Accepted manuscript

Temporal patterns of energy intake and physical activity and cross-sectional associations with body weight status in children and adolescents: results from the Portuguese National Food, Nutrition and Physical Activity Survey 2015–2016

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Keywords: feeding behaviour; eating patterns; energy intake; chrononutrition; physical activity; paediatric obesity.

This peer-reviewed article has been accepted for publication but not yet copyedited or typeset, and so may be subject to change during the production process. The article is considered published and may be cited using its DOI
10.1017/S0007114524002861
The British Journal of Nutrition is published by Cambridge University Press on behalf of The Nutrition Society

https://doi.org/10.1017/S0007114524002861 Published online by Cambridge University Press

ABSTRACT

Temporal energy intake (EI) and physical activity (PA) patterns may be associated with obesity. We aimed to derive and characterise temporal EI and PA patterns and assess their cross-sectional association with weight status in participants aged 6–14 years from the Portuguese National Food, Nutrition and Physical Activity Survey 2015-2016. We extracted times and EI of all eating occasions from two 1-day food diaries/24-hour recalls, while types and times of PA from 4-day PA diaries. We derived EI patterns (n=714) and PA patterns (n=595), using, respectively, a hierarchical and K-means cluster analysis, considering the average proportion of total daily EI (%TEI) and PA intensity (%TPA), within each 2-hour interval across the 24-hour day. We assessed the association between patterns and Overweight or obesity (Body Mass Index z-score ≥+1 Standard Deviation) using adjusted logistic regressions [Odds ratio (95% Confidence Interval)]. Three EI patterns were identified and labelled based on the 2-h interval of %TEI peaks: 1–“Early afternoon & Early evening”; 2–“Early afternoon & Late evening”, and 3–"Late morning, Early and Mid-afternoon & Late evening”. EI Pattern 3 vs. 1 was negatively associated with *overweight or obesity* [0.49 (0.26,0.92)]. PA pattern 1–“Late Morning, Mid-afternoon & Early evening” vs. Pattern 2–“Late afternoon”, was not associated with weight status [0.95 (0.65,1.38)]. A daily EI pattern characterised by more and even %TEI peaks at earlier daytime periods, instead of less, may be negatively associated with overweight or obesity amongst Portuguese 6-to-14-year-olds, whereas the identified PA patterns might have no relationship.

List of abbreviations: CI, confidence interval; d, day; g, grams; h, hours; IAN-AF, National Food, Nutrition and Physical Activity Survey 2015-2016; EI, energy intake; IQR, interquartile range, kJ, kilojoule; METs, metabolic equivalent of tasks; min, minutes; MSF, midpoint of sleep on free days; MSFsc, midpoint of sleep on free days corrected; OR, odds ratio; PA, physical activity; SD, standard deviation; TEI, total daily energy intake; TPA, total daily physical activity intensity.

https://doi.org/10.1017/S0007114524002861 Published online by Cambridge University Press

https://doi.org/10.1017/S0007114524002861 Published online by Cambridge University Press

INTRODUCTION

The obesity epidemic constitutes one of the major public health concerns of the 21st century[1]. School-age children and adolescents experience important physiological, anatomical, and behavioural changes. Paediatric obesity and related behaviours, including poor dietary and physical activity habits, are especially concerning given its tracking into adulthood[1,2]. Their cumulative health impacts are associated with an increased risk for chronic diseases and disability-adjusted life years[1,2]. Dietary intake and physical activity (PA) are crucial modifiable risk factors, yet prevention strategies have mostly focused on diet composition, PA intensity and frequency[3].

Chronobiology studies suggest that the alignment of an individual's internal circadian clock system with the 24-hour (h) light/dark cycle is crucial to metabolism homeostasis and weight regulation[4]. Biological clocks govern and are synchronised by physiological and behavioural rhythms, including sleeping and feeding[5]. If food intake and locomotor activity occur later than the favoured daytime/light period[5], internal rhythmicity is affected[6]. Age, sex, family practices, school and extracurricular activities schedules, and other dietary and activity behaviours also influence food intake and PA timings[7].

Later eating patterns, marked by concentrating meals or energy intake (EI) during the evening/nighttime instead of earlier times, may disrupt appetite and nutrient and energy metabolism, increasing the risk of obesity[5]. Different measures (mealtimes and/or EI) and criteria to define "later" eating rhythms/patterns (periods or time cut-offs) have contributed to their inconsistent association with higher adiposity in adults[8,9] and children[10].

Although physiological responses to exercise may vary with the time of day of its practice[11], few studies in adults found an association between PA patterns, based on the times and intensity of all activities, and weight status[9,12]. Notwithstanding the potential effect of feeding and PA rhythms in modulating the risk of paediatric obesity, evidence of the relationship between the temporal distribution of EI and PA with adiposity in paediatric age remains uncertain and scarce.

Hence, the present study aimed to derive and characterise daily temporal EI and PA patterns, based on a cluster analysis of the EI and PA intensity within each 2-h interval across the 24-h day; and to assess their cross-sectional association with weight status, based on Body Mass Index, in children and adolescents. Considering biological plausibility and current literature limitations, we hypothesise that at least two EI and PA

clusters will emerge, and the one with a later vs. earlier distribution will be positively associated with a worse weight status.

METHODS

Study design and participants

The present study used data from the Portuguese National Food, Nutrition and Physical Activity Survey 2015-2016 (IAN-AF)[13,14]. Briefly, this cross-sectional survey used the National Health Registry sampling frame and applied a multi-stage sampling to obtain a representative sample of the general Portuguese population aged between three months and 84 years. First, in each of the seven Portuguese NUTS II geographic regions, Health Units were randomly selected, weighted by the number of registered health users. Then, within each Unit, individuals were randomly selected, with a fixed number by sex and age group. A total of 5811 participants completed both interviews of the survey, of whom 1327 were aged three months to 9 years, and 632 were aged 10 to 17 years.

For the present study, the inclusion criteria were school-age participants aged between 6 to 14 years, with two valid dietary intake assessments, and height and weight measurements (n=714). For the EI patterns analyses, we included 714 participants. For the analyses concerning PA patterns, additional inclusion criteria were to have a minimum of three PA diaries, with at least 12 h of reported activities, on two weekdays and one weekend day, resulting in a sub-sample of 595 participants. Considering that PA diaries were only applied amongst 6–14-year-olds in the IAN-AF and to ensure a homogenous age range for the present study, we selected this inclusion criterion, with no requirement regarding the proportions of children (6–9 years) and adolescents (10–14 years).

Data collection

The variables of interest in our study were obtained through extraction from the IAN-AF's dataset and/or as a result of our further calculations/categorisations. In the IAN-AF, data collection was conducted between October 2015 and September 2016, covering the annual four seasons and every day of the week, through two personal computer-assisted interviews (8-15 days apart) performed by trained interviewers, using the "You eAT&Move" platform developed for the survey[13,14]. Data on dietary intake, physical activity, health, and sociodemographic variables were collected.

In our study, to capture the temporal dimension of EI and PA patterns, we analysed the distribution of TEI and TPA across every 2-h clock time interval within 24-h, similar to

https://doi.org/10.1017/S0007114524002861 Published online by Cambridge University Press

previous works[15–17]. We adopted this approach to avoid the limitations of using meal definitions or arbitrary clock time cutoffs to define early/later patterns. The 2-h intervals allowed us to obtain a feasible number of clusters (vs.1-h) and enough information to capture distribution differences (vs. larger intervals). We considered these intervals were also adequate considering the average Portuguese meal schedules and frequency[18], as well as parents' work and school schedules. Throughout, all clock times are in the 24-h format (h:min).

Dietary intake assessment

In the IAN-AF, dietary intake was assessed according to European guidelines[19] by two non-consecutive 1-day (d) food diaries filled by the children's main caregiver, and by two non-consecutive 24-h recalls administered to adolescents accompanied by caregivers. On each day, from 00:00 to 23:59, participants had to report the wake-up time, types and quantities of every consumed food and beverage (including recipes) per eating occasion, plus their respective place and time. Eating occasions included three main meals which could only be reported once per day, namely breakfast, lunch, and dinner, and snacks consumed before and after each of the main meals. All data were computerised using the "eAT24" software[20] linked with FoodEX2[21] and an extended version of the Portuguese Food Composition Table that included recipes[22] to estimate energy and nutrient intake.

Temporal energy intake patterns

To obtain one of the independent variables of interest in our study, namely the temporal EI patterns, we performed three steps: 1) for each participant and each dietary intake report, we extracted the clock times and EI of each eating occasion and the total daily energy intake (TEI) (in kilojoules (kJ)); 2) for each participant, using the two reports, we computed the daily average proportion of TEI (%TEI) ingested at each 2-h intervals of the 24-h day (00:00-23:59), and 3) considering these data we performed a hierarchical cluster analysis with the complete linkage method[23]. This method does not allow the user to predefine an expected number of clusters, instead, it generates a cluster tree by sequentially agglomerating similar data points into larger groups (clusters). We trimmed the cluster tree based on the Dunn Index[24], obtaining three clusters (i.e., patterns).

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Diet-related covariates

We considered the below-cited dietary-related covariates to characterise participants and/or assess them as potential confounders or effect modifiers in the logistic regression analysis. Participants' diet quality was assessed using a previously developed and validated index in Portuguese children, termed healthy eating index[25,26]. This index indicates adherence to a healthier diet and was based on the WHO's paediatric dietary recommendations[27] for nine food groups, including five considered *"healthier": Cereals and potatoes*; *Dairy; Fruit and Vegetables, including legumes*; and *White meat, fish, and eggs;* and four *"less healthy": Red Meat and processed meat*; *Salty snacks*; *Sugar-sweetened beverages*; *Sugar and honey*, and *Sweets.* For each group, the average daily consumption was calculated (grams(g)/d), and respective quartiles were obtained, for children and adolescents. Each quartile was scored between 1 to 4 points, in an ascending order for healthier food groups, and a descending order for the less healthy. We re-categorised consumption of *Salty snacks* and *Sugar and honey* as *No* (0g/d) vs. *Yes* (> 0g/d), scoring 2 and 1 points, respectively, given the prevalence of non-consumers. The summed-up score ranged from 9 to 32 points.

We estimated each participant's average TEI (kJ/d) and respective contributions of proteins, carbohydrates, and fats (%TEI), plus dietary fibre intake (g/d).

We computed the daily average number of all reported eating occasions (n/d), and categorised breakfast-skipping as *No* (reported on both days) or as *Yes* (reported on ≤1 day). To further characterise time parameters of the patterns, we calculated the daily average clock times of several parameters: i) breakfast, lunch, and dinner (main meals); ii) first and last EI occasions (not necessarily breakfast and dinner); iii) the 'eating midpoint' which is the midpoint of the eating window ([clock time of last EI occasion– clock time of first EI occasion] / 2)[28]; and iv) clock times of achievement of 25%, 50% and 75% of TEI, which represent the cumulative distribution of TEI in quartiles.

Furthermore, we categorised participants based on the type of days of their dietary intake reports. Most had both reports on weekdays (n=515, 72%); 24% (n=169) had one on a weekday and one on a weekend day, while 4% (n=30) had both reports on weekends.

https://doi.org/10.1017/S0007114524002861 Published online by Cambridge University Press

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Physical activity assessment

In the IAN-AF, adolescents and children's main caregivers were asked to fill a 4-day PA diary, adapted from Bouchard's 3-day activity record[29], over two consecutive weekdays and two weekend days. Briefly, in a logbook they had to describe the main activity performed every 15-minute interval from 00:00 to 23:59. Using the "Move" module, PA intensity of each activity was estimated by multiplying its duration by the metabolic equivalents of task (METs)[30,31]. The total daily PA intensity (TPA), in METs-hours, corresponded to the sum of the PA intensity of all activities, excluding sleep. Additionally, all participants and/or parents answered questions about participants' PA behaviours.

Temporal physical activity patterns

To obtain temporal PA patterns, the second independent variable of interest in our study, we performed four steps: 1) for each day and participant, we extracted the start and end times of each reported activity and the respective PA intensity (kilojoules (kJ)), and the TPA; 2) we assigned a unique clock time to each activity (excluding sleep), correspondent to the halfway between its start and end times (e.g., the activity "watching television while seated" reported between 14:00 and 15:30 was assigned 14:45); 3) for each participant, considering the total number of PA diaries we computed their daily average proportion of TPA (%TPA) expended at each 2-h interval of the 24-h day, and 4) considering these data, we performed a k-means cluster analysis[32]. This method produces a partition of the data into a particular number of groups while decreasing the variability within clusters and increasing the variability between clusters[32]. Two to 10 clusters were tested, and the best partition of data was evaluated based on a set of 30 different indices[33], resulting in two clusters.

Physical activity-related covariates

We considered a set of PA-related covariates. The practice of extracurricular structured PA (*No/Yes*) - hereby defined simply as "Structured PA" - was assessed by the question "Does the child/Do you practice regular PA or sports (apart from physical education classes)?". For the sub-sample with PA diaries, we computed the daily average TPA (METs/d) of all reported PA diaries, and the daily average time spent (h/d) on PA with a moderate-to-vigorous intensity (METs $\geq$3) and in sedentary behaviour (METs $\leq$1.5)[34]. To further explore the temporal distribution of PA, in cumulative terms, we calculated

the daily average clock times of the following parameters: first and last reported activities (excluding sleep), the midpoint of the PA window, and the achievement of 25%, 50%, and 75% of TPA, using PA diaries.

Anthropometric assessment

Anthropometry was performed by trained staff according to standard procedures[35] without shoes and with light clothing. Weight was measured to the closest 0.1 kilogram, using a digital scale (SECA® 813, Hamburg, Germany), and height was measured to 0.1 centimetre, using a portable wall stadiometer (SECA® 213, Hamburg, Germany). Sex-specific Body Mass Index-for-age z-scores (zBMI) were calculated using specific software and categorised according to the World Health Organization (WHO) criteria as: underweight (<-2 Standard deviations (SD)), normal weight (-2≤ SD <+1), overweight (+1≤ SD ≤+2) or obesity (>+2 SD)[36].

Body weight status

Participants' body weight status based on the WHO's zBMI categories, constituted our study's dependent variable (outcome). Within our sample (n=714), the prevalences of underweight, normal weight, overweight, and obesity were respectively: 1.3%, 60.5%, 25.4%, and 12.9%. Given the low prevalence of underweight, and previous studies usually aggregate categories, we re-categorised weight status as *Underweight or normal weight* (zBMI ≤+2 SD) and *Overweight or obesity* (zBMI >+2 SD).

Sociodemographic covariates

We considered participants' sex, age (as a continuous variable), age group (children or adolescents), and the reported household size, which was categorised based on the number of living members as three; four to five, or above five. Maternal current working status was self-reported and categorised as *Employed* or *Unemployed or other* (*including student/retired/disabled*). Parental education level was defined by the parent reporting the highest level of schooling completed and it was re-categorised as *Secondary or lower* (none to secondary level, which corresponds to 0–12 years) or *Tertiary education* (post-secondary level, correspondent to >12 years).

https://doi.org/10.1017/S0007114524002861 Published online by Cambridge University Press

https://doi.org/10.1017/S0007114524002861 Published online by Cambridge University Press

Sleep and chronotype covariates

Individuals' wake-up and bedtimes and circadian rhythms are related to temporal PA and EI patterns[7,37,38]. For the sub-sample with PA diaries, we were able to extract the start and the end clock times of the activity "sleep" on weeknights (Sunday to Friday) and weekend nights (Friday to Sunday) to calculate sleep duration for each day and participant. We also estimated participants' chronotype, which is a biological construct used as a proxy to assess individual differences in their circadian system[37,39]. According to Roenneberg, an individual's chronotype can be estimated using the Munich ChronoType Questionnaire, by determining the midpoint of sleep on free days (MSF), as the halfway point between sleep onset and sleep offset clock times on days without social (school) obligations, and correcting it for sleep debt accumulated during school days, which originates the midpoint of sleep on free days corrected (MSFsc)[37,39]. Following these principles, in our study we had to adapt the formulas, assuming weekends were free days, without interferences in sleep times, and that the reported sleep start and end times in the PA diaries were proxies of sleep onset and end times[38], respectively. We calculated the MSFsc (in h:min) for 591 participants, through a computation process detailed elsewhere[38] that implied data imputation for missing sleep start times.

Ethics Approval Statement

The IAN-AF 2015–2016 survey was conducted in accordance with the Declaration of Helsinki and was approved by the National Data Protection Committee (May 26, 2015), the Ethical Committee of the Institute of Public Health of the University of Porto (March 13, 2015), and the Ethical Commissions of each one of the Regional Administrations of Health.

Informed Consent Statement

Informed consent was obtained from all subjects involved in IAN-AF 2015-2016. Participants were asked to provide written informed consent according to the Ethical Principles for Medical Research expressed in the Helsinki Declaration of the World Medical Association, and national law. Written consent was signed by legal representatives of children, and by adolescents and their legal representatives. All parties involved signed a declaration of confidentiality and good practice.

https://doi.org/10.1017/S0007114524002861 Published online by Cambridge University Press

Statistical analysis

We characterised the sample included in EI patterns analyses and the sub-sample of PA patterns analyses and compared the sub-sample with excluded individuals.

Continuous variables were summarised by the mean and standard deviation (SD), if the distribution was considered normal through visually inspecting histograms and Q-Q plots, or by the median and interquartile range (IQR). Differences were assessed by One-way Analysis of Variance (ANOVA) or T-Student's test, or by the respective nonparametric tests Kruskal-Wallis or Independent-Samples Mann-Whitney U test, appropriately. Categorical variables were summarised by frequency (n) and column proportions (%) and differences were assessed using Pearson's Chi-square test.

Temporal EI patterns and PA patterns were characterised separately by a plot depicting the daily average %TEI ingested / %TPA expended by each 2-h clock time interval across the 24-h day (from 00:00 until 23:59). To supplement these, we summarised and compared the %TEI between each EI pattern, and of %TPA between PA patterns. We labelled each pattern based on the 2-h intervals at which the highest values %TEI/TPA ("peaks") occurred. First, within the 24-h day we identified three daytime periods, namely morning (06:00–11:59), afternoon (12:00–17:59), and evening (18:00–21:59) - which we further split into 2-h periods (early, [mid], and late) – and one nighttime period (22:00–05:59). Each pattern could have more than one peak if there were similarly high values of %TEI/TPA at different intervals, which we compared using Related samples Friedman's Two-way Analysis of Variance by ranks.

Participant's characteristics were summarised and compared according to their EI and PA Patterns, separately.

The associations of EI and PA patterns (Pattern 1 as the reference category) with weight status (*Overweight or obesity* as the response) were assessed separately by logistic regression analysis. For the independent variable EI patterns, we fitted four nested models: a crude model (Model 1), and adjusted models for sex, age, and parental education (Model 2), healthy eating index score (Model 3), and structured PA practice (Model 4). For PA patterns, we conducted Models 1, 2, and 3, plus models adjusted for the time spent in sedentary behaviour (Model 4) and MSFsc (Model 5). Results are expressed as Odds ratio (OR) and the respective 95% confidence intervals (CI). There was no significant interaction effect for age, age group (or MSFsc, assessed only for PA patterns).

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As supplementary analyses, we compared PA-related covariates across EI patterns for the subsample included in PA Patterns. We also explored the potential role of weekdays/weekends and circannual periods[38,40] (summer school holidays vs. other) reported on dietary intake reports in the EI/PA Patterns. We assessed the association between the types of days of dietary intake reports and EI patterns. For participants with diaries on weekdays and weekends (n=169), we compared daily average clock times of EI parameters computed with and without weighing for the type of day (as we applied in this study). We categorised each participant's period of dietary intake reports and PA diaries, as S*ummer school holidays* if ≥1 day occurred between June 9th and September 15th, 2016, or *Other,* if none did. Then, we assessed the associations between the period of dietary intake/PA reports and EI/PA patterns, and between the period of both reports and weight status.

EI and PA patterns were derived in R statistical computing program, version 3.4.1 (R Foundation for Statistic Computing, Austria, 2010), and the remaining analyses were conducted in Statistical Package for the Social Sciences, version 26.0 (IBM SPSS Statistics for Windows, IBM Corp., Armonk, NY).

RESULTS

Participant characteristics

The sample included in EI patterns (n=714, 51% girls) had a mean age of 10 (SD=2.7) years, zBMI of 0.70 (SD=1.11), and 38.2% had overweight or obesity. Most practiced extracurricular structured PA (61%), had employed mothers (82%), and parents with a secondary or lower level of education (64%). The sub-sample included in PA patterns (n=595) vs. EI patterns had similar characteristics. However, compared to participants without PA diaries (n=119), the proportion of overweight or obesity was lower (36% vs. 47%, p=0.03) (**Table 1**).

Temporal EI patterns

Three temporal EI patterns were identified within this study, whose distribution of %TEI across the 24-h day is presented in **Figure 1** and detailed in **Table S1**. EI Pattern 1 – "Early afternoon & Early evening", showed two peaks providing around 25% of TEI, at two 2-h intervals: 12:00–13:59 and 18:00–19:59. Pattern 2 – "Early afternoon & Late evening", was the most prevalent (70% of the sample) and also had two %TEI peaks (24 and 26%) at 12:00–13:59 and 20:00–21:59. Pattern 3 – "Late morning, Early and Mid-afternoon & Late evening" (10%, n=72) had four similar %TEI peaks, ranging

from 14% to 19%, three occurred at 2-h intervals between 10:00 and 15:59, and the last at 20:00-21:59. Although participants with Pattern 3 presented later main meals, they achieved 50% of TEI (15:00 vs. 14:35 and 14:22, p=0.009) and 75% of TEI earlier than other Patterns (**Table 2**). Overweight or obesity was less prevalent in participants with Pattern 3 vs. Pattern 1 and 2 (28% vs. 44% and 38%, p=0.06) (**Table 2**).

Table S2 comparing PA-related covariates between EI patterns for the sub-sample (n=595), showed that participants with EI Pattern 1 vs. 2 had earlier sleep start and end times on weekdays. EI patterns were not associated with participants' type of days of dietary reports (chi-square test p=0.95). Additionally, **Table S3** showed that most average EI parameters calculated with or without weighting for weekdays and weekends were similar (n=169). EI pattern 2 vs. 1 was associated with summer school holidays vs. other periods (**Table S4**).

Temporal PA patterns

The daily distribution of %TPA of the two identified temporal PA patterns is depicted in **Figure 2** and detailed in **Table S5**. PA Pattern 1 – "Late morning, Mid-afternoon & Early evening", was presented by most of the sub-sample (64%) and had three peaks of %TPA, ranging from 15% to 16%, at 10:00–11:59, 14:00–15:59, and 18:00–19:59. PA Pattern 2 – "Late afternoon", had a single %TPA peak of 21% at 16:00–17:59. Pattern 2 achieved quartiles of TPA at later clock times and had later sleep start and end times (**Table 3**). Participants who presented PA Pattern 2, vs. Pattern 1, were older, less practiced structured PA, and had a lower TPA, greater sedentary behaviour, lower diet quality, and later caloric midpoint, while both presented similar EI Patterns and weight status (**Table 3**). The proportion of participants with PA reports on summer holidays was higher for PA Pattern 2 (p<0.001) (**Table S6**).

Association between EI and PA patterns and BMI category

EI Pattern 3 – "Late morning, Early and Mid-afternoon & Late evening" vs. Pattern 1 – "Early afternoon & Early Evening", was negatively associated with having overweight or obesity (zBMI ≥+1 SD) even after adjustments (Model 4: OR=0.49; 95%CI:0.26, 0.92). PA pattern 1 vs. Pattern 2 – "Late afternoon" was not associated with overweight or obesity in crude and adjusted models, even for MSFsc (Model 5: OR=0.93; 95%CI:0.65,1.34) (**Table 4**).

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DISCUSSION

Temporal EI patterns

In our study, three EI patterns emerged, and Pattern 3 was negatively associated with having overweight or obesity. Compared with Pattern 1 – "Early afternoon & Early Evening" and Pattern 2 – "Early afternoon & Late evening"; Pattern 3 – "Late morning, Early and Mid-afternoon & Late evening", had more and lower %TEI at their peaks (19% compared to around 25% of TEI). Additionally, Pattern 3 had a higher %TEI ingested in the mid-morning and mid-afternoon, contributing to an earlier achievement of 50% of TEI, i.e. caloric midpoint[41], and 75% of TEI. Therefore, Pattern 3 could be interpreted as having a relatively earlier overall distribution of TEI and it was associated with a lower risk of having overweight or obesity, compared to Pattern 1.

Although mechanisms are not fully clear, concentrating EI towards later in the day may be associated with adiposity due to the mismatch between the fasting-feeding and light-dark cycles and the biological clocks of the circadian system (chrono-disruption)[6]. In turn, metabolic processes and hormonal levels may become dysregulated, leading to an increased appetite, especially for highly palatable foods, insulin resistance, lower energy expenditure, and fat accumulation[6,42,43]. Biologically, food intake synchronises molecular peripheral clocks ubiquitous in the body, such as in the pancreas and muscles. These clocks intercommunicate with the central hypothalamic clock, the suprachiasmatic nuclei, via neuro-humoral signals. Peripheral clocks are implicated in crucial physiological processes, including the secretion of insulin and hormones involved in energy and nutrient metabolism, appetite, and the reward system[6]. Behaviourally, individuals with a later EI concentration may exhibit obesity-related behaviours, such as a greater screen-time, breakfast-skipping, and a lower diet quality, which was the opposite of what we found, as well as a later chronotype or bedtimes[44] and shorter sleep duration[7].

Previous epidemiological studies have reported inconsistent results on the association between later eating rhythms and overweight or obesity, in children[10,45–49] and in adults[8,9,50]. The divergency of results may be affected by methodological heterogeneity in the assessment methods and in the criteria to define eating rhythms as "later", which encompasses mealtimes[47], the absolute EI or the %TEI ingested at different meals[49], at time-intervals[45,48] or clock time cut-offs[46]. Since few works in children have focused on time-based EI patterns, studies with related exposures will be discussed, although they are not directly comparable.

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In adults, a cross-sectional study with a similar analysis found that participants with a pattern marked by consistent and moderate peaks exhibited a lower risk of obesity[15]. The %TEI ingested after 20:00 was cross-sectionally positively associated with a higher BMI in children and adolescents[46]. In young adults, having a "later" caloric midpoint (≥15:30, the sample's median) was associated with a higher BMI[50]. In a national UK survey, adolescents with an EI pattern marked by earlier main meals and night snacks, compared to slightly later main meals but no night snacks, had higher BMI[47]. As the results for mealtimes and %TEI across time may differ, the importance of analysing both parameters for main meals and snacks is highlighted. Results for EI Pattern 3 vs. 1 are in line with those of studies focusing on EI Patterns characterised by timing, magnitude, and frequency of %TEI peaks[15], or on single and less detailed measures of the temporal daily distribution of %TEI[46,50]. EI Pattern 3 had more and even peaks of %TEI, occurring during earlier periods within the daytime, which contributed to an earlier caloric midpoint, compared to Pattern 1. The distribution of EI of Pattern 3 was also more even across all 2-h intervals, compared with Patterns 1 and 2, where the magnitude of peaks diverged more, which is in line with previous work[15]. However, if only the times of eating occasions or meals were being compared across patterns, which were later for Pattern 3, our findings would disagree with works that suggested later mealtimes were linked to higher adiposity[47]. Pattern 2, "Early afternoon & Late evening", was not associated with having overweight or obesity compared to EI Pattern 1 or 3 (data not shown). Patterns 2 and 1 had a similar magnitude of %TEI peaks, while Patterns 2 and 3 had a similar caloric midpoint and last EI occasion, which might have contributed to the null association. Likewise, some works found no significant associations of EI patterns, defined by the %TEI within 2–3-hour intervals across 06:00 to 24:00[48] or by the %TEI at different meals[49] with BMI, in 2–16 year-olds.

Additionally, the %TEI at each 2-h interval should not be mistaken for meals. In Patterns 1 and 2, peaks coincided mainly with the reported times of lunch and dinner. However, for Pattern 3, which exhibits a higher variance in data, the first three peaks could coincide either with breakfast or lunch times. This reinforces the relevance of analysing the distribution of %TEI across the 24-h day besides mealtimes.

Temporal PA patterns

Two PA patterns were identified. Although PA Pattern 2 – "Late afternoon", was marked by a concentration of PA later in the day, compared with PA Pattern 1 – "Late morning,

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Mid-afternoon & Early evening" no evidence of an association between these PA patterns and weight status was found.

The endogenous response induced by PA, including in the metabolism of energy and nutrients, food intake, and sleep may vary across the 24-hour day[11], although evidence from studies in adults is still conflicting and lacking in paediatric age. While a lower percentage of activity counts before noon was cross-sectionally associated with a higher BMI[12], a study that applied a cluster analysis found that a PA pattern with higher activity counts performed throughout the day, early (8:00–11:00) or late (16:00–21:00), exhibited lower BMI compared with patterns with lower activity counts[16]. Comparison with our results is hindered by the different age ranges and methodological heterogeneity to derive temporal PA clusters, as we used PA diaries to estimate the %TPA within 2-h intervals, whereas previous research used accelerometers to assess the absolute PA counts during the morning/evening periods[12,16].

We obtained only two clusters, and none was a "morning" pattern, contrasting to the previous studies in adults. The timing of PA of 6-to-14-year-olds is mostly influenced by school schedules[31] and differences in PA patterns may become more noticeable in older individuals. The reduced variability of patterns and a lower prevalence of overweight or obesity in this sub-sample (vs. individuals without PA diaries) may have contributed to the null association. Nonetheless, participants with PA Pattern 2 presented a clustering of overweight-related behaviours (lower diet quality, higher sedentarism, and later sleep times), previously reported since a young age[51], and thus, should be investigated to help identify risk factors for obesity.

Strengths and limitations

Strengths of this study include the harmonised methodology to collect dietary intake by trained staff with a background in Nutrition Sciences or Dietetics[19] and the classification of earlier/later patterns in understudied populations without criteria based on mealtimes or cut-off definitions. Limitations include possible measurement errors and social desirability bias of dietary intake, including differential under/overreporting according to meals or weight status[47,52]. Also, we used PA diaries to estimate PA intensity which are less accurate than objective methods.

Cluster misclassification of individuals is a possible limitation. Two dietary intake/three PA reports may be insufficient to accurately measure the temporal daily distribution of EI and PA due to intraindividual variability[53]. Each eating occasion's reported clock time was allocated at a 2-h interval, although it could be close to consecutive intervals,

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contributing to the %TEI at one interval vs. the other. Nevertheless, we assessed %TEI differences between every 2-h interval to ensure similar peaks would be considered.

EI patterns were not associated with different types of days of dietary intake reports presented by participants. Although EI Pattern 2 vs. 1 was more prevalent in participant's filling reports during summer holidays, there was no difference between EI pattern 1 and 3. Additionally, participants with dietary reports (or PA diaries) on "summer holidays" vs. "other periods" had similar characteristics, including covariates and weight status, although sample size was reduced for some comparisons. Therefore, we do not expect this to have biased our association results. Moreover, our results should be interpreted with caution given the sample size of Pattern 3, due to a higher variance of data and a lower precision of the estimate. Given the cluster approach to derive temporal EI and PA patterns, one cannot disentangle the exact factors that explain their association with weight status, e.g. consistency of the magnitude, the temporal distribution of peaks, or the %TEI/TPA attained at a specific time-cut-off. Nevertheless, we discussed and postulated the plausibility of our findings, considering current knowledge.

We cannot exclude the potential role of unmeasured covariates or those with high missingness in our results, such as pubertal stage, chronotype, and sleep in EI patterns' analyses. The sub-sample of PA patterns' analyses had a lower prevalence of overweight or obesity compared to excluded individuals, limiting the generalizability of results.

Lastly, the cross-sectional study design does not allow the establishment of a causal association between patterns and BMI within this population.

Therefore, future research on temporal patterns should include larger samples with older adolescents, a higher number of observations during weekly and annual variations, and explore jointed patterns of EI and PA applying accelerometry[9].

Conclusion

Having a temporal EI pattern with more and even peaks of proportions of total daily energy intake throughout the day, mostly concentrated at earlier daytime periods, compared to less and higher peaks at later times, may be associated with a lower risk of overweight and obesity, in Portuguese youth aged 6 to 14 years. In contrast, the identified temporal PA patterns do not appear to be linked with body weight status. Investigating the distribution of caloric intake and PA intensity across the 24-h day,

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besides clock times of main meals, and the associated overweight-related behaviours may be important to identify risk factors for paediatric obesity.

ACKNOWLEDGMENTS

The IAN-AF2015-2016 had institutional support from the General Directorate of Health (DGS), the Regional Health Administration Departments, the Central Administration of the Health System (ACSS) and the European Food Safety Authority (CFT/EFSA/DCM/2012/01-C03). The researchers acknowledge all these institutions, and all persons involved in all phases of the survey, as well as the participants.

Regarding financial support, this work was supported by Fundação para Ciência e Tecnologia, I.P. (FCT) through projects with the references UIDB/04750/2020, (https://doi.org/10.54499/UIDB/04750/2020) and LA/P/0064/2020 (https://doi.org/10.54499/LA/P/0064/2020). S.C. is supported by a Studentship for Doctoral Research (UI/BD/150784/2020, https://doi.org/10.54499/UI/BD/150784/2020) funded by FCT within the Collaboration Protocol for Financing the Multiannual Plan for Scholarships for Doctoral Students signed between FCT and EPIUnit. S.V. is supported by national funds through FCT, under the programme "Stimulus of Scientific Employment-Individual Support" within the contract 10.54499/2021.01096.CEECIND/CP1686/CT0001 (https://doi.org/10.54499/2021.01096.CEECIND/CP1686/CT0001). The IAN-AF 2015-2016 had funding from the EEA Grants Programme "Public Health Initiatives" (PT06 - 000088SI3). The funding institutions had no role in the design, analysis or writing of this article.

The authors' contributions to the manuscript were: Conceptualization S.C., I.S. and S.V.; Data curation, S.C., I.S., and D.C.; Methodology, S.C., I.S., S.V; Software, D.C.; Formal analysis, D.C. (in R), S.C. and I.S. (in SPSS); Data interpretation, all authors; Writing-original draft, S.C. and I.S.; Writing-review and editing, all authors; Validation, D.C. and S.V.; Visualization, S.C., I.S. and D.C; Supervision, S.V. All authors have read and agreed to the submitted and published versions.

All authors declare no conflicts of interest.

The authors confirm that the data supporting the findings of this study are available within the article and supplementary material.

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SUPPLEMENTARY MATERIAL

Additional supporting information can be found online in the Supplementary material.

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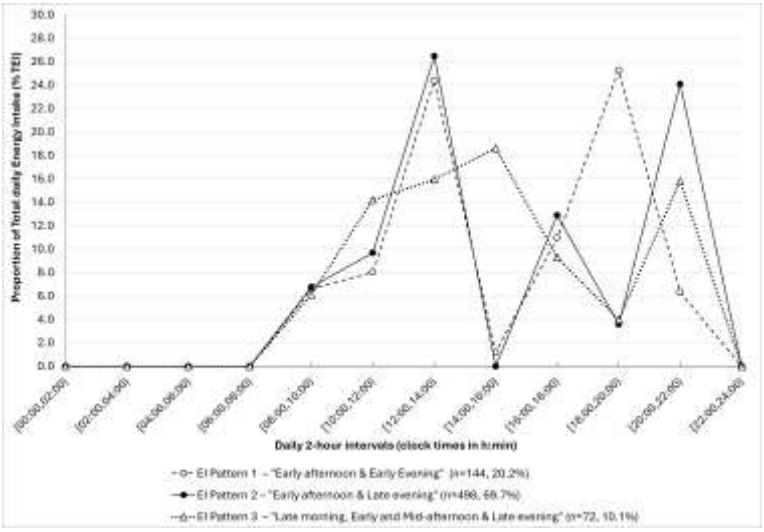

Figure 1: Daily average proportion of total daily energy intake (%TEI) ingested at each 2-hour interval across the 24-hour day (from 00:00 until 23:59), for the three identified Temporal EI patterns (n=714). The median values of %TEI are presented due to the non-normal distribution of the variables. The bounds of each 2-hour interval are in local clock times (h:min). EI Pattern 1 – "Early afternoon & Early Evening" (n=144, 20.2%) (white circles and dashed line). EI Pattern 2 – "Early afternoon & Late evening" (n= 498, 69.7%) (black circles and full line). EI Pattern 3 – "Late morning, Early and Mid-afternoon & Late evening" (n=72, 10.1%) (triangle and dotted line).

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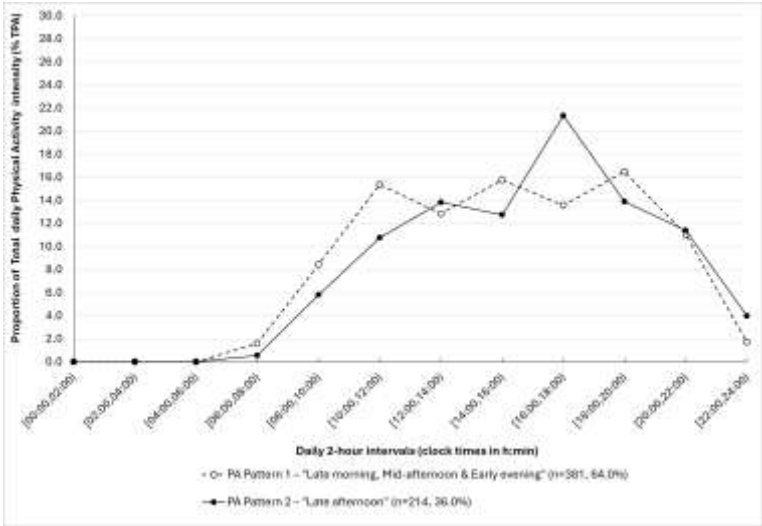

Figure 2: Daily average proportion of total daily physical activity intensity (%TPA) expended at each 2-hour interval across the 24-hour day (from 00:00 until 23:59), for the two identified Temporal PA patterns (n=595). The median values of %TPA are presented due to the non-normal distribution of the variables. The bounds of each 2-hour interval are in local clock times (h:min). PA Pattern 1 – "Late morning, Mid-afternoon & Early evening" (n=381, 64.0%) (white circles and dashed line). Pattern 2 – "Late afternoon" (n=214, 36.0%) (black circles and full line).

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Table 1. Participants' characteristics according to the sample of Temporal Energy Intake (EI) patterns (n=714), sub-sample of Temporal Physical Activity (PA) patterns (n=595), and comparison with Excluded individuals (n=119).

	Sample of EI patterns		**Sub-sample of PA patterns**		**Excluded individuals**		
	n 714		*n* 595		*n* 119		
	n* mean	**% SD***	***n* mean***	**% SD***	***n* mean***	**% SD***	**p-value †**
Sex							0.50
Female	362	50.7	305	51.3	57	47.9	
Male	352	49.3	290	48.7	62	52.1	
Age, y	10.0	2.69	10.1	2.68	9.8	2.69	0.29
Age group							0.36
Children	297	41.6	243	40.8	54	45.4	
Adolescents	417	58.4	352	59.2	65	54.6	
Household size‡							0.95
2-3 members	192	29.6	158	29.3	34	30.9	
4 members	361	55.6	301	55.8	60	54.5	
>4 members	96	14.8	80	14.8	16	14.5	
Parental education level‡							0.11
Secondary or lower	452	63.9	369	62.6	83	70.3	
Tertiary	255	36.1	220	37.4	35	29.7	
Maternal age, y‡	40.3	5.31	40.5	5.00	39.3	5.99	0.060
Maternal working status‡							0.34
Employed	578	81.8	486	82.4	92	78.6	
Unemployed or other	129	18.2	104	17.6	25	21.4	
z-score Body Mass Index (zBMI), SD	0.70	1.11	0.68	1.10	0.83	1.17	0.16
Weight status							0.030
Underweight or normal weight	441	61.8	378	63.5	63	52.9	
Overweight or obesity	273	38.2	217	36.5	56	47.1	
Temporal EI patterns							0.005
EI Pattern 1 – Early afternoon & Early evening	144	20.2	107	18	37	31.1	
EI Pattern 2 – Early afternoon & Late evening	498	69.7	426	71.6	72	60.5	

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EI Pattern 3 – Late morning, Early and Mid-afternoon & Late evening	72	10.1	62	10.4	10	8.4	
Healthy eating index score, points	20.4	3.39	20.4	3.40	20.7	3.36	0.44
TEI, kJ	7958	2169.0	7941	2164.0	8037	2202.5	0.67
Temporal PA patterns	NA	NA	595		NA	NA	
PA Pattern 1 – Late morning, Mid-afternoon & Early evening	NA	NA	381	64.0	NA	NA	
PA Pattern 2 – Late afternoon	NA	NA	214	36.0	NA	NA	
Structured PA‡							0.78
No	271	38.8	225	38.6	46	38.6	
Yes	427	61.2	358	61.4	69	61.4	

Abbreviations: EI, energy intake; PA, physical activity; n, frequency; %, proportion; SD, standard deviation; y, years; zBMI, z-score body mass index; TEI, total daily energy intake; kJ, kilojoule; NA, not applicable, because there was no data available.

* Data are presented as frequency (n) and column proportions (%) for categorical variables and as mean and standard deviation (SD) for continuous variables.

† P-value for Pearson's Chi-square test for categorical variables, and Student's t-test for continuous variables, conducted between the Sub-sample PA patterns and Excluded individuals.

‡ Based on n=616 for the Sample of EI patterns, n=514 for the Sub-sample of PA patterns, and n=102 for Excluded individuals, due to missing data. For categorical variables, the sum of each category did not add up to the *n* of each column due to missing data.

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Table 2. Participants' characteristics according to Temporal Energy Intake (EI) patterns (n=714).

	EI Pattern 1 – Early afternoon & Early evening		**EI Pattern 2 – Early afternoon & Late evening**		**EI Pattern 3 – Late morning, Early and Mid-afternoon & Late evening**		
	n 144		*n* 498		*n* 72		
	n* mean or median	**% SD or IQR***	***n* mean or median***	**% SD or IQR***	***n* mean or median***	**% SD IQR***	**p-value†**
Sex							0.51
Female	67	46.5	259	52	36	50	
Male	77	53.5	239	48	36	50	
Age, y	10.0	2.88	9.93	2.64	10.6	2.56	0.18
Age group							0.13
Children	62	43.1	213	42.8	22	30.6	
Adolescents	82	56.9	285	57.2	50	69.4	
Maternal age, y‡	39.1	5.34	40.7	5.18	39.6	5.74	0.007
Parental education level							0.029
Secondary or lower	101	70.6	299	60.8	52	72.2	
Tertiary	42	29.4	193	39.2	20	27.8	
Maternal working status‡							0.46
Employed	119	82.6	404	82.3	55	76.4	
Unemployed or other	25	17.4	87	17.7	17	23.6	
zBMI, SD	0.93	1.14	0.67	1.11	0.49	1.01	0.01
Weight status							0.06

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Underweight or normal weight	80	55.6	309	62.0	52	72.2	
Overweight or obesity	64	44.4	189	38.0	20	27.8	
Healthy eating index score, points	20.3	3.46	20.6	3.37	19.6	3.28	0.05
TEI, kJ	7920	2087.0	7950	2159.4	8083	2412.9	0.87
Protein, %TEI	17.3	3.27	17.1	3.32	16.3	3.47	0.11
Carbohydrates, %TEI	50.6	5.89	51.4	6.15	52.0	5.46	0.25
Free sugars, %TEI	12.4	6.07	12.1	5.97	14.1	6.75	0.04
Fiber, g/d[§]	14.2	5.91	15.4	6.56	14.3	6.14	0.25
Fat, %TEI	30.2	5.74	29.6	5.72	29.8	5.24	0.52
Trans fatty acids, %TEI[§]	0.43	0.26	0.43	0.29	0.45	0.40	0.49
Saturated fatty acids, %TEI	11.0	2.6	10.66	2.66	11.1	2.61	0.08
Daily frequency of eating occasions, n/d	6	1.1	6	1.3	6	1.4	0.97
Breakfast-skipping							0.61
No	130	90.3	462	92.8	66	91.7	
Yes	14	9.7	36	7.2	6	8.3	
Breakfast, h:min[§]	08:12	1:15	08:29	1:30	08:37	1:44	0.01
Lunch, h:min[§]	12:47	0:42	12:55	0:42	13:13	1:24	0.001
Dinner, h:min	19:30	0:48	20:17	0:45	20:25	0:48	<0.001
First EI	08:15	1:21	08:30	1:32	08:31	1:42	0.56

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occasion, h:min§							
25% of TEI, h:min§	12:00	1:57	11:55	1:45	11:47	2:30	0.99
50% of TEI, h:min§	15:00	2:11	14:35	2:42	14:22	2:06	0.009
75% of TEI, h:min§	19:00	1:30	19:08	2:07	18:00	2:03	<0.001
Last EI occasion, h:min§	20:45	1:30	21:00	1:00	21:00	1:51	0.11
Eating midpoint, h:min§‖	14:37	1:18	14:45	1:04	14:49	1:22	0.19
Structured PA‡							0.23
No	61	43.3	179	36.8	31	44.3	
Yes	80	56.7	308	63.2	39	55.7	

Abbreviations: EI, energy intake; n, frequency; %, proportion; SD, standard deviation; IQR, interquartile range; y, years; zBMI, z-score body mass index; PA, physical activity; TEI, total daily energy intake; kJ, kilojoule; g, grams; d, day; h, hours; min, minutes.

* Data are presented as frequency (n) and column proportions (%) for categorical variables; as mean and standard deviation (SD) for continuous variables or as the median and interquartile range (IQR), if these were non-normally distributed.

† P-values for Pearson's Chi-square test for categorical variables, and One-way ANOVA or Kruskal-Wallis test for continuous variables.

‡ Based on n=616, due to missing data. For categorical variables, the sum of each category did not add up to the *n* of each column due to missing data.

§ Variables for which median (IQR) and p-value for Kruskal-Wallis test are presented.

‖ Calculated as (clock time of last EI occasion – clock time of first EI occasion) / 2.

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Table 3. Participants' characteristics according to Temporal Physical Activity (PA) patterns (n=595).

	PA Pattern 1 – Late morning, Mid-afternoon & Early evening		**PA Pattern 2 – Late afternoon**		
	n 381		*n 214*		
	n* mean or median	**% SD or IQR***	***n* mean or median***	**% SD or IQR***	**p-value†**
Sex					0.42
Female	200	52.5	105	49.1	
Male	181	47.5	109	50.9	
Age, y	9.8	2.68	10.4	2.66	0.008
Age group					0.14
Children	164	43.0	79	36.9	
Adolescents	217	57.0	135	63.1	
Maternal age, y‡	40.0	5.15	41.4	5.01	0.002
Parental education‡					0.62
Secondary or lower	228	60.5	141	66.5	
Tertiary	149	39.5	71	33.5	
Maternal work status‡					0.62
Employed	310	81.8	176	83.4	
Unemployed or other	69	18.2	35	16.6	
zBMI, SD	0.66	1.09	0.70	1.12	0.69
Weight status					0.99
Underweight or normal weight	242	63.5	136	63.6	
Overweight or obesity	139	36.5	78	36.4	
Temporal EI Patterns					0.74
EI Pattern 1 – Early afternoon & Early evening	72	18.9	35	16.4	
EI Pattern 2 – Early	270	70.9	156	72.9	

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afternoon & Late evening					
EI Pattern 3 – Late morning, Early and Mid-afternoon & Late evening	39	10.2	23	10.7	
Healthy eating index score, points	20.7	3.28	19.8	3.52	0.001
TEI, kJ	7937	2218.8	7950	2067.7	0.95
Protein, %TEI	17.1	3.26	17.0	3.33	0.72
Carbohydrates, %TEI	51.0	6.24	51.3	5.89	0.61
Free sugars, %TEI	11.8	5.86	13.0	5.89	0.02
Fiber, g/d[§]	15.2	7.24	15.2	5.68	0.24
Fat, %TEI	30.0	5.78	30.0	5.66	0.91
Trans fatty acids, %TEI[§]	0.4	0.31	0.5	0.31	0.38
Saturated fatty acids, %TEI	10.8	2.51	10.7	2.86	0.62
Daily frequency of eating occasions, n/d	6.2	1.25	6.1	1.49	
Breakfast-skipping					0.004
No	361	94.8	189	88.3	
Yes	20	5.2	25	11.7	
Breakfast, h:min[§]	8:22	1:12	8:37	1:55	0.004
Lunch, h:min[§]	12:52	0:45	13:00	0:51	0.002
Dinner, h:min[§]	20:15	0:42	20:22	0:52	0.04
First EI occasion, h:min[§]	8:30	1:22	8:30	2:00	0.31
25% of TEI, h:min[§]	11:47	1:52	12:12	1:52	0.01
50% of TEI, h:min[§]	14:40	2:22	14:45	2:45	0.05
75% of TEI, h:min[§]	19:00	2:00	19:15	2:05	0.11
Last EI occasion, h:min[§]	20:45	1:15	21:02	1:15	<0.001
Eating midpoint, h:min[§‖]	14:41	1:01	14:53	1:15	0.006
Structured PA[‡]					0.004
No	127	34.2	98	46.2	
Yes	244	65.8	114	53.8	
Total Daily Physical activity	21.5	8.28	22.9	8.40	0.03

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intensity (TPA), METs/d[§]					
Time spent in Moderate to vigorous intensity PA, h:min	1:32	1:12	1:44	1:27	0.08
Time spent in Sedentary behaviour, h:min	6:33	1:53	6:57	2:21	0.03
First PA occasion, h:min[§]	08:22	1:05	08:38	1:38	0.004
25% of TPA, h:min[§]	11:56	1:01	12:38	1:24	<0.001
50% of TPA, h:min[§]	15:16	1:09	15:56	1:05	<0.001
75% of TPA, h:min[§]	18:24	0:58	18:35	1:15	<0.001
Last PA occasion, h:min[§]	21:48	0:56	22:08	1:05	<0.001
Midpoint of PA time window, h:min[§]	15:04	0:47	15:12	1:10	0.001
Sleep start weekdays, h:min[§]	22:00	0:57	22:27	1:11	<0.001
Sleep end weekdays, h:min[§]	07:35	1:00	08:00	2:22	<0.001
Sleep duration weekdays, h:min[§]	09:45	1:15	09:48	1:40	0.42
Sleep start weekends, h:min	22:30	1:15	23:00	1:49	<0.001
Sleep end weekends, h:min	09:05	1:01	09:58	1:16	<0.001
Sleep duration weekends, h:min	10:34	1:09	10:56	1:28	0.001
Midpoint of sleep on free days corrected (MSFsc), h:min	03:26	0:48	04:03	1:11	<0.001

Abbreviations: PA, physical activity; n, frequency; %, proportion; SD, standard deviation; IQR, interquartile range; y, years; zBMI, z-score body mass index; TPA, total

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daily physical activity intensity; METs, metabolic equivalent of tasks; d, day; h, hours; min, minutes; TEI, total daily energy intake; kJ, kilojoule; g, grams; EI, energy intake.

* Data are presented as frequency (n) and column proportions (%) for categorical variables; as mean and standard deviation (SD) for continuous variables or as the median and interquartile range (IQR), if these were non-normally distributed.

† P-value for Pearson's Chi-square test for categorical variables, and Student's t-test or Mann-Whitney U test for continuous variables.

‡ Maternal age based on n=514, due to missing data. Sleep on weekends and MSFsc-related variables based on n=591 due to missing data. For categorical variables, the sum of each category did not add up to the *n* of each column due to missing data.

§ Variables for which median (IQR) and P-value for Mann-Whitney U test are presented.

‖ Calculated as (clock time of last EI occasion – clock time of first EI occasion) / 2.

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Table 4. Associations of Temporal Energy Intake (EI) patterns and Physical Activity (PA) patterns with weight status (Underweight or normal weight vs. Overweight or obesity) by ordinal logistic regression analysis (OR (95%CI)).

	Overweight or obesity †														
	Model 1 (Crude)‡			**Model 2**‡			**Model 3**‡			**Model 4**‡§			**Model 5**‖		
Independent variables	OR	95% CI Lower Bound	95% CI Upper bound	OR	95% CI Lower Bound	95% CI Upper bound	OR	95% CI Lower Bound	95% CI Upper bound	OR	95% CI Lower Bound	95% CI Upper bound	OR	95% CI Lower Bound	95% CI Upper bound
Temporal EI patterns (n=714)*														NA	
EI Pattern 1 – Early afternoon & Early evening (n=144)	1			1			1			1					
EI Pattern 2 – Early afternoon & Late evening (n=498)	0.77	0.52	1.11	0.77	0.53	1.12	0.77	0.53	1.13	0.79	0.54	1.16			
EI Pattern 3 – Late morning, Early and Mid-afternoon & Late evening (n=72)	0.48	0.26	0.89	0.48	0.26	0.88	0.46	0.25	0.86	0.49	0.26	0.92			
Temporal PA patterns (n=595)*															
PA Pattern 1 – Late morning, Mid-afternoon & Early evening (n=381)	1			1			1			1			1		
PA Pattern 2 – Late afternoon (n=214)	1	0.71	1.41	0.99	0.7	1.36	0.95	0.67	1.36	0.93	0.65	1.34	0.95	0.65	1.38

Abbreviations: OR, odds ratio; CI, confidence interval; EI, energy intake; NA, not applicable, due to missing data on the covariate Midpoint of sleep on free days corrected for sleep debt (MSFsc); PA, physical activity.

* For analyses with EI and PA patterns as independent variables, Pattern 1 was the reference category, thus OR=1. The sample size of each model may not add up to 714 for EI Patterns, or 595 for PA Patterns, due to missing data on adjustment covariates.

† The outcome was modelled with *Overweight or obesity* as the response.

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‡ Models included the following variables (and sample size), respectively for EI and PA Patterns: *Model 1*: EI/PA Patterns (n=714/n=595); *Model 2:* Model 1 adjusted for Sex, Age, and Parental education (n=707/ n=589); *Model 3*: Model 2 plus healthy eating index (n=707/ n=589); *Model 4* for EI Patterns: Model 3 plus Structured PA (n=691).

§ *Model 4* for PA Patterns: Model 3 plus time spent in sedentary behaviour (n=585).

|| *Model 5* for PA Patterns: Model 4 plus MSFsc (n=574).

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