U.PORTO

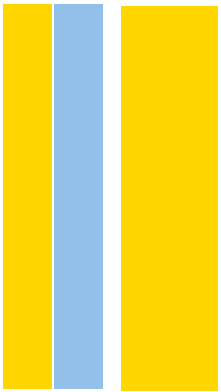
MASTER
PUBLIC HEALTH

The effect of sleeping habits and problems at 4 and at 7 years of age on cardiometabolic health

Raisa Rios Lodi Guedes

M

2020



Raisa Rios Lodi Guedes

The effect of sleeping habits and problems at 4 and at 7 years of age on cardiometabolic health

Dissertação de candidatura ao grau de Mestre em Saúde Pública apresentada à Faculdade de Medicina da Universidade do Porto e ao Instituto de Ciências Biomédicas Abel Salazar.

Orientadora: Prof. Doutora Liane Correia Costa
Co-Orientadora: Prof. Doutora Ana Cristina Santos

Porto, 2020

O presente trabalho foi elaborado no âmbito da coorte Geração XXI, desenvolvido no Departamento de Epidemiologia Clínica, Medicina Preditiva e Saúde Pública da Faculdade de Medicina do Porto e no Instituto de Saúde Pública da Universidade do Porto.

A sua realização só foi possível com a participação das crianças e famílias que aceitaram este desafio, às quais agradecemos a sua disponibilidade.

O projeto Geração 21 é apoiado pelo Fundo Europeu de Desenvolvimento Regional (FEDER) através do Programa Operacional para a Competitividade e Internacionalização e por financiamento nacional da Fundação para a Ciência e Tecnologia (FCT), Ministério da Ciência, Tecnologia e Ensino Superior e da Unidade de Investigação em Epidemiologia - Instituto de Saúde Pública da Universidade do Porto (EPIUnit) (UIDB / 04750/2020), Administração Regional de Saúde do Norte (Departamento Regional do Ministério da Saúde) e Fundação Calouste Gulbenkian.

Esta dissertação tem por base um manuscrito, no qual colaborei ativamente na formulação das hipóteses, análise e interpretação dos dados e fui responsável pela escrita da sua primeira versão e participei em todas as revisões posteriores:

- The effect of sleeping habits and problems at 4 and at 7 years of age on cardiometabolic health in a Portuguese birth cohort.

AGRADECIMENTOS

Table of Contents

List of tables

Abbreviations

BMI - Body mass index

BP - Blood Pressure

95% CI - 95% Confidence interval

DBP - Diastolic blood pressure

HDLc - High-density Lipoprotein Cholesterol

HOMA-IR - Homeostatic Model Assessment for Insulin Resistance

ICIDS-3 - International Classification of Sleep Disorders

LDLc - Low-density Lipoprotein Cholesterol

NREM - Non-rapid Eye Movement

OSA - Obstructive sleep apnea

PSQ - Pediatric Sleep Questionnaire

REM - Rapid Eye Movement

SBP - Systolic Blood Pressure

SD - Standard Deviation

SRBD - Sleep-Related Breathing Disorder

TG - Triglycerides

WHO - World Health Organization

Resumo

Hábitos e problemas de sono parecem ter impacto na morbimortalidade cardiovascular na idade adulta, mas a evidência desta associação durante a infância é ainda muito escassa. No presente estudo, pretendeu-se caracterizar hábitos e problemas do sono, em idade pré-escolar e escolar, e estudar o seu impacto no índice de massa corporal (IMC) e em outras variáveis cardiometabólicas.

Nesta tese foram analisados dados provenientes da coorte de nascimentos Geração XXI, uma coorte de base populacional, com recrutamento inicial entre 2005 e 2006, em todas as maternidades públicas da área Metropolitana do Porto (n= 5), tendo sido incluídas 8647 crianças (91,4% de todas as famílias convidadas a participar). Aos 4 anos de idade foi realizada a primeira avaliação global da coorte, com 86% de participação, e aos 7 anos de idade uma segunda avaliação, com 80% de participação. Foram elegíveis para o presente estudo todas as crianças com informação sobre o IMC aos 7 anos e com informação acerca de variáveis de sono, recolhidas aos 4 e/ou aos 7 anos, com uma amostra final de 5529 crianças. Entrevistadores treinados aplicaram um questionário estruturado sobre características demográficas e socioeconômicas, saúde da criança, hábitos e problemas do sono aos 4 e 7 anos de idade. Foi ainda recolhida informação acerca de diversas variáveis cardiometabólicas, nomeadamente antropometria, pressão arterial (PA), perfil lipídico, glicose e insulina, aos 7 anos de idade.

A duração do sono (sono noturno e duração total do sono diário) foi registada aos 4 anos e a duração do sono durante a semana e fim-de-semana foi registrada aos 7 anos. Aos 4 anos, foram considerados como problemas de sono duração de sono curta (dormir menos que 10 horas de sono noturno), atraso no início do sono, despertares noturnos e parassómnias da fase de sono de movimento oculares não-rápidos (NREM) (especificamente sonambulismo e terrores do sono). Aos 7 anos, foram considerados como problemas de sono, a duração de sono curto (dormir menos que 9 horas de sono noturno), a presença de irregularidade de sono (diferença de mais de uma hora no horário de dormir entre dias úteis e fins de semana) e distúrbios respiratórios relacionados com o sono (SRBD) e sonolência diurna, os dois últimos avaliados pela aplicação do *Pediatric Sleep Questionnaire* (PSQ), previamente validado em Portugal. As associações entre variáveis foram analisadas através de testes T, testes de Qui-quadrado e modelos de regressão linear multivariada.

Aos 4 anos, a média (desvio padrão, DP) da duração diária do sono noturno foi de 11,2 (1,00) horas; 4343 (78,5%) das crianças apresentaram algum problema de sono e 9,1% das crianças apresentaram alguma parassómnia NREM. Verificou-se que a ocorrência de duração de sono curto aos 4 anos tinha sido mais frequente nas crianças com sobrepeso ou obesidade aos 7 anos (34.8% vs 31.2%, p=0.007). Aos 7 anos, a duração média (DP) do sono noturno diário foi de 10,0 (0,6) horas; 1044 (57,7%) das crianças apresentaram algum problema de sono e 13,5% das crianças apresentaram SRBD. A presença de SRBD foi significativamente maior entre crianças com sobrepeso/obesidade aos 7 anos de idade (17,5% vs 11,6%, p <0,001).

Aos 4 anos, em modelos ajustados para sexo e escolaridade materna (Modelo 2), verificou-se que os níveis de colesterol LDL aumentaram 0,96 mg/dL (intervalo de confiança a 95%, IC 0,13 a 1,79, p = 0,022) por hora de sono noturno diário, enquanto os níveis de PA sistólica diminuíram 0,32 mmHg (IC -0,63 a 0,00, p = 0,047) por hora de sono total diário. A presença de despertares noturnos associou-se a valores mais elevados de *z-score* de IMC (em 0,09 (IC 0,03 a 0,16, p = 0,004); nos modelos adicionalmente ajustados para *z-score* de IMC (Modelo 2 + *z-score* de IMC), o aumento nos valores de triglicerídeos (TG) foi de 3,09 mg/dL (IC 0,70 a 5,48), p=0,011). A presença de parassómnias NREM associou-se a valores de PA mais elevados. Este efeito, após ajuste para o IMC, apenas permaneceu significativo para PA diastólica, aumentando 0,72 mmHg (IC 0,05 a 1,40, p=0,035) na presença de sonambulismo e/ou terrores noturnos.

Aos 7 anos de idade, em modelos ajustados para sexo e escolaridade materna (Modelo 2), verificou-se que o *z-score* de IMC diminuiu em 0,09 (IC -0,16 a -0,02, p=0,012) por hora de sono diário durante a semana e em 0,07 (IC -0,12 a -0,03, p=0,001) por hora de sono noturno diário no fim de semana, e que os níveis de *Homeostatic Model Assessment for Insulin Resistance* (HOMA-IR) diminuíram significativamente em 0,06 (IC -0,11 a -0,00, p=0,022) por hora de sono diário durante a semana. A irregularidade do sono associou-se a valores mais elevados de HOMA-IR (0,09 (IC 0,02 a 0,17, p=0,012). A presença de SRBD associou-se a valores de *z-score* de IMC mais elevados (0,39 (IC 0,23 a 0,55, p<0,001), HOMA-IR superior (0,14 (IC 0,03 a 0,25, p=0,011) e valores mais elevados de PA (PA sistólica 1,80 mmHg (IC 0,60 a 2,99, p=0,003) e PA diastólica 1,01 mmHg (IC 0,02 a 1,99, p=0.044). Nos modelos adicionalmente ajustados para o *z-score* de IMC (Modelo 2 + *z-score* de IMC), a presença de SRBD aos 7 anos associou-se a níveis mais baixos de colesterol HDL em 1,97 mg/dL (IC -3,54 a -0,39,

p=0,014). A sonolência diurna associou-se a valores da PA superiores (PA sistólica 1,04 mmHg (IC 0,51 a 1,57), p<0,001), (PA diastólica 0,85 mmHg (IC 0,38 a 1,32), p<0,001). Em conclusão, no presente estudo os problemas de sono aos 4 e 7 anos de idade associaram-se a IMC mais elevado e a níveis também mais elevados de diversos fatores de risco cardiometabólico, nomeadamente PA, resistência à insulina e lípidos no sangue aos 7 anos de idade. Além disto, foi ainda encontrada uma associação negativa entre a duração do sono e IMC. São necessários estudos longitudinais que ajudem a clarificar os efeitos a longo prazo de hábitos desadequados e problemas de sono durante a infância e do potencial efeito de cada um destes factores na saúde cardiovascular ao longo da vida.

Palavras-chave
Sono; Crianças; IMC; Lipídos; Pressão arterial; Factores de risco cardiometabólicos; Duração do sono; Problemas de sono; Distúrbios do sono

Abstract

Previous research demonstrated that sleep habits and problems have an impact on the cardiovascular morbimortality in adulthood, but evidence on this association during childhood is much scarcer. In the present study, we aimed to characterize the sleep habits and problems of preschool and school-aged children and to study their impact on body mass index (BMI) and on other cardiometabolic variables during childhood.

This thesis includes data from the Generation XXI cohort, a prospective population-based birth cohort with initial recruitment between 2005 and 2006 from all public maternity units in the Porto area (n=5), Portugal. Of the invited families, 91.4% agreed to participate (n=8647 children). At 4 years of age the first follow-up was performed, with 86% of participation, and at 7 years of age the second follow-up was performed, with 80% of participation, being eligible for the present study all children with BMI assessment at the 7-years follow-up and with sleep-related information provided at any of these visits. After exclusions, a final subsample of 5529 children was included. Trained interviewers administered a structured questionnaire on demographic and socioeconomic characteristics, children's health, sleeping habits and problems at 4 and 7 years of age and several cardiometabolic variables were assessed at 7 years of age, such as anthropometrics, blood pressure (BP), lipid profile, glucose and insulin. Sleep duration, both night sleep duration and total sleep duration, was recorded at 4 years of age and sleep duration, at weeknights and at weekend nights, was recorded at 7 years of age. Short sleep duration (sleep less than 10 hours at night), sleep onset delay, night awakenings and Non-rapid Eye Movement (NREM) parasomnias disorders (namely sleepwalking and sleep terrors) were defined as sleep problems at 4 years of age. Short sleep duration (sleep less than 9 hours at night), sleep irregularity (difference of more than one hour between weekdays and weekends bedtimes) and sleep-related breathing disorders (SRBD) and daytime sleepiness, the last 2 assessed by the application of the Pediatric Sleep Questionnaire (PSQ), previously validated in Portugal, were defined as sleep problems at 7 years of age. Associations between variables were analyzed using T-tests and Qui-square tests and multiple linear regression models were computed.

At 4 years of age, the mean (standard deviation, SD) daily night sleep duration was 11.2 (1.0) hours; 4343 (78.5%) of children presented any sleep problem and 9.1% of children presented a NREM parasomnia. Short sleep duration reported at 4 years of age was significantly higher among overweight/obesity children at 7 years of age (34.8% vs 31.2%, p=0.007). At 7 years of age, the mean (SD) daily night sleep duration was 10.0

(0.6) hours; 1044 (57.7%) of children presented any sleep problem and 13.5% of children presented SRBD. The presence of SRBD was significantly higher among overweight/obese children at 7 years of age (17.5% vs 11.6%, p<0.001).

Considering the sleep variables at 4 years of age, in the models adjusted for sex and classes of maternal education (Model 2), the levels of low-density lipoprotein cholesterol (LDLc) significantly increased by 0.96 mg/dL (95% confidence interval, CI 0.13 to 1.79, p=0.022) per hour of daily night sleep, while the levels of systolic blood pressure (SBP) decreased by 0.32 mmHg (CI -0.63 to 0.00, p=0.047) per hour of daily total sleep. Night awakening was associated with higher BMI z-score values (by 0.09 (CI 0.03 to 0.16, p=0.004). In the models additionally adjusted for BMI z-score (Model 2 + BMI z-score), triglycerides (TG) values increased by 3.09 mg/dL (CI 0.70 to 5.48), p=0.011). The BP values were positively associated with the presence of NREM parasomnias, but only the increase in diastolic blood pressure (DBP) values remained significant after adjustment for BMI z-score, with DBP increasing by 0.72 mmHg (CI 0.05 to 1.40, p=0.035) in the presence of any of this sleep problems group.

Considering the sleep variables at 7 years of age, in the models adjusted for sex and classes of maternal education (Model 2), BMI z-score significantly decreased by 0.09 (CI -0.16 to -0.02, p=0.012) per hour of daily week night sleep and by 0.07 (CI -0.12 to -0.03, p=0.001) per hour of daily weekend night sleep, and the levels of Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) significantly decreased by 0.06 (CI -0.11 to -0.00, p=0.022) per hour of daily week night sleep. Sleep irregularity was associated with higher HOMA-IR values (by 0.09 (CI 0.02 to 0.17, p=0.012). The presence of SRBD was associated with higher BMI z-score (by 0.39 (CI 0.23 to 0.55, p<0.001), higher HOMA-IR (by 0.14 (CI 0.03 to 0.25, p=0.011) and higher BP values, with SBP increasing by 1.80 mmHg (CI 0.60 to 2.99, p=0.003) and DPB by 1.01 mmHg (CI 0.02 to 1.99, p=0.044). In the models additionally adjusted for BMI z-score (Model 2 + BMI z-score), SRBD at 7 years of age were also associated with significantly lower levels of high-density lipoprotein cholesterol (HDLc) by 1.97 mg/dL (CI -3.54 to -0.39, p=0.014). Daytime sleepiness increase BP values by 1.04 mmHg (CI 0.51 to 1.57, p<0.001) for SBP and 0.85 mmHg (CI 0.38 to 1.32, p<0.001).

Sleep problems at both 4 and 7 years-old were associated with higher BMI and higher levels of cardiometabolic risk variables, namely BP, insulin resistance and blood lipids, at 7 years of age. Moreover, a negative association was found between sleep duration and BMI. Longitudinal studies are necessary for a better understanding of the long-term

effects of poor sleep during childhood and the potential differential effect of the diverse types of sleep problems on the cardiovascular health later in life.

Keywords
Sleep; Children; BMI; Blood Lipids; Blood pressure; Cardiometabolic markers; Sleep duration; Sleep problems; Sleep Disorders

Background

Sleeping is a complex and active process, with several physiological processes involved, which allows us to process what we experienced during the day and also provides us time to recover from daytime physical activities[1]. Childhood is a particularly sensitive period of time in what concerns to sleep patterns, organization, and structure establishment [1,2], and is also a period where several transient difficulties can emerge[2]. Additionally, some chronic sleep problems are also known to start during childhood[2].

The scientific literature presents evidence of several problems known to be related with poor sleep quality [2–6]. It is known to exist an impact on learning, memory processes, school performance, and general well-being[2–6]. Moreover, even modest sleep length restrictions have been associated with alterations on children's behaviors and cognitive function[7]. There is also evidence that sleep problems can increase parental stress and have an impact on the well-being of the family, as a whole[3]. Despite all this, a secular trend towards sleep deprivation, especially in children, has been consistently reported in the literature[8–10]. The promotion of healthy sleeping habits can be seen as a strategic priority in public health[4,5,11–13]. The recognition of sleep problems as a central concern for population health[4,13] is especially important on the pediatric age group, where the neurodevelopmental consequences and the risk of psychiatric and non-psychiatric disorders development might be more worrying and more capable of negatively affecting their path towards a healthy adult life[5,11,12].

Sleep Health

The concept of "sleep health" is fairly recent[13], and its creation is linked with the concept of health proposed by the World Health Organization (WHO)[14], in which health is described as a state of well-being, rather than the mere absence of disease, and placed in the context of the society, not only at an individual level[13]. Therefore, the understanding of sleep health as a whole is changing, from a concept focused on sleep disorders and sleep duration to a broader concept entailing well-being promotion[4,13], where sleep is considered as part of the daily activities and does not occur in isolation from the other activities of the day[4]. With all these changes, it has been increasingly recognized that sleep duration is not the only characteristic contributing to optimal health and well-being, but that several other characteristics of sleep, such as sleep quality, timing, and variability also have important roles as health outcomes predictors[4,5,13].

The sleep characteristics are usually referred to as sleep domains and are conceptualized as follows[5,13]:

- Sleep duration – total sleep obtained in 24 h;
- Sleep continuity or efficiency – ability to fall asleep and return to sleep upon waking;
- Timing – placement of sleep within the 24-hour day, referring to bedtime, naps and waking hours;
- Alertness/daytime sleepiness or drowsiness – alertness as the ability to maintain attentive wakefulness, to remain alert, without sleep at inappropriate times and sleepiness or drowsiness as its opposite;
- Satisfaction or quality – subjective assessment of sleep ("good" or "bad") and feeling upon awake as rested or not;
- Regularity – consistency of sleep and wake times during a certain period.

In fact, these sleep domains and characteristics are usually used to conceptualize the definition of "sleep health", as in the 2014 Buysse's definition, who states that "*Sleep health is a multidimensional pattern of sleep-wakefulness, adapted to individual, social, and environmental demands, that promotes physical and mental well-being. Good sleep health is characterized by subjective satisfaction, appropriate timing, adequate duration, high efficiency, and sustained alertness during waking hours"*[13].

Sleep duration and quality during childhood

Sleep duration, quality, and architecture are known to change with growth, especially in the first years of life[1]. Sleep architecture is considered to include 2 stages of sleep, the rapid eye movement sleep (REM) and the non-rapid eye movement sleep (NREM) stages, which together form a sleep cycle. Healthy individuals usually have many sleep cycles per night. REM sleep is a moment of intense cerebral activity and is usually the stage where most of the dreams are experienced[12], while NREM sleep is a state of restorative sleep, with typical electroencephalographic waveforms, characterized by low brain activity. NREM sleep is divided into three stages, from stage 1, the lightest sleep stage, to stage 3, the deepest[1]. In childhood, the existence of sleep stages, similar to those of adults, is believed to start to occur at approximately 6 months of age[12].

The ideal sleep duration is known to drastically change with the growth and aging process. As an infant, considering the 24-hours of a day, a total sleep time of around 14 to 17 hours a day is required, with decreasing normal ranges, of 8 to 10 hours a day during adolescence. Until the age of 5, regular naps during the day, as a measure to ensure that children have the required total sleep duration, are recommended by several scientific associations and entities, including the Portuguese Society of Pediatrics [11,12,15,16].

In 2015, the National Sleep Foundation, after a multidisciplinary expert panel and a systematic literature review, formulated the most frequently cited age-specific sleep duration recommendations[17]: 10–13 hours of sleep for preschoolers (3–5 years old) and 9–11 hours of sleep for school-children (6–13years old)[7,17]. Additionally, it is recommended that children should go to bed no later than 21:00 hours, maintain a regular sleep schedule with consistent bedtimes, wake-times (i.e., no more than 30–60 min variations in sleep schedules)[7] and naptimes, if age-appropriated.

Pediatric sleep problems and disorders

The presence of sleep problems is intrinsically related to sleep quality, and is frequently used as an accurate measure of sleep quality itself[18]. The reported prevalence of clinically relevant sleep problems in the pediatric age ranges from 20 to 30%[1,2,19]. It is estimated that approximately 70% of children experience at least one sleep-related problem at least one night a week, and that around 50% report it every day[20,21]. Sleep problems are very common and frequently associated with behavioral disorders[1]. The most frequently reported sleep problems are difficulties in sleep initiation (e.g., sleep onset delay), in sleep maintenance (e.g., night awakening) or early morning awakenings[19]. A recent review shows that, in preschool children, the prevalence of difficulties in sleep initiation and night awakening is estimated to range from 15 to 30%[12] and, in school-aged children, the prevalence of sleep problems is estimate to be around 37%, with 10% of children experiencing sleep-onset delay and anxiety, and 10% experiencing daytime sleepiness[12]. Besides considering sleep duration, we will also study the impact of some of these sleep problems, such as night awakening and sleep onset delay, at 4 years of age, and sleep irregularity and daytime sleepiness, at 7 years of age.

Sleep disorders are usually divided in 7 major categories, as described on the International Classification of Sleep Disorders, 3rd edition (ICIDS-3)[22,23]: Insomnia (3 types, chronic insomnia disorder, short-term insomnia disorder, and other insomnia disorder); Sleep-related breathing disorders (SRBD) (4 major groups, central sleep apnea syndromes, obstructive sleep apnea (OSA) disorders, sleep-related hypoventilation disorders and sleep-related hypoxemia disorder); Central disorders of hypersomnolence (including narcolepsy, idiopathic hypersomnia, Kleine-Levin syndrome and other hypersomnias); Circadian rhythm sleep-wake disorders; Parasomnias (3 clusters: NREM related, REM related, and others); Sleep-related

movement disorders and Other sleep disorders. Each disorder has specific diagnostic criteria, for adults and children.

In the present thesis, we will mainly focus on 2 types of sleep disorders: Parasomnias, specifically 2 of the NREM-related parasomnias, (sleep terrors and somnambulism/sleepwalking) evaluated at 4 years of age, as reported by the main caregiver; and SRBD, evaluated at 7 years of age, with the application of the validated Portuguese version of the Paediatric Sleep Questionnaire, a 22-item scale specifically developed and used for research purposes aiming to study the presence of childhood SRBD[24]. These 2 types of sleep disorders are very common in children, with Parasomnias in general having a prevalence approaching 90% of children and sleepwalking prevalence reaching 60% if both parents are also affected[25]; and obstructive sleep apnea, the most common disorder in the group of SRBD, estimated to occur in 1% to 5% of children[26]. We will consider sleep disorders within the group of sleep problems.

Cardiovascular Risk and Sleep Health

Childhood obesity has been identified as one of the most important risk factors for developing cardiovascular diseases in later life in several recent reviews and meta-analyses studies[27–30], thus representing a major public health concern and naturally an important theme for scientific research. Childhood obesity, especially, has become a global epidemic in the last decades, with well-known associations with several adverse health outcomes in later childhood and adulthood. It is known to track into adulthood and to be associated with several cardiovascular risk factors. In fact, childhood obesity has numerous consequences on the cardiovascular system. The accumulation of fat leads to the development of several metabolic changes and promotes changes in traditional cardiovascular risk factors, such as hypertension, dyslipidemia and glucose intolerance/insulin resistance, which later in life might increase the risk of cardiovascular events and premature mortality[31].

Sleep has been recognized to impact cardiovascular risk through a complex, and still not completely understood, relationship[32–34]. In fact, sleep is being increasingly considered one of the 3 pillars, along with diet and physical activity, of a healthy lifestyle[4,33], and as an important modifiable lifestyle component that can ultimately affect both eating and activity behaviors[4,33]. In adults, among other effects, poor sleep is known to interfere with weight gain[34], blood pressure (BP)[33], and insulin resistance[33]. Adults with OSA or

insomnia are at significantly greater risk for cardiovascular and cerebrovascular diseases, among them atherosclerosis, coronary heart disease, heart failure, hypertension, and stroke and metabolic disorders, especially obesity, type 2 diabetes mellitus, and dyslipidemia, according to a recent scientific statement from the American Heart Association[35]. Moreover, the association between short sleep duration and obesity in adults is already well established and it has also been suggested that weight management is hindered in sleep deprived adults[36]. In children, more recently, evidence has also pointed to the existence of similar effects. A review analyzing the association of health indicators with physical activity, sedentary behavior and sleep, in children 5-17 years old, showed that children with a combination of high physical activity/high sleep/low sedentary behavior presented a better profile of adiposity and cardiometabolic health, when compared with those with low PA/low sleep/high sedentary behavior[37]. Another review analyzing sleep and cardiometabolic risk, in children and adolescents, also concluded that inadequate sleep and increased abdominal adiposity were associated with decreased insulin sensitivity and high BP[33]. Despite some recent evidence in the pediatric age group, so far studies mainly focused on the impact of sleep duration on adiposity and weight gain, and the association with other risk factors is still scarcely studied[32,38–40]. In fact, even in adults, sleep disturbances, other than sleep duration, have been scarcely explored[32,33,39]. As previously stated, as the concept of "sleep health" gains more importance, it has been acknowledged that other characteristics of sleep, such as sleep quality, timing, and variability, might also have an important role contributing as predictors of health outcomes[4,5,13].

Thus, in the current era of both increase in cardiovascular risk in children, mostly associated with the increasing trends of overweight and obesity[41], and a secular trend towards sleep deprivation[8–10], the role of poor sleep as a modifiable variable that is related to cardiometabolic risk assumes particular importance.

In the present study, on a population-based birth cohort, we aimed to characterize the sleeping habits, which include the sleep domains of sleep duration and regularity, daytime sleeping and bedtime habits, and common sleep problems of prepubertal children and to study their potential impact on body mass index (BMI) status and on other cardiometabolic variables, such as BP, insulin resistance and blood lipids, during childhood.

Aims

In the present study, we aimed to characterize the sleeping habits and common sleep problems of preschool and school-aged children and to study their potential impact on their nutritional status and on several cardiometabolic variables.

As specific objectives, we aimed:

1. To characterize the sleeping habits, which include the sleep domains of sleep duration and regularity and daytime sleeping (daily night sleep duration, both nighttime and total, and afternoon nap, bedtime, wakeup time, afternoon nap performance) and bedtime habits (nighttime sleep at own bedroom, familiar presence at bedtime, use of transitional object, and reading stories routine) at 4 years old, and of sleep duration and regularity (daily night sleep duration at weekdays and weekends) at 7 years of age;

2. To characterize the presence of common sleep problems, namely short sleep duration, sleep onset delay, night awakening and NREM parasomnias, at 4 years of age, and short sleep duration, sleep irregularity, SRBD and daytime sleepiness at 7 years of age;

3. To test the association of sleep duration and sleep problems at 4 and at 7 years of age with the presence of overweight/obesity at 7 years of age;

4. To test the association of sleep duration and sleep problems at 4 and at 7 years of age with cardiometabolic variables (BP, insulin resistance and blood lipids) at 7 years of age.

Paper I

The effect of sleeping habits and problems at 4 and at 7 years of age on cardiometabolic health in a Portuguese birth cohort

Abstract

Background

Previous research demonstrated that sleep habits and problems have an impact on the cardiovascular morbimortality in adulthood but evidence on this association during childhood is much scarcer. In the present study, we aimed to characterize the sleep habits and problems of preschool and school-aged children and to study their impact on body mass index (BMI) and on other cardiometabolic variables during childhood.

Methods

This study used data from a population-based birth cohort, Generation XXI. 8647 children participated in the initial recruitment and, respectively, 86% and 80% participated in the follow-up visits at 4 and 7 years of age. After exclusions, a final subsample of 5529 children was included. Trained interviewers administered a structured questionnaire on socioeconomic, health and sleeping characteristics at 4 and at 7 years of age and several cardiometabolic variables were assessed at 7 years of age, such as anthropometrics, blood pressure (BP), lipid profile, glucose and insulin. Sleep duration, night and total, was recorded at 4 years of age and sleep duration, at weeknights and at weekend nights, at 7 years. Short sleep duration, sleep onset delay, night awakenings and non-rapid eye movement (NREM) parasomnias were defined as sleeping problems at 4 years of age. Short sleep duration, sleep irregularity, sleep-related breathing disorders (SRBD) and daytime sleepiness, the last 2 assessed by the Pediatric Sleep Questionnaire (PSQ), previously validated in Portugal, were defined as sleeping problems at 7 years of age. Associations between variables were analyzed using T-tests and Qui-square tests and multiple linear regression models were computed.

Results

At 4 years of age, the mean (standard deviation, SD) daily night sleep duration was 11.2 (1.00) hours; 4343 (78.5%) of children presented at least one sleep problem and 9.1% of children presented a NREM parasomnia. At 7 years of age, the mean (SD) daily night sleep duration was 10.0 (0.6) hours; 1044 (57.7%) of children presented sleep problems.

At 4 years of age, in the models adjusted for sex and classes of maternal education (Model 2), the levels of low-density lipoprotein cholesterol (LDLc) significantly increased by 0.96 mg/dL (95% confidence interval, CI 0.13 to 1.79, p=0.022) per hour of daily night sleep, while the levels of systolic blood pressure (SBP) decreased by 0.32 mmHg (CI -0.63 to 0.00, p=0.047) per hour of daily total sleep. Night awakening was associated with

higher BMI z-score values (by 0.09 (CI 0.03 to 0.16, p=0.004)). In the models additionally adjusted for BMI z-score (Model 2 + BMI z-score), TG values increased by 3.09 mg/dL (CI 0.70 to 5.48, p=0.011). The BP values were positively associated with the presence of NREM parasomnias, with diastolic blood pressure (DBP) increasing by 0.72 mmHg (CI 0.05 to 1.40, p=0.035).

At 7 years of age, in models adjusted for sex and classes of maternal education, BMI z-score decreased by 0.09 (CI -0.16 to -0.02, p=0.012) per hour of weeknight sleep and by 0.07 (CI -0.12 to -0.03, p=0.001) per hour of weekend night sleep, as the levels of Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) (by 0.06 (CI -0.11 to -0.00, p=0.022)) per hour of weeknight sleep. Sleep irregularity was associated with higher HOMA-IR values (by 0.09 (CI 0.02 to 0.17, p=0.012)). SRBD was associated with higher BMI z-score (by 0.39 (CI 0.23 to 0.55, p<0.001)), HOMA-IR (by 0.14 (CI 0.03 to 0.25, p=0.011)) and BP values, with SBP increasing by 1.80 mmHg (CI 0.60 to 2.99, p=0.003) and DPB by 1.01 mmHg (CI 0.02 to 1.99, p=0.044). In Model 2 + BMI z-score, SRBD were also associated with lower levels of high-density lipoprotein cholesterol (HDLc) by 1.97 mg/dL (CI -3.54 to -0.39, p=0.014). Daytime sleepiness increased BP values by 1.04 mmHg (CI 0.51 to 1.57, p<0.001) for SBP and 0.85 mmHg (CI 0.38 to 1.32, p<0.001).

Conclusions
Sleeping characteristics both at 4 and 7 years-old, were associated with higher BMI and higher levels of cardiometabolic risk variables at 7 years of age. Longitudinal studies are necessary for a better understanding of the long-term effects of poor sleep during childhood and later in life.

Keywords
Sleep; Children; BMI; Blood Lipids; Blood pressure; Cardiometabolic markers; Sleep duration; Sleep problems; Sleep Disorders

Introduction

Sleep is considered essential for both mental and physical health, especially during childhood and adolescence. Several factors are known to affect the duration and quality of sleep and recent research has highlighted a rising trend towards sleep deprivation during childhood[1–3]. Infancy is a sensitive time regarding the creation of patterns, organization, and structure establishment of sleep[4,5], and where several transient difficulties can emerge[5].

Sleep has been recognized to impact cardiovascular risk through a complex, and still not completely understood, relationship[6–8]. In fact, sleep is being increasingly considered one of the 3 pillars, along with diet and physical activity, of a healthy lifestyle, and as an important modifiable lifestyle component that can ultimately affect both eating and activity behaviors[7,9]. In adults, among other effects, poor sleep, considered when sleep is of low quality and/or quantity, is known to interfere with weight gain[8], blood pressure (BP)[7], and insulin resistance[7]. Short sleep duration seems to be associated with excessive body fat gain and development of insulin resistance, which possibly through the inflammatory milieu that arises is believed to at least partially explain the increased cardiovascular risk of individuals with poor sleep[10].

Childhood obesity has become a global epidemic in the last decades with well-known associations with several adverse health outcomes in later childhood and adulthood[11]. Childhood obesity is known to track into adulthood and to be associated with several cardiovascular risk factors, which later in life might increase the risk of cardiovascular events and premature mortality[6,11,12].

Despite the effects of poor sleep on the cardiometabolic risk have been recently recognized, characteristics other than sleep duration, such as sleep quality, timing, and variability, have been scarcely explored[6,7,10]. In the last years, the concept of “sleep health” has gained importance, with the recognition of sleep as a promoter of both physical and mental well-being[9,13] and as an health predictor[6]. Further, it has been acknowledged that these other less studied characteristics of sleep might have an important role contributing as predictors of health outcomes[9,13,14]. Additionally, chronic sleep problems are known to start during childhood[5], which could establish a vicious cycle of poor sleep and cardiovascular risk increase.

Thus, in the current era of both increase in cardiovascular risk in children, mostly associated with the increasing trends of overweight and obesity[15], and a secular trend towards sleep deprivation[1–3], the role of poor sleep as a modifiable variable that is related to cardiometabolic risk assumes particular importance. In the present study, in a population-based birth cohort, we aimed to characterize sleep habits and some common

sleep problems of preschool and school-aged children and to study their potential impact on body mass index (BMI) and on other cardiometabolic variables, such as BP, insulin resistance and blood lipids.

Materials and Methods

Study design and sample

This study was based on the Portuguese birth cohort, Generation XXI. A total of 8647 newborns were enrolled in this cohort. The recruitment occurred between April 2005 and August 2006, in all the 5 public maternity units of the metropolitan area of Porto, Portugal, where mothers were invited to participate after delivery. At birth, 91.4% of the invited mothers accepted to participate.

Between April 2009 and August 2011, a follow-up was conducted for children with 4 years of age, and 7459 children were evaluated (86% participation rate). At the 4-year-old's follow-up, trained interviewers applied a structured questionnaire focused on child and mother's health, allowing the collection of demographic and socioeconomic data, caregivers' information, child's lifestyle, sleeping habits, and maternal socioeconomic characteristics.

Between April 2012 and March 2014, another follow-up evaluation was conducted for children with 7 years of age, with 6889 children (80% participation rate) evaluated and, of those, 5842 children were evaluated by face-to-face interviews, physical examination, and blood withdrawal, at the study site.

All the families were invited to participate in the follow-up evaluations, by a telephone contact.

For the present analysis, only the firstborn child of twins, triplets, and quadruplets were included (exclusion of 152 non-firstborn twins). Children with severe neurologic or developmental disorders, that reported sleeping periods over 18 hours/day (n=2), and children with missing data on BMI at 7 years of age were also excluded (n=2964). Finally, 5529 children were included in the present analysis.

Data Collection and variable definition

Sleeping habits and associated characteristics at 4 and 7 years of age were evaluated by a parent-administered questionnaire, which in 88% was answered by mothers. At 4 years of age, sleeping habits, included the sleep domains of sleep duration, regularity and daytime sleeping (daily night sleep duration, both nighttime and total, and afternoon nap, bedtime, wakeup time, afternoon nap performance) and bedtime habits (nighttime sleep at own bedroom, familiar presence at bedtime, use of transitional object, and reading stories routine); at 7 years of age sleep duration and regularity (daily night sleep duration at weekdays and weekends) were evaluated.

At both ages, daily nighttime sleep duration was calculated in hours considering children's bedtime and morning waking time. At 4 years of age, daily total sleep duration was calculated adding the afternoon nap to the daily night sleep duration, also in hours. Bedtime was considered the specific hour that the children went to bed and wake up time the specific hour that the children got out of bed, as reported. Afternoon nap was calculated dividing the total weekly naptime duration reported by 7, reaching the total daily naptime duration. Afternoon nap performance was considered as reported if the child did not have naps at 4 years of age (not currently), had naps between 1 and 6 days of the week (several times in a week) and had naps the 7 days of the week (everyday). At 7 years old, night sleep duration was calculated for weekdays (from Monday to Friday) and for weekend days (Saturday and Sunday).

Regarding bedtime habits, at 4 years of age, the following characteristics, considered as dichotomous variables, were recorded and analyzed: nighttime sleep at own bedroom; familiar presence at bedtime, use of transitional object, and reading stories routine before falling asleep.

The sleep problems evaluated were short sleep duration, sleep onset delay, night awakening and NREM parasomnias, at 4 years of age, and short sleep duration, sleep irregularity, SRBD and daytime sleepiness at 7 years of age. Short nighttime sleep duration was defined as <10 hours of sleep at 4 years-old and <9 hours of sleep at 7 years old during the week[16–18]. Sleep irregularity was defined if a difference of more than one hour was reported between weekdays and weekends bedtimes[16].

Sleep onset delay was considered present when, in a usual day, the child was reported to need more than 20 minutes to fall asleep. Night awakening was considered present if the child was reported to wake-up during the night at least once a month, in the last 6 months. The child was considered to have a NREM parasomnia if either sleepwalking or night terrors were reported to have occurred at least once. The frequency of night awakening and NREM parasomnia episodes was also recorded.

At 7 years of age, the validated Portuguese version of the Pediatric Sleep Questionnaire (PSQ) with a 22-item scale was applied[19]. A total of 4360 answered sleep-related questions and questions from the PSQ, at 7 years of age, but only 1891 children had complete information to all questions of the questionnaire. The original cut-off value >0.33, that is, at least 8 positive answers from the total of 22, was used as an indicator of the presence of sleep-related breathing disorder (SRBD)[20]. Daytime sleepiness was considered present if the answers given to any of the PSQ's group B questions, composed of 4 questions, B1 ("Does your child wake up feeling unrefreshed in the morning?"), B2 ("Does your child have a problem with sleepiness during the day?"), B4

("Does your child has a teacher or other supervisor commented that your child appears sleepy during the day?") or B6 ("Is it hard to wake your child up in the morning?") were positive[20].

As demographic and socioeconomic variables, maternal age (categorized as ≤25 or >25 years of age), maternal and paternal education level (number of schooling years, and categorized for maternal educational level as ≤6, 7-9, 10-12 and >12 years of schooling), paternal working condition (employed or other situation, which included unemployed, stay at home, student and retired fathers) and average monthly household income (≤1000, 1001-1500, >1500€/month) were recorded. All socioeconomic variables considered for analysis were those collected at baseline (enrollment at birth), except for average monthly household income, which was considered the one reported at the 7-years follow-up. Birthweight and gestational age (in weeks) at birth were also recorded.

At 7 years of age, body weight was determined to the nearest 0.1 kg in a digital scale (Tanita®) and height was determined in the upright position to the nearest 0.1 cm with a wall stadiometer (Seca®). BMI was described both as kg/m² and z-score. BMI-for-age values were classified according to the World Health Organization (WHO) z-score table standardized internationally, sex, and age-specific[21] and recoded into nonoverweight (BMI ≤1 standard Deviation, SD) and overweight/obese (BMI >1 SD), according with WHO references[22]. BP was evaluated with an aneroid sphygmomanometer (Elite® 92125) with an adequately sized cuff, by a trained examiner, twice with a 5-minute interval between measurements, with the subject in a seated position and the antecubital fossa supported at heart level, after at least a 5-minute rest. When the difference between the 2 determinations was larger than 5 mmHg for systolic (SBP) or diastolic BP (DBP) a third measurement was taken. The mean of the values was considered.
Venous blood samples were obtained after 8 to 12 hours of overnight fast, for lipid profile (total cholesterol, low-density lipoprotein cholesterol (LDLc), high-density lipoprotein cholesterol (HDLc), and triglycerides (TG)), high-sensitivity C-reactive protein (hs-CRP), fasting plasma glucose and insulin. All determinations were performed in the Clinical Pathology Department of Centro Hospitalar São João, Porto, Portugal. The basal insulin resistance was assessed using the homeostasis model assessment of insulin resistance (HOMA-IR), calculated by multiplying fasting glucose (mg/dL) by fasting insulin (μU/mL) and then dividing this value by 405[23].

Statistical Analysis

Sample characteristics are presented as counts and proportions for categorical variables and as mean and standard deviations (SD) for continuous variables. Comparison between categorical variables was performed using Chi-square tests. Comparisons of continuous variables between groups were assessed using Students' T-tests or ANOVA tests for normally distributed variables and using Mann-Whitney tests or Kruskall-Wallis tests, respectively, for variables with a distribution different from the normal.

Regression coefficients (β) and 95% confidence intervals (CI) were estimated by linear regression models (one for each sleeping variable), with each cardiometabolic variable (BMI z-score, SBP, DBP, HOMA-IR, HDLc, LDLc and TG) as the dependent variable. The models include each sleeping variable at a time and, in all cases, Model 1 is additionally adjusted for sex, Model 2 is additionally adjusted for sex and classes of maternal education and, when duly signed (Model 2 + BMI z-score), also for BMI z-score. All p-values are two-sided. The significance level was set at $p<0.05$. The statistical analysis was made using Statistical Package for Social Sciences (SPSS) for Windows, version 26.0.

Ethics

The Geração XXI study was approved by the local institutional Ethics Committee and by the National Data Protection Commission. The study complies with national legislation and the Helsinki Declaration. Written informed consent (approved by the Ethics Committee) was obtained from parents or legal guardians.

Results

A total of 5529 children were included in the present analysis, of which 2679 (48.5%) were girls. Table 1 describes the general demographic and socioeconomic characteristics and the cardiometabolic variables at 7 years of age, in the whole sample and by classes of BMI (nonoverweight, n=3348 (62.4%), and overweight, n=2081(37.6%)). The mean (SD) maternal age at birth was 29.6 (5.3) years and maternal education was grouped in 4 categories, as follows: 6 years or less, 7 to 9 years, 10 to 12 years, more than 12 years of education, with 28.4% of mothers presenting more than 12 years of education. The monthly household income was above 1000€ in more than 70% of the sample. Overweight and obese children were significantly heavier at birth but also presented a higher gestational age; their mothers were significantly younger and their parents, both mother and father, were less educated.

Regarding, cardiometabolic variables at 7 years of age, the mean (SD) BMI of the included sample was 17.1 (2.5) (kg/m^2) (15.5 (1.0) vs. 19.7 (2.1), $p<0.001$, in the nonoverweight and overweight groups, respectively). Office BP values, glucose, insulin, Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) and blood lipids were all significantly higher in the overweight group of children, except for HDLc which is significantly lower in this group.

At 4 years of age, 4343 (78.5%) of children were reported to have at least one of the sleeping problems considered, including short nighttime sleep duration. When disregarding sleep duration, 3896 (70.5%) still reported at least one sleeping problem. The sleeping habits and sleep problems at 4 years of age, by BMI classes, are presented in Table 2. The mean (SD) reported total sleep duration was 11.2 hours (1.00), 10.1 (0.8) during nighttime and 1.2 (0.7) in the afternoon naps, with 1758 (32.1%) of children presenting short nighttime sleep (10 hours or less); 57.6% of children were reported to nap, but only 24.5% napped every day. Most of the children slept in their bedrooms during nighttime sleep (alone or with siblings) (73.6%), but almost half of the sample needed familiar presence at bedtime (48.8%) or a transitional object (47.6%). Night awakenings were reported in 60.5% of children, sleep onset delay in 27.9% and NREM parasomnias (sleepwalking and/or night terrors) in 9.1%. Differences in the sleeping habits or sleep problems were found between nonoverweight and overweight children when considering short nighttime sleep duration (31.2% vs 34.8%, p=0.007, in nonoverweight and overweight, respectively), bedtime (21:25 (2.48) vs 21:10 (3.3), p=0.020, in nonoverweight and overweight, respectively) and afternoon nap performance

(41.0% vs 44.7% not having naps, p=0.020, in nonoverweight and overweight, respectively).

At 7 years of age, 1044 (57.7%) of children were reported to have at least one of the sleep problems considered, including short nighttime sleep duration. When disregarding sleep duration, 1027 (56.7%) still reported at least one sleeping problem. The sleeping habits and sleep problems at 7 years of age, by BMI classes, are presented in Table 3. The mean (SD) daily night sleep duration reported was 10.0 (0.6) hours on weekdays and 10.5 (1.0) hours on weekends, with significantly shorter night sleep duration on weekends among overweight children (10.5 (0.9) vs. 10.4 (1.0) hours, p=0.018, in nonoverweight and overweight children, respectively). SRBD, according to the PSQ score, were present in 13.5% of the total sample but in a significantly higher proportion of overweight children when compared to their nonoverweight counterparts (17.5% vs. 11.6%, p<0.001, in overweight and nonoverweight children, respectively).

The mean change in each cardiometabolic variable, at 7 years of age, by the presence of each sleeping variable, at 4 and at 7 years of age, are presented in Table 4. Considering the sleeping variables at 4 years of age, in the models adjusted for sex and classes of maternal education (Model 2), the levels of LDLc significantly increased by 0.96 mg/dL (CI 0.13 to 1.79, p=0.022) per hour of daily night sleep and this association remained significant after adjustment for BMI z-score, while the levels of SBP significantly decreased by 0.32 mmHg (CI -0.63 to 0.00, p=0.047) per hour of daily total sleep but this association was lost after adjustment for BMI z-score. In the models adjusted for sex and classes of maternal education (Model 2), night awakening was associated with higher BMI z-score values (by 0.09 (CI 0.03 to 0.16, p=0.004)). In the models, further adjusted for BMI z-score (Model 2 + BMI z-score), night awakening at 4 years of age was also associated with lower TG values (by -2.21 (CI -4.40 to -0.02) mg/dL, p=0.048). TG values increased by 3.38 mg/dL (CI 0.96 to 5.80, p=0.006) in the presence of sleep onset delay (Model 2), even after adjustment for BMI z-score (3.09 (CI 0.70 to 5.48), p=0.011). The BP values were positively associated with the presence of NREM parasomnias, but only the increase in DBP values remained significant after adjustment for BMI z-score, with DBP increasing by 0.72 mmHg (CI 0.05 to 1.40, p=0.035) in the presence of any of this sleep problems group.

Considering the sleep variables at 7 years of age, in the models adjusted for sex and classes of maternal education (Model 2), BMI z-score significantly decreased by 0.09 (CI -0.16 to -0.02, p=0.012) per hour of daily weeknight sleep and by 0.07 (CI -0.12 to -0.03, p=0.001) per hour of daily weekend night sleep, and the levels of HOMA-IR significantly

decreased by 0.06 (CI -0.11 to -0.00, p=0.022) per hour of daily week night sleep. Sleep irregularity was associated with higher HOMA-IR values (by 0.09 (CI 0.02 to 0.17, p=0.012)). The presence of SRBD was associated with higher BMI z-score (by 0.39 (CI 0.23 to 0.55, p<0.001)), higher HOMA-IR (by 0.14 (CI 0.03 to 0.25, p=0.011)) and higher BP values, with SBP increasing by 1.80 mmHg (CI 0.60 to 2.99, p=0.003) and DPB by 1.01 mmHg (CI 0.02 to 1.99, p=0.044). In the models additionally adjusted for BMI z-score (Model 2 + BMI z-score), SRBD at 7 years of age were also associated with significantly lower levels of HDLc by 1.97 mg/dL (CI -3.54 to -0.39, p=0.014). Daytime sleepiness increased BP z-score values by 1.04 (CI 0.51 to 1.57, p<0.001) for SBP and 0.85 (CI 0.38 to 1.32, p<0.001) for DBP. Both HOMA-IR and TG values were negatively associated with the presence of daytime sleepiness, and remained significant after adjustment for BMI z-score, with HOMA-IR decreasing by 0.10 (CI -0.17 to -0.02, p=0.009) and TG decreasing by 2.76 mg/dL (CI -5.36 to -0.16, p=0.037) in the presence of daytime sleepiness.

Discussion

Our results reveal that short sleep duration and sleep problems are present in around three-quarters of our sample, at 4 years of age, and that some of these, such as night awakening, are associated with higher BMI z-scores at the age of 7, and with a worst cardiometabolic profile at this age, independently of BMI. Specifically, we reported that higher LDLc levels are associated with longer night sleep duration, higher levels of TG are associated with sleep onset delay and higher values of DBP are associated with NREM parasomnias. At the 7-year-old evaluation, we found that shorter night sleep duration was associated with higher BMI z-score values and higher insulin resistance. Sleep irregularity was also shown to be associated with higher insulin resistance and daytime sleepiness with higher BP values. At this age, we found that overweight children presented significantly more SRBD than their normal weight counterparts. Additionally, the presence of SRBD was associated with higher BP values, more insulin resistance and lower HDLc values, the latter being independent of the BMI status.

Despite recommendations for the improvement of children's sleep duration and quality and the growing number of studies indicating the importance of sleep[6,17], there is a secular trend towards decreased sleep duration in children[2]. The relationship between poor sleep quality and other health problems in the pediatric age has been acknowledged in the recent years[6,18,24], namely with increased cardiometabolic risk[10,12]. Nonetheless, most of the existing studies have mainly focused on the impact of poor sleep, specially sleep duration, in the cardiovascular risk of older children and adolescents[6,10,12], which underlines the importance of our study focusing in other sleep characteristics, from early ages, and evaluating their impact on the cardiometabolic profile of prepubertal children. Over the last decade, the relationship between sleep characteristics and obesity has been the most assessed and the one yielding the strongest evidence, in the context of cardiometabolic risk. In our study, we found higher BMI z-score values among those who had reported night awakening at the age of 4 and among those with shorter sleep duration at the age of 7. Regarding the sleep duration association with nutritional status, our results seem to agree with the previous evidence available. For instance, in a 2018 systematic review which analyzed the association of sleep with hypertension and cardiovascular risk, in participants under 21 years of age, of the 15 studies that evaluated obesity, 13 reported a significant association with sleep caractheristics[10]. Specifically, 8 studies reported a negative association between sleep duration and the presence of obesity or any measure of obesity, such as BMI or BMI z-score[10]. Similarly, another systematic review evaluating the association between sleep characteristics and

cardiovascular risk, including participants under 24 years of age, reported that 8 out of 16 longitudinal studies included found that shorter sleep duration was associated with higher BMI values, greater increases in BMI during follow-up, or greater risk of overweight/obesity over time, compared to longer sleep duration. Finally, another systematic review of prospective cohort studies, published in 2017, specifically focusing on the association between sleep duration and obesity in children and adolescents, found that in 9 out of 14 studies included, stated that reduced sleeping periods were associated with higher subsequent BMI values and 6 studies reported that sleep duration was negatively associated with BMI gain, further reinforcing the conclusion that short sleep duration increases the risk of childhood obesity[25].

Notwithstanding the growing number of studies on the impact of sleep on cardiovascular health, sleep characteristics other than sleep duration have been much less considered so far. On a meta-review on children's sleep and health, when considering the reviews about adiposity, only 7 out of the 20 reviews examined other characteristics of sleep than sleep duration, and all reported limited data about sleep quality[6]. A previous study reported that short sleep duration increased the severity of obesity, considered night awakening as one of the characteristics of sleep duration[27]. Night awakening, usually considered as a poor sleep quality variable, was also found to be associated with higher BMI z-score values in our study. We also found an association between BMI status and the presence of SRBD, at 7 years of age, but considering the recognized importance of breathing disorders on cardiometabolic health, this association will be further discussed below.

Regarding the association between sleep characteristics and other cardiometabolic variables, we reported significant associations between blood lipids and sleep variables. When considering LDLc levels, we found a positive association with daily night sleep duration, at 4 years of age. In the literature, results on the association between sleep and LDLc are heterogeneous[7,12]. A 2016 systematic review assessed the association between sleep characteristics and cardiovascular risk in participants under 24 years of age. They found a positive association between poor sleep quality and LDLc but not with non-HDLc and a positive association between LDLc and short sleep duration in secondary students[12]. A study analyzing the association between sleep duration and cardiometabolic risk in Iranian children reported that boys aged 10 to 14 with longer sleep duration presented higher total cholesterol levels but, in contrast with our results, no association was found specifically regarding LDLc[28]. Another systematic review that focused specifically on sleep duration and dyslipidemia in adolescents, reported that,

from 7 studies included, 2 studies found that shorter sleep duration was associated higher total cholesterol and LDLc[29].

We also found that TG levels were significantly higher among children who reported sleep onset delay, at 4 years of age. Interestingly, the levels of TG were lower among children who reported daytime sleepiness and breathing problems, at 7 years of age. Again, the results of previous studies on this subject are heterogeneous[7,10]. The systematic review previously cited, which analyzed the association between sleep characteristics and cardiovascular risk on participants under 24 years, found that from 4 studies, 2 presented a negative association between TG levels and sleep duration; no studies analyzed the association between TG and sleep quality variables[12]. The meta-review on children's sleep and health found that, of 7 reviews evaluating blood lipids, one reported that longer sleep was associated with higher triglyceride levels and only 2 examined other sleep characteristics than sleep duration[6]. From all the studies reviewed, only one considered sleep regularity/irregularity and reported a significant association with TG in obese children[6,30]. Assuming that sleep onset delay can have a variable impact on sleep timing and thus on sleep regularity[30], we can assume that our finding of higher TG levels among children with this sleep problem, is in line with these previous findings. The relatively few studies on sleep quality seem to favor the existence of an association with higher TG levels but more studies are needed to better clarify this association[6].

Regarding BP levels, we reported that children with NREM parasomnias presented higher SBP and DBP values, in sex and maternal education-adjusted models, but only the association with DBP remained significant after adjustment for BMI z-score. To the best of our knowledge, no previous studies analyzed the association of sleepwalking and sleep terrors with cardiovascular risk. NREM parasomnias are presumed to occur because of the incomplete transition between wakefulness and stages of sleep[31], and is comorbidity commonly described in association with SRBD[32]. A previous study examined sleep stages and reported that morning BP was greater among obese adolescents with poorer sleep quality, as characterized by shorter periods spent in deep sleep stages (when NREM sleep occurs), regardless of BMI[33]. The same study also stated that there is increasing evidence in the literature that, in adults, sleep disruption with reduction of deeper sleep stages contributes to a higher risk of hypertension[33]. Daytime sleepiness was also shown to significantly increase BP levels, both SBD and DBP, in all the models fitted, independently of BMI. In line with these findings, in one study of US children, aged 11-12 years old, daytime sleepiness was also associated with a higher risk of hypertension[34]. Despite some mixed results, overall, 3 systematic reviews associating

sleep and cardiovascular characteristics in children provide fairly consistent evidence on the existence of a relationship between sleep and high BP, with both SBP and DBP appearing to be related to sleep quality[6,10,35].

Intriguingly, in our study, daytime sleepiness also presented a significant negative association with HOMA-IR, independently of BMI. No other specific evidence relating daytime sleepiness and HOMA-IR seems to exist, but negative associations between HOMA-IR and sleep quality had already been described in few previous studies. In one systematic review on sleep, hypertension and cardiovascular risk in children, one of the studies included reported that sleep deprivation was associated with lower HOMA-IR and another reported a negative association between HOMA-IR and sleep efficiency, in a sample of overweight/obese adolescent girls[10]. Some authors hypothesize that the association with insulin resistance might vary with sleep architecture, quality, and timing, characteristics still less studied[6,10].

In our study, we also found that children with SRBD presented significantly higher BP levels. In adults, the relationship of SRBD and cardiovascular risk in well established, with moderate and severe SRBD shown to be associated with an elevated cardiovascular risk, namely incidence of stroke[36]. A longitudinal study that evaluated polysomnography results in children aged 6, and 5 years later, at 11 years old, reported a trend between the presence of SRBD and higher SBP[37]. A systematic review focusing on the impact of obstructive sleep apnea (OSA), the more common of the SRBD group and cardiovascular risk in children, concluded that there is an association between OSA and changes in BP in the pediatric population[38]. Nonetheless, it should be stressed the confounding effect of BMI on this association, since frequently children with OSA also present higher BMI[38]. In fact, our data showed that the positive association between SRBD and SBP and DBP was not independent of BMI z-score levels. It has been argued that OSA and BMI might have a concurrent effect on diurnal SBP and DBP, shown by a number of case-control studies[38]. A longitudinal study in preadolescent children, initially without OSA, showed that participants who were overweight at the initial visit were significantly more likely to develop OSA, stressing the association of childhood obesity on the development of OSA[39]. In the previously cited review, it was stated that studies analyzing BP dipping, the physiological BP decrease expected to occur during nighttime, known to have a protector effect on the cardiovascular system, reported that the dipper BP profile was frequently absent in children with OSA, which might be another explanation for the increased cardiovascular risk in children with SRBD, independently of obesity[38]. We also reported a positive association between SRBD and insulin resistance, and this effect remained significant after adjustment for BMI. In a pediatric cohort study, which included children aged 5 to 12 years, sleep fragmentation was,

independently of obesity, positively associated with insulin resistance. The presence of obesity increased the odds for insulin resistance and severity of the disease[40]. In the earlier cited review, it was shown that artificially induced sleep fragmentation was associated with decreased morning insulin sensitivity and, in a study in healthy adults, intermittent hypoxia was shown to decrease insulin sensitivity, leading to the conclusion that the sleep fragmentation associated with OSA might also play a role in the disruption of insulin regulation[38]. In fact, another pediatric study also reported an association between sleep fragmentation and adverse glucose metabolism profile in obese adolescents with SRBD[41].

The major strength of our study is the fact that we present an analysis of a large population-based cohort data, for which longitudinal data not only on sleep duration but also on sleep quality and sleep problems was available. The impact of sleep characteristics at 2 different ages on several cardiometabolic variables was analyzed, allowing to appreciate the prospective impact of sleep problems on the risk of a later adverse cardiovascular profile. As recommended in the recent literature, we conceptualized sleep as a component of the 24-hour-day and as a multidimensional construct but it should be acknowledged that the estimation of sleep duration by parents' reports limits the quality of the data, since it is susceptible of recall and social desirability bias. Nonetheless, it has been suggested that self-reported studies seem to be biased toward underestimation[6,12], which suggest that a larger effect would have been observed if objective sleep measures could have been used. Furthermore, considering the observational nature of our study, one cannot exclude bias from unmeasured confounders but the consistency of our results with previously published studies is in favor of the reported associations to be meaningful and worthy of being reported.

In conclusion, we report a significant effect of several sleep characteristics on cardiometabolic variables in school-aged children. Our data allow us to reinforce the hypothesis that poorer sleep health acts upon cardiometabolic health, possibly increasing cardiovascular risk throughout life. The identification of a modifiable risk factor with impact on cardiovascular outcomes, as sleep has come to be considered, assumes a particular public health interest in the quest for approaches to reduce the burden of cardiovascular diseases, the most common cause of morbidity, mortality, and disability with high health care costs[28]. Further research is needed to clarify the role of sleep duration, quality, timing, and variability on health during childhood, and to investigate the long-term impact of poor sleep health.

Funding

Generation XXI was supported by the European Regional Development Fund (ERDF) through the Operational Programme Competitiveness and Internationalization and national funding from the Foundation for Science and Technology (FCT), Portuguese Ministry of Science, Technology and Higher Education and by the Unidade de Investigação em Epidemiologia - Instituto de Saúde Pública da Universidade do Porto (EPIUnit) (UIDB/04750/2020), Administração Regional de Saúde Norte (Regional Department of Ministry of Health) and Fundação Calouste Gulbenkian.

Conflicts of interest

None.

Acknowledgments

The authors gratefully acknowledge the families enrolled in Generation XXI for their kindness, all members of the research team for their enthusiasm and perseverance and the participating hospitals and their staff for their help and support.

References

1. Matricciani L, Bin YS, Lallukka T, et al. Past, present, and future: trends in sleep duration and implications for public health. *Sleep Heal*. 2017;3(5):317-323. doi:10.1016/j.sleh.2017.07.006
2. Matricciani L, Olds T, Petkov J. In search of lost sleep: Secular trends in the sleep time of school-aged children and adolescents. *Sleep Med Rev*. 2012;16(3):203-211. doi:10.1016/j.smrv.2011.03.005
3. Brambilla P, Giussani M, Pasinato A, et al. Sleep habits and pattern in 1-14 years old children and relationship with video devices use and evening and night child activities. *Ital J Pediatr*. 2017;43:1-11. doi:10.1186/s13052-016-0324-x
4. Bathory E, Tomopoulos S. Sleep Regulation, Physiology and Development, Sleep Duration and Patterns, and Sleep Hygiene in Infants, Toddlers, and Preschool-Age Children. *Curr Probl Pediatr Adolesc Health Care*. 2017;47(2):29-42. doi:10.1016/j.cppeds.2016.12.001
5. Blunden S, Lushington K, Lorenzen B, Ooi T, Fung F, Kennedy D. Are sleep problems under-recognised in general practice? *Arch Dis Child*. 2004;89(8):708-712. doi:10.1136/adc.2003.027011
6. Matricciani L, Paquet C, Galland B, Short M, Olds T. Children's sleep and health: A meta-review. *Sleep Med Rev*. 2019;46:136-150. doi:10.1016/j.smrv.2019.04.011
7. Quist JS, Sjödin A, Chaput JP, Hjorth MF. Sleep and cardiometabolic risk in children and adolescents. *Sleep Med Rev*. 2016;29:76-100. doi:10.1016/j.smrv.2015.09.001
8. Sluggett L, Wagner SL, Harris RL. Sleep Duration and Obesity in Children and Adolescents. *Can J Diabetes*. 2019;43(2):146-152. doi:10.1016/j.jcjd.2018.06.006
9. Matricciani L, Bin YS, Lallukka T, et al. Rethinking the sleep-health link. *Sleep Heal*. 2018;4(4):339-348. doi:10.1016/j.sleh.2018.05.004
10. Fobian AD, Elliott L, Louie T. A Systematic Review of Sleep, Hypertension, and Cardiovascular Risk in Children and Adolescents. *Curr Hypertens Rep*. 2018;20(5):1-11. doi:10.1007/s11906-018-0841-7
11. Twig G, Yaniv G, Levine H, et al. Body-Mass Index in 2.3 Million Adolescents and Cardiovascular Death in Adulthood. *N Engl J Med*. 2016;374(25):2430-2440. doi:10.1056/NEJMoa1503840
12. Matthews KA, Pantesco EJM. Sleep characteristics and cardiovascular risk in children and adolescents: An enumerative review. *Sleep Med*. 2016;18:36-49. doi:10.1016/j.sleep.2015.06.004

13. Buysse DJ. Sleep Health: Can We Define It? Does It Matter? *Sleep*. 2014;37(1):9-17. doi:10.5665/sleep.3298
14. de Almondes KM, Leonardo MEM. Study protocol of sleep education tool for children: Serious game “Perfect Bedroom: Learn to sleep well.” *Front Psychol*. 2018;9(JUN):1-13. doi:10.3389/fpsyg.2018.01016
15. Jaquith BC, Harris MA, Penprase B. Cardiovascular disease risk in children and adolescents. *J Pediatr Nurs*. 2013;28(3):258-266. doi:10.1016/j.pedn.2012.11.003
16. Allen SL, Howlett MD, Coulombe JA, Corkum P V. ABCs of SLEEPING: A review of the evidence behind pediatric sleep practice recommendations. *Sleep Med Rev*. 2016;29:1-14. doi:10.1016/J.SMRV.2015.08.006
17. Ohayon M, Wickwire EM, Hirshkowitz M, et al. National Sleep Foundation’s sleep quality recommendations: first report. *Sleep Heal*. 2017;3(1):6-19. doi:10.1016/j.sleh.2016.11.006
18. Hirshkowitz M, Whiton K, Albert SM, et al. National Sleep Foundation’s updated sleep duration recommendations: Final report. *Sleep Heal*. 2015;1(4):233-243. doi:10.1016/j.sleh.2015.10.004
19. Certal V, de Lima FF, Winck JC, Azevedo I, Costa-Pereira A. Translation and cross-cultural adaptation of the Pediatric Sleep Questionnaire into Portuguese language. *Int J Pediatr Otorhinolaryngol*. 2015;79(2):175-178. doi:10.1016/j.ijporl.2014.12.002
20. Chervin RD, Hedger K, Dillon JE, Pituch KJ. Pediatric sleep questionnaire (PSQ): Validity and reliability of scales for sleep-disordered breathing, snoring, sleepiness, and behavioral problems. *Sleep Med*. 2000;1(1):21-32. doi:10.1016/S1389-9457(99)00009-X
21. De Onis M, Onyango AW, Borghi E, Siyam A, Nishida C, Siekmann J. Development of a WHO growth reference for school-aged children and adolescents. *Bull World Health Organ*. 2007;85(9):660-667. doi:10.2471/BLT.07.043497
22. Obesity and overweight. https://www.who.int/en/news-room/fact-sheets/detail/obesity-and-overweight. Accessed July 3, 2020.
23. Matthews DR, Hosker JR, Rudenski AS, Naylor BA, Treacher DF, Turner RC. *Homeostasis Model Assessment: Insulin Resistance and Fl-Cell Function from Fasting Plasma Glucose and Insulin Concentrations in Man*. Vol 28.; 1985.
24. Kaczor M, Sk Als K I M, Skalski M. Prevalence and consequences of insomnia in pediatric population. *Psychiatr Pol*. 2016;50(3):555-569. doi:10.12740/PP/61226
25. Li L, Zhang S, Huang Y, Chen K. Sleep duration and obesity in children: A

systematic review and meta-analysis of prospective cohort studies. *J Paediatr Child Health*. 2017;53(4):378-385. doi:10.1111/jpc.13434

26. Fatima Y, Doi SAR, Mamun AA. Sleep quality and obesity in young subjects: a meta-analysis. *Obes Rev*. 2016;17(11):1154-1166. doi:10.1111/obr.12444
27. Navarro-Solera M, Carrasco-Luna J, Pin-Arboledas G, González-Carrascosa R, Soriano JM, Codoñer-Franch P. Short Sleep Duration Is Related to Emerging Cardiovascular Risk Factors in Obese Children. *J Pediatr Gastroenterol Nutr*. 2015;61(5):571-576. doi:10.1097/MPG.0000000000000868
28. Azadbakht L, Kelishadi R, Khodarahmi M, et al. The association of sleep duration and cardiometabolic risk factors in a national sample of children and adolescents: The CASPIAN III Study. *Nutrition*. 2013;29(9):1133-1141. doi:10.1016/j.nut.2013.03.006
29. De Azevedo Abreu G, Barufaldi LA, Bloch KV, Szklo M. Revisão sistemática sobre duração do sono e dislipidemia em adolescentes: Avaliando inconsistências. *Arq Bras Cardiol*. 2015;105(4):418-425. doi:10.5935/abc.20150121
30. Becker SP, Sidol CA, Van Dyk TR, Epstein JN, Beebe DW. Intraindividual variability of sleep/wake patterns in relation to child and adolescent functioning: A systematic review. *Sleep Med Rev*. 2017;34:94-121. doi:10.1016/j.smrv.2016.07.004
31. Singh S, Kaur H, Singh S, Khawaja I. Parasomnias: A Comprehensive Review. *Cureus*. 2018;10(12). doi:10.7759/cureus.3807
32. Guilleminault C, Palombini L, Pelayo R, Chervin RD. Sleepwalking and sleep terrors in prepubertal children: what triggers them? *Pediatrics*. 2003;111(1). doi:10.1542/peds.111.1.e17
33. Hannon TS, Tu W, Watson SE, Jalou H, Chakravorty S, Arslanian SA. Morning blood pressure is associated with sleep quality in obese adolescents. *J Pediatr*. 2014;164(2):313-317. doi:10.1016/j.jpeds.2013.10.011
34. Peach H, Gaultney JF, Reeve CL. Sleep Characteristics, Body Mass Index, and Risk for Hypertension in Young Adolescents. *J Youth Adolesc*. 2014;44(2):271-284. doi:10.1007/s10964-014-0149-0
35. Jiang W, Hu C, Li F, Hua X, Zhang X. Association between sleep duration and high blood pressure in adolescents: a systematic review and meta-analysis. *Ann Hum Biol*. 2018;45(6-8):457-462. doi:10.1080/03014460.2018.1535661
36. St-Onge MP, Grandner MA, Brown D, et al. Sleep Duration and Quality: Impact on Lifestyle Behaviors and Cardiometabolic Health: A Scientific Statement from the American Heart Association. *Circulation*. 2016;134(18):e367-e386. doi:10.1161/CIR.0000000000000444

37. Archbold KH, Vasquez MM, Goodwin JL, Quan SF. Effects of sleep patterns and obesity on increases in blood pressure in a 5-year period: Report from the Tucson children's assessment of sleep apnea study. *J Pediatr*. 2012;161(1):26-30. doi:10.1016/j.jpeds.2011.12.034
38. Smith DF, Amin RS. OSA and Cardiovascular Risk in Pediatrics. *Chest*. 2019;156(2):402-413. doi:10.1016/j.chest.2019.02.011
39. Bazzano LA, Hu T, Bertisch SM, et al. Childhood obesity patterns and relation to middle-age sleep apnoea risk: The Bogalusa heart study. *Pediatr Obes*. 2016;11(6):535-542. doi:10.1111/ijpo.12103
40. Koren D, Gozal D, Philby MF, Bhattacharjee R, Kheirandish-Gozal L. Impact of obstructive sleep apnoea on insulin resistance in nonobese and obese children. *Eur Respir J*. 2016;47(4):1152-1161. doi:10.1183/13993003.01430-2015
41. Lesser DJ, Bhatia R, Tran WH, et al. Sleep fragmentation and intermittent hypoxemia are associated with decreased insulin sensitivity in obese adolescent Latino males. *Pediatr Res*. 2012;72(3):293-298. doi:10.1038/pr.2012.73

Tables

Table 1 – Demographic and socioeconomic characteristics and cardiometabolic variables, at 7 years of age, by body mass index classes

	All	Nonoverweight	Overweight	*p*
Socioeconomic characteristics				
Birthweight (g)	3175 (506)	3134 (520)	3243 (476)	0.006
Gestational age (weeks)	38.6 (1.7)	38.5 (1.8)	38.7 (1.6)	<0.001
Maternal age (years)[1]	29.6 (5.3)	29.7 (5.3)	29.3 (5.3)	0.005
Maternal education (years)[1]				<0.001
≤ 6	1126 (20.5%)	686 (60.9%)	440 (39.1%)	
7-9	1274 (23.2%)	750 (58.9%)	524 (41.1%)	
10-12	1537 (27.9%)	937 (61.0%)	600 (39.0%)	
>12	1563 (28.4%)	1055 (67.5%)	508 (32.5%)	
Paternal education (years)[1]	10.4 (4.1)	10.7(4.1)	10.0(4.0)	<0.001
Monthly household income[2] (€)				<0.001
>1000	1565 (29.1%)	946 (28.1%)	619 (30.7%)	
1001-2000	2562 (47.6%)	1567 (46.5%)	995 (49.4%)	
≥2001	125 (23.4%)	85 (25.4%)	40 (19.9%)	
Paternal working conditions[1]				0.279
Employed	2768 (94.1%)	1716 (93.7%)	1052 (94.7%)	
Other	174 (5.9%)	115 (6.3%)	59 (5.3%)	
7-years-old evaluation				
Anthropometry				
Weight (kg)	26.3 (5.3)	23.4 (2.7)	31.2 (4.8)	<0.001
Height (cm)	123.7 (5.3)	122.4 (5.0)	125.7 (5.2)	<0.001
BMI (kg/m²)	17.1 (2.5)	15.5 (1.0)	19.7 (2.1)	<0.001
BMI z-score	0.7 (1.2)	-0.0 (0.7)	1.9 (0.7)	<0.001
Office blood pressure				
Systolic BP (mmHg)	105.5 (8.9)	103.4 (8.2)	109.0 (8.8)	<0.001
Diastolic BP (mmHg)	70.0 (7.5)	68.8 (7.4)	72.0 (7.3)	<0.001
Analytic variables				
Glucose (mg/dL)	83.1 (7.7)	82.7 (8.1)	83.9 (6.9)	<0.001
Insulin (mg/dL)	5.6 (4.2)	4.6 (3.4)	7.2 (4.9)	<0.001
HOMA-IR	1.1 (0.9)	0.9 (0.8)	1.5 (1.1)	<0.001
HDL-cholesterol (mg/dL)	55.9 (10.6)	56.5 (10.8)	55,0 (10.3)	<0.001
LDL-cholesterol (mg/dL)	99.6 (23.8)	98.3 (23.5)	101.9 (24.2)	<0.001
Triglycerides (mg/dL)	69.0 (35.8)	65.8 (31.8)	74.4 (41.1)	<0.001
hs-CRP (mg/L)	1.2 (3.8)	1.1 (4.0)	1.5 (3.59)	<0.001

The values presented are mean (standard deviation) or n (%).
[1] Reported at birth.
[2] Reported at 7 years of age.

BMI, body mass index; BP, blood pressure; HDL, high-density lipoprotein; HOMA-IR: Homeostatic Model Assessment for Insulin Resistance, hs-CRP: high-sensitivity C-reactive Protein; LDL cholesterol, low-density lipoprotein.

Table 2 - Sleeping habits and problems at 4 years of age, by body mass index classes.

	All	Nonoverweight	Overweight	*p*
Sleeping habits				
Daily night sleep duration (hours)				
Total	11.2 (1.0)	11.2 (0.9)	11.1 (1.0)	0.148
Nighttime	10.1 (0.8)	10.1 (1.0)	10.1 (0.8)	0.099
Afternoon nap	1.2 (0.7)	1.2 (0.6)	1.2 (0.7)	0.981
Bedtime	21:20 (3:0)	21:25 (2.4)	21:10 (3.3)	0.020
Wakeup time	7:58 (0:5)	7:59 (0.4)	7.6 (1.0)	0.941
Afternoon nap performance				0.020
Not currently	2291 (42.4%)	1386 (41.0%)	905 (44.7%)	
Several times in a week	1785 (33.1%)	1155 (34.2%)	630 (31.1%)	
Everyday	1324 (24.5%)	836 (24.8%)	488 (24.1%)	
Nighttime sleep at own bedroom	3979 (73.6%)	2507 (74.2%)	1472 (72.7%)	0.214
No familiar presence at bedtime	2487 (51.2%)	1576 (51.3%)	911 (51.0%)	0.849
Use of transitional object	2310 (47.6%)	1462 (47.7%)	848 (47.6%)	0.993
Reading stories routine	3631 (74.9%)	2298 (75.0%)	1333 (74.8%)	0.756
Sleep problems				
Any sleep problem	4343 (78.5%)	2698 (80.3%)	1645 (82.0%)	0.129
Short nighttime sleep duration	1758 (31.8%)	1054 (31.2%)	704 (34.8%)	0.007
Night awakening	3269 (60.5%)	2011 (59.5%)	1258 (62.2%)	0.054
Sleep onset delay	1503 (27.9%)	912 (27.0%)	591 (29.3%)	0.077
NREM Parasomnias	500 (9.1%)	310 (9.0%)	190 (9.2%)	0.848
Sleepwalking	60 (1.2%)	38 (1.2%)	22 (1.2%)	0.999
Night terrors	478 (8.6%)	292 (8.5%)	186 (8.9%)	0.547

The values presented are mean (standard deviation) or n (%).

Table 3 - Sleeping habits and problems at 7 years of age, by body mass index classes.

	All	Nonoverweight	Overweight	p
Sleeping habits				
Daily night sleep duration (hours)				
Total (weekdays)	10.0 (0.6)	10.0 (0.6)	10.0 (0.6)	0.079
Total (weekends)	10.5 (1.0)	10.5 (0.9)	10.4 (1.0)	0.018
Bedtime (weekdays)	21:41 (0:5)	21:40 (0:5)	21:43 (0.5)	0.190
Wakeup time (weekdays)	7:46 (0:3)	7:47 (0:3)	7:46 (0:3)	0.690
Sleep problems				
Any sleep problem	1044 (57.7%)	662 (56.0%)	382 (60.8%)	0.048
Short nighttime sleep duration	92 (3.2%)	48 (2.7%)	44 (4.2%)	0.026
Sleep Irregularity	772 (26.3%)	483 (25.6%)	289 (27.7%)	0.208
Sleep-related breathing disorders[1]	265 (13.5%)	151 (11.6%)	114 (17.5%)	<0.001
Daytime sleepiness[2]	1183 (21.4%)	753 (21.8%)	430 (20.7%)	0.302
B1 - Child wakes up very sleepy in the morning	922 (31.0%)	598 (31.1%)	324 (30.9%)	0.892
B2 - Child experience sleepiness during the day	150 (5.1%)	99 (5.2%)	51 (4.9%)	0.704
B4 - Teacher or caretaker report child sleepiness during the day	96 (3.2%)	58 (3.0%)	38 (3.6%)	0.400
B6 – Child is hard to wake up in the morning	789 (26.5%)	501 (26.0%)	288 (27.3%)	0.447

The values presented are mean (standard deviation) or n (%).
[1] Sleep-related breathing disorders were considered present when the score in the Pediatric Sleep Questionnaire Score was >0.33.
[2] The presence of daytime sleepiness was considered when the answer to any of the questions presented (B1, B2, B4, B6) was “yes”.

Table 4 - Mean change in each cardiometabolic variable, at 7 years of age, by the presence of each sleep variable, at 4 and at 7 years of age.

	BMIz	SBP	DBP	HOMA-IR	HDLc	LDLc	TG
Sleep variables at 4 years	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
Daily night sleep duration (hours)							
Model 1	-0.03 (-0.06 to 0.00)	-0.05 (-0.32 to 0.21)	-0.00 (-0.24 to 0.22)	-0.01 (-0.05 to 0.02)	0.18 (-0.18 to 0.56)	0.97 (0.14 to 1.80)*	0.39 (-0.85 to 1.64)
Model 2	-0.02 (-0.06 to 0.00)	-0.09 (-0.37 to 0.17)	-0.01 (-0.25 to 0.21)	-0.01 (-0.05 to 0.02)	0.19 (-0.17 to 0.56)	0.96 (0.13 to 1.79)*	0.38 (-0.86 to 1.63)
Model 2 + BMIz	-	-0.01 (-0.27 to 0.24)	0.02 (-0.20 to 0.25)	-0.00 (-0.04 to 0.02)	0.16 (-0.20 to 0.53)	1.03 (0.20 to 1.85)*	0.55 (-0.68 to 1.79)
Daily total sleep duration (hours)							
Model 1	-0.01 (-.06 to 0.02)	-0.25 (-0.5 to 0.5)	-0.22 (-0.48 to 0.04)	-0.00 (-0.04 to 0.03)	-0.10 (-0.52 to 0.31)	0.45 (-0.49 to 1.40)	0.43 (-0.88 to 1.75)
Model 2	-0.02 (-0.06 to 0.02)	-0.32 (-0.63 to -0.00)*	-0.23 (-0.50 to 0.03)	-0.00 (-0.04 to 0.03)	-0.12 (-0.55 to 0.29)	0.47 (-0.48 to 1.42)	0.38 (-0.94 to 1.71)
Model 2 + BMIz	-	-0.25 (-0.55 to 0.03)	-0.19 (-0.45 to 0.06)	0.00 (-0.03 to 0.03)	-0.14 (-0.57 to 0.27)	0.52 (-0.42 to 1.46)	0.48 (-0.82 to 1.79)
Night Awakening							
Model 1	0.08 (0.01 to 0.14)*	0.16 (-0.30 to 0.64)	-0.03 (-0.44 to 0.37)	0.02 (-0.04 to 0.08)	0.30 (-0.35 to 0.96)	0.70 (-0.75 to 2.16)	-1.83 (-4.03 to 0.37)
Model 2	0.09 (0.03 to 0.16)*	0.23 (-0.25 to 0.72)	-0.00 (-0.42 to 0.40)	0.02 (-0.03 to 0.09)	0.25 (-0.40 to 0.91)	0.54 (-0.92 to 2.01)	-1.85 (-4.06 to 0.36)
Model 2 + BMIz	-	-0.02 (-0.48 to 0.42)	-0.15 (-0.55 to 0.25)	0.00 (-0.05 to 0.06)	0.32 (-0.33 to 0.97)	0.40 (-1.06 to 1.86)	-2.21 (-4.40 to -0.02)*
Sleep Onset Delay							
Model 1	0.07 (-0.00 to 0.14)	-0.02 (-0.54 to 0.48)	0.14 (-0.30 to 0.59)	0.04 (-0.02 to 0.11)	-0.43 (-1.14 to 0.28)	0.14 (-1.45 to 1.74)	3.25 (0.84 to 5.65)*
Model 2	0.06 (-0.00 to 0.13)	-0.03 (-0.56 to 0.49)	0.14 (-0.31 to 0.58)	0.04 (-0.02 to 0.11)	-0.44 (-1.15 to 0.27)	0.29 (-1.30 to 1.89)	3.38 (0.96 to 5.80)*
Model 2 + BMIz	-	-0.21 (-0.70 to 0.27)	0.04 (-0.39 to 0.48)	0.02 (-0.4 to 0.08)	-0.38 (-1.09 to 0.33)	0.18 (-1.41 to 1.78)	3.09 (0.70 to 5.48)*
NREM Parasomnias							
Model 1	0.02 (-0.08 to 0.13)	0.78 (-0.01 to 1.59)	0.74 (0.5 to 1.44)*	0.08 (-0.02 to 0.18)	0.95 (-0.14 to 2.05)	0.39 (-2.05 to 2.84)	2.24 (-1.44 to 5.94)
Model 2	0.04 (-0.06 to 0.15)	0.84 (0.02 to 1.66)*	0.78 (0.94 to 1.48)*	0.09 (-0.01 to 0.20)	0.92 (-0.18 to 2.02)	0.18 (-2.27 to 2.64)	2.30 (-1.40 to 6.01)
Model 2 + BMIz	-	0.72 (-0.03 to 1.49)	0.72 (0.05 to 1.40)*	0.07 (-0.02 to 0.17)	0.97 (-0.12 to 2.07)	0.09 (-2.35 to 2.53)	2.04 (-1.62 to 5.71)
Sleep variables at 7 years							
Night sleep duration week (hours)							
Model 1	-0.07 (-0.14 to -0.01)*	-0.29 (-0.81 to 0.22)	-.026 (-0.70 to 0.17)	-0.05 (-0.10 to -0.00)*	0.47 (-0.24 to 1.18)	1.17 (-0.36 to 2.71)	1.66 (-0.58 to 3.91)
Model 2	-0.09 (-0.16 to -0.02)*	-0.38 (-0.91 to 0.14)	-.033 (-0.76 to 0.10)	-0.06 (-0.11 to -0.00)*	0.51 (-0.20 to 1.22)	1.21 (-.033 to 2.75)	1.51 (-0.73 to 3.77)
Model 2 + BMIz	-	-0.13 (-0.63 to 0.35)	-0.20 (-0.62 to 0.22)	-0.05 (-0.09 to -0.00)*	0.44 (-0.26 to 1.15)	1.32 (-0.20 to 2.86)	1.80 (-0.42 to 4.04)
Night sleep duration weekend (hours)							
Model 1	-0.06 (-0.10 to -0.01)*	-0.15 (-0.49 to 0.17)	0.02 (-0.25 to 0.30)	-0.00 (-0.03 to 0.03)	0.24 (0.22 to 0.70)	0.09 (-0.91 to 1.10)	0.40 (-1.05 to 1.87)
Model 2	-0.07 (-0.12 to -0.03)*	-0.28 (-0.62 to 0.05)	-0.04 (-0.33 to 0.23)	-0.00 (-0.03 to 0.02)	0.29 (-0.17 to 0.76)	0.13 (-0.87 to 1.15)	0.21 (-1.26 to 1.69)
Model 2 + BMIz	-	-0.06 (-0.38 to 0.25)	0.06 (-0.20 to 0.34)	0.00 (-0.02 to 0.03)	0.24 (-0.22 to 0.70)	0.22 (-0.78 to 1.23)	0.42 (-1.03 to 1.89)
Sleep Irregularity							
Model 1	0.05 (-0.04 to 0.16)	0.37 (-0.38 to 1.12)	-0.05 (-0.68 to 0.56)	0.10 (0.02 to 0.17)*	-0.01 (-1.05 to 1.02)	-0.81 (-3.07 to 1.43)	1.5 (-1.72 to 4.86)
Model 2	0.04 (-0.05 to 0.14)	0.22 (-0.53 to 0.98)	-0.15 (-0.77 to 0.47)	0.09 (0.02 to 0.17)*	-0.00 (-1.04 to 1.04)	-0.67 (-2.93 to 1.59)	1.40 (-1.90 to 4.71)
Model 2 + BMIz	-	0.11 (-0.58 to 0.81)	-0.20 (-0.81 to 0.39)	0.06 (-0.00 to 0.12)	0.04 (-0.99 to 1.07)	-0.75 (-3.00 to 1.50)	1.21 (-2.06 to 4.49)
Presence of SRBD							
Model 1	0.40 (0.24 to 0.56)**	2.00 (0.80 to 3.19)**	1.14 (0.16 to 2.13)*	0.14 (0.03 to 0.25)*	-2.30 (-3.87 to -0.73)*	0.02 (-3.43 to 3.47)	1.06 (-3.93 to 6.06)
Model 2	0.39 (0.23 to 0.55)**	1.80 (0.60 to 2.99)*	1.01 (0.02 to 1.99)*	0.14 (0.03 to 0.25)*	-2.25 (-3.82 to -0.68)*	0.34 (-3.12 to 3.80)	1.10 (-3.91 to 6.12)
Model 2 + BMIz	-	0.73 (-0.38 to 1.85)	0.46 (-0.50 to 1.42)	0.05 (-0.04 to 0.15)	-1.97 (-3.54 to -0.39)*	-0.28 (-3.75 to 3.18)	-0.39 (-5.38 to 4.59)
Daytime sleepiness							
Model 1	-0.03 (-0.11 to -0.04)	0.90 (0.34 to 1.46)*	0.80 (0.31 to 1.28)*	-0.12 (-0.20 to -0.04)*	-0.55 (-1.33 to 0.22)	-0.69 (-2.43 to 1.04)	-3.06 (-5.68 to -0.44)*
Model 2	-0.02 (-0.10 to 0.04)	0.95 (0.37 to 1.52)*	0.80 (0.32 to 1.29)*	-0.12 (-0.20 to -0.04)*	-0.55 (-1.34 to 0.22)	0.58 (-2.32 to 1.16)	-2.96 (-5.59 to -0.34)*
Model 2 + BMIz	-	1.04 (0.51 to 1.57)**	0.85 (0.38 to 1.32)**	-0.10 (-0.17 to -0.02)*	-0.59 (-1.38 to 0.18)	-0.50 (-2.24 to 1.23)	-2.76 (-5.36 to -0.16)*

The values presented are regression coefficients (β) and 95% confidence intervals, as estimated by linear regression models (one for each sleep variable), with each cardiometabolic variable (BMI z-score, SBP, DBP, HOMA-IR, HDL, LDL and TG) as the dependent variable. The models include each sleep variable at a time and, in all cases, Model 1 is additionally adjusted for sex, Model 2 is additionally adjusted for sex and classes of maternal education and, when duly signed (Model 2 + BMIz), also for BMI z-score.

BMI, body mass index; BP, blood pressure; DBP: diastolic blood pressure, HDL, high-density lipoprotein; HOMA-IR: Homeostatic Model Assessment for Insulin Resistance, LDL cholesterol, low-density lipoprotein, SBP: systolic blood pressure, SRBD: Sleep related breathing disorders.

* *p* value <0.005; ** *p* value <0.001

[1] Sleep-related breathing disorders were considered present when the score in the Pediatric Sleep Questionnaire Score was >0.33.

[2] The presence of daytime sleepiness was considered when the answer to any of the questions presented (B1, B2, B4, B6) was "yes".

Conclusion

Several domains of sleep, as sleep duration and different specific sleep problems, both at 4 and 7 years of age were shown to be associated with higher BMI and cardiovascular risk at 7 years old. Our data allow us to reinforce the hypothesis that poorer sleep health acts upon cardiometabolic health, possibly increasing cardiovascular risk throughout life. At 4 years of age, we found that night awakening was associated with higher BMI z-score and a worst cardiometabolic profile at the age of 7, also that higher levels of TG were associated with sleep onset delay and higher values of DBP with NREM parasomnias. At 7 years old, we found that shorter night sleep duration was associated with higher BMI z-score and higher insulin resistance. Sleep irregularity was also shown to be associated with higher insulin resistance and daytime sleepiness with higher BP values. The presence of SRBD was also shown to be associated with higher BP values, more insulin resistance and lower HDLc values.

From a public health perspective, the study the role of children's sleep on cardiometabolic health and the establishment of future behaviors, is of utmost importance in the promotion sleep health literacy among parents and caregivers, which can have an important preventive impact as to the development of chronic diseases, notably cardiovascular disease, into adulthood. Moreover, in a broader approach, not only cardiometabolic health but also immediate and long-term children's well-being, might be meaningfully influenced by the effort to aware parents and caregivers of the importance of prepuberal sleep behaviors and problems.

Nonetheless, it is important to acknowledge that further research is needed to clarify the role of sleep duration, quality, timing, and variability on health during childhood, and to investigate the long-term impact of poor sleep health.

References

1. Bathory E, Tomopoulos S. Sleep Regulation, Physiology and Development, Sleep Duration and Patterns, and Sleep Hygiene in Infants, Toddlers, and Preschool-Age Children. *Curr Probl Pediatr Adolesc Health Care*. 2017;47(2):29-42. doi:10.1016/j.cppeds.2016.12.001
2. Blunden S, Lushington K, Lorenzen B, Ooi T, Fung F, Kennedy D. Are sleep problems under-recognised in general practice? *Arch Dis Child*. 2004;89(8):708-712. doi:10.1136/adc.2003.027011
3. Byars KC, Yeomans-Maldonado G, Noll JG. Parental functioning and pediatric sleep disturbance: An examination of factors associated with parenting stress in children clinically referred for evaluation of insomnia. *Sleep Med*. 2011;12(9):898-905. doi:10.1016/j.sleep.2011.05.002
4. Matricciani L, Bin YS, Lallukka T, et al. Rethinking the sleep-health link. *Sleep Heal*. 2018;4(4):339-348. doi:10.1016/j.sleh.2018.05.004
5. de Almondes KM, Leonardo MEM. Study protocol of sleep education tool for children: Serious game “Perfect Bedroom: Learn to sleep well.” *Front Psychol*. 2018;9(JUN):1-13. doi:10.3389/fpsyg.2018.01016
6. Blunden S, Rigney G. Lessons learned from sleep education in schools: A review of dos and don’ts. *J Clin Sleep Med*. 2015;11(6):671-680. doi:10.5664/jcsm.4782
7. Allen SL, Howlett MD, Coulombe JA, Corkum P V. ABCs of SLEEPING: A review of the evidence behind pediatric sleep practice recommendations. *Sleep Med Rev*. 2016;29:1-14. doi:10.1016/J.SMRV.2015.08.006
8. Matricciani L, Bin YS, Lallukka T, et al. Past, present, and future: trends in sleep duration and implications for public health. *Sleep Heal*. 2017;3(5):317-323. doi:10.1016/j.sleh.2017.07.006
9. Matricciani L, Olds T, Petkov J. In search of lost sleep: Secular trends in the sleep time of school-aged children and adolescents. *Sleep Med Rev*. 2012;16(3):203-211. doi:10.1016/j.smrv.2011.03.005
10. Brambilla P, Giussani M, Pasinato A, et al. Sleep habits and pattern in 1-14 years old children and relationship with video devices use and evening and night child activities. *Ital J Pediatr*. 2017;43:1-11. doi:10.1186/s13052-016-0324-x
11. Carter JC, Wrede JE. Overview of Sleep and Sleep Disorders in Infancy and Childhood. *Pediatr Ann*. 2017;46(4):e133-e138. doi:10.3928/19382359-20170316-02
12. Ophoff D, Slaats MA, Boudewyns A, Glazemakers I, Van Hoorenbeeck K, Verhulst SL. Sleep disorders during childhood: a practical review. *Eur J Pediatr*. 2018;177(5):641-648. doi:10.1007/s00431-018-3116-z
13. Buysse DJ. Sleep Health: Can We Define It? Does It Matter? *Sleep*. 2014;37(1):9-17. doi:10.5665/sleep.3298
14. Organization WH. Preamble to the Constitution of the World Health Organization as adopted by the International Health Conference, New York, 19-22 June, 1946 ; signed on 22 July 1946 by the representatives of 61 States (Official Records of the World Health Organization, no. 2, p. 100) and entered into force on 7 April 1948. *http://www.who.int/governance/eb/who_constitution_en.pdf*. 1948.
15. Paruthi S, Brooks LJ, D’Ambrosio C, et al. Recommended amount of sleep for pediatric populations: A consensus statement of the American Academy of Sleep Medicine. *J Clin Sleep Med*. 2016;12(6):785-786. doi:10.5664/jcsm.5866
16. Vasconcelos A, Prior C, Estevão H, Loureiro HC, Ferreira R, Paiva T. Recomendações sps-spp: prática da sesta da criança. *Soc Port Pediatr*. 2017:1-8. http://www.spp.pt/UserFiles/file/Noticias_2017/VERSAO PROFISSIONAIS DE SAUDE_RECOMENDACOES SPS-SPP SESTA NA CRIANCA.pdf.
17. Hirshkowitz M, Whiton K, Albert SM, et al. National Sleep Foundation’s updated

sleep duration recommendations: Final report. *Sleep Heal*. 2015;1(4):233-243. doi:10.1016/j.sleh.2015.10.004
18. Ohayon M, Wickwire EM, Hirshkowitz M, et al. National Sleep Foundation's sleep quality recommendations: first report. *Sleep Heal*. 2017;3(1):6-19. doi:10.1016/j.sleh.2016.11.006
19. Allen SL, Howlett MD, Coulombe JA, Corkum P V. ABCs of SLEEPING: A review of the evidence behind pediatric sleep practice recommendations. *Sleep Med Rev*. 2016;29:1-14. doi:10.1016/j.smrv.2015.08.006
20. Majnoni d'Intignano B. Fin du fin de l'ISF ? *Commentaire*. 2017;Numéro157(1):65. doi:10.1016/j.smrv.2015.08.006
21. Koulouglioti C, Cole R, Moskow M, McQuillan B, Carno MA, Grape A. The longitudinal association of young children's everyday routines to sleep duration. *J Pediatr Heal Care*. 2014;28(1):80-87. doi:10.1016/j.pedhc.2012.12.006
22. Sateia MJ. International classification of sleep disorders-third edition highlights and modifications. *Chest*. 2014;146(5):1387-1394. doi:10.1378/chest.14-0970
23. Darien I. *American Academy of Sleep Medicine . International Classification of Sleep Disorders*. 3rd ed. American Academy of Sleep Medicine; 2014.
24. Certal V, de Lima FF, Winck JC, Azevedo I, Costa-Pereira A. Translation and cross-cultural adaptation of the Pediatric Sleep Questionnaire into Portuguese language. *Int J Pediatr Otorhinolaryngol*. 2015;79(2):175-178. doi:10.1016/j.ijporl.2014.12.002
25. Hintze JP, Paruthi S. Sleep in the Pediatric Population. *Sleep Med Clin*. 2016;11(1):91-103. doi:10.1016/j.jsmc.2015.10.010
26. Carter KA, Hathaway NE. *Common Sleep Disorders in Children*. Vol 89.; 2014. www.aafp.org/afp. Accessed July 16, 2020.
27. Lloyd LJ, Langley-Evans SC, McMullen S. Childhood obesity and adult cardiovascular disease risk: A systematic review. *Int J Obes*. 2010;34(1):18-28. doi:10.1038/ijo.2009.61
28. Juonala M, Magnussen CG, Berenson GS, et al. Childhood Adiposity, Adult Adiposity, and Cardiovascular Risk Factors. *N Engl J Med*. 2011;365(20):1876-1885. doi:10.1056/NEJMoa1010112
29. Reilly JJ, Kelly J. Long-term impact of overweight and obesity in childhood and adolescence on morbidity and premature mortality in adulthood: Systematic review. *Int J Obes*. 2011;35(7):891-898. doi:10.1038/ijo.2010.222
30. Park MH, Falconer C, Viner RM, Kinra S. The impact of childhood obesity on morbidity and mortality in adulthood: a systematic review. *Obes Rev*. 2012;13(11):985-1000. doi:10.1111/j.1467-789X.2012.01015.x
31. Twig G, Yaniv G, Levine H, et al. Body-Mass Index in 2.3 Million Adolescents and Cardiovascular Death in Adulthood. *N Engl J Med*. 2016;374(25):2430-2440. doi:10.1056/NEJMoa1503840
32. Matricciani L, Paquet C, Galland B, Short M, Olds T. Children's sleep and health: A meta-review. *Sleep Med Rev*. 2019;46:136-150. doi:10.1016/j.smrv.2019.04.011
33. Quist JS, Sjödin A, Chaput JP, Hjorth MF. Sleep and cardiometabolic risk in children and adolescents. *Sleep Med Rev*. 2016;29:76-100. doi:10.1016/j.smrv.2015.09.001
34. Sluggett L, Wagner SL, Harris RL. Sleep Duration and Obesity in Children and Adolescents. *Can J Diabetes*. 2019;43(2):146-152. doi:10.1016/j.jcjd.2018.06.006
35. St-Onge MP, Grandner MA, Brown D, et al. Sleep Duration and Quality: Impact on Lifestyle Behaviors and Cardiometabolic Health: A Scientific Statement from the American Heart Association. *Circulation*. 2016;134(18):e367-e386. doi:10.1161/CIR.0000000000000444
36. St-Onge M-P. Sleep-obesity relation: underlying mechanisms and consequences for treatment. *Obes Rev*. 2017;18:34-39. doi:10.1111/obr.12499
37. Saunders TJ, Gray CE, Poitras VJ, et al. Combinations of physical activity,

sedentary behaviour and sleep: Relationships with health indicators in school-aged children and youth. *Appl Physiol Nutr Metab*. 2016;41(6):S283-S293. doi:10.1139/apnm-2015-0626
38. Li L, Zhang S, Huang Y, Chen K. Sleep duration and obesity in children: A systematic review and meta-analysis of prospective cohort studies. *J Paediatr Child Health*. 2017;53(4):378-385. doi:10.1111/jpc.13434
39. Fobian AD, Elliott L, Louie T. A Systematic Review of Sleep, Hypertension, and Cardiovascular Risk in Children and Adolescents. *Curr Hypertens Rep*. 2018;20(5):1-11. doi:10.1007/s11906-018-0841-7
40. Azadbakht L, Kelishadi R, Khodarahmi M, et al. The association of sleep duration and cardiometabolic risk factors in a national sample of children and adolescents: The CASPIAN III Study. *Nutrition*. 2013;29(9):1133-1141. doi:10.1016/j.nut.2013.03.006
41. Jaquith BC, Harris MA, Penprase B. Cardiovascular disease risk in children and adolescents. *J Pediatr Nurs*. 2013;28(3):258-266. doi:10.1016/j.pedn.2012.11.003

SEDE ADMINISTRATIVA

FACULDADE DE **MEDICINA**

INSTITUTO DE **CIÊNCIAS BIOMÉDICAS ABEL SALAZAR**