INTRODUCTION

Organisms from bacteria to humans have evolved internal timekeeping mechanisms (circadian clocks) that structure biological processes within the 24-hour cycles of the environment. The circadian clock actively uses signals in the environment, called zeitgebers, to synchronize or entrain. This process creates a precise and appropriate internal temporal structure. The primary zeitgeber for human entrainment is light. Yet, the light signal is dynamic, influenced by season as well as by individual behaviors. We wished to explore how seasonal and socially dictated (workweek)
light exposures impact objective measures of entrainment in humans (eg, sleep timing estimated via actimetry, dim-light melatonin onset, DLMO).

Dim-light melatonin onset is an estimation of phase of entrainment, typically occurring 2-3 hours before sleep onset.10-12 Light exposure both suppresses melatonin secretion and leads to a phase shift in its timing.13,14 Given that the light environment changes systematically and sometimes drastically throughout the year, we wondered how this impacts measures of entrainment in humans. Studies that have assessed DLMO or peak of the melatonin rhythm across seasons describe conflicting results.12,15-19 These discrepant findings might derive from assessing the melatonin rhythm in different months within the same season or in different conditions and latitudes, or from relatively small sample sizes (range in cited studies: 6-16 participants). Many countries also adopt daylight saving time (DST), which must be considered in interpreting results.20 In another series of experiments, the phase of melatonin expression was shown to shift over a (simulated) weekend, an effect which could be prevented by exposure to light.19,21-24 We therefore performed a field study measuring the timing of sleep, DLMO, and light exposure in the same individuals in summer and winter as well as on work and free days. In addition, we assessed chronotype, as an estimation of inter-individual differences in phase of entrainment by the circadian clock.

Sleep timing was estimated from actimetry (see Section 2) for 10 days around the 21st of June (longest photoperiod) and the 21st of December (shortest photoperiod) in the same individuals (N = 33) without any restriction or modification to their habitual lifestyle. Chronotype (here, entrained phase of sleep) was assessed using the Munich ChronoType Questionnaire (MCTQ).25 DLMO was assessed on the evenings of a workday and of a work-free day in winter (between 02.12.16 and 18.12.16). Throughout the manuscript, all clock times will be reported in standard time (UCT +1) and in 24-hour format. In summer, photoperiod (day length) ranged between 16:49 and 16:58 hours in length (sunrise: 4:05-4:12 hours; sunset: 21:02-21:05 hours). In winter, photoperiod duration was between 7:31 and 7:51 hours (sunrise: 8:27-8:44 hours; sunset 16:16-16:19 hours).

2 METHODS

2.1 Study design

The study was conducted in Groningen (53°13’N/6°33’E), The Netherlands, according to the principles of the Medical Research Involving Human Subjects Act (WMO, 2012), and the Declaration of Helsinki (64th WMA General Assembly, Fortaleza, Brazil, October 2013). The Medical Ethical Committee of the University Medical Center Groningen approved the study. The participants signed a written informed consent and received financial compensation for taking part in the study.

The protocol lasted 10 days, starting on a Friday and ending the following week on a Sunday (Figure 1). The same protocol was used in summer (between 17.06.16 and 03.07.16; under daylight saving time [DST]) and winter (between 02.12.16 and 18.12.16). Throughout the manuscript, all clock times will be reported in standard time (UCT +1) and in 24-hour format. In summer, photoperiod (day length) ranged between 16:49 and 16:58 hours in length (sunrise: 4:05-4:12 hours; sunset: 21:02-21:05 hours). In winter, photoperiod duration was between 7:31 and 7:51 hours (sunrise: 8:27-8:44 hours; sunset 16:16-16:19 hours).

2.2 Participants and questionnaires

Participants were recruited via online posts and flyers. Participants had regular weekly schedules (at least four workdays per week), had not performed shift work in the past 5 years nor traveled across more than two time zones during the month before the study started, and were healthy (ie, free of any medication). Females were selected only if they made use of any type of hormonal contraceptives to avoid possible fluctuations in melatonin levels depending on the phase of the menstrual cycle.26 A total of 35 participants (20 females; mean age 29 years ± SD 4.9, age range: 22-40) completed the first part of the study in

![FIGURE 1](image_url) Study design. The study design was within-subjects with the same protocol run in summer (between 17.06.16 and 03.07.16) and in winter (between 02.12.16 and 18.12.16). The protocol lasted 10 d, always starting on a Friday and ending the following week on a Sunday. Here, we show a typical working week with five workdays (color-coded in dark gray) between Monday and Friday and two work-free days (color-coded in light gray) on Saturdays and Sundays. At the beginning of each study period, a set of questionnaires to assess chronotype, social jetlag, depressive symptoms, and subjective sleep quality was administered to the participants (MCTQ, BDI-II, PSQI). During each study period, sleep was assessed via sleep diaries (filled in daily by the participants) and by actigraphy (actimeters worn continuously at the nondominant wrist). On two evenings, saliva samples were collected at home to later determine DLMO on workdays (on a Wednesday or Thursday) and on work-free days (on Sundays).
summer. In winter, there was one dropout and one participant changed working schedules resulting in a final sample size of 33 participants (18 females; mean age 29 years ± SD 4.9, age range: 23-40). Chronotype was assessed with the MCTQ25 as the midpoint of sleep on work-free days (MSFsc) corrected for sleep debt accumulated on workdays (MSFscw). Participants additionally filled in two more questionnaires to assess depressive symptoms and subjective sleep quality (Beck Depression Inventory, BDI-II and Pittsburgh Sleep Quality Index, PSQI; data presented in Supporting Information).27,28

2.3 | Circadian phase (DLMO)

For DLMO assessment, participants collected saliva samples each hour for 6 hours at home. They started 5 hours before and ended one hour after habitual sleep onset (MCTQ weighted average of sleep onset on workdays and on work-free days). Subjects remained in dim light and wore a pair of blue-light-blocking glasses (89%-99.9% filter of blue light between 400 and 500 nm, general decrease of light intensity: 50% AugenLichtSchutz). Light exposure during melatonin collection was assessed via actimeters worn at the nondominant wrist (MotionWatch 8; CamNtech). The median light exposure during the 6 hours of saliva sample collection averaged across participants ranged between 4 lux in winter and 19 lux in summer (winter workdays: 4 lux ± SD 3, winter work-free days: 4 lux ± SD 4, summer workdays: 13 lux ± SD 12, summer work-free days: 19 lux ± SD 30; individual median light levels during saliva sample collection are reported in Table S5). Participants were instructed not to use toothpaste or consume coffee, tea, alcohol, chocolate, banana, or food with artificial additives during the saliva collection protocol. The collections were performed on a workday (Wednesday or Thursday) and on a work-free day (Sunday) evening both in winter and in summer. The saliva samples were collected using Salivettes (Sarstedt), refrigerated overnight and then sent to the laboratory by mail. Upon arrival, samples were frozen at −80°C. Melatonin was determined by saliva melatonin radioimmunoassay (RIA) test kits (Bühlmann). DLMO was calculated by linear interpolation between the time points before and after melatonin concentrations crossed and stayed above the threshold of 3 pg/mL. The lower limit detection of the kit was below 0.5 pg/mL. The intra-assay coefficient of variability was 13.33% (low melatonin) and 12.66% (high melatonin), while the inter-assay coefficient of variability was 9.76% (low melatonin) and 12.11% (high melatonin).

2.4 | Actigraphy and light recordings

Sleep times were assessed via actimeters, which were worn continuously on the nondominant wrist (MotionWatch 8; CamNtech; mean of samples recorded with 1-second interval stored each 30 seconds). Activity counts were later binned (10 minutes) and analyzed with ChronoSapiens (version 9) to detect sleep episodes.29 MotionWatches continuously measure white light intensities in lux with the same sampling interval as the activity data (for more information and the statistical analyses of the light data, see Supporting Information). Participants kept a daily sleep diary in order to validate actigraphy (see Supporting Information).

2.5 | Statistical analyses

Statistical analyses were performed using R software (R version 4.0.0).30 Linear mixed models (R packages “lme4” and “lmerTest”)31,32 with participant “id” as random factor were used to explore the influence of sex, age, chronotype (MSFscw), weekday (workday vs. work-free days), and season (summer vs. winter) on DLMO, sleep onset, sleep offset, sleep duration, and the phase angle difference between DLMO and sleep (onset and offset). BDI-II scores and PSQI scores were not significantly associated with any of the outcome variables and therefore removed from the models. For sleep timing and duration, daily values were analyzed in the model. Continuous variables (age and MSFscw) were centered around the mean. The following interaction terms were tested in separate models: weekday*MSFsc, season*MSFscw and weekday*season. Here, we report only the models with the first interaction term (weekday*MSFsc), since the other two interactions were nonsignificant in all analyses. The unstandardized coefficients (b) estimated by the models together with the associated p-values are reported in the Results (for more detailed specifications of the models and the coefficients interpretation see Tables 2-4). The coefficients estimated by the models are expressed in decimal hours (eg, 0.5 = 30 minutes). Multicollinearity was assessed by calculating the VIF values (variance inflation factor). In addition, the residuals were plotted to assess their normality (assumption of normally distributed errors) and variance (assumption of constant variance or homoscedasticity). To compare the variance in DLMO, sleep timing, sleep duration, and phase angle difference between DLMO and sleep between summer and winter, we ran the Pitman-Morgan test for paired samples (R package “PairedData”).33 Figures were prepared in R (package “ggplot2”).34

3 | RESULTS

The “background” variables assessed with the MCTQ, BDI-II, and PSQI in summer and in winter are reported in Table 1 (see Supporting Information for more details). Only
### TABLE 1

Chronotype (MSFsc), social jetlag (SJL), depressive symptoms (BDI-II), subjective sleep quality (PSQI), DLMO, sleep timing, and phase angle between DLMO and sleep in summer and in winter, and on workdays and on work-free days.

| Outcome variable       | Summer                         | Winter                         | Workdays | Work-free days | Workdays | Work-free days |
|------------------------|--------------------------------|--------------------------------|-----------|----------------|-----------|----------------|
|                        | N | Mean ± SD | Median | Range       | N | Mean ± SD | Median | Range       | N | Mean ± SD | Median | Range       |
| MSFsc (hh:mm)          | 30 | 3:14 ± 1:02 | 3:08   | 1:24-6:02 | 24 | 4:14 ± 1:01 | 4:11   | 2:02-6:14 |
| SJL (hh:mm)            | 30 | 1:18 ± 0:46 | 1:08   | 0:14-3:08 | 24 | 1:23 ± 0:49 | 1:15   | 0:00-2:53 |
| BDI-II score           | 33 | 5.94 ± 5.33 | 6      | 0-22       | 33 | 5.15 ± 5.24 | 3      | 0-16       |
| PSQI score             | 33 | 4.39 ± 2.21 | 4      | 1-9        | 33 | 3.67 ± 1.41 | 4      | 0-8        |

**Notes:** Mean (± standard deviation), median, and range of MSFsc, SJL, BDI-II score, and PSQI score assessed in summer and in winter are reported. Mean (± standard deviation) and range of DLMO, phase angle difference between DLMO and sleep onset/offset, sleep onset, sleep offset, and sleep duration assessed in summer and winter, separately for workdays and work-free days are also reported. The phase angle difference was calculated as sleep onset/offset-DLMO. Therefore, positive values indicate that DLMO occurred before sleep onset/offset. Clock times are expressed in Standard Time (UCT +1) and in 24-h format. Missing data for MSFsc and SJL derive from the use of an alarm clock on work-free days. Missing data for DLMO and the phase angles derive from the failure to determine DLMO because of too low or too high melatonin concentrations (either melatonin never rose above the 3 pg/mL threshold or was already above the threshold when sample collection started). Missing data for sleep variables derive from a malfunctioning activity monitor.
TABLE 2 Model dim-light melatonin onset

| Predictors                                      | Estimates | CI            | T-test | P     | df  |
|------------------------------------------------|-----------|---------------|--------|-------|-----|
| (Intercept)                                    | −3.70     | −4.10, −3.30  | −18.11 | <.001 | 34.44 |
| Sex [male]                                     | 0.17      | −0.38, 0.73   | 0.61   | .548  | 27.27 |
| Age (y)                                        | −0.10     | −0.16, −0.04  | −3.42  | <.001 | 27.64 |
| Weekday [work-free day]                       | 0.45      | 0.26, 0.65    | 4.53   | <.001 | 66.75 |
| Season [winter]                                | 0.92      | 0.71, 1.13    | 8.53   | <.001 | 71.48 |
| Chronotype (MSFsc; hours)                     | 0.14      | −0.10, 0.39   | 1.14   | .257  | 73.27 |
| Weekday [work-free day]*Chronotype (MSFsc; hours) | 0.21      | 0.01, 0.41    | 2.10   | .040  | 66.55 |

Random effects

|                |           |              |
|----------------|-----------|--------------|
| $\sigma^2$    | 0.25      |              |
| $\tau_{0 \text{id}}$ | 0.49     |              |
| Observations  | 101       |              |
| Marginal $R^2$/conditional $R^2$ | .454/.818 |              |

Notes: Results of the mixed model predicting DLMO from sex, age, MSFsc, weekday, and season. Confidence intervals (CI) and P-values associated with the coefficients estimated by the model are displayed (significant P-values are highlighted with bold characters). T test statistics and degrees of freedom (estimated with the Satterthwaite method) are also reported. Age and MSFsc were mean centered. For those unfamiliar with interpreting regression results, the intercept (−3.70) indicates the timing of DLMO for an individual of average chronotype and age. The confidence interval (CI) is also reported. The marginal $R^2$ indicate the proportion of variance explained by only the fixed factors in the model, whereas the conditional $R^2$ includes also the proportion of variance explained by both fixed and random factors. Missing observations derive either from missing DLMOs (8 observations out of 132) or missing chronotypes (3 participants in summer and 9 in winter used an alarm clock on work-free days precluding chronotype determination).

chronotype and the use of an alarm clock on work-free days varied with season. Chronotype was later by 1 hour in winter ($b = 0.947$, $P < .0001$), and 27% of the participants used an alarm clock on work-free days in winter compared to 9% in summer (McNemar’s Chi-squared = 4.5, df = 1, $P = .034$, continuity correction: $P = .077$). Beware that all times are expressed in standard time throughout the manuscript. Since data in summer were collected under DST, such a 1-hour difference means that the timing was different in relation to sun time but the same in relation to social time (DST in summer and ST in winter). Here, the reported differences between winter and summer are likely driven by both seasonal changes in photoperiod as well as DST (social activities are moved 1 hour earlier in summer relative to sun time thus changing behavioral light exposure).

The primary outcomes, DLMO and the timing of sleep (assessed via actimeters), are also summarized in Table 1. For the sleep outcomes determined from sleep diaries, see Table S1. The results based on activity presented in the following paragraphs were confirmed when looking at sleep estimated from the sleep diaries (see Results in the Supporting Information, Figures S1-S3 and Tables S2 and S3). Overall, there was a good correspondence between sleep data assessed via actimeters and via sleep diaries (Figures S4 and S5).

3.1 Demographics and DLMO/sleep

Our regression models on all six primary outcome variables (DLMO, sleep onset, sleep offset, sleep duration, phase angles DLMO-sleep onset/offset) showed expected effects of age and sex. DLMO was earlier in older individuals ($b = −0.103$, $P = .0020$; Table 2), but did not differ between males and females ($P > .05$; Table 2). For each one-year increase in age, our model predicts an advance in DLMO of 3.1 minutes. Sleep onset—but not offset—was generally later in male participants (by 30 minutes, $b = 0.500$, $P = .0072$; Table 3). As a consequence, males slept less compared to females (21 minutes, $b = −0.360$, $P = .0478$; Table 3). Sleep offset—but not onset—was slightly earlier in older individuals advancing by 2.5 min/year of life ($b = −0.044$, $P = .0331$; Table 3). Nonetheless, there was no significant decrease in sleep duration from younger to older individuals ($P > .05$, Table 3). The phase angle difference between DLMO and sleep was greater in older individuals (DLMO-sleep onset:
### Table 3: Models sleep timing and duration (data collected via actimeters)

| Predictors                       | Sleep onset Estimates | CI     | T-test | P  | df | Sleep end Estimates | CI     | T-test | P  | df | Sleep duration Estimates | CI     | T-test | P  | df |
|----------------------------------|----------------------|--------|--------|----|----|---------------------|--------|--------|----|----|--------------------------|--------|--------|    |----|
| (Intercept)                      | −1.20                | −1.47, −0.94 | −8.77 | <.001 | 54.88 | 6.15 | 5.88, 6.42 | 44.46 | <.001 | 40.50 | 7.35 | 7.09, 7.62 | 53.95 | <.001 | 50.50 |
| Sex [male]                       | 0.50                 | 0.16, 0.84 | 2.90 | .007 | 28.68 | 0.14 | −0.22, 0.50 | 0.78 | .445 | 26.55 | −0.36 | −0.70, −0.02 | −2.07 | .048 | 27.95 |
| Age (y)                          | −0.02                | −0.06, 0.01 | −1.28 | .211 | 28.49 | −0.04 | −0.08, −0.01 | −2.25 | .033 | 26.41 | −0.02 | −0.06, 0.02 | −1.07 | .294 | 27.75 |
| Weekday [work-free day]          | 1.07                 | 0.84, 1.29 | 9.22 | <.001 | 472.76 | 1.66 | 1.47, 1.85 | 17.24 | <.001 | 466.64 | 0.59 | 0.37, 0.80 | 5.32 | <.001 | 471.15 |
| Season [winter]                  | 0.58                 | 0.34, 0.81 | 4.81 | <.001 | 472.71 | 1.02 | 0.82, 1.22 | 10.00 | <.001 | 483.12 | 0.44 | 0.21, 0.66 | 3.80 | <.001 | 475.48 |
| Chronotype (MSFsc; hours)        | 0.25                 | 0.06, 0.44 | 2.58 | .012 | 73.55 | 0.25 | 0.06, 0.43 | 2.61 | .011 | 61.55 | 0.02 | −0.17, 0.21 | 0.23 | .817 | 69.42 |
| Weekday [work-free day]*Chronotype (MSFsc; hours) | 0.63                 | 0.39, 0.87 | 5.13 | <.001 | 473.23 | 0.59 | 0.39, 0.79 | 5.73 | <.001 | 467.16 | −0.04 | −0.27, 0.19 | −0.31 | .758 | 471.66 |
| Random effects                   |                      |        |        |    |    |                     |        |        |    |    |                      |        |        |    |    |
| σ²                               | 1.60                 |        |        |    |    |                     |        |        |    |    |                     |        |        |    |    |
| τ₀₀                              | 0.10₀₀              |        |        |    |    |                     |        |        |    |    |                     |        |        |    |    |
| Observations                     | 494                 |        |        |    |    |                     |        |        |    |    |                     |        |        |    |    |
| Marginal R²/conditional R²       | 0.313/0.354         | 0.514/0.577 |        |        |    | 1.45 | 0.12₀₀ | 0.12₀₀ |    |    |                     |        |        |    |    |
| Notes: The results of the mixed models predicting sleep timing and duration from sex, age, MSFsc, weekday (significant P-values are highlighted with bold characters), and season are reported. For an explanation of the statistical outputs reported here and their interpretation see Table 2. The reference categories of the categorical predictors were female, workday, summer. The residual variance σ², the variance explained by the random factor “id,” the number of observations (data points; 20 per participants), and the marginal and conditional R² are also reported. Missing observations derive either from a malfunctioning actimeter or from missing chronotypes (3 participants in summer and 9 in winter used an alarm clock on work-free days precluding chronotype determination).
### TABLE 4  Models phase angle between DLMO and sleep (data collected via actimeters)

| Predictors                        | DLMO-sleep onset | DLMO-sleep end |
|-----------------------------------|------------------|----------------|
|                                   | Estimates   CI   | T-test | P       | df  | Estimates CI   | T-test | P       | df  |
| (Intercept)                      | 2.56 2.14, 2.98 | 11.90 | **<.001** | 53.30 | 9.92 9.48, 10.35 | 44.42 | **<.001** | 42.61 |
| Sex [male]                       | 0.34 −0.18, 0.86 | 1.28  | .210    | 28.70 | −0.05 −0.62, 0.53 | −0.16 | .877    | 27.29 |
| Age (y)                          | 0.07 0.02, 0.13 | 2.51  | **0.018** | 28.53 | 0.06 −0.00, 0.12 | 1.94  | .063    | 27.16 |
| Weekday [work-free day]          | 0.60 0.26, 0.94 | 3.47  | **0.011** | 68.98 | 1.20 0.91, 1.50 | 7.98  | **<.001** | 66.65 |
| Season [winter]                  | −0.40 −0.76, −0.04 | −2.19 | **0.031** | 79.95 | 0.04 −0.28, 0.36 | 0.25  | .802    | 76.11 |
| Chronotype (MSFsc; hours)        | 0.01 −0.29, 0.31 | 0.05  | .960    | 67.64 | 0.01 −0.29, 0.31 | 0.06  | .954    | 60.67 |
| Weekday [work-free day]*Chronotype (MSFsc; hours) | 0.39 0.04, 0.74 | 2.20  | **0.031** | 68.66 | 0.33 0.03, 0.63 | 2.13  | **0.037** | 66.45 |

**Random effects**

|                       | σ²     | τ₀₀   | Observations | Marginal \( R^2 \) / conditional \( R^2 \) |
|-----------------------|--------|-------|--------------|------------------------------------------|
|                       | 0.74   | 0.25_id | 100          | .289/.470                               |
|                       | 0.56   | 0.42_id | 100          | .338/.621                               |

**Notes:** The results of the mixed models predicting the phase angle difference between dim-light melatonin onset (DLMO) and sleep (onset and offset) from sex, age, MSFsc, weekday, and season are reported (significant P-values are highlighted with bold characters). For an explanation of the statistical outputs reported here and their interpretation see Table 2. The reference categories of the categorical predictors were female, workday, and summer. The residual variance \( \sigma^2 \), the variance explained by the random factor “id,” the number of observations (data points; 4 per participants), and the marginal and conditional \( R^2 \) are also reported. Missing observation derive either from missing DLMOs (eight observations out of 132), from a malfunctioning actimeter or from missing chronotypes (three participants in summer and 9 in winter used an alarm clock on work-free days precluding chronotype determination).

**FIGURE 2**  Seasonal variation in dim-light melatonin onset (DLMO), sleep timing, and their phase angle relationship. The circular plot shows the distribution of the variables DLMO (gray), sleep onset (blue), and sleep offset (green) in summer and in winter for all 33 participants. Photoperiod is indicated in yellow. For visualization purposes, data were aggregated by season; that is, the variables were averaged over workdays and work-free days. The straight, colored lines indicate the median of the respective variable. All three variables occurred later in winter compared to summer (\( P < .0001 \)). The phase angle difference between variables can also be observed (phase angle DLMO-sleep onset smaller in winter; \( P = .0314 \))

**FIGURE 3**  Weekly variation in dim-light melatonin onset (DLMO), sleep timing, and their phase angle relationship. The circular plot shows the distribution of the variables DLMO (gray), sleep onset (blue), and sleep offset (green) on workdays and on work-free days for all 33 participants. For visualization purposes, data were aggregated by weekday; that is, the variables were averaged over summer and winter. The straight, colored lines indicate the median of the respective variable. All three variables occurred later on work-free days compared to workdays (\( P < .0001 \)). The phase angle difference between variables can also be observed (phase angle between DLMO and sleep greater on work-free days; DLMO-sleep onset: \( P = .0009 \); DLMO-sleep offset: \( P < .0001 \))
3.2 | Season and DLMO/sleep

We found a clear seasonal effect on DLMO and sleep timing. DLMO was later in winter compared to summer by close to 1 hour ($b = 0.922$, $P < .0001$; Figure 2, Table 2). Similarly, sleep timing (onset and offset) was later in winter: Our model predicts that participants delayed their sleep onset on average by 35 minutes ($b = 0.576$, $P < .0001$; Figure 2, Table 3) and their sleep offset by 1 hour ($b = 1.017$, $P < .0001$; Figure 2; Table 3), resulting in a significant extension of almost 30 minutes of sleep in winter ($b = 0.436$, $P = .0002$; Table 3). The phase angle difference between DLMO and sleep onset—but not offset—was smaller in winter by 24 minutes compared to summer ($b = -0.400$, $P = .0314$; Figure 2, Table 4).

3.3 | Weekday and DLMO/sleep

Sleep timing is known to be influenced by the weekly schedule, with many people sleeping earlier and shorter during the workweek. It is not clear whether DLMO also varies between workdays and work-free days in a working population. We found that DLMO was later on work-free days by almost 30 minutes compared to workdays ($b = 0.450$, $P < .0001$; Figure 3, Table 2). Notably, this effect was stronger for later chronotypes whose sleep timing changes more drastically between work and work-free days. Our model predicts that a delay in chronotype by 1 hour corresponds to an additional weekend delay in DLMO of 13 minutes ($b = 0.213$, $P = .0399$; Figure 4A, Table 2). Similarly, sleep timing was later on work-free days (sleep onset: $b = 1.068$, $P < .0001$; sleep offset: $b = 1.661$, $P < .0001$; Figure 3, Table 3), and the
The effect was also modulated by chronotype. Late chronotypes showed a greater difference in their sleep timing between workdays and work-free days, with a delay in chronotype by 1 hour corresponding to an additional weekend delay in sleep timing of about 35 minutes (sleep onset: $b = 0.632$, $P < .0001$; sleep offset: $b = 0.587$, $P < .0001$; Figure 4B,C, Table 3). In terms of sleep duration, participants slept longer on work-free days ($b = 0.587$, $P < .0001$; Figure 3, Table 3)—as expected—but no significant interaction with chronotype emerged ($P > .05$; Figure 4D, Table 3). Finally, the phase angle difference between DLMO and sleep (onset and offset) was also greater on work-free days (DLMO-sleep onset: $b = 0.602$, $P = .0009$; DLMO-sleep offset: $b = 1.202$, $P < .0001$; Figure 3, Table 4). Again, this effect was stronger for later chronotypes, with a delay in chronotype by 1 hour leading to an additional weekend increase in phase angle of about 20 minutes (DLMO-sleep onset: $b = 0.390$, $P = .0310$; DLMO-sleep offset: $0.327$, $P = .0371$; Figure 4E,F, Table 4).

### 3.4 Variance in outcomes

The variance in DLMO between individuals was significantly greater in winter compared to summer ($t (25) = -4.37$, $P = .0002$). The same was true for the variance of the phase angle difference between DLMO and sleep (onset: $t (25) = -2.96$, $P = .0066$; offset: $t (25) = -1.86$, $P = .0754$). The variance in sleep timing and duration was not significantly different between summer and winter (all $P > .05$).

### 3.5 Light exposure

Experienced light intensities (assessed via actimeters worn at the wrist) were on average 10 times higher in summer compared to winter (Figure 5; see Figures S6 and S7 for light exposure plotted separately in early and late chronotypes, on workdays and on work-free days). As a consequence, season and light exposure explained a similar portion of the variance in DLMO. Time above 1000 Lux and daily light exposure were both significant predictors of DLMO only when season was not entered in the same model (1000 Lux: $b = -0.023$, $P < .0001$; daily light exposure: $b = -0.699$, $P = .001$, Model 2, Table S4). Both longer exposure above 1000 Lux and average exposure to higher light intensities predicted earlier DLMOs. Average daily light exposure additionally interacted with weekday ($b = -1.350$, $P = .006$; Model 4, Table S4), indicating that the weekend delay in DLMO ($b = 1.530$, $P = .002$, Model 4, Table S4) can be counteracted by increased light exposure during the weekend. More precisely, one-unit increase (on a log scale) in average light exposure on work-free days reduced the weekend delay in DLMO by around 1 hour 20 minutes. Finally, the timing of first exposure to light levels above 100 Lux also predicted DLMO, with earlier first exposure resulting in earlier DLMOs (model without season: $b = 0.321$, $P < .0001$).

### 4 DISCUSSION

The aim of this study was to better understand how the tension between the social and biological temporal structures is interpreted by the circadian clock in humans in summer and winter. To achieve this, we assessed a phase marker of the circadian clock, DLMO, in different environmental conditions encountered by millions of people on a weekly basis.

DLMO is considered the gold standard to estimate phase of entrainment in humans and requires melatonin assessments to be done in dim light ($<10$ lux). In some of our participants, home light levels during melatonin sampling exceeded this limit to some extent in summer (31 out of 124
observations; note that light levels were assessed via actimeters worn at the wrist). However, participants wore blue-light blocking glasses, which reduce blue light at the eye by 89%-99.90% and overall light intensities by 50%. This measure and the results of our additional analyses on the influence of light levels on DLMO underscore the validity of our in-home DLMO assessments (for more details see Supporting Information).

Using DLMO, entrainment in summer was compared to winter, including separate analyses for work and work-free days. This design thus incorporates considerations of the zeitgeber (light) environment, which varies substantially due to natural conditions (season) and due to self-exposure (based on core daily activities). From previous studies, we know that humans entrain to sun time rather than social time. We therefore used standard time, as it most closely reflects sun time in summer and winter with midnight and midday falling close to mid-scotoperiod and mid-photoperiod. Our hypothesis was that DLMO would be earlier in summer because of the combined effect of a stronger zeitgeber and the imposed shift in social time during DST. We further expected that DLMO would be stable between work and work-free days despite transient changes in light exposure. Rapid light-dependent phase shifts in DLMO have so far only been shown in highly controlled laboratory conditions or after imposed changes in sleep schedules.

4.1 The influence of demographics on DLMO and sleep

Both DLMO and sleep showed expected variations depending on age and sex. The timing of DLMO and sleep advanced with increasing age (DLMO 6 minutes and sleep offset 2.5 minutes earlier/year of life), and male participants slept later and less (30 minutes), as previously shown. The influence of age and sex on the phase angle difference between DLMO and sleep is less clear. We found that the phase angle was greater in older individuals (4 min greater/year of life). Other studies have found smaller phase angles in older individuals when comparing more extreme age groups (e.g., ~20 to 65 years old) as found in our sample. Studies looking at sex effects found both a greater phase angle in women compared to men or the opposite. We did not find a significant effect of sex in our sample, maybe due to a lack of power. However, the magnitude and direction of the sex effect, with the phase angle between DLMO and sleep onset on average 20 minutes larger in men, is in line with previous studies on sex differences in intrinsic circadian period and its association with phase angle difference. Altogether, it has been suggested that there is an optimal range for these two variables (DLMO and sleep time) to occur in order to promote good sleep and health. On average, DLMO occurs 2-3 hours before sleep onset in healthy individuals, while this interval tends to be shorter in patients with insomnia, mood disorders, or older individuals with sleep problems.

4.2 The influence of season on DLMO and sleep

The circular plots reveal how the timing of DLMO and sleep varied between seasons in relation to sun time (sunrise, midday, sunset). The phase angle between DLMO and sunrise and between DLMO and sunset changed substantially between seasons (4-6 hours difference), whereas the phase angle between DLMO and midday remained stable (only a 1-hour difference). Our data therefore suggest that midday and midnight are the most stable reference points for entrainment compared to sunrise and sunset, suggesting parametric entrainment in humans—at least in our sample.

Overall both DLMO and sleep (onset and offset) occurred earlier in summer compared to winter. The difference in timing between summer and winter was for all variables—except for sleep onset—close to 1 hour. Since clock time was converted to and expressed as standard time, this 1-hour difference suggests that by late June the participants fully adapted to the 1-hour shift imposed by DST in March, confirming previous studies. Japan is one of the few countries without DST. In 1992, Honma and colleagues found that the melatonin peak was earlier in summer compared to winter. Based on entrainment theories and previous studies, an earlier melatonin rhythm in summer is to be expected because the zeitgeber is stronger (higher and longer light exposure). Our data and that of others concerning light exposure in different seasons are in agreement: Light intensities were 10 times higher, and light exposure above 1000 Lux was longer in summer compared to winter (although light exposure in winter might have been underestimated; see paragraph on the study limitations).

The phase angle difference between DLMO and sleep onset, but not between DLMO and sleep offset, was greater in summer (actigraphy data). The data collected with the sleep diaries showed, however, no significant effect of season. As discussed previously, there may be an optimal relationship in the timing of DLMO and sleep for good sleep and health. This recommendation may need to incorporate seasonal biology or, alternatively, seasonal changes that occur in a light environment that is heavily influenced by artificial light and social schedules.

In line with previous studies, we found that sleep duration was longer in winter compared to summer. In addition, the use of an alarm clock on work-free days increased in
winter (27% of the participants used an alarm clock in winter compared to 9% in summer). The same result was recently found in a larger cohort. Lower light levels and shorter photoperiod may facilitate sleep extension in winter.

Finally, season influenced the variance between individuals in the phase angle difference between DLMO and sleep. The variance was higher in winter compared to summer. This result is in line with previous studies that have shown how the distribution of circadian parameters in a given population (eg, chronotype and DLMO) is greater when individuals are exposed to weak zeitgebers. Interestingly, the variance in sleep timing was not significantly different between seasons, suggesting that DLMO may be more affected by the strength of the zeitgeber (light).

### 4.3 Influence of weekly schedule on DLMO and sleep

We observed later and longer sleep episodes on work-free days—especially in late chronotypes. This is no surprise as it has been often reported in working populations. However, we found that DLMO was later on work-free days and that this effect correlated with chronotype. When considering the earliest and latest third of our chronotype distribution, early chronotypes had similar DLMOs between workdays and work-free days, whereas late chronotypes had significantly delayed DLMOs (on average 44 minutes) over the weekend. To the best of our knowledge, this is the first observation of a chronotype-dependent shift in the phase of DLMO within a workweek in a population where no restriction on sleep timing or light exposure was imposed. Similarly, the phase angle difference between DLMO and sleep onset varied according to weekday, being greater on work-free days, and with the effect being stronger for later chronotypes. Overall, DLMO, sleep time, and their phase relationship varied according to the weekly schedule and chronotype.

In support of our findings, experimental studies where sleep timing and/or duration were manipulated to simulate a typical weekend with later and longer sleep yielded the same results. The magnitude of the DLMO delay was similar to our observations, ranging from 30 minutes to 1 hour. In these studies, the authors suggested that a delay in DLMO following a change in the sleep schedule was probably related to differences in light exposure. In our study, late chronotypes were exposed to lower light intensities throughout the day, especially in summer (see Figure S6). Increasing light exposure on work-free days, especially in the early hours, could be a potentially effective countermeasure to the observed delay in DLMO. Similar differences in light exposure between chronotypes and between workdays and work-free days have been reported in other studies.

### 4.4 Study limitations

As for many other field studies, light exposure was assessed with actimeters worn at the wrist, which can lead to an underestimation of retinal light exposure especially in winter (ie, long sleeves might cover the sensor). We did not collect any information about the spectral composition of the light exposure, which has been shown to vary depending on time of day and time of year and, therefore, limits our interpretation of the effects of light on DLMO and sleep. In addition, our conclusions might not hold at different latitudes from the one studied here (53°13′N) because of differences in photoperiod depending on latitude.

The average median light exposure during sample collection across participants was 19 lux on work-free days in summer, 13 lux on workdays in summer, and 4 lux on both work-free days and workdays in winter—detected at the wrist not considering that the blue-light-blocking glasses worn by the participants decrease light intensities by 50%. Given that participants received 10-times higher light intensities throughout the day during summer, the slightly higher light exposures during the summer collections possibly did not affect melatonin levels as participants were likely less sensitive to evening light in summer (as shown by studies on prior light history, eg). Furthermore, we ran additional analyses to confirm that our seasonal and weekly effects are stable despite the different light levels during collections, which demonstrated, for example, that light exposure during saliva sample collections did not predict DLMO and that the results reported in Table 2 remained stable when this variable was added as covariate to the model (season: b = 0.926, P < .0001, weekday: b = 0.441, P < .0001). Median light levels during melatonin sample collection per participant (Table S5), correlations between DLMOs and median light levels during melatonin sampling (Figure S8), individual raw melatonin profiles (Figure S9), and raw melatonin profiles averaged across participants (Figure S10) can be found in the Supporting Information.

We also point out that chronotype, as estimated from sleep timing in the field, may not only be influenced by the circadian clock but also by sleep homeostatic forces—despite basic adjustments performed to limit the homeostatic influence. Indeed, we hope that our findings lead to experiments that address if the discrepancy between DLMO and phase of sleep at various time points may be explained by the sleep homeostat.

Another limitation is that we did not record which type of hormonal contraceptive our female participants used. However, the most common method of hormonal contraception in the Netherlands is oral contraception (40% of women), while only 5%-10% of women use an intrauterine device (IUD). In general, there are conflicting results on effects of menstrual phase and hormonal contraception on melatonin...
phase and levels, which requires further research to define a consensus on study protocols involving female participants and melatonin assessments.54

Finally, the observational nature of this study does not allow inferring any causal relationship between the variables studied.

5 | CONCLUDING REMARKS

We assessed DLMO and sleep in 33 working individuals in a longitudinal study design. DLMO and sleep were earlier in summer compared to winter, consistent with exposure to longer and stronger light intensities in summer, as well as the 1-hour shift of social activities imposed by DST in March. Our results therefore confirm previously elaborated properties of the circadian clock entrainment, such as an earlier phase of entrainment as well as a reduced between-individuals variance in phase of entrainment when stronger zeitgebers are present.2,5,53,56

In addition, our results pose new questions on the role of DLMO as phase marker to assess entrainment of the circadian clock in humans. The finding that DLMO shifted over the weekend suggests that DLMO in the real world is not as stable a phase marker as thought to be. There are at least two possibilities which may explain this observation: (a) DLMO is a reliable phase marker of the circadian clock and individuals (mainly late chronotypes) change their phase of entrainment each week as they move between weekdays and work-free days; (b) DLMO is an output of the circadian clock that is allowed to vary (like the sleep-wake cycle) between workdays and work-free days, while the circadian clock keeps a relatively stable phase of entrainment. With our data, we cannot determine which of these two (or other) possibilities is correct. However, our data clearly show that the recent history of sleep timing, self-exposure to light and other variables will impact DLMO. Similar results have been shown in highly controlled laboratory conditions, in which advancing the sleep-wake cycle paired with exposure to strong (10 000 Lux)—but not moderate (150 Lux)—light intensities led to an advance in DLMO.65 Interestingly, a large individual variation in adaptation to the advancing protocol was observed, which could be in part explained by chronotype. In our study, we also observed greater differences in DLMO between individuals variance in phase of entrainment when stronger zeitgebers are present.2,5,53,56

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

AUTHOR CONTRIBUTIONS

MM and GZ designed and planned the study. GZ collected and analyzed the data, prepared the figures, and wrote the manuscript under the supervision of MM. EW contributed to data analysis and figure preparation. Both MM and EW revised the manuscript. All authors have approved the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.

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