Clinical Nutrition 49 (2025) 165–177

Contents lists available at ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Original article

Nutritional profile of the diet according to circadian clock genes in the European Prospective Investigation into Cancer and Nutrition (EPIC) chronodiet study

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ARTICLE INFO

Article history:
Received 27 September 2024
Accepted 21 April 2025

Keywords:
Chronobiology
Circadian clock
Anthropometry
Nutrition
Diet habits
Genetic association analyses

SUMMARY

Background & aims: Circadian rhythms seem to impact both dietary intake and metabolism, depending on the individual's chronotype. We aimed to explore whether the nutritional composition of meals throughout the day is influenced by genetics linked to the circadian clock and chronotype within the "European Prospective Investigation into Cancer and Nutrition (EPIC) chronodiet" study.
Methods: The study population comprised 3,183 subjects with information on diet and twelve genetic variants of six genes (*PER1, PER2, PER3, CRY1, NR1D1, CLOCK*). The associations between the variants with chrononutrition variables (macronutrients and serving sizes of each meal) were evaluated using linear regression, considering an additive genetic model, and adjusting for sex, age and center, among others. The β coefficients, 95 % confidence intervals (CI), and p-values corrected for multiple comparisons were estimated. A genetic risk score (GRS) that was associated to the evening/late chronotype as well as overweight/obesity in a previous study, the chronotype-GRS, was tested for its association with chrononutrition variables.
Results: The nutritional profile of the diet differed according to the individual's chronotype, with evening/late chronotypes exhibiting an unbalanced intake during breakfast and dinner compared to the intermediate and early chronotypes (e.g., percentage of fats consumed at breakfast relative to the total fat intake: 13 % and 9 %, respectively). However, significant differences were not encountered by the chronotype-GRS. In multivariate analyses, individual associations between the genetic variants and the

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https://doi.org/10.1016/j.clnu.2025.04.023

nutrients revealed some nominal associations (e.g., rs1801260 and rs2070062 with carbohydrates at breakfast: β = −0.06 to 0.08). Higher scorings of the chronotype-GRS were inversely associated with the intake of proteins and carbohydrates (β = −0.46 and −0.41; nominal p-value<0.006; corrected = 0.25) during breakfast. Also, there was an inverse association between the chronotype-GRS and the breakfast's portion size (β = −0.3; nominal p-value = 0.03; corrected = 0.1).

Conclusions: Genetic susceptibility to an evening-like chronotype prone to overweight/obesity seems to be associated with a smaller serving size during breakfast, with lower protein and carbohydrate content.

1. Introduction

Chrononutrition is an emerging field in nutritional research that focuses on studying how circadian rhythms affect dietary intake and metabolism throughout the day [1]. The circadian rhythm follows an approximate 24-hour cycle, regulating a wide range of physiological functions and behavioral aspects including shift work, meal timing and dietary intake, amongst others [2,3]. The circadian rhythm also determines the chronotype, which ranges from more morning to more evening-like types, and which influences the individual's preference for the timing of activities such as sleeping and eating [4]. Moreover, there are several clock genes (e.g., *PER1, PER2, PER3, CRY1, NR1D1, CLOCK*) that regulate circadian rhythms, implying that these rhythms are influenced, at least in part, by genetic factors [5]. Also, genetic scores comprising a number of genetic loci belonging to clock gene variants have been associated with the evening-like chronotype in some studies [3,6,7].

Recent research has shown that synchronizing food intake with circadian rhythms can have a significant impact on metabolism regulation and overall well-being, including gut health [8,9]. It is also well-known that metabolism and response to food intake vary throughout the day, whereby earlier meal timing has been suggested to mitigate adverse metabolic effects [10]. However, the so-called chronodisruption affecting the circadian rhythm and, ultimately, eating behaviors, can lead to negative health effects [8,11]. Therefore, chrononutrition pursues the idea that meal timing and the distribution of calories and nutrients over the day should align with the body's natural biological rhythms [1]. Indeed, it has been demonstrated that this relationship is crucial for preventing obesity, type 2 diabetes, and cardiovascular diseases [11–15]. Some studies have also suggested that proper synchronization of nutrient intake with circadian rhythms may have beneficial effects on cognitive function and sleep quality [16,17]. Moreover, the timing of caloric intake has been observed to influence satiety, appetite control, and regulation of blood glucose and insulin levels, [13]. For instance, it has been reported that individuals who consume a higher proportion of calories and proteins at breakfast experience less weight gain or Metabolic Syndrome compared to those with an unbalanced diet [14,18]. Diets rich in proteins at dinner have been also related to a reduced obesity risk [1,19], whereas skipping breakfast has been associated to obesity and metabolic risk in some studies [8,13]. Some of these studies have linked breakfast skipping to certain genetic variants of clock genes (e.g., rs228697 in *PER3*) and circadian rhythm-related genes (e.g., *METTL4*), which in turn are associated with a higher body mass index (BMI) [3,20]. There are also some studies that have suggested a connection between the chronotype, chrononutrition and disease outcomes associated with metabolic deregulation, including obesity [11,21]. The evening-like chronotype, for instance, has been found to be more prone to irregular eating, breakfast skipping or consuming less energy intake early in the day, late meal intake, lower intakes of fruits and vegetables, or higher intakes of fats, saturated fats and carbohydrates (sucrose) at dinner, likely increasing the risk of adiposity and other metabolic risk factors [4,21,22].

In light of the above, the timing and nutritional composition of meals may vary according to each person's chronotype. However, no study has examined whether meal timing and corresponding nutrient intake are related to the genetics of the circadian clock and an individual's chronotype. In a previous study that we conducted within the "EPIC-Spain chronodiet" study, we found that 12 circadian clock gene variants when combined in a genetic risk score (GRS) were associated with the evening-like chronotype, and that genetic predisposition to this chronotype was related to meal timing (breakfast skipping, and later lunch and dinner times) [3]. In this previous study, it was also shown that this chronotype-GRS was associated with risk of overweight and obesity in early and late adulthood, underscoring the connection between clock genes, chronotype, and obesity. However, the influence of the nutritional composition of the diet on this risk phenotype, particularly in relation to circadian genes, was not explored.

Thus, the aim of this study was to further explore the connection between chronotype, genetics and chrononutrition variables to clarify the circadian genes–diet interaction, using genetic, dietary and nutritional data from the EPIC-Spain chronodiet study.

2. Methods

2.1. Study design

Cross-sectional study conducted within a sample of the Spanish EPIC (European Prospective Investigation into Cancer and Nutrition) cohort, "the EPIC-Spain chronodiet study". EPIC-Spain is part of the EPIC-Europe study, which is a cohort study designed to explore the role of dietary, lifestyle, environmental and genetic factors in the development of cancer and other chronic diseases. Further details can be found in other studies [23–25].

2.2. Study population

In the EPIC-Spain study, a total of 41,440 individuals were recruited between 1992 and 1996 from five regions in Spain (Asturias, Guipuzkoa, Navarra, Granada and Murcia). Detailed descriptions of the participants and their characteristics can be found elsewhere [25,26]. All enrolled in the study voluntarily, and the study was approved by the ethical review boards of the International Agency for Research on Cancer (IARC) and the Ethics Committee of the Bellvitge University Hospital.

Regarding the "EPIC-Spain chronodiet study", the participants were contacted to provide information on lifestyle and other variables between 2017 and 2019. The study population and study protocol have been described elsewhere [3,14,27]. The participants signed an informed consent and the study was also approved by the Ethics Committees of the participating centers and the Bellvitge University Hospital.

2.3. Assessment of chronotype, sleep patterns, and obesity measures

As described previously, several variables were collected within the EPIC-Spain chronodiet study [3,14,27]. For instance, chronotype data was collected by means of the Munich Chronotype Questionnaire (MCTQ) [28], and information on sleeping patterns was collected via validated questionnaires [29]. Details on variables derived from these questionnaires are described elsewhere [3,14,27]. In brief, mid-sleep time corrected for sleeping on working and weekend days (MSFc) was calculated to derive the individual's chronotype [30]. In particular, the MSFc was used to classify the participants into five different chronotypes considering sex-specific cut-off points (percentiles 2.5 %, 10 %, 90 % and 97.5 %): extreme early type, slight early type, normal or moderate type, slight late type, and extreme late type [30].

Regarding anthropometric measurements, they included weight and height, waist and hip circumference. As previously described in other EPIC studies, these measurements were taken in a standardized manner [3,23]. BMI was calculated as weight in kilograms divided by the square of height in meters. Participants were classified as non-obese (BMI<30 kg/m2) and obese (BMI≥30 kg/m2), and as normal weight (BMI<25 kg/m2) and overweight/obese (BMI≥25 kg/m2). Based on waist circumference (WC), participants were classified into three categories: normal and moderately increased (<102 cm and <88 cm), and abdominal obese (≥102 cm and ≥88 cm), men and women, respectively.

Information on laboral physical activity, non-laboral physical activity—including leisure-time, housework, and vigorous activity, were also collected, and used further to derive MET-hours/week, as the sum of recreational and vigorous activities.

2.4. Assessment of chrononutrition variables

Information on dietary intake was retrieved in the EPIC-Spain chronodiet study through a validated diet history, which served to derive data on food consumption in grams/day (g/d), number of meals and timing of each meal. As aforementioned, the relationship between the chronotype, its genetic determinants and these chronodiet variables has been explored previously [3]. In the present study, we derived further information on chrononutrition variables from the dietary questionnaire with regard to macronutrient intake per meals. Total energy intake and the intake of macronutrients was calculated using several Spanish food composition tables [31,32]. In addition, for every meal, we obtained the caloric intake and its contribution to the total dietary energy intake, as well the macronutrient profile of the meal (intake of fats, carbohydrates and proteins). Therefore, we dealt not only with the crude intake of these nutrients, but also with their relative intake (RI) with respect to the energy intake of each meal (RI per meal (%) = (intake of the nutrient in Kcal/energy intake in Kcal)*100), and with respect to the total daily intake (RI per nutrient (%) = (intake of the nutrient in grams in a given meal/total daily intake of that nutrient in grams)*100). The RI was in this way expressed as the contribution of the macronutrient to the energy intake per meal (the caloric profile), and the contribution of the macronutrient to the nutrient's overall intake (the daily contribution), respectively. In addition, information on proteins of animal and of plant origin, fiber and sugars, and on fatty acids (saturated, monounsaturated and polyunsaturated) was derived. The same was done for the overall daily intake. To account for serving sizes in each meal, we considered (energy from meal/total energy intake) * 2000 Kcal [14].

2.5. Circadian genes

As detailed elsewhere [3], there were 3,484 participants who provided blood samples for whom information on genetic data could be obtained. The genotyping was performed for 6 key circadian genes (*CLOCK:* clock circadian regulator, *CRY1:* Cryptochrome Circadian Regulator 1 or *MTERF2*, *NR1D1*: Nuclear Receptor Subfamily 1 Group D or Rev-erb-α, *PER1, PER2 and PER3*: Period Circadian Regulator 1, 2 and 3), that included 12 genetic variants (i.e., single nucleotide polymorphisms, SNPs). These variants were selected based on previous chronobiology and obesity-related studies [12,33–40]. As for quality controls, in brief, filters were applied to both SNPs and individual data. Participants with genotypic data showing a missing rate >10 % (sample call rate) were excluded leaving 3,183 individuals available for analyses. Assumptions such as the Hardy–Weinberg equilibrium (HWE) was verified and a minor allele frequency higher than 5 % was considered. Genotypes were coded to "0", for major homozygotes (reference), "1" for heterozygotes, and "2" for homozygotes bearing the minor allele or risk allele. Characteristics of these variants, the genotypes and allele frequencies are shown elsewhere [3].

2.6. Statistical analysis

Descriptive statistics by chronotype and genetically defined chronotype were conducted and included median and interquartile range (IQR) for continuous variables, and absolute and relative frequencies for the categorical ones. ANOVA and Kruskal–Wallis tests (for continuous, normal or non-normal data, respectively) or $\chi 2$ test (for categorical data), respectively, were applied to evaluate differences in the distribution of the data by these strata, as appropriate. Post-hoc analyses for comparisons of three or more groups were applied through the Tukey and Dunn tests. The $\chi 2$ test was used to test genotype frequencies in all subjects for HWE. As mentioned earlier, the HWE assumption was met.

Non-normally distributed outcome variables (all nutrient variables, in g/d or in percentages) according to Kolmogorov–Smirnov test were logarithmically transformed. Linear regression analyses were performed to test for associations between every SNP (independent variables) and the intake of the nutrients (dependent variables). Beta (β) coefficients and corresponding 95 % Confidence Intervals (CI) were derived from these analyses. Additive genetic models of inheritance were considered. Thus, for every one-unit increase in risk alleles in each SNP we expect the continuous outcomes to change by "100*(exp(β)-1)" percent or exp(β) units.

Several multivariable-adjusted models were fitted to control for potential confounders. The first model (M1) was adjusted for sex, age in years, and center. As described elsewhere [3], population stratification or genotyping batch effects were considered unlikely. Indeed, the EPIC-Spain study population could be considered genetically homogenous [41]. Nonetheless, any influence of potential genetic ancestry in our analyses was minimized by adjustment for center. The second model (M2) was further adjusted for energy intake. In a third model (M3), we considered adjustment for lifestyle factors including some variables related to chronobiology, namely energy intake, educational level (primary school, technical school, secondary school, or university degree), physical activity (MET-hours/week), and smoking status (never, former, and current smoker). Analyses were performed overall, and separately for men and women.

2.7. SNP-based genetic risk score associated with the evening/late chronotype

As described in the previous EPIC-Spain chronodiet study [3], weighted genetic risk score (GRS) was generated based on the selected genetic variants. Minor alleles of each genetic variant were weighted by their effect sizes on the evening/late chronotype *(vs* moderate and early), using the per allele β coefficients derived from multivariate regression models adjusted for age, sex and center [42]. Absolute values of the coefficients were considered as weights. The resulting chronotype-like GRS (evening/late vs moderate and early), was tested for its association with the chrononutrition variables (e.g., nutrients per meal) in order to assess whether genetic susceptibility to chronotype could subsequently lead to a certain dietary profile: chronotype-GRS→chrononutrition. Furthermore, we considered tertiles and predefined cutpoints (<0.3, 0.3 to 0.6 and >0.6) of this GRS for descriptive analyses.

In sensitivity analyses, we excluded SNPs in LD (R2>0.8) from the GRS to minimize multi-collinearity effects in regression models [43].

All p-values were two-sided, with $p < 0.05$ indicating statistical significance. P-values were also corrected for multiple comparisons by the Benjamini-Hochberg method [44]. Statistical analyses were performed with the R program version 4.0.1 (The R Foundation for Statistical Computing) [45]. We used the R packages for PredictABLE for the GRS [46], and *SNPassoc* for the association analyses [47].

3. Results

3.1. Characteristics of the study population: meal timing and nutrient intake per meal

The characteristics of the study population by the individual's chronotype and with reference to meal timing variables are shown in Supplemental Table 1 and Supplemental Table 2.

Table 1 shows the nutrient composition of the diet, overall and by meal, as well as by chronotype. The extreme-late chronotype featured a significantly higher animal-based protein intake. However, post-hoc tests did not show significant differences between the chronotypes. By meal occasions, the most relevant differences ($p < 0.05$) were observed at breakfast and dinner, where the extreme-late chronotype exhibited the lowest energy intake at breakfast (314 Kcal, with intake of fats and plant-based proteins being relatively low) compared to the slight early and moderate chronotype (Dunne's test, $p < 0.05$), but the highest intake at dinner (727 Kcal, Dunne's test, $p < 0.05$: compared to all other chronotypes), at the expense of carbohydrates, sugars, fats and animal-based proteins. Post-hoc tests revealed significant differences by carbohydrates at dinner between the groups. This pattern was opposite to the extreme-early chronotype (e.g., 367 Kcal at breakfast and 586 Kcal at dinner). Serving sizes of these meals standardized to 2000 kcal intake, also differed between the chronotypes. The evening/late chronotype showed the lowest serving size during breakfast while the highest during dinner ($p < 0.05$), this difference being significant for the extreme-late chronotype compared to the moderate and slight chronotypes (Dunne's test, $p < 0.05$). In comparison to the nutrient intake in the overall diet (% of proteins, carbohydrates and fats), as shown in Table 2, variations were observed in the intake of proteins, carbohydrates, and fats during breakfast and dinner with respect to the RI per nutrient, across the different chronotypes. Overall, at breakfast, the intake of proteins, carbohydrates and fats was lower in the extreme late chronotype compared to the slight early and moderate chronotype (Tukey's test $p < 0.05$). As for dinner, the extreme late chronotype showed the highest intake of proteins (Tukey's test $p = 0.03$: compared to all other), carbohydrates (Tukey's test $p = 0.002$: compared to the extreme early chronotype) and fats (Tukey's test $p = 0.02$: compared to the slight early chronotype). However, the caloric profile of the diet in each meal, i.e., the total energy intake or RI per meal, was consistent across the groups, except for proteins during lunch ($p = 0.02$); however, Tukey's test did not show statistically significant differences between the chronotypes. The caloric intake (Table 2 and Supplemental Fig. 1), was found to be highest among individuals with extreme and early chronotypes during breakfast but lowest during dinner within these groups ($p = 0.001$; Tukey's test $p = 0.003$ at breakfast and 0.001 at dinner).

Table 3 shows the nutrient composition of the diet, overall and by meal, by the chronotype-GRS. This genetically-proxied chronotype was found to be more prevalent in the extreme-late chronotype compared to the extreme-early type ($p = 0.03$). However, the variations of the nutritional composition of the diet by the GRS across tertiles were less pronounced and mostly non-significant, compared to the pattern observed above by the questionnaire-defined chronotypes. More specifically, as shown in Supplemental Table 3, the breakfast's energy intake did not vary by the GRS's tertiles ($p = 0.97$), possibly because the third tertile is likely to reflect the moderate, late and extreme-late phenotypes. In the highest GRS tertile, participants appeared to have lower intakes of fiber and plant-based proteins, both overall and during dinner. The caloric profile of the diet per meal did also not differ by these groups (Supplemental Table 4). For predefined cutpoints of the GRS, as shown in Tables 3 and 4, the mean intake of sugars or percentage of carbohydrates taken during lunch tended to be higher, in the third GRS group, i.e., the evening-like chronotype. No significant differences were noted for other nutrients (fiber, etc.) neither by the questionnaire-defined chronotypes nor by this chronotype-GRS.

3.2. Associations between the genetic variants and nutrient intake by meal occasion

In analyses evaluating the association between each SNP and the crude intake of the nutrients per meal (Supplemental Tables 5–8), no significant associations were observed overall, with only a few nominal associations emerging. For instance, the variants rs228697 (gene *PER3*) and rs2287161 (gene *CRY1*) tended to be positively associated with intake of sugars ($p = 0.01$; corrected $p = 0.06$, in multivariate adjusted models). By meal occasions, we observed that during breakfast (Fig. 1 and Supplemental Table 6), only the variants rs1801260 ($p = 0.01$) and rs2070062 ($p = 0.04$) of the *CLOCK* gene were negatively associated at the nominal levels with the intake of carbohydrates (corrected $p = 0.1$). At lunch (Supplemental Table 7), no SNP was significantly associated with the intake of energy or that of proteins, carbohydrates, sugars, fiber, fats or fatty acids. At dinner (Supplemental Table 8), the aforementioned *CLOCK* gene variants were associated with a higher intake of sugars ($p = 0.04$; corrected $p = 0.2$), whereas another *CLOCK* gene variant (rs3749474) was associated with a reduced intake of this nutrient ($p = 0.02$; corrected $p = 0.2$). Of note, results were similar, i.e., non-significant, when the relative intake, either as the RI per meal (caloric profile) or the RI in the overall diet, was considered (data not shown). Adjustment for covariates (M3), including energy intake (M2), had a minor effect on the associations shown in M1 (Supplemental Tables 5–8).

3.3. Associations between the chronotype-GRS and nutrient intake by meal occasion

Table 5 shows results on the association between the GRS and the crude intake of all macronutrients at breakfast, lunch and dinner. Increasing scores of the genetically-proxied evening/late

Table 1
Characteristics of the study population (3,183 individuals of the EPIC-Spain chronodiet study) by chronotype (5 types) and chrononutrition variables.

	1: Extreme early; N = 81		2: Slight early; N = 222		3: Moderate; N = 2,505		4: Slight late; N = 234		5: Extreme late; N = 79		p-value[a]
	Median	[p25; p75]	Median	[p25; p75]	Median	[p25; p75]	Median	[p25; p75]	Median	[p25; p75]	
Total intake per day											
Energy Kcal	2420	[2205; 2721]	2385	[2180; 2737]	2426	[2166; 2733]	2506	[2167; 2789]	2449	[2224; 2815]	0.303
Carbs (g)	245	[215; 290]	255	[224; 288]	252	[222; 286]	256	[221; 291]	259	[231; 301]	0.424
Sugars (g)	92.6	[79.4; 121]	91.7	[77.5; 113]	92.7	[75.5; 111]	90.0	[74.7; 108]	94.4	[79.8; 122]	0.558
Fiber (g)	40.4	[32.4; 46.7]	37	[29.1; 44.2]	36.7	[30.5; 43.8]	35.8	[30.6; 42.7]	35.1	[28.7; 43.0]	0.286
AB-prot (g)	69.9	[57.6; 82.0]	67.8	[55.0; 81.9]	67.9	[56.8; 81.3]	69.9	[60.2; 84.5]	73.6	[60.2; 90.6]	**0.035**
PB-prot (g)	44	[39.6; 50.3]	44.5	[38.4; 51.8]	44	[37.3; 50.7]	43.1	[38.2; 51.1]	41.7	[37.3; 52.3]	0.943
Fats (g)	103	[85.8; 114]	98.7	[82.6; 117]	98.9	[84.8; 117]	102	[86.2; 120]	98.6	[81.5; 114]	0.515
SFA (g)	26.2	[21.4; 30.6]	25.2	[20.4; 32.5]	26.2	[21.3; 32.5]	27.5	[22.1; 33.9]	26.5	[20.0; 35.2]	0.335
MUFA (g)	46.8	[39.9; 51.5]	44.3	[36.3; 53.0]	44.8	[37.4; 53.4]	45.8	[39.1; 53.5]	43.5	[35.9; 52.4]	0.406
PUFA (g)	19	[14.2; 23.3]	17.4	[13.6; 22.6]	17.4	[13.7; 22.7]	17.9	[14.1; 22.4]	17.7	[14.0; 22.9]	0.666
Breakfast											
Energy Kcal	367	[260; 486]	379	[281; 504]	362	[265; 467]	348	[248; 458]	314	[228; 401]	**0.006**
Serving size	296	[236; 381]	313	[236; 404]	296	[222; 384]	286	[202; 375]	261	[177; 336]	**0.002**
Carbs (g)	49.2	[32.6; 73.9]	49	[37.9; 67.2]	49.2	[36.0; 65.3]	46.9	[33.7; 65.0]	44.9	[30.7; 57.9]	0.103
Sugars (g)	26.5	[13.9; 42.2]	22.9	[15.0; 33.8]	23.2	[15.7; 33.7]	23.0	[13.7; 33.0]	22.3	[13.5; 29.4]	0.165
Fiber (g)	3.21	[1.75; 6.26]	3.08	[1.91; 5.59]	3.24	[1.83; 5.30]	2.69	[1.45; 5.08]	2.88	[1.75; 4.43]	0.067
AB-prot (g)	6.6	[3.30; 9.60]	6.55	[3.20; 9.60]	6.6	[3.64; 9.15]	6.6	[4.00; 8.63]	6.6	[3.50; 8.11]	0.934
PB-prot (g)	5.8	[2.46; 8.23]	6.39	[3.98; 8.80]	5.93	[3.60; 7.94]	5.34	[3.24; 7.51]	5.62	[3.32; 7.20]	**0.008**
Fats (g)	11.2	[5.72; 18.6]	12	[6.36; 17.7]	10.7	[5.97; 17.2]	10.4	[5.61; 16.2]	8.38	[4.15; 11.9]	**0.012**
SFA (g)	2.9	[1.64; 5.33]	3.65	[1.75; 5.63]	3.23	[1.73; 5.51]	3.45	[1.61; 5.97]	2.59	[1.03; 4.02]	**0.039**
MUFA (g)	4.26	[1.60; 7.60]	4.35	[2.07; 8.20]	4.13	[1.80; 7.25]	3.84	[1.60; 6.88]	2.83	[1.44; 4.96]	**0.012**
PUFA (g)	1.21	[0.52; 3.04]	1.34	[0.70; 2.77]	1.22	[0.67; 2.31]	1.15	[0.60; 1.98]	1.02	[0.38; 1.77]	**0.037**
Lunch											
Energy Kcal	1139	[922; 1265]	1115	[918; 1325]	1155	[959; 1360]	1160	[991; 1368]	1127	[994; 1351]	0.348
Serving size	938	[772; 1074]	928	[784; 1038]	947	[833; 1063]	939	[827; 1061]	926	[830; 1031]	0.308
Carbs (g)	113	[92.9; 132]	114	[88.3; 138]	116	[94.3; 138]	117	[95.8; 140]	124	[98.8; 146]	0.294
Sugars (g)	23.6	[15.5; 29.9]	26.7	[18.9; 34.1]	27.2	[20.4; 35.1]	26.9	[20.4; 34.0]	25.6	[18.8; 39.8]	**0.042**
Fiber (g)	22.6	[17.2; 27.9]	22	[16.3; 26.9]	22.3	[17.6; 27.7]	22.5	[17.4; 27.0]	20.5	[17.2; 26.0]	0.586
AB-prot (g)	31.3	[27.0; 43.4]	33.9	[27.2; 40.9]	33.8	[26.8; 41.2]	34	[27.7; 42.3]	35.8	[27.4; 41.6]	0.789
PB-prot (g)	25.1	[19.7; 29.7]	24.2	[19.4; 30.2]	24.9	[19.8; 30.5]	24.8	[20.2; 29.8]	24.6	[19.4; 30.5]	0.884
Fats (g)	45.2	[36.6; 56.6]	44	[36.2; 55.7]	45.8	[35.9; 57.8]	48.2	[38.9; 56.5]	44.6	[38.0; 52.3]	0.403
SFA (g)	10.5	[8.10; 13.7]	9.88	[7.71; 13.5]	10.6	[7.96; 14.0]	11	[8.50; 14.1]	11.4	[8.05; 13.2]	0.303
MUFA (g)	22.2	[17.1; 27.5]	22.6	[17.6; 27.4]	22.6	[17.5; 28.3]	23	[18.5; 27.9]	20.8	[18.7; 25.1]	0.598
PUFA (g)	7.25	[5.72; 9.51]	7.4	[5.65; 9.37]	7.51	[5.84; 9.78]	7.88	[6.36; 9.77]	7.51	[5.82; 9.56]	0.210
Dinner											
Energy Kcal	586	[431; 748]	598	[465; 764]	614	[479; 772]	619	[472; 790]	727	[541; 891]	**0.003**
Serving size	495	[355; 570]	496	[398; 593]	511	[407; 615]	515	[382; 602]	586	[454; 674]	**0.001**
Carbs (g)	46.6	[32.6; 61.0]	53.6	[40.8; 69.7]	53.5	[40.6; 68.2]	52	[38.6; 66.6]	58.7	[48.5; 76.2]	**0.001**
Sugars (g)	15.4	[9.24; 23.1]	19.3	[13.4; 26.0]	18.4	[11.6; 25.8]	17.1	[9.78; 24.9]	20.2	[13.1; 31.2]	**0.025**
Fiber (g)	6.37	[3.98; 9.43]	6.66	[4.51; 9.45]	6.74	[4.59; 9.59]	6.53	[4.31; 9.65]	7.52	[5.27; 10.4]	0.314
AB-prot (g)	20.9	[14.7; 28.5]	20.1	[12.8; 28.3]	21.4	[15.2; 29.1]	23.1	[16.2; 31.1]	25.2	[16.7; 34.2]	**0.005**
PB-prot (g)	9.08	[6.04; 11.7]	9.02	[6.34; 12.2]	8.99	[6.45; 11.6]	8.84	[6.51; 11.8]	9.51	[7.47; 12.1]	0.289
Fats (g)	25.7	[19.4; 38.8]	26.3	[18.1; 35.7]	27.9	[19.2; 38.0]	28.2	[18.4; 40.1]	33	[21.9; 44.8]	**0.035**
SFA (g)	6.56	[4.62; 9.52]	6.89	[4.60; 9.96]	7.13	[4.98; 10.1]	7.36	[5.03; 10.7]	7.68	[5.59; 11.9]	0.095
MUFA (g)	12	[7.64; 16.8]	11.2	[7.39; 16.0]	11.8	[7.94; 16.9]	12.5	[8.00; 17.6]	12.5	[9.25; 18.5]	0.163
PUFA (g)	4.62	[2.82; 6.74]	4.08	[2.46; 7.04]	4.52	[2.83; 7.22]	4.8	[2.85; 8.23]	5.93	[3.35; 8.50]	**0.026**

Significant p-values are highlighted in bold.
Significant post-hoc tests for: Breakfast (Energy: 2–5, 3–5; Serving size: 2–5, 3–5; Carbs: 1–5, 2–5, 3–5; PB-prot: 2–3, 2–5; Fats and fatty acids: 2–5, 3–5), and Dinner (Energy: 1–5, 2–5, 3–5, 4–5; Serving size. 2–5, 3–5, 4–5; Carbs: 2–5, 3–5, 4–5; Fats and PUFA: 2–5).
Abbreviations: Carbs (Carbohydrates), SFA (saturated fatty acids), MUFA (mono-unsaturated fatty acids), PUFA (poly-unsaturated fatty acids), PB-prot (plant-based protein), AB-prot (animal-based protein). Serving size refers to energy contribution per meal in overall diet per 2000 Kcal.
1 Numbers do not sum up due to missing data for chronotype in 62 participants.
[a] p-values derived from Kruskal–Wallis tests, where appropriate, accounting for pairwise comparison correction.

chronotype was significantly associated with a lower serving size at breakfast ($\beta = -0.28$, $p = 0.03$). However, this finding did not remain significant after correction for multiple comparison (corrected $p = 0.1$). No further significant associations were observed.

Table 6 shows associations for relative intake of the macronutrients per meal. Interestingly, the relative intake of proteins and carbohydrates during breakfast, with respect to the energy intake in this meal, was significantly reduced for increased values of this chronotype-GRS ($\beta = -0.4$ for both proteins and carbohydrates, $p = 0.005–0.007$, corrected $p = 0.1$). There were no significant associations with the relative intake of these macronutrients at lunch or dinner. Adjustment of energy intake (M2) or other lifestyle variables (M3: smoking, physical activity or educational level) did not influence these associations (data not shown).

3.4. Subgroup and sensitivity analyses

No variations in the results were observed when restricting the GRS analyses to SNPs that were not in LD with each other (data not shown). Also, we did not observe major differences in analyses stratified by sex (data not shown).

4. Discussion

In this study, conducted within the EPIC-Spain chronodiet study, we investigated the association between circadian-related SNPs, the individuals' chronotypes, and nutrient intake patterns. More specifically, we focused on analyzing nutrient intake at each meal rather than meal timing, according to genetic variation in the

Table 2
Characteristics of the study population (3,183 individuals of the EPIC-Spain chronodiet study) by chronotype, the caloric profile of the diet (RI per meal) and the contribution of the nutrient intake to the overall diet (RI per nutrient).

	1: Extreme early type; N = 81		2: Slight early type; N = 222		3: Moderate type; N = 2,505		4: Slight late type; N = 234		5: Extreme late type; N = 79		
	Mean	(SD)	Mean	(SD)	Mean	(SD)	Mean	(SD)	Mean	(SD)	p-value[a]
Breakfast											
% of total E	16.4	(10.1)	16	(7.1)	15.3	(6.8)	14.6	(7.1)	12.4	(5.8)	**0.001**
% proteins[b]	14.6	(6.5)	14.4	(6.1)	14.7	(5.8)	14.7	(6.0)	16.6	(7.7)	0.062
% carbs[b]	57	(15.5)	55.7	(14.6)	56.3	(13.0)	56	(14.6)	58.8	(13.6)	0.457
% fats[b]	28	(15.0)	29	(15.1)	28.3	(13.5)	28	(13.8)	24.4	(15.0)	0.121
% proteins[c]	12.3	(8.5)	11.9	(5.5)	11.5	(5.4)	11.1	(5.6)	9.8	(4.5)	**0.021**
% carbs[c]	21.9	(11.3)	20.7	(9.1)	20.3	(8.7)	19.4	(9.4)	16.9	(7.9)	**0.002**
% fats[c]	13.3	(11.9)	13.5	(9.6)	12.5	(8.7)	11.6	(8.0)	9.21	(6.7)	**0.002**
Lunch											
% of total E	46.1	(10.1)	46	(9.6)	47.2	(8.6)	47.1	(8.7)	46.4	(6.8)	0.224
% proteins[b]	21.4	(3.41)	21.5	(4.4)	20.8	(3.5)	20.9	(3.3)	20.9	(3.6)	**0.025**
% carbs[b]	39.5	(7.8)	40.1	(6.7)	40.1	(6.8)	40	(6.0)	41.8	(5.8)	0.207
% fats[b]	36.8	(6.67)	36.1	(6.0)	36.3	(6.4)	36.6	(5.6)	34.7	(5.2)	0.183
% proteins[c]	52	(11.8)	52.9	(9.5)	52.5	(8.9)	52.7	(9.4)	51.4	(8.6)	0.717
% carbs[c]	44.9	(10.8)	44.2	(10.4)	45.7	(9.5)	46	(9.6)	46.3	(8.5)	0.176
% fats[c]	45.9	(14.1)	46.2	(13.4)	47	(11.6)	47.2	(11.4)	46	(10.0)	0.689
Dinner											
% of total E	24	(9.1)	25.1	(8.5)	25.7	(7.8)	25.3	(7.9)	28.8	(7.7)	**0.001**
% proteins[b]	21.2	(5.6)	20	(6.0)	20.6	(5.3)	20.8	(5.5)	20.6	(5.7)	0.433
% carbs[b]	35.4	(16.2)	38	(12.1)	35.9	(11.4)	35.3	(12.2)	35.7	(11.2)	0.106
% fats[b]	41.1	(13.9)	39.4	(11.5)	40.6	(10.5)	41.1	(10.2)	40.7	(9.9)	0.498
% proteins[c]	26.6	(10.5)	26.6	(9.4)	27.9	(8.5)	27.9	(8.9)	30.8	(8.6)	**0.004**
% carbs[c]	19.5	(8.9)	22.1	(7.9)	21.6	(7.5)	21	(7.4)	23.9	(8.4)	**0.003**
% fats[c]	27.1	(12.9)	27.6	(12.2)	29	(11.7)	28.8	(11.4)	33.3	(11.1)	**0.003**

Significant p-values are highlighted in bold.
Significant post-hoc tests for: Breakfast (% Energy: 1–5; % proteins: 2–5, 3–5; % carbs: 1–5, 2–5, 3–5; % fats: 1–5, 2–5, 3–5) and Dinner (% Energy: 1–5, 2–5, 3–5; % proteins: 1–5, 2–5, 3–5; % carbs: 1–5; % fats: 1–5, 2–5, 3–5).
Abbreviations: E (energy), Carbs (Carbohydrates).
1 Numbers do not sum up due to missing data for chronotype in 62 participants.
2 The caloric profile of the diet does not sum up since the contribution of alcohol intake was not taken into consideration.
[a] p-values derived from ANOVA tests, accounting for pairwise comparison correction. Mean and (SD) are shown.
[b] RI per meal.
[c] RI per nutrient.

circadian clock and by chronotype. It is worth highlighting that our study is the first to investigate whether genetic variants associated with the circadian rhythm, individually and in combination, influence eating patterns from a nutritional perspective.

Our findings revealed non-significant associations between the SNPs and nutrient intake, except for certain variants of the *CLOCK* gene and carbohydrate intake at breakfast. Notably, a genetically-proxied evening/late chronotype, the chronotype-GRS, which was built based on selected circadian-related SNPs in a previous study [3], showed a nominal association with reduced food intake at breakfast, driven by a lower protein and carbohydrate intake during this meal. However, these associations did not remain significant after correcting for multiple comparisons, and thus should be interpreted with caution. While we cannot draw definitive conclusions, we considered it as a potential finding given the candidate nature of our study. Nevertheless, although candidate studies often explore specific hypotheses, statistical rigor is crucial to avoid false positives [44]. Besides, the lack of significance after correction could be explained by the complexity of the interactions between genetic and environmental factors influencing food intake and meal timing. Therefore, further replication in independent cohorts is needed to confirm the observed associations.

In our previous study [3], an association between the chronotype-GRS and obesity was demonstrated, as well as an association with later meal timing and breakfast skipping. Within the EPIC-study [14,27], we have also evidenced associations between the breakfast's portion size and carbohydrate content with metabolic disorders, underscoring the importance of the nutritional profile of breakfast in preventing obesity during adulthood.

The results of the current study agree with previous findings, particularly in the context of chronotype and nutrient intake. Studies have shown that individuals with morning-like chronotypes tend to have a greater appetite in the morning, which leads them to consume a larger breakfast, whereas those with evening-like chronotypes exhibit reduced hunger in the morning, potentially leading them to skip breakfast or opt for a lighter meal [1,4]. This observation is consistent with our study, where the genetically-proxied evening/late chronotype was associated with reduced food intake at breakfast, especially with lower protein and carbohydrate intake. In fact, our results suggest that this specific chronotype might be at a higher risk of obesity due to disrupted eating patterns, such as skipping or consuming smaller breakfasts. Thus, our findings reinforce the connection between evening chronotypes and metabolic risk factors, in line with evidence from other studies linking evening chronotype to obesogenic behaviors, such as irregular eating patterns, breakfast skipping, and the consumption of high-energy or nutrient-poor foods [48,49]. The genetic link with the above has been accounted for in only a few studies. For instance, in a Spanish study of 420 individuals, some genetic variants of *CLOCK* genes (e.g., rs4580704) were related to late eaters, as well as to more evening types, less energetic breakfasts and slower weight loss patterns [15], too. Nevertheless, this genetic variant did not play a significant role in our study. Overall, the association between *CLOCK* gene variants and traits of obesity is still unclear [4,7,13]. The results of our study indicate that the evening chronotype could be prone to an imbalanced nutritional profile, particularly at breakfast, which may contribute to the development of obesity over time. This finding is supported by

Table 3
Characteristics of the study population (3,183 individuals of the EPIC-Spain chronodiet study) by the genetically-proxied chronotype (predefined cutpoints of the GRS) and chrononutrition variables.

	Group 1 GRS; N = 791		Group 2 GRS; N = 2,001		Group 3 GRS; N = 391		p-value[a]
	Median	[p25; p75]	Median	[p25; p75]	Median	[p25; p75]	
Total intake per day							
Energy Kcal	2415	[2173; 2758]	2435	[2172; 2747]	2422	[2149; 2710]	0.590
Carbs (g)	253	[223; 288]	252	[220; 286]	253	[225; 289]	0.474
Sugars (g)	93.4	[76.5; 112]	91.8	[75.4; 111]	94.1	[79.0; 112]	0.165
Fiber (g)	37.3	[30.5; 44.5]	36.6	[30.0; 43.8]	36.4	[30.7; 43.5]	0.200
AB-prot (g)	67.9	[56.8; 82.0]	68.6	[57.2; 81.8]	68.3	[55.8; 81.4]	0.851
PB-prot (g)	44.5	[37.5; 51.5]	43.8	[37.4; 50.5]	42.7	[37.5; 50.5]	0.336
Fats (g)	98.1	[84.5; 118]	100	[85.4; 117]	97.2	[82.0; 113]	0.110
SFA (g)	25.5	[21.2; 33.1]	26.5	[21.4; 32.4]	25.8	[20.8; 32.8]	0.746
MUFA (g)	44.5	[37.4; 53.3]	45.3	[37.8; 53.6]	44.3	[36.5; 52.1]	0.103
PUFA (g)	17.6	[13.7; 22.9]	17.6	[13.9; 22.6]	16.9	[13.0; 22.0]	0.063
Breakfast							
Energy Kcal	362	[260; 462]	358	[266; 461]	375	[265; 482]	0.527
Serving size	294	[216; 384]	295	[220; 379]	305	[228; 401]	0.250
Carbs (g)	49.3	[35.8; 66.1]	49	[36.1; 64.2]	49.1	[33.8; 68.9]	0.897
Sugars (g)	22.8	[15.6; 33.5]	23.7	[15.4; 33.6]	22.5	[15.2; 34.3]	0.927
Fiber (g)	3.13	[1.83; 5.21]	3.19	[1.78; 5.31]	3.32	[1.83; 5.50]	0.754
AB-prot (g)	6.6	[3.41; 9.44]	6.6	[3.43; 9.06]	6.49	[4.43; 9.55]	0.685
PB-prot (g)	6.02	[3.52; 7.86]	5.86	[3.58; 7.94]	5.87	[3.43; 8.18]	0.902
Fats (g)	10.3	[5.62; 16.8]	10.7	[5.89; 17.1]	11.3	[6.20; 18.1]	0.317
SFA (g)	3.3	[1.75; 5.30]	3.14	[1.61; 5.47]	3.61	[1.79; 5.74]	0.175
MUFA (g)	3.94	[1.71; 7.38]	4.06	[1.74; 7.15]	4.35	[2.00; 7.31]	0.592
PUFA (g)	1.15	[0.61; 2.32]	1.22	[0.65; 2.30]	1.27	[0.68; 2.40]	0.333
Lunch							
Energy Kcal	1142	[982; 1363]	1156	[957; 1357]	1159	[943; 1344]	0.883
Serving size	953	[836; 1055]	943	[825; 1063]	941	[832; 1062]	0.812
Carbs (g)	117	[96.1; 139]	115	[93.2; 139]	117	[96.9; 138]	0.224
Sugars (g)	27.5	[20.8; 35.7]	26.7	[19.8; 34.5]	27.8	[21.1; 35.0]	**0.041**
Fiber (g)	22.5	[17.7; 28.1]	22.1	[17.3; 27.4]	22.4	[17.7; 27.7]	0.182
AB-prot (g)	33.9	[26.9; 41.6]	34	[27.0; 41.5]	33.2	[26.5; 40.4]	0.382
PB-prot (g)	25.1	[19.9; 30.7]	24.6	[19.7; 30.4]	25.3	[19.8; 30.0]	0.319
Fats (g)	45.7	[36.8; 57.8]	45.9	[36.0; 57.8]	45.4	[35.3; 56.6]	0.597
SFA (g)	10.5	[8.07; 14.5]	10.6	[8.01; 13.9]	10.6	[7.80; 13.8]	0.626
MUFA (g)	22.4	[17.9; 28.3]	22.6	[17.5; 28.3]	22.5	[17.4; 27.1]	0.734
PUFA (g)	7.59	[5.99; 9.88]	7.56	[5.83; 9.78]	7.39	[5.72; 9.36]	0.334
Dinner							
Energy Kcal	615	[493; 769]	618	[470; 780]	593	[466; 771]	0.675
Serving size	516	[409; 612]	511	[403; 618]	503	[408; 609]	0.871
Carbs (g)	52.9	[40.2; 67.2]	53.5	[40.1; 68.9]	53.8	[41.2; 69.4]	0.769
Sugars (g)	18.2	[11.2; 25.7]	18.4	[11.8; 25.8]	19.2	[11.4; 26.1]	0.602
Fiber (g)	6.77	[4.64; 10.0]	6.72	[4.57; 9.59]	6.7	[4.44; 9.32]	0.790
AB-prot (g)	21.6	[15.4; 30.0]	21.4	[15.1; 28.9]	21.5	[14.5; 29.8]	0.733
PB-prot (g)	8.93	[6.58; 11.8]	9.08	[6.40; 11.8]	8.67	[6.22; 11.4]	0.355
Fats (g)	28	[19.4; 38.5]	28	[19.1; 37.8]	26.3	[18.0; 37.3]	0.225
SFA (g)	7.16	[5.16; 9.98]	7.1	[4.94; 10.1]	6.95	[4.84; 10.3]	0.656
MUFA (g)	12.2	[8.00; 17.2]	11.9	[7.98; 16.8]	10.9	[7.47; 16.8]	0.222
PUFA (g)	4.77	[2.84; 7.67]	4.55	[2.81; 7.23]	4.3	[2.64; 6.93]	0.113

Significant p-values are highlighted in bold.
Abbreviations: Carbs (Carbohydrates), SFA (saturated fatty acids), MUFA (mono-unsaturated fatty acids), PUFA (poly-unsaturated fatty acids), PB-prot (plant-based protein), AB-prot (animal-based protein). Serving size refers to energy contribution per meal in overall diet per 2000 Kcal.
1 Numbers do not sum up due to missing data for chronotype in 62 participants.
[a] p-values derived from Kruskal–Wallis tests, where appropriate, accounting for pairwise comparison correction.

other studies reporting associations between chronotype, breakfast skipping and weight gain or obesity risk [3,4,22,27,48].

Recently, a causal link through Mendelian Randomization analyses has been shown between circadian gene variants, meal timing and breakfast skipping, the evening chronotype and obesity [20]. In our previous research, [3], we also provided insight on the interaction between genetics, the individual's chronotype, the meal timing and obesity. Thus, it is plausible that the evening/late chronotype is at a higher obesity risk due to an unbalanced nutritional profile at breakfast and/or breakfast skipping. Indeed, it has been suggested that a high-energy breakfast diet has synchronizing actions on circadian clock genes, leading to improved glucose metabolism, postprandial glycemic excursions, weight loss and reduced appetite in type 2 diabetes mellitus patients and healthy adults [18,50,51]. This also supports the notion that proper meal timing, particularly breakfast, could be crucial for preventing metabolic disorders such as obesity, and highlights the relevance of chrononutrition in personalized nutrition strategies.

4.1. Associations between the chronotype, the SNPs and the nutrient intake

Associations between chronotype and timing of energy and nutrient intakes in adults were examined among 1,854 adults of the FINRISK and FINDIET studies [21]. In these studies, the evening-like type presented a lower energy intake by 10 am, i.e., at breakfast, as well as a lower intake of carbohydrates, proteins and fat, meanwhile at dinner (after 8 pm), the pattern was reversed. The dietary

Table 4
Characteristics of the study population (3,183 individuals of the EPIC-Spain chronodiet study) by the genetically-proxied chronotype (predefined cutpoints of the GRS), the caloric profile of the diet (RI per meal) and the contribution to the overall diet (RI per energy intake).

	Group 1 GRS; N = 791		Group 2 GRS; N = 2,001		Group 3 GRS; N = 391		p-value[a]
	Mean	(SD)	Mean	(SD)	Mean	(SD)	
Breakfast							
% of total E	15.1	(6.8)	15.3	(7.1)	15.6	(6.6)	0.526
% proteins[b]	14.9	(5.8)	14.7	(6.0)	14.6	(5.6)	0.690
% carbs[b]	56.9	(13.0)	56.2	(13.5)	55.6	(13.6)	0.211
% fats[b]	27.7	(13.7)	28.2	(13.8)	29	(13.7)	0.294
% proteins[c]	11.5	(5.6)	11.5	(5.5)	11.9	(5.5)	0.418
% carbs[c]	20.2	(8.7)	20.3	(8.9)	20.2	(8.7)	0.954
% fats[c]	12.2	(8.6)	12.5	(9.0)	13.1	(8.8)	0.237
Lunch							
% of total E	47.3	(8.6)	47	(8.8)	47.3	(8.1)	0.659
% proteins[b]	20.9	(3.7)	20.9	(3.5)	20.7	(3.1)	0.506
% carbs[b]	40.3	(6.6)	39.9	(6.7)	40.9	(6.7)	**0.020**
% fats[b]	36.3	(6.1)	36.4	(6.4)	35.7	(6.4)	0.124
% proteins[c]	52.6	(8.9)	52.6	(9.1)	52.5	(9.1)	0.966
% carbs[c]	46	(9.6)	45.5	(9.7)	45.8	(8.7)	0.419
% fats[c]	47	(11.7)	46.9	(11.8)	47.2	(11.7)	0.895
Dinner							
% of total E	25.6	(7.5)	25.7	(8.1)	25.5	(7.8)	0.889
% proteins[b]	20.7	(5.5)	20.5	(5.3)	20.6	(5.3)	0.839
% carbs[b]	35.5	(12.1)	36	(11.4)	37	(11.9)	0.104
% fats[b]	41.1	(10.9)	40.5	(10.5)	39.7	(10.4)	0.093
% proteins[c]	27.9	(8.4)	27.8	(8.8)	28	(9.1)	0.960
% carbs[c]	21.3	(7.4)	21.8	(7.7)	21.7	(7.9)	0.335
% fats[c]	29.1	(11.4)	28.9	(12.0)	28.7	(11.7)	0.855

Significant p-values are highlighted in bold.
Abbreviations: E (energy), Carbs (Carbohydrates).
1 Numbers do not sum up due to missing data for chronotype in 62 participants.
2 The caloric profile of the diet does not sum up since the contribution of alcohol intake was not taken into consideration.
[a] p-values derived from ANOVA tests, accounting for pairwise comparison correction. Mean and (SD) are shown.
[b] RI per meal.
[c] RI per nutrient.

information relied on 48 hour-recalls and the chronotype was assessed by a short screener. Our study confirms that the evening chronotype as compared with the morning type, has a lower energy and macronutrient intake during breakfast. Earlier studies also suggested differences by chronotype with regard to the intake of fatty foods at dinner [52]. However, findings regarding the association between evening chronotypes and the intake of specific foods or nutrients has been inconsistent across various studies [4].

Few studies have also examined the impact that genetic variants of the circadian clock have on macronutrient intake [52–54]. For instance, the study by Garaulet *et al.* in 2014, conducted among 2,214 Spanish and North American subjects, identified a specific circadian gene variant, *REV-ERB-ALPHA1* rs2314339 [53], which was associated with obesity and monounsaturated fatty acid intake. In another Spanish study of 898 subjects, the wild type allele of the rs3749474 variant of the *CLOCK* gene was associated with a reduced evening carbohydrate intake [54]. Interestingly, risk alleles of this genetic variant were also associated with a reduced intake of sugars at dinner in our study, albeit at a nominal level. However, the impact of circadian gene variants on specific patterns of carbohydrate, protein, and fat intake throughout the day remains unknown. Our study addressed the examination of multiple genetic variants of the circadian system with the daily intake of macronutrients and specific meal occasions, thereby enhancing the existing knowledge on this topic. In addition to the aforementioned result, we have identified two genetic variants of the *CLOCK* gene (rs1801260 and rs2070062) that appear to be associated with a reduced intake of carbohydrates at breakfast, suggesting that this gene–diet interaction may influence eating preferences at this mealtime. While this result lost statistical significance after multiple testing correction, this finding may have significant implications, especially considering recent studies indicating that a low-carbohydrate diet, especially at breakfast, can induce various metabolic dysfunctions [1,27,55,56].

4.2. Associations between the genetically-proxied chronotype and the nutrient intake

The combination of all circadian gene variants in a GRS, previously associated with obesity and related factors, including meal timing and breakfast skipping, and the evening/late chronotype in the EPIC-Spain chronodiet study [3], was used to examine whether genetic predisposition to this chronotype plays a role in determining the diet's nutrient profile. The genetics of the individual's chronotype has been unraveled in some other studies [5,6,20], which established associations between genetic variation of the chronotype in relation to body weight [5,6], or food timing [20]. However, none of them explored further whether genetic variants of the circadian clock may influence metabolic responses to food and nutrient intake. Our chronotype-GRS tended to be associated with an unhealthier dietary pattern at breakfast, which could in turn lead to circadian rhythm misalignment and obesity during adulthood. For the above given reasons, eating more calories, carbohydrates and proteins at breakfast could be a beneficial dietary intervention for obesity prevention.

This study's strengths lie in its substantial sample size, comprehensive data collection, assessment of the individual's chronotype by both questionnaire and genetic data, as well as the availability of structured dietary data categorized by meals from each participant. This enabled us to focus specifically on breakfast

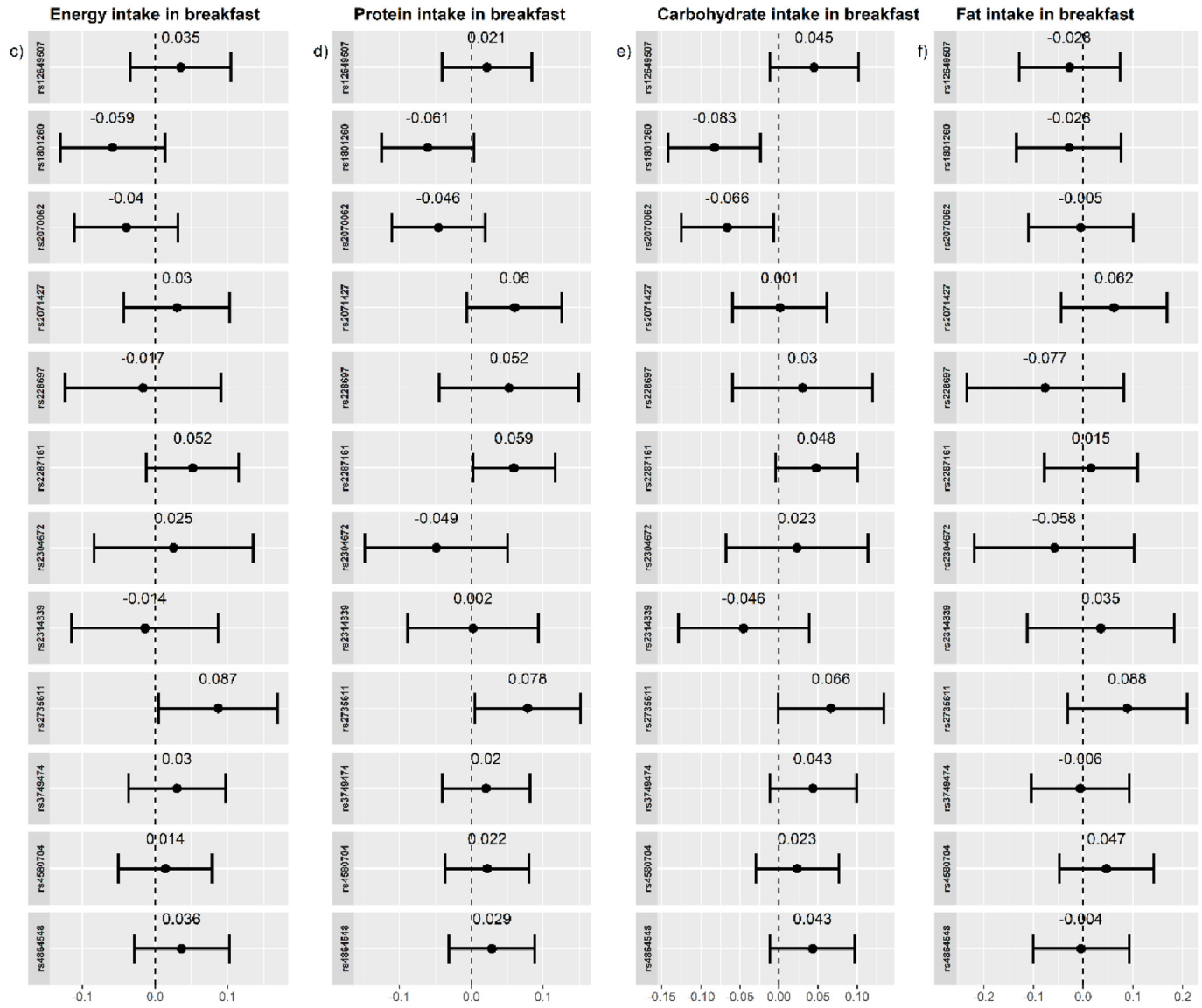

Fig. 1. Coefficient plot of the association between individual SNPs and the overall caloric intake in breakfast (c), and that supplied by macronutrients (d to f), among 3,183 individuals of the EPIC-Spain chronodiet study. Coefficient plots of the association between SNPs and dietary variables (Energy intake in Kcal/day and macronutrients in g/day) among 3183 individuals of the EPIC-Spain chronodiet study. Point estimates for β coefficients and horizontal lines for 95 % CI (x axis) are provided for every SNP (y axis). Specifically, the x-axis scale represents the magnitude and direction of the association, with negative values indicating a decrease and positive values an increase in the dietary variable associated with each SNP. Thus, the point estimates reflect the increase or decrease in the dietary variable per unit increase in the number of risk alleles.
Multivariable linear regression adjusted for age in years, sex (male, female) and center (Asturias, Gipuzkoa, Navarra, Granada and Murcia), total energy intake (Kcal/d) – except in analyses involving energy intake as Y variable, physical activity (Mets-h/week), educational level (none, primary, secondary, technical studies, higher education), smoking habit (never, former and smoker). Additive genetic model. Dietary variables were log-transformed to approximate a normal distribution. Corrected p-values for multiple testing are not shown. All were >0.05.

choices, which, as mentioned earlier, have been linked to various outcomes in numerous studies. The chronotype-GRS allowed us to evaluate the cumulative effect of all circadian clock genetic variants, which individually showed a non-significant effect on the chrononutrition variables. By using this GRS, which was previously associated with obesity [3], we were able to demonstrate that genetic susceptibility to this chronotype results in reduced consumption of proteins and carbohydrates during breakfast. Thus, our study is the first to investigate a link between an individual's chronotype, genetic predisposition, and the nutritional preferences at breakfast, lunch, and dinner. Additionally, it is the first to demonstrate that a reduced relative contribution of proteins and carbohydrates during breakfast among evening/late chronotypes may be associated with a less healthy phenotype (for example, obesity) within this group. Although multiple comparison corrections may not have been entirely necessary given that this is a candidate association study, we applied them to minimize the risk of false positives.

There are also some limitations of this study to note. The first one concerns the limited ability to apply our findings to other populations due to the specific characteristics of our study sample. While errors in measurement are common in nutritional studies, we tried minimize this issue by using the diet history method and food composition tables tailored to the Spanish diet [24]. The effect sizes of the chronotype-GRS were calculated using data from our study population, which means that our results could be too closely

Table 5
Association between the genetically-proxied chronotype and the chrononutrition variables in the EPIC-Spain chronodiet study.

	Model 1					Model 2				
	β	LCI	UCI	R^2	p-value	β	LCI	UCI	R^2	p-value
Breakfast										
Energy Kcal	−0.308	−0.658	0.042	0.009	0.084	−0.295	−0.645	0.054	0.011	0.098
Serving size	−0.283	−0.629	0.063	0.011	**0.026**	−0.294	−0.640	0.052	0.013	**0.030**
Carbs (g)	−0.312	−0.638	0.015	0.006	0.061	−0.298	−0.624	0.028	0.009	0.073
Sugars (g)	−0.100	−0.515	0.316	0.006	0.639	−0.083	−0.498	0.332	0.009	0.694
Fiber (g)	−0.393	−1.337	0.551	0.016	0.415	−0.378	−1.322	0.567	0.016	0.433
AB-prot (g)	−0.352	−1.694	0.990	0.021	0.607	−0.331	−1.673	1.012	0.021	0.629
PB-prot (g)	−0.323	−0.804	0.157	0.008	0.187	−0.307	−0.787	0.173	0.010	0.210
Fats (g)	−0.008	−0.519	0.503	0.016	0.975	0.015	−0.496	0.525	0.019	0.956
SFA (g)	−0.261	−1.001	0.479	0.010	0.489	−0.228	−0.966	0.511	0.013	0.546
MUFA (g)	−0.064	−0.844	0.716	0.017	0.872	−0.039	−0.819	0.740	0.019	0.921
PUFA (g)	0.002	−0.735	0.739	0.008	0.996	0.030	−0.706	0.767	0.011	0.936
Lunch										
Energy Kcal	−0.049	−0.161	0.063	0.065	0.392	−0.021	−0.126	0.085	0.173	0.700
Serving size	−0.029	−0.134	0.077	0.020	0.598	−0.025	−0.130	0.081	0.023	0.647
Carbs (g)	−0.037	−0.149	0.074	0.060	0.511	−0.009	−0.114	0.096	0.168	0.861
Sugars (g)	−0.066	−0.203	0.071	0.041	0.343	−0.044	−0.177	0.090	0.088	0.522
Fiber (g)	−0.078	−0.193	0.038	0.044	0.188	−0.051	−0.161	0.059	0.136	0.363
AB-prot (g)	−0.028	−0.235	0.180	0.024	0.795	−0.006	−0.212	0.200	0.043	0.954
PB-prot (g)	−0.057	−0.168	0.053	0.054	0.309	−0.030	−0.134	0.074	0.162	0.576
Fats (g)	−0.072	−0.185	0.042	0.070	0.214	−0.040	−0.145	0.065	0.204	0.454
SFA (g)	−0.057	−0.179	0.064	0.079	0.356	−0.023	−0.135	0.090	0.214	0.690
MUFA (g)	−0.067	−0.182	0.048	0.069	0.255	−0.036	−0.143	0.072	0.194	0.516
PUFA (g)	−0.101	−0.222	0.020	0.060	0.102	−0.071	−0.185	0.043	0.168	0.224
Dinner										
Energy Kcal	−0.021	−0.109	0.067	0.159	0.635	0.005	−0.076	0.085	0.293	0.908
Serving size	−0.001	−0.081	0.079	0.085	0.981	0.001	−0.080	0.081	0.085	0.990
Carbs (g)	0.023	−0.089	0.136	0.069	0.684	0.045	−0.064	0.154	0.131	0.418
Sugars (g)	−0.066	0.070	0.343	−0.203	0.071	0.895	−0.086	0.109	0.133	0.810
Fiber (g)	0.086	−0.181	0.352	0.021	0.530	0.111	−0.154	0.376	0.037	0.411
AB-prot (g)	−0.150	−0.514	0.213	0.016	0.418	−0.116	−0.477	0.245	0.032	0.529
PB-prot (g)	−0.130	−0.370	0.110	0.031	0.287	−0.104	−0.341	0.133	0.052	0.392
Fats (g)	−0.056	−0.197	0.085	0.098	0.437	−0.020	−0.153	0.113	0.206	0.770
SFA (g)	−0.053	−0.224	0.117	0.047	0.540	−0.020	−0.185	0.144	0.111	0.808
MUFA (g)	−0.130	−0.341	0.081	0.050	0.228	−0.089	−0.293	0.115	0.115	0.392
PUFA (g)	−0.102	−0.330	0.126	0.084	0.381	−0.061	−0.282	0.161	0.138	0.591

Linear regression models. Dietary variables were log-transformed to approximate a normal distribution. Additive genetic model.
Model 1: Adjusted for age in years, sex (male, female) and center (Asturias, Gipuzkoa, Navarra, Granada and Murcia).
Model 2: Model 1, additionally adjusted for total energy intake (kcal/d).
Corrected p-values for multiple testing are not shown. All were >0.05 in GRS analyses. Significant p-values at the nominal level are highlighted in bold. Upper (UCI) and lower (LCI) 95 % confidence intervals, and the R-squared (R^2), are shown.
Abbreviations: Carbs (Carbohydrates), SFA (saturated fatty acids), MUFA (mono-unsaturated fatty acids), PUFA (poly-unsaturated fatty acids), PB-prot (plant-based protein), AB-prot (animal-based protein).

fitted to this population. However, the circadian clock gene variants used for constructing the chronotype-GRS have consistently shown associations with chronobiological aspects and obesity [12]. Our previous study even demonstrated an association between this GRS and obesity/overweight across different life stages [3].

In conclusion, genetic susceptibility to an evening-like chronotype seems to be associated with a smaller serving size during breakfast, with reduced protein and carbohydrate content, whereas the nutritional composition of other meals in the daily diet is not influenced by the individual's genetically determined chronotype. These findings highlight the potential for designing personalized nutritional interventions that specifically target breakfast composition. For example, dietary recommendations could focus on optimizing the protein and carbohydrate intake during breakfast in individuals predisposed to an evening-like chronotype. By aligning meal composition with individual chronotype patterns, it may be possible to enhance satiety, regulate appetite, and improve overall energy balance throughout the day, which are key factors in obesity prevention.

Author contributions

All authors meet the criteria for authorship. They have read and approved the last version of the manuscript. Contributors: Writing original draft: EMM prepared the initial draft of the manuscript and protocol. EMM and AAS conducted the data analyses. JRQ and MJS provided substantial intellectual input. Writing, review & editing: All authors contributed to critical revisions of the manuscript and approved the final version for submission. Conceptualization and funding acquisition: JRQ. Project administration: PJ and MJS. The

Table 6
Association between the genetically-proxied chronotype and with the caloric profile of the diet (RI per meal) and the contribution to the overall diet (RI per energy intake) in the EPIC-Spain chronodiet study.

	Model 1					Model 2				
	β	LCI	UCI	R^2	p-value	β	LCI	UCI	R^2	p-value
Breakfast										
% of total E	−0.228	−0.535	0.080	0.012	0.147	−0.239	−0.546	0.069	0.014	0.128
% proteins[a]	−0.456	−0.774	−0.139	0.010	**0.005**	−0.462	−0.779	−0.144	0.010	**0.004**
% carbs[a]	−0.412	−0.709	−0.114	0.007	**0.007**	−0.415	−0.712	−0.117	0.008	**0.006**
% fats[a]	−0.063	−0.567	0.442	0.012	0.808	−0.055	−0.560	0.450	0.012	0.831
% proteins[b]	−0.309	−0.642	0.024	0.016	0.069	−0.318	−0.651	0.016	0.017	0.062
% carbs[b]	−0.288	−0.598	0.023	0.007	0.069	−0.296	−0.606	0.014	0.008	0.061
% fats[b]	0.034	−0.474	0.541	0.015	0.897	0.027	−0.481	0.534	0.015	0.919
Lunch										
% of total E	−0.029	−0.123	0.065	0.025	0.547	−0.026	−0.120	0.069	0.027	0.593
% proteins[a]	−0.004	−0.093	0.084	0.017	0.924	−0.006	−0.095	0.082	0.018	0.887
% carbs[a]	0.016	−0.077	0.109	0.003	0.739	0.019	−0.074	0.112	0.006	0.688
% fats[a]	−0.019	−0.111	0.074	0.010	0.693	−0.011	−0.104	0.081	0.021	0.808
% proteins[b]	−0.034	−0.127	0.060	0.024	0.483	−0.031	−0.124	0.063	0.026	0.522
% carbs[b]	−0.027	−0.125	0.071	0.026	0.588	−0.021	−0.119	0.077	0.032	0.671
% fats[b]	−0.033	−0.138	0.072	0.022	0.539	−0.031	−0.136	0.074	0.022	0.565
Dinner										
% of total E	−0.001	−0.081	0.079	0.085	0.981	0.001	−0.080	0.081	0.085	0.990
% proteins[a]	0.009	−0.053	0.072	0.031	0.773	0.004	−0.058	0.066	0.043	0.896
% carbs[a]	0.045	−0.041	0.130	0.055	0.304	0.040	−0.045	0.125	0.060	0.353
% fats[a]	−0.035	−0.115	0.045	0.031	0.394	−0.025	−0.103	0.054	0.060	0.541
% proteins[b]	0.012	−0.075	0.099	0.080	0.792	0.013	−0.074	0.100	0.080	0.773
% carbs[b]	0.034	−0.066	0.133	0.031	0.508	0.033	−0.066	0.133	0.031	0.513
% fats[b]	−0.017	−0.144	0.110	0.079	0.793	−0.011	−0.138	0.116	0.083	0.869

Linear regression models. Dietary variables were log-transformed to approximate a normal distribution. Additive genetic model.
Model 1: Adjusted for age in years, sex (male, female) and center (Asturias, Gipuzkoa, Navarra, Granada and Murcia).
Model 2: Model 1, additionally adjusted for total energy intake (kcal/d).
Corrected p-values for multiple testing are not shown. All were >0.05 in GRS analyses. Significant p-values at the nominal level are highlighted in bold. Upper (UCI) and lower (LCI) 95 % confidence intervals, and the R-squared (R^2), are shown.
Abbreviations: E (energy), Carbs (Carbohydrates).
[a] RI per meal.
[b] RI per nutrient.

corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria were omitted.

Data availability statement

The data of this study is preserved by the EPIC-Spain research group. Data are subject to data sharing agreements and are not publicly available.

Funding

This study was supported by the Spanish “Acción Estratégica en Salud” (AES), Instituto de Salud Carlos III (PI15/00347; PI15/01752; PI15/00579; PI15/02181; PI15/01658), and the Marató TV3 (201604-10).

Conflict of interest

The authors declare no conflicts of interest.

Acknowledgements

The authors are thankful to the study participants of the EPIC-Spain chronodiet study. We also acknowledge the collaboration of Biobank Network of the Region of Murcia, BIOBANC-MUR, registered on the Registro Nacional de Biobancos with registration number B.0000859. BIOBANC-MUR is supported by the “Instituto de Salud Carlos III” (project PT20/00109), by the “Instituto Murciano de Investigación Biosanitaria “Pascual Parrilla” (IMIB)”, and by “Consejería de Salud de la Region de Murcia”. Also, we acknowledge the Basque Biobank, which belongs to the Basque Government, Department of Health, with registration number B.0000140, and the BIOBANCO del Principado de Asturias with registration number B.0000827. The genotyping was performed at the Spanish National Cancer Research Centre, in the Human-Genotyping lab, a member of CeGen, PRB3 and is supported by grant PT17/0019, of the PE IþDþi 2013e2016, funded by ISCIII and ERDF. We thank the CERCA Program/Generalitat de Catalunya for institutional support. Funding for open access charge: Universidad de Granada / CBUA.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2025.04.023.

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