

Perinatal Photoperiod and Childhood Cancer: Pooled results from 182,856 individuals in the International Childhood Cancer Cohort Consortium (I4C)

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Abstract

Experimental evidence suggests that perinatal light imprinting of circadian clocks and systems may affect downstream physiology and cancer risk in later life. For humans, the predominant circadian stimulus is the daily light-dark cycle. Herein, we explore associations between perinatal photoperiod characteristics (photoperiod: duration of daylight as determined by time-of-year and location) and childhood cancer risk. We use pooled data on 182,856 mothers and babies from prospective birth cohorts in six countries (Australia, Denmark, Israel, Norway, UK, USA,) within the International Childhood Cancer Cohort Consortium (I4C). Cox proportional hazards regression was used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs). In line with predicted differential dose-responses, restricted cubic splines indicate a non-linear, non-monotonic relationship between perinatal mean daily photoperiod (0-24 hours) and childhood cancer risk. In a restricted analysis of 154,121 individuals who experienced 3rd trimester photoperiods exclusively within the 8-16 hours range, the relative risk of developing childhood cancer decreased by 9% with every hour increase in 3rd trimester mean daily photoperiod [HR: 0.91 (95% CIs: 0.84-0.99)].

In conclusion, in this first study of photoperiod and childhood cancer, we detected an inverse [‘protective’] linear association between 3rd trimester mean daily photoperiod and childhood cancer risk in the 8-16 hour set of the total study population. Limited statistical power impeded investigation of risks with individuals exposed to more extreme photoperiods. Future studies are needed to confirm differential photoperiod-associated risks, and further investigations into the hypothesized circadian imprinting mechanism are warranted.

Key words: perinatal, light, circadian, childhood cancer, leukaemia, cohort, PLICCS

Introduction

Circadian (from Latin *circa diem*, ‘about a day’) rhythms are found in almost every cell in the human body providing temporal organisation to physiology. The environmental light/dark cycle (LD) is the predominant entraining agent or zeitgeber (from German, ‘time giver’) of the human circadian system – aligning internal time with environmental time. Disruption of circadian rhythms can be associated with sleep, mood, metabolic and other disorders (Czeisler 2015, Smolensky et al. 2016, Smolensky et al. 2015, West et al. 2017), and according to the International Agency for Research on Cancer (IARC) “probably” with cancer (Ward et al. 2019, Stevens et al. 2007).

Perinatal light imprinting of circadian clocks and systems (PLICCS) has been described in animal models (Richter et al. 2018, Salazar et al. 2018; for a systematic review up to 2016, see Lewis and Erren 2017a). Differential enduring effects on circadian rhythmicity of clock genes in individual neurons in the suprachiasmatic nuclei (SCN, the ‘master’ clock in the brain) are observed in animal models following different perinatal LD exposures (Ciarleglio et al. 2011, Tackenberg and McMahon 2018). Following this, downstream effects on circadian physiology and behaviour are observed (Lewis and Erren 2017a, Richter et al. 2018, Salazar et al. 2018). It was hypothesized that such imprinting could contribute to development of mood disorders and cancers (Ciarleglio et al. 2011, Erren et al. 2011). While PLICCS mechanism and effects *per se* may be difficult to assess in humans given the complex nature of required studies, associations between perinatal photoperiod (photoperiod: duration of daylight determined by time-of-year and location) and disease risk may be detectable. For instance, recent epidemiological studies suggest a possible link between perinatal photoperiod and mood disorders in humans (Bauer et al. 2015, Devore et al. 2018). Review and synthesis of epidemiological studies provide support that time-of-year of birth (month/season) *or* latitude may be associated with childhood cancer risk (Lewis and Erren 2017a). Importantly, though, daily photoperiods which depend on both time of year *and* latitude have not

been studied together in regard to childhood cancer (Lewis and Erren 2017a). To address this empirical gap, herein we provide first epidemiological explorations of the associations between perinatal photoperiod and childhood cancer using pooled data from prospective cohorts within the International Childhood Cancer Cohort Consortium (I4C) (Tikellis et al. 2018).

Hypothesis

Time-of-year of birth and location of birth, together determining perinatal photoperiod, contribute to cancer development through *Perinatal Light Imprinting of Circadian Clocks and Systems* (PLICCS and cancer hypothesis – Lewis and Erren [2017b]).

Predictions

Based on a review of experimental evidence (Lewis and Erren 2017a), photoperiods in the 3rd trimester of pregnancy, and the first 3-months post-birth, are predicted to be associated with childhood cancer risks. Two animal experiments offer evidence to arrive at dose-response predictions for such photoperiod-associated cancer risks: Compared to photoperiods with 16 hours of light, 8 hours were associated with higher risks of later life aberrant neural activity and clock gene expression in the SCN following a circadian challenge in mice [=“detrimental”] (Ciarleglio et al. 2011). Compared to 12 hours of light, 24 hours or constant light disrupted the developing biological clock in mice [=“detrimental”] (Ohta et al. 2006). Reconciling the hypothesized PLICCS mechanism and the photoperiod dose aspects, predictions 1-3 [P1-3] are:

P1 Photoperiods in two perinatal time windows; namely, the 3rd trimester of pregnancy, and the first 3-months post-birth, are associated with childhood cancer risks (Lewis and Erren 2017a).

P2 Photoperiod-associated risks of childhood cancer do not follow linear dose-response relationships from 0 to 24 hours of light; rather, increased or decreased risks are to be expected within ‘to-be-identified’ ranges of photoperiod.

Additionally, constant laboratory light-dark cycles are not the same as environmental photoperiods that increase or decrease daily between the spring and autumn equinoxes. Individuals may experience different percentages of perinatal time windows within or outside of the ‘to-be-identified’ ranges. Thus:

P3 Photoperiod-associated risks of childhood cancer can be affected by individuals’ experience of different percentages of perinatal time windows outside of the ‘to-be-identified’ ranges of photoperiod.

We explore the validity of P1, P2, and P3 using two metrics representing perinatal photoperiod characteristics, namely, mean daily photoperiod and maximum photoperiod change.

Methods

The I4C

The I4C was established in 2005 to investigate childhood cancer aetiologies with prospectively collected data – something that has been lacking in the field (Tikellis et al. 2018). The I4C currently pools data on some 190,000 mothers and their babies from six temporally diverse (recruitment years ranging between 1959 and 2009) and geographically diverse birth cohorts; namely, the Avon

Longitudinal Study of Parents and Children (ALSPAC – UK), the Collaborative Perinatal Project (CPP – USA), the Danish National Birth Cohort (DNBC – Denmark), the Jerusalem Perinatal Study (JPS – Israel), The Norway Mother, Father and Child Cohort Study (MoBa – Norway), and the Tasmanian Infant Health Study (TIHS - Australia) cohorts (Table 1) (Tikellis et al. 2018). The DNBC and MoBa each contribute a random 10% sample of their total cohorts and all cases in a case-cohort design (Tikellis et al. 2018). Cancer was ascertained using national or regional cancer registries (or by examination of diagnostic summaries and death records for the CPP) (Tikellis et al. 2018). Harmonisation and pooling of I4C data was performed at the Murdoch Childrens Research Institute (Australia) with further organisation and analyses of data for the current study performed at the University Hospital of Cologne (Germany) (Tikellis et al. 2018).

[Table 1 near here]

Ethics

Ethical approval for cohort studies was obtained from all requisite local research ethics committees based on informed consent and in accordance with the Declaration of Helsinki where applicable. The current study was approved by the I4C Steering Committee and the Ethics Commission of the Medical Faculty of the University of Cologne. The study conforms to standards put forward by (Portaluppi et al. 2010).

Data Description

This study employed a pooled cohort analysis of data on all cancer cases and non-cancer cohort participants who had information on (1) time-of-year of birth (date/month), (2) perinatal location or place of birth (from which closest latitude could be determined), and (3) sex. Individuals with

Down syndrome, which is associated with a particularly high risk of childhood leukaemia, were excluded (Seewald et al. 2012). Childhood cancer was classified as a diagnosis before 15 years of age. We calculated time to cancer onset as date of birth (or midpoint of month of birth) to date of first cancer diagnosis and censored all non-cases at date of last known follow-up, age 15 years or age reached by the time of the last cancer registry linkage, or date of death – whichever occurred first. Note, some individuals, for instance, in the weighted MoBa or DNBC samples, who may have emigrated or died are considered as non-cases censored at age 15 or age at last linkage to a cancer registry. However, as childhood cancer is a rare disease and the number of individuals whose time at risk is not properly defined is also considered to be low, we consider the impact of this on final results to be negligible.

Two perinatal time windows of interest were previously predicted based on a review of experimental evidence for PLICCS (Lewis and Erren 2017a): the 3rd trimester of pregnancy, and the first 3-months post-birth. Each individual was assigned (i) a mean daily photoperiod score and (ii) a maximum photoperiod change score (maximum daylight hours – minimum daylight hours) for the two predicted time windows of interest: The metrics were developed using the solar calculator provided by the United States Naval Observatory Astronomical Applications Department (<http://aa.usno.navy.mil/index.php>), which provides daily photoperiod information based on time-of-year of birth and location. Cohort specific details regarding time and location of birth can be found in the supplementary material.

Based on hypothesized PLICCS associations with childhood cancer risk, the following covariates were also included in adjusted models: sex, gestational age, birthweight, maternal and paternal education (as proxies for socioeconomic status/potential lifestyle differences), direction of photoperiod change in the time window of interest, and photoperiod metrics from the adjacent perinatal time window of interest (Fig. 1).

[Fig.1 near here]

Given the sinusoidal nature of photoperiod across the year for a given latitude and the fact that a year is 12 months in length, adjusting for photoperiod metrics in a non-adjacent perinatal 3-month time window (i.e. the 2nd trimester and the first 3-months post-birth) will introduce collinearity and small-sample bias (Rothman et al. 2008). Moreover, including more than one adjacent perinatal photoperiod in the adjusted models does not add more information because time-of-year relationship to photoperiod and duration of the adjacent time windows (i.e. 2x3 months=6 months) are sufficient to predict other perinatal photoperiods. Missing data in the covariates (Table 1) were addressed by multiple imputation using chained equations (chains=20, burn-in=10) based on variables collected by the I4C, including those not deemed to be biologically plausible covariates (see supplementary material). Convergence of chains was assessed by examining stationarity after the burn-in period. All multiple imputations included cancer, time to event, and sex as regular variables as these were pertinent to the hypothesis and final statistical analyses. Data were imputed for each cohort separately as factors specific to each cohort may impact the imputation of covariates. Overall, 37.9% of gestational age data, 0.4% of birthweight data, 6.85% of mother's education data, and 13.85% of father's education data was imputed (Table 1).

Analysis

The distribution of time-to-event was analysed by Cox proportional hazards regression for both metrics, both time-windows of interest, for all cancer types, and for blood or solid cancers, with and without adjustment for covariates. The proportional hazards assumption was checked by investigating Schoenfeld residuals (Schoenfeld 1982). Pooled models were stratified by cohort.

Sampling weights (factor of 10) were applied to non-cases in the DNBC and MoBa to account for their case-cohort design contribution of data to the I4C (Tikellis et al. 2018). Forest plots were used to display the findings. Heterogeneity between the Scandinavian and non-Scandinavian cohorts was assessed by computing the I^2 statistic using an interaction term between these groups in Cochran's Q. Linearity in the log hazard was visually inspected and supported using restricted cubic splines and comparing the non-spline linear Cox regression with quadratic and cubic fits to determine if either of the latter may be more appropriate. The restricted cubic splines involve a transformation of the log hazard, split by 'knot' points, defining piecewise polynomials. Separate curves are fit to each segment except at the tail ends where the splines are forced linear. In essence, the restricted cubic splines are used to inspect and characterize the shape and strength of the association of interest, here the log hazards as a function of the photoperiod metrics. Insofar, we use splines to visualize deviations from linearity in our associations of interest alongside the statistical assessment of whether linear, quadratic, or cubic Cox regression models provide best fit. According to Amrhein et al. (2019b), "Often, it is better to simply describe observed associations and their uncertainties (e.g. by giving point and interval estimates and plotting raw data)". Thus, *inter alia*, we visually inspected the linearity of the log hazard using restricted cubic splines; imprecision of spline estimation is reported by 95% confidence interval curves (Desquilbet and Mariotti 2010). Restricted cubic splines were also used to assess appropriate limits of mean daily photoperiod for inclusion into the statistical models. All spline models were computed following Stata's default (i.e. determined by the percentiles recommended by Harrell [2001]) and centred at mean daily photoperiod of 12-hours. All analyses were conducted with Stata, Version 15.1 (StataCorp, LLC, College Station, TX, USA). Hazard ratios (HR) with corresponding 95% confidence intervals (CIs) are reported. Focusing on 95%CIs, we follow Rothman in that "the advantage of confidence intervals over significance tests is that confidence intervals shift the

interpretation from a qualitative judgement about the role of chance as first (and sometimes only) interpretative goal to a quantitative estimation of the biologic measure of effect” (Rothman 1986). For a recent emphasis of this approach, see also (Amrhein et al. 2019a). Depending on whether the null hypothesis value [equal to “1”] falls outside or inside the confidence interval, results may be considered statistically significant at the 0.05 level or not (Rothman 1986). We did not adjust for multiplicity because comparisons were highly correlated (i.e. due to nested photoperiods and related metrics) and to guard against loss of statistical power (Rothman 2014).

Results

Table 1 describes the cohort-specific characteristics of the six cohorts used in this analysis. In total 182,856 mothers and babies contributed an average of 4604 days of follow-up and 2,319,245 person-years. Male sex is greater in all cohorts except the JPS. Gestational age is similar across the cohorts and average birthweight ranges from 3109 grams (TIHS) to 3561 grams (in DNBC) (Table 1).

Mean daily photoperiod in the 3rd trimester

Using all data from the six cohorts pooled, no pronounced overall change in risk is detected with increasing mean daily photoperiod in the 3rd trimester using Cox regression analysis (Table 2). However, the pattern of HRs of four individual cohorts (TIHS, ALSPAC, CPP, and JPS) smaller than 1 is compatible with decreasing risk with increasing 3rd trimester mean daily photoperiod (albeit their 95% CIs include the 1). Hazard ratios from the other two cohorts (DNBC and MoBa) are close to 1 and the confidence intervals are more centred around the 1 suggesting no change in risk with increasing 3rd trimester mean daily photoperiod (Table 2). The addition of an interaction

term for Scandinavian (DNBC and MoBa) vs. non-Scandinavian cohorts yields an interaction HR of 1.11 (95% CIs: 1.02-1.20). This difference of 11% in the effect of 3rd trimester mean daily photoperiod on childhood cancer risk suggests heterogeneity. The I^2 for 6 individual cohorts is 45.4% ($p=0.103$). While there are no substantial differences in cancer incidence rates between the Scandinavian and non-Scandinavian cohorts, the Scandinavian cohorts encompass a broader range of more extreme photoperiods including 0 hours (polar night) and 24 hours (midnight sun). Adjusted and unadjusted models showed similar results; hence, only unadjusted results are reported.

[Table 2 near here]

Visual inspection of the restricted cubic spline developed using all data from the 6 cohorts suggests a potential non-linear and non-monotonic relationship between 3rd trimester mean daily photoperiod and childhood cancer risk (Fig. 2A). Furthermore, the association between exposure and risk may be different for individuals who experienced photoperiods outside an approximately 8-16-hours range compared to individuals who experienced photoperiods within this range. However, too few study individuals and cases outside this range – evinced by the wide 95% CIs of the spline – impedes consideration of photoperiod associations with childhood cancer risk that includes these more extreme photoperiods.

[Fig. 2 near here]

The HRs and associated 95% CIs for the pooled and individual cohorts of those constituting the restricted set who did not experience the extremes of photoperiod, thereby including only those

with 3rd trimester daily photoperiods exclusively within the 8-16-hours range (range of interest is approximated from the spline) are presented in Fig. 3A. The pooled HR indicates a 9% decrease in risk for every hour increase in 3rd trimester mean daily photoperiod with the associated 95%CIs below 1. All cohorts that reach convergence (all except MoBa) give rise to HRs below 1 (albeit, the 95%CIs include the 1) except DNBC, which now contributes the lowest number of study individuals in this restricted set analysis. Analysis of MoBa does not reach convergence, likely due to too few study individuals and cases. Including individuals with increasing percentages of their 3rd trimester time windows with daily photoperiods outside the 8-16-hour range resulted in HRs closer to 1 and a more even spread of the 95%CIs either side of the 1 (Table 2).

[Fig. 3 near here]

Visual inspection of the restricted cubic spline for the 8-16 hours restricted set (Fig. 4A) supports this linear relationship between individual mean photoperiod length during the 3rd trimester and childhood cancer risk detected by the Cox regression analysis (Table 2). The section of the spline corresponding to below the 10-hour mark indicates higher risk than that section around the 12-hour mark and more-so compared to around the 14-hour mark. As splines allow insight into potential non-linear structures, the slight “bump” around the 12-hour mark and the “bump” around the 14-hour mark that results in an increasing gradient could be artefact, the latter possibly emphasized as restricted cubic splines are forced linear at the ends. This would be in line with the fact that quadratic and cubic Cox regression non-spline models (data not shown) are not better fits than the non-spline linear model for explaining the relationship between relative risk of childhood cancer and 3rd trimester individual mean daily photoperiod. Alternatively, they may reflect a true and rather steep decrease in risk from 12- to 14-hours. This decrease is in the range of many data points

and may have generated the on average decreased HR in the linear Cox regression modelling.

[Fig. 4 near here]

Following this signal of a potential photoperiod association with all cancer types in the restricted set, we separately assessed solid cancers and blood cancers. Decreasing risk with increasing 3rd trimester mean daily photoperiod is observed for both blood and solid cancer types (Fig. 5), with both the HR and 95% CIs below 1 for blood cancer (Fig 5A).

[Fig. 5 near here]

Mean daily photoperiod in the first 3 months post-birth

Similar to the 3rd trimester, a potential non-linear, non-monotonic relationship between mean daily photoperiod in the first 3 months post-birth and childhood cancer is indicated by visual inspection of the restricted cubic spline (Fig, 2B), but too few study individuals and cases outside the 8-16 hour range impedes further investigation of the study population as a whole. In contrast to the 3rd trimester time window, no linear association is suggested from the splines of the restricted set of daily photoperiods experienced during the first 3 months post birth (Fig. 3B and Fig. 4B).

Maximum photoperiod change in the 3rd trimester and first 3 months post-birth

There was no clear association between individual maximum photoperiod change and relative risk of all cancers or either sub-type in either time window for either the study population as a whole or for the 8-16 hour restricted set as determined by assessment of individual cohorts, pooled HRs and 95% CIs, and restricted cubic splines (supplementary Fig. S1, Table S1, and Table S2).

Discussion

This is the first epidemiological study of hypothesized links between perinatal photoperiods and childhood cancer. We assessed associations between two photoperiod metrics and childhood cancer risks in two perinatal time windows of interest. We did so in the I4C study population of 182,856 individuals as a whole, and in a substantial restricted set of 154,121 individuals with perinatal daily photoperiods exclusively within the 8-16 hours range. We detected an inverse or ‘protective’ association between 3rd trimester mean daily photoperiod and childhood cancer risk in the restricted set using Cox regression, supported visually by restricted cubic splines, which was present for both blood cancers and solid cancers.

Contrary to our expectation for the 3 months post-birth time window, ‘null’ associations with childhood cancer risks were statistically detected and this is supported by splines. As to why this is so remains unclear. A potential explanation may be that our susceptible time window predictions are based on limited evidence from animal models, and there may be differences in development between species.

Visual inspection of splines for the study population as a whole indicates potential non-linear and non-monotonic relationships (i.e. positive, ‘null’, or negative associations in distinct ranges of photoperiod) might exist in both the prenatal and postnatal window. However, too few study individuals and cases exposed to extreme photoperiods impedes more detailed consideration.

Similarly, with the maximum change in photoperiod metric, the hazard ratio point estimates were close to 1 and the even spread of confidence intervals on either side of the 1 was visually supported by the splines. Yet, as this is a first exploration of photoperiod associations with childhood cancer risk, we suggest the photoperiod change metric should be included in further

studies. Epidemiologically, a possible association of perinatal photoperiod change with lifetime depression was recently suggested by Devore et al. (2018).

Our findings are, for the most part, compatible with our chronobiology-derived predictions. Regarding prediction 1, empirical evidence supports that photoperiods in the 3rd trimester of pregnancy, but not in the first 3-months post-birth, are associated with childhood cancer risks. Regarding prediction 2, an inverse linear ‘protective’ association was statistically detected between 3rd trimester mean daily photoperiod and childhood cancer risks in the restricted set. Regarding predictions 2 and 3, when decreasing restricted set inclusion constraints, thereby including more individuals with increasing percentages of their 3rd trimesters at more extreme photoperiods, the inverse linear association weakened (as a pattern, the HRs moved closer to 1 and the 95% CIs were more evenly spread either side of the devore1). When considering the individual cohorts, the point estimates for the risk associations in DNBC and MoBa (cohorts with most individuals exposed to more extreme photoperiods) went in a different direction to the other four cohorts (P2 and P3). The 3rd trimester mean daily photoperiod spline for the study population as a whole is visually compatible with a non-linear and non-monotonic association with childhood cancer risk (P2); albeit, this is weak evidence without the ability to perform more detailed statistical testing and given the forced linear ends of splines. By the same rationale, it would be remiss to discuss the weak evidence of gradient in the 3 months post-birth (P1) spline in terms of our predictions. Limited data impeded detailed consideration of the 0-24 hour range as a whole or zoning in on photoperiod ranges outside of 8-16-hours. On the other hand, individual assessment of cohorts and of the pooled restricted set with gradually loosening of confinement provides – at least some – support for our chronobiology-derived predictions when considering all study individuals.

No previous study has combined latitude with time-of-year of birth as one determinant of a potential cancer co-causal environmental exposure (Lewis and Erren 2017a). Of those that detect

time-of-year of birth cancer risk differences, winter-spring births generally present with higher risks (Basta et al. 2010, Crump et al. 2015b, Crump et al. 2015a, Feltbower et al. 2001, Higgins et al. 2001, Makino et al. 2011, Meltzer et al. 1996, Nyari et al. 2008, Sorensen et al. 2001). As winter-spring birth implies a shorter 3rd trimester photoperiod, these findings are compatible with the findings of the current study.

Regarding geographical resolution of photoperiod, we anticipate the role of altitude to be negligible with no perinatal locations involving particularly mountainous regions. Moreover, daily differences within half a degree of latitude are for the most part very small (minutes of photoperiod); thus, lost resolution with rounding to the nearest latitude is expected to be negligible. We expect the perinatal location information used in this study (including postcodes, geo-coordinates, hospital of birth, or recruitment location of the cohort more generally e.g. Bristol) does not differ substantially from more precise perinatal location information after rounding to the nearest latitude.

Importantly, given the temporal (recruitment periods spanning five decades) and geographic diversity within the I4C, we consider it unlikely that other environmental variables confound our observations. Environmental factors that typically come to mind such as UV (and Vitamin D) and temperature only correlate with photoperiod when either latitude or time-of-year is held constant. Even then, photoperiod is predictably increasing or decreasing with each day whereas other environmental factors can fluctuate significantly on a day-to-day basis, across longitude (which does not affect daily photoperiod), and this variability increases further with geographical and temporal diversity. Similarly, we consider pesticide exposure and infectious agents also unlikely to be confounders that associate with the exposure as they are unlikely to be consistent across the geographical and temporal diversity within the I4C that will also include high variability in local climatic and weather-related factors; however, they cannot be completely ruled

out.

Regarding the hypothesized PLICCS mechanism, inability to account for circulating circadian factors or “temporal information” provided via breast milk may be considered a limitation. Although some I4C cohorts collected breastfeeding information, there was insufficient detail on timing across all cohorts for respective analyses. Similarly, it is unknown whether mothers took medication with possible effects on the foetal circadian system (e.g. melatonin). Additionally, this study assumes sufficient photoperiod information reaches the developing circadian systems of each foetus/infant on a sufficient number of days (brief exposures of mothers to natural light in the mornings and evenings and even through windows can be expected to suffice) (Simonneaux 2011). Furthermore, we assume most pregnant women in the 3rd trimester and mothers with newborns no more than 3 months old base their “schedules” around the daily photoperiod for the majority of the time windows of interest. That we observe a significant association between perinatal photoperiod and childhood cancer, and consider other factors less likely to be driving the association (for reasons outlined above), is in line with this rationale. Importantly, natural photoperiod impacts on the circadian time structure of individuals can be observed in ecological studies despite the presence of artificial light (Roenneberg et al. 2007, Shochat et al. 2019). This is likely due to the higher intensity of daylight being of greater zeitgeber strength (Duffy et al. 1996). In a similar fashion, natural photoperiod can be a co-causal agent in the current study.

Of note, photoperiod scores are inverted 6 months before or after any targeted time window (e.g. 16:8LD will be 8:16LD 6 months later). Thus, we cannot rule out that the signal of association with 3rd trimester mean daily photoperiod in the population restricted set is not, in fact, the inverse association with the 1st trimester mean daily photoperiod. In our view, this is unlikely given the prediction of relevant time windows for the hypothesized PLICCS mechanism from experimental evidence and given the evidence that the human circadian system is significantly developing in the

predicted perinatal time window(s).

In conclusion, we detected an inverse [‘protective’] linear association between the mean daily photoperiod in the 3rd trimester of pregnancy and childhood cancer risk for individuals who experience 3rd trimester daily photoperiods exclusively in the 8-16 hours range. Future epidemiological work should explore (a) possible confounding by other environmental factors, (b) inclusion of ranges with more extreme photoperiods, (c) exploration of differences in association between perinatal photoperiod and specific types of cancers, and (d) extension to other disease endpoints. Finally, further future experimental investigations into the hypothesized circadian imprinting mechanism are warranted.

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Author Contributions

TE conceived the hypothesis (2011) and organized the project thereafter. PL, TE, and JVG designed the study. PL, MH, LF, and PM conducted analyses. PL, MH, LF, PM, and TE interpreted results. PL and TE wrote the manuscript draft. LF and ALP contributed to the epidemiological aspects of the discussion. RF contributed to the light-associated aspects of the discussion. I4C representatives contributed data for the analyses. All authors commented on the manuscript draft, approved the submission, and are accountable for the work. TE and PL contributed equally to this study.

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Disclosure of Interest

All authors declare no conflict of interest.

Data Availability

Please contact Gabriella Tikellis at the Murdoch Childrens Research Institute (Australia) regarding availability of the International Childhood Cancer Cohort Consortium (I4C) data. E: gabriella.tikellis@mcri.edu.au

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Table 1: Descriptive Characteristics

	ALSPAC	CPP	DNBC^a	JPS	MoBa^a	TIHS	Total^a
Latitude	52°N	30-45°N	54.5-57°N	32°N	58.5-70.3°N	41-43°S	-
Recruitment	1991-92	1959-65	1996-2002	1964-76	1999-2009	1987-1995	1959-2009
Livebirths (n)	14,049	56,690	9,596	90,079	11,295	10,629	192,338
After exclusion ^b	14,033	48,533	9,585	90,044	11,037	10,624	183,856
Cancer cases (n)	24	50	202	172	233	32	713
After exclusion ^{bc}	22	50	200	172	218	27	689
Leukaemia	<5	16	63	41	72	4	199
Lymphoma	n/a	5	19	31	9	4	68
Blood ^d	<5*	21	82	72	81	8	267*
Solid	>17*	29	118	100	137	19	422*
Follow-up Time (days, mean ± SD)	5474 ± 114	2447 ± 808	5356 ± 544	5472 ± 151	4519 ± 806	5364 ± 266	4604 ± 1400
Person Years (nearest whole year)	210,467	325,405	140,651	1,349,951	136,640	156,130	2,319,245
Sex							
Male	51.71%	50.53%	50.87%	48.49%	50.38%	68.79%	50.69%
Female	48.29%	49.47%	49.13%	51.51%	49.62%	31.21%	49.31%
Gestational Age							
Weeks, mean ± SD	39.36 ± 2.03	39.4 ± 2.98	39.93 ± 1.96	39.6 ± 2.3	39.31 ± 2.07	38.47 ± 2.73	39.38 ± 2.6
(Missing)	(0.01%)	(0.96%)	(0.09%)	(76.78%)	(0.38%)	(0.29%)	(37.9%)
Birthweight							
Grams, mean ± SD	3386 ± 572	3160 ± 545	3561 ± 591	3252 ± 541	3542 ± 615	3109 ± 769	3263 ± 580
(Missing)	(1.28%)	(0.17%)	(0.58%)	(0.46%)	(0.09%)	-	(0.4%)
Mother ≥ 12 years education							
Yes	62.05%	41.37%	45.78%	40.85%	83.56%	81.20%	47.76%
No	26.62%	56.81%	24.14%	52.33%	6.68%	18.52%	45.39%
(Missing)	(11.33%)	(1.82%)	(30.08%)	(6.82%)	(9.76%)	(0.27%)	(6.85%)
Father ≥ 12 years education							
Yes	55.78%	35.51%	30.34%	45.03%	77.44%	70.18%	45.97%
No	29.47%	41.14%	37.43%	47.67%	9.03%	21.35%	40.18%
(Missing)	(14.75%)	(23.35%)	(32.23%)	(7.3%)	(13.53%)	(8.47%)	(13.85%)

ALSPAC = Avon Longitudinal Study of Parents and Children (UK); DNBC = Danish National Birth Cohort (Denmark); CPP = Collaborative Perinatal Project (USA); JPS = Jerusalem Perinatal Study (Israel); MoBa = Norwegian Mother, Father and Child Cohort Study (Norway); TIHS = Tasmanian Infant Health Study (Australia); SD = standard deviation; n/a = not available due to data protection issues associated with small numbers;

^a The DNBC and MoBa individuals utilised in this study were in case-cohort design (10% of total cohort and all cancer cases). ^b Exclusion criteria includes presence of Down syndrome, insufficient information on date or month of birth, perinatal location or place of birth (to the closest latitude), and/or sex; ^c Individuals for whom cancer diagnosis occurred after 15 years were counted as non-cases;

^d Blood cancers = leukaemias and lymphomas. *Data protection issues associated with small numbers precluded differentiation of lymphomas from solid cancers in the ALSPAC cohort (n<5).

Table 2: Hazard ratios (HR) and 95% confidence intervals (CI) computed by Cox proportional hazards regression on pooled data of all cancers stratified by cohort for 3rd trimester individual mean daily photoperiod. The percentage indicates how much of the time window includes photoperiods outside of the 8-16 hour range.

Percentage of 3rd trimester	Pooled HR (CIs)	ALSPAC HR (CIs)	CPP HR (CIs)	DNBC HR (CIs)	JPS HR (CIs)	MoBa HR (CIs)	TIHS HR (CIs)
0%	0.91 (0.84-0.99)	0.65 (0.28-1.53)	0.89 (0.76-1.04)	1.05 (0.52-2.12)	0.93 (0.83-1.05)	*	0.87 (0.70-1.08)
5%	0.92 (0.84-0.99)	0.89 (0.59-1.35)	-	1.27 (0.82-2.00)	-	*	-
10%	0.92 (0.85-1.00)	-	-	1.20 (0.89-1.61)	-	0.79 (0.42-1.49)	-
15%	0.93 (0.86-1.00)	-	-	1.15 (0.91-1.45)	-	0.95 (0.59-1.52)	-
20%	0.94 (0.87-1.01)	-	-	1.02 (0.84-1.22)	-	1.18 (0.86-1.63)	-
40%	0.96 (0.91-1.01)	-	-	1.02 (0.93-1.13)	-	1.00 (0.89-1.12)	-
60%	0.99 (0.95-1.03)	-	-	1.03 (0.97-1.10)	-	1.00 (0.94-1.07)	-
80%	1.00 (0.97-1.03)	-	-	1.02 (0.97-1.07)	-	1.01 (0.97-1.06)	-
100%	1.00 (0.98-1.03)	-	-	1.02 (0.97-1.06)	-	1.01 (0.98-1.05)	-

ALSPAC = Avon Longitudinal Study of Parents and Children (UK); DNBC = Danish National Birth Cohort (Denmark); CPP = Collaborative Perinatal Project (USA); JPS = Jerusalem Perinatal Study (Israel); MoBa = Norwegian Mother, Father and Child Cohort Study (Norway); TIHS = Tasmanian Infant Health Study (Australia)

*No convergence, no cases in these cohorts, or too few cases/individuals to provide realistic estimates

- No further individuals are included and so the HRs and CIs remain as they are.

Table 3: Cancer cases and non-cases for all cancers and by type, and maximum and minimum photoperiods experienced by an individual in each cohort for the 3rd trimester after exclusion of individuals who experience a daily photoperiod outside the 8-16-hours range in this time-window. Maximum and minimum photoperiods experienced by an individual in each cohort for the 3rd trimester in full dataset are also shown for comparison.

	Cohorts					
	ALSPAC	CPP	DNBC	JPS	MoBa	TIHS
Maximum individual mean daily photoperiod (hours)	12.93	15.24	12.94	14.02	12.15	14.98
Minimum individual mean daily photoperiod (hours)	10.98	9.17	11.03	10.29	12.10	9.34
All cancer cases	7	50	30	172	6	27
Blood cancers	0	20	11	72	2	8
Solid cancers	7	30	19	100	4	19
Non-cases	3,652	48,483	1,054	89,872	171	10,597
Full dataset						
Maximum individual mean daily photoperiod (hours)	15.987	15.24	16.97	14.02	23.31	14.98
Minimum individual mean daily photoperiod (hours)	8.50	9.17	7.61	10.29	1.26	9.34

ALSPAC = Avon Longitudinal Study of Parents and Children (UK); DNBC = Danish National Birth Cohort (Denmark); CPP = Collaborative Perinatal Project (USA); JPS = Jerusalem Perinatal Study (Israel); MoBa = Norwegian Mother, Father and Child Cohort Study (Norway); TIHS = Tasmanian Infant Health Study (Australia)

Figure Captions

Fig. 1 Directed acyclic graph with hypothesized mechanism and potentially relevant covariates. Yellow boxes indicate the hypothesized main effect pathway from perinatal latitude and time-of-year as determinants of perinatal photoperiod which affect PLICCS and subsequent risk of childhood cancer. The green box indicates potentially relevant covariates that were considered in this study.

Fig. 2 Restricted cubic splines of log HR and associated CIs for all cancer cases and participants in all cohorts pooled plotted as a function of (A) 3rd trimester and (B) first 3-months post-birth individual mean daily photoperiods. Log HR is centered at mean daily photoperiod = 12 hours.

Fig. 3 Forest plots of HR and associated CIs for all childhood cancers in each cohort and pooled data stratified by cohort for (A) 3rd trimester and (B) first 3-months post-birth individual mean daily photoperiods for individuals who experience photoperiods exclusively inside the 8-16-hour range in the respective time-windows. As inclusion of other covariates did not improve the model fits; the results above are unadjusted. Data in the MoBa cohort were limited to such an extent that the analysis did not reach convergence; thus, MoBa is missing from the above plots.

Fig. 4 Restricted cubic splines of log HR and associated CIs for all cohorts pooled plotted as a function of (A) 3rd trimester and (B) first 3-months post-birth individual mean daily

photoperiods for individuals who experience photoperiods exclusively inside the 8-16 hour range in the respective time-windows. Log HR is centered at mean daily photoperiod = 12 hours.

Fig. 5 Forest plots of HR and associated CIs for for (A) blood cancers and (B) solid cancers in all cohorts and pooled data stratified by cohort for 3rd trimester individual mean daily photoperiods for individuals who experience photoperiods exclusively inside the 8-16 hour range in the respective time-windows. As inclusion of other covariates did not improve the model fits, the results above are unadjusted. Data in the MoBa cohort were limited to such an extent that the analysis did not reach convergence; thus, MoBa is omitted from the above plots. For (A), ALSPAC did not contain any blood cancer cases in the 8-16 hour range; thus ALSPAC is omitted from (A).