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Childhood Sleep Duration and Quality in Relation to Leptin Concentration in Two Cohort Studies

Caroline E. Boeke, ScD[1,2]; Amy Storfer-Isser, PhD[3]; Susan Redline, MD, MPH[4-7]; Elsie M. Taveras, MD, MPH[8,9]

[1]Department of Epidemiology, Harvard School of Public Health, Boston, MA; [2]Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital, Boston, MA; [3]Statistical Research Consultants, LLC, Schaumburg, IL; [4]Division of Sleep Medicine, Department of Medicine, Brigham and Women's Hospital, Boston, MA; [5]Division of Pulmonary Medicine, Department of Medicine, Beth Israel Deaconess Medical Center, Boston, MA; [6]Harvard Medical School, Boston, MA; [7]Department of Medicine, Brigham and Women's Hospital, Boston, MA; [8]Division of General Academic Pediatrics, Department of Pediatrics, Massachusetts General Hospital for Children, Boston, MA; [9]Departments of Pediatrics and Population Medicine, Harvard Medical School, Boston, MA

Study Objectives: Poor sleep in childhood is associated with increased obesity risk, possibly by affecting appetite-regulating hormones such as leptin. We examined short- and long-term sleep duration and quality in relation to leptin in two US pediatric cohorts.

Design: Analysis of data from two prospective cohort studies.

Setting: Population-based. Adolescent polysomnography assessments performed in a clinical research unit.

Patients or Participants: Children in Project Viva (n = 655) and adolescents in the Cleveland Children's Sleep & Health Study (n = 502).

Interventions: N/A.

Measurements and Results: In Project Viva, mothers reported average child sleep duration annually from infancy through age 7, and we measured leptin at ages 3 and 7. In the Cleveland Children's Sleep & Health Study, we collected self-reported sleep duration, polysomnography-derived measures of sleep quality, and fasting leptin at ages 16-19. In sex-stratified linear regression analyses adjusted for sociodemographic characteristics and adiposity, chronic curtailed sleep was associated with lower leptin at age 7 in girls; a one-unit decrease in sleep score was associated with a 0.08 decrease in log leptin (95%CI: 0.01,0.15). The association was stronger in girls with greater adiposity (P = 0.01). Among adolescents, shorter sleep was associated with lower leptin in males; each one-hour decrease in sleep duration was associated with a 0.06 decrease in log leptin (95%CI: 0.00, 0.11). Sleep duration was not associated with leptin at other ages. Sleep quality indices were not associated with leptin.

Conclusions: Our results suggest possible age-specific sexual dimorphism in the influence of sleep on leptin, which may partly explain inconsistencies in the literature.

Keywords: Sleep, leptin, obesity, youth

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INTRODUCTION

Given the increasing prevalence of childhood obesity in recent decades, it is important to identify modifiable risk factors in early life. Short sleep has been identified as a risk factor for obesity in children as well as adults.[1-4] Although the mechanisms by which poor sleep increases obesity risk are unclear, it is possible that poor sleep impacts glucose regulation, inflammatory markers, and appetite regulating hormones.[5] Leptin is a satiety hormone of particular interest as it appreciably influences each of these systems.[6] It has been speculated that curtailed sleep duration may contribute to leptin dysregulation, which in turn adversely impacts energy balance through insulin-glucose regulation, inflammatory processes, and energy homeostasis.[7] Leptin exposure typically reduces energy intake and increases energy expenditure, leading to weight *loss*. However, reduced sensitivity to the appetite-regulating effects of leptin, or leptin resistance, may occur, whereby the body does not respond to leptin's signaling mechanisms[6,8]; in such cases, leptin may be associated with weight *gain* or no change in weight.

Studies of sleep duration in relation to leptin in adults have yielded mixed findings.[9] Laboratory studies of healthy young men have found that sleep restriction was associated with lower leptin[10,11] or had no association with leptin,[12,13] while several studies in women[14,15] have reported an inverse association between sleep duration and leptin. Findings from observational studies that included adult males and females are also inconsistent, whereby shorter sleep duration was associated with lower leptin,[16,17] higher leptin[7,18,19] or was not associated with leptin.[20] Although the inconsistencies among these studies may partly relate to differences in experimental procedures such as ad libitum eating or controlled caloric intake, it is possible that differences in subject characteristics, including adiposity, age and sex, as well as differences in leptin sensitivity across study populations contribute to these discordant findings.[9]

Less is known about the relation of sleep duration and leptin in pediatric as compared to adult populations. A study of youth aged 6-19 years found that shorter weekday sleep duration was associated with higher leptin among girls but not boys,[21] while no association was found between sleep duration and leptin in a study of 13- to 17-year-old adolescents.[22] Still less is known about how sleep quality affects leptin in youth. A self-reported

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Caroline Boeke and Amy Storfer-Isser contributed equally to this manuscript as co-first authors.

Address correspondence to: Caroline E. Boeke, Department of Epidemiology, Harvard School of Public Health; 677 Huntington Avenue, Boston, MA 02115; Tel: (617) 525-2102; Fax: (617) 525-2008; E-mail: caroline.boeke@mail.harvard.edu

measure of sleep quality and self-reported sleep duration were not significantly related to leptin among 14- to 18-year-old adolescent females.[23]

In the current study, we utilized data from two relatively large cohort studies that together provided both cross-sectional and prospective information on sleep duration in relation to leptin in pre-pubertal (infancy to age 7) and post-pubertal (age 16 to 19 years) youth. Additionally, we assessed leptin in relation to indices of sleep architecture obtained by overnight polysomnography in the postpubertal cohort.

METHODS

Study Populations

We utilized data from two cohorts that jointly provided information during childhood and adolescence, with one cohort including infants and pre-pubertal children (Project Viva) and the other including late adolescents (Cleveland Children's Sleep and Health Study). Project Viva is a prospective cohort study of pre- and perinatal influences on maternal, fetal, and child health outcomes and the Cleveland Children's Sleep and Health Study (Cleveland Cohort) is a community-based urban cohort study designed to evaluate sleep measures and health outcomes in children born full-term and preterm. From 1999-2002, Project Viva recruited pregnant women at in-person visits in the first trimester of pregnancy from 8 obstetric offices of Harvard Vanguard Medical Associations, a multisite group practice in eastern Massachusetts.[24] Mother-child in-person visits occurred at 6 months, 3 years, and 7 years. Annually, participants completed questionnaires, and throughout investigators obtained other data from medical records. The number of live births to study participants was 2,128. Of the 2,128 mother-child pairs, 702 children had blood measurements at age 7 and of those, 655 had at least one sleep measurement. Institutional review boards of Harvard Pilgrim Health Care, Brigham and Women's Hospital, and Beth Israel Deaconess Medical Center approved the study protocols and all mothers provided written informed consent.

The Cleveland Children's Sleep and Health Study (Cleveland Cohort), recruited children who had been born at three hospitals in the Cleveland area between 1988 and 1993. As previously described,[25] all 907 children that participated in the middle childhood examination at ages 8-11 years were eligible to participate in a late adolescent examination conducted approximately 8 years later (at age 16-19 years). Of the 517 adolescents that were studied at this examination, 502 had leptin data and were included in these analyses; blood samples were not collected at the middle childhood examination and therefore leptin was not available for that time point. The protocol for the late adolescent examination was approved by University Hospitals of Cleveland Institutional Review Board. Informed written consent was obtained from participants aged 18 and older; for participants under age 18 years, the adolescent's legal guardian provided informed written consent, and the adolescent provided assent.

Field and Laboratory Procedures – Project Viva

Mothers reported child average sleep duration in a 24-h period at 6 months and annually from 1 through 7 years of age on questionnaires. At 6 months postpartum, the questionnaire asked: (1) "In the past month, on average, for how long does your baby nap during the morning," (2) "In the past month, on average, for how long does your baby nap during the afternoon," and (3) "In the past month, on average, how many hours does your baby sleep during the night." Subsequent questionnaires asked on average how many hours the child slept in a usual 24-h period in the past month. At 2, 3, and 4 years, the response categories were < 9 h, 9 h/day, 10 h/day, 11 h/day, 12 h/day, 13 h/day, and ≥ 14 h/day. At 1, 5, and 6 years, mothers wrote in the number of hours. At 7 years, mothers wrote in the number of hours on weekdays and weekend days separately. To calculate an age-specific weighted average of sleep duration from ages 6 months to 2 years, we created a cumulative sleep duration score via a sum that was weighted by the interval of time between the data collection of all 3 data points and divided the sum by 2. To estimate adequate sleep duration over time, we created a sleep duration score by taking the sum of the following measures: 1 point for sleep 12+ h at age 6 months to 2 years, 0 points for < 12 h; 2 points for 11+ h at age 3-5 years, 1 point for 10-11 h, 0 points for < 10 h; 2 points for 10+ h at age 6-7 years, 1 point for 9-10 h, 0 points for < 9 h.

At ages 3 and 7 years, the children provided non-fasting, venous whole blood samples. Research assistants immediately refrigerated plasma samples after collection and transferred them to a storage laboratory within 24 hours, where samples were spun and blood components were separated into aliquots for storage in liquid nitrogen. Plasma leptin was measured using a radioimmunoassay (Linco Research Inc, St Charles, Missouri, US).[26]

To assess child adiposity, weight at birth was obtained from hospital records. At 3 and 7 years, trained research assistants measured child height with a calibrated stadiometer (Shorr Productions, Olney, Maryland, US),[27] weight with a calibrated scale (age 3: Seca model 881, Seca Corp, Hanover, Maryland, US; age 7: Tanita model TBF-300A, Tanita Corporation of America, Inc., Arlington Heights, Illinois, US), and subscapular and triceps skinfold thicknesses with Holtain calipers (Holtain Ltd, Crosswell, Wales). At age 7, research assistants measured child dual energy X-ray absorptiometry (DXA) total body fat mass using a Hologic model Discovery A scanner (Hologic, Bedford, Massachusetts, US); a single investigator (CEB) checked all scans and defined body regions for analysis with QDR version 12.6 software. We calculated birth weight-for-gestational age z-score using national reference data from the United States Natality datasets.[28] Body mass index (BMI) was calculated by dividing weight in kilograms by height in meters squared, and age-sex-adjusted BMI z-scores and percentiles using the 2000 Centers for Disease Control and Prevention growth charts.[29,30] Using questionnaires and interviews designed specifically for this study, we asked mothers about their education, household income, and child's race/ethnicity, television watching, and physical activity.[8,24] Preterm labor was defined as birth at less than 37 weeks.

Field and Laboratory Procedures – The Cleveland Cohort

Overnight polysomnography (PSG), physiological, and anthropometric assessments were performed with a standardized protocol at a dedicated clinical research unit when the

adolescent was free from acute illness.[25,31] Adolescents and their primary caregivers also completed demographic, sleep, and health questionnaires during the examination, which began at approximately 17:00 and ended the following day at 11:00. All research staff (nurses, nutritionists and research assistants) received training, followed a detailed written protocol, and were supervised by a lead nutritionist and the principal investigator (Dr. Redline).

Full-channel overnight polysomnography was performed over a single recording night (E-Series [Compumedics Ltd, Abbotsford, Victoria, Australia]). As previously described,[32] sleep stages and arousal were scored using standard criteria.[33,34] The obstructive apnea-hypopnea index (OAHI) was summarized as the total number of obstructive apneas and hypopneas associated with ≥ 3% desaturations per hour of sleep. Other PSG-based summary measures of sleep architecture included sleep efficiency (percentage of the sleep period spent asleep), number of arousals per hour (arousal index), and percentage of sleep time in stage N3 (slow wave sleep) and REM sleep. Daytime sleepiness, an additional measure of sleep quality, was assessed with a modified version of the Epworth Sleepiness Scale.[35] Finally, we calculated weekday sleep duration using adolescent-reported usual weekday bedtime and wake time.

At approximately 07:00 the morning after the PSG and an overnight fast, venipuncture was performed by trained and certified research nurses. Blood was immediately processed and aliquots of EDTA plasma were frozen until assayed in batch at the University of Vermont Clinical Biochemistry Research Laboratory. Leptin was assayed using Millipore Bio-Rad Luminex Flow Cytometry assay (interassay CV: 1.8 to 11.3%).

Trained research nutritionists used a rigid stadiometer (Holtain, Pembrokeshire, UK) and a digital scale (Health-o-meter, Shelton, Connecticut) to measure height and weight, respectively. BMI and BMI z-score were calculated using the methodology described previously for Project Viva. Total body fat (including percentage of fat free body mass) was estimated by calculating resistance/reactance using bioelectrical impedance analysis (Quantum X; accuracies +1 ohm), and BMI, using Cyprus 1.2 Body-Composition Analysis software (Clinton Twp., Michigan, US) and pediatric prediction equations.[36] The adolescent's primary caregiver completed questionnaires specifically designed for this study to ascertain highest level of education, annual household income, and the adolescent's race/ethnicity.

Statistical Analysis

Subject characteristics, sleep measures, and leptin were summarized using descriptive statistics. The distribution of leptin was right-skewed and was transformed using the natural logarithm to achieve approximate normality. As there is evidence to suggest that the association of sleep duration and leptin may vary by sex,[19,21,37] we used sex-stratified multivariable linear regression analyses to evaluate the relation of sleep duration and quality to log leptin.

In Project Viva, we assessed the following relationships: (a) mean child sleep duration from 6 months to 2 years and leptin at age 3 years; (b) sleep duration and leptin at ages 3 and 7 years (cross-sectional associations); (c) sleep duration score from infancy through age 7 years and leptin at age 7 years; and (d) change in sleep duration from ages 3 to 7 years and change in leptin from ages 3 to 7 years. Using a multiple imputation algorithm in SAS statistical analysis software, we created 50 multiply imputed datasets to fill in any missing data on the 2,128 children in the cohort. We limited the analysis to the 655 individuals with sleep and leptin data available as described previously. In the Cleveland cohort, data from the late adolescent examination was used to examine the cross-sectional association of leptin with each PSG summary measure as well as self-reported sleep duration and daytime sleepiness. Complete case analyses were used as less than 1% of the data were missing.

Analyses of data from both cohorts included fitting sex-stratified linear regression models adjusted for a priori potential confounders: age, race/ethnicity, income, parental education, and preterm status (Cleveland Cohort only). Furthermore, as leptin is produced by adipose tissue and is highly correlated with adiposity, we additionally adjusted for both BMI z-score and body fat to assess the relation between sleep and leptin independent of adiposity. In Project Viva, we ran sensitivity analyses in which we adjusted for TV watching and physical activity, but adjusting for these additional variables did not substantially change our results so we did not include these variables in our final models. In the Cleveland Cohort, we ran sensitivity analyses excluding those with obstructive sleep apnea (OAHI3 ≥ 5).

Given that leptin is produced by adipose cells, and individuals with more body fat may have reduced leptin sensitivity, we assessed whether the association between sleep and leptin varied by adiposity in exploratory analyses. The two-way interaction between the sleep exposure and adiposity was examined by adding a sleep × BMI or sleep × body fat interaction term to the regression model. If the interaction term was significant ($P < 0.05$), we also assessed the associations by BMI percentile and body fat categories including BMI < 5th, 5th to < 85th, and ≥ 85th percentile and body fat at the ~20th, ~50th and ~80th percentiles based on age- and sex-specific normative values from the U.S. population.[38] Results were summarized using point estimates and 95% confidence intervals. All analyses were conducted using SAS version 9.2 or higher (SAS Institute, Cary, North Carolina).

RESULTS

Subject characteristics for the two cohorts are displayed in Table 1. In each cohort, approximately half of the sample was male, about one-third was non-Caucasian, and the proportion of obese participants was between 10.2% (7-year-old males) and 22.9% (17-year-old males). In Project Viva, mean (SD) sleep duration in infancy (6 months to 2 years) and at 7 years were 12.2 (0.9) and 9.8 (0.7) h/day, respectively. The median value of leptin was 1.4 ng/mL at age 3 and 3.1 ng/mL at age 7; leptin was higher in females than males. Participants in the Cleveland cohort were 17.7 (0.4) years old on average and reported sleeping 7.9 (1.7) h on average on weeknights. Findings from overnight PSG indicated that values for sleep architecture were consistent with normative data on adolescents [41]; 3% of adolescents had obstructive sleep apnea (OAHI ≥ 5) and 11% had mild sleep apnea (OAHI 2-4.9). The median value of leptin was 9.6 ng/mL, and the median value was higher in females (16.4) than males (2.9).

Table 1—Participant characteristics by cohort and child sex

	Project Viva		Cleveland Cohort	
Participant & Family Characteristics	**Females (n = 310)**	**Males (n = 345)**	**Females (n = 249)**	**Males (n = 253)**
Age, years, mean (SD)				
3 year study visit	3.3 (0.3)	3.3 (0.3)		
7 year study visit	7.8 (0.8)	7.9 (0.8)		
Late adolescent study visit			17.7 (0.5)	17.7 (0.4)
Child race/ethnicity				
African American	19.7%	18.9%	36.2%	34.0%
Hispanic	2.9%	5.0%	3.6%	3.2%
Caucasian	62.8%	61.3%	56.6%	58.9%
Other	14.6%	14.9%	3.6%	3.9%
Annual household income				
< $20,000	3.6%	7.8%	12.4%	13.8%
$20,000-$39,999	15.8%	9.7%	12.9%	15.0%
$40,000-$69,999	18.3%	21.4%		
$40,000-$74,999			22.5%	22.5%
≥ $70,000	62.1%	60.9%		
≥ $75,000			42.2%	37.6%
No answer			10.0%	11.1%
Mother's education*				
High school or less	10.8%	9.6%	21.3%	24.9%
Some college / vocational school	19.5%	25.0%	34.9%	32.4%
College degree	37.9%	36.3%	20.5%	22.5%
Graduate degree	31.8%	29.1%	23.3%	20.2%
Preterm	3.9%	7.5%	44.2%	43.1%
Birthweight, grams, mean (SD)	3,483 (498)	3,563 (576)		
Adiposity				
BMI z-score at 3 years, mean (SD)	0.46 (1.02)	0.51 (1.04)		
BMI z-score at 7 years, mean (SD)	0.45 (1.06)	0.35 (0.97)		
BMI z-score - late adolescent study visit, mean (SD)			0.56 (0.96)	0.64 (1.13)
Obese (BMI ≥ 95th percentile)**	16.8%	10.2%	16.1%	22.9%
Sum of subscapular and triceps skinfolds at 3 years, mm, mean (SD)	17.4 (4.3)	15.9 (3.9)		
DXA total body fat mass at 7 years, kg, mean (SD)	8.3 (4.2)	6.6 (3.4)		
DXA fat mass index at 7 years, kg/m^2, mean (SD)	5.0 (2.1)	3.9 (1.7)		
Bioimpedance % body fat, mean (SD)			31.0 (6.6)	22.1 (7.7)
Leptin Measures, median (IQR)				
Age 3, ng/mL	1.6 (0.8, 3.7)	1.3 (0.6, 2.8)		
Age 7, ng/mL	3.6 (1.5, 12.0)	2.7 (0.9, 8.6)		
Late adolescent study visit, ng/mL			16.4 (9.7, 30.1)	2.9 (1.4, 9.6)
Sleep Duration, mean (SD)				
Average sleep duration from 6 months to 2 years, hours/day	12.2 (1.2)	12.1 (1.2)		
Average sleep duration at 3 years, hours/day	11.1 (1.1)	11.1 (1.2)		
Average sleep duration at 7 years, hours/day	9.8 (0.9)	9.8 (0.9)		
Sleep duration score, 0 (low)-5 (high)	3.6 (1.4)	3.4 (1.5)		
Weeknight sleep duration late adolescent study visit, hours/day			7.9 (1.6)	8.0 (1.8)
Weekend sleep duration late adolescent study visit, hours/day			9.3 (1.6)	9.2 (1.8)
Polysomnography Measures: Late Adolescent Study Visit				
OAHI, median (IQR)			0.22 (0.00, 0.59)	0.71 (0.24, 1.88)
Sleep efficiency, median (IQR)			90.1 (84.3, 94.6)	88.1 (81.2, 93.1)
% time in stages 3-4 sleep, mean (SD)			24.1 (8.1)	22.2 (7.6)
% time in REM sleep, mean (SD)			20.3 (4.8)	21.1 (5.5)
Arousal Index, median (IQR)			7.5 (5.8, 9.8)	8.3 (6.6, 11.4)
Daytime Sleepiness: ESS, mean (SD)			7.9 (3.7)	7.2 (3.9)

SD, standard deviation; IQR, interquartile range. *In the Cleveland Cohort, this was the education of the primary caregiver, who was usually the mother. **In Project Viva, this refers to the age 7 visit.

Table 2—Sleep duration and quality in relation to the adjusted change in plasma leptin concentration

	Females		Males	
	Slope	**95% CI**	**Slope**	**95% CI**
Project Viva*				
	Age 3 Ln leptin (ng/mL)			
Infant sleep duration, per h/day	-0.000	-0.090, 0.090	-0.043	-0.127, 0.042
Age 3 sleep duration, per h/day	-0.044	-0.138, 0.050	-0.051	-0.123, 0.022
	Age 7 Ln leptin (ng/mL)			
Age 7 sleep duration, per h/day	0.070	-0.037,0.178	0.022	-0.078, 0.123
Childhood sleep duration score (0-5)	**0.084**	**0.014, 0.154**	-0.012	-0.075, 0.050
	Change in Ln leptin from 3 to 7 years (ng/mL)			
Change in sleep from 3 to 7 years, per h/day	-0.067	-0.180, 0.046	0.016	-0.073, 0.105
Cleveland Cohort**				
	Age 17 Ln leptin (ng/mL)			
Self-report: weekday sleep duration, per h/night	0.001	-0.038, 0.040	0.053	-0.003, 0.109
Self-report: weekend sleep duration, per h/night	0.003	-0.035, 0.041	**0.056**	**0.000, 0.112**
PSG sleep duration	-0.025	-0.088, 0.039	0.002	-0.082, 0.085
ln OAHI	0.036	-0.045, 0.116	0.076	-0.019, 0.171
Sleep efficiency	-0.003	-0.011, 0.004	0.001	-0.009, 0.010
% time in stages 3-4 sleep	0.001	-0.007, 0.009	0.002	-0.010, 0.015
% time in REM sleep	-0.001	-0.014, 0.012	-0.011	-0.029, 0.007
Arousal index	-0.002	-0.021, 0.017	0.003	-0.021, 0.027
Daytime sleepiness	-0.003	-0.020, 0.013	-0.019	-0.044, 0.006

*Project Viva models adjusted for race/ethnicity, age at leptin measurement, household income, maternal education, and adiposity (infant sleep models: birthweight, BMI z-score and sum of skinfolds at age 3; age 3 sleep models: BMI z-score and sum of skinfolds at age 3; age 7 sleep models: BMI z-score and DXA fat mass index at age 7; sleep score models: BMI z-score and DXA fat mass index at age 7; change in sleep models: BMI z-score and sum of skinfolds at age 3, change in BMI z-score and change in sum of skinfolds from ages 3 to 7). All models that were adjusted for sum of skinfolds were additionally adjusted for height. The sleep duration score was defined by taking the sum of the following measures: 1 point for sleep 12+ h at age 6 months to 2 years, 0 points for < 12 h; 2 points for 11+ h at age 3-5 years, 1 point for 10- < 11 h, 0 points for < 10 h; 2 points for 10+ h at age 6-7 years, 1 point for 9- < 10 h, 0 points for < 9 h. **Cleveland Cohort models adjusted for race/ethnicity, age at leptin measurement, preterm birth status, household income, caregiver education, BMI z-score, and bioelectric impedance percent body fat. Note: Values in bold are significant at P < 0.05

Prepubertal Children (Project Viva)

The results of the regression analyses for males and females are shown in Table 2. In univariate analyses, sleep duration was not associated with leptin at either time period. In analyses adjusted for potential confounders, neither infant sleep duration nor sleep duration at age 3 years were associated with leptin at age 3 in boys or girls. No relation was observed between change in sleep duration between ages 3-7 years and change in leptin during that time period. Findings were also null in cross-sectional analyses of the relation of sleep duration and leptin at age 7 years for both sexes. However, in multivariable-adjusted models, low cumulative sleep duration scores, indicative of chronic sleep curtailment in infancy and childhood, were associated with lower leptin at age 7 years among females only. A one-unit decrease in cumulative sleep duration score was associated with a 0.084 decrease in log leptin (95% CI: 0.014, 0.154). BMI z-score and fat mass index most strongly confounded this association; adjusting for these variables revealed a positive association between sleep duration score and age 7 leptin that was not apparent in univariate analysis. Adjusting for race and education further strengthened the positive association. There was a significant interaction between cumulative sleep duration score and child fat mass index in girls (P = 0.01). The positive association between sleep duration and leptin was stronger in girls with greater adiposity; among overweight or obese girls (n = 94), a one-unit decrease in cumulative sleep duration sleep score was associated with a 0.156 decrease in log leptin (95% CI: 0.011, 0.302). Cumulative sleep duration was not associated with leptin at age 7 in boys.

Postpubertal Children (Cleveland Cohort)

Among adolescent males, shorter weekday and weekend sleep duration were associated with lower leptin; each one-hour decrease in weekend sleep duration was associated with a 0.056 decrease in log leptin (95% CI: 0.000, 0.112), with a similar association observed for weekday sleep duration (Table 2). Among adolescent females, sleep duration was not associated with leptin. None of the PSG-based sleep quality measures or subjective daytime sleepiness were related to leptin in adolescent males or females. In exploratory analyses, in females only, there was a significant interaction between body fat and sleep efficiency in relation to leptin (P = 0.01). Post hoc analyses revealed an inverse association between sleep efficiency and leptin among adolescent females with low (28%) body fat, whereby a 10% increase in sleep efficiency was associated with a 0.090 decrease in log leptin (95% CI: -0.178, -0.006; P = 0.04); the association between sleep efficiency and leptin among females with high (40%) body fat was positive but of lower magnitude and was not statistically significant (β = 0.097, 95% CI: -0.025, 0.220, P = 0.12). No association was observed for among females with average (34%) body fat (β = 0.003, 95% CI: -0.075, 0.080; P = 0.95). We observed a similar pattern of results in sensitivity analyses that excluded adolescents with obstructive sleep apnea (OAHI3 ≥ 5).

DISCUSSION

In two large pediatric populations, we observed sex- and age-dependent associations between sleep and leptin. Chronic sleep curtailment in infancy and childhood was associated with lower leptin at age 7 years only in females, especially those

with greater adiposity. However, sleep duration at other periods examined was not associated with leptin at ages 3 or 7. In cross-sectional analyses, shorter sleep duration was associated with lower leptin in postpubertal males. Measures of sleep architecture were not associated with leptin among adolescent males or females. Our finding of a 0.08 difference in log leptin concentration is equivalent to a ~1.1 ng/mL increase in leptin for each one unit increase in child sleep duration score (7-year-old girls) or one hour increase in daily sleep (adolescent males). Taken together, our results suggest that there is a modest positive association between sleep duration and leptin in some subgroups of children and adolescents, and associations likely differ by sex and age, and possibly by level of adiposity.

Our findings extend the limited body of literature on sleep and leptin in youth,[21-23] as, to our knowledge, only three prior studies have assessed this relation. The Kiel Obesity Prevention study reported an inverse association of sleep duration and leptin among girls but not boys.[21] It is important to note that this study included both children and adolescents aged 6.1-19.9 years in their analyses, which may have obscured age-dependent associations. The AFINOS study of adolescents aged 13-17 years reported no association of sleep duration with leptin and did not find that BMI modified this association, although the small sample size likely limited the statistical power to detect effect modification.[22] In a cross-sectional study of 62 lean and 64 obese 14- to 18-year-old Saudi girls, neither self-reported sleep duration nor sleep quality were associated with fasting leptin,[23] which is consistent with findings from our study, although again the sample size was relatively small. However, our finding that shorter sleep duration was associated with lower leptin in adolescent males is consistent with the results of laboratory studies of young adult males[10,11,39] and several cohort studies.[16,17]

It is possible that leptin levels may differ in response to chronic as compared to acute sleep deprivation. This could explain why no association was observed in most prior observational cross-sectional studies, while we observed an association with our sleep score variable in Project Viva. Additionally, the discordant results across these previous studies may be partially explained by differences in participant age, sex and adiposity and/or methodological differences (i.e., different amounts of measurement error in the sleep variable in different populations). Our study was unique in that we examined cumulative habitual sleep duration across infancy and early childhood in relation to leptin at age 7 years. This cumulative exposure is likely to have less measurement error than the age-specific sleep exposures because it is less affected by short-term variability in sleep duration. The fact that we observed a stronger association of child 7-year leptin with cumulative sleep duration score than with sleep duration at age 7 may be a result of a more precise measure of chronic sleep curtailment or differential associations of contemporaneous and longer-term sleep curtailment with leptin.

There are many factors that may explain differences in the association of sleep duration with leptin across childhood/adolescence and in boys and girls, including changes in body size and shape, changes in sleep duration and quality from childhood to adolescence, pubertal changes in leptin concentration, and sensitivity to leptin. In childhood, leptin tends to be slightly higher in girls than boys, and leptin levels rise gradually with age. In girls, leptin levels continue to rise through puberty, whereas, in boys there is a decrease in leptin during puberty.[40] The sexual dimorphism in leptin concentrations continues into adulthood; leptin is several-fold higher in women compared to men, and differences in body fat do not fully explain this difference.[41] Leptin is likely one of several hormonal factors that allow puberty to begin, rather than a primary stimulant of the pubertal process.[42] In addition, since the actions of leptin are primarily through hypothalamic receptors influencing energy balance, it is possible that age and/or pubertal factors influence the central actions of leptin on appetite regulation.

There is previous evidence to suggest that the association of sleep measures and appetite-regulating hormones varies by sex.[9] Previous studies found that short sleep duration was associated with higher leptin only among girls,[21] and was more strongly associated with elevated leptin among women compared to men.[19] Additionally, St-Onge and colleagues[37] reported that short sleep duration was associated with increased total ghrelin concentrations only in men, and glucogen-like peptide 1 (GLP-1) was decreased in the short sleep duration condition only in women, although in this study leptin did not differ by sleep duration in men or women.[37]

Our findings that curtailed sleep was associated with decreased leptin in some age-sex-specific groups may suggest that chronic sleep deprivation could be related to weight gain through reduced leptin concentration, at least among those who are leptin-sensitive. However, we cannot exclude the possibility that the participants in our study are not leptin sensitive. There is some evidence to suggest that children/adolescents in this age range may have some level of leptin resistance. In Project Viva, we found that higher leptin concentration at age 3 was associated with *greater* weight gain from ages 3 to 7 and greater adiposity at age 7.[8] Similarly, higher basal leptin concentrations were associated with greater gains in BMI z-score 2-3 years later among obese girls age 7-18 years.[43] The findings from these prior studies suggest that even among youth, the body may not respond appropriately to leptin. If our population is leptin-resistant, it is hard to interpret what an association between sleep and leptin means. Unfortunately, leptin sensitivity is not measurable at this time. More research on leptin sensitivity/resistance is needed to fully interpret our findings.

Measures of sleep architecture and sleep disturbances, including the apnea hypopnea index, sleep efficiency, arousal index, sleep architecture and self-reported daytime sleepiness were not associated with leptin in adolescent males or females. However, in exploratory analyses, we found a negative association of sleep efficiency and leptin among lean adolescent females and a positive (albeit non-significant) association of sleep efficiency and leptin among heavy adolescent females. Since high sleep efficiency in the laboratory may reflect the influence of sleep homeostatic pressure in chronically sleep-deprived adolescents, the findings in lean adolescents is consistent with an effect of chronic sleep insufficiency on leptin levels.

Our analysis, which to our knowledge is the first to look at short- and long-term child sleep duration and sleep quality during adolescence in relation to leptin, has a number of strengths. Using data from two pediatric cohort studies, we were able to examine sleep in relation to leptin during important

developmental periods: early childhood and late adolescence. In the younger cohort, we prospectively collected detailed information on sleep habits, including bedtime and wake time, every year during early childhood. Thus we were able to assess the short-term association between sleep and leptin by looking at the cross-sectional relation between the variables collected, as well as long-term average sleep duration in the first 7 years of life with leptin in early childhood. In adolescents, we had objective measures of sleep quality from overnight PSG. Additionally, sex- and age-stratified analyses adjusted for multiple measures of adiposity as well as other confounders enabled us to examine the independent association of sleep and leptin. However, our results need to be interpreted in light of several limitations. Our findings are observational and thus do not have a direct causal interpretation. We used parent- or adolescent-reported sleep duration, which has more measurement error than objective measures of sleep duration and could influence the findings towards the null. Additionally, our results are not adjusted for multiple comparisons as we had a priori reasons to examine each association; however, this increases the chance of type 1 error and thus these associations require further examination in other populations. It is possible that our significant findings in our main analysis and particularly our exploratory analyses are due to chance. Leptin sensitivity, which cannot be measured, could potentially moderate the relation of sleep duration and leptin levels along with downstream effects of leptin on weight and other appetite-regulating hormones, glucose regulation and inflammation.

In two large prospective cohort studies, we found a modest prospective positive association between childhood sleep duration and leptin at age 7 years in females, particularly those with greater adiposity, and a positive cross-sectional association between sleep duration and leptin in adolescent males. Further research is needed to explore the relation of sleep and leptin during the peripubertal period as well as changes in leptin sensitivity and potential variation in leptin resistance during childhood and adolescence. Additionally, it would be interesting to examine how sleep and leptin relate to sex hormones and metabolism in youth. Our results underscore the potential role of age and sex differences in sleep physiology and hormone regulation as well as differences in leptin production in overweight and obese children.

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