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Short Sleep Duration as a Risk Factor for Obesity in Childhood Is Associated with Increased Leptin, Ghrelin, and Orexin Levels

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Abstract ***Objectives:*** To explore the association between short sleep duration, obesity and the role of appetite-related hormones in 10 to 15 years old children. ***Design:*** An observational study was conducted from January 2003 to June 2010, with epidemiological survey, anthropometry, and laboratory tests. One-way logistic and linear logistic methods were used for data analysis. ***Participants:*** A total of 311 obese children aged from 10 to 15 years and 300 healthy children with normal body mass index at the equivalent ages. ***Measurements and Results:*** Life styles of all participants were surveyed by questionnaires. Fasting serum samples were collected for detection of leptin, ghrelin and orexin from 114 obese and 49 healthy children randomly selected from the patient pool. Sleep duration of obese children, usually less than 9 hours, was 1 hour shorter than that of the healthy controls (P<0.05). Compared to the children sleeping less than 8 hours, the odd ratio for susceptibility to obesity was 0.015 (95% confidence interval: 0.005-0.051) for those sleeping more than 10 hours. Linear regression revealed that shorter sleep was associated with higher serum leptin, ghrelin, and orexin levels. If children's sleep time was deprived by one hour, the level of leptin, ghrelin and orexin will increase 7.894 ng/ml, 21.716 ng/ml, and 2.409 ng/ml respectively (P<0.01). ***Conclusions:*** Children at the age of 10 to 15 years have short sleep in common and those who do not sleep sufficiently are susceptible to obesity. Serum leptin, ghrelin, and orexin levels in children were elevated when they sleep less and consequently this increased the risk of obesity.

Key words Body mass index; Ghrelin; Leptin; Obesity, childhood; Orexin; Short sleep duration

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Introduction

Short sleep is the perception of inadequate sleep, either in amount or quality, leading to unsatisfactory normal duration of sleep caused by subjective or objective environment. Chronic hyposomnia will develop when one is exposed to long-term inadequate sleep. In China, with the fast social and economical development as well as the accelerated pace of life, more and more people are voluntarily suffering from a situational sleep restriction associated with exogenous events, particularly in case of stress from work or study. A close "mirror-reflection relationship" between the rapid rise in incidence of obesity and the propensity of inadequate sleep was noted among the US adults. The obesity prevalence almost doubled along with the prevalence of inadequate sleep.[1] However there was limited data on Chinese children's sleep state and to

our best knowledge there is no study on the relationship among the short sleep duration, childhood obesity and appetite regulating neuropeptides in China. This study was performed to study this relationship and the associated changes of appetite regulating neuropeptides.

Methods

Participants

Three hundred and eleven obese children and adolescents aged between 10 and 15 years old who were referred to our endocrinology department with the complaint of obesity from January 2003 to June 2010 were included into our study.

Inclusion criteria: (I) Subjects were eligible if they were healthy, and had a weight that was above the 97th percentile, and exceeded 20% of their standard weight for their age and sex based on the national reference data in 2005, consistent with an accepted diagnosis of obesity in Chinese children;[2] (II) In order to exclude subjects with endocrine or metabolic disease, liver and kidney function, thyroid function, adrenocorticotropic hormone and cortisol levels were measured and evaluated. Computed tomography or magnetic resonance imaging scans were performed to exclude hypothalamic and pituitary disease. Ultrasound was used to detect liver and adrenal lesions in all subjects. Chromosome examination was also performed when necessary. That is to say all those with underlying endocrine or genetic disease were excluded from our study.

A total of 311 (220 boys and 91 girls) children were eligible and enrolled, with a mean body mass index (BMI) of 28.34±3.79 kg/m^2 and a mean age of 11.75±1.45 years. Among them, 81 boys and 33 girls (BMI: 28.53±3.64 kg/m^2 and mean age: 11.75±2.29 years) were randomly selected for detecting serum levels of leptin, ghrehin and orexin.

The control group consisted of aged matched normal school students undergoing annual health examination whose BMI was below the 85th centile for all Chinese children in 2005 and whose weight ranged between the 25th and the 75th centile for Chinese children in 2005.[2] Informed consent was obtained both from the children and their parents/supervisors. Three hundred children were included (179 boys and 121 girls), with a mean age of 11.28±1.28 years and a mean BMI of 17.42±1.65 kg/m^2. Among them, 49 children (20 boys and 29 girls, mean BMI 16.86±1.79 kg/m^2, mean age 11.62±1.12 years) were randomly selected for testing serum leptin, ghrelin, and orexin levels.

Variables

Record of Sleep Duration: For the obese children, their sleep duration was recorded by using a 7-day sleep diary reported by the parents on first attendance or through phone interview. The control subjects were asked to complete a self-administered questionnaire under the guidance of the research team when they attended the physical check. Sleep duration included the period from sleep onset to sleep end (recorded from Monday to Sunday respectively) and sleep period during daytime like nap. The total sleep time in one week was divided by 7 to obtain the average daily sleep duration.

Measurements: Weight, height, waist and hip circumference (both at widest circle: midway between lowest rib and superior iliac crest hip) were measured at a standing posture. Blood pressure (BP) measurements were made using the right arm, with the subject in a quiet sitting position. BP was measured twice and the average recorded. The precision for weight, height and blood pressure were 0.1 kg, 0.1 cm, and 0 mmHg, respectively.

Questionnaire: "Life Style of Children in Zhejiang Province" was handed out to the 300 children with normal BMI and the 311 obese children. The content of the questionnaires included sleep duration, parents' weight and height total family income every month, education level of parents, health state at birth, breast-fed (Y/N), sport-activities, hours spent on television or computer, and dietary habits.

Hormone Assays: Fasting blood samples were taken from 114 obese children and 49 normal children and centrifuged for isolation of serum (which was stored at -80^{o}C). Leptin was measured by using a leptin enzyme-linked immunoassay kit (ELISA; Shenzhen Innogent Bioscience Inc.; batch number: 070622; *detection range*: 156-10000 pg/ml) and ghrelin by an ELISA kit (Phoenix Pharmaceuticals Inc.; batch number: 425302; *detection range*: 0.1-1.5 ng/ml). For orexin detection, plasma samples were obtained with presence of sodium citrate and aprotinin, and then stored at -80^{o}C. Orexin was determined by using a quantitative ELISA kit (Phoenix Pharmaceuticals Inc.; batch number: 425630; *detection range*: 0.13-1.65 ng/ml). All procedures were carried out according to manufacturer's instructions.

Definition of Inadequate Sleep: We regarded sleep less than 8 hours that had taken place for at least one year as inadequate sleep for 10-15 years old students based on the recommendation by the Chinese Ministry of Education and Ministry of Health in 1979 which defined sleep duration of 10 hours, 9 hours, 8 hours or more as adequate for

primary school students, middle school students and high school students respectively.

Statistical Analysis

All quantitative data with normal distribution were expressed as mean±SD. Data with skewed distribution were expressed with median, first quartile and third quartile that represent their central tendency and discrete tendency. Fisher's exact test, ANOVA, one-way logistic regression and linear regression methods were used in this study. A *P*-value less than 0.05 was defined as significant. Statistical analyses were processed under SPSS 16.0.

Results

The General Characteristics of the Participants

There were significant differences of mean sleep hours (8.75±1.21 vs 9.54±0.89 hours), BMI (28.34±3.79 vs 17.42±1.65 kg/m^2) between the obese group and the control group (P<0.05), while there was no significant difference of maximal sleep hours (12.61 vs 12.31 hours, minimal sleep hours (5.41 vs 6.12 hours) and mean age (11.75± 1.45 vs 11.28±1.28 years) (Table 1).

Effect of Lifestyle and Behaviour on Obesity

The univariate analysis showed that there were significant differences of parent's BMI ≥25 kg/m^2, parental education below primary level, total family income ≥5000 RMB, birth weight ≥4 kg, sleep duration <9 hours, spending on TV, computer or video games more than 3 hours every day, daily exercise less than 0.5 hour, taking fast food almost every day, taking no breakfast almost every day, taking snacks almost every night and bedtime after 23:00 between the obese group and the control group (P<0.05 respectively). There were no significant difference between the obese and healthy in comparison of age and gender, P>0.05 (Table 2).

Sleep Duration and Obesity

According to our data, 148 (24.22%) of the total 611 participants had a sleep duration ≥10 hours, and 48 (0.08%) less than 8 hours. Further analysis revealed that the proportion of control and obese groups who had a daily

Table 1 The general information of the obese and healthy

Category	Obese group	Control group	χ^2	*P* value
N	**311**	**300**		
Age (year)	11.75±1.45	11.28±1.28	9.57	0.056
Body mass index (kg/m^2)	28.34±3.79	17.42±1.65	25.29	0.002
Sleep hours (hour)	8.75±1.21	9.54±0.89	26.12	0.001
Longest sleep hours (hour)	**12.61**	**12.31**	**5.34**	**0.120**
Shortest sleep hours (hour)	**5.41**	**6.12**	**12.87**	**0.045**

Table 2 Comparison of risk factors for obesity-related lifestyle between the obese and healthy

Risk factors	Control group	Obese group	χ^2	*P* value
Either parent's body mass index ≥25 kg/m^2	0.370 (111/300)	0.627 (195/311)	47.823	<0.001
Either parental education < primary school graduate	0.167 (50/300)	0.309 (96/311)	20.385	<0.001
Total family income every month ≥5000 RMB	0.427 (128/300)	0.669 (208/311)	42.727	<0.001
Birth weight ≥4 kg	0.080 (24/300)	0.167 (52/311)	12.260	<0.001
Sleep duration every day <9 hours	0.160 (48/300)	0.717 (223/311)	133.855	<0.001
Spending on TV, computer or videogames more than 3 hours every day	0.067 (20/300)	0.232 (72/311)	37.998	<0.001
Daily exercise less than 0.5 hour	0.463 (139/300)	0.994 (309/311)	273.291	<0.001
Taking fast food almost every day	0.010 (3/300)	0.630 (196/311)	312.101	<0.001
Taking no breakfast almost every day	0.017 (5/300)	0.408 (127/311)	157.968	<0.001
Taking snacks almost every night	0.070 (21/300)	0.695 (216/311)	293.298	<0.001
Bedtime after 23:00 every day	0.083 (25/300)	0.209 (65/311)	18.265	<0.001

Comparison of age and gender between the obese and healthy, P>0.05

sleep ≥10 hours were 38.33% (115/300) and 10.61% (33/311), respectively. In the obese group, 15.11% (46/311) less than 8 hours, significantly different from the proportion of 0.67% (2/300) with less than 8 hours sleep in the control group.

The obese children were further divided in 3 subgroups by BMI: A with 23 kg/m^2 ≤BMI<25 kg/m^2, B with 25 kg/m^2 ≤BMI<30 kg/m^2, and C with BMI ≥30 kg/m^2, respectively. Sleep hours of subgroup A, B and C were 8.89±0.87 hours, 8.76±0.98 hours and 8.26±1.05 hours respectively. All the 3 subgroups had significantly shorter sleep duration compared with the control (9.53±0.89 hours) (P<0.05). Using Nemenyi method, we found significant difference between the above 4 groups, except for that between A and B subgroup (Table 3).

The one-way logistic regression analysis showed that sleep duration was a risk factor for childhood obesity, that is, the prevalence of obesity increased with the reduction of sleep hours. The odd ratio of obesity for those with daily sleep duration of 10 hours or more was 0.015 (95% confidential interval 0.005-0.051) compared with those who had shorter sleep (less than 8 hours daily) (Table 4).

Sleep Duration and Metabolism-relevant Hormones

All the subjects who received hormone assessment were divided into two groups, the group of ≥10 hours daily (32 children) and the group of less than 8 hours daily (26 children). We made comparison between the two groups with respect to BMI and relevant hormones (leptin, ghrelin and orexin) and behaviour of food intake at night. The subgroup of less than 8 hours, compared with the subgroup of 10 hours or more, presented a rise of 18.97%, 13.39%, 58.74%, and 34.62% in their BMI, serum leptin, ghrelin and orexin, respectively. Meanwhile, a dramatically higher rate of taking night snacks was observed in the subgroup with sleep of less than 8 hours daily. Further analysis revealed that all the subjects who received hormone detection were further divided in four subgroups by hours daily: the subgroup 1 (≥10 hours daily, obese group); the subgroup 2 (≥10 hours daily control group); the subgroup 3 (less than 8 hours daily ,obese group; the subgroup 4 (less than 8 hours daily, control group). Using Nemenyi method, we found that the subgroup 1 and 3 (less than 8 hours sleep, control group and obese group) had higher leptin, ghrelin, orexin and night snack intake rate than the subgroup 2 (more than 10 hours sleep, control group) (P<0.01); the subgroup 3 (less than 8 hours daily, obese group) had higher BMI, leptin, ghrelin orexin and night snack intake rate than the subgroup 1 (more than 10 hours sleep, obese group) (P<0.01); but leptin, ghrelin and orexin had no difference between the subgroup 4 (<8 hours, control group) and the subgroup 1 (≥10 hours, obese group) (Table 5).

Linear regression analysis showed a relationship between sleep duration and serum leptin, ghrelin and orexin levels. The reduced sleep hours was associated with higher leptin, ghrelin and orexin (P<0.01). The children' sleep time reduced by every one hour, the level of leptin, ghrelin and orexin would increase 7.894 ng/ml, 21.716 ng/ml and 2.409 ng/ml respectively (Table 6).

Discussion

Sleep as one of the fundamental human physiological functions plays a significant role in energy balance. Good and adequate sleep is essential for children's physical and psychological development. However, in the modern society the changes in lifestyle have extensively affected children's sleep duration. Over the past 40 years, Americans self-reported morpheus time has reduced by 1-2 hours, and in the past eight years, the number of Americans who sleep less than six hours a night jumped from 13% to 20%, and those who reported sleeping eight hours or more dropped from 38% to 28%.[3] According to the data collected by Shen et al[4] and Ma et al[5] respectively for the sleep habits of one-

Table 3 Comparison of sleep hours between the obese and healthy

	Control group	Obese group			F value/χ2	*P* value*
		Subgroup A	Subgroup B	Subgroup C		
N	300	56	188	67		
Sleep hours (hour)	9.53±0.89	8.89±0.87†	8.76±0.98†	8.265±1.05†‡§	87.850	<0.001

*Analysis of variance

The obese children were further divided in 3 subgroups by BMI: A with 23 kg/m^2 ≤BMI<25 kg/m^2; B with 25 kg/m^2 ≤BMI<30 kg/m^2; and C with BMI ≥30 kg/m^2

Nemenyi method: †compared with control group, p<0.001; ‡compared with Subgroup A, p<0.001; §compared with Subgroup B, p<0.001

month infant to 5-year-old children in Shanghai and 6-year-old or older residents in eastern China, even the infants and younger children were suffering from sleep loss. Our findings are consistent with previous studies and found that 10- to 15-year-old children did not sleep adequately in Zhejiang, eastern China, as only 24.22% of them had sleep of 10 hours or more daily while up to 0.08% of them had sleep of less than 8 hours daily.

Several recent large researches reported a U-shaped curvilinear relationship between sleep duration and obesity in adults and a negative relation in children.[6-8] A survey of the Wisconsin Sleep Cohort[9] revealed a negative correlation between BMI and sleep duration in adults. Chaput et al[10] surveyed the pupils in Quebec province of Canada and revealed that sleep loss was the first cause for childhood overweight and obesity. We found that the children with obesity usually slept less than 9 hours daily, about 1 hour less than those with normal BMI. The proportion of adequate sleeper (≥10 hours) was much lower in the obese children than that in the controls (10.61% vs 38.33%), that is to say, the number of children with sleep loss (<8 hours) was markedly larger (15.11% vs 0.67%). The sleep duration in children with BMI ≥30 kg/m^2 was shorter than that in those with BMI <30 kg/m^2. The one-way logistic regression analysis showed that shorter sleep duration was a risk factor for obesity and that children with sleep of less than 8 hours

Table 4 Comparison of sleep hours between the obese and healthy

Sleep hours		<8 hours	8-10 hours	≥10 hours
Parameter estimate		-2.648	-4.18	
Standard error			0.598	0.617
Wald χ2		83.983	19.57	45.863
P value		<0.001	<0.001	<0.001
Odd ratio			0.071	0.015
Odd ratio (95% CI)	Upper		0.022	0.005
	Lower		0.229	0.051

Table 5 Sleep duration is associated with BMI, metabolic hormone levels and snack-taking

Sleep hours	≥10 hours		<8 hours		*u* value/χ^2	P value
	Obese group	Control group	Obese group	Control group		
N	12	20	24	2		
Body mass index (kg/m^2)*	26.31 (2.11)	16.52§ (1.84)	32.47†‡§ (3.35)	17.26†§ (2.91)	32.870	<0.001
Leptin (ng/ml)**	35.14 (22.85, 58.42)	12.68§ (2.98, 53.71)	47.51†‡§ (27.50, 40.47)	29.61†‡ (28.06, 31.15)	9.791	0.004
Ghrelin (ng/ml)**	28.28 (1.84, 56.38)	7.76§ (1.73, 19.99)	92.80†‡§ (38.71, 155.92)	22.83†‡ (22.67, 23.00)	28.291	<0.001
Orexin (ng/ml)**	11.86 (3.46, 27.91)	8.10§ (3.85, 12.29)	18.14†‡§ (6.35, 28.65)	12.40†‡ (12.35, 12.46)	8.032	0.012
Night food intake (%)	16.67	0.00§	70.83†‡§	50†‡§	10.414	0.006#

*mean (standard deviation); **median (first quartile, third quartile); #Fisher's exact test

Nemenyi method: †compared with obese subgroup (<8 hours), p<0.001; ‡compared with control subgroup (≥10 hours), p<0.001; §compared with obese subgroup (≥10 hours), p<0.001;

The rate of food intake at night is obtained by dividing the number of persons who almost had snacks daily by total number in each group.

Table 6 Association between sleep duration and hormones by linear regression analysis

		Leptin		Ghrelin		Orexin	
		CT	Sleep duration	CT	Sleep duration	CT	Sleep duration
Partial regression coefficient	B	104.64	–7.894	236.154	–21.716	33.424	–2.409
	SE	9.879	1.101	15.296	1.705	3.412	0.380
Standard regression coefficient		–	–0.492	–	–0.709	–	–0.447
t value		10.592	–7.170	15.439	–12.739	9.796	–6.334
P value		0.000	0.000	0.000	0.000	0.000	0.000

B: partial regression coefficient; SE: standard error; CT: constant term

were more susceptible to obesity. These findings are consistent with Chaput's results[10] and suggest a causative relationship between sleep curtailment and obesity. In addition, we found that late bedtime was associated with obesity in children. Bedtime after 23:00 accounted for 20.90% in the obese children and 8.30% in the healthy ones. How to account for this difference? The likely explanation is that growth-relevant hormones (such as growth hormone) can promote the development of human body as well as fat metabolism. Since growth hormone is generally secreted in a pulsating mode in the deep sleeping phase, the postponement of bedtime may interfere with the hormone secretion and cause fat accumulation.[11] However, these questions remain to be elucidated by further investigations.

How is sleep curtailment related to obesity? Hormone homeostasis is achieved during good sleep as a result of sufficient secretion of various hormones. Recent evidences have shown[12,13] that inadequate sleep can lead to imbalance of energy and obesity by changing food intake behaviour and minimising energy consumption. Leptin, ghrelin and orexin, as components of the adipose-central neuron system axis, are responsible for regulating appetite and energy expenditure.[14] In our study, we found that the obese children with sleep duration of less than 8 hours had an 18.97% increase in BMI, 13.39% in the level of leptin, 58.74% in ghrelin and 34.62% in orexin, compared with those who had adequate sleep. Among the 163 participants, those with sleep loss were also associated with elevated leptin, ghrelin and orexin. All these suggested that levels of leptin, ghrelin and orexin may contribute to the sleep-loss caused obesity.

Leptin, a protein product of ob gene, is released by adipose tissue and functions as an anorexigenic hormone. Hunger or excessive food intake will induce a sharp reduction or elevation in serum leptin. As exogenous leptin has presented a weight-losing effect in ob/ob mice, a same effect may be deduced in human being if the leptin level is inversely related to body fat. However, increased leptin level has been observed in most obese individuals, indicating leptin resistance.[15] Ghrelin, a recently discovered brain-gut peptide that is mainly produced by the human stomach, act as the internal ligand for somastatin receptors and stimulates appetite in human being. Ghrelin levels increase before meals and decrease after meals.[16] Ghrelin is regarded as the counterpart of leptin as it has presented many effects including stimulation of food intake, enhancement of carbohydrate expenditure, increasing fat accumulation, promotion of gastric motility and acid secretion, and reducing spontaneous activities. Taheri et al[9] and Spiegel et al[12] found that adult sleep curtailment is associated with decreased leptin levels, elevated ghrelin levels, increased BMI and a 24% increase in hunger sensation. The increase in hunger sensation was strongly correlated with the increase in the ghrelin-to-leptin ratio. In our study, we also observed that the behaviour of taking night snacks increased in the obese children with sleep curtailment. It was as high as 70.83% in less than 8 hours sleepers and as low as 16.67% in more than 10 hours ones (out of the 114 obese children with hormonal assessment). The decrease in sleep hours was associated with the increase in appetite. However, our finding of leptin levels during sleep restriction is not consistent with that of Taheri's findings in adults. In our study, the level of leptin during sleep loss was elevated with the increase of BMI. The children' sleep time reduced by every one hour, the level of leptin and ghrelin would increase 7.894 ng/ml and 21.716 ng/ml respectively. At the same time, we found that the level of leptin in the obesity children with insufficient sleep was dramatically higher than that of obese children with adequate sleep and the control healthy group (whatever their sleep is sufficient or not). Besides, the healthy children with insufficient sleep also showed higher level of leptin than the healthy children who had sufficient sleep. The level of leptin was even close to that in the obesity children with sufficient sleep. which indicated that sleep curtailment affected the serection of leptin. The different changes of leptin level may be due to the difference in study subjects. Taheri's subjects were healthy adults with acute sleep restriction, while ours were obese children with chronic sleep loss. In our subjects, leptin resistance might have taken place, and therefore leptin might not play an important role in the pathogenesis of obesity in the children with sleep restriction. As we can see in this study that leptin levels increased obviously in the children with short sleep duration and weight gaining.

Orexins are the common names given to a pair of highly excitatory neuropeptide hormones (orexin A and B), derived from cleavage of a single precursor protein. Orexins are produced by neurons in the lateral and posterior hypothalamus and released into ventricular circulation by which they indirectly act on distant target cells after combination to the G-protein coupled receptors (OX_1R and OX_2R). The orexin system was initially suggested to be primarily involved in the stimulation of food intake and wakefulness. The discovery that exogenous orexin strikingly promotes food intake in rats and the level of prepro-orexin mRNA increases in fasting rats indicated a major role for orexin in the regulation of food intake. Spiegel et al[17] discovered that compared with those who

had slept 12 hours, healthy young men who were deprived of sleep for 4 hours experienced a relative rise in rapid eye movement (REM) phase in spite of the absolute reduction in both phases of sleep. Kigashchenko et al[18] found that more orexins were produced under the condition of wakefulness and in the REM phase than in the slow wave sleep phase. These findings indicated that sleep restriction may lead to increase of orexin levels. In another research,[19] the mRNA level of prepro-orexin in the hypothalamus did not change obviously during the 4-hour sleep restriction, but increased as the restriction duration is prolonged. If sleep restriction amounted to 8 hours, the mRNA levels in the hypothalamus and cerebral cortex would rise markedly. High orexin level may be necessary to the maintenance of wakefulness during sleep restriction and responsible for high appetite. Our data revealed a high orexin level in obese children with less sleep (while children' sleep time was reduced one hour, the level of orexin increased 2.409 ng/ml), suggesting a role of orexin in the correlation between sleep loss and obesity in children.

In conclusion, our findings demonstrated that the elevated ghrelin and orexin contributed to the increase in food intake and that the resistance of the anorexigenic hormone leptin further promoted this activity. Our incautious attitude towards sleep may partly contribute to our bulging bodies.[20] Sleep duration has been recognised as an easily amendable risk factor for obesity, which may play an important role in prevention and treatment of obesity.

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