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Title: Fragmentation of daily rhythms associates with obesity and cardiorespiratory fitness in adolescents: the HELENA study

Authors: Marta Garaulet[1], Antonio Martinez-Nicolas[1], Jonatan R Ruiz[2,3], Kenn Konstabel[4], Idoia Labayen[5], Marcela González-Gross[6], Ascensión Marcos[7], Dénes Molnar[8], Kurt Widhalm[9], Jose Antonio Casajús[10a,b], Stefaan De Henauw[11], Anthony Kafatos[12], Christina Breidenassel[13], Michael Sjöström[3], Manuel J Castillo[14], Luis A Moreno [10a,c,15], Juan A Madrid[1], Francisco B Ortega[2,3], on behalf of the HELENA study group*.

* The authors of this paper take sole responsibility for its content. See Supplemental Material for a complete list of the HELENA study group members.

Affiliations

[1] Chronobiology laboratory, Department of Physiology, University of Murcia and Research Biomedical Institute of Murcia (IMIB), Murcia, Spain.

[2] PROFITH "PROmoting FITness and Health through physical activity" research group, Department of Physical Education and Sports, Faculty of Sport Sciences, University of Granada, Spain.

[3] Department of Biosciences and Nutrition, Karolinska Institutet, Huddinge, Sweden.

[4] Department of Chronic Diseases, Centre of Health and Behavioral Sciences, National Institute for Health Development, Tallinn, Estonia.

[5] Department of Nutrition and Food Science, University of the Basque Country, Vitoria, Spain.

[6] Department of Health and Human Performance, Facultad de Ciencias de la Actividad Física y del Deporte, Universidad Politécnica de Madrid, Madrid, Spain.

[7] Immunonutrition Research Group, Department of Metabolism and Nutrition, Instituto del Frio, Institute of Food Science, Technology and Nutrition (ICTAN), Spanish National Research Council (CSIC), Madrid, Spain.

[8] Department of Paediatrics, Medical Faculty, University of Pécs. Jzsef A, Pécs, Hungary.

[9] Division of Clinical Nutrition and Prevention, Department of Pediatrics, Medical University of Vienna, Vienna, Austria.

[10] [a]GENUD "Growth, Exercise, NUtrition and Development" Research Group, Universidad de Zaragoza, Spain. [b]Faculty of Health and Sport Science (FCSD), Department of Physiotherapy and Nursing, Universidad de Zaragoza, Huesca, Spain. [c]Faculty of Health Science (FCS), Department of Physiotherapy and Nursing, Universidad de Zaragoza, Zaragoza, Spain.

[11] Department of Public Health, Ghent University, Ghent, Belgium.

[12] Preventive Medicine and Nutrition Unit, University of Crete, School of Medicine, Heraklion, Crete, Greece.

[13] Institut für Ernährungs- und Lebensmittelwissenschaften— Humanernährung, Rheinische Friedrich-Wilhelms Universität, Bonn, Germany.

[14] Department of Medical Physiology, Faculty of Medicine, University of Granada, Granada, Spain.

[15] Visiting Professor, Department of Preventive Medicine, Faculty of Medicine, University of Sao Paulo, Brazil.

Corresponding author: Marta Garaulet. Department of Physiology. Faculty. University of Murcia. Campus de Espinardo, s/n. 30100. Murcia, Spain. Phone: +34 868 88 39 30. Fax: +34 868 88 39 63. E-mail: garaulet@um.es.

Key terms: Daily, Rest-activity rhythms, Obesity, Cardiorespiratory fitness, Adolescents

ABSTRACT

Background and aims: Chronobiology studies periodic changes in living organisms and it has been proposed as a promising approach to investigate obesity. We analyze the association of the characteristics of the rest-activity rhythms with obesity, cardiorespiratory fitness and metabolic risk in adolescents from nine European countries.

Methods: 1044 adolescents (12.5 to 17.5 y) were studied. Circadian health was evaluated by actigraphy with accelerometers (Actigraph GT1M). Characteristics of the daytime activity such as fragmentation (intradaily variability), estimated acrophase, and 10 h mean daytime activity index were obtained. Body composition was assessed using Bioelectrical-Impedance-Analysis, skinfold thickness, air-displacement-plethysmography and Dual-energy-X-ray-Absorptiometry. Cardiorespiratory fitness (VO_{2max}) and metabolic risk were studied.

Results: Highly fragmented activity rhythms were associated with obesity and central adiposity ($P<0.05$). Obese adolescents had ~3 times higher odds of having a high fragmentation of daytime activity compared to normal weight adolescents OR (95% CI) = 2.8 (1.170, 6.443). A highly fragmented rhythm was also related to lower cardiorespiratory fitness and higher metabolic risk ($P<0.05$) so those adolescents classified as low fitness showed a significantly higher fragmentation of daytime activity than those included in the high fitness group ($P<0.0001$). Other characteristics of the rhythms such as smaller 10 h daytime mean activity index and delayed estimated acrophase were also related to obesity and metabolic risk ($P<0.05$).

Conclusions: Our results indicate that the daily organization of the rest-activity cycle is more fragmented in obese and less fit adolescents and correlates with higher metabolic

risk. This fact reinforces our hypothesis that disturbances in daily rhythms can be considered as sensitive markers of poorer adolescent's health.

INTRODUCTION

Chronobiology, the science that studies periodic changes in living organisms, has been recently proposed as a new and promising approach to investigate obesity and related metabolic disorders [1]. Alterations of the circadian system may contribute to obesity and its complication [2, 3].

Previous authors have indicated that the fragmentation of the rhythm, which gives an indication of the frequency of changes between high and low activity, may be a health indicator in adults [4]. Indeed, a high fragmentation of the activity rhythms has been related to mortality risk, and to cerebral alterations, cardiovascular disease, cognitive impairment, depressive symptoms, sleepiness, aging, and obesity [4, 5]. The precise mechanisms linking obesity and metabolic risk to the disruption of the circadian system (chronodisruption (CD)) are not well understood. Studies have suggested that scheduled physical activity (PA) can alter circadian rhythms acting as an "input" of the circadian timing system [6]. Studies examining the association of circadian system health and obesity are still scarce [7] and, to our knowledge, there has been no study undertaken to assess the impact of CD on obesity and metabolic risk in adolescents.

Another relevant factor closely related to obesity and metabolic alterations is physical fitness, particularly cardiorespiratory fitness [8]. It has been proposed as a powerful marker of health status in adolescence [9] and later in life [10]. Cardiorespiratory fitness already at adolescence predicts myocardial infarction later in life [11], supporting the notion of cardiorespiratory fitness as an early cardiovascular risk factor. Likewise, cardiorespiratory fitness has been considered as an additional

component of the metabolic syndrome and included in metabolic risk score calculations [12].

PA may be also understood as an "output" of the circadian system machinery. Indeed, one of the approaches to assess the circadian system health consists of measuring daily changes patterns from resting to activity. Actigraphy is considered the method of choice for evaluating circadian disorders such as delayed- or advanced-sleep-phase-syndrome, free-running-syndrome and irregular circadian rhythms [13] taking into account that it can be recorded in free-living conditions and during several consecutive days [14].

The aim of the current study is to analyze the association of the characteristics of the daytime rest-activity rhythms with obesity, cardiorespiratory fitness and metabolic risk in European adolescents from nine different countries.

MATERIALS/SUBJECTS AND METHODS

Participants

A total of 3,528 adolescents aged 12.5 to 17.5 years participated in the study from 10 European cities: Athens and Heraklion in Greece, Dortmund in Germany, Gent in Belgium, Lille in France, Pecs in Hungary, Rome in Italy, Stockholm in Sweden, Vienna in Austria, and Zaragoza in Spain. Data collection took place between 2006 and 2007. The general HELENA inclusion criteria were: to speak the language of the participating country, not to be involved in another clinical trial, not to have any acute infection when inclusion and to have valid data for age, sex and body mass index (BMI) [15]. In addition, for the purpose of the present study, only those participants with valid accelerometer data and a large registration period (see description of actigraphy below) were included in this study, i.e. N=1044.

Ethics

As previously reported (Ref), all the parents/guardians signed a consent form and all the adolescents gave their assent to participate in the study. The study was undertaken following the ethical guidelines of the Declaration of Helsinki 1964 (revised Edinburgh 2000) and the current specific legislation concerning clinical research in humans in each of the participating countries. The protocol was approved by human research review committees at the centers involved.

Actigraphy

Adolescents were asked to wear an accelerometer (GT1M Actigraph, USA) for 7 consecutive days during waking hours except when engaged in water-based activities or activities with major risk for harm caused by accelerometer. The time sampling interval (epoch) was set at 15 seconds. We excluded from the analysis bouts of 20 continuous minutes of activity with intensity counts of 0, considering these periods to be non-wearing time. Monitor wearing time was calculated by subtracting non- wear time from the total registered time for the day. A recording of more than 20,000 counts/min, was considered a potential malfunction of the accelerometer, and the value was excluded from the analyses. Moderate-to-vigorous PA (MVPA) was defined using the cut-off point of ≥2000 counts/min [12, 16]. Average PA was computed as the total number of counts divided by total wearing time in minutes and expressed as counts/min.

In previous articles from the HELENA study [16], at least 3 days of recording with a minimum of 8h of registration *per* day were necessary for the adolescent to be included in the study. However, for the purpose of the present study we needed longer registration time in order to study rhythms in days (waking hours) as complete as possible. Consequently, we set a specific inclusion criterion for accelerometer compliance, which was to have a minimum of 12h per day and to have a minimum of 2

147 weekdays plus 1 weekend day. Compliance was high and the participants had a median
148 value of 5 days and 14h of valid register time.

149 *Description of the circadian-related variables obtained*

150 We calculated non-parametric indexes described by Van Someren *et al*. [17] to
151 characterize activity patterns.

152 a) *Fragmentation of daytime activity, measured by* Intradaily Variability (IV), which
153 gives an indication of the frequency of changes between high and low activity. Its
154 values oscillated between 0, when the wave was perfectly sinusoidal, and 2, when
155 the wave was as Gaussian noise.
156 b) *10h mean daytime activity index (M10),* computed as the total number of counts
157 during the 10h of maximum daytime activity divided by registered time during this
158 period (mean of 10 hours).
159 c) *Estimated acrophase* (TM10), which refers to the time of maximum activity in the
160 daily rhythm.

161 *Anthropometry and body composition*

162 Height (m), weight (kg), waist circumference (cm) and 6 skinfold thicknesses
163 (mm, tri- ceps, biceps, subscapular, suprailiac, thigh, and calf) were measured, and BMI
164 and waist-to-height ratio were computed. In addition, adolescents were classified
165 according to the sex-and-age specific BMI cut-points proposed by the International
166 Obesity Task Force [18]. Four methods were used to assess total adiposity: 1) skinfold
167 thicknesses according to the equation proposed by Slaugther [19] which has shown to
168 be the most accurate in adolescents; 2) Bioelectrical Impedance Analysis (BIA), 3) air-
169 displacement plethysmography (ADP); and 4) Dual-energy X-ray Absorptiometry
170 (DXA) as described elsewhere. Fat mass index (FMI, calculated as fat mass (kg)
171 divided by the height (m^2)) was used in the analyses as an indicator of total adiposity,

since it has been proposed as a better indicator of total adiposity than percent body fat. Skinfold thickness and BIA were measured in the whole sample, while ADP and DXA only in adolescents from Zaragoza, Spain. Central or abdominal adiposity was estimated by waist and waist-to-height and truncal and abdominal adiposity by DXA.

For certain analyses, adolescents were classified according to the sex-and-age specific BMI cut-points proposed by the International Obesity Task Force [18, 20] that corresponds to Underweight (UW)<18.5 in adults; Normoweight (NW): 18.5 to 25; Overweight (OW): 25-30 and Obese (OB) >30 in adults.

Cardiorespiratory fitness

Detailed information on fitness protocols have been published elsewhere [21]. As previously reported [22], cardiorespiratory fitness was assessed by the 20 m shuttle-run test [23] and maximum oxygen consumption (VO_{2max}, $mL \cdot kg^{-1} \cdot min^{-1}$) was estimated using the equation reported by Léger *et al*. [23]. Low fitness was defined according to the cut-points proposed by the FITNESSGRAM [24].

Metabolic risk factors

We computed two well established cardiometabolic risk scores as proposed by Andersen *et al*. [12] and by Martínez-Vizcaino *et al*. [25]. As previously reported [22], the cardiometabolic risk score proposed by Andersen and colleagues is an average value computed from the sex- and age-specific z-scores of sum of four skinfolds, homoeostasis model assessment (HOMA index), systolic blood pressure, triglyceride, total cholesterol/high density lipoprotein cholesterol (HDLc) and cardiorespiratory fitness (VO_{2max}; this score was inverted multiplying by -1) [12]; and the score proposed by Martínez-Vizcaino and colleagues is an average value computed from the sex- and

196 age-specific z-scores of waist circumference, fasting insulin, triglyceride/HDLc and
197 mean arterial pressure [25].

198 *Socio economic factors*

199 As previously reported [22] we assessed the socioeconomic factors by using: a)
200 the family-affluence scale (FAS) which describes family consumption based on 4 items:
201 own bedroom, number of cars in the family, number of PCs in the home and internet
202 access; and b) maternal education which categorizes as university and non-university
203 level.

204 *Statistical methods*

205 General characteristics of the population were expressed by mean ± SD.
206 Significant differences between sexes were analyzed by student's t test. Partial
207 correlation analyses, ANCOVA analyses and logistic regression analyses were also
208 performed to assess the association of fragmentation (IV) with obesity (total and central
209 adiposity), fitness, and metabolic risk. Analyses were performed after controlling for
210 age, sex and socioeconomic factors.

211 Additional adjustment for registration time did not alter substantially the results.
212 Associations with estimated acrophase were adjusted for average PA (counts per
213 minute). Analyses with M10 and IV score were not adjusted by PA level because these
214 parameters were included in the equations ($IV = \frac{n\sum_{i=2}^{n}(x_i - x_{i-1})^2}{(n-1)\sum_{i=1}^{n}(x_i - \bar{x})^2}$ where n is the total
215 sample size, $\bar{x}$ is the mean activity level and x_i represents each individual activity
216 value). Males and females were studied together because no interaction was found with
217 gender (P>0.1)

RESULTS

The different characteristics of the subjects are shown in Table 1. Boys showed more robust rhythms than girls, with higher 10h mean daytime activity-index (M10) and lower fragmentation. However, estimated acrophase (the time of maximum activity) was delayed in boys as compared to girls.

The proportions of obesity in the total population was ~5%, being higher among boys than girls ($P<0.05$). Total and abdominal adiposity showed significant differences towards higher values in girls than boys. Parameters of metabolic risk such as total triglycerides, cholesterol, and blood pressure were also found to be significantly higher in girls than boys (Table 1).

Nevertheless, PA parameters and cardiorespiratory fitness were significantly higher among boys. Individual rhythms representing a comparison between high (Figure 1A) and low fragmentation are shown in Figure 1(Figure 1B). Examples of individual high and low estimated acrophases are represented in Figure 2 (Figures 2A and 2B) and high and low 10h mean daytime activity index (M10) rhythms in Figures 2C and 2D).

When analysis of correlations was performed between the fragmentation of daytime activity (IV) and obesity parameters (Table 2), the results indicated that there was a positive and significant correlation between both parameters towards a higher fragmentation of daytime activity rhythm with obesity as assessed by BMI, total adiposity (FMI measured by anthropometry, BIA, ADP, and DXA) and central adiposity (i.e. waist, waist-to-height ratio and truncal and abdominal adiposity as measured by DXA).

Interestingly, a higher fragmentation of daytime activity (IV) and a lower 10h mean daytime activity index (M10) were also related to lower cardiorespiratory fitness

and higher metabolic risk in this European adolescent population. The results showed no significant correlations for individual metabolic risk factors except for HDLc values, which were negatively correlated with IV and positively with M10 (Table 2).

Moreover, we observed negative and significant correlations between the M10 and BMI, and total and central adiposity variables (Table 2). Estimated acrophase (TM10) was positively associated with waist (P<0.05) and the same trend was found for Waist-to-Height Ratio and FMI (BIA). No significant correlations were found between estimated acrophase differences among week days and weekends and adiposity indicators (Data not shown).

When the population was classified by BMI categories in underweight (UW), normal weight (NW), overweight (OW) and obese (O), a significant association between fragmentation of daytime activity (IV) and obesity, was found (P=0.039). Post hoc analyses revealed that the main differences were found between obese adolescents and the other three groups (UW, NW, and OW) (Figure 3A). In the same line, after logistic regression analyses it was demonstrated that obese adolescents had 2.8 higher odds of having a high fragmentation of daytime activity compared to normal weight adolescents OR (95% CI)=2.8 (1.170, 6.443). Differences in fragmentation of daytime activity (IV) according to cardiorespiratory fitness levels are shown in Figure 3B. Adolescents classified as low fitness showed a significantly higher fragmentation of daytime activity than those included in the high fitness group (P<0.0001).

DISCUSSION

In the present study performed in adolescents from nine different European countries, more fragmented rhythms as assessed by actigraphy were associated with total and central adiposity determined by 4 different methods for analysis of body

composition, including DXA, ADP and BIA. Obese adolescents had ~3 times higher odds of having a high fragmentation of daytime activity as opposed to normal weight adolescents. Interestingly, a higher fragmentation was also related to lower cardiorespiratory fitness and higher metabolic risk in this European adolescent population. To a lesser extent, other characteristics of the rhythms such as smaller 10h mean daytime activity index and delayed estimated acrophase were also related to obesity and metabolic risk.

As far as we know, this study is the first to be performed in youth and our results obtained with the accelerometer support that fragmentation of daytime activity was related to obesity. Results are in agreement with a study performed previously in adult women in which obesity was related to a higher intradaily variability (IV), which suggests a greater fragmentation of the rest-activity rhythm as compared with their normal-weight counterparts [26]. Other studies performed in large adult populations have also found a positive association between obesity, metabolic risk and fragmentation of the rhythm [4]. Moreover, our group has demonstrated in previous studies that an increase in rhythm fragmentation with obesity and metabolic risk is related to a decrease in the amplitude of melatonin secretion: a biological sign of chronodisruption [26]. However, as a cross-sectional design, our correlational study does not allow for conclusions on causal directions between rest–activity rhythms and obesity. Moreover, the nature of the relation between the circadian fragmentation and obesity/metabolic risk is still unknown. Previous results from our group indicate that **the genetics** of our internal clock, particularly *CLOCK* 3111T/C, is related to the fragmentation of the rhythm independently to obesity [27]. Furthermore, our genetic studies using classical twin models, demonstrate that fragmentation of the rhythm is partly driven by genetic factors in a 53% [28]. In general, genetic studies start to reveal

certain genetic basis for the link between the circadian clock and obesity. For instance, in animal models, mice with *Clock* gene disruptions are prone to develop obesity [29]; and in humans, most of the identified genetic variants at *CLOCK* are associated with a higher BMI while several of them associated with obesity and/or metabolic syndrome [30-32]. On the other hand, recent findings in chronobiology reveal that **disrupting the normal behavioral (sleep-wake) cycles** can also cause metabolic dysfunction, contributing significantly to obesity [7]. For example, shift work, sleep deprivation and nighttime light exposure are associated with increased adiposity [3].

In the current European adolescent population, a higher fragmentation was also related to a lower **cardiorespiratory fitness.** Previous studies have observed that the effect produced by exercise on the circadian rhythm (input) depended on the level of physical fitness. Likewise, those individuals who presented a higher cardiorespiratory fitness displayed a marked circadian rhythm of left ventricular systole; on the contrary, in those individuals with lower cardiorespiratory fitness the circadian rhythm was not evident [33]. Cardiorespiratory fitness is already in children and adolescents one of the main markers of cardiovascular health. Our current data are agreement with previous studies performed in older men that have demonstrated that measures of decreased circadian activity rhythm robustness are associated with an increased risk of CVD events. Despite the fact that CVD events occur most frequently in the fifth decade of life, precursors of CVD have their origin in the years of childhood and adolescence [34].

Indeed, in the present population a higher fragmentation of the rhythm was related to a higher **metabolic risk score**. More specifically, from the total plasma lipids analyzed, HDL was the one significantly associated. The circadian system regulates metabolic activity in all tissues and organs. Many studies have found that circadian rhythm and metabolic risk influence one another and that the disruption of circadian

rhythms can increase the metabolic risk [7]. Some studies in rodents have shown that the deletion of the circadian system clock genes results in metabolic alterations [29]. In addition, recent evidence indicates that the molecular circadian clock located in peripheral tissues is affected by the time of exercise, which suggests that PA provides relevant information of timing for the synchronization of circadian clocks located in different tissues of our body. Indeed, the lack of rest-activity contrast can contribute to metabolic disturbances and lead to CD [7].

Apart from the fragmentation of daytime activity, other characteristics of the rhythms such as the **10h mean daytime activity index (M10)** were also related to obesity, cardiorespiratory fitness and metabolic risk in the studied population. VO_{2max} was associated with M10 indicating that a higher cardiorespiratory fitness level measured by VO_{2max} was related with higher M10. If we consider that adolescents had a hypothetical activity next to 0 during the nighttime, M10 would be indicative of the rhythm amplitude. Results are in agreements with Atkinson *et al*. [35] who proved that subjects with higher fitness levels had higher amplitudes in body temperature as opposed to those who presented lower fitness levels. Researchers have surmised that exercising routinely can stabilize the day-to-day daily habits of a subject, thereby providing them with a more consistent circadian rhythm. In the same line, in animal models, to exercise at the right time can enhance the amplitude of the rhythm of the SCN and it is also beneficial for other rhythmic functions controlled by the SCN [36]. Moreover, scheduled locomotor activity can entrain circadian behavior in circadian mutants [37] while it also operate the magnitude and phasing of the circadian-regulated outputs of heart rate and body temperature [38]. Although the precise nature of the exercise-circadian system feedback signal is unknown, it involves a variety of physiological systems [37].

The current results indicate that obesity, particularly central obesity, is related to a delayed estimated **acrophase** in rest-activity rhythms, which shows that those adolescents that performed PA at later hours along the day were more likely to be obese. Although weaker than those found with fragmentation, these results are interesting taking into account that late chronotypes in adolescence have been related to less healthy and more irregular lifestyles [39].

One of the **strengths** of the current study is that we analyzed rest-activity rhythms in a large adolescent population from 10 European cities. The number and variety of subjects increases the generalizability of the results. Another strength is the use of objective measurements to assess daily rest and activity cycles by using actigraphy, the method that has been chosen for the evaluation and diagnosis of circadian disorders. The fact that records have included only daytime measures may be considered as a limitation. However, one derived advantage is that the findings demonstrate that fragmentation of daytime activity during the day is *per se* associated with obesity, even without considering night time in the analyses, as has been previously suggested for mortality risk in adult populations. Nevertheless, future studies performed in adolescents will confirm or contrast our finding when recording 24h patterns (including day and night data). A third strength is that body fat was measured by four different methods and results are consistent throughout the several techniques included. Finally, we analyzed activity rhythms non-parametrically, in order to avoid assumptions about the underlying shape of their daily rhythm. In spite of these strengths, this study is also limited considering that all data were cross-sectional, which precludes studying the direction and causality of the associations. Further longitudinal and intervention studies should be also performed.

Some recommendations to improve the daily rhythms in the adolescents are: a) to be active for longer periods and in a more constant way during the day to decrease the fragmentation of the rhythms. The use of pedometer along the day to achieve at least 9000 steps/day may help to achieve this goal [40]; b) to exercise at the right time to enhance the amplitude of the rhythm of the SCN [36]. It has been described that to perform physical activity in the morning may maintain an optimal circadian system health [6]. By contrast, to perform physical activity during the evening (at 9 PM) is characterized by a lower amplitude and acrophase delay in peripheral body temperature a marker of circadian-health [6].

We conclude that the daily organization of the rest-activity cycle is more fragmented in obese adolescents. Low cardiorespiratory fitness and traditional metabolic risk factors were also associated with disturbances in the daily organization of the rest-activity cycle, suggesting that those who are more physically fit and metabolically healthier have a more robust activity rhythm. Future intervention studies should show if changing lifestyle in adolescent populations is effective in reducing circadian disturbances associated with obesity and fitness.

Declarations

List of abbreviations

PA: Physical Activity; IV: Intradaily variability; b) M10: 10h mean daytime activity index; TM10: Estimated acrophase; BMI: Body Mass Index; BIA: Bioelectrical Impedance Analysis; ADP: Air-Displacement Plethysmography; DXA: Dual-energy X-ray Absorptiometry.

Ethics approval and consent to participate

All the parents/guardians signed a consent form and all the adolescents gave their assent to participate in the study. The study was undertaken following the ethical guidelines of the Declaration of Helsinki 1964 (revised Edinburgh 2000) and the current specific legislation concerning clinical research in humans in each of the participating countries. The protocol was approved by human research review committees at the centers involved.

Consent for publication

This manuscript describes original work and is not under consideration by any other journal. All authors approved the manuscript and this submission. All authors have read and agree to the publication of the manuscript, and that the manuscript has not been submitted elsewhere.

Competing interests

The authors have no conflict of interest

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420

421 **Authors' Contributions**

422 "SDH, JAM and FBO designed research; MG, LAM, JAM and FBO conducted
423 research; IL, MGG, AM, DM, KW, JAC, SDH, AK, CB, MS and MJC contributed by
424 providing databases necessary for research; MG, AMN, JRR and KK analyzed data;
425 MG, AMN, JAM and FBO wrote the paper; MG and FBO had primary responsibility
426 for final content. All authors read and approved the final manuscript."

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TABLE LEGEND

Table 1. Characteristics of the study sample.

Table 2. Significant partial correlations of circadian markers with adiposity, cardiorespiratory and metabolic risk markers.

FIGURE LEGEND

Figure 1. Two examples of activity plots for high and low fragmentation (intradaily variability). Plots show 6 days activity rhythms of two study adolescents represented as counts of activity in 1 minute for high fragmentation (A) and low fragmentation (B).

Figure 2. Four examples of activity mean waveforms for late and early estimated acrophase and high and low 10h mean daytime activity index (M10). In the left part are represented late and early estimated acrophases measured by TM10 mean waveforms (A and B, respectively). Right section shows mean waveforms for high and low 10h mean daytime activity index (M10) (C and D, respectively).

Figure 3. Differences in fragmentation of daytime activity (Intradaily Variability) according to weight status (panel A) and to cardiorespiratory fitness levels (panel B). Adolescents were classified according to the sex-and-age specific BMI cut-points proposed by the International Obesity Task Force [18, 20] that corresponds to Underweight (UW)<18.5 in adults; Normoweight (NW): 18.5 to 25; Overweight (OW): 25-30 and Obese (OB) >30 in adults,

Table 2.

	Fragmentation (IV)		10h mean activity index (M10)		Estimated acrophase (TM10)*	
	r	*P*	r	*P*	r	*P*
Weight	.053	.108	**-.067**	**.040**	.045	.168
BMI	**.095**	**.004**	**-.088**	**.007**	.041	.212
Waist	**.073**	**.026**	**-.066**	**.045**	**.072**	**.028**
Waist to Height Ratio	**.097**	**.003**	**-.068**	**.038**	.062	.059
FMI (Skinfolds)	**.119**	**.000**	**-.129**	**.000**	.029	.383
FMI (BIA)	**.093**	**.005**	**-.100**	**.002**	.057	.083
Sum of 6 skinfolds	**.122**	**.000**	**-.153**	**.000**	.033	.310
FMI (BodPod)	**.200**	**.006**	**-.221**	**.002**	.061	.407
FMI (DXA)	**.155**	**.031**	**-.200**	**.005**	.090	.215
Trunk fat (DXA)	**.193**	**.007**	**-.254**	**.000**	.097	.180

Cardiorespiratory fitness (VO2max ml/kg/min)	**-.205**	**.000**	**.239**	**.000**	.003	.939
HDLc	**-.184**	**.001**	**.248**	**.000**	.045	.405
Metabolic risk score 1: Andersen	**.135**	**.030**	**-.180**	**.004**	-.024	.697
Metabolic risk score 2: Martinez-Vizcaino	.090	.101	**-.120**	**.029**	.043	.435

Controlled for age, sex and socioeconomic factors (Family-affluence scale and maternal education). Characters in bold represent significant correlations. The cardiometabolic risk score 1 is an average value computed from the sex- and age-specific z-scores of sum of four skinfolds, homoeostasis model assessment (HOMA index), systolic blood pressure, triglyceride, total cholesterol/HDL ratio and cardiorespiratory fitness (VO_{2max}; this score was inverted multiplying by -1); and the score 2 is an average value computed from the sex- and age-specific z-scores of waist circumference, fasting insulin, triglyceride/HDL cholesterol ratio and mean arterial pressure.

* This model was additionally controlled for average physical activity (counts per minute)

Table 1.

	All			Boys			Girls			
	N	Mean	SD	N	Mean	SD	N	Mean	SD	P
Age (y)	1044	14.5	1.2	511	14.6	1.2	533	14.5	1.2	0.220
Height (m)	1044	1.65	9.30	511	1.68	9.80	533	1.61	7.20	0.000
Obesity (n, %)	1044	51, 4.9		511.	31, 6.1		533.0	20, 2.8		0.194
Circadian variables										
10 h mean activity index (TM10)	1044	621.9	233.8	511	710.1	246.5	533	537.3	185.2	0.000
Fragmentation (IV)	1044	0.5	0.1	511	0.49	0.1	533	0.57	0.1	0.000
Estimated acrophase (h)	1044	14.6	2.5	511	14.8	2.4	533	14.5	2.5	0.021
Acrophase difference (week-ends – weekdays; h)	1044	1.0	2.9	511	1.1	2.9	511	1.0	2.9	0.909
Obesity parameters										
Weight (kg)	1044	57.6	12.1	511	60.4	13.4	533	54.9	10.0	0.000
BMI (kg/m^2)	1044	21.0	3.6	511	21.1	3.6	533	21.0	3.5	0.897
Waist (cm)	1035	71.5	8.4	508	73.5	8.7	527	69.6	7.6	0.000
Waist to Height Ratio	1035	0.43	0.05	508	0.44	0.05	527	0.43	0.05	0.170

Sum of 6 skinfolds (mm)	1000	87.0	37.9	490	75.0	37.3	510	98.6	34.6	0.000
FMI (Skinfolds)	1018	5.0	2.9	489	4.4	3.3	529	5.6	2.5	0.000
FMI (BIA)	1033	4.3	2.6	505	3.3	2.3	528	5.3	2.6	0.000
FMI (BodPod)	197	5.0	2.5	98	4.2	2.7	99	5.8	2.0	0.000
FMI (DXA)	200	5.3	2.2	113	4.5	2.1	87	6.3	1.8	0.000
Trunk fat (DXA)	200	20.7	7.7	113	17.3	6.9	87	25.1	6.4	0.000
Metabolic and Cardiovascular risk factors										
Glucose (Mmol/L)	360	5.1	0.4	169	5.2	0.4	191	5.0	0.4	0.000
Insulin (mIU/L)	351	10.3	7.2	165	10.1	8.5	186	10.5	6.0	0.530
Homoeostasis model assessment (HOMA index)	351	2.4	1.8	165	2.4	2.1	186	2.3	1.4	0.944
Triglycerides (TG, mmol/L)	360	0.78	0.39	169	0.71	0.35	191	0.83	0.41	0.003
High-density lipoprotein cholesterol (HDL, mmol/L)	360	1.45	0.27	169	1.41	0.25	191	1.48	0.28	0.008
Low-density lipoprotein cholesterol (LDL, mmol/L)	360	2.45	0.63	169	2.36	0.59	191	2.53	0.65	0.006
Total cholesterol (TC, mmol/L)	360	4.18	0.68	169	4.00	0.59	191	4.34	0.71	0.000
Systolic blood pressure (mmHg)	1029	119.0	13.0	501	122.9	13.5	528	115.4	11.3	0.000
Diastolic blood pressure (mmHg)	1029	67.6	8.8	501	67.0	8.9	528	68.2	8.6	0.032

Metabolic Scores										
Cardiometabolic risk (Andersen score)	272	-0.07	0.68	137	-0.15	0.67	135	0.005	0.68	0.066
Cardiometabolic risk score (Martinez score)	348	-0.03	0.68	164	-0.07	0.66	184	0.01	0.69	0.256
Physical Activity										
Valid time (min)	1044	866.9	80.4	511	873.6	84.6	533	860.5	75.7	0.009
Actimetry days recorded	1044	5.3	1.2	511	5.3	1.2	533	5.2	1.2	0.059
Average PA (counts per minute)	1044	419.8	137.4	511	469.8	149.0	533	371.8	104.9	0.000
Moderate Vigorous PA (minutes)	1044	63.1	24.6	511	71.7	25.8	533	54.9	20.2	0.000
Cardiorespiratory fitness (VO_{2max}, $mL \cdot kg^{-1} \cdot min^{-1}$)	873	42.9	7.5	434	46.9	6.9	439	39.1	5.8	0.000
Low fitness (VO_{2max}) (n, %)	873	243, 27.8		434	141, 32.5		439	102, 23.2		0.002
Socioeconomic status										
Maternal education (University level, n, %)	1000	368.0	36.8	489	191.0	39.1	511	177.0	34.6	0.350
Family Affluence Scale (Low/Middle/high, n, %)	1034	127/575/ 332	12.3/55.6/ 32.1	504	45/295/ 164	8.9/58.5/ 32.5	530	82/280/ 168	15.5/52.8/ 31.7	0.005

Figure 1

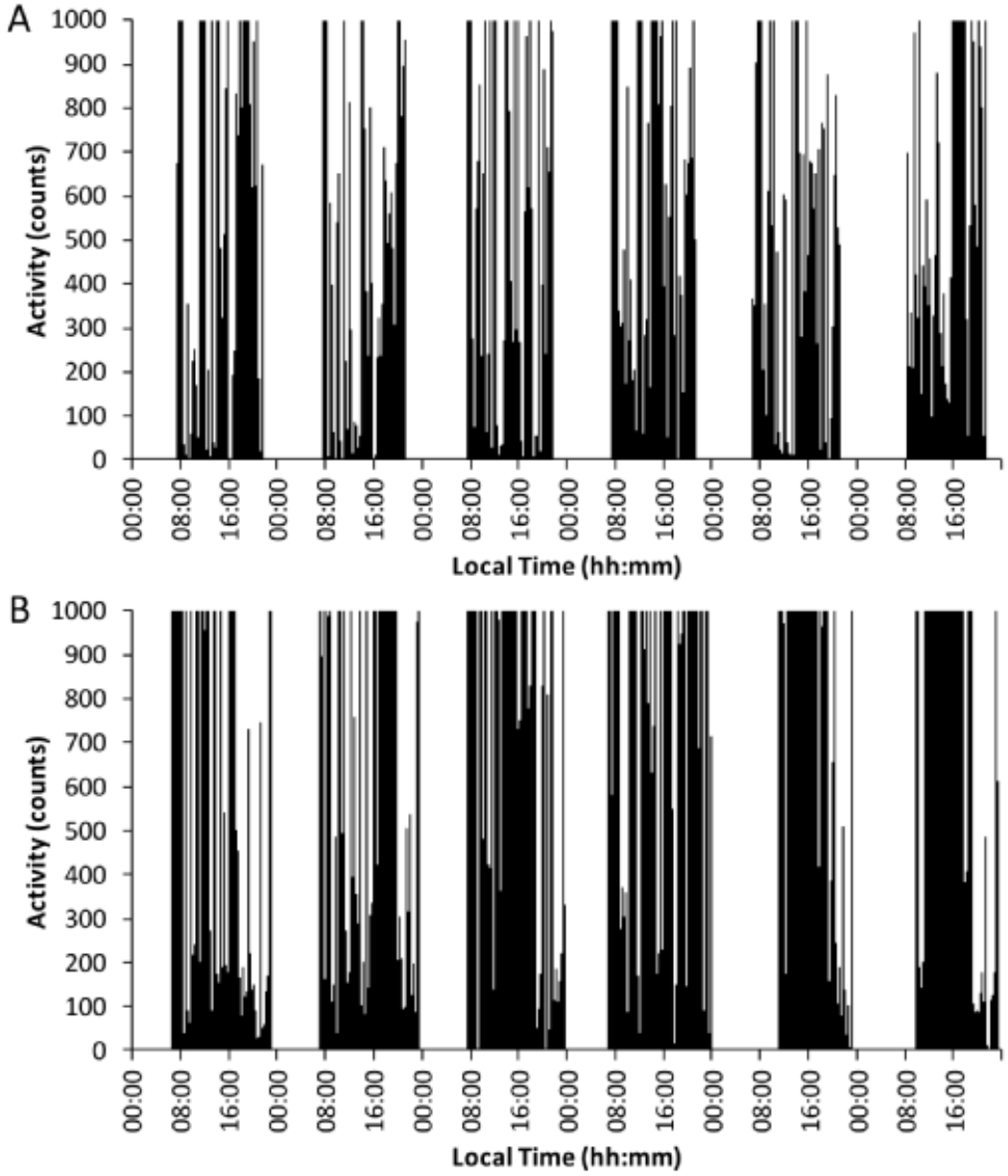

A
B
Activity (counts)
0
100
200
300
400
500
600
700
800
900
1000
00:00
08:00
16:00
Local Time (hh:mm)

Figure 2

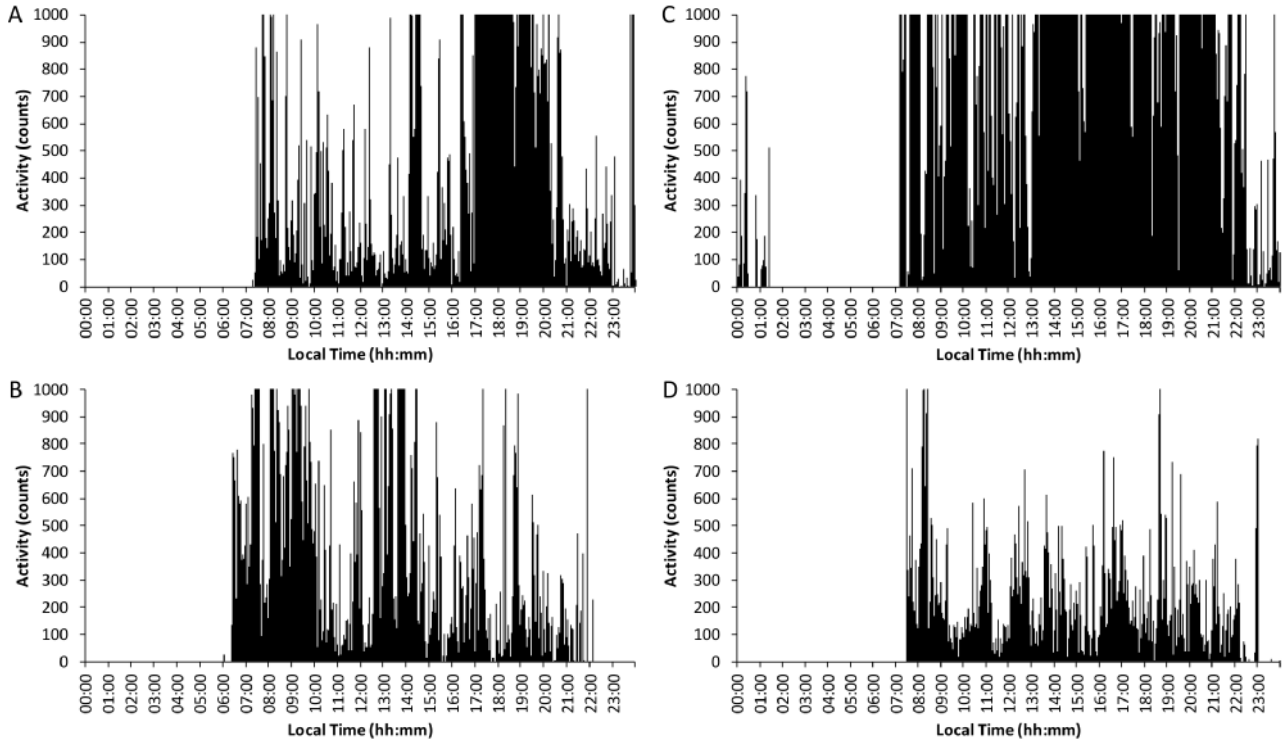
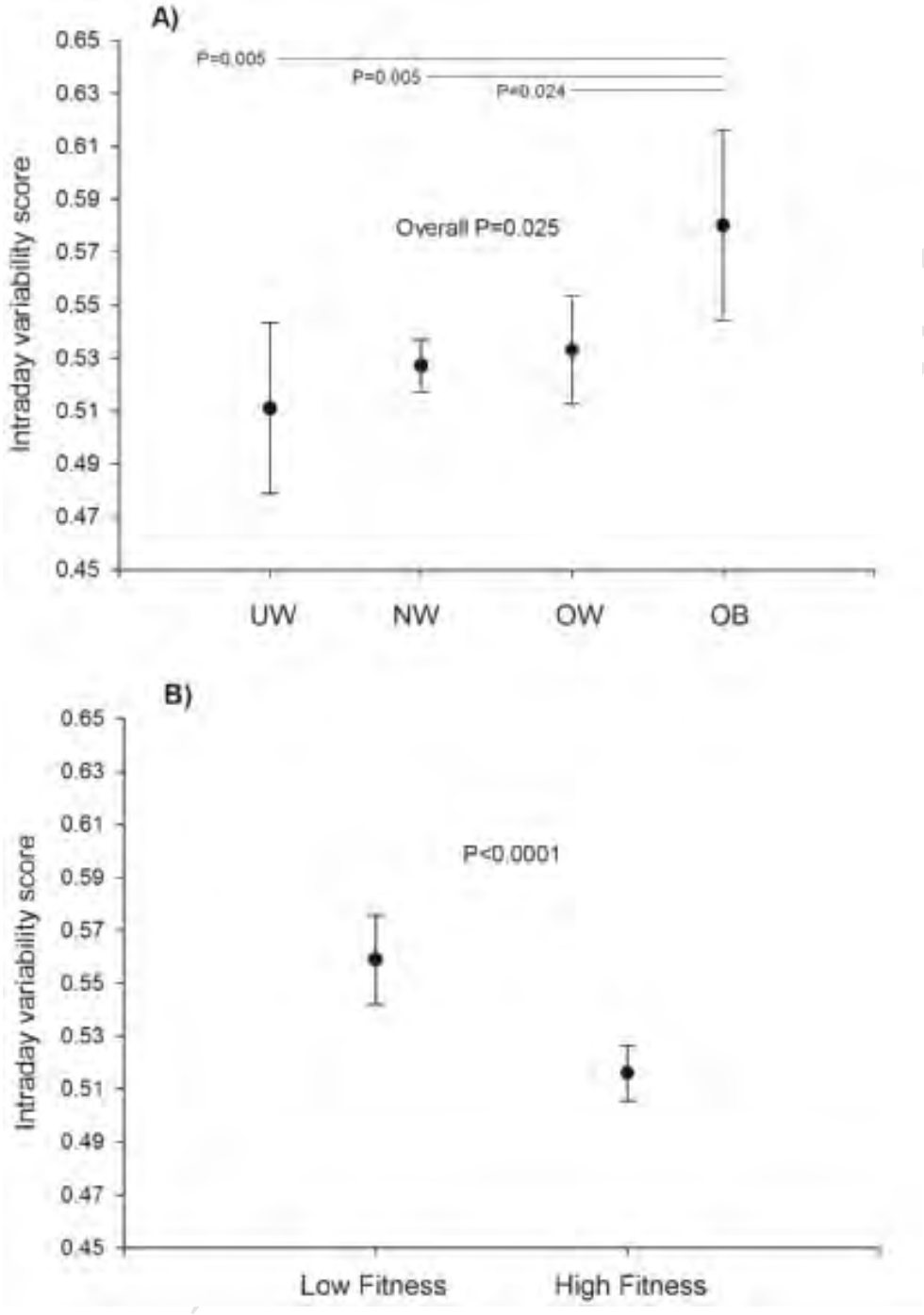


Figure 3


A)
P=0.005
P=0.005
P=0.024
Overall P=0.025
Intraday variability score
0.65
0.63
0.61
0.59
0.57
0.55
0.53
0.51
0.49
0.47
0.45
UW
NW
OW
OB
B)
P<0.0001
Intraday variability score
0.65
0.63
0.61
0.59
0.57
0.55
0.53
0.51
0.49
0.47
0.45
Low Fitness
High Fitness