Circadian health differs between boys and girls as assessed by non-invasive tools in school-aged children

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SUMMARY

**Background & aims:** Assessment of circadian health is confined to adults. However, understanding circadian status of school-aged children is necessary due to its health implications. The aim was to develop 1) a protocol to assess circadian function in school-aged children by combining the best non-invasive tools previously validated in adults; 2) a score to capture circadian function in children including food timing. This protocol will allow to explore gender differences and to compare the circadian function of school-aged children with adults from the same Mediterranean area.

**Methods:** Healthy children (8–12 y) from 3 schools in a Mediterranean area of Spain were recruited (n = 248; 125 males and 123 females). Several non-invasive tools were used: a) 7-day-diaries of food timing and food intake, physical-activity and sleep, b) Munich-chronotype-self-reported-questionnaire; c) cortisol and melatonin saliva determinations; d) 7-day-rhythms of wrist temperature (T), activity (A), position (P) and the integrative variable TAP e) 7-day-light exposure.

**Results:** We have constructed the first school-aged children population for the assessment of circadian function (ONTIME-Jr) and a new circadian score has been developed. Among circadian-related measures, TAP was the most suitable and reliable to determine circadian system characteristics. Circadian function was better in girls than in boys [circadian score (AU) Mean ± SD (girls, 1216 ± 153 vs. 1159 ± 173 boys, P = 0.012)], and also in school-aged children than in adults from the same Mediterranean area [Circadian-Function-Index: children 0.47 ± 0.06 vs. adults 0.45 ± 0.06 P = 0.001].

**Conclusions:** A new protocol, including TAP and food timing, demonstrated to be reliable in assessing circadian function in children. These non-invasive techniques provide the wherewithal for paediatricians to assess circadian function in clinical practice.


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1. Introduction

The assessment of circadian health is confined mostly to adults [1–4]. Nevertheless, understanding circadian status in school-aged children is necessary due to its health implications. The school timetable influences children’s lives by affecting external synchronizers of the biological clock such as changes from fasting to eating (i.e., food timing) and from resting to activity, light exposition and sleep duration [5–7]. Inadequate exposure to these body clock synchronizers may disrupt the circadian system function and contribute to the risk of developing metabolic diseases [8–10].

One of the main challenges in chronobiology when studying circadian function is to implement non-invasive techniques that...
capture daily rhythms under free-living conditions. These techniques provide the wherewithal for paediatricians to assess circadian function in clinical practice.

Objective measurements to assess circadian function include saliva determinations of circadian-related hormones [5,13] and the assessment of the 24 h rhythms in body temperature and motor activity [11–14], among others. However, 24 h rhythms data may be affected by environmental masking factors and the presence of artifacts [3] (i.e., device failures due to removal for showering or placement in a hot surface affecting the data recorded, etc.). Integrative variables that include daily rhythms of wrist temperature (T), motor activity (A), and body position (P), such as TAP are suggested to minimize such effects [2]. TAP has been used in adult populations to evaluate the heritability of circadian health [3] and to assess chronodisruption in pathologies such as metabolic syndrome or obesity in adults [4]. To our knowledge, TAP has never been used in school-aged children to assess circadian health.

The main purpose of the current work is to develop a protocol to assess circadian function in school-aged children by combining the best non-invasive tools previously validated in adults. This protocol will allow us to (i) detect chronobiological aspects in children; (ii) identify underlying mechanisms of chronodisruption; (iii) to explore gender differences; and, (iv) to compare the circadian function of school-aged children with adults from the same Mediterranean area. We also aim to develop a score to capture circadian system function in this age group.

2. Materials and methods

2.1. Subjects

2.1.1. Children

Healthy children (8–12 y) from three schools in a Mediterranean area of Spain were recruited (n = 248; 125 males and 123 females) (Table 1). Two urban schools, one public and one private, and one rural public school were chosen to provide a representative population sample of this area of Spain. Subjects with chronic illness, or those with fever during the week of the assessment were excluded (n = 6). The study was conducted from October 1, 2014, to June 1, 2016. Testing for each child required 7 days. Approval for this study was obtained by the Ethics Committee of the University of Murcia. Written consent to participate was provided by the parents. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

2.1.2. Adult population

Certain parameters of the children population were compared with an adult population composed of 177 healthy women from the same Mediterranean area. The average age was 39.92 ± 12.44, the average weight was 75.16 ± 14.49 kg and BMI was 28.54 ± 5.43 kg/m² (Mean ± SD).

2.2. Anthropometric and body composition measurements

Anthropometric measurements were collected on the first day of the week of study. Body weight was valued in barefooted subjects wearing light clothes using a digital scale accurate to the nearest 0.1 kg. Height was determined using a portable stadiometer (rank, 0.14–2.10). The subjects were positioned upright, relaxed and with the head in the Frankfort plane. These data were used to calculate the body mass index (BMI) according to the formula: weight (kg)/height (m²). Total body fat was determined by bioelectrical impedance, using TANITA TBF-300 (Tanita Corporation of America, Arlington, Hills, IL) equipment.

2.3. Circadian tools

2.3.1. Self-reported diaries of food timing and intake, physical activity and sleep

Children and parents completed diaries adapted for the appropriate age group [15]. Food intake diary included: a 7-day food record that specified time of food intake, type of food and amount of food eaten and physical activities diary included: type, time, intensity and duration of every activity performed during 7 days [16]. Sleep diary included: a) nocturnal sleep (bedtime, number of awakenings during the sleep, and awake time); b) time and duration of naps [16].

2.3.2. Chronotype

An age appropriate Spanish version of the Munich Chronotype Questionnaire (MCTQ) was used [17] and sleep phase and sleep duration for weekdays and free-days was calculated. Social jet lag defined as the difference in the meantime of sleep between weekend (MSF) and weekdays (MSW); (Social jet lag = MSF – MSW) was also calculated.

2.3.3. Cortisol and melatonin saliva determinations

Salivettes (Sarstedt, Barcelona, Spain) were used for three salivary samples of cortisol, collected before breakfast (9:00 h), before lunch (14:00 h) and after dinner (23:00 h). The morning/night ratio, a suitable marker of chronodisruption, was calculated [18]. Melatonin samples were collected at night (01:00 h) and before lunch (14:00 h) and were measured by radioimmunoassay.
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