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Original article

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.

Trial registration: Chronobiology and childhood obesity (ONTIME-Jr: Obesity, Nutrigenetics, Timing and Mediterranean, Junior). ClinicalTrials.gov ID: NCT02895282, October 2014.

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].

Abbreviations: T, Temperature; A, Activity; P, Position; MCTQ, Munich Chronotype Questionnaire; CFI, Circadian Function Index; TAP, Temperature, Activity, Position; BMI, Body mass index; ONTIME-Jr, Obesity, Nutrigenomics, Timing, Mediterranean, Junior.

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Glossary

Acrophase time at which the peak of a rhythm occurs
Amplitude difference between the peak (or nadir) and the mean value of a wave
Circadian Function Index (CFI) a numerical index that determines the circadian robustness, based on three circadian parameters: Interday stability (IS), Intraday variability (IV) and Relative amplitude (RA). CFI oscillates between 0 (absence of circadian rhythmicity) and 1 (robust circadian rhythm)
Circadian rhythms occurring or functioning in cycles of approximately 24 h
Cosinor a procedure for the analysis of biological rhythms based on the fitting of a cosine wave to the raw data
Eveningness characteristic of being most active and alert during the evening
Fragmentation of the rhythm frequency of changes of the circadian variable studied
Interday stability (IS) determines the constancy of the 24 h rhythmic pattern over days. A stable rhythm is characterized by a 24-h profile that remains very similar from day to day
Intraday variability (IV) characterizes rhythm fragmentation
M5 average of the five consecutive hours of maximum values and its timing (TM5), used for the 24 h rhythms of peripheral temperature
M10 average of the ten consecutive hours of maximum values and its timing (TM10), used for the 24 h rhythms of activity and position
Mesor average value around which the variable oscillates in a cosine wave
Percentage of rhythmicity (PR) percentage of variance of data explained by the sinusoidal function
Photoperiod duration of the illuminated segment of a light–dark cycle
Rayleigh test to assess the acrophase distribution within a period of successive days. This test provides an r vector with its origin at the center of a circumference of radius one. The r vector length (between 0 and 1) is proportional to the degree of phase homogeneity during the period analyzed and can be considered to be a measure of the rhythm's phase stability during successive days
Relative amplitude (RA) difference between the maximum (or minimum) value of the cosine function and mesor
Sleep episodes interval of sleep that may be voluntary or involuntary
Social jet lag syndrome that occurs when our body's biological clock and our actual sleep schedules don't match up.
Synchronizera stimulus capable of resetting a pacemaker or synchronizing a self-sustaining oscillation

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.

Table 1
General characteristics of the children studied.

		Total	Boys	Girls
		n = 248	n = 125	n = 123
Age (years)	8	28	12	16
	9	78	33	45
	10	78	45	33
	11	49	25	24
	12	15	10	5
Race	White	227	114	113
	Other	21	11	10
Weight (kg)		38.5 ± 9.4	39.3 ± 10.3	37.8 ± 8.4
Height (cm)		142 ± 9	143 ± 10	142 ± 9
BMI (kg/m^2)		18.8 ± 3.3	19.0 ± 3.4	18.7 ± 3.1
Body fat (%)		21.4 ± 7.6	19.4 ± 7.7	23.4 ± 7.5

Age and race (n). Anthropometric data (means ± standard deviation).

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 40 ± 12 y, the average weight was 75.2 ± 14.5 kg and BMI was 28.5 ± 5.4 kg/m^2 (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^2 (m). Total body fat was determined by bioelectrical impedance, using TANITA TBF-300 (Tanita Corporation of America, Arlington Heights, 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.

2.3.4. Wrist temperature, physical activity and body position

Children wore a wristwatch during 7 days of study in a non-dominant hand. This wristwatch integrated two different sensors:

1) *Temperature sensor* for determining wrist temperature rhythms [14] (Thermochron iButton DS1921H, Dallas, Maxim, Dallas, TX) programmed to collect information every 5 min. To mitigate environmental temperature masking of wrist temperature, the study was conducted between October and May, avoiding extreme environmental temperatures typical of Southern Spain in summer.
2) *Accelerometer sensor* (G Acceleration Data Logger UA-004-64; Onset Computer, Bourne, MA, USA) that measures physical activity and body position rhythms programmed to record data every 30 s. Activity was calculated as degrees of change in X, Y and Z axes. *Position* was calculated as the angle between X-axis of the accelerometer and the horizontal plane, where 0° represents the arm in a horizontal position and 90° when vertical [2].

Calculation of TAP: The integrative variable TAP was obtained by calculating the 95th and 5th percentiles for each variable. Wrist temperature values were inverted because Activity and Position values were opposites so that the maximum values for all 3 variables occurred at the same time of day. The mean of 3 normalized variables was calculated. Zero corresponded to complete rest and 1 to periods of high movement [2].

Luxmeter: A HOBO Pendant Light Data Logger UA-002-64 (Onset Computer, Bourne, Massachusetts, USA) was programmed to collect light information continuously every 30 s for 7 days. Children were instructed to wear HOBO as a pendant over their clothing and to leave on a bedside table when asleep [19].

2.4. Statistical methods

2.4.1. To characterize temperature, activity, position and TAP

We calculated the following parameters using parametric and non-parametric methods:

a) *Cosinor's analysis* [20] was applied to calculate: minimum; mesor, amplitude, acrophase, percentage of rhythmicity and Rayleigh test (see glossary).
b) *Non-parametric analyses* [21] were performed to calculate: interday stability, intraday variability and relative amplitude. A Circadian Function Index (CFI) was calculated as the average of these three parameters (see glossary) [21]. M5 and its timing for temperature (TM5) and M10 and its timing (TM10) for activity were also determined.

All of the rhythmic parameters were obtained using an integrated package for temporal series analysis *Kroniwizard* (https://kronowizard.um.es/kronowizard) (Chronobiology Laboratory, University of Murcia, Spain, 2015).

2.4.2. To calculate a circadian score

For each participant a weight average score which includes circadian-related variables with assigned weights was calculated. For this purpose, and as a first step, a factor analysis was executed.

This methodology determines whether a large number of biomarkers of the circadian system evaluated could be replaced by a smaller number of underlying patterns or factors and to know how

they contributed to explain the general process of circadian system function.

Factor analysis searches for patterns, or factors, that have eigenvalues greater than one. For this analysis, an orthogonal rotation method was used. Gamma is pre-assigned to 1 because varimax rotation was performed. Rotated factor loadings (pattern) are coefficients of the factor after rotation and show the degree to which a given variable is represented in a particular factor.

Factor analysis was applied to all those variables that have been previously demonstrated to be good markers of the circadian system status in previous studies which comprise every variable described in the method section.

Supplemental Table 2, shows the result of the factor analysis. Five patterns emerged that accounted for ~50% of variance. Thus, different combinations of these factors could generate 50% of the total information in circadian system function data. The rotated factor-loading pattern for the five principal factors is shown in Supplemental Table 2. Factors 1 and 2 explained ~28% of variance and loaded highest and positively by the characteristics the daily rhythm of TAP (integrated variable of temperature, activity and position), while factor 3 explained ~10% of variance, loaded on cortisol measurements. Factors 4 and 5 explained ~13% of the variance and loaded on timing of food intake (breakfast and dinner).

These five factors of the factor analysis were considered to determine a *final Circadian Score* (Supplemental Table 3). For each participant, the score of Factor 1 was calculated multiplying the value of CFI TAP by the loading (0.96); plus, the value of TAP amplitude by its loading 0.96 and the value of the percentage of rhythmicity (PR) by its loading (0.92); and so forth in order to include all the characteristics of the daily rhythm of TAP present in the first factor, (Supplemental Table 3).

Similar calculations were performed for the scores of the five factors. To obtain a final Circadian Score, we performed the weighted mean of the first five factors (multiplying by the % of variance of each factor) using the following equation.

Final Circadian score = (F1 score*% variance F1) + (F2 score*% variance F2) + (F3 score*% variance F3) + (F4 score*% variance F4) + (F5 score*% variance F5)

A similar Circadian Score has been previously used and validated in an adult population (70 women) from the same Mediterranean area to capture the status of their circadian system [22].

2.4.3. To discriminate circadian function between girls and boys

Circadian-related data obtained by the non-invasive tools used were compared between girls and boys by Students' t-Test. Furthermore, a discriminate function analysis [23,24] was used to determine whether circadian characteristics could reliably classify subjects on the basis of gender. In this study, circadian variables were treated as predictors. Univariate F-tests were then calculated in order to determine the importance of each independent variable in forming the discriminant functions. Examining the Wilk's Lambda values for each of the predictors determined how important the independent variable was to the discriminant function, with smaller values representing greater importance.

2.4.4. To compare wrist temperature patterns between children and adults

Circadian-related variables obtained from 24 h rhythms of wrist temperature and the daily pattern were compared between children and adults by Student's t-Test.

All statistical analyses were performed using SPSS version 20.0 (SPSS, Chicago, Illinois, USA). Values of $P < 0.05$ were considered to be statistically significant.

3. Results

3.1. Participation rate and percentage of achievement of the new protocol

The characteristics of the population are described in Table 1 and the participation rate for each school is presented in Supplemental Table 1. Urban schools had the highest participation rates (urban 65–95% *vs* rural 41%). Complete 7-day recordings of wrist temperature, physical activity and body position ranged from 96 to 100%. The cortisol samples at 14:00 h were the easiest to obtain (92%), while we had more difficulties in collecting the melatonin samples at 1:00 h (72%). Completion rates of self-reported questionnaires were highest for MCTQ (97%) and lowest for the 7-day food record (92%) (Supplemental Table 1).

3.2. Circadian score

Data obtained from each of the non-invasive techniques used were pooled in a factor analysis which identified five independent factors that explained 50% of total variance (Supplemental Tables 2 and 3). Most of the variables located in the first factor were derived from TAP 24 h rhythms, with CFI in the first position. The circadian score obtained ranged from 542 to 1555 (AU) in the total population studied.

3.3. Gender differences in circadian function

The circadian score calculated from factor analysis was significantly higher in girls than in boys (Table 2). TAP proved to be sensitive in detecting gender differences in circadian-related characteristics demonstrating better CFI in girls *vs* boys (Table 2).

Twenty-four hours TAP rhythms displayed higher values of interday stability, amplitude and relative amplitude, Rayleigh, and percentage of rhythmicity (see glossary) in girls than in boys (Table 2). Girls had significantly higher temperatures, especially between 00:20 h and 7:10 h ($P < 0.05$) (Fig. 1A) and had a significant increase in M5 ($P < 0.05$). Girls also had significantly higher minimum temperature compared to boys ($P < 0.05$) (Table 2). Girls demonstrated greater activity (°/min) (average, mesor, M10), mainly in the evening hours, from 16:00 h to 21:30 h (Fig. 1B), lower fragmentation of rhythm (as assessed by intraday variability) and better circadian function (CFI) than boys ($P < 0.05$) (Table 2).

Girls had significant higher cortisol morning/night ratio than boys ($P < 0.05$) (Table 2). Nevertheless, girls had a later breakfast and presented a more evening-type chronotype than boys with a later midpoint of sleep (15 min later) as determined by the MCTQ punctuation ($P = 0.003$). In addition, social jet lag was higher in girls (0:54) than in boys (0:44) (hh:mm) ($P = 0.008$). Discriminate function analysis showed that 77% of the children studied were correctly classified by gender using the following measures: TAP amplitude, two activity-derived variables (average and intraday variability) and cortisol morning/night ratio.

3.4. Children demonstrated a different temperature pattern and better circadian function than adults

Wrist temperature curve exhibited a biphasic pattern of sleep-time rhythm in children that was not present in adults (Fig. 1E). Children showed significantly higher values than adults ($P < 0.05$) for the following temperature rhythm parameters: a) amplitude; b)

Table 2
Differences between girls and boys in circadian-related variables.

	Total	SD	Boys	SD	Girls	SD	*P-value*
	N = 248		N = 125		N = 123		
Circadian variables							
Circadian Score	1188	165	1159	173	1216	153	**0.012**
TAP							
Average (AU)	0.43	0.03	0.43	0.04	0.44	0.02	**0.001**
Mesor (AU)	0.43	0.03	0.43	0.04	0.44	0.02	**0.001**
IS (AU)	0.59	0.10	0.58	0.11	0.61	0.09	**0.012**
Amplitude (AU)	0.26	0.04	0.25	0.04	0.27	0.04	**<0.001**
Rayleigh (AU)	0.89	0.19	0.86	0.22	0.91	0.15	**0.023**
PR	42	10	40	11	4	9	**0.002**
M10 (AU)	0.62	0.04	0.60	0.04	0.63	0.03	**<0.001**
RA (AU)	0.59	0.09	0.58	0.09	0.61	0.08	**0.044**
CFI	0.66	0.06	0.65	0.07	0.67	0.06	**0.011**
Temperature							
Minimum (°C)	30.14	0.84	30.02	0.75	30.26	0.90	**0.028**
M5 (°C)	34.44	0.85	34.32	0.84	34.57	0.84	**0.021**
Activity							
Average (°/min)	47.27	5.96	46.07	6.09	48.45	5.62	**0.002**
Mesor (°/min)	47.32	5.97	46.15	6.07	48.48	5.66	**0.002**
Amplitude (°/min)	39.30	6.20	37.93	6.46	40.67	5.63	**0.001**
IV	0.86	0.06	0.87	0.06	0.85	0.06	**0.007**
PR	22.05	5.12	20.90	5.17	23.18	4.84	**<0.001**
M10 (°/min)	75.25	9.62	73.12	9.92	77.36	8.87	**0.001**
RA (°/min)	0.85	0.44	0.84	0.04	0.86	0.04	**0.001**
CFI	0.60	0.04	0.59	0.04	0.61	0.04	**0.003**
Position							
IV	0.93	0.18	0.88	0.18	0.97	0.16	**0.001**
TM10 (h)	16:26	2:28	16:06	2:37	16:45	2:15	**0.040**
CFI	0.35	0.06	0.36	0.06	0.34	0.06	**0.022**
Cortisol concentrations **(nmol/l)**							
09:00 h	14.22	6.17	13.92	6.51	14.52	5.84	0.458
14:00 h	7.16	3.70	7.37	3.24	6.97	4.10	0.396
23:00 h	4.20	3.09	4.41	2.93	3.99	3.24	0.298
Morning-night ratio*	0.91	0.31	0.87	0.34	0.95	0.27	**0.033**
Morning/night ratio*	0.66	0.46	0.59	0.42	0.73	0.49	**0.025**
Melatonin concentrations **(pg/ml)**							
14:00 h	7.97	7.25	8.22	8.33	7.72	6.02	0.593
1:00 h	30.11	22.90	31.44	26.70	28.80	18.50	0.374
Night-day ratio*	1.22	0.40	1.22	0.40	1.22	0.38	0.981
Night/day ratio*	0.58	0.31	0.58	0.33	0.58	0.29	0.847
Dietary composition							
Energy (kcal)	2054	391	2135	381	1973	386	**0.002**
Proteins (g)	73	15	76	16	69	13	**0.001**
Carbohydrates (g)	223	58	231	57	214	58	**0.028**
Lipids (g)	101	23	104	27	98	18	0.058
Energy (kcal/kg)	56.43	16.92	57.96	16.62	54.90	17.14	0.176
Proteins (g/kg)	1.98	0.61	2.05	0.58	1.93	0.63	0.142
Carbohydrates (g/kg)	6.13	2.17	6.29	2.18	5.96	2.15	0.248
Lipids (g/kg)	2.78	0.90	2.83	0.96	2.73	0.84	0.432
Food timing (hh:mm)							
Breakfast time	8:30	0:24	8:26	0:24	8:34	0:24	**0.011**
Lunch time	14:28	0:19	14:27	0:19	14:29	0:19	0.367
Dinner time	21:03	0:31	21:03	0:31	21:04	0:31	0.721
Sleeping diary (hh:mm)							
Sleep bedtime	22:35	0:44	22:32	0:42	22:37	0:46	0.389
Sleep awake	8:10	0:24	8:08	0:22	8:12	0:27	0.273
Sleep duration	9:27	0:31	9:27	0:38	9:26	0:43	0.731
MCTQ (hh:mm)	3:58	0:39	3:50	0:36	4:05	0:40	**0.003**
Social Jet Lag (hh:mm)	0:49	0:28	0:44	0:28	0:54	0:28	**0.008**

Data were expressed as mean ± SD (SD: standard deviation), *(log). Arbitrary Units: AU; IS: interday stability; IV: intraday variability; PR: percentage of rhythmicity; M5: average of the five consecutive hours of maximum values and its timing (TM5); M10: average of the ten consecutive hours of maximum values and its timing (TM10); RA: relative amplitude; and CFI: circadian function index; MCTQ: Munich Chronotype Questionnaire. The bold values represents significant differences ($P < 0.05$).

percentage of rhythmicity; and c) CFI (Table 3). Lower values in children were obtained for fragmentation of the rhythm ($P < 0.05$). Adults showed a later acrophase, that was delayed ~ 1 h ($P < 0.05$) (Table 3).

4. Discussion

The purpose of the study was to develop a protocol to assess circadian function in school-aged children by combining the best non-invasive tools previously validated in adults. A new circadian score was developed to obtain a chronobiological characterization of school-aged children. Among circadian-related measures, the integrative variable TAP was most suitable and reliable to determine circadian system characteristics in school-age children. In general, circadian function was better in girls than in boys, and also in school-aged children than in adults from the same Mediterranean area.

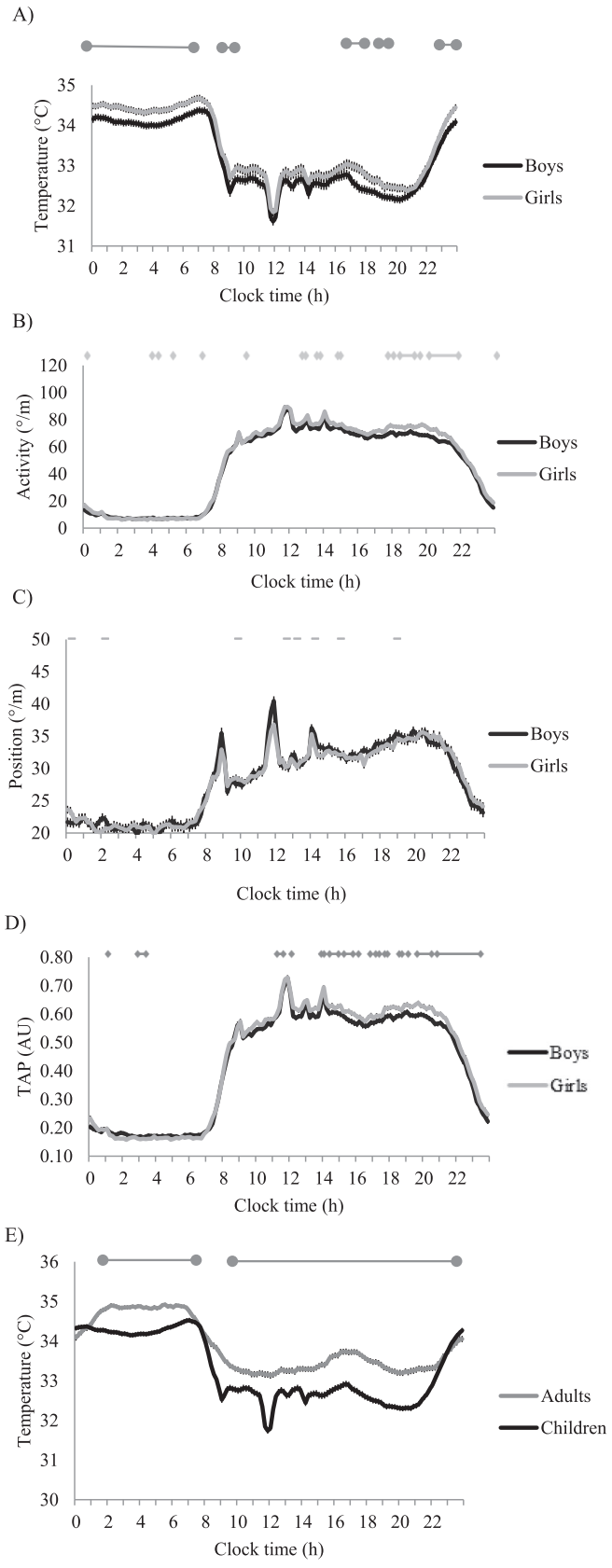

Fig. 1. Daily patterns of temperature, activity, position and TAP in children and adults. Each curve represents the wrist temperature's daily patterns recorded over a seven-day period. A–D, represent the differences between boys and girls. E, depicts the differences between children and adults. The upper line represents the hours at which the pattern differs significantly ($P < 0.05$).

Table 3
Significant differences between school-aged children and adult population in temperature circadian-related variables.

	Adults	SD	Children	SD	*P-value*
	N = 177		N = 248		
Temperature Circadian variables					
Mesor (°C)	33.70	0.71	33.25	0.94	0.001
Amplitude (°C)	0.93	0.50	1.09	0.55	0.002
Acrophase (hh:mm)	4:54	4:51	3:48	2:49	0.004
PR	19.28	12.58	23.30	15.43	0.004
IV	0.20	0.09	0.10	0.04	0.001
CFI	0.45	0.06	0.47	0.06	0.001

All indexes are expressed as mean ± SD (SD: standard deviation). PR: Percentage of rhythmicity; IV: Intraday variability; and CFI: Circadian function index.

We have constructed the first and most complete population of school-aged children to assess circadian function (ONTIME-Jr) which includes circadian-related data obtained by multiple circadian tools such as self-reported diaries and chronotype questionnaires, salivary samples, two different wrist sensors to test the integrative variable TAP [2,16] and a luxmeter as a pendant around the neck. All these tests were non-invasive and have been designed to assess circadian function without affecting normal living condition. Previous studies in the assessment of circadian function were mainly performed in adults and were limited by the number of circadian markers, e.g. chronotype through questionnaires [17,25], or salivary melatonin and cortisol levels, or sleep patterns [5], skin temperature [12,13] and activity rhythms through accelerometers [11], but to our knowledge all these non-invasive tools had never been used together in the same population of children.

Results from the present study show that the integrative variable TAP and the morning/night cortisol ratio best explained sample variability in circadian-related characteristics, as determined by factor analysis. Prior studies of adults suggest that TAP is more reliable and less subject to environmental artifacts than individual variables like temperature or activity separately [2].

Of the measures included in TAP, CFI was the first factor to explain variability. Circadian Function Index (CFI) summarizes regularity and fragmentation of rhythm, which provides information about the circadian system and facilitates objective evaluation of chronodisruption [2]. High CFI indicates more regular day-to-day rhythms, as demonstrated by a) higher interday stability; b) higher Rayleigh values, with more stable phase between days; and c) higher amplitude which shows an elevated difference between morning and evening values. Circadian Function Index was significantly higher in girls than boys suggesting that girls have better circadian function, a finding confirmed by the newly developed circadian score obtained from factor analysis that was also higher in girls than in boys. Compared with a control population of adults from the same Mediterranean area, data suggest better circadian function in school-aged children than in adults.

Another test used in the current protocol was salivary cortisol. Girls had a higher morning/night ratio than boys, indicating a normal diurnal curve [26]. Cortisol is a corticosteroid with a robust circadian profile. Previous studies performed in adults have demonstrated a similar decrease in amplitude of cortisol in men as compare to women [27], so these results could be inherent to sex. Nevertheless, we cannot discard the hypotheses that these lower amplitude in boys compared to girls, could be related to circadian impairment [26]. In adults, diminished daily amplitude in the cortisol pattern has been associated with central obesity (characteristic of men), metabolic disturbances in blood pressure, and regulation of plasma glucose and lipids [26,28]. Further studies will help to elucidate if cortisol morning/night ratio associates with metabolic disturbances in children.

The level of physical activity, and the fragmentation of activity rhythm, which is the frequency of changes between high and low activity, showed differences between girls and boys. Girls were more active than boys as demonstrated by the significantly higher values obtained in activity average, mesor and M10. In addition, girls showed a less fragmented activity rhythm than boys, with lower intraday variability values. Previous authors suggest that fragmentation of rhythm is a health indicator in adults [29,30]. High fragmentation of activity rhythms is related to mortality risk, cardiovascular disease, cognitive impairment, depression symptoms, and obesity [29,30]. Fragmentation of activity rhythm has also been related to obesity and central adiposity in adolescents [11].

Eveningness has been defined as the characteristic of being most active and alert during the evening, and it is considered as a trait of puberty. Adolescents experience a delay in circadian clocks that causes them to sleep and wake later than younger children [11]. In the current study girls had a higher tendency to eveningness than boys. Previous studies suggest that girls achieve eveningness at an earlier age than boys, although at this age chronotype is not yet fully defined in boys [31].

4.1. Differences between children and adults

The present study suggests that wrist temperature rhythm differs between early and later stages of life with a better circadian function in children than in adults from the same Mediterranean area. School-aged children exhibited a biphasic pattern in temperature rhythm during night sleep while adults had a monophasic pattern [32]. Significantly longer sleep episodes in children compared to adults, could explain the differences [33]. Similar biphasic patterns have been previously shown in adults who expanded their sleep episodes from 7 to 11 h *per* day [33].

A comparison of circadian-related measures from 7-day records of wrist temperature rhythm between children and adults demonstrated less fragmented rhythms and higher amplitude and percentage of rhythm in children than in adults. Decreased amplitude has previously been related to aging [34]. Circadian Function Index was also higher in children than adults, which suggests a better circadian rhythm. Our data agree with previous studies that indicate that aging is associated with progressive deterioration of circadian rhythms [35,36].

Results from the present study suggest that the test protocol and the newly developed circadian score can assess circadian function in children. Nevertheless, other physiological factors may be implicated in the differences found in the circadian-related variables, further studies are needed to determine whether childhood pathologies, such as overweight and obesity, are linked to this circadian score and if school and physical activity schedules and artificial light exposure are linked with circadian rhythm alterations.

From the current work, several recommendations could be suggested for future studies in similar children populations:

a) To use a more secure and waterproof design of wrist watches. In the current study sensors were placed in specially designed wrist watches to be worn safely and comfortably. Nevertheless, some of the temperature devices were detached from abrupt movements while playing (4%) and some accelerometers (3%) failed because of water vapor from sweat entering the device.
b) Replace the 7-day food record with a simpler test e.g. a smartphone application.
c) Further studies should investigate the specific role of the melatonin ratio in the circadian system function in children. In the current study, melatonin was not able to capture circadian variability or to assess differences between girls and boys, as previously described [5]. However, this could be due to the small successful collection rate. Indeed, melatonin sampling at 01:00 h was difficult to obtain and in several cases insufficient quantity of saliva was collected (28%). This finding needs to be explored in a bigger population, with a higher number of samples.
d) Because we decided to analyze the lux parameter only when the study was already started, only 20 children were included in the analyses of light. In order to make any conclusion about the validity of this measurement, it is necessary to expand these analyses.

In conclusion, we have developed a new circadian score using non-invasive measurements, including TAP, which seems to be reliable to assess circadian system in school-aged children under normal living conditions. Children had better circadian function than adults. Furthermore girls seem to have a better circadian function than boys. Further studies should deep in the origin of these sexual differences. Chronobiological tools should be included in a comprehensive approach to the paediatric patient.

Statement of authorship

Marta Garaulet: conceptualized and designed the study and drafted the initial manuscript. Gloria Maria Barraco and Nuria Martínez-Lozano: performed the data and samples collection, contributed to draft the initial manuscript and critically reviewed the manuscript. Claudia Vales-Villamarín and Carmen Blaya: performed the data and samples collection. Juan Antonio Madrid and Rafael Rios: critically reviewed the manuscript for important intellectual content. Paul Fardy, contributed to write the manuscript. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Conflicts of interest

The authors have no conflicts of interest to disclose.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.clnu.2018.03.001.

References

[1] Ortiz-Tudela E, Martinez-Nicolas A, Diaz-Mardomingo C, Garcia-Herranz S, Pereda-Perez I, Valencia A, et al. The characterization of biological rhythms in mild cognitive impairment. BioMed Res Int 2014;2014:524971.
[2] Ortiz-Tudela E, Martinez-Nicolas A, Campos M, Rol MA, Madrid JA. A new integrated variable based on thermometry, actimetry and body position (TAP) to evaluate circadian system status in humans. PLoS Comput Biol 2010;6(11). e1000996.
[3] Lopez-Minguez J, Colodro-Conde L, Bandin C, Ordonana JR, Garaulet M, Madrid JA. Application of multiparametric procedures for assessing the heritability of circadian health. Chronobiol Int 2016;33(2):234–44.
[4] Bandin C, Martinez-Nicolas A, Ordovas JM, Ros Lucas JA, Castell P, Silvente T, et al. Differences in circadian rhythmicity in CLOCK 3111T/C genetic variants in moderate obese women as assessed by thermometry, actimetry and body position. Int J Obes (Lond) 2013;37(8):1044–50.

[5] Carissimi A, Martins AC, Dresch F, da Silva LC, Zeni CP, Hidalgo MP. School start time influences melatonin and cortisol levels in children and adolescents – a community-based study. Chronobiol Int 2016;33(10):1400–9.
[6] Carissimi A, Dresch F, Martins AC, Levandovski RM, Adan A, Natale V, et al. The influence of school time on sleep patterns of children and adolescents. Sleep Med 2016;19:33–9.
[7] School start times for adolescents. Pediatrics 2014;134(3):642–9.
[8] Lopez-Minguez J, Gomez-Abellan P, Garaulet M. Circadian rhythms, food timing and obesity. Proc Nutr Soc 2016;75(4):501–11.
[9] Garaulet M, Ordovas JM, Madrid JA. The chronobiology, etiology and pathophysiology of obesity. Int J Obes (Lond) 2010;34(12):1667–83.
[10] Garaulet M, Madrid JA. Chronobiology, genetics and metabolic syndrome. Curr Opin Lipidol 2009;20(2):127–34.
[11] Garaulet M, Martinez-Nicolas A, Ruiz JR, Konstabel K, Labayen I, Gonzalez-Gross M, et al. Fragmentation of daily rhythms associates with obesity and cardiorespiratory fitness in adolescents: the HELENA study. Clin Nutr 2016;36(6):1558–66.
[12] Pronina TS, Orlova NI, Rybakov VP. Circadian rhythm of skin temperature of children during puberty. Fiziologiia cheloveka 2015;41(2):74–84.
[13] Pronina TS, Rybakov BP. Features of the circadian rhythm of temperature of the skin at children of 8-9 years and young men and girls. Fiziologiia cheloveka 2011;37(4):98–104.
[14] Sarabia JA, Rol MA, Mendiola P, Madrid JA. Circadian rhythm of wrist temperature in normal-living subjects A candidate of new index of the circadian system. Physiol Behav 2008;95(4):570–80.
[15] Azevedo C, Sousa I, Paul K, MacLeish M, Mondejar M, Sarabia J, et al. Teaching chronobiology and sleep habits in school and university. Mind, Brain Educ 2008;2(1):34–47.
[16] Garaulet M, Madrid JA. Methods for monitoring the functional status of the circadian system in dietary surveys studies: application criteria and interpretation of results. Nutr Hosp: organo oficial de la Sociedad Espanola de Nutricion Parenteral y Enteral 2015;31(Suppl 3):279–89.
[17] Roenneberg T, Kuehnle T, Juda M, Kantermann T, Allebrandt K, Gordijn M, et al. Epidemiology of the human circadian clock. Sleep Med Rev 2007;11(6):429–38.
[18] Myers BL, Badia P. Changes in circadian rhythms and sleep quality with aging: mechanisms and interventions. Neurosci Biobehav Rev 1995;19(4):553–71.
[19] Martinez-Nicolas A, Ortiz-Tudela E, Madrid JA, Rol MA. Crosstalk between environmental light and internal time in humans. Chronobiol Int 2011;28(7):617–29.
[20] Campuzano A, Cambras T, Vilaplana J, Canal MM, Carulla M, Diez-Noguera A. Period length of the light-dark cycle influences the growth rate and food intake in mice. Physiol Behav 1999;67(5):791–7.
[21] Van Someren EJ, Swaab DF, Colenda CC, Cohen W, McCall WV, Rosenquist PB. Bright light therapy: improved sensitivity to its effects on rest-activity rhythms in Alzheimer patients by application of nonparametric methods. Chronobiol Int 1999;16(4):505–18.
[22] Corbalan-Tutau MD, Gomez-Abellan P, Madrid JA, Canteras M, Ordovas JM, Garaulet M. Toward a chronobiological characterization of obesity and metabolic syndrome in clinical practice. Clin Nutr 2015;34(3):477–83.
[23] Klecka WR. Discriminant analysis. Quantitative applications in the social sciences series. CA: Sage Publications.; 1980.
[24] Ruiz-Lozano T, Vidal J, de Hollanda A, Canteras M, Garaulet M, Izquierdo-Pulido M. Evening chronotype associates with obesity in severely obese subjects: interaction with CLOCK 3111T/C. Int J Obes (Lond) 2016;40(10):1550–7.
[25] Horne JA, Ostberg O. A self-assessment questionnaire to determine morningness-eveningness in human circadian rhythms. Int J Chronobiol 1976;4(2):97–110.
[26] Garcia-Prieto MD, Tebar FJ, Nicolas F, Larque E, Zamora S, Garaulet M. Cortisol secretary pattern and glucocorticoid feedback sensitivity in women from a Mediterranean area: relationship with anthropometric characteristics, dietary intake and plasma fatty acid profile. Clin Endocrinol 2007;66(2):185–91.
[27] Larsson CA, Gullberg B, Rastam L, Lindblad U. Salivary cortisol differs with age and sex and shows inverse associations with WHR in Swedish women: a cross-sectional study. BMC Endocr Disord 2009;9:16.
[28] Corbalan-Tutau D, Madrid JA, Nicolas F, Garaulet M. Daily profile in two circadian markers "melatonin and cortisol" and associations with metabolic syndrome components. Physiol Behav 2014;123:231–5.
[29] Luik AI, Zuurbier LA, Hofman A, Van Someren EJ, Tiemeier H. Stability and fragmentation of the activity rhythm across the sleep-wake cycle: the importance of age, lifestyle, and mental health. Chronobiol Int 2013;30(10):1223–30.
[30] Oosterman JM, van Someren EJ, Vogels RL, Van Harten B, Scherder EJ. Fragmentation of the rest-activity rhythm correlates with age-related cognitive deficits. J Sleep Res 2009;18(1):129–35.
[31] Tonetti L, Fabbri M, Natale V. Sex difference in sleep-time preference and sleep need: a cross-sectional survey among Italian pre-adolescents, adolescents, and adults. Chronobiol Int 2008;25(5):745–59.
[32] Rosenwasser AM. Functional neuroanatomy of sleep and circadian rhythms. Brain Res Rev 2009;61(2):281–306.
[33] Wehr TA. In short photoperiods, human sleep is biphasic. J Sleep Res 1992;1(2):103–7.
[34] Dijk DJ, Duffy JF, Czeisler CA. Contribution of circadian physiology and sleep homeostasis to age-related changes in human sleep. Chronobiol Int 2000;17(3):285–311.
[35] Batinga H, Martinez-Nicolas A, Zornoza-Moreno M, Sanchez-Solis M, Larque E, Mondejar MT, et al. Ontogeny and aging of the distal skin temperature rhythm in humans. Age (Dordr) 2015;37(2):29.
[36] Turek FW, Penev P, Zhang Y, van Reeth O, Zee P. Effects of age on the circadian system. Neurosci Biobehav Rev 1995;19(1):53–8.