ± 9.3 [ab]	176.9 ± 10.2 [b]	175.6 ± 9.4 [b]	175.6 ± 10.7 [ab]	**<0.001**
BMI, kg/m^2	22.2 ± 3.0	22.6 ± 3.5	22.9 ± 3.5	22.6 ± 3.4	22.6 ± 3.4	0.162
Overweight/obesity, n(%)						0.349
No	326 (83.8)	277 (83.9)	101 (76.5)	277 (81.5)	59 (83.1)	
Yes	63 (16.2)	53 (16.1)	31 (23.5)	63 (18.5)	12 (16.9)	
BMI measurements, n(%)						NA
Examination						
Questionnaire	389 (100.0)	330 (100.0)	132 (100.0)	340 (100.0)	71 (100.0)	
Season, n(%)						**0.047**
Spring	110 (28.3)	91 (27.6)	31 (23.5)	99 (29.1)	18 (25.4)	
Summer	88 (22.6)	83 (25.2)	47 (35.6)	105 (30.9)	19 (26.8)	
Autumn	86 (22.1)	83 (25.2)	29 (22.0)	81 (23.8)	18 (25.4)	
Winter	105 (27.0)	73 (22.1)	25 (18.9)	55 (16.2)	16 (22.5)	
Total energy intake, kcal/day	1720.3 ± 611.5	1799.2 ± 678.5	1776.0 ± 622.6	1801.6 ± 725.2	1887.0 ± 595.5	0.331
Sedentary behavior, hours	8.7 ± 1.5 [a]	8.5 ± 1.6 [ab]	8.1 ± 1.5 [bc]	8.2 ± 1.5 [bc]	7.8 ± 1.5 [c]	**<0.001**
MVPA, minutes	45.3 ± 24.2	47.9 ± 23.0	44.8 ± 23.4	46.5 ± 24.5	45.4 ± 20.1	0.565
Parental highest education, n(%)						**0.015**
Low/medium	88 (22.6)	103 (31.2)	47 (35.6)	93 (27.4)	24 (33.8)	
High	301 (77.4) [a]	227 (68.8) [ab]	85 (64.4) [b]	247 (72.6) [ab]	47 (66.2) [ab]	

The results are presented as mean ± standard deviation or n (%) (number(percentage)). Abbreviations: BMI, body mass index; MVPA, moderate-to-vigorous physical activity; GINIplus, German Infant Study on the influence of Nutrition Intervention PLUS environmental and genetic influences on allergy development; LISA, Influence of Lifestyle factors on the development of the Immune System and Allergies in East and West Germany.

Overweight/obesity: BMI z-score >1 for adolescents; BMI ≥25 kg/m^2 for adults according to World Health Organization.

The number of participants with available total energy intake: n=1063 in adolescence; n=1019 in young adulthood.

*: Post hoc tests after one-way analysis of variance or Chi-square test, with Bonferroni adjustment. Sharing the same letter (a b c) are considered not significantly different (adjusted *P*-values < 0.05). *P*-values < 0.05 were highlighted in bold.

Table S5. Number of participants with repeated data in sleep clusters in adolescence and young adulthood

Sleep clusters in adolescence	Sleep clusters in young adulthood				
	Good sleep	Delayed sleep phase	Sleep irregularity and variability	Fragmented sleep	Prolonged sleep latency
Total (n=636)					
Good sleep	119 (0.50)	73 (0.31)	16 (0.07)	27 (0.11)	4 (0.02)
Delayed sleep phase	33 (0.31)	42 (0.40)	6 (0.06)	21 (0.20)	4 (0.04)
Sleep irregularity and variability	11 (0.21)	16 (0.30)	5 (0.09)	16 (0.30)	5 (0.09)
Fragmented sleep	31 (0.16)	27 (0.14)	30 (0.15)	94 (0.48)	12 (0.06)
Prolonged sleep latency	8 (0.18)	12 (0.27)	7 (0.16)	15 (0.34)	2 (0.05)
Male (n=243)					
Good sleep	32 (0.42)	24 (0.32)	6 (0.08)	14 (0.18)	0 (0)
Delayed sleep phase	5 (0.16)	15 (0.47)	2 (0.06)	9 (0.28)	1 (0.03)
Sleep irregularity and variability	0 (0)	7 (0.44)	2 (0.13)	5 (0.31)	2 (0.13)
Fragmented sleep	13 (0.13)	18 (0.18)	16 (0.16)	43 (0.43)	9 (0.09)
Prolonged sleep latency	5 (0.25)	6 (0.30)	1 (0.05)	7 (0.35)	1 (0.05)
Female (n=393)					
Good sleep	87 (0.53)	49 (0.30)	10 (0.06)	13 (0.08)	4 (0.02)
Delayed sleep phase	28 (0.38)	27 (0.36)	4 (0.05)	12 (0.16)	3 (0.04)
Sleep irregularity and variability	11 (0.30)	9 (0.24)	3 (0.08)	11 (0.30)	3 (0.08)
Fragmented sleep	18 (0.19)	9 (0.09)	14 (0.15)	51 (0.54)	3 (0.03)
Prolonged sleep latency	3 (0.13)	6 (0.25)	6 (0.25)	8 (0.33)	1 (0.04)

The results are presented as number (proportion) within the group of sleep clusters in adolescence.

Table S6. Comparisons of identified sleep clusters by two methods in participants with repeated data in adolescence and young adulthood (*N*=636)

Sleep clusters identified at each follow-up using all available data at that time	Sleep clusters re-identified only among participants with repeated data (N=636)				
	Good sleep	Delayed sleep phase	Sleep irregularity and variability	Fragmented sleep	Prolonged sleep latency
Sleep clusters in adolescence					
Good sleep	**203**	28	2	6	0
Delayed sleep phase	1	**104**	1	0	0
Sleep irregularity and variability	0	0	**49**	2	2
Fragmented sleep	0	10	0	**184**	0
Prolonged sleep latency	1	2	1	3	**37**
Sleep clusters in young adulthood					
Good sleep	**187**	3	0	12	0
Delayed sleep phase	53	**116**	0	1	0
Sleep irregularity and variability	0	15	**45**	4	0
Fragmented sleep	0	11	0	**161**	1
Prolonged sleep latency	0	2	0	0	**25**

The results are presented as number of participants within the group of sleep clusters identified by two methods. The number of participants remained in the same cluster were highlighted in bold. The Cohen's kappa of clustering results identified by the two methods was 0.87 in adolescence and 0.78 in young adulthood, both statistically significant (*P*-values < 0.001).

Table S7. Sex-interaction and sex-stratified associations of sleep clusters with BMI and overweight/obesity

Sleep clusters	**Male**			**Female**			**Sex-interaction**
BMI	**Observations**	**β [95%CI]**	***P*-value**	**Observations**	**β [95%CI]**	***P*-value**	***P*-value**
Good sleep	257	Ref		572	Ref		
Delayed sleep phase	234	0.08 [-0.31, 0.47]	0.693	341	0.04 [-0.30, 0.37]	0.837	0.325
Sleep irregularity and variability	114	0.34 [-0.18, 0.86]	0.204	148	-0.15 [-0.58, 0.28]	0.482	0.146
Fragmented sleep	407	0.39 [-0.00, 0.78]	0.051	357	0.26 [-0.12, 0.64]	0.180	0.652
Prolonged sleep latency	90	0.69 [-0.07, 1.45]	0.075	89	0.43 [-0.21, 1.07]	0.191	0.719
Overweight/obesity	**Cases /Observations**	**OR [95%CI]**	***P*-value**	**Cases /Observations**	**OR [95%CI]**	***P*-value**	***P*-value**
Good sleep	14.8%	Ref		15.9%	Ref		
Delayed sleep phase	17.9%	1.15 [0.74, 1.81]	0.530	15.2%	0.96 [0.70, 1.32]	0.801	0.655
Sleep irregularity and variability	21.9%	1.48 [0.87, 2.52]	0.151	17.6%	0.97 [0.62, 1.52]	0.894	0.324
Fragmented sleep	20.4%	1.51 [1.01, 2.26]	**0.046**	14.8%	1.08 [0.75, 1.56]	0.686	0.183
Prolonged sleep latency	27.8%	2.20 [1.26, 3.84]	**0.005**	15.7%	1.12 [0.56, 2.23]	0.757	0.088

Adjusted for time of follow-ups, age, study, study center, parental highest education, BMI measurement methods, season, sedentary behavior, moderate-to-vigorous physical activity, and total energy intake. *P*-values < 0.05 were highlighted in bold. Abbreviations: BMI, body mass index; CI, confidence interval; OR, odds ratio.

Table S8. The interactions of sleep clusters with time of follow-ups on BMI and overweight/obesity in total population and by sex

Sleep clusters	Total		Male		Female	
BMI	**β [95%CI]**	***P*-value**	**β [95%CI]**	***P*-value**	**β [95%CI]**	***P*-value**
Good sleep	Ref		Ref		Ref	
Delayed sleep phase	-0.04 [-0.42, 0.34]	0.837	-0.51 [-1.08, 0.06]	0.080	0.27 [-0.22, 0.76]	0.273
Sleep irregularity and variability	-0.10 [-0.55, 0.35]	0.657	0.31 [-0.39, 1.01]	0.384	-0.42 [-1.06, 0.23]	0.204
Fragmented sleep	0.19 [-0.12, 0.51]	0.225	0.18 [-0.28, 0.65]	0.443	0.52 [0.10, 0.94]	**0.015**
Prolonged sleep latency	0.68 [0.16, 1.21]	**0.011**	0.84 [-0.03, 1.72]	0.060	0.73 [0.11, 1.35]	**0.021**
Time of follow-ups	-0.45 [-1.94, 1.04]	0.556	0.50 [-1.68, 2.68]	0.654	-0.67 [-2.61, 1.27]	0.499
Delayed sleep phase * Time of follow-ups	0.21 [-0.31, 0.73]	0.425	1.09 [0.35, 1.83]	**0.004**	-0.46 [-1.13, 0.22]	0.184
Sleep irregularity and variability * Time of follow-ups	0.41 [-0.25, 1.06]	0.226	0.15 [-0.79, 1.08]	0.757	0.54 [-0.40, 1.48]	0.261
Fragmented sleep * Time of follow-ups	0.16 [-0.26, 0.59]	0.454	0.47 [-0.14, 1.09]	0.134	-0.47 [-1.01, 0.07]	0.088
Prolonged sleep latency * Time of follow-ups	-0.34 [-1.39, 0.70]	0.519	-0.40 [-1.92, 1.12]	0.605	-0.72 [-2.16, 0.71]	0.324
Overweight/obesity	**OR [95%CI]**	***P*-value**	**OR [95%CI]**	***P*-value**	**OR [95%CI]**	***P*-value**
Good sleep	Ref		Ref		Ref	
Delayed sleep phase	1.05 [0.71, 1.55]	0.811	0.77 [0.40, 1.47]	0.423	1.37 [0.84, 2.23]	0.208
Sleep irregularity and variability	0.83 [0.49, 1.42]	0.503	0.73 [0.33, 1.63]	0.442	0.89 [0.44, 1.81]	0.750
Fragmented sleep	1.20 [0.86, 1.68]	0.279	1.15 [0.72, 1.85]	0.557	1.22 [0.74, 2.01]	0.428
Prolonged sleep latency	1.95 [1.20, 3.18]	**0.007**	2.29 [1.20, 4.34]	**0.012**	1.50 [0.67, 3.37]	0.329
Time of follow-ups	0.65 [0.16, 2.73]	0.557	0.52 [0.06, 4.27]	0.544	0.60 [0.08, 4.59]	0.620
Delayed sleep phase * Time of follow-ups	0.92 [0.54, 1.57]	0.758	2.69 [1.08, 6.73]	**0.034**	0.53 [0.27, 1.04]	0.066
Sleep irregularity and variability * Time of follow-ups	1.75 [0.89, 3.45]	0.106	4.43 [1.51, 13.02]	**0.007**	1.19 [0.48, 2.96]	0.701
Fragmented sleep * Time of follow-ups	1.03 [0.65, 1.63]	0.915	2.23 [1.05, 4.74]	**0.037**	0.81 [0.43, 1.52]	0.519
Prolonged sleep latency * Time of follow-ups	0.54 [0.23, 1.28]	0.162	0.96 [0.30, 3.05]	0.938	0.52 [0.13, 2.07]	0.357

Adjusted for sex (only for total population), age, study, study center, parental highest education, BMI measurement methods, season, sedentary behavior, moderate-to-vigorous physical activity, and total energy intake. *P*-values < 0.05 were highlighted in bold. Abbreviations: BMI, body mass index; CI, confidence interval; OR, odds ratio.

Table S9. Cross-sectional associations of sleep clusters with BMI and overweight/obesity in adolescence and young adulthood, respectively

Sleep clusters	Total			Male			Female		
Adolescence									
BMI	**Numbers**	**β [95%CI]**	***P***	**Numbers**	**β [95%CI]**	***P***	**Numbers**	**β [95%CI]**	***P***
Good sleep	440	Ref		154	Ref		286	Ref	
Delayed sleep phase	245	-0.12 [-0.58, 0.34]	0.614	101	-0.47 [-1.24, 0.30]	0.228	144	0.10 [-0.49, 0.69]	0.738
Sleep irregularity and variability	130	0.44 [-0.15, 1.02]	0.141	54	0.37 [-0.58, 1.33]	0.442	76	0.46 [-0.28, 1.20]	0.226
Fragmented sleep	424	0.41 [0.00, 0.81]	0.050	249	0.30 [-0.33, 0.92]	0.349	175	0.45 [-0.11, 1.00]	0.114
Prolonged sleep latency	108	1.03 [0.39, 1.67]	**0.002**	53	1.13 [0.15, 2.12]	**0.024**	55	0.87 [0.01, 1.73]	**0.047**
Overweight/obesity	**Prevalence**	**OR [95%CI]**	***P***	**Prevalence**	**OR [95%CI]**	***P***	**Prevalence**	**OR [95%CI]**	***P***
Good sleep	15.0%	Ref		18.8%	Ref		12.9%	Ref	
Delayed sleep phase	16.7%	0.98 [0.63, 1.52]	0.931	16.8%	0.76 [0.39, 1.51]	0.437	16.7%	1.21 [0.68, 2.14]	0.517
Sleep irregularity and variability	15.4%	0.98 [0.56, 1.72]	0.956	14.8%	0.69 [0.29, 1.67]	0.413	15.8%	1.27 [0.61, 2.64]	0.517
Fragmented sleep	17.2%	1.18 [0.80, 1.74]	0.394	20.5%	1.14 [0.67, 1.95]	0.622	12.6%	1.17 [0.65, 2.09]	0.608
Prolonged sleep latency	25.0%	1.99 [1.16, 3.41]	**0.013**	34.0%	2.21 [1.04, 4.70]	**0.040**	16.4%	1.58 [0.69, 3.65]	0.279
Young adulthood									
BMI	**Numbers**	**β [95%CI]**	***P***	**Numbers**	**β [95%CI]**	***P***	**Numbers**	**β [95%CI]**	***P***
Good sleep	389	Ref		103	Ref		286	Ref	
Delayed sleep phase	330	0.22 [-0.26, 0.70]	0.373	133	0.75 [-0.00, 1.50]	0.052	197	-0.00 [-0.63, 0.62]	0.990
Sleep irregularity and variability	132	0.51 [-0.15, 1.17]	0.128	60	0.83 [-0.12, 1.78]	0.088	72	0.50 [-0.40, 1.40]	0.274
Fragmented sleep	340	0.33 [-0.16, 0.82]	0.184	158	0.71 [-0.02, 1.44]	0.059	182	0.10 [-0.55, 0.75]	0.764
Prolonged sleep latency	71	0.31 [-0.53, 1.14]	0.473	37	0.47 [-0.64, 1.58]	0.408	34	0.20 [-1.02, 1.43]	0.745
Overweight/obesity	**Prevalence**	**OR [95%CI]**	***P***	**Prevalence**	**OR [95%CI]**	***P***	**Prevalence**	**OR [95%CI]**	***P***
Good sleep	16.2%	Ref		8.7%	Ref		18.9%	Ref	
Delayed sleep phase	16.1%	0.93 [0.62, 1.40]	0.736	18.8%	2.24 [0.98, 5.12]	0.055	14.2%	0.69 [0.42, 1.15]	0.155
Sleep irregularity and variability	23.5%	1.43 [0.87, 2.37]	0.161	28.3%	3.84 [1.53, 9.64]	**0.004**	19.4%	0.99 [0.50, 1.96]	0.988
Fragmented sleep	18.5%	1.12 [0.75, 1.67]	0.567	20.3%	2.62 [1.17, 5.85]	**0.019**	17.0%	0.83 [0.50, 1.37]	0.458
Prolonged sleep latency	16.9%	1.04 [0.52, 2.07]	0.918	18.9%	2.16 [0.72, 6.49]	0.171	14.7%	0.77 [0.28, 2.12]	0.612

Adjusted for sex (only for total population), age, study, study center, parental highest education, BMI measurement methods (only for adolescence), season, sedentary behavior, moderate-to-vigorous physical activity, and total energy intake. *P*-values < 0.05 were highlighted in bold. Abbreviations: BMI, body mass index; CI, confidence interval; OR, odds ratio.

Table S10. Associations of sleep clusters with BMI and overweight/obesity in participants with repeated two time-points data

Sleep clusters	**N=636, 1272 observations**	**Model**	
BMI	**Observations**	**β [95%CI]**	***P*-value**
Good sleep	441	Ref	
Delayed sleep phase	176	0.07 [-0.24, 0.37]	0.666
Sleep irregularity and variability	117	0.01 [-0.40, 0.41]	0.973
Fragmented sleep	367	0.22 [-0.13, 0.58]	0.211
Prolonged sleep latency	71	0.50 [-0.17, 1.18]	0.140
Overweight/obesity	**Cases/Observations**	**OR [95%CI]**	***P*-value**
Good sleep	15.4%	Ref	
Delayed sleep phase	16.3%	1.05 [0.75, 1.46]	0.795
Sleep irregularity and variability	18.8%	1.12 [0.71, 1.77]	0.616
Fragmented sleep	15.8%	1.15 [0.81, 1.65]	0.431
Prolonged sleep latency	22.5%	1.78 [0.95, 3.35]	0.074

Adjusted for time of follow-ups, sex, age, study, study center, parental highest education, BMI measurement methods, season, sedentary behavior, moderate-to-vigorous physical activity, and total energy intake. Abbreviations: BMI, body mass index; CI, confidence interval; OR, odds ratio.

Table S11. Associations of sleep clusters with BMI and overweight/obesity, excluding participants with missing total energy intake information

Sleep clusters	**N=1630, 2082 observations**	**Model**	
BMI	**Observations**	**β [95%CI]**	***P*-value**
Good sleep	699	Ref	
Delayed sleep phase	457	0.10 [-0.21, 0.41]	0.540
Sleep irregularity and variability	189	-0.09 [-0.50, 0.31]	0.644
Fragmented sleep	598	0.26 [-0.08, 0.59]	0.130
Prolonged sleep latency	139	0.29 [-0.28, 0.86]	0.325
Overweight/obesity	**Cases/Observations**	**OR [95%CI]**	***P*-value**
Good sleep	15.2%	Ref	
Delayed sleep phase	16.0%	0.99 [0.74, 1.33]	0.965
Sleep irregularity and variability	16.4%	1.04 [0.70, 1.55]	0.839
Fragmented sleep	17.2%	1.23 [0.93, 1.64]	0.151
Prolonged sleep latency	21.6%	1.55 [0.95, 2.54]	0.078

Adjusted for time of follow-ups, sex, age, study, study center, parental highest education, BMI measurement methods, season, sedentary behavior, moderate-to-vigorous physical activity, and total energy intake. *P*-values < 0.05 were highlighted in bold. Abbreviations: BMI, body mass index; CI, confidence interval; OR, odds ratio.

Table S12. Associations of sleep clusters with BMI and overweight/obesity, excluding participants with parent-reported BMI in adolescence

Sleep clusters	**N=1890, 2459 observations**	**Model**	
BMI	**Observations**	**β [95%CI]**	***P*-value**
Good sleep	786	Ref	
Delayed sleep phase	549	0.03 [-0.24, 0.30]	0.824
Sleep irregularity and variability	251	0.12 [-0.22, 0.45]	0.504
Fragmented sleep	704	0.30 [0.01, 0.59]	**0.041**
Prolonged sleep latency	169	0.63 [0.10, 1.15]	**0.019**
Overweight/obesity	**Cases/Observations**	**OR [95%CI]**	***P*-value**
Good sleep	16.0%	Ref	
Delayed sleep phase	16.2%	0.98 [0.75, 1.27]	0.873
Sleep irregularity and variability	19.9%	1.13 [0.80, 1.59]	0.483
Fragmented sleep	18.5%	1.23 [0.94, 1.59]	0.127
Prolonged sleep latency	23.1%	1.67 [1.11, 2.51]	**0.014**

Adjusted for time of follow-ups, sex, age, study, study center, parental highest education, season, sedentary behavior, moderate-to-vigorous physical activity, and total energy intake. *P*-values < 0.05 were highlighted in bold. Abbreviations: BMI, body mass index; CI, confidence interval; OR, odds ratio.

Table S13. Associations of BMI and overweight/obesity with sleep clusters identified by sleep characteristics, excluding the Friday night and Saturday night

Sleep clusters	**N=1973, 2609 observations**	**Model**	
BMI	**Observations**	**β [95%CI]**	***P*-value**
Good sleep	944	Ref	
Delayed sleep phase	486	0.15 [-0.10, 0.39]	0.244
Sleep irregularity and variability	249	0.33 [-0.04, 0.70]	0.079
Fragmented sleep	729	0.27 [0.00, 0.53]	**0.047**
Prolonged sleep latency	201	0.74 [0.28, 1.19]	**0.002**
Overweight/obesity	**Cases/Observations**	**OR [95%CI]**	***P*-value**
Good sleep	15.6%	Ref	
Delayed sleep phase	15.2%	1.05 [0.80, 1.38]	0.715
Sleep irregularity and variability	21.3%	1.43 [1.03, 1.97]	**0.032**
Fragmented sleep	17.6%	1.20 [0.94, 1.55]	0.148
Prolonged sleep latency	23.4%	1.93 [1.34, 2.78]	**<0.001**

Adjusted for time of follow-ups, sex, age, study, study center, parental highest education, BMI measurement methods, season, sedentary behavior, moderate-to-vigorous physical activity, and total energy intake. *P*-values < 0.05 were highlighted in bold. Abbreviations: BMI, body mass index; CI, confidence interval; OR, odds ratio.

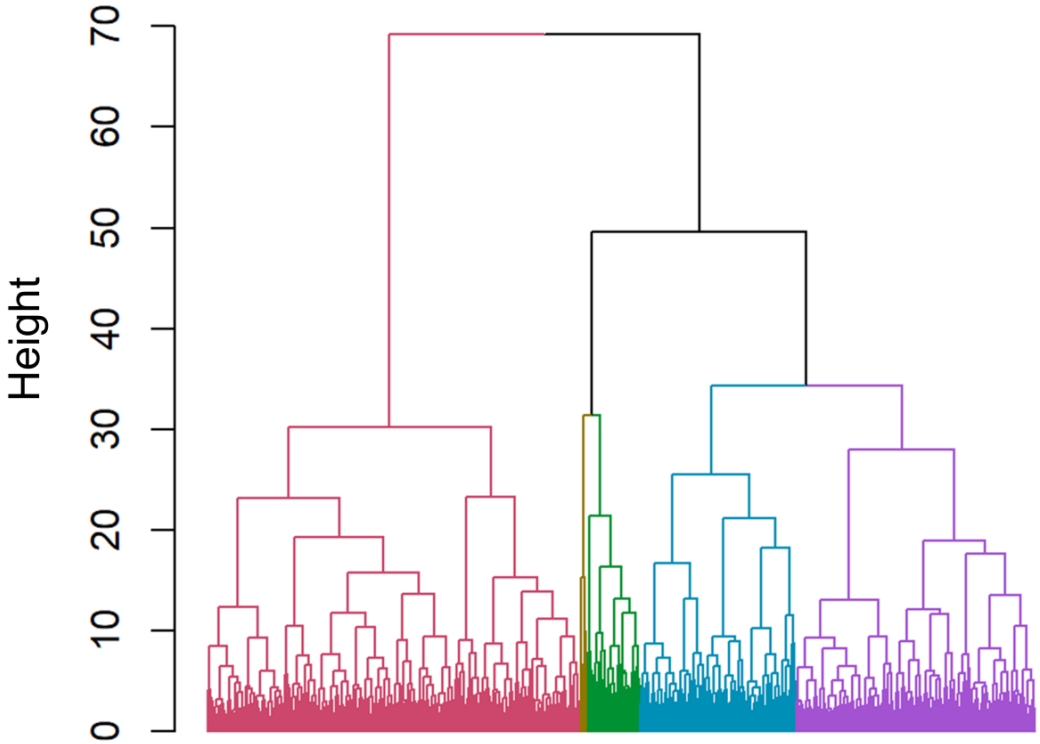

Fig. S1. Hierarchical clustering dendrogram based on 12 sleep characteristics from the 20-year data.

Fig. S1 illustrates the dendrogram of the hierarchical clustering from the 20-year data, using Euclidean distance and Ward's linkage across 12 standardized sleep characteristics. The y-axis of the dendrogram displays the "Height", which represents the distance used to cluster the objects. In this agglomerative approach, each observation begins as its own cluster and is subsequently grouped with similar objects in the early stages. As the number of clusters decreases, the height increases, reflecting greater heterogeneity among clusters. Five different colors denote the final five clusters.

Note, the hierarchical clustering dendrogram based on 12 sleep characteristics from the 15-year data has been published previously [1].

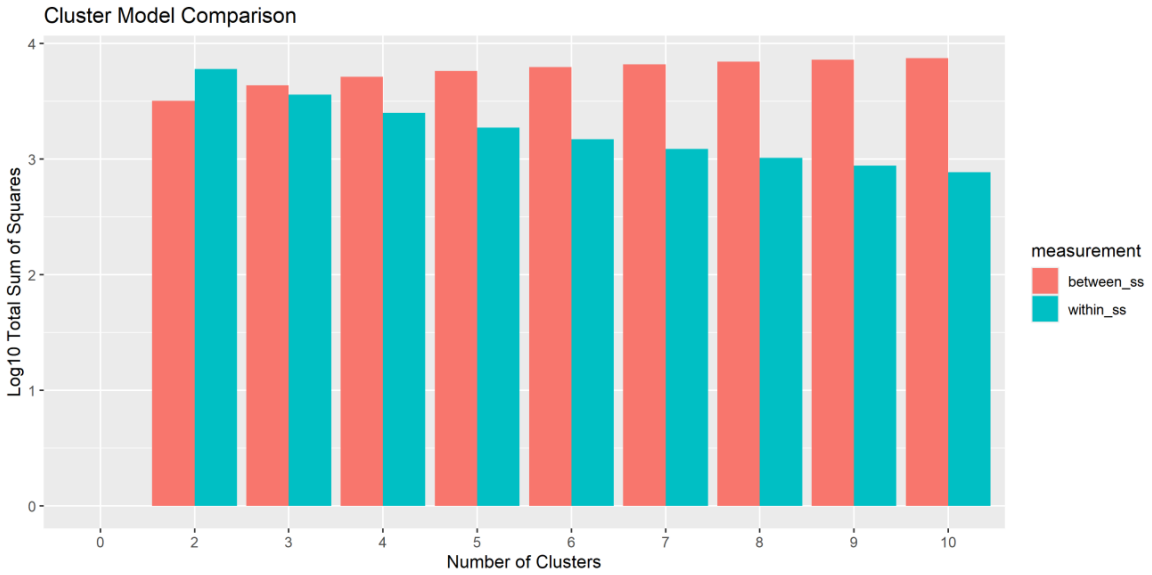

Fig. S2. Sum of squares method for k-means cluster model comparison from the 20-year data.

Fig. S2 presents the visualization of the sum of squares method, which is used to determine the optimal number of clusters. This method evaluates the within-cluster sum of squares, representing cluster compactness (blue color, the smaller the better), and the between-cluster sum of squares, representing the separation among clusters (red color, the larger the better). The figure suggests that choosing five clusters is the appropriate option.

Note, you can find the sum of squares method for k-means cluster model comparison from the 15-year data in previous paper [1].

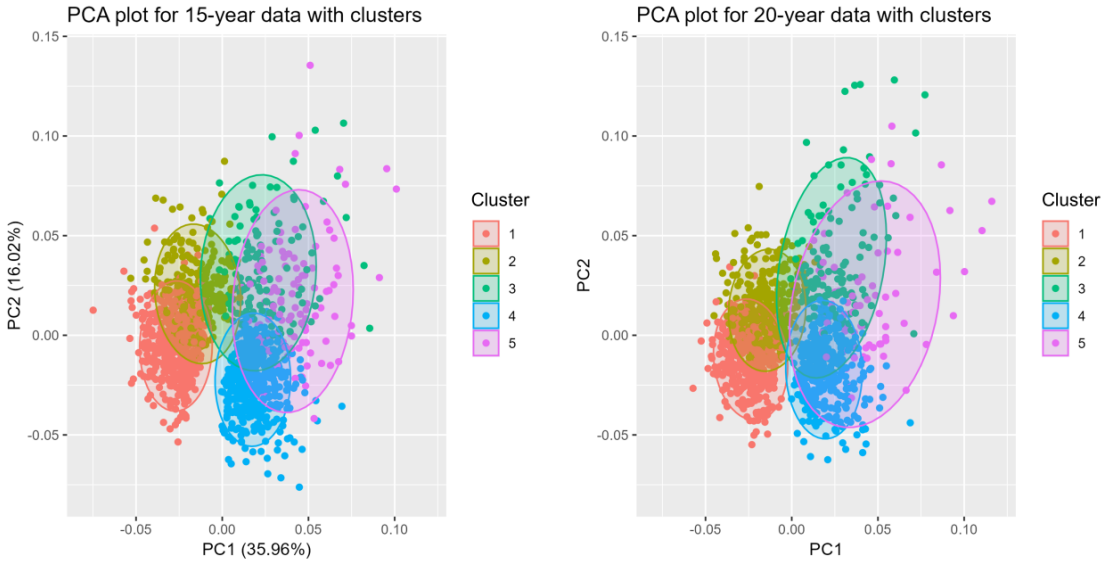

Fig. S3. Principal component analysis (PCA) plots for the validation of clustering results from 15-year data on 20-year data. The five sleep clusters at both data are: 1, "good sleep" cluster; 2, "delayed sleep phase" cluster; 3, "sleep irregularity and variability" cluster; 4, "fragmented sleep" cluster; 5, "prolonged sleep latency" cluster. The x-axis is the first principal component (PC), and the y-axis is the second PC.

In the **left plot** of **Fig. S3**, we display the 15-year data in the first two PCs, with each cluster represented by a distinct color, and ellipses drawn around the cluster centers. Cluster 1 (red) is distinctly separated from clusters 4 (blue) and 5 (purple) along the first PC. Clusters 1 (red), 2 (brown) and 3 (green) show some overlaps. There are some overlaps among Clusters 2 (brown), 3 (green), 4 (blue), and 5 (purple).

In the **right plot** of **Fig. S3**, we project the 20-year data onto the PCs of 15-year data via scaling and rotating, to compare more directly the PCA plot for 20-year data with the PCA plot for 15-year data (instead of calculating the PCA anew for the 20-year data). The distribution of 5 clusters is similar for both 15-year and 20-year data. For example, Cluster 1 (red) has a clear separation from Cluster 5 (purple) by the first PC, although there is small overlap with Cluster 4 (blue).

The validation of the PCA plots followed a published framework [2].

References

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2. Ullmann T, Hennig C, Boulesteix AL. Validation of cluster analysis results on validation data: A systematic framework. WIREs Data Mining and Knowledge Discovery. 2021;12(3): e14444.

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List of all scientific publications to date

Wang M, Flexeder C, Harris CP, Kress S, Schikowski T, Peters A, Standl M. Accelerometry-assessed sleep clusters and obesity in adolescents and young adults: a longitudinal analysis in GINIplus/LISA birth cohorts. *World Journal of Pediatrics*. Published online January 4, 2025. https://doi.org/10.1007/s12519-024-00872-5.

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