The influence of meal timing and residential artificial light-at-night in the development of non-communicable diseases

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Summary

During the last century, the exponential human and economic growth, accompanied by the wide development of electric lightning, has forced the shift towards a 24-h society in which people eat around the clock and are exposed to artificial light-at-night (ALAN). However, studies investigating the influence of meal timing, nighttime fasting duration, and exposure to residential ALAN on the development of non-communicable diseases (NCDs) are scarce. The aim of this doctoral thesis is to investigate (i) whether daily eating/fasting cycles and (ii) exposure to residential ALAN can influence the development of NCDs.

Data from the Spanish MultiCase-Control study (MCC-Spain) and two prospective studies, the French NutriNet-Santé, and the Genomes for Life (GCAT) Catalan cohorts, were analysed to address this aim. We found that a longer nighttime fasting duration may be associated with a lower prostate cancer risk when the fast is broken early in the morning (≤8:30 AM). Although we did not observe an association with nighttime fasting duration, breakfast was also crucial in relation to breast cancer risk. Having a later first meal of the day was associated with a higher risk of breast cancer, especially among premenopausal women.

A later first meal of the day was also associated with a higher risk of type 2 diabetes (T2D). These results also indicated that extending the nighttime fasting duration may be associated with a lower T2D risk only when the fast is broken early in the morning (<8 AM). A later

first meal of the day was similarly linked to a higher risk of cardiovascular diseases (CVD) as well as a late last meal of the day, especially cerebrovascular diseases. The impact of later meal timings on CVD was worse for women than for men. Finally, exposure to higher levels of residential ALAN, was associated with prevalent hypertension and incident hypercholesterolemia.

Overall, the findings of this thesis suggest that aligning nutritional behaviours with circadian rhythms, by eating during daytime and especially having an early first meal of the day, may help to prevent NCDs. Extending the nighttime fasting duration may be beneficial only if this fast period is broken early in the day, together with an early last meal of the day. Additionally, reducing the exposure to residential ALAN, a source of circadian disruption, may also reduce the risk of developing this group of diseases.

Resumen

Durante el último siglo, el crecimiento humano y económico exponenciales, acompañados del amplio desarrollo de la iluminación eléctrica, han forzado el cambio hacia una sociedad de 24 horas en la que las personas comen a cualquier hora del día y están expuestas a luz artificial nocturna (LAN). Sin embargo, son escasos los estudios que investigan la influencia del horario de las comidas, del ayuno nocturno y de la exposición a LAN residencial en el desarrollo de las enfermedades no transmisibles (ENT). El objetivo de esta tesis doctoral es investigar (i) si los ciclos diarios de alimentación/ayuno y (ii) la exposición a LAN residencial pueden influir en el desarrollo de las ENT.

Para ello se han analizado datos del estudio español MCC-Spain y de dos estudios prospectivos, las cohortes de NutriNet-Santé y GCAT. Encontramos que una mayor duración del ayuno nocturno puede estar asociada con un menor riesgo de cáncer de próstata cuando el ayuno nocturno se rompe temprano en la mañana (≤8:30AM). Aunque no observamos una asociación con la duración del ayuno nocturno, el des -ayuno también fue crucial en relación con el riesgo de cáncer de mama. Realizar la primera comida del día más tarde se asoció con un mayor riesgo de cáncer de mama, especialmente en las mujeres premenopáusicas.

Una primera comida del día más tardía también se asoció con un mayor riesgo de diabetes de tipo 2 (DT2). Estos resultados también indicaron que prolongar la duración del ayuno nocturno puede

asociarse a un menor riesgo de DT2 sólo cuando se rompe el ayuno a primera hora de la mañana (<8AM). Una primera comida del día más tardía se relacionó de forma similar con un mayor riesgo de enfermedades cardiovasculares y una última comida tardía con un mayor riesgo de este grupo de enfermedades, especialmente las cerebrovasculares. El impacto de un horario de comidas más tardío fue peor para las mujeres que para los hombres. Por último, la exposición a niveles más altos de LAN residencial, se asoció a hipertensión prevalente y a hipercolesterolemia incidente.

En general, los hallazgos de esta tesis sugieren que alinear los comportamientos nutricionales con los ritmos circadianos, comiendo durante el día y especialmente haciendo una primera comida del día temprana, puede ayudar a prevenir las ENT. Prolongar la duración del ayuno nocturno puede ser beneficioso sólo si este periodo de ayuno se rompe a primera hora del día, junto con una última comida temprana. Además, reducir la exposición a LAN residencial, una fuente de alteración circadiana, también puede reducir el riesgo de desarrollar este grupo de enfermedades.

Resum

Durant l'últim segle, el creixement humà i econòmic exponencials, acompanyats de l'ampli desenvolupament de la il·luminació elèctrica, han forçat el canvi cap a una societat de 24 hores en què les persones mengen a qualsevol hora del dia i estan exposades a llum artificial nocturna (LAN). No obstant, són escassos els estudis que investiguen la influència de l'horari dels àpats, del dejuni nocturn i de l'exposició a la LAN residencial en el desenvolupament de les malalties no transmissibles (MNT). L'objectiu d'aquesta tesi doctoral és investigar (i) si els cicles diaris d'alimentació/dejú i (ii) l'exposició a LAN residencial poden influir en el desenvolupament de les MNT.

Per fer-ho, s'han analitzat dades de l'estudi espanyol MCC-Spain i de dos estudis prospectius, les cohorts del NutriNet-Santé i del GCAT. Trobem que un dejuni nocturn perllongat podria estar associat amb un menor risc de càncer de pròstata quan el dejuni es trenca d'hora al matí (≤8:30AM). Encara que no observem una associació amb la durada del dejuni nocturn, l'esmorzar també va ser crucial en relació amb el risc de càncer de mama. Realitzar el primer àpat del dia més tard es va associar amb un major risc de càncer de mama, especialment en les dones premenopàusiques.

Un primer àpat del dia més tardà també es va associar amb un risc major de diabetis tipus 2 (DT2). Aquests resultats també van indicar que perllongar la durada del dejuni nocturn es podria associar a un menor risc de DT2 només quan es trenca el dejuni a primera hora del matí (<8AM). Un primer àpat del dia més tardà es va relacionar de manera similar amb un risc major de malalties cardiovasculars i un

darrer àpat tardà amb un risc més alt d'aquest grup de malalties, especialment les cerebrovasculars. L'impacte d'un horari més tardà d'àpats va ser pitjor per a les dones que per als homes. Finalment, l'exposició a nivells més alts de LAN residencial es va associar a hipertensió prevalent i a hipercolesterolèmia incident.

En general, les troballes d'aquesta tesi suggereixen que alinear els comportaments nutricionals amb els ritmes circadians, menjant durant el dia i especialment fent un primer àpat del dia temprà, pot ajudar a prevenir les MNT. Perllongar la durada del dejuni nocturn només pot ser beneficiós només si aquest període de dejuni es trenca a primera hora del dia, juntament amb un darrer àpat del dia temprà. A més, reduir l'exposició a LAN residencial, una font d'alteració circadiana, també pot reduir el risc de desenvolupar aquest grup de malalties.

Preface

This doctoral thesis has been developed at the Barcelona Institute of Global Health (ISGlobal) from October 2019 to September 2022 under the supervision of Prof. Manolis Kogevinas and Dr. Dora Romaguera. The PhD candidate did a 4-month research stay (from September to December 2021) at the Nutritional Epidemiology Research Team (EREN), in Paris under the supervision of Dr. Mathilde Touvier and Dr. Bernard Srour. The present doctoral thesis consists of five articles of original research contributions first-authored by the PhD candidate (2 published, 2 under review and 1 as an advanced draft). This thesis complies with the procedures and regulations of the Biomedicine PhD program of the Department of Medicine and Life Sciences of the Universitat Pompeu Fabra, Barcelona, Spain.

The present thesis contributes to understanding the health impact of mild circadian disruption in the general population, mainly arising from mistimed nutritional behaviours, but also from exposure to artificial light-at-night in the residence. The introduction revises the current evidence on this research question, both from animal and human studies, and identifies the gaps in research that justify the main objective of this doctoral thesis. *Paper I* and *Paper II* explore the association of nighttime fasting duration with breast and prostate cancer with special attention to the time of breakfast and were conducted with data from the MCC-Spain. *Paper III* and *Paper IV* were conducted within the NutriNet-Santé cohort, a large longitudinal study in France, and examine the association of daily

eating / fasting timings in relation with the risk of developing T2D and CVDs. The last article enclosed in this thesis (*Paper V)*, investigates the exposure to artificial light-at-night in relation with cardiometabolic risk factors and diseases and was conducted with data from the GCAT study, a large longitudinal study established in Catalonia. In the discussion section, the methodology of the included articles is considered.

For all the publications, the PhD candidate was responsible for designing the analysis plan and conducting the analyses which included different statistical methods applied in epidemiological studies. The PhD candidate was also responsible for interpreting the results, reporting them in scientific publications and disseminating the findings to the scientific community in national and international congresses, as well as the general population through several multimedia channels. Aside from doing research the PhD candidate undertook several courses to improve her understanding of complex epidemiological situations as well as to improve her writing and statistical skills. The PhD candidate co-authored as well two investigations. One on melatonin and sex hormone-related changes in night shift workers that contributed to a better understanding of circadian disruption in this population and its association with cancer (Harding et al., 2022). She co-authored as well a protocol for a scoping review on the effects of ALAN on human health (Deprato et al., 2022). In this work she will be mainly contributing to the cancer outcomes. Additionally, the candidate has worked as a reviewer in a paper on daily eating duration and fatal cancer (Meth et al., 2022) and has been involved in teaching activities (see Appendices).

This PhD thesis was funded by a MINECO (Ministry of Economy in Spain) fellowship (PRE2019-089038) and by the FIS Grant PI17/01388 and was conducted, to a large extent, during the COVID-19 pandemic. Some of the initial sub-objectives stated in the thesis protocol were planned to be addressed with a validation study conducted within the GCAT study. The objective of this study was to validate several exposures including exposure to artificial light-at-night and to natural daylight and also timing of physical activity and meals. The PhD candidate wrote the protocol, coordinated and conducted the field work for this study. The field work involved the contact with the participants, the administration of the questionnaires and two visits to the participants' residence to perform different measurements. This was done from November 2019 to March 2020 when the COVID-19 pandemic and lock-down forced to stop it. By then the study was only conducted in 50 participants. The following waves of COVID-19 did not allow to continue this validation study and, therefore, the PhD candidate had to adapt and modify the thesis protocol accordingly to this new situation.

Abbreviations

ALAN	Artificial light-at-night
BMI	Body-mass index
CHD	Coronary heart diseases
CI	Confidence interval
CVD	Cardiovascular disease
eTRE	Early time-restricted eating
FFQ	Food frequency questionnaire
HbA1c	Haemoglobin A1c
HDL	High-density lipoprotein cholesterol
HR	Hazard ratio
IARC	International Agency for Research on Cancer
IF	Intermittent fasting
IQR	Interquartile range
ipRGC	Intrinsically-photosensitive retinal ganglion cell
LDL	Low-density lipoprotein cholesterol
LED	Light-emitting diode
NCD	Non-communicable disease
OR	Odds ratio
RCT	Randomised controlled trial
SCN	Suprachiasmatic nucleus
T1D	Type 1 diabetes
T2D	Type 2 diabetes
TRE	Time-restricted eating
TRF	Time-restricted feeding
WHR	Waist-to-hip ratio

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1. INTRODUCTION

1.1. Global burden of non-communicable diseases

In 2019, the Global Burden of Diseases (GBD) project estimated that 4 out of the 5 most common causes of death worldwide were non-communicable diseases (NCDs), led by cardiovascular diseases (CVDs) and including neoplasms and diabetes (Institute for Health Metrics and Evaluation (IHME), 2019). According to the World Health Organisation (WHO), every year 41 million deaths globally (71%) can be attributed to NCDs (World Health Organisation, 2021). Given the global burden of NCDs, it is of great global health interest to improve the understanding and prevention of risk factors contributing to the development of this group of diseases.

Classic modifiable risk factors for NCDs include tobacco and alcohol consumption, unhealthy diet, physical inactivity and air pollution (World Health Organisation, 2021). Recently, the alteration of circadian rhythms has been put under the spotlight in relation to several NCDs including cancer, CVDs and type 2 diabetes (T2D). Mounting evidence suggests that alterations in the circadian system through mistimed 24-hour activities characteristic of the modern Western society could play an important role in the rising burden of NCDs. Night shift work or jet lag are classic examples of strong circadian alteration, but we can find other sources of milder circadian disruption such as mistimed nutritional behaviours or exposure to artificial light-at-night (ALAN).

1.2. The circadian system

Life on earth has evolved to adapt and anticipate daily environmental oscillations resulting from the 24-hour rotation of planet Earth. Natural selection has driven the development of a system, known as the circadian system, which is conserved across all organisms, from primitive cyanobacteria to humans (Honma, 2018). The term circadian derives from the Latin "circa diem" and means "about a day". Circadian rhythms are corporal changes that occur in our body with a periodicity of about 24-hours.

This system enables organisms to be prepared to access food when available and to store supplies and undergo repairment processes during the hours of fasting, improving metabolic fitness and survival. The natural selection of circadian rhythms enabled organisms to efficiently anticipate periodic events such as availability of food, light and temperature. This anticipation is more evident in photosynthetic organisms that obtain energy from the sun, but is also conserved in energetic cycles in higher organisms (Takahashi, 2017).

We can find circadian rhythms in daily activities such as sleep, oscillations in body temperature or alertness. A clear example of a circadian rhythm can be also found in the endocrine glands. Hormone synthesis and release, key factors in the communication between organs, are highly rhythmic and predictable over the 24h period (Gamble et al., 2014). This can be explained by the strong circadian regulation of this system. Melatonin and cortisol are two hormones produced under a stringent circadian regulation (Gamble et al., 2014).

Melatonin is characteristically low in light conditions and is produced in the pineal gland in response to darkness during the night. This hormone triggers key endocrine responses to induce and maintain sleep. The production of cortisol is regulated through the hypothalamic-pituitary-adrenal axis and is also subject to time-of-day-dependent control. Cortisol levels peak in the morning (7-8AM) to prepare the body for anticipating increased activity like the increased energetic demand (Kalsbeek et al., 1996).

1.2.1. Organisation and importance of the circadian system

Circadian rhythms are endogenous, which means that they function without any external time-giving input. Without external inputs, or in *free-running* conditions, circadian rhythms happen with a periodicity of a little bit more of 24 hours (Obert et al., 2000). The self-sustained nature of circadian rhythms reflects the existence of an intrinsic circadian clock. In 2017, Hall, Rosbash and Young were awarded with the Nobel Prize in Physiology or Medicine, for their discovery of the circadian clock (Huang, 2018). Overall, the molecular functioning of the circadian clock consists of an auto-regulatory mechanism composed of inter-locking transcriptional-translational feedback loops (Gutierrez Lopez et al., 2021). The rhythmic synthesis of specific proteins, called clock proteins, establish intra- and extracellular temporal signals (Challet, 2019). The circadian clock in mammalian cells is presented in Figure 1.

The master clock of the circadian system is located in the suprachiasmatic nucleus (SCN) in the brain and there are peripheral clocks in virtually all tissues of the human body including the liver, pancreas, gut, adipocytes or muscles (Richards & Gumz, 2012).

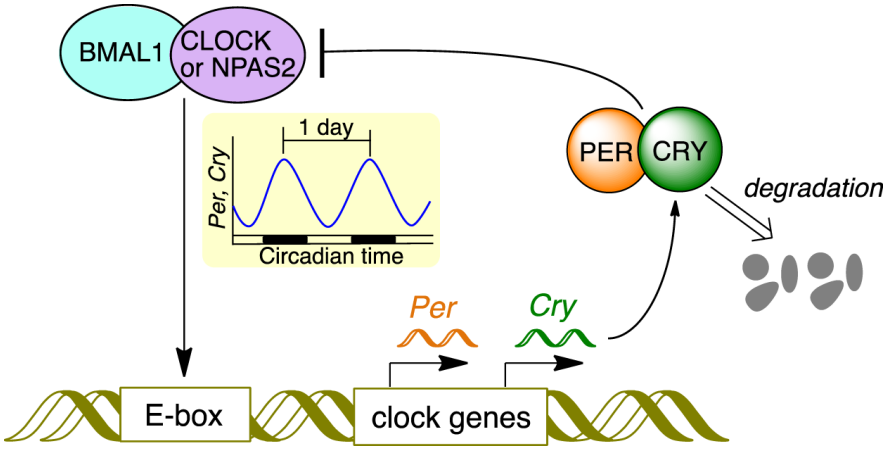

Figure 1. Autoregulatory machinery of the circadian clock in mammals (Minegishi et al., 2018). Figure 1, p. 2 of original source.

The mammalian circadian system is responsible for regulating multiple physiological activities in the body including hormonal secretion, immune regulation, metabolic and redox homeostasis, inflammatory response and DNA damage repair (Fagiani et al., 2022). The circadian system optimises metabolic performance, by anticipating food intake, synchronised cell division and immune response (Woller & Gonze, 2021).

1.2.2. Light-induced circadian synchronisation

Although circadian rhythms have a self-sustained nature, they are resynchronised every day to environmental inputs or “time givers”

which are called *zeitgebers* (Pickel & Sung, 2020). This process of circadian synchronisation is known as entrainment.

The main synchroniser of the circadian system is the natural light/dark cycle. Aside from the visual processing of light, which enables us to see, the retina is also responsible for its non-image forming photoreception. Specifically, light is captured by intrinsically photosensitive retinal ganglion cells (ipRGCs) in the retina which express melanopsin (Fonken & Nelson, 2014). Signals are sent to the SCN through the retinohypothalamic tract.

The master clock communicates time-of-day information to peripheral clocks through the sympathetic and parasympathetic nervous system, the core body temperature and through hormones (e.g., cortisol, melatonin) (Patke et al., 2019). There is a reciprocal crosstalk between the master clock in the SCN and peripheral clocks across the body (Lee & Wisor, 2021). Once the SCN receives photic inputs, rapid changes in cellular activities occur, including the induction of circadian genes. The SCN projects output pathways mainly to the serotonin-producing raphe nuclei, which regulates wakefulness, and to the pineal gland, regulating the production of melatonin, the sleep hormone (Blume et al., 2019).

1.2.3. Food-induced circadian synchronisation

Aside from the inputs received from the master clock in the SCN, peripheral clocks can also respond to non-photic zeitgebers from the environment including food intake, exercise or temperature. Light has only indirect effects on peripheral clocks, but food intake is the most powerful zeitgeber of peripheral clocks (Pickel & Sung, 2020).

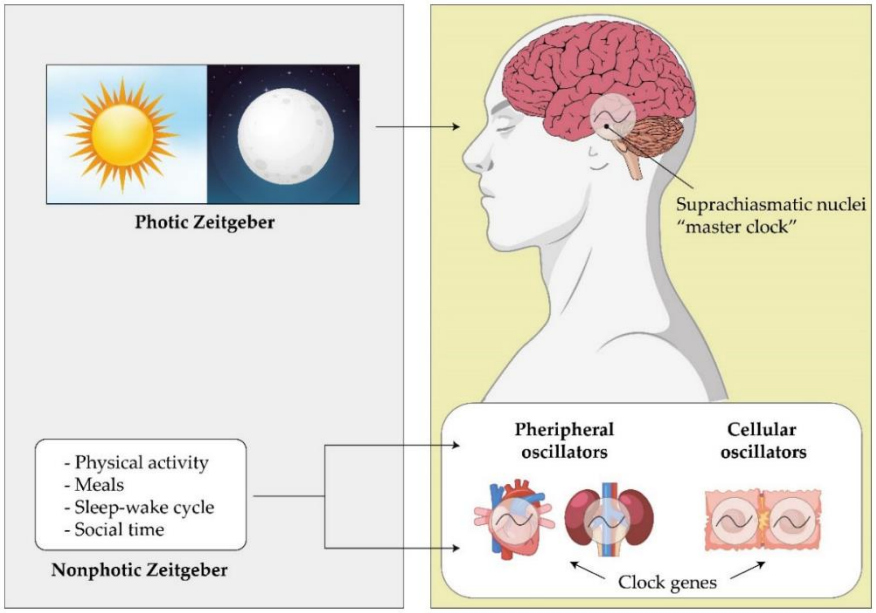

Figure 2. Synchronisation of the circadian system by external inputs (Rahi´c et al., 2021). Figure 2, p.67 of original source.

Genes involved in energy metabolism are under circadian control (Marcheva et al., 2013). Research in mice shows that mutations in the Clock gene induces the attenuation of feeding rhythm, alteration of gluconeogenesis, lipid homeostasis, impaired insulin sensitivity and obesity (Oishi et al., 2006; Turek et al., 2005). Bmal1 knockout mice, another key clock gene, also present alterations in glucose and lipid homeostasis (Rudic et al., 2004; Shimba et al., 2011).

In animal models, it has been shown that the production of insulin and insulin-like growth factor 1 (IGF-1), in response to food intake, are key signals to reset peripheral circadian clocks throughout the body (Crosby et al., 2019). Restricting eating to the inactive phase can lead to a phase shift of peripheral clocks in the pancreas, heart,

liver, kidney and skeletal muscle. This zeitgeber effect of food on peripheral clocks do not affect the SCN and it is independent of light (Crosby et al., 2019; Damiola et al., 2000; Hara et al., 2001; Schibler et al., 2003; Stokkan et al., 2001). This independency of central and peripheral entrainment has been demonstrated since restricted eating can synchronise the liver clock in mice with an impaired SCN (Hara et al., 2001).

The peripheral clock responding more rapidly to food intake is the liver, a key organ in metabolism (Damiola et al., 2000). Additionally, the most effective meal in determining the liver clock phase is breakfast as it is the first meal after nightly fasting (Hirao et al., 2010). Breakfast consumption controls the expression of clock genes and allows a normal fluctuation of the circadian clock (Jakubowicz et al., 2017).

From an evolutionary, perspective it is justified that tissues respond differently to *zeitgeber* because of the organ-specific primary function and environmental inputs to anticipate. Peripheral clocks regulate local physiological processes such as glucose and lipid homeostasis, digestion/absorption, colonic motility, immunity and secretion of hormones (Richards & Gumz, 2012).

It is becoming more evident that there is a time-of-day-dependent optimal metabolism. Nutrients exhibit circadian fluctuations in their capacities to be digested, absorbed and metabolised (Aoyama & Shibata, 2020). Gastric emptying (Goo et al., 1987) and gastrointestinal motility (Rao et al., 2001) are optimal in the circadian

morning. The digestion of nutrients is also maximised during the active phase through the production of bile acids (Han et al., 2015) and intestinal absorption (Hussain & Pan, 2015). Additionally, gut microbiota displays daily rhythms facilitating energy metabolism during the day and during the night (Thaiss et al., 2014). Diet-induced thermogenesis reaches its peak during the morning and decreases throughout the day (Morris et al., 2015).

There is clear evidence for diurnal variations in glucose tolerance and whole-body insulin sensitivity, both peaking in the early morning and declining throughout the day (Poggiogalle et al., 2018; Saad et al., 2012; Stenvers et al., 2019). This is strongly mediated by the rhythm in nutrient-induced secretion of insulin in pancreatic β-cells which is stronger in the circadian morning (Perelis et al., 2015, 2016). Postprandial responses to triacylglycerol (TG) and amino acids are dependent on time-of-day, similarly, with an optimal intestinal absorption and metabolism earlier than later in the day (Aoyama & Shibata, 2020).

One meal can have very different responses depending on when during the day it is consumed, showing that circadian clocks regulate energy metabolism and that meal timing is a decisive factor in nutrient handling. In the light of recent findings on food-induced circadian synchronisation, chrononutrition has emerged as a field of nutritional sciences that studies the relationship between food intake, the circadian system and health (Tahara & Shibata, 2013).

1.2.4. Circadian misalignment

Industrialisation and the wide development of artificial light have forced the shift towards a 24-h society in which people work night shifts to increase productivity (around 20% globally, (Hernández-García et al., 2020)), have mistimed nutritional behaviours and are exposed to ALAN. Travelling to geographical regions with different time-zones can also result in an abrupt shift of the sleep/wake cycle and a misalignment of the circadian system (Vosko et al., 2010).

Receiving mistimed inputs from *zeitgebers* can alter the circadian system leading to circadian misalignment or chronodisruption (Rajaratnam & Arendt, 2001). Given the broad implications of the circadian system, dysregulations in this system can have wide implications for our health (Figure 3). The consequences of chronodisruption have been widely studied in night shift workers (Q. J. Wu et al., 2022), since this population is exposed to several mistimed inputs including exposure to ALAN, mistimed nutritional behaviours and disturbances of the sleep cycle.

In 2007, and again in 2019, the International Agency for Research on Cancer (IARC) identified night shift work involving circadian disruption as a probably carcinogen for breast, colon and prostate cancer (IARC Monographs Vol 124 group, 2019; Straif et al., 2007). Night shift work has been also linked to many cardiometabolic disturbances including CVDs (Vyas et al., 2012; Q. J. Wu et al., 2022), T2D (Q. J. Wu et al., 2022), obesity (M. Sun et al., 2018) and metabolic syndrome (Cheng et al., 2021; F. Wang et al., 2014).

Aside from the severe circadian desynchronization in night shift workers, there are also other causes of milder circadian misalignment in the general population. Being exposed to light during the evening or while sleeping can alter this system. Similarly, the discordance between the eating behaviour and the activity in the central clock in the SNC can uncouple peripheral clocks and lead to circadian misalignment.

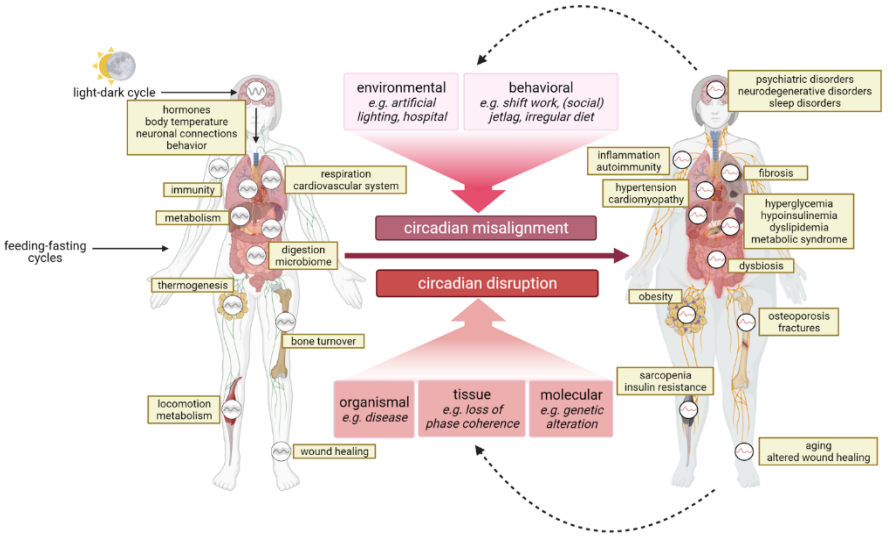

Figure 3. Circadian misalignment and consequences (Kramer et al., 2022). Figure 1, p.2 of original source.

Key messages

- The circadian system orchestrates the **optimal** functioning of multiple **physiological functions** across all organisms.
- Circadian rhythms function in a **self-sustained manner**, under the control of the **circadian clock**, but they can be **resynchronised** every day by external "time givers" or ***zeitgebers*** which allow the **adaptation** to the environment.
- The natural daily **light/dark cycle** is the main synchroniser of the **master circadian clock in the brain**, but **food intake** is the most powerful *zeitgeber* of **peripheral clocks**.
- There is a **time-of-day-dependent metabolism** and **meal timing** is a **decisive** factor in **nutrient handling**.
- Mistimed external inputs, such exposure to ALAN or mistimed eating patterns, can lead to **chronodisruption**.

1.3. Circadian nutritional behaviours

The shift towards a 24h society has forced the extension of the daily eating window, prolonging it towards later eating times. Additionally, the speeded modern lifestyle, the perception of lacking time in Western societies, and the current surge in fasting practices have forced the change of nutritional behaviours characterised by meals skipping, especially breakfast (Pendergast et al., 2016). The daily eating/fasting cycle is a key synchroniser of the circadian system and mistimed nutritional behaviours can have deleterious health effects.

Throughout this thesis we will refer to circadian nutritional behaviours as the time-related behaviours in eating patterns, capturing when we eat and when we fast on a daily basis. These behaviours include the starting time of the daily eating window (breakfast or first meal of the day), the ending time of the daily eating window (last meal of the day) and the nighttime fasting duration.

In this section we will first summarise the current evidence from animals and humans exploring the influence of the first and the last meal of the day on the development of NCDs and we will cover cardiometabolic risk factors, overweight and obesity, T2D, CVDs and cancer. Then, in a separate section we will go through the more recent and less explored case of nighttime fasting duration in relation with NCDs.

1.3.1. Research in animal models

A mice model of a delayed first meal of the day (for 6 hours) showed that skipping the analogous breakfast altered the peak time of clock genes expression, which are involved in lipid metabolism, resulting in increased lipogenesis and weight gain (C. Yoshida et al., 2012). Similarly, a 4h delayed breakfast timing protocol for rats showed increased adipose tissue weight and body weight gain (Shimizu et al., 2018). Moreover, there was a delayed peak of serum non-esterified fatty acids, bile acids and insulin and, probably as a consequence, a delayed circadian oscillation of the hepatic clock and lipid metabolism-related genes. Delaying the first active-phase meal resulted as well in a delay on the surge in body temperature, which could have contributed to the observed weight gain through decreased energy expenditure. In 2021, results from the same rat model indicated that delaying breakfast led to an increased concentration of hepatic lipids and adipose tissue weight without changes in food intake (D. Kim et al., 2021).

On the other hand, eating late in the evening can affect the phase of peripheral clocks (Kuroda et al., 2012). Feeding nocturnal rodents restrictedly during the day for a week can alter the phase of the circadian expression of clock genes (Damiola et al., 2000; Hara et al., 2001; Stokkan et al., 2001). Mice fed with a high fat diet during the rest phase during two weeks gained significantly more weight than mice fed during the active phase (Arble et al., 2009). There was no difference in energy intake between both groups and the changes persisted after 4 weeks of the intervention. In rats, mimicked late-

night-eating also resulted in hepatic lipid accumulation, microbial dysbiosis and systemic inflammation in peripheral tissues (Ni et al., 2019). Overall, suggesting that mistimed eating during the natural rest phase can lead to obesity and metabolic disturbances.

1.3.2. Research in humans

1.3.2.1. Cardiometabolic risk factors

Energy intake seems to have a time-of-day dependent impact on cardiometabolic health. Skipping breakfast has been associated with an impaired cardiometabolic risk profile in a cross-sectional analysis with data from the National Health and Nutrition Examination Survey (NHANES, 1999-2006) (Deshmukh-Taskar et al., 2013). Specifically, breakfast skippers were more likely to have an elevated blood pressure, total cholesterol and serum insulin. It has also been suggested that skipping breakfast can lead to a metabolic inflexibility through a higher postprandial insulin and increased fat oxidation and that this can result in low-grade inflammation and alter glucose homeostasis (Nas et al., 2017).

The change towards a more diurnal nutritional behaviour, in a trial in healthy men habitually omitting breakfast, resulted in an advance of the circadian phase of the cardiac autonomic nervous system (Yoshizaki et al., 2013). Furthermore, participants in the early mealtime group (with three meals at 8AM, 1PM and 6PM compared to the control group at 1PM, 6PM and 11PM) showed significantly decreased levels of triglycerides, total and low-density lipoprotein (LDL) cholesterol.

Habitual breakfast omission concomitant with late-night-dinner has been associated with metabolic syndrome (OR = 1.17, 95% CI 1.08 – 1.28) and proteinuria (OR = 1.37, 95% CI 1.24 – 1.52), a key risk factor in the development of CVDs and chronic kidney disease (Kutsuma et al., 2014). Similarly, a cohort study with 3.9 years of follow-up, showed that nighttime eating was linked to an increased risk of metabolic syndrome in women, but not in men (J. Yoshida et al., 2018). This study reported as well an association with dyslipidaemia in both men and women.

In a weight loss trial, 82 women were randomly assigned to an early evening meal (7PM – 7:30PM) or a late evening meal group (10:30PM – 11PM) (Madjd et al., 2021). After 12 weeks of programme, compared to women consuming a late evening meal, those consuming an early evening meal showed a reduction in body-mass index (BMI), waist circumference, total cholesterol, triacylglycerols and homeostasis model assessment of insulin resistance. In line with these results, a randomized crossover trial in 20 healthy volunteers, showed that a late dinner (10PM versus 6PM) resulted in a 4-hour shift in the postprandial period, nocturnal glucose intolerance and altered fatty acid oxidation and mobilization (C. Gu et al., 2020). Moreover, these alterations were more pronounced in earlier sleepers assessed with overnight polysomnography.

Only two observational studies have explored the association between daily times of the first and last eating occasion and cardiometabolic risk factors. A 1-year prospective study of 116 women, showed that a delay in the averaged timing of first eating

occasion (per 30-min delay) was associated with a worse cardiovascular health score (measured with the American Heart Association Life's Simple 7 score), a higher diastolic blood pressure and a higher fasting glucose level (Makarem et al., 2020). Similarly, cross-sectional data from the NHANES (2005-2016) showed that every additional hour delay in the time of first meal of the day was linked to higher C-reactive protein levels, insulin and glucose and to lower levels of high-density lipoprotein (HDL) cholesterol (Wirth et al., 2021). Additionally, every additional hour in the time of last meal of the day was associated with higher levels of glycated haemoglobin (HbA1c) and lower LDL cholesterol levels.

1.3.2.2. Overweight and obesity

The relationship between breakfast and obesity remains a controversial topic. In a systematic review and meta-analysis of 36 cross-sectional and 9 cohort studies, skipping breakfast was consistently associated with an increased risk of overweight and obesity (OR = 1.30, 95% CI 1.16 – 1.47 and OR = 1.48, 95% CI 1.36 – 1.62, respectively) (Ma et al., 2020). Contrary to these results, a meta-analysis of randomized controlled trials (RCTs) concluded that there was no evidence supporting the regular consumption of breakfast as a weight loss strategy (Sievert et al., 2019). However, all the included studies had a high risk of bias, mainly due to a lack of blinding, and results should be interpreted with caution.

Previous research in humans has also suggested that eating late-at-night, in misalignment with the circadian clock, is associated with

increased risk for obesity. The habit of eating late-at-night has been associated with overweight and obesity in two cross-sectional studies (Berg et al., 2009; Okada et al., 2019) and a prospective cohort study with 8,153 adult individuals (J. Yoshida et al., 2018). Late-night eating has been also associated with a delayed body-temperature phase, a reduced amplitude of the circadian rhythm of cortisol, with inflammatory markers, higher waist circumference and obesity in children (Martínez-Lozano et al., 2020).

1.3.2.3. Type 2 diabetes

Nutritional behaviours are key factors in the prevention and management of T2D and, hence, the circadian component has been also studied in relation with this metabolic disease. Results from a crossover study with 18 healthy adult individuals and 18 adult individuals with diabetes, showed that skipping breakfast altered clock and clock-controlled gene expression and resulted in an increased postprandial glycaemic response (Jakubowicz et al., 2017). These results were observed both in the healthy group and the group with diabetes. Similarly, in a crossover trial, 22 individuals with diabetes were randomly assigned to two test days: one with breakfast, lunch and dinner and one without breakfast (Jakubowicz et al., 2015). Compared to the test day with breakfast, in the no breakfast day participants showed a 30-min delay in the insulin peak after lunch and dinner and a lower intact glucagon-like peptide-1 (iGLP-1). Overall, this resulted in postprandial hyperglycaemia when skipping breakfast (Jakubowicz et al., 2015). Likewise, an observational study with 5,479 adults with normal HbA1c levels showed that skipping

breakfast for 3 times or more per week was associated with an increased risk of a poor glycaemic control after four years of follow-up (OR = 2.11, 95% CI 1.04 – 4.26) (Iwasaki et al., 2019).

Results from a systematic review and meta-analysis with 6 prospective cohort studies reported an increased risk of T2D for participants ever skipping breakfast compared to those never skipping it (RR = 1.33, 95% CI 1.22 – 1.46) (Ballon et al., 2019). This association showed some degree of mediation by BMI. In the 6 included cohort studies breakfast was assessed through a questionnaire at baseline that included a question about frequency of eating breakfast throughout the week (Byrne et al., 2016; Mekary et al., 2012; Odegaard et al., 2013; Sugimori et al., 1998; Uemura et al., 2015).

Only one study has explored the association between timing of breakfast and risk of T2D in older adults (aged ≥ 65 years) (Carew et al., 2022). In this prospective study, breakfast consumption was not associated with T2D, but the habit of having a later breakfast (after 9AM versus 7AM to 9AM) was associated with a reduced risk of T2D (adjusted HR = 0.71, 95% CI 0.51 – 0.99). This was only seen in participants with impaired fasting glucose at baseline. The inverse association between time of breakfast and the null association between breakfast consumption and risk of T2D are in disagreement with previous evidence (Ballon et al., 2019). In one of the studies included in the meta-analyses by Ballon and colleagues on breakfast and T2D (Ballon et al., 2019), the association between an irregular breakfast consumption and T2D was only present in participants aged

under 65 (Mekary et al., 2013). Taken together this shows some degree of effect modification of the association between breakfast and T2D by age possibly explained by the differential nutritional needs in older adults (Carew et al., 2022).

On the other hand, eating late-at-night has been also linked to predictors in the development of T2D such as nocturnal glucose intolerance or insulin resistance (C. Gu et al., 2020; Madjd et al., 2021). A large-scale cross-sectional study in more than 60 thousand individuals, reported that the habit of late-night-dinner eating was associated with hyperglycaemia (Nakajima & Suwa, 2015). One hypothesis explaining these results is that food intake concomitant with high melatonin levels (characteristic of the rest period) may result in glucose intolerance. In fact, results from a randomised cross-over trial showed that late dinner (11PM) concurrent with high endogenous melatonin levels can result in impaired glucose tolerance (Lopez-Minguez et al., 2018). However, no study has ever explored the link between time of last eating episode of the day and risk of T2D.

1.3.2.4. Cardiovascular diseases

Few epidemiological studies have explored the link between circadian nutritional behaviours and CVDs. In a meta-analysis of 6 cohort studies, compared to regular breakfast consumption, skipping breakfast has been associated with the risk of CVD (RR = 1.22, 95% CI 1.10 – 1.35) and all-cause mortality (RR = 1.25, 95% CI 1.11 – 1.40) (H. Chen et al., 2020). In the included cohort from the Health

Professionals Follow-up Study, men who reported eating late-at-night had a higher risk of coronary heart diseases (CHD) compared to participants not eating at night (RR = 1.55, 95% CI 1.05 – 2.29) (Cahill et al., 2013). In an adult population that included 7,771 individuals free of CVD, during a mean 3.19 years, self-reported night-eating frequency was associated with arterial stiffness (X. Zhang et al., 2020). This investigation reported as well the presence of effect modification by sex, showing a stronger association in women.

In line with these results, a retrospective analysis of a nationwide epidemiological database explored the association between eating behaviours and CVDs (Kaneko et al., 2021). Multivariate models were adjusted by age, sex, BMI, waist circumference, hypertension, T2D, dyslipidaemia and cigarette smoking. Participants reporting non-optimal behaviours characterised by skipping breakfast (≥ 3 days /week), late-night dinner (having dinner < 2 h before their bedtime for ≥3 days /week) and bedtime snacking (eating snacks after dinner for ≥3 days /week) had a statistically significant higher risk of stroke and heart failure compared to those with optimal behaviours.

1.3.2.5. Cancer

Night shift work that involves circadian disruption is a probable carcinogen for cancers of the breast prostate and colon (IARC Monographs Vol 124 group, 2019; Straif et al., 2007). Following the IARC classification, some studies started to be conducted to investigate whether milder circadian disruption in the general

population is also linked with these cancers. As we have seen, circadian nutritional behaviours have multiple implications in human health. Late-night-eating and skipping breakfast could be linked to inflammation and metabolic and circadian disturbances (Martínez-Lozano et al., 2020; Ni et al., 2019), which could potentially be linked to cancer.

Prostate and breast cancer risk have been scarcely studied in relation with meal timings. Data from the MCC-Spain study, suggested that compared to a time interval of 1hr or less between dinner and sleep, extending this interval to more than 2 hours was associated with a decreased risk of prostate and breast cancer combined (OR = 0.80, 95% CI 0.67 – 0.96) (Kogevinas et al., 2018). The association for a long-time interval between dinner and sleep was more pronounced in individuals with a morning chronotype, which is human trait that shows preference for timing of daily activities more towards the morning or the evening. In the same study, compared to a dinner at 10PM or later, a dinner before 9PM was associated with a reduced risk of both cancers (OR = 0.82, 95% CI 0.67 – 1.00).

Consistent with these results, data from the French NutriNet-Santé cohort study on 41,389 adults showed that late eaters (having dinner after 9:30PM) were more likely to develop prostate (HR = 2.20, 95% CI 1.28 – 3.78) and breast cancer (HR = 1.48, 95% 1.02 – 2.17) (Srour et al., 2018). In this study it was also investigated the longitudinal associations with number of eating episodes, time of first meal and macronutrient composition of the last meal of the day.

However, no association was observed between any of these other nutritional behaviours and cancer risk.

1.3.2.6. General remarks

There is clear evidence that meal timing is a decisive factor in nutrient handling, that food intake is an important synchronizer of peripheral circadian clocks and that the first meal of the day is an important trigger of this system. Skipping breakfast, defined as the weekly frequency of breakfast consumption, affect key predictors of cardiometabolic diseases and has been linked to T2D and CVDs. Similarly, the frequency of eating late-at-night has been linked to obesity, CVDs and to a less extent cancer. However, the definition of these behaviours in observational studies has been generally based on whether the participant reported eating or not a specific meal (i.e., breakfast) without taking into consideration the time of the day at which this meal was taken, leading to classification bias (for example, eating at 11AM could be defined as having breakfast or not having breakfast). Only one study has explored specifically the time of breakfast in relation with T2D in older adults, but no other study has investigated the daily times of the first and last eating occasion and the risk of developing NCDs. Therefore, it remains unclear when is the optimal time to eat in the day in relation with the risk of this group of diseases.

1.3.3. Nighttime fasting

Even though circadian patterns in nutritional behaviours should be investigated in their totality, I have a separate section on nighttime

fasting, because this is a fairly new research area with limited evidence.

Fasting has been commonly practiced for cultural, spiritual and religious reasons in ancient societies (Mandal et al., 2022). Recently, diets based on the manipulation of timing have gained great attention in media and research (Freire, 2020). Intermittent fasting (IF) consists of alternating periods of unrestricted eating and regular periods of fasting which can range from hours to days (Welton et al., 2020). Time-restricted eating (TRE) is a form of IF that promotes consistently restricting the daily length of the eating window to less than 12 hours, by extending the nighttime fasting duration (Schuppelius et al., 2021).

The first studies on this topic were conducted in rodents under the term of time-restricted feeding (TRF). Compared to mice fed *ad-libitum* (as often as desired), mice under TRF (for eight hours during the active phase) have shown reduced body weight, hyperinsulinemia, inflammation and cholesterol levels (Hatori et al., 2012; Sherman et al., 2012). The protective effects of TRF can be maintained when mimicking the *ad libitum* food access during the weekends, a typical behaviour of western societies (Chaix et al., 2014). Furthermore, this timed feeding has shown the ability to restore the expression phase of circadian clock genes (Sherman et al., 2012).

The increased research interest in time-based diets also promoted the initiation of intervention trials in humans. A meta-analysis of RCTs

showed that IF reduced body weight, waist circumference and fat mass more effectively than non-intervention diet (L. Gu et al., 2022). However, the main limitation in this meta-analysis is that multiple fasting regimens were compared including TRE, but also alternate-day fasting and other IF schemes of more than 1 day.

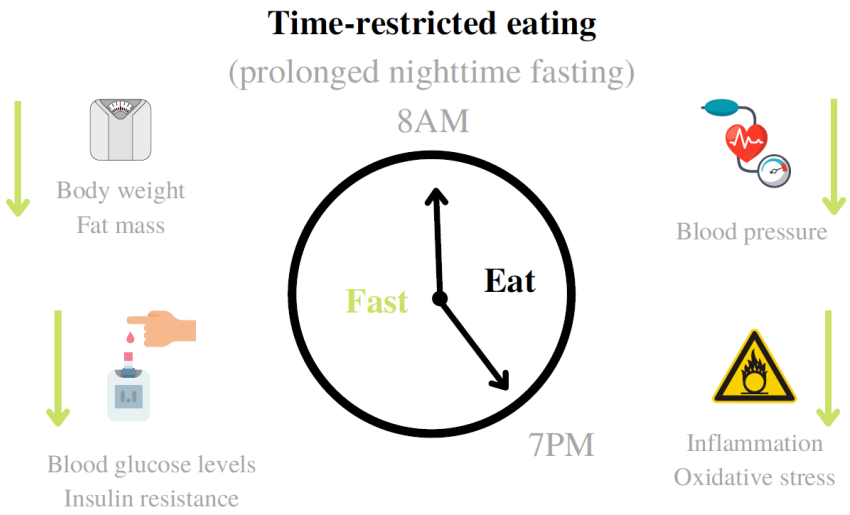

Figure 4. Time-restricted eating and cardiometabolic outcomes.

TRE is the fasting regimen most related with circadian rhythms because it has a daily basis and it shows promising results reducing body weight, fat mass, blood glucose levels, insulin resistance, inflammation, oxidative stress and blood pressure (Figure 4) (Schuppelius et al., 2021). Although, the potential benefits of TRE could be explained by the reduction in energy intake (D. Liu et al., 2022), several trials have also demonstrated positive effects of TRE independently of caloric restriction (Schuppelius et al., 2021).

Few observational studies have explored the association between fasting practices and cardiometabolic diseases (Currenti et al., 2021; Makarem et al., 2020; Wirth et al., 2021). A cross-sectional analysis of 1,936 adults with nutritional data from food frequency questionnaires (FFQ), showed that compared to participants with a daily eating window of more than 10 hours, those with a shorter eating window were less likely to have overweight/obesity, hypertension and dyslipidaemias (Currenti et al., 2021). Habitual nighttime fasting duration has also been investigated in relation with cardiometabolic endpoints in two observational studies (Makarem et al., 2020; Wirth et al., 2021). Both studies reported that longer nighttime fasting periods were positively associated with cardiometabolic risk factors. However, in both studies models were not adjusted for the time of first meal of the day, something that in the same studies was reported to be also associated with cardiometabolic endpoints.

One important limitation of the current evidence on TRE is that there is a wide spectrum of regimens with periods of fasting from 13 to 20 hours and that it remains unclear when is best to start and finish these periods (Schuppelius et al., 2021). It is not the same to start the eating window at 8AM and finish it at 6PM in the afternoon than starting at 12AM and finish it at 10PM. A 14-week parallel-arm RCT, showed that energy restriction in combination with early TRE (eTRE) (8-hour eating window from 7AM to 3PM) was more effective for losing weight and improving diastolic blood pressure than in combination with a non-TRE regimen (Jamshed et al., 2022). Data from a recently published RCT in 90 healthy individuals has shown that an eTRE (8h

period, between 6AM and 3PM) was more efficient in improving insulin sensitivity compared to a mid-day TRE (8h period, between 11AM and 8PM) (Xie et al., 2022). Additionally, only the early regimen was able to reduce total body mass, improve fasting glucose, reduce inflammation and increase the diversity of the gut microbiota.

Very few studies have investigated the effects of TRE on cancer in rodent models and data are inconsistent. In mouse models of obesity-driven postmenopausal breast cancer, compared to mice fed *ad libitum*, TRF to 8 hours inhibited tumour initiation, progression and metastasis independently or daily energy intake and of weight loss (Das et al., 2021). Results from this study also suggested that this was partly mediated through reduced insulin signalling. In line with these results, restricting feeding to the active phase in mice mitigated the mammary tumorigenesis induced by a high-fat diet (Sundaram & Yan, 2018). However, in a mouse model of renal cancer, TRF did not significantly reduce tumour growth or weight (Turbitt et al., 2020).

Nighttime fasting duration in relation with cancer has been also explored scarcely in observational studies. Within the prospective Women's Healthy Eating and Living study, compared to women practicing a nighttime fasting of 13 hours or more, those with a shorter fasting duration had a higher risk of breast cancer recurrence (HR = 1.36, 95% CI 1.05 – 1.76) (Marinac et al., 2016). Results from the French NutriNet-Santé cohort showed that nighttime fasting duration was not associated neither with the risk of breast nor prostate cancer (Srour et al., 2018). In the Uppsala Longitudinal Study of Adult Men, the daily eating duration and the day-to-day variability in

the timing of eating were explored in relation with fatal cancer risk in community-dwelling men (Meth et al., 2022). Compared to men reporting on average a daily eating duration of less than 11 hours, those reporting a daily eating duration of 13 hours had an increased risk of fatal cancer risk (HR = 2.33, 95% CI 1.22 – 4.44). Moreover, those with a higher variability in the timing of eating had a higher risk.

In contrast with the nutritional behaviour of our modern society, usually defined by a delayed and extended daily eating window, recent evidence suggests that time-based diets offer a potential strategy to prevent cardiometabolic diseases. There is an increasing attention within the scientific community on the promising results of TRE for cardiometabolic health. However, no previous epidemiological study has explored the association between prolonged nighttime fasting duration, or in other words TRE, and incident cardiometabolic diseases such as T2D and CVDs. Moreover, data on prolonged nighttime fasting duration and cancer are very limited in humans.

Key messages

- **Circadian nutritional behaviours** are the **time-related** behaviours in **eating patterns**, capturing when we eat and fast on a daily basis.
- Nowadays there is a shift towards **later circadian nutritional behaviours** and **extended** daily eating periods in **Western societies**.
- The habit of **skipping breakfast** throughout the week has been **associated with NCDs** including T2D, obesity and CVDs.
- In animal models a **delaying the time of the first meal** of the day leads to **alterations** in **circadian** regulation, **lipid** metabolism and **energy** expenditure.
- **Very few** population studies have **explored** the influence of the **time of the first meal** of the day on the risk of NCD.
- The habit of **late-night eating** has been, similarly, associated with **NCD risk**, but the **definition** of late-night eating is **controversial** as it is related with cultural patterns.
- Extending the **nighttime fasting duration**, or practicing time-restricted eating, could be a **potential strategy** to improve circadian nutritional behaviours and **prevent NCD**.
- **There is a lack of studies** investigating the influence of the **time of first and last meal of the day** and the **nighttime fasting** duration on the risk of developing **NCDs**.

1.4. Exposure to artificial light-at-night

During the last century, the wide development of electric lighting, exponential human growth, transportation and economic activity have contributed to the acceleration of light pollution (Bennie et al., 2014). The industrial development, together with the wide-spread use of light-emitting devices have turned nights brighter (Ishihara et al., 2021).

Light is the main *zeitgeber* of the circadian master clock and, therefore, exposure to this environmental input in misalignment with the internal circadian clock can have wide consequences for our health. For many years, rods and cones, the main photoreceptors in visual responses to light, have been considered also responsible for circadian photoentrainment (Fu et al., 2005). At the beginning of the 21st century, this assumption was rejected after the discovery of the ipRGCs in the retina (Figure 5), the main photoreceptors in the circadian system (Berson et al., 2002). Melanopsin is the photopigment sensing light in the ipRGCs and is sensitive in a shorter spectral wavelength than cones and rods (Brown et al., 2022). Therefore, photometric measures of light in the melanopsin-sensitivity portion might be more appropriate than those describing photopic illuminance to assess the chronodisrupting effects of ALAN (Brown et al., 2022).

As it is the case of air pollution, light pollution is widespread across the globe and will continue to increase since it is linked to the exponential population and economic growth. ALAN is present both in indoor settings, mainly affecting humans, and in outdoor settings,

affecting biodiversity and also humans involved in night activities outdoors (Svechkina et al., 2020). In outdoor settings, ALAN can come from different anthropogenic sources including the public street lightning but also from vehicles and buildings.

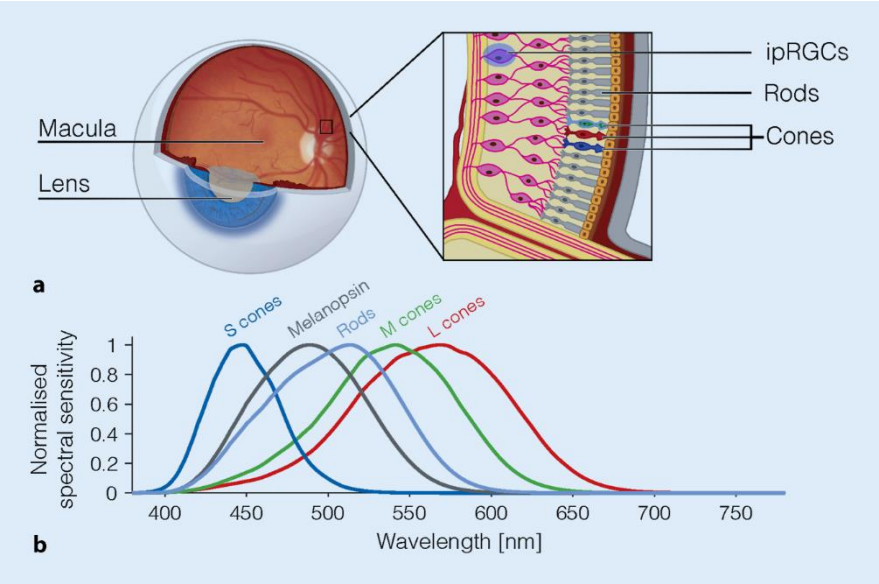

Figure 5. Graphic representation of photoreceptors in the retina and their spectral sensitivity (Blume et al., 2019). Figure 3, p. 150 of original source.

Street lighting constitute a crucial part of urban designing since it conveys security, quality and comfort to the public environment. For many decades, public street lightning was mainly composed by high pressure sodium and metal halide lamps (Gutierrez-Escolar et al., 2015). Many European cities, including Barcelona (Ajuntament de Barcelona, n.d.), are now changing the public street lightning towards the use of light-emitting diode (LED) lamps to move towards a more sustainable and efficient urban environment (Garcia-Saenz et al.,

2020). However, this alternative lighting technology may have a greater impact on the human circadian system than lamps with green or yellow wavelengths (Jo et al., 2021). This is because light emitted by LEDs has the most efficient wavelength to supress melatonin production (Davies & Smyth, 2018).

1.4.1. Research in animal models

Evidence in nonhuman animals demonstrates that exposure to ALAN can induce carcinogenity (IARC Monographs Vol 124 group, 2019). Mice exposed to continuous light (24h) had a higher risk of developing spontaneous lung carcinoma, leukaemia and hepatocarcinoma compared to mice under a standard light/dark regimen (Anisimov et al., 2004). Another study in mice demonstrated that an 8-hour advance in the light-dark schedule could result in an increased incidence of hepatocellular carcinoma (Kettner et al., 2016). Mechanisms supporting the association between ALAN and cancer include oxidative stress, suppression of the immune response, inflammation, epigenetic alterations, changes in the endocrine function including melatonin suppression, changes in telomere length and disruption of clock genes (IARC Monographs Vol 124 group, 2019).

Alterations in the light/dark cycle can hamper metabolic health. Exposing mice to ALAN results in an impaired glucose tolerance and decreased plasma insulin independently of caloric intake (Fonken et al., 2010; Masís-Vargas et al., 2019; Opperhuizen et al., 2017). Similarly, dim ALAN can alter the balance of lipogenic pathways resulting in lipid accumulation in the liver of rats (Okuliarova et al.,

2020). Mistimed light exposure disturbs the circadian rhythm amplitude, impairs circadian rhythmicity in food intake and energy expenditure, induces body weight gain and abolishes the normal circadian fluctuation in insulin sensitivity in mice (Coomans et al., 2013).

Constant light exposure (24 h/day) can also exert cardiovascular effects. In a study in rats, exposure to light for 4 weeks resulted in a weakened cardiac function potentially related with an observed sympathetic hyperactivity (Jing et al., 2020). Moreover, exposure to low-intensity ALAN led to increased systolic blood pressure, plasma insulin and hepatic triglyceride levels in a model of spontaneously hypertensive rats (Rumanova et al., 2019).

1.4.2. Research in humans

Mistimed exposure to ALAN disrupts circadian rhythms and can impair the production of melatonin and oestrogens, alter the metabolic function, damage DNA and can result in oxidative stress and inflammation (Figure 6) (Rumanova et al., 2020; Russart & Nelson, 2018).

High levels of residential ALAN exposure, in the blue light spectrum and assessed from satellite images, have been associated with a higher risk of breast, prostate and colorectal cancer in the MCC-Spain study (Garcia-Saenz et al., 2018, 2020). Exposure to ALAN, both from indoor and outdoor settings, has been associated with a higher breast cancer risk (RR = 1.11, 95% CI, 1.07 – 1.15) in a meta-analysis of 10 cohort and 7 case-control studies (Urbano et al., 2021). In this

study, results showed a slightly stronger association among premenopausal women and among women with oestrogen receptor positive. Residential ALAN exposure, assessed from satellite images, has also been associated with cancer of the thyroid in a longitudinal study after 12.8 years of follow-up (D. Zhang et al., 2021). Similarly, night shift work has been linked to a different degree with cancers of the prostate, lung, pancreas, bladder, colon and rectum and non-Hodgkin's lymphoma, with melatonin suppression as a result of exposure to ALAN, the mostly cited mechanistic hypothesis (Parent et al., 2012).

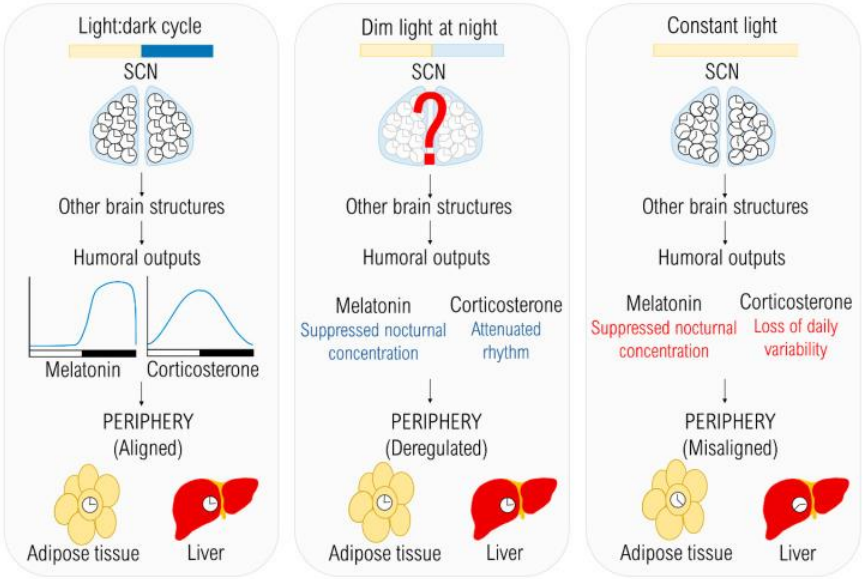

Figure 6. Consequences of exposure to dim and constant light-at-night (Rumanova et al., 2020). Figure 1, p.10 of original source.

Aside from the described oncogenic associations, exposure to ALAN has also been linked with cardiometabolic health in humans. In a

cross-sectional study with data on 528 elderly individuals, participants with a higher ALAN exposure in their bedroom (participants exposed on average to ≥3 lux compared to <3 lux) had a worse metabolic profile defined by a higher BMI, triglyceride levels and LDL levels and by a lower HDL level (Obayashi et al., 2013). Furthermore, participants exposed to greater ALAN levels were more likely to be obese and to have dyslipidaemia. These associations were independent of classic demographic and socioeconomic confounders.

In this same population, Obayashi and colleagues (2014) showed that individuals in the bedroom ALAN group had a higher nighttime systolic and diastolic blood pressure (Obayashi et al., 2014). Exposure to ALAN can suppress the production of melatonin, which could be potentially associated with blood pressure (Scheer et al., 2004). However, the results by Obayashi and colleagues were independent of overnight urinary melatonin excretion and sleep quality (Obayashi et al., 2014). Another explanation could be through the regulation of catecholamines levels (Hannemann et al., 2021).

A laboratory study with 20 young adults showed that a single night of exposure to room light during sleep could increase insulin resistance probably through increased sympathetic nervous system activation (Mason et al., 2022). Data from 678 elderly participants, showed that after a median follow-up of 3.5 years, individuals exposed to higher bedroom light levels (≥ 5 lux on average) had a higher incidence rate for diabetes (Obayashi et al., 2020).

Exposure to ALAN has also been associated with higher odds of overweight and obesity (OR = 1.13, 95% CI 1.10 – 1.16 and OR = 1.22, 95% CI 1.07 – 1.38) in a meta-analysis of observational studies including 3 longitudinal studies and 9 cross-sectional studies (Lai et al., 2020). However, in neither of the two observational studies on residential ALAN, other urban exposures such as air pollution or noise levels at night were considered and the whole visual spectrum was considered (Abay & Amare, 2018; Koo et al., 2016).

Others studies have also explored this association. In a longitudinal study with data from 239,781 adults who were not obese, participants with the highest residential ALAN exposure had a higher risk of developing overweight and obesity (D. Zhang et al., 2020). Exposure to residential ALAN has also been associated with overweight and obesity in a cross-sectional study which included 47,990 children and adolescents (Lin et al., 2022). In the latter study, authors reported that associations were independent of air pollution or green spaces, something that has been suggested that could be highly correlated with ALAN and that has been poorly studied in other studies on light (Huss et al., 2019).

Only one observational study has explored the association between residential ALAN, assessed from satellite images, and risk of CHD (S. Sun et al., 2021). After a median follow-up of 11 years, each interquartile range increase in outdoor nighttime illumination (60 nW/cm^2 /sr) was associated with a 11% higher risk of CHD hospitalisations (HR, 95% CI 1.03 – 1.18) and a 10% higher risk of CHD deaths (1.00 – 1.22).

Mounting evidence indicates that exposure to light in misalignment with the internal circadian clock can lead to metabolic disturbances. However, large-scale studies investigating the association between residential ALAN, measured from high resolution images, and cardiometabolic diseases considering concomitant urban exposures are lacking. Additionally, photometric measures of the whole visual range have been generally used to assess these associations and might not be the most appropriate to examine the chronodisrupting effects of ALAN.

Key messages

- Acceleration of **light pollution** in urban settings has importantly increased the brightness of our nights.
- **Exposure to ALAN** induces discordance with the master circadian clock in the brain and **chronodisruption**.
- Research in animals demonstrates that **ALAN** can **impair glucose** and **lipid homeostasis** and **cardiovascular** regulation.
- Evidence in humans suggests that ALAN exposure could be linked with obesity, CVDs, T2D and cancer.
- Urban exposures such as **air pollution** or night road **noise have rarely been considered**, while concerning light exposure assessment, **only measures of photopic illuminance** have been usually assessed.
- Measures of **light in the melanopsin-sensitivity** portion might be the **more appropriate** to assess the **chronodisrupting** effects of ALAN and **studies** examining its relation **with cardiometabolic outcomes are lacking**.

2. RATIONALE

NCDs are major contributors to global morbidity and mortality, causing more than two thirds of deaths worldwide. The increasing number of deaths from NCDs is concomitating with the aging of the population. Although, there is a genetic component in the development of these diseases, human behaviours have been extensively linked to the development of NCDs . Following, the discovery of the circadian clock and also the classification of night shift work involving circadian disruption as a probable carcinogen, a new research interest arose in the scientific community to investigate whether the timing of key 24-hour activities in the general population could also influence the risk of developing NCDs. Light and food intake are the main synchronisers of the master and peripheral clocks in the circadian system and have been both put on the spotlight.

The wide establishment of ALAN in urban settings and the shift towards a 24h society has forced the extension of daily eating behaviours implying late-night-eating. Additionally, the perception of lacking time in Western societies and the current surge in fasting practices, sometimes by skipping breakfast, have led to a delay of eating behaviours. However, there is limited scientific evidence supporting fasting practices as a way to improve health, and specifically studies exploring when in the day is best to fast. Data from animal models suggest that prolonging nighttime fasting duration could be related with a reduced cancer risk. However, very

few studies have addressed this question in humans and especially considering the time when this period of fast is broken.

Dietary aspects, such as the quality and quantity of food intake, have been also widely studied as a modifiable risk factor for cardiometabolic diseases, however, there is less evidence in relation with the timing of food intake. Data from clinical trials and observational studies suggest that skipping breakfast and eating late-at-night may be associated with T2D and CVDs. However, less is known about daily timings of eating and fasting in relation with the development of NCDs.

Finally, in the field of circadian research, several studies have also suggested that mistimed exposure to ALAN in the general population can have wide consequences for health. In outdoor settings, ALAN exposure can come from many different sources. Public street lightning, or residential ALAN, is an important anthropogenic pollutant contributing to night brightness. Some studies have explored the influence of residential ALAN on NCDs risk, however, other harmful aspects characteristic of the urban environment (such as air or noise pollution), which could be partly explaining the effects of residential ALAN exposure on human heath, have been usually dismissed. Moreover, evidence on this topic has generally explored photopic luminance and has not considered the luminance specifically in the melanopsin sensitivity band, the most relevant band in terms of circadian disruption.

3. OBJECTIVES

The main objective of the present doctoral thesis is to investigate how mild circadian disruption in the general population, through mistimed daily eating/fasting and light/dark cycles relate with the risk of developing NCDs.

The specific objectives of the thesis are:

1. To examine the association of circadian nutritional behaviours, defined by the habitual daily eating/fasting cycle, with cancer and cardiometabolic diseases.
    a. Association of nighttime fasting duration and time of the first meal of the day with prostate (***Paper I***) and breast cancer risk (***Paper II***).
    b. Relationship of time of first and last meal of the day, number of eating occasions and nighttime fasting duration with risk of T2D (***Paper III***) and CVDs (***Paper IV***).
2. To assess the link between exposure to residential ALAN, through photopic and melanopic light measurements, and cardiometabolic disease risk (***Paper V***).

The objectives of this thesis changed slightly due to the COVID-19 pandemic and the forced stop of the validation study within the GCAT cohort as mentioned in the preface.

4. METHODS

In this section, the main methods of *Papers I-V* are briefly explained with an overview of the study design, setting and participants and details on the exposure and outcome assessment. Statistical analyses are explained with further detail in each of the papers in this thesis.

4.1. Design, setting and participants

The present doctoral thesis examines data from three different studies: the multicase-control (MCC-Spain) study (*Paper I and II)*, the French NutriNet-Santé cohort study (*Paper III and IV)* and the Genomes for Life (GCAT) cohort study (*Paper V)*.

4.1.1. The MCC-Spain study

The MCC-Spain is a population-based multicase-control study that was conducted between 2008 and 2013 in 12 provinces of Spain (Figure 7) to investigate etiological factors of common cancers (Castaño-Vinyals et al., 2015). Cases were recruited from 23 collaborating hospitals. Simultaneously, controls were randomly selected from administrative registries of primary health care centres in the same area and were invited to participate. Recruitment criteria were to be aged 20-85 years old and to had resided in the area of recruitment for at least 6 months before inclusion. A total of 10,106 individuals were recruited with 1,738 breast, 1,112 prostate, 2,140 colorectal, 459 gastric and 559 chronic lymphocytic leukaemia cancer cases and 4,098 controls. Data on prostate and breast cancer cases were used for *Papers I and II* respectively.

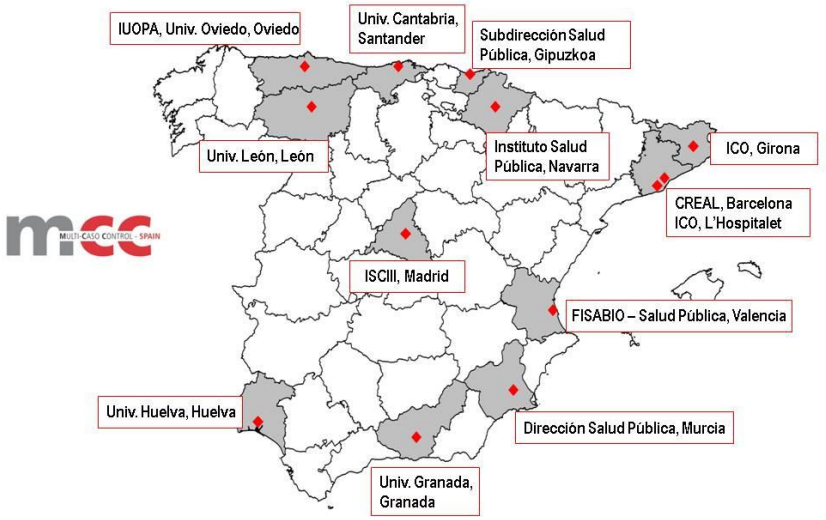

Figure 7. Collaborating hospitals in the MCC-Spain study (https://www.mccspain.org/)

At inclusion, participants were requested to answer an epidemiological questionnaire in a face-to-face interview administered by trained personnel. They were asked questions on sociodemographics, medical history, weight and height, physical activity and other lifestyle factors. After the questionnaire, participants were also requested to answer a semi-quantitative FFQ at home. Participants' energy intake was then estimated using the Centro de Enseñanza Superior de Nutrición y Dietética (CESNID) food composition table (Moreiras et al., 2003). Later in time, participants were recontacted and were requested to answer an additional questionnaire on circadian information through a telephonic interview. Participants were asked about their behaviours

at mid-age and also the year previous to the inclusion in the MCC-Spain study.

4.1.2. The NutriNet-Santé study

The NutriNet-Santé study (https://etude-nutrinet-sante.fr/) is an ongoing web-based prospective study that was established in 2009 in France to investigate and better understand the relationship between nutrition and health. Recruitment was done through several multimedia campaigns and targeted adult individuals. The protocol of the NutriNet-Santé study was registered at ClinicalTrials.gov under the identifier NCT03335644 and has been previously published (Hercberg et al., 2010).

At inclusion participants answered a set of questionnaires on diet (see more information in 4.2. Exposure assessment section), sociodemographic and lifestyle information, anthropometric measurements, physical activity and health status. Then, every year participants responded to the same questionnaires. Health events were self-reported through the website and were additionally ascertained through the linkage with medico-administrative databases of the Système National d'Information Interrégimes de l'Assurance Maladie (SNIIRAM) and with CépiDC, the French national mortality registry. Data from baseline in 2009 to September 2021 with more than 100,000 individuals were used for *Papers III and IV*.

4.1.3. The GCAT study

The Genomes for Life (GCAT) study, a prospective study in Catalonia, was launched in 2014 to investigate the interplay between environmental and genetic factors in the development of chronic NCDs (Obón-Santacana et al., 2018). Active invitations to the study were done through the Blood and Tissue Bank, a public agency of the Catalan Department of Health, but participation was open to any volunteer residing in Catalonia. At baseline, volunteers were asked to attend a recruitment centre and answer a computer-based epidemiological questionnaire with sociodemographic information, including address of residence, and also on lifestyle and health factors. Additionally, during this initial visit participants underwent anthropometry measurements including weight, height and blood pressure and gave a blood sample. The follow-up of the GCAT study is done actively every two years through email and passively through the linkage to electronic health records of the Catalan Public Healthcare System.

4.2. Exposure assessment

The main objective of the present thesis was to explore the association of mild circadian disruption in the general population, resulting from mistimed daily eating/fasting cycles and exposure to artificial light-at-night, with important NCDs. Therefore, this section is divided into (i) exposure assessment of circadian nutritional behaviours in the MCC-Spain study for *Papers I and II* and in the NutriNet-Santé study for *Papers III and IV* and (ii) ALAN assessment in the GCAT study for *Paper V*.

4.2.1. Circadian nutritional behaviours

In the MCC-Spain study

Within the MCC-Spain study, time of last meal was already explored and published elsewhere (Kogevinas et al., 2018). In *Papers I and II*, nighttime fasting duration and time of the first meal of the day were investigated to build a more complete picture of the association between circadian nutritional behaviours and cancers of the prostate and breast. In the MCC-Spain study, meal timing was assessed in the circadian interview that was answered by the participants a bit later in time, after a median time of 3 years. Participants were asked about habits at mid-age (40 years) and habits corresponding to the previous year of being included in the study.

Questions about the habitual time of breakfast and dinner or after dinner snack during weekdays and weekends were asked in this circadian questionnaire. Meal timings corresponding to habits at mid-age were considered in the main analyses to avoid the potential effects of reverse causation and to capture the long latency period in the development of cancer. Similarly, habits corresponding to weekdays were selected because these are more representative of daily lifestyle behaviours. The time of breakfast was considered as the time of the first meal of the day except for participants reporting not having breakfast, in which case time of lunch was used instead. Nighttime fasting duration was calculated as the time elapsed between the last eating episode and the first eating episode the

following day, considering the time of after-dinner snacks among participants reporting this type of behaviour.

In the NutriNet-Santé study

In the NutriNet-Santé study, diet was assessed through sets of 3 non-consecutive 24-hours food records and included information on 2 working days and 1 non-working day. Every 6 months participants were asked to repeat this set of records to vary the season of completion. For *Papers III and IV* circadian nutritional behaviours, including daily time of first meal and last meals, were averaged from the available records during the first two years of follow-up. Although, it is not a circadian nutritional behaviour *per se*, because it is not time-related, we also explored the daily number of eating occasions. For this variable, the intake of any food or beverage of at least 1 kcal was considered (to exclude water drinking) and it was also averaged from the records of the first two years of follow-up. Nighttime fasting duration was calculated as explained for the MCC-Spain study, assuming that daily behaviours remained similar.

4.2.2. Artificial light-at-night

To assess ALAN exposure, in *Paper V*, a nighttime image of Barcelona taken by the Crew Earth Observation program of the International Space Station (ISS) was used (ISS035E023385, 18th of April of 2013 at 22:10:46 GMT). This image was taken with the digital Single-Lens Reflex cameras, providing information of the visual range on the radiances in the red, green and blue (RGB) spectral bands. Using previously described regressions (de Miguel et

al., 2019; Sánchez de Miguel et al., 2019) and from the calibrated RGB spectral bands, two physiologically-relevant sensitivity bands were estimated. These were the spectral radiance in the visual photopic band and the melanopic spectral radiance. The first one representing the perception of brightness and the latter one the radiance able to stimulate melanopsin, the main light-detecting photopigment involved in circadian regulation (Berson et al., 2002).

To estimate the environmental exposure to residential ALAN in every pixel of the map, radiances were converted to horizontal illuminance and melanopic illuminance in lux. As a last step, these estimates were assigned to the participants' addresses through the Geographic Information System (GIS).

4.3. Outcome assessment

This thesis explores several NCDs and, consequently, this section has been divided into (i) cancer which was explored in *Papers I and II* within the MCC-Spain study and (ii) cardiometabolic outcomes examined in *Papers III and IV* with data from the NutriNet-Santé and *Paper V* within the GCAT study.

4.3.1. Cancer

Prostate and breast cancer were investigated in *Paper I and II*, respectively. In the MCC-Spain study, cases were identified through periodical visits to the collaborating hospitals and were recruited as soon as possible after diagnosis. Histologically-confirmed cases of cancers of the prostate (International Classification of Diseases 10th Revision [ICD-10]: C61, D07.5) and the breast (ICD-10: C50, D05.1,

D05.7) with no prior history of the disease were included. Controls were invited to participate following a frequency-matching approach to ensure that in each of the collaborating regions and for each case there was at least one control of the same 5-year age interval and sex.

From medical records, clinical information on prostate cancer aggressiveness, assessed through the Gleason score, and on breast cancer subtype was collected. Breast cancer subtypes included tumours with hormonal receptors (either for oestrogens or progesterone), tumours with over expression of the human epidermal growth factor 2 (HER2) and negative tumours for both aspects.

4.3.2. Cardiometabolic outcomes

In the NutriNet-Santé study

Cardiometabolic diseases were ascertained in the NutriNet-Santé study and were the main outcomes in *Paper III* (T2D) and *Paper IV* (CVDs). In this longitudinal web-based cohort, participants were asked to report any new major health event, medications and treatments in the yearly health questionnaire, in a check-up every 6 months and at any time through the website. Participants were also asked to provide medical records to support the declaration of a health event. Additionally, NutriNet-Santé data were linked to medico-administrative databases of national health insurance (SNIIRAM) with information on medication and medical consultation history. Finally, to censor deaths during follow-up, a linkage to the French national mortality registry (CépiDC) was also done.

The ICD-10 code E11 was used to identify cases of T2D. The International Classification of Diseases-Clinical Modification codes (ICD-CM, 10th revision) was used to identify cases of CVD. *Paper IV* included incident cases of stroke (I64), transient ischemic attack (G45.8, G45.9) and these were grouped as cerebrovascular diseases. Myocardial infraction (I21), angina pectoris (I20.1, I20.8, I20.9), acute coronary syndrome (I20.0, I12.4) and angioplasty (Z95.8) were also included and grouped as coronary heart diseases.

In the GCAT study

In *Paper V*, two types of analyses were conducted to assess the association between exposure to ALAN and cardiometabolic health. First, cross-sectional analyses explored the relationship with cardiometabolic risk factors including systolic blood pressure, diastolic blood pressure, BMI, waist-to-hip ratio (WHR) and HbA1c. These measurements were done at the baseline visit by trained personnel. From cross-sectional analyses we also explored the association with prevalent cardiometabolic diseases defined by these clinical markers: General obesity (BMI >30 kg/m^2) and abdominal obesity (WHR males ≥ 1.0 and females ≥ 0.85) were categorised into obese and non-obese according to the WHO guidelines. Hypertension was defined as a systolic blood pressure ≥ 140 mmHg or a diastolic blood pressure ≥ 90 mmHg. Finally, we categorised as diabetes if HbA1c levels were of 6.5% or above.

Secondly, in *Paper V*, the association with incident cardiometabolic diseases was also investigated. For this part of the analysis, the

linkage with an extraction of electronic health records up to 2017 was used. In this records, diseases were classified according to the 9th Revision of the International Classification of Diseases [ICD-9] and codes included: diabetes mellitus (T1D, T2D, not specified and prediabetes: 250, 250.01, 250.02, 250.00, 250.0, 79029), angina pectoris (413.9, 413.0, 413.1, 4111), myocardial infraction (410, 410.10, 410.11, 410.41, 410.42, 410.51, 410.81, 410.90, 410.91, 410.4, 410.3, 410.71), stroke (434.01, 434.11, 434.10, 434.90, 434.91, 431, 430, 4321, 433.91, 433.90, 433.30, 433.21, 433.11, 433.10, 433.00, 435.0, 435.1, 435.3, 435.8, 435.9, ,436,437.0, 437.1, 437.2, 437.3, 437.8, 437.9, 438.0, 438.11, 438.21, 438.6, 438.7, 438.82, 438.85, 438.89), hypertension (401, 401.0, 401.1, 401.9) and hypercholesterolemia (2720). Self-reported prevalent cases at baseline questionnaire were excluded for this analysis.

Note: The current time of follow-up presented in this part of the analyses of *Paper V* is very short and these results will be updated with a new electronic health records extraction that we are expecting to receive after summer 2022. This new extraction will include information from 2012 to 2021 and, therefore, the follow-up time will be increased by 4 more years. Once the incident results are updated, we will proceed with the submission of the paper.

5. RESULTS

Paper I. The Association of Nighttime Fasting Duration and Prostate Cancer Risk: Results from the Multicase-Control (MCC) Study in Spain

Paper II. Association of time of breakfast and nighttime fasting duration with breast cancer risk in the Multicase-Control Study in Spain (MCC-Spain)

Paper III. Associations of meal timing, number of eating occasions and nighttime fasting duration with type 2 diabetes risk in the NutriNet-Santé cohort

Paper IV. Meal timing, number of eating occasions and nighttime fasting duration in relation with cardiovascular disease risk: Results from the Prospective NutriNet-Santé cohort

Paper V. Exposure to residential artificial light-at-night and cardiometabolic disease risk: an urban perspective from the Catalan GCAT cohort study

5.1. Paper I

Palomar-Cros A, Espinosa A, Straif K, Pérez-Gómez B, Papantoniou K, Gómez-Acebo I, Molina-Barceló A, Olmedo-Requena R, Alguacil J, Fernández-Tardón G, Casabonne D, Aragonés N, Castaño-Vinyals G, Pollán M, Romaguera D, Kogevinas M. The Association of Nighttime Fasting Duration and Prostate Cancer Risk: Results from the Multicase-Control (MCC) Study in Spain. Nutrients. 2021;13(8):2662. doi: 10.3390/nu13082662

Supplementary material:
https://drive.google.com/drive/folders/1CyGg6xjA1BJPxeFEAxMR0xf8jt-8uHuv?usp=sharing

nutrients

MDPI

Article

The Association of Nighttime Fasting Duration and Prostate Cancer Risk: Results from the Multicase-Control (MCC) Study in Spain

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Abstract: Nighttime fasting has been inconclusively associated with a reduced risk of cancer. The purpose of this study was to investigate this association in relation to prostate cancer risk. We examined data from 607 prostate cancer cases and 848 population controls who had never worked in night shift work from the Spanish multicase-control (MCC) study, 2008–2013. Through an interview, we collected circadian information on meal timing at mid-age. We estimated odds ratios (OR) and 95% confidence intervals (CI) with unconditional logistic regression. After controlling for time of breakfast, fasting for more than 11 h overnight (the median duration among controls) was associated with a reduced risk of prostate cancer compared to those fasting for 11 h or less (OR = 0.77, 95% 0.54–1.07). Combining a long nighttime fasting and an early breakfast was associated with a lower risk of prostate cancer compared to a short nighttime fasting and a late breakfast (OR = 0.54, 95% CI 0.27–1.04). This study suggests that a prolonged nighttime fasting duration and an early breakfast may be associated with a lower risk of prostate cancer. Findings should be interpreted cautiously and add to growing evidence on the importance of chrononutrition in relation to cancer risk.

Keywords: prostate cancer; prolonged nighttime fasting; early time-restricted feeding; circadian rhythms; breakfast; chrononutrition

1. Introduction

Prostate cancer is the most frequently diagnosed cancer in men and ranks as the third cause of cancer mortality in Spain [1]. Several non-modifiable risk factors for prostate cancer have been identified, including age, ethnicity and a family history of prostate cancer [2]. In 2019, following a first evaluation in 2007 of shift work that involves circadian disruption, the International Agency for Research on Cancer (IARC) classified night shift work as probably carcinogenic to humans, supported by limited evidence in humans for prostate, breast and colon cancer [3,4]. Circadian rhythms allow the adaptation to daily environmental changes and regulate multiple physiological activities in the organism following cycles of 24 h [5]. In the IARC report, several mechanistic hypotheses were proposed to explain the evaluated association, including chronic inflammation, hormonal alterations, cell proliferation and immunosuppression [3]. In the general population, circadian disruption as a result of exposure to artificial light-at-night (ALAN) [6,7], sleep duration [8–10] and mutations in clock genes [11], has also been associated with prostate cancer.

Although the light captured by the retina is the main synchronizer of the central circadian system, the feeding–fasting cycle also plays a major role in the regulation of peripheral clocks. Late-night eating has been associated with an increased prostate cancer risk in a study in France [12]. Similarly, previous results from the multicase-control (MCC) study in Spain found that having a long-time interval between supper and sleep was associated with a statistically significant reduced risk of prostate cancer [13]. This protective association was more pronounced in individuals with a morning chronotype, a human attribute believed to have a genetic basis that reflects a personal preference for timing of activity [13]. The morning chronotype was defined as having a mid-sleep time before 3:35 AM, based on the distribution among controls [13]. Results from the same study showed that having an early supper was associated with a reduced risk of prostate cancer, but results were not statistically significant [13]. Another study in adults reported an association between nighttime snacking and increased body fatness [14], the latter classified as a probable risk factor for prostate cancer [15,16].

Various fasting regimens have been associated with weight loss and have been recently popularized [17]. Intermittent fasting is a form of fasting that consists of restricting the feeding window to 8 h, therefore prolonging the nighttime fasting duration [17]. Prolonged nighttime fasting has also been associated with a reduction in waist circumference, blood glucose (HbA1c) levels and systemic inflammation, which may influence cancer risk [18,19]. Results from the prospective Women's Healthy Eating and Living study suggested that fasting for 13 h or more reduced the risk of breast cancer recurrence compared to a shorter fasting period [20]. However, data from the NutriNet-Santé prospective cohort study showed that duration of fasting overnight was not significantly associated with the risk of developing prostate cancer [12]. Although nighttime fasting has been associated with a lower risk of cancer outcomes, skipping breakfast has been linked with an increased risk of low-grade inflammation [21].

Food behaviors, including time of supper and interval between supper and sleep, were previously examined in the MCC study [13]. However, the associations with nighttime fasting duration and time of breakfast were not evaluated. Given the current surge in fasting as a new food behavior, in this study we investigate whether prolonged nighttime fasting is associated with a reduced prostate cancer risk Additionally, we consider whether the time window of this period of fasting and the time of breakfast play an important role in this association.

2. Methods

2.1. Study Population

The MCC study (http://www.mccspain.org, accessed on 6 April 2021) is a multicase-control study conducted in 12 provinces of Spain between 2008 and 2013 [22]. Included in this analysis were individuals between the ages of 20 and 85 with histological confirmation of prostate cancer. As soon as possible after diagnosis, cancer cases were frequency matched based on age, sex and area with population controls. In each of the recruitment areas, administrative records from primary healthcare centers were reviewed to randomly select controls residing there for a minimum period of 6 months. For this study, participants who did not respond to the follow-up circadian interview (N = 522) were excluded (Figure 1). To avoid confounding, subjects ever doing night shift work were excluded (N = 535), with night shift work defined as working partly or entirely between midnight and 6 AM for 3 nights or more per month [13]. Participants with missing data on nighttime fasting (N = 93) were also excluded. In this analysis, 607 prostate cancer cases and 848 population controls from 7 different regions (Madrid, Barcelona, Asturias, Huelva, Cantabria, Valencia and Granada) were selected. Each of the Ethics committees of the included centers reviewed and approved the study protocol. All subjects signed a written informed consent.

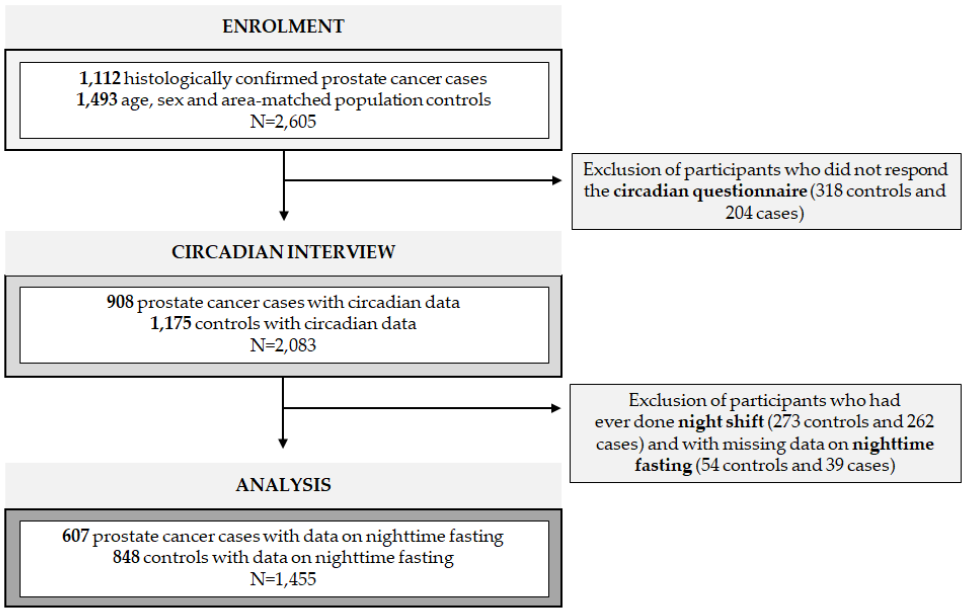

Figure 1. Flow chart of study population. N= sample size.

2.2. Data Collection

A questionnaire was answered by participants in a face-to-face interview with a trained interviewer [22]. We requested information on age, sociodemographic factors, family history of prostate cancer, smoking, sleep duration, sleep problems and height and weight one year before the interview, from which the body mass index (BMI) was calculated as kg/m^2. Exposure to indoor ALAN was assessed in the questionnaire through a Likert scale ranging from total darkness, almost dark and dim light to quite illuminated [6]. Outdoor ALAN exposure was also assessed but only for the cities of Barcelona and

Madrid, where satellite images were available [6]. Light exposure was modeled and attributed to participants' geocoded residences through Geographic Information System [6].

Following the interview, information on daily energy intake was gathered through a semi-quantitative food frequency questionnaire answered at home and estimated using the Centro de Enseñanza Superior de Nutrición y Dietética (CESNID) food composition table [23]. Ten percent of the participants did not respond to the food frequency questionnaire. A World Cancer Research Fund/American Institute of Cancer Research (WCRF/AICR) score was constructed [24]. The WCRF/AICR score ranged from 0 to 6 and included information on body fatness, physical activity, foods and drinks that promote weight gain, plant foods, animal foods and alcoholic drinks. This variable was further categorized into sex-specific tertiles based on the distribution in the control group [24].

Circadian data, including information on meal frequencies, duration and timings, were assessed through a telephone interview conducted 6 months to 5 years after the initial interview (median time 3 years) [13]. In this paper, we refer to these variables as dietary circadian variables. Participants reported their dietary circadian variables on weekdays and weekends at 40 years of age and during the year prior to the initial interview. Participants younger than 40 years of age were only asked for the information on the year prior to recruitment. Participants were also asked questions on sleep and timing of physical activity and answered the Munich Chronotype Questionnaire [13]. Chronotype was estimated as the standard mid-sleep time on free days, Mid-sleep time on free days (MSF) = [sleep onset on a free day + (sleep duration on free day/2)], and mid-sleep time on free days corrected for oversleeping on free days (MSFcorr) = MSF − [sleep duration on a free day − (sleep duration on a working day/2)]. Further details have been described elsewhere [13].

2.3. Exposure and Outcome Assessment

Nighttime fasting duration was defined as the period elapsed between the last eating episode before going to sleep and the first episode the following day. In the previous paper from MCC, we investigated time of supper [13], but now time of last intake also considered after supper snacks. For participants reporting not having breakfast, lunch was considered as breakfast, understood as the wider concept of breaking the nightly fast. The cut-off point for nighttime fasting distribution was set at 11 h (the median distribution among controls), defining a categorical variable with two levels: 11 h or less of fast, the reference category, versus more than 11 h of fast. We based the main analysis on patterns of weekdays and at 40 years of age, because weekdays are more representative of daily lifestyle habits, and this also helped avoid having missing data on weekend patterns. We used data at 40 years of age to avoid potential reverse causation since it has been reported that cancer can cause anorexia (loss of appetite), thus affecting eating patterns [25].

Clinical information on prostate cancer aggressiveness determined by the Gleason score was recorded from medical records. We did not use the new Gleason grading system because of small numbers in some groups [26]. We classified cases into two groups: low-grade aggressive prostate cancer (Gleason score = 6 or 3 + 4) and high-grade aggressive prostate cancer (Gleason score 4 + 3 or higher), as it has been previously reported in the literature [27].

2.4. Statistical Analyses

We compared the distribution of characteristics in prostate cancer cases and controls and also prostate cancer risk factors according to nighttime fasting duration in controls. To evaluate significant differences, chi-square tests and *t*-tests were applied to calculate the *p*-value in categorical and continuous variables, respectively.

We assessed the correlation between nighttime fasting and the other dietary circadian variables (time of supper, interval between supper and sleep and time of breakfast) using Spearman's correlation coefficient. We examined the linearity of the association between nighttime fasting duration (continuous variable, in hours) and prostate cancer

using generalized additive models (GAM). We applied ANOVA, including the smoothing terms versus the linear term model, to test this association's linearity.

Odds ratios (OR) and 95% confidence intervals (CI) were estimated with unconditional logistic regression using categorical exposure data. The crude model was adjusted for age (continuous, years), center (Madrid, Barcelona, Asturias, Huelva, Cantabria, Valencia, Granada) and for educational level (less than primary school, primary school, secondary school, university). We included educational level in the crude model because population controls had a higher level of education compared to cases [22]. Moreover, in our study population, education was significantly associated both with the exposure and the outcome (see Tables 1 and 2).

Table 1. Basic characteristics of study population.

	Controls (*N* = 848)	**Cases (*N* = 607)**	***p*-Value**
	Mean (SD) or *N* (%)	Mean (SD) or *N* (%)	
Age	66.0 (8.4)	65.6 (7.0)	0.372
BMI	27.5 (3.6)	27.5 (3.6)	0.967
Normal weight (<25 kg/m^2)	225 (26.5)	153 (25.2)	
Overweight (≥25 to <30 kg/m^2)	439 (51.8)	320 (52.7)	0.850
Obese (≥30 kg/m^2)	184 (21.7)	134 (22.1)	
Educational level			
Less than primary school	115 (13.6)	104 (17.1)	
Primary school	247 (29.1)	244 (40.2)	<0.001
Secondary school	264 (31.1)	142 (23.4)	
University	222 (26.2)	117 (19.3)	
Family history of prostate cancer			
No	793 (93.5)	506 (83.4)	<0.001
Yes	55 (6.5)	101 (16.6)	
Smoking			
Never	242 (28.5)	171 (28.2)	
Ex-smoker	434 (51.2)	325 (53.5)	0.571
Current smoker	172 (20.3)	111 (18.3)	
Chronotype			
Morning	419 (50.5)	306 (50.5)	
Intermediate	303 (36.6)	224 (37.0)	0.974
Evening	107 (12.9)	76 (12.5)	
Unknown	19	1	
WCRF/AICR score			
Low adherence [a]	307 (40.1)	203 (37.3)	
Medium adherence [b]	262 (34.2)	232 (42.6)	0.004
High adherence [c]	196 (25.6)	109 (20.0)	
Unknown	83	63	
Diabetes			
No	671 (79.3)	518 (85.6)	0.003
Yes	175 (20.7)	87 (14.4)	
Unknown	2	2	
Indoor ALAN exposure			
Total darkness	147 (17.4)	87 (14.4)	
Almost dark	348 (41.2)	211 (34.9)	0.001
Dim light	261 (30.9)	204 (33.7)	
Quite illuminated	88 (10.4)	103 (17.0)	

Unknown	4	2	
Breakfast			
No	17 (2.0)	3 (0.5)	0.001
Only weekends	3 (0.4)	12 (2.0)	
Only weekdays	15 (1.8)	16 (2.6)	
Always	809 (95.9)	575 (94.9)	
Unknown	4	1	
Time of breakfast			
8:30 AM or before	466 (55.0)	314 (51.7)	0.227
After 8:30 AM or skip breakfast	382 (45.0)	293 (48.3)	
Time of last intake			
10 PM or later	294 (34.7)	227 (37.4)	0.350
9:00 to <10 PM	439 (51.8)	311 (51.2)	
Before 9 PM	115 (13.6)	69 (11.4)	
Supper/sleep interval			
1 h or less	186 (22.2)	169 (28.1)	0.034
From >1 to ≤2 h	327 (39.0)	223 (37.0)	
More than 2 h	326 (38.9)	210 (34.9)	
Unknown	9	5	

ALAN = artificial light at night; BMI = body mass index; *N* = sample size; NA = not applicable; SD = standard deviation; WCRF/AICR = World Cancer Research Fund / American Institute for Cancer Research. [a] Men (0.25–3); Women (0.5–3.5). [b] Men (3.25–4); Women (3.75–4.25). [c] Men (4.25–6); Women (4.5–6).

Table 2. Distribution of prostate cancer risk factors according to nighttime fasting duration in controls.

	≤11 h of Fast (*N* = 474)	**>11 h of Fast (*N* = 374)**	***p*-Value**
	Mean (SD) or *N* (%)	Mean (SD) or *N* (%)	
Age	65.5 (8.5)	66.5 (8.2)	0.070
BMI	27.4 (3.5)	27.6 (3.8)	0.384
Normal weight (<25 kg/m^2)	121 (25.5)	104 (27.8)	0.198
Overweight (≥25 to <30 kg/m^2)	258 (54.4)	181 (48.4)	
Obese (≥30 kg/m^2)	95 (20.0)	89 (23.8)	
Educational level			
Less than primary school	50 (10.5)	65 (17.4)	<0.001
Primary school	115 (24.3)	132 (35.3)	
Secondary school	161 (34.0)	103 (27.5)	
University	148 (31.2)	74 (19.8)	
Family history of prostate cancer			
No	445 (93.9)	348 (93.0)	0.727
Yes	29 (6.1)	26 (7.0)	
Smoking			
Never	140 (29.5)	102 (27.3)	0.191
Ex-smoker	230 (48.5)	204 (54.5)	
Current smoker	104 (21.9)	68 (18.2)	
Chronotype			
Morning	219 (47.1)	200 (54.9)	0.080
Intermediate	181 (38.9)	122 (33.5)	
Evening	65 (14.0)	42 (11.5)	
Unknown	9	10	
WCRF/AICR score			
Low adherence [a]	164 (38.2)	143 (42.6)	0.432
Medium adherence [b]	154 (35.9)	108 (32.1)	

High adherence [c]	111 (25.9)	85 (25.3)	
Unknown	45	38	
Diabetes			
No	387 (81.8)	284 (76.1)	0.052
Yes	86 (18.2)	89 (23.9)	
Unknown	1	1	
Indoor ALAN exposure			
Total darkness	63 (13.3)	84 (22.6)	0.005
Almost dark	203 (43.0	145 (39.0)	
Dim light	151 (32.0)	110 (29.6)	
Quite illuminated	55 (11.7)	33 (8.9)	
Unknown	2	2	
Breakfast			
No	1 (0.2)	16 (4.3)	<0.001
Only weekends	NA	3 (0.8)	
Only weekdays	8 (1.7)	7 (1.9)	
Always	465 (98.1)	344 (93.0)	
Unknown	NA	4	
Time of breakfast			
8:30 AM or before	417 (88.0)	49 (13.1)	<0.001
After 8:30 AM or skip breakfast	57 (12.0)	325 (86.9)	
Time of last intake			
10 PM or later	215 (45.4)	79 (21.1)	<0.001
9:00 to <10 PM	233 (49.2)	206 (55.1)	
Before 9 PM	26 (5.5)	89 (23.8)	
Supper/sleep interval			
1 h or less	110 (23.6)	76 (20.4)	0.553
From >1 to ≤2 h	178 (38.1)	149 (40.1)	
More than 2 h	179 (38.3)	147 (39.5)	
Unknown	7	2	

ALAN = artificial light at night; BMI = body mass index; *N* = sample size; SD = standard deviation; WCRF/AICR = World Cancer Research Fund / American Institute for Cancer Research. a Men (0.25–3); Women (0.5–3.5). b Men (3.25–4); Women (3.75–4.25). c Men (4.25–6); Women (4.5–6).

Since there is limited prior evidence of determinants for both the exposure and the outcome, we followed a mixed criterion for the selection of further potential confounders. First, on the basis of the limited prior knowledge, we built a directed acyclic graph using Daggity (Figure S1) [28]. The variables considered were family history of prostate cancer (yes, no), adherence to a healthy lifestyle through the WCRF/AICR score (high, medium, low), indoor ALAN (total darkness, almost dark, dim light, quite illuminated), outdoor ALAN (continuous, cd/m^2), sleep problems (yes, no) and duration (7 h or less, more than 7 h), number of daily eating episodes (three or less, more than three), time of the last intake (before 9 PM, 9:00 to <10 PM, 10 PM, or later) and the interval between the time of supper and sleep (1 h or less, from >1 to 2 h, more than 2 h). "Time of breakfast" was categorized in two levels (8:30 AM or before, after 8:30 or skip breakfast). Skipping breakfast has been associated with negative metabolic outcomes, but in our study only 3 cases reported never having breakfast. We also considered timing of physical activity (inactive, 8 AM–10 AM, 10 AM–12 PM, 12 PM–7 PM, 7 PM–11 PM, any other pattern), since it has been recently reported that morning exercise may be associated with a lower risk of prostate cancer compared to exercising later in the day [29]. Additionally, we considered diabetes (yes, no) because its treatment can include changes in dietary habits and it has been inconsistently suggested as a protective factor for prostate cancer [30–32]. Finally, we included BMI (continuous, kg/m^2), smoking (never, ex-smoker, current smoker) and chronotype (morning, intermediate, evening).

In the adjusted model we included variables strongly associated with both the exposure and outcome in our study population (see Tables 1 and 2) and variables changing the association between nighttime fasting and prostate cancer risk by more than 10% (Table S1). These variables were diabetes and indoor ALAN for the first criterion and breakfast time as the second criterion. Less than 1% of data was missing in the selected covariates. Multicollinearity was assessed in the adjusted model through the variance inflation factor to control for redundancy.

We explored whether there was evidence of effect modification of the association of interest by chronotype, healthy lifestyle (assessed with the WCRF/AICR score) and time of breakfast, including an interaction term in each of the models and examining results from a likelihood ratio test. We also investigated the combined association of nighttime fasting duration and time of breakfast with prostate cancer risk. The association between nighttime fasting and prostate cancer aggressiveness was examined with a multinomial logistic regression model. The statistical package R-4.0.0 (The R Project for Statistical Computing, Vienna) was used for these analyses.

2.5. Sensitivity Analyses

We explored the role of nighttime fasting on days off and working days by combining nighttime fasting data from weekdays and weekends (at 40 years of age). We used a weighted mean giving a weight of 5/7 to weekdays and of 2/7 to weekends. We also explored eliminating point outliers of the exposure distribution to exclude the most extreme patterns. We considered as potential point outliers any observation below the Q1-(1.5*IQR) and above Q3 + (1.5*IQR). Nine (0.6%) and 35 observations (2.4%) were identified and excluded, respectively. To test the robustness of our results, exposure the previous year to the circadian interview was also considered, despite the potential bias due to reverse causation. This model was adjusted for time of breakfast corresponding to the year before the circadian interview and we assumed that exposure to indoor ALAN did not change during these years.

The time elapsed between the inclusion in the study and the exposure assessment from the circadian interview was long for some participants. Therefore, we did a sensitivity analysis including only those participants that answered the interview 3 years or less after the baseline questionnaire.

3. Results

3.1. Study Population

Characteristics of the included prostate cancer cases (N = 607) and matched controls (N = 848) are shown in Table 1. Educational level (p-value < 0.001), family history of prostate cancer (p-value < 0.001) and poor adherence to the WCRF/AICR recommendations (p-value = 0.004) were significantly associated with prostate cancer. Having a shorter time interval elapsed between supper and sleep (p-value = 0.034), not having breakfast consistently (p-value = 0.001) and being exposed to indoor artificial light at night (p-value = 0.001) were significantly associated with prostate cancer. Cases were less likely to have diabetes (p-value = 0.003).

3.2. Nighttime Fasting and Prostate Cancer Risk

The median nighttime fasting duration in the control group was 11 h (interquartile range 10–12). The distribution among controls of prostate cancer risk factors according to categories of nighttime fasting duration is presented in Table 2. Highly educated subjects tended to fast fewer hours overnight (p-value < 0.001). Participants with diabetes and less exposed to indoor ALAN tended to have more extended nighttime fasting periods (p-value = 0.052 and 0.005, respectively). Participants who never had breakfast or had the first intake after 8:30 AM and the last intake before 9 PM tended to fast for more hours (p-value < 0.001 for both variables).

A low positive correlation was observed between nighttime fasting duration and time elapsed between supper and sleep (Figure S2A, with a Spearman's correlation coefficient of 0.12). Time of supper and nighttime fasting duration showed a low negative correlation (Figure S2B, with a Spearman's correlation coefficient of −0.36). High correlation was found between nighttime fasting duration and time of breakfast (Figure S2C, Spearman's correlation coefficient of 0.83).

The GAM showed a slight reduction of prostate cancer risk with more extended nighttime fasting (Figure S3A). When adjusting this model for time of breakfast, the reduction in the prostate cancer risk associated with fasting was more evident (Figure S3B). In the crude logistic regression model, fasting for more than 11 h overnight was associated with a slightly non-significant reduced risk of prostate cancer compared to those fasting for 11 h or less (OR = 0.92, 95% CI 0.73–1.16, Table 3). After adjusting for confounders, the model showed that a more extended nightly fast was linked to a more potent reduction of prostate cancer risk (adjusted model, OR = 0.77, 95% CI 0.54–1.07, Table 3). In this model, having breakfast after 8:30 AM was associated with a non-significant increased risk of prostate cancer (OR = 1.30, 95% CI 0.92–1.85) compared to having breakfast at 8:30 AM or before. We did not find important collinearity in the adjusted model between the two diet time-related variables.

Table 3. Association of nighttime fasting and prostate cancer risk.

Nighttime Fasting	Controls *N* (%)	Cases *N* (%)	OR (95% CI) [a]	OR (95% CI) [b]
≤11 h	474 (55.9)	342 (56.3)	Ref	Ref
>11 h	374 (44.1)	265 (43.7)	0.92 (0.73–1.16)	0.77 (0.54–1.07)

N = sample size; OR = odds ratio; 95% CI = 95% confidence interval. [a] Adjusted for age, center and education. [b] Adjusted for age, center, education, diabetes (missing for 2 controls and 2 cases), indoor ALAN exposure (missing for 4 controls and 2 cases) and time of breakfast.

We also tried categorizing the exposure into three categories based on the distribution of this variable in the control group: 10 h or less (reference category), more than 10 h to 12 h, and more than 12 h (Table S2). In this analysis, fasting for more than 10 h to 12 h was associated with a slightly higher prostate cancer reduced risk than the most extended fasting category. This difference was dissipated when adjusting for time of breakfast, suggesting that this difference could be explained by the adverse effects of postponing breakfast.

3.3. Prostate Cancer Risk Stratified by Time of First Intake

The prostate cancer risk reduction with a prolonged nightly fast was moderately stronger among those individuals having their breakfast at 8:30 or before (adjusted model, OR= 0.60, 95% CI 0.33–1.04) compared to those having it later on the day or skipping it (OR= 0.90, 95% CI 0.58–1.39, Table 4). Despite this pattern, confidence intervals overlapped and the interaction in the adjusted model was not significant (p-value = 0.26, 1 degree of freedom).

Table 4. Association of nighttime fasting and prostate cancer risk stratified by time of breakfast.

Nighttime fasting	Controls *N* (%)	Cases *N* (%)	OR (95% CI) [a]	OR (95% CI) [b]
Breakfast at 8:30 AM or before				
≤11 h	417 (89.5)	293 (93.3)	Ref	Ref
>11 h	49 (10.5)	21 (6.7)	0.66 (0.37–1.13)	0.60 (0.33–1.04)
Breakfast after 8:30 AM or skip breakfast				
≤11 h	57 (14.9)	49 (16.7)	Ref	Ref
>11 h	325 (85.1)	245 (83.3)	0.87 (0.57–1.33)	0.90 (0.58–1.39)

N = sample size; OR = odds ratio; 95% CI = 95% confidence interval. [a] Adjusted for age, center and education. [b] Adjusted for age, center, education, diabetes (missing for 2 controls and 2 cases), indoor ALAN exposure (missing for 4 controls and 2 cases) and chronotype (missing for 19 controls and 1 case).

3.4. Association Combining Nighttime Fasting Duration and Time of Breakfast with Prostate Cancer Risk

When combining both nighttime fasting duration and time of breakfast we observed that the combination associated with a lower risk of prostate cancer was having a long nighttime fasting and an early breakfast compared to a short nighttime fasting and a late breakfast (OR = 0.54, 95% CI 0.27–1.04, Table 5). This association was maintained even after adjusting for time of last intake and interval between supper and sleep.

Table 5. Association of nighttime fasting and time of breakfast with prostate cancer risk.

	Controls N (%)	Cases N (%)	OR (95% CI) [a]	OR (95% CI) [b]
Short nighttime fasting (≤11 h) and late breakfast (>8:30 AM)	56 (6.6)	49 (8.1)	Ref	Ref
Short nighttime fasting (≤11 h) and early breakfast (≤8:30 AM)	418 (49.3)	293 (48.3)	0.88 (0.57–1.36)	0.89 (0.57–1.39)
Long nighttime fasting (>11 h) and late breakfast (>8:30 AM)	325 (38.3)	244 (40.2)	0.87 (0.57–1.33)	0.88 (0.57–1.36)
Long nighttime fasting (>11 h) and early breakfast (≤8:30 AM)	49 (5.8)	21 (3.5)	0.58 (0.30–1.11)	0.54 (0.27–1.04)

N = sample size; OR = odds ratio; 95% CI = 95% confidence interval. [a] Adjusted for age, center and education. [b] Adjusted for age, center, education, diabetes (missing for 2 controls and 2 cases) and indoor ALAN exposure (missing for 4 controls and 2 cases).

3.5. Prostate Cancer Risk Stratified by Chronotype

The association of prolonged fasting period overnight and reduced prostate cancer risk was slightly more pronounced in morning chronotype individuals (adjusted model, OR = 0.70, 95% CI 0.47–1.04) compared to intermediate and particularly evening ones (OR= 0.80, 95% CI 0.50–1.27; OR = 0.99, 95% CI 0.51–1.92, Table 6). However, the likelihood ratio test for interaction in the adjusted model showed a *p*-value = 0.60 with 2 degrees of freedom. On the other hand, we observed no effect modification by adherence to the WCRF/AICR score (Table S3).

Table 6. Association of nighttime fasting and prostate cancer risk stratified by chronotype (missing for 19 controls and for 1 case).

Nighttime Fasting	Controls N (%)	Cases N (%)	OR (95% CI) [a]	OR (95% CI) [b]
Morning chronotype				
≤11 h	219 (52.3)	170 (55.5)	Ref	Ref
>11 h	200 (47.7)	136 (44.5)	0.84 (0.61–1.15)	0.70 (0.47–1.04)
Intermediate chronotype				
≤11 h	181 (59.7)	130 (58.0)	Ref	Ref
>11 h	122 (40.3)	94 (42.0)	0.97 (0.67–1.41)	0.80 (0.50–1.27)
Evening chronotype				
≤11 h	65 (60.7)	41 (53.9)	Ref	Ref
>11 h	42 (39.3)	35 (46.1)	1.17 (0.63–2.18)	0.99 (0.51–1.92)

N = sample size; OR = odds ratio; 95% CI = 95% confidence interval. [a] Adjusted for age, center and education. [b] Adjusted for age, center, education, diabetes (missing for 2 controls and 2 cases), indoor ALAN exposure (missing for 4 controls and 2 cases) and time of first intake.

3.6. Relative Risk among Cancer Subtypes

We evaluated the association between fasting overnight and aggressiveness of prostate cancer (Gleason score) in a multinomial logistic regression model. The association of prolonged fasting overnight and reduced prostate cancer risk was similar in cases diagnosed with a more aggressive prostate cancer subtype (Gleason score of 4 + 3 or higher)

and in those with a Gleason score of 6 or 3 + 4 (adjusted model, OR = 0.78, 95% CI 0.53–1.14 and OR = 0.71, 95% CI 0.42–1.19, Table 7). The differences among cancer subtypes were non-significant (*p*-value = 0.97).

Table 7. Association of nighttime fasting and prostate cancer relative risk by cancer subtypes (missing for 17 cases).

Nighttime Fasting	Controls N (%)	Cases N (%)	RRR (95% CI) [a]	RRR (95% CI) [b]
Low aggressiveness				
≤11 h	474 (55.9)	246 (56.7)	Ref	Ref
>11 h	374 (44.1)	188 (43.3)	0.91 (0.71–1.18)	0.78 (0.53–1.14)
High aggressiveness				
≤11 h	474 (55.9)	86 (55.1)	Ref	Ref
>11 h	374 (44.1)	70 (44.9)	0.92 (0.63–1.33)	0.71 (0.42–1.19)

N = sample size; RRR = Relative risk ratio; 95% CI = 95% confidence interval. [a] Adjusted for age, center and education. [b] Adjusted for age, center, education, diabetes (missing for 2 controls and 2 cases), indoor ALAN exposure (missing for 4 controls and 2 cases) and time of first intake.

3.7. Sensitivity Analyses

Further adjustment of the model with other potential confounders did not change the estimates (Table S1). Combining data from weekdays and weekends did not result in different estimates than on weekdays only (data not shown). Removing the outliers of the exposure variable distribution also had a minimal effect on estimates (adjusted model, OR = 0.76, 95% CI 0.54–1.06, Table S4). The GAM in this reduced dataset showed the same pattern (see Figure S3C,D).

The median nighttime fasting duration reported one year before the inclusion in the study in the control group was 11.50 h (interquartile range 10.50–12.50). Models built with data on nighttime fasting during weekdays reported one year before diagnosis or before inclusion in the study for the controls changed the direction of the association (adjusted model OR = 1.13, 95% CI 0.88–1.46, Table S5). Analyzing the cancer subtypes, we observed that the relative risk ratio for aggressive prostate cancer cases (adjusted RRR = 1.23, 95% CI 0.82–1.84, Table S6) was slightly higher than for less aggressive cases (adjusted RRR = 1.10, 95% CI 0.83–1.46, Table S6).

In a model that excluded participants answering the circadian interview more than 3 years later from the baseline interview strengthened the findings (participants interviewed within 3 years: OR = 0.68, 95% CI 0.41–1.13; all participants: OR = 0.77, 95% CI 0.54–1.07).

4. Discussion

This is one of the first studies to examine the association between nighttime fasting duration and cancer risk. These findings suggest a possible lower risk of prostate cancer with longer overnight fasting time, especially when feeding is synchronized with the appropriate circadian timing by having an early breakfast.

From an evolutionary perspective, the benefits of a prolonged period of fast overnight are plausible. Life on earth has evolved to adapt and anticipate daily environmental oscillations resulting from the 24 h rotation of our planet. Natural selection drove the development of circadian rhythms enabling the daily optimization of energy acquisition, including sunlight (for photosynthetic organisms) or food [33]. The circadian clock permitted organisms to be prepared and aroused to access food when available and store supplies and undergo repairment processes during fasting hours, improving metabolic fitness and survival [33]. The extensive discussion on the benefits of nighttime fasting in mass media (e.g., Twitter) that are frequently based on this evolutionary perspective is not, however, backed by reliable population data.

Prolonged periods of fast overnight have been linked to a significant improvement in glycemic control and inflammation biomarkers, potentially explaining a lower risk of

prostate cancer [18,19]. Our results are consistent with those of the prospective WHEL study, showing that fasting for less than 13 h was associated with a 36% increased hazard of breast cancer recurrence than a more extended fasting period [20]. Similarly to what we found, the prospective study from the NutriNet-Santé cohort showed that each hour increase in time of first intake was associated with a 17% increase in the hazard of developing PCa (HR = 1.17, 95% CI 0.96–1.43) [12]. However, contrary to what we found, even adjusting for this confounding effect of time of breakfast, nighttime fasting duration was associated with a slight increase in the hazard of developing prostate cancer in this study population (HR= 1.12, 95% CI 0.93–1.34) [12]. Differences between findings of epidemiological studies might be explained because of varying methods, sample sizes and time intervals examined.

It has been previously reported that time of supper [12,13] and time elapsed between supper and sleep [13] are associated with breast and prostate cancer. To disentangle these effects from the effects of nighttime fasting, we inspected their correlation and we explored adjustment of the crude model for these variables. We found low correlation between nighttime fasting duration and time of supper and with time interval between supper and sleep. Similarly, when we explored adjusting our basic model for time of last intake and for time interval between supper and sleep, the estimates did not significantly change. These results show that the potentially protective association between a prolonged nightly fasting period and reduced prostate cancer risk might be complementary to the protective association of having a larger time interval between supper and sleep.

Fasting regimens that promote long periods of fasting overnight, even by skipping breakfast, have become increasingly popular. Our results suggest that the time of breakfast is confounding the association between nighttime fasting and prostate cancer risk and that elongating the nightly period of fast by skipping breakfast might be counterproductive. Nighttime fasting is most beneficial when the eating window starts early in the morning, a form of intermittent fasting known as early time-restricted feeding [34]. Time-of-day variations in the benefits of nightly fasting can be explained by fluctuations of the circadian rhythms, which can also vary across chronotypes. The optimal time for food intake might be in the early hours of the day when diet-induced thermogenesis, glucose tolerance, insulin sensitivity, pancreatic beta-cell responsiveness and oxidation of fatty acids are higher [35].

Our study shows that in morning chronotype individuals, the association between a prolonged period overnight and a prostate cancer risk reduction is slightly larger than other chronotypes. This association was independent of the time of the first intake. On the contrary, in evening chronotype individuals, long nighttime fasting was not associated with lower prostate cancer risk. This may be explained by a higher frequency of chronic sleep deprivation and social jet-lag among late chronotypes, especially when working on early morning work schedules [36].

Reverse causation might explain the differences observed between the analyses of habits at 40 years of age and those shortly before disease or inclusion in the study. A possible explanation may be that eating and sleep habits one year before diagnosis might be reflecting a cancer-related loss of appetite or other early disease-related changes in lifestyle [25].

The strengths of this research include the novelty of the research question, the large sample size, the adjustment for a wide variety of well-measured confounders and the validated questionnaires to assess diet and the individual chronotype. As limitations, one of the primary concerns is that this assessment is subject to recall bias [13]. We tried to minimize this bias by requesting data on the timing of diet at mid-life. Additionally, in a sensitivity analysis, we explored excluding those participants with a longer time elapsed between inclusion in the study and exposure assessment in the circadian interview and, therefore, more prone to recall bias. After excluding these participants, estimates for our association of interest were strengthened. Meal timings and diet quality were assessed in one time point and these are behaviors prone to fluctuations. Future studies should

include multiple time assessments to increase the validity of the results. A further inherent limitation of studies on prostate cancer is the lack of knowledge of this cancer's non-genetic risk factors that could result in uncontrolled confounding. Finally, circadian patterns, including sleep timing and duration, light exposure and meal timings, are all interconnected. To improve public health recommendations, a future approach could be to analyze these behaviors in an integrated manner.

5. Conclusions

To conclude, this is one of the first studies with epidemiological data examining the association between nighttime fasting duration and prostate cancer risk and to consider the time window of this period of fasting. Complementing the previous results from the MCC study that showed a beneficial association between an early supper and a long supper–sleep time interval, our results suggest that lengthening the fasting period could be associated with a lower risk of this cancer, especially when having an early breakfast. This research highlights the importance of considering a circadian perspective, such as the time window of nighttime fasting and combined effects of meal timing aspects, in studies evaluating dietary and sleep determinants of cancer.

Supplementary Materials: The following are available online at www.mdpi.com/article/10.3390/nu13082662/s1. Figure S1. Directed acyclic graph representing the potential association between nighttime fasting duration and prostate cancer risk; Figure S2. Scatter plot for each of the correlations among controls; Figure S3. Generalized additive model examining the linearity of the association between nighttime fasting duration (continuous variable, in hours) and prostate cancer risk; Table S1. Examination of further adjustment with potential confounders; Table S2. Association of nighttime fasting duration with prostate cancer risk. Exposure variable categorized into three levels based on the distribution of this variable in the control group; Table S3. Association of nighttime fasting and prostate cancer risk stratified by adherence to the WCRF/AICR score; Table S4. Association of nighttime fasting duration with prostate cancer risk excluding outliers; Table S5. Association of nighttime fasting duration with prostate cancer risk based on information on dietary habits the year before the circadian interview; Table S6. Prostate cancer relative risk by cancer subtypes with information on dietary habits the year before the circadian interview.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data can be provided by contacting the corresponding author and following permission by the participating centres in the MCC Spain study. The computing code can be provided by the corresponding author.

Conflicts of Interest: None declared.

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5.2. Paper II

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Association of time of breakfast and nighttime fasting duration with breast cancer risk in the multicase-control study in Spain

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Circadian nutritional behaviors, defined by the daily eating/fasting cycle, have been linked with breast cancer. This study aimed to further disentangle the association of nighttime fasting duration and time of breakfast with breast cancer risk. We analyzed data from 1,181 breast cancer cases and 1,326 population controls from the Spanish multicase-control study (MCC-Spain),

2008–2013. We collected circadian nutritional behaviors at mid-age *via* a telephonic interview. We applied logistic regression to estimate odds ratios (OR) and 95% confidence intervals (CIs) for the association of nighttime fasting duration and time of breakfast with breast cancer risk in all women and stratified by menopausal status. Models were adjusted for age, center, education, family history of breast cancer, age at menarche, number of children, breastfeeding, age at first child, body mass index (BMI), contraceptive use, and hormonal replacement therapy (HRT). A later time of breakfast was associated with a non-significant increased risk of breast cancer (OR = 1.05, 95% CI: 0.95–1.16, per hour increase). This association was stronger among premenopausal women, among whom each hour later, the time of breakfast was associated with an 18% increase in breast cancer risk (OR = 1.18, 95% CI: 1.01–1.40). The association was not observed in postmenopausal women. We did not observe an association between nighttime fasting duration and breast cancer risk after adjusting for the time of breakfast. In this study, late breakfast was associated with increased breast cancer risk, especially among premenopausal women, compared with early breakfast. Aside from nutritional quality, circadian nutritional behaviors should be further studied in relation to cancer.

KEYWORDS

meal timing, circadian nutritional behaviors, nighttime fasting duration, breakfast, breast cancer risk, chrononutrition, circadian rhythms

Introduction

Female breast cancer was the most commonly diagnosed cancer and the fifth leading cause of cancer-related mortality worldwide in 2020 (1). It was first proposed during the 1970s that the disruption of circadian rhythms could influence breast cancer risk (2). On the basis of a growing body of evidence, the International Agency for Research on Cancer (IARC) classified circadian rhythm disruption, resulting from night shift work, as probably carcinogenic for cancer of the breast, prostate, and colon (3, 4). Circadian rhythms regulate multiple physiological activities including hormonal secretion, immune regulation, and cellular cycle (5). Several external factors or *zeitgebers* can synchronize the circadian rhythms including the daily light-dark and feeding-fasting cycles (5).

The emerging field of chrononutrition studies the relationship between the timing of nutritional behaviors, circadian rhythms, and health (6–8). Some studies have shown that circadian nutritional behaviors, or meal timings, may be associated with breast cancer risk and progression (9–11). A prolonged nightly period of fasting has been associated with reduced systemic inflammation (12), a putative risk factor for breast cancer. Fasting for less than 13 h overnight has been associated with increased odds of breast cancer recurrence compared with a longer nightly fasting period (11). Contrarily, a study from the French cohort NutriNet-Santé showed no association between the length of the nightly fasting period and the risk of breast cancer (10).

The nightly fasting interval can be elongated either by having an early dinner or by having a late breakfast. Results from the multicase-control study (MCC-Spain) and the NutriNet-Santé cohort showed that having an early dinner was associated with a reduced risk of breast cancer compared with a late dinner (9, 10). In contrast, previous studies indicate that skipping breakfast, or delaying the first meal, can lead to metabolic and inflammatory deregulation (13–17). It has also been inconsistently linked with weight gain (14, 18, 19). The omission of breakfast has also been associated with increased cancer-related and all-cause mortality (20). Finally, data from the NutriNet-Santé cohort shows a non-significant association between a later breakfast and a higher risk of breast cancer (10). These analyses were not stratified by menopausal status.

Some cross-sectional studies have suggested an association between a prolonged nightly fasting period and a reduction in potential breast cancer risk factors (12, 21). However, the evidence is scarce and inconclusive. Moreover, the association with the time of breakfast and with consideration of menopausal status remains unclear. This analysis builds on previous results within the MCC-Spain study in relation to the time of

dinner and the time interval between dinner and sleep (9) to build a more integrated understanding of the circadian nutritional behaviors as a whole. This study investigates whether circadian nutritional behaviors, specifically nighttime fasting duration and time of breakfast, are associated with breast cancer risk.

Materials and methods

Study design and population

The multicase-control MCC-Spain study[1] is a large population-based case-control study of 5 common tumors, which was conducted in Spain between 2008 and 2013 (22, 23). Histologically confirmed cancer cases were recruited from 23 collaborating hospitals in 12 Spanish provinces. Simultaneously, controls were randomly selected from the primary healthcare centers located within the catchment area and were frequency-matched to cases by age, sex, and region. All participants were aged 20–85 years and had resided in the catchment area for 6 months or more prior to recruitment. For each of the included centers, the ethics committees reviewed and approved the study protocol. Before being included in this study, participants signed an informed consent form (22).

In this analysis, only breast cancer was examined. A total of 3,648 women were eligible for this analysis, including 1,738 breast cancer cases and 1,910 population controls. We excluded 360 women who reported ever working on the night shift and 7 with missing menopausal status (**Figure 1**). We excluded night shift workers, to focus our analysis mainly on the circadian disruption specifically related to nutritional behaviors and to avoid potential confounding with this other source of circadian disruption. We considered night shift work as working entirely or partly between 00:00 and 6:00 for 3 nights or more per month (9). We also excluded 661 women who did not respond to the circadian questionnaire and 113 who had missing information on nighttime fasting (**Figure 1**). Finally, 1,181 breast cancer cases and 1,326 population controls were included in these analyses.

Data collection and variable assessment

Trained personnel administered an epidemiological questionnaire in a face-to-face interview. The questionnaire included information on socio-demographics, personal and family medical history, reproductive factors, medication, weight and height (corresponding to the year prior to study inclusion), recreational physical activity, and smoking (22). Using the Ainsworth classification (24), we assigned a physiological measure of energy expenditure (Metabolic Equivalent of Task, MET) to all recreational physical activities reported and we calculated the equivalent MET hour/week. We excluded data on physical activity corresponding to the 2 years before the interview to avoid any changes caused by the disease. We calculated body mass index (BMI) from self-reported weight and height. We also provided the participants with a previously validated food frequency questionnaire (FFQ) that was self-administered to evaluate nutritional behaviors over the previous year (22). The overall response rate was 88%. The questionnaire included an assessment of alcohol consumption between 30 and 40 years of age. Daily energy intake (kcal/day) and past daily consumption of ethanol (g/day) were estimated separately using the Centro de Enseñanza Superior de Nutrición y Dietética (CESNID) food composition table (25). As a proxy of a healthy diet, we also considered daily consumption of vegetables and fresh fruits (g/day).

Cases were classified into three subtypes based on pathology records, namely, (1) tumors with hormonal receptors either for estrogens or progesterone (labeled as positive hormonal receptors), (2) tumors with overexpression of the human epidermal growth factor 2 (HER2 +), and (3) tumors without hormonal receptors nor overexpression of HER2 (triple negative).

In total, 6 months to 5 years after enrollment in the study (median time 3 years), a telephonic interview was performed to assess circadian nutritional behaviors, timing of physical activity, and sleep patterns (questionnaire available on the study website; see text footnote 1). This interview also included a question on bedroom light during sleep assessed with a four-digit Likert scale (a) total darkness, (b) almost dark, (c) dim light, and (d) quite illuminated. Chronotype is the individual preference for the timing of circadian activity and has a genetic basis (26). This was also assessed in the circadian interview. Participants were asked to report their behaviors at mid-age (40 years of age) and the year before their inclusion in the study. Circadian nutritional behavior questions assessed the frequency of consumption of main meals and usual timing during weekdays and weekend days. We conducted the main analyses with behaviors at mid-age to avoid potential reverse causation from the more recent behaviors. We asked the participants about the frequency of breakfast consumption as never having breakfast, having breakfast only on weekends, having breakfast only on weekdays, and always having breakfast. Sleep duration was calculated as the difference between the time of turning off the lights and the time of awakening on weekdays and weekends. Nighttime fasting duration was calculated as

1 http://www.mccspain.org

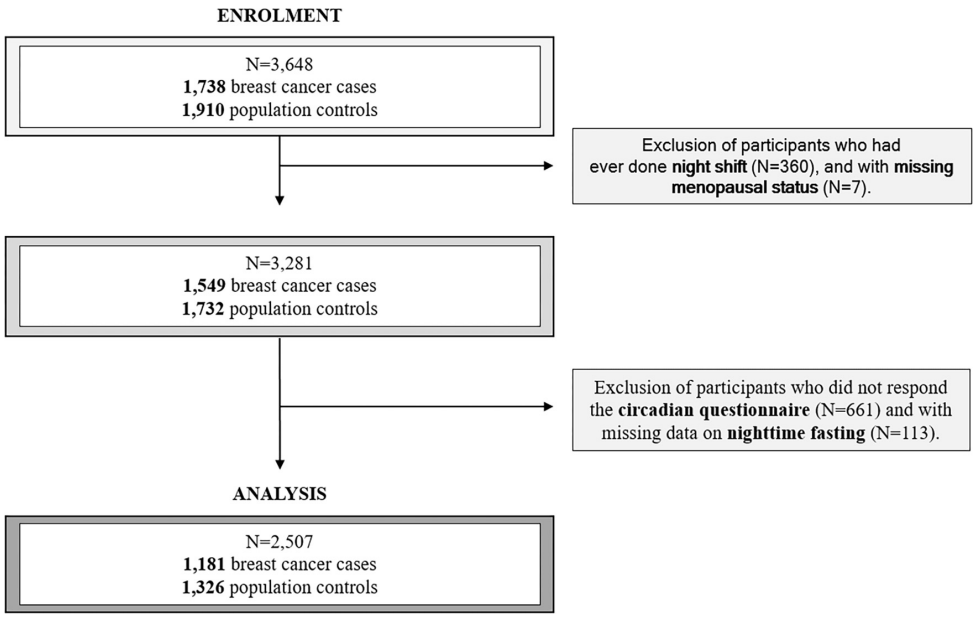

FIGURE 1
Participant flow diagram.

the time elapsed between the last meal and breakfast the following day. For those participants that reported never having breakfast (1%) or having it only on weekends (< 1%), the time of lunch was considered as breakfast, understood as the broader concept of the time when the nightly fast was broken.

Statistical analyses

We compared basic characteristics among cases and controls and in premenopausal and postmenopausal women separately.

To investigate the associations between nighttime fasting duration, time of breakfast, and breast cancer risk, we built logistic regression models and estimated odds ratios (ORs) and 95% confidence intervals (CIs). Models were adjusted for age (continuous, years), center (Madrid, Barcelona, Navarra, Gipuzkoa, Leon, Asturias, Huelva, Cantabria, Valencia, and Gerona), and educational level (less than primary school, primary school, secondary school, university). We also adjusted all models for well-established breast cancer risk factors: family history of breast cancer (no, yes), age at menarche (continuous, years), number of children (nulliparous, 1 or 2 children, 3 or more children), breastfeeding (parous women no breastfeeding, breastfeeding up to 6 months, breastfeeding 6–24 months, breastfeeding for more than 24 months, and nulliparous women), age at the first child (less than 20 years, from 20 to 35 years, more than 35 years, and nulliparous women), BMI 1 year before inclusion to the study (continuous, kg/m^2), contraceptive use (never, ever), and hormonal replacement therapy (HRT) and menopausal status (premenopausal women, postmenopausal women who ever used HRT, and postmenopausal women who never used HRT). All the covariates included in the main model had less than 3% of missing values; therefore, we applied a complete case analysis. We initially explored separate models for the two exposures (nighttime fasting duration and time of breakfast) and then a model mutually adjusting both circadian nutritional behaviors.

There are well-established molecular and etiological differences between premenopausal and postmenopausal breast cancer (27), a pattern that has been replicated also for the effect of night shift work (28). We checked whether there was evidence of effect modification of the association between time of breakfast and nighttime fasting duration with breast cancer risk, by menopausal status by including an interaction term in the adjusted model and conducting a likelihood ratio test. We did follow the same procedure for chronotype and for HRT history (ever vs. never) among postmenopausal women.

We inspected the linearity of the association between nighttime fasting duration, time of breakfast, and breast cancer risk by building generalized additive models (GAMs). To test for linearity, we conducted an ANOVA comparing two models with the exposure of interest included with or without the smoothing term. None of the models showed a significant departure from linearity; therefore, we considered exposure variables as continuous. We further categorized nighttime fasting duration and time of breakfast according to the median point in controls: 11.00 h (interquartile range, IQR 10.00–12.00) and 8:00 a.m. (IQR 7:30–9:00 a.m.), respectively. We explored the correlation among both exposures and also with other circadian behaviors including those already examined in the previous MCC-Spain study on mistimed eating patterns (9).

Finally, in a multinomial logistic regression, we investigated the association of nighttime fasting duration and time of breakfast with the risk of breast cancer subtype, reporting relative risk (RR) ratios and examining differences between subtypes with the Wald test.

In sensitivity analyses, we explored adjustment for other lifestyle factors (daily alcohol intake, physical activity, daily caloric intake, and daily consumption of fruits and vegetables) and other potential risk factors for breast cancer including socioeconomic status, smoking, and age at menopause. We also explored further adjustment with other circadian behaviors including time of dinner, interval between dinner and sleep, indoor light-at-night, sleep duration, and chronotype. To investigate the potential influence of recall bias, we examined the association between nighttime fasting duration and time of breakfast with breast cancer risk using data reported for the year previous to diagnosis (or enrollment for controls) and checked the correlation with behaviors at mid-age. Finally, we explored the joint effects of nighttime fasting and breakfast timing in a model combining both exposures.

The statistical package R 4.0.5 was used to perform these analyses (R Foundation for Statistical Computing, Vienna, Austria).[2]

Results

Study population

The characteristics of our study population are shown in Table 1. The mean age of cases was 55 years (11.6 *SD*) and of controls 58 years (12.5 *SD*). Overall, cases were more likely to have a family history of breast cancer, to be premenopausal

TABLE 1 Main characteristics of the study population.

	Controls ($N = 1,326$) mean (*SD*) or *N* (%)	Cases ($N = 1,181$) mean (*SD*) or *N* (%)
Age (years)	58.4 (12.5)	55.4 (11.6)
BMI (kg/m^2)	25.7 (4.7)	25.9 (0.187)
Education		
Less than primary school	193 (14.6)	137 (11.6)
Primary school	412 (31.1)	403 (34.1)
Secondary school	438 (33.0)	407 (34.5)
University	283 (21.3)	234 (19.8)
Score socioeconomic		
Low	357 (27.8)	334 (28.3)
Medium	696 (54.2)	660 (55.9)
High	232 (18.1)	187 (15.8)
Family history of breast cancer		
Yes	124 (9.4)	175 (14.8)
No	1,202 (90.6)	1,006 (85.2)
Diabetes		
No	1,222 (92.4)	1,104 (93.9)
Yes	100 (7.6)	72 (6.1)
Age at menarche (years)	12.8 (1.6)	12.7 (1.5)
Number of children		
Nulliparous	236 (17.8)	243 (20.6)
1–2 children	745 (56.3)	694 (58.8)
3 children or more	342 (25.9)	243 (20.6)
Age at first child		
First child < 20 years old	52 (4.8)	46 (4.9)
First child 20–35 years old	957 (88.1)	811 (86.9)
Parous ≥ 35 years old	77 (7.1)	76 (8.1)
Breastfeeding		
Parous without breastfeeding	166 (15.3)	141 (15.5)
Parous breastfeeding for less than 6 months	294 (27.2)	269 (29.6)
Parous breastfeeding for 6–24 months	496 (45.8)	423 (46.5)
Parous breastfeeding for more than 24 months	126 (11.6)	76 (8.4)
Contraceptive use		
Never	648 (48.9)	603 (51.1)
Ever	677 (51.1)	577 (48.9)
Menopausal status		
Premenopausal	386 (29.1)	436 (36.9)
Postmenopausal	940 (70.9)	745 (63.1)
Hormonal replacement therapy		
Never	1,178 (92.0)	1,071 (92.5)
Ever	102 (8.0)	87 (7.5)
Smoking		
Never smoker	778 (58.7)	662 (56.1)
Past smoker	292 (22.0)	311 (26.4)
Current smoker	256 (19.3)	207 (17.5)
Daily alcohol intake (g ethanol)	6.0 (10.1)	6.9 (12.7)
Daily caloric intake (Kcal)	1,717.8 (537.4)	1,831.6 (610.5)
Daily consumption of vegetables and fruits (g)	557.0 (264.4)	555.8 (300.2)
Physical activity[a]		
Inactive	516 (38.9)	500 (42.3)
Poorly active	255 (19.2)	203 (17.2)
Moderately active	167 (12.6)	147 (12.4)
Very active	387 (29.2)	331 (28.0)

(Continued)

2 http://www.R-project.org/

TABLE 1 (Continued)

	Controls ($N = 1,326$) mean (SD) or N (%)	Cases ($N = 1,181$) mean (SD) or N (%)
Chronotype		
Morning	508 (38.8)	426 (36.5)
Intermediate	528 (40.3)	464 (39.7)
Evening	273 (20.9)	278 (23.8)
Sleep duration (hours)	6.9 (1.3)	7.1 (1.3)
Breakfast		
Never	9 (0.7)	21 (1.8)
Only weekends	10 (0.8)	6 (0.5)
Only weekdays	20 (1.5)	24 (2.0)
Always	1,279 (97.0)	1,129 (95.7)
Time of breakfast (start time, a.m.)	8.4 (1.4)	8.5 (1.4)
Nighttime fasting duration (hours)	11.0 (1.6)	11.1 (1.6)

BMI, body mass index; N, sample size; SD, standard deviation.
[a]Physical activity was classified according to the annual mean of METS h/week. Inactive = 0 METS h/week; poorly active = 0.0001–8 METS h/week; moderately active = 8.0001–16 METS h/week; very active = more than 16.0001 METS h/week.

women, to have less children, and to have a higher past consumption of alcohol and daily energy. Sleep differed between controls and cases with a duration of 6.9 h (1.3 *SD*) and 7.1 h (1.3 *SD*), respectively. Only 9 controls (0.7%) reported never having breakfast, whereas 21 cases (1.8%) skipped breakfast. Moreover, the time of breakfast was later for cases (8.5, 1.4 *SD*) compared with controls (8.4, 1.4 *SD*). Nighttime fasting duration was similar between both groups (11.0, 1.6 *SD* controls and 11.1, 1.6 *SD* cases).

We found no correlation among controls between the time of breakfast and the time of last meal (Spearman's correlation coefficient 0.09, **Supplementary Figure 1**) nor with the interval between dinner and time going to sleep (Spearman's correlation coefficient 0.06). We found a high correlation between nighttime fasting duration and time of breakfast (Spearman's correlation coefficient 0.8).

We explored characteristics of cases and controls by menopausal status (**Supplementary Table 1**). Postmenopausal cases tended to have a higher BMI compared with postmenopausal controls (27.1 vs. 26.3 kg/m^2). Among premenopausal women, BMI was similar among both groups. Premenopausal cases had a longer nighttime fasting duration and a later breakfast compared with controls (11.0 vs. 10.6 and 8.6 vs. 8.2, respectively). In postmenopausal women, there were no differences in neither of these two nutritional circadian behaviors.

Association of nighttime fasting duration and breast cancer risk

In all women, we observed no association between nighttime fasting duration and breast cancer risk after adjusting for the time of breakfast (**Table 2**, OR = 1.01, 95% CI: 0.93–1.11). For premenopausal women, we observed an association between nighttime fasting duration and breast cancer risk (OR = 1.11, 95% CI: 1.01–1.21), but no association was observed after adjusting for time of breakfast (OR = 0.99, 95% CI: 0.86–1.14). We observed the same tendency, in the GAMs (**Figures 2A–C**). Among postmenopausal women, we did not observe an association between nighttime fasting duration and breast cancer risk (**Table 2**, OR = 1.04, 95% CI: 0.93 – 1.17, model adjusted for time of breakfast). The absence of an

TABLE 2 Logistic regression models investigating the association between nighttime fasting and time of breakfast with breast cancer risk.

	All women			
	Controls N (%) or mean (SD)	Cases N (%) or mean (SD)	OR (95% CI)[a]	OR (95% CI)[b]
Nighttime fasting				
Continuous (hours)	11.0 (1.6)	11.1 (1.6)	1.05 (0.99–1.10)	1.01 (0.93–1.11)
≤11.00 h[c]	744 (60.4)	646 (57.9)	*Ref*	*Ref*
>11.00 h	488 (39.6)	470 (42.1)	1.12 (0.94–1.33)	1.02 (0.83–1.27)
Time of breakfast				
Continuous	8.4 (1.4)	8.5 (1.4)	1.06 (1.00–1.13)	1.05 (0.95–1.16)
≤8.00 a.m.[c]	648 (52.6)	518 (46.4)	*Ref*	*Ref*
>8.00 a.m.	584 (47.4)	598 (53.6)	1.27 (1.08–1.51)	1.25 (1.02–1.54)
Premenopausal women				
Nighttime fasting				
Continuous (hours)	10.6 (1.5)	11.0 (1.8)	1.11 (1.01–1.21)	0.99 (0.86–1.14)
≤11.00 h	265 (69.7)	262 (61.9)	*Ref*	*Ref*
>11.00 h	115 (30.3)	161 (38.1)	1.31 (0.96–1.78)	0.97 (0.66–1.43)
Time of breakfast				
Continuous	8.2 (1.3)	8.6 (1.6)	1.18 (1.06–1.31)	1.18 (1.01–1.40)
≤8.00 a.m.	227 (59.7)	205 (48.5)	*Ref*	*Ref*
>8.00 a.m.	153 (40.3)	218 (51.5)	1.53 (1.13–2.07)	1.40 (0.98–2.00)
Postmenopausal women				
Nighttime fasting				
Continuous (hours)	11.2 (1.7)	11.2 (1.5)	1.02 (0.95–1.09)	1.04 (0.93–1.17)
≤11.00 h	479 (56.2)	384 (55.4)	*Ref*	*Ref*
>11.00 h	373 (43.8)	309 (44.6)	1.06 (0.86–1.32)	1.07 (0.82–1.38)
Time of breakfast				
Continuous	8.5 (1.5)	8.5 (1.3)	1.01 (0.93–1.09)	0.97 (0.85–1.11)
≤8.00 a.m.	421 (49.4)	313 (45.2)	*Ref*	*Ref*
>8.00 a.m.	431 (50.6)	380 (54.8)	1.22 (0.98–1.52)	1.26 (0.97–1.62)

[a]Adjusted for age, center, education, family history of breast cancer, menarche, number of children, BMI, contraceptive use, hormonal replacement therapy, menopausal status, breastfeeding, and age of the first child.
[b]Same as a. Models for both exposures were mutually adjusted.
[c]Categorizations in both exposures were performed according to the median point among controls. N, sample size; OR, odds ratio; SD, standard deviation. The p-value for interaction between the time of breakfast and menopause = 0.021.

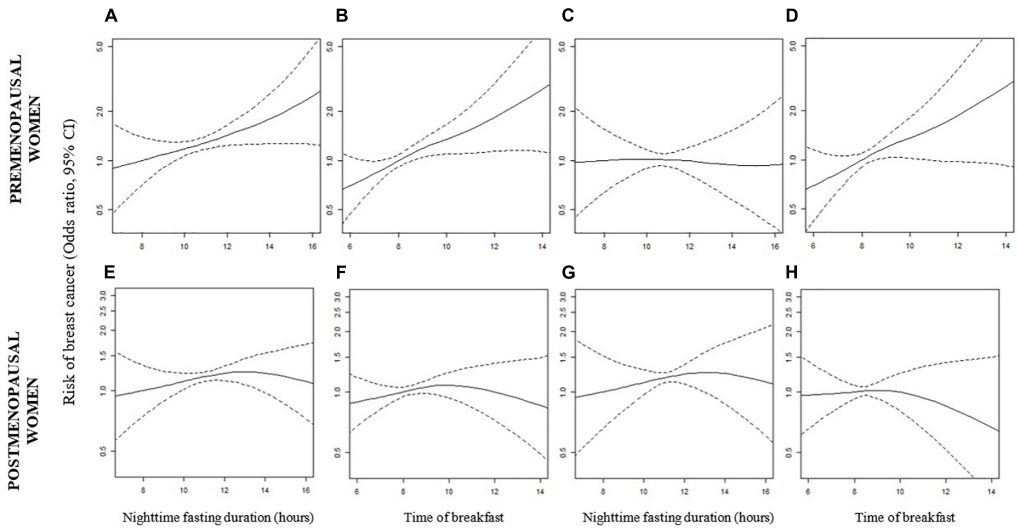

FIGURE 2
Generalized additive models showing the association between nighttime fasting duration, time of breakfast, and breast cancer risk in premenopausal **(A–D)** and postmenopausal women **(E–H)**. Models were adjusted for age, center, education, family history of breast cancer, menarche, number of children, BMI, contraceptive use, hormonal replacement therapy, breastfeeding, and age of the first child. In models **(C)**, **(D)**, **(G)**, and **(H)**, nighttime fasting duration and time of breakfast were mutually adjusted.

association was also observed in Figures 2E,G. There was no significant evidence of effect modification by menopausal status.

Association of time of breakfast and breast cancer risk

In all women, having the first meal after 8 a.m. was associated with a 25% increase in the risk of having breast cancer compared with breakfast before 8 a.m. (Table 2, OR = 1.25, 95% CI: 1.02–1.54) after adjusting for nighttime fasting. In the continuous model, this association was weaker and non-significant (OR = 1.05, 95% CI: 0.95–1.16). This pattern was stronger for premenopausal women: the OR for having breakfast after 8 a.m. was 1.40 (95% CI: 0.98–2.00), while each hour later in the time of breakfast was associated with an 18% increase in the risk of having breast cancer (OR = 1.18, 95% CI: 1.01–1.40). Both models were adjusted for nighttime fasting duration. This association was linear (Figure 2D, *p*-value from ANOVA test = 0.35). In postmenopausal women, the pattern was less clear with a slightly increased risk observed in the categorical analysis but without a clear dose response (OR per hour later in breakfast = 0.97, 95% CI: 0.85–1.11) (Table 2). Figures 2F,H showed the same pattern. Finally, in a model combining our main exposures, we observed that an early breakfast (8 a.m. or before) was associated with a slightly reduced risk independently of the nighttime fasting duration (Supplementary Table 2). This was observed in all women and stratified by menopausal status.

We found a statistically significant effect modification of the association between the time of breakfast and breast cancer risk by menopausal status (*p*-value for interaction = 0.021). We found no effect modification by chronotype (*p*-value for interaction = 0.9) nor by HRT history (ever vs. never) among postmenopausal women (*p*-value for interaction = 0.5).

Cancer subtype

In a multinomial logistic regression model, we explored the association of both nighttime fasting duration and time of breakfast with the RR of each breast cancer subtype. Among all women, we did not observe significant differences in nighttime fasting duration or time of breakfast and different cancer subtypes (Table 3). Among premenopausal women, later time of breakfast (per hour increase) was associated with a higher risk of HER2 + tumors compared with positive hormonal receptors and triple-negative (Table 3, RR = 1.38, 95% CI: 1.03–1.85, RR = 1.19, 95% CI: 0.99–1.42, and RR = 1.11, 95% CI: 0.69–1.78, *p*-value from Wald test = 0.03). No differences were observed for postmenopausal women by subtype.

TABLE 3 Multinomial logistic regression model investigating the association between nighttime fasting and time of breakfast with breast cancer risk subtype.

	Controls	HER2 +		+ Hormonal receptors		Triple-negative	
	Mean (*SD*) or *N* (%)	Mean (*SD*) or *N* (%)	RR (95% CI)[a]	Mean (*SD*) or *N* (%)	RR (95% CI)[a]	Mean (*SD*) or *N* (%)	RR (95% CI)[a]
All women							
Nighttime fasting (hours)	11.0 (1.6)	11.5 (1.6)	1.13 (0.96–1.34)	11.0 (1.6)	0.98 (0.89–1.08)	10.9 (1.6)	0.96 (0.75–1.22)
Time of breakfast	8.4 (1.4)	8.8 (1.6)	1.07 (0.89–1.28)	8.5 (1.3)	1.04 (0.93–1.17)	8.4 (1.4)	1.01 (0.75–1.35)
Total	1,232 (54.8)	200 (8.9)		740 (32.9)		75 (3.3)	
Premenopausal women							
Nighttime fasting (hours)	10.6 (1.5)	11.5 (1.8)	1.09 (0.84–1.41)	10.8 (1.7)	0.94 (0.81–1.10)	10.7 (1.6)	0.93 (0.63–1.37)
Time of breakfast	8.2 (1.3)	9.1 (1.8)	1.38 (1.03–1.85)	8.5 (1.4)	1.19 (0.99–1.42)	8.5 (1.5)	1.11 (0.69–1.78)
Total	380 (49.5)	71 (9.3)		290 (37.8)		26 (3.4)	
Postmenopausal women							
Nighttime fasting (hours)	11.2 (1.7)	11.4 (1.5)	1.19 (0.95–1.48)	11.2 (1.5)	1.02 (0.89–1.16)	11.0 (1.6)	1.02 (0.74–1.41)
Time of breakfast	8.5 (1.5)	8.7 (1.5)	0.92 (0.72–1.18)	8.5 (1.2)	0.98 (0.84–1.14)	8.4 (1.4)	0.91 (0.62–1.32)
Total	852 (57.6)	129 (8.7)		450 (30.4)		49 (3.3)	

[a]Models were adjusted for age, center, educational status, family history of breast cancer, menarche, number of children, BMI, contraceptive use, hormonal replacement therapy, menopausal status, breastfeeding, and age of the first child. Models were mutually adjusted for both exposures. Controls were considered as the reference group.
N, sample size; RR, relative Risk; SD, standard deviation.

Sensitivity analyses

Adjustment for lifestyle factors and other potential breast cancer risk factors did not importantly change our estimates (Supplementary Tables 3, 4). We explored further adjustment of our models with other circadian behaviors (Supplementary Table 5). In all women, the additional adjustment of time of breakfast with the time of last meal (without nighttime fasting) strengthened the association between time of breakfast and breast cancer risk (OR = 1.05, 95% CI: 0.94–1.16 to 1.06 and 95% CI: 1.00–1.14). None of the other estimates in these models importantly changed after adjustment (Supplementary Table 5).

We also investigated the associations of interest using the behaviors reported as corresponding to the year prior to baseline. We observed associations between a later time of breakfast (per hour increase) and breast cancer in all, premenopausal and postmenopausal women (Supplementary Table 6, OR = 1.19, 95% CI: 1.08–1.31, OR = 1.21, 95% CI: 1.03–1.43, and OR = 1.18, 95% CI: 1.04–1.33, respectively). In none of the strata, nighttime fasting duration was associated with breast cancer risk after considering the time of breakfast (Supplementary Table 6).

We observed some differences in the correlation between data reported as corresponding to 40 years of age and to the previous year. For premenopausal women, the correlation was high for the time of breakfast (rho = 0.83), time of last meal (rho = 0.86), and nighttime fasting duration (rho = 0.82). For postmenopausal women, we found a low correlation for the time of breakfast (rho = 0.44), a moderate correlation for the time of last meal (rho = 0.52), and a low correlation for nighttime fasting duration (rho = 0.44).

Discussion

This is one of the first epidemiological studies to investigate the association between circadian nutritional behaviors and breast cancer risk. We found that having a late breakfast was associated with an increased risk of breast cancer compared with an earlier breakfast. This pattern was stronger among premenopausal women and was observed across all chronotypes. We observed an association between nighttime fasting and breast cancer, especially among premenopausal women, which disappeared after adjusting for the time of breakfast.

There is few evidence available on circadian timing of diet and cancer risk. To the best of our knowledge, only one other epidemiological study examined the association between the time of breakfast and breast cancer risk (10). The results of this prospective study showed that each hour later in breakfast was associated with a 13% increase in the hazards of developing breast cancer risk (HR = 1.13, 95% 0.99–1.29, *p*-value 0.07) (10). In this French cohort, non-cases were younger (aged 45 years, 14.5 *SD*) compared with controls in this analysis (aged 58 years, 12.5 *SD*). Results were not stratified for menopausal status, which according to our results might be an effect modifier in this association. These and other factors such as the prospective study design in the NutriNet-Santé cohort or the exposure assessment could explain the differences between

the results from the NutriNet-Santé study and the results presented in this study.

Two cross-sectional studies have suggested that prolonged nighttime fasting could reduce systemic inflammation and improve glycemic control, both potential breast cancer risk factors (12, 21). A prospective cohort study showed that elongating the nighttime fasting period could reduce breast cancer recurrence (11). In this study, the mean nighttime fasting duration was 12.5 (1.7, SD) h. Differences between our results and the results by Marinac et al. (11) could be also explained by the mean nighttime fasting of the study population. Although models were adjusted for a binary variable of eating after 8 PM, the time of breakfast was not considered. As suggested in our study and in a previous analysis from the MCC-Spain study, the time of breakfast was confounding the association between nighttime fasting duration and risk of cancer (29). In line with our results, in the NutriNet-Santé study nighttime fasting duration was not associated with breast cancer after adjusting for the time of the first meal. In the French cohort, the mean nighttime fasting duration was 11.9 (1.2, *SD*).

Several hypotheses could explain the association between the time of breakfast and breast cancer risk. Breakfast skipping might be compensated with a higher intake later on the day (6). This could be also the case for a later time of breakfast, but even after adjusting for daily caloric intake, no changes were observed. Similarly, regular breakfast consumption has been linked with healthier lifestyle behaviors (6). It could be that the observations for early breakfast are also indicative of a "healthy user bias." We explored adjustment for alcohol intake, vegetable and fruit intake, and physical activity, and we did not observe important changes.

The association is biologically plausible. Delaying breakfast could be associated with worse glycemic control (15), lipid profile (17), inflammation (16), and alterations in the cortisol rhythm (30), which may then lead to breast cancer risk (11, 12, 31, 32). In animal models, it has been shown that skipping the analogous breakfast, delaying the first active-phase meal by 4 h, can be associated with increased visceral fat (33), increased hepatic lipid accumulation (34), and with a phase delay in the expression of circadian genes in the liver and fat tissue (35, 36). A randomized clinical trial showed that skipping breakfast acutely altered the regulation of clock and clock-controlled genes (37). Supporting this hypothesis, the downregulation of clock genes has been correlated with breast cancer (38).

The differences in menopausal status could be explained by an increased susceptibility of breast tissue to circadian disruption in earlier life stages (28). It could also be that the potential for recall bias is greater among postmenopausal women (older women) with a long time elapsed since exposure. In fact, the correlation between mid-age behaviors and the previous year to the inclusion in this study was lower in postmenopausal women. The strongest association between the time of breakfast and breast cancer risk in premenopausal women was observed in HER2 + cases. However, there is less evidence on differences in circadian parameters and breast cancer subtypes (3). Further studies are needed to confirm and understand these differences.

The main strengths of this study are the large sample size of the study population, which enabled the stratification of our results by menopausal status, and the detailed information on circadian nutritional behaviors. This investigation gives new insights and future questions on the impact of chrononutrition in cancer, an emerging field of study that deserves more attention. The main limitation of this study is the potential for recall bias since women were asked for behaviors at 40 years of age. Circadian nutritional behaviors were assessed at one single time point, which may affect the validity of these exposures. Finally, given the observational nature of the study and its design, residual confounding cannot be completely ruled out, and causal interpretation of these findings should be taken with caution.

Our results suggest that delaying circadian nutritional behaviors, specifically having a late breakfast, is associated with increased breast cancer risk, especially among premenopausal women. Together with our previous study on other circadian timing aspects, this study suggests that when to eat may also be an important aspect of healthy nutritional behaviors, influencing cancer risk. If these results are confirmed by prospective studies and clinical trials, public nutritional recommendations may consider including timing aspects aside from the quality and quantity components of the diet.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by CEIC 2008/3123/I. The patients/participants provided their written informed consent to participate in this study.

Author contributions

BP-G, EA, TF, PA, IG-A, VM, JA, GF-T, AM-B, RM-G, NA, GC-V, MG, AM, MP, and MK performed the data acquisition. AP-C and AE performed the data curation. MP and MK carried funding acquisition. DR and MK performed the supervision of the study. AP-C wrote the first draft of the manuscript. All authors contributed to the study conception and design,

commented on previous versions of the manuscript and read and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.941477/full#supplementary-material

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5.3. Paper III

Palomar-Cros A, Srour B, Andreeva V, Fezeu L, Bellicha A, Kesse-Guyot E, Hercberg S, Romaguera D, Kogevinas M, Touvier M. Associations of meal timing, number of eating occasions and nighttime fasting duration with type 2 diabetes risk in the NutriNet-Santé cohort. *Under review in Int J Epidemiol.*

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Associations of meal timing, number of eating occasions and nighttime fasting duration with type 2 diabetes risk in the NutriNet-Santé cohort

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ABSTRACT

Background

Food intake plays a pivotal role in regulating circadian rhythms, which modulate glucose and lipid homeostasis. However, studies investigating the association of meal timing and type 2 diabetes (T2D) risk are lacking. The objective of the present study is to investigate the longitudinal associations of meal timing, number of eating occasions and nighttime fasting duration with risk of T2D.

Methods

103,312 adults (79% women, mean age at baseline=42.7 (SD=14.6)) from the NutriNet-Santé cohort (2009-2021) were included. Participants' meal timings and frequency were assessed using repeated 24h dietary records and averaged from the two first years of follow-up (5.7 records/participant). Associations of meal timing, number of eating occasions and nighttime fasting duration with risk of T2D were assessed by multivariable Cox proportional hazard models adjusted for known risk factors.

Results

During a median follow-up of 7.3 years, 963 new cases of T2D were ascertained. Compared to participants habitually having a first meal before 8AM, those eating after 9AM had a higher risk of T2D (HR=1.59, 1.30 – 1.94). Time of last meal was not associated with T2D risk. Each additional eating episode was associated with a lower

risk of T2D, (HR=0.95, 0.90-0.99). Nighttime fasting duration was not associated with T2D risk, except in participants having breakfast before 8AM and fasting for more than 13 hours overnight (HR=0.47, 0.27-0.82).

Conclusions

In this large prospective study, a later first meal was associated with a higher risk of T2D. If confirmed in other largescale studies, an early breakfast should be considered in preventing T2D.

Keywords. Meal timing, type 2 diabetes mellitus, circadian rhythms, cohort, breakfast, fasting

Key Messages

- Although meal timing exerts a key role in the regulation of circadian rhythms and glucose and lipid homeostasis, it remains unclear which are the optimal daily timings for eating and fasting to prevent type 2 diabetes (T2D).
- In this large prospective study with data from 103,312 participants, habitually having a first meal later in the day (after 8AM) was associated with a higher risk of T2D.
- These results show that beyond the nutritional quality of the diet, having an early first meal may be associated with a lower T2D risk and, if confirmed, could lead to promising lifestyle interventions, using chrononutrition to prevent T2D.

INTRODUCTION

In 2019, 463 million individuals were living worldwide with diabetes, a number expected to double by 2045 [1]. The development of type 2 diabetes is mostly determined by modifiable risk factors including an unhealthy diet, physical inactivity or smoking [2]. Recently, meal timing has been added to the list.

Chrononutrition is an emerging field that studies the interplay between timing of food intake, circadian rhythms and health [3]. Circadian rhythms are involved in virtually all functions of the body and are regulated by the circadian clock, which is mainly synchronised by light but also by food [4]. The correct functioning of this system is crucial to ensure an optimal metabolism [5]. Glucose tolerance and insulin sensitivity follow a circadian rhythmicity reaching its maximum in the morning [6]. Circadian misalignment induced by night shift work or later eating times has been associated with several chronic diseases including cancer [7,8], obesity [9] and diabetes [10].

It has been shown that beta cell responsiveness and insulin sensitivity are better at breakfast than dinner [11]. Data from RCTs suggest a link between late dinner and nocturnal glucose intolerance [12] and insulin resistance [13], predictors in the development of type 2 diabetes [14]. Skipping breakfast has been associated in observational studies with worse glycaemic control [15], increased LDL cholesterol and serum insulin levels [16] in healthy individuals. Results from a randomised study showed as well an increased daily

average blood glucose [17]. Skipping breakfast has been also linked to obesity in a meta-analysis of observational studies [18]. In 2018, a meta-analysis of prospective cohort studies reported an association between breakfast omission and risk of type 2 diabetes (Relative risk = 1.22, 95% CI 1.12 – 1.34), partly mediated by BMI [19].

Similarly, a reduced number of daily meals has been inconsistently associated with an increased type 2 diabetes risk in cohort studies [20,21]. Only one study has explored the association between breakfast timing and risk of type 2 diabetes, showing a lower risk of type 2 diabetes for those participants having breakfast after 9AM [22]. However, this study was conducted in adults aged 65 years or more, and assessed meal timing with a questionnaire at baseline. Multiple RCTs have linked time restricted eating (TRE), implying an elongated nighttime fasting duration, with a reduction of body weight and fat, an improved glycaemic profile and insulin sensitivity [23].

In this prospective study, we aim to evaluate the association of meal timing, number of eating occasions and nighttime fasting duration, assessed using comprehensive and repeated daily food records, with risk of type 2 diabetes.

METHODS

Study population

The NutriNet-Santé study (https://etude-nutrinet-sante.fr/) is an ongoing web-based cohort established in 2009 in France to

investigate the relationship between nutrition and health [24]. Recruitment targets volunteers aged 18 or more with access to internet, through vast multimedia campaigns. The International Research Board of the French Institute for Health and Medical Research (IRB Inserm n° 0000388FWA00005831) and the "Comité National Informatique et Liberté" (CNIL n°908450 and n° 909216) approved the NutriNet-Santé study [24]. The study protocol is registered at https://clinicaltrials.gov/: NCT03335644. Electronic informed consent is requested and can be withdrawn at any point of the study.

Data collection

Dietary data

Dietary intakes were assessed every 6 months through online 24h dietary records for 3 non-consecutive days (2 working days and 1 non-working day). We used dietary records from the first two years of follow-up. In these records, participants were asked to report any food or beverage taken during that day and the time of meal. The validity of these dietary records has been previously tested against an interview by a dietician [25] and against biomarkers of nutritional status [26,27]. Then, we used food composition tables to estimate daily energy intake, alcohol and macronutrients [28]. We calculated these values as an individual daily average. We identified energy under-reporters with the method proposed by Black [29].

Exposure variables

To estimate time of first meal, time of last meal, and number of eating occasions, we computed averages across all available food records from the two first years of follow-up. For the number of eating occasions, we considered as an eating episode any food or beverage intake of at least 1 kcal (to exclude water). We calculated nighttime fasting duration as the difference between the last and the first eating episode, assuming that daily behaviours remain similar. We then explored different categorizations for the most consistently reported fasting/eating schemes [23], as a nighttime fasting duration of 12 hours or less, 12 to 13 hours, and more than 13 hours.

Covariates

At inclusion, participants filled an initial set of online questionnaires on diet, socio-demographics and lifestyle [30], anthropometrics [31,32], physical activity (validated IPAQ questionnaire) [33] and health status (with information on personal and family medical history, medication use and menopausal status). Volunteers were asked to fill these questionnaires every year. During the follow-up (2014), 37,042 participants responded to an optional sleep questionnaire [34]. Two variables were used for sub-sample analyses: nighttime total sleep time and bedtime.

Case ascertainment

Participants' health status was reported at enrolment and reassessed every six months by a check-up questionnaire. At any time during follow-up, participants could also declare a health event, a new

treatment, or a medical exam via a dedicated and secured online platform. In addition, data were linked to medico-administrative databases of the SNIIRAM, providing detailed information about the reimbursement of medication and medical consultations. Details on type 2 diabetes ascertainment are provided in Supplementary Method S1. Mortality cases were identified using a linkage to CépiDC, the French national mortality registry. In this study, all incident type 2 diabetes diagnoses up to September 2021 were considered.

Statistical analyses

We included participants who completed at least three 24h-dietary records during their first two years of follow-up; we excluded under-reporters, those with prevalent diabetes at baseline, and those who did report a first meal after 3PM or a last meal before the same hour (as a proxy of night shift) (Figure 1).

Time of first meal, time of last meal and number of eating occasions were modelled as continuous variables (per hour and per meal increase). We explored their correlation with Spearman's rank correlation (Supplementary Figure S1). We categorised these variables as an approximation of the tertiles in the population for readability purposes. The exact tertiles were [5AM,7.7AM], (7.7AM,8.5AM], >8.5AM for time of first meal, [3PM,8PM], (8PM,8.7PM], >8.7PM for time of last meal and [1.3,4], (4,5.3], >5.3 for number of eating occasions. To examine the association between these nutritional behaviours and risk of type 2 diabetes, we built

cause-specific Cox proportional hazards models and estimated HR and 95% CI.

Death and incident cases of type 1 diabetes were considered as competing events. Participants contributed person-time until date of type 2 diabetes diagnosis, competing event, last connection or September 30th, 2021, whichever occurred first.

Models were adjusted for age (timescale), sex (women, men), educational level (less than high school degree, <2 years after high school degree, ≥2 years after high school degree), BMI at baseline (continuous, kg/m^2), family history of type 2 diabetes (no, yes), alcohol intake (continuous, g/day), daily energy intake excluding alcohol (continuous, kcal/day), smoking (current, former, never), physical activity (low, moderated, elevated, as defined by the IPAQ [33]), number of dietary records (continuous) and number of meals (continuous). We also added a healthy and a Western dietary pattern (continuous), obtained from principal component analysis computation based on 20 predefined food groups (Supplementary Method S2). We did a second model mutually adjusting time of first meal, time of last meal and number of eating occasions. We verified the assumptions of proportional hazards by plotting the Schoenfeld Residuals and of linearity by introducing spline terms in Cox models.

Less than 1% of participants had missing data on the covariates, except for physical activity with 14% of missing data. We applied mean and mode imputation for continuous and categorical variables,

respectively. We also explored multiple imputation using the multivariate imputation by chained equations (MICE) method (Supplementary Table S1).

Then, we tested whether the nighttime fasting duration was associated with type 2 diabetes. We built Cox models with the continuous and categorical variable of nighttime fasting duration. Models were adjusted for the same confounders as before.

We tested for effect modification by time of first meal by introducing an interaction term between nighttime fasting duration and time of first meal. We conducted likelihood ratio test to compare this model with the version without the interaction. Finally, we calculated the specific estimates for those breaking the nighttime fasting at 8AM or before, i.e., early time restricted eating (eTRE), and those breaking it after 8AM, i.e., late time restricted eating (lTRE). This threshold was chosen as it was the most frequently used time in studies on eTRE [23].

Sensitivity analyses

We explored further adjustment of the main model for intake of saturated fatty acids, sodium, sugar and fiber during the first meal. We also explored adjusting for daily consumption of red and processed meat, sugary drinks, fruits and vegetables, nuts, whole grain, yoghurt, caffeine, ultra-processed food, region of residence and profession. We also tested a model without baseline BMI and total energy intake to explore their confounding effect and a model

with weight change during follow-up to account for its potential mediator role.

We investigated excluding cases of type 2 diabetes diagnosed during the first 2 years of follow-up (to limit reverse causality). Among the subset that responded the sleep questionnaire we selected individuals who reported going to bed anytime between 6pm and 8am (N= 37,042) to exclude potential night shift workers. We repeated our models among this sub-sample and we explored including bedtime and total sleep duration (minutes/24 hours).

Finally, we tested for effect modification of the association between time of first meal and risk of type 2 diabetes by: obesity, sex, education, smoking status and chronotype (morning, intermediate, evening). Analyses were conducted using the statistical package R-4.0.0 (The R Project for Statistical Computing, Vienna, Austria).

RESULTS

In these analyses, we included 103,312 participants (78.9% women) with a mean age at baseline of 42.7 ± 14.6 years. Up to the 30th of September of 2021 (703,752.9 person-years; median follow-up time, 7.3 years; Q1 to Q3, 3.2 to 10.1 years), 963 cases of type 2 diabetes were identified. During the first two years of follow-up, participants responded to a mean of 5.7 (SD 3.0, max 18.0) dietary records.

The baseline characteristics of the study population, according to the averaged time of first meal of the day, are shown in Table 1. Overall,

participants reporting an habitual first meal before 8AM were older (mean age at baseline = 46.6 [SD, 13.1]) compared to participants having a first meal between 8AM and 9AM (42.5 [14.8]) and those having it after 9AM (33.9 [13.4]). Compared to the other two groups, participants in the group with the earliest first meal were more likely to have lower physical activity levels, lower education levels and an earlier last meal of the day. Additionally, they were less likely to be current smokers and to have a family history of type 2 diabetes.

Meal timing, number of eating occasions and risk of type 2 diabetes

Compared to participants reporting a first meal before 8AM, those reporting a first meal after 9AM had an increased risk of developing type 2 diabetes (HR = 1.62, 95% CI 1.33 – 1.97, P for trend < 0.001, Table 2). Similarly, participants reporting a late last meal (after 9PM) had an increased risk compared to participants reporting a last meal before 8PM (HR = 1.28, 95% CI 1.06 – 1.54, P for trend 0.05).

When nutritional behaviours were mutually adjusted, the association of time of first meal remained stable (HR = 1.59, 95% CI 1.30 – 1.94, P for trend < 0.001, for a first meal after 9AM compared to a first meal before 8AM). However, the association between time of last meal did not persist.

In this model, eating for more than 5 occasions per day was associated with a lower risk of type 2 diabetes compared to 4 or less occasions (HR = 0.80, 95% CI 0.68 – 0.95, P for trend 0.02).

There was no clear evidence for non-linear associations (Figure 2).

Nighttime fasting duration and risk of type 2 diabetes

Extending the nighttime fasting duration was not associated with type 2 diabetes (Table 3). We found an effect modification of this association by time of first meal (p-value for interaction <0.001). Our results suggest that a nighttime fasting of more than 13 hours would be inversely associated with type 2 diabetes risk only when fasting is broken at 8AM or before (HR = 0.47, 95% CI 0.27 – 0.82, compared to a fasting duration of 12 hours or less, P for trend=0.3).

Sensitivity analyses

The associations between meal timing and type 2 diabetes risk remained stable in sensitivity analyses (Supplementary Table S1). In addition, we found no evidence of effect modification of the association between time of first meal and risk of type 2 diabetes. The association between daily number of eating occasions and risk of type 2 diabetes was no longer evident after adjusting for daily intake of caffeine, after considering sleep and excluding cases ascertained during the first 2 years of follow-up (Supplementary Table S1).

DISCUSSION

To our knowledge, this is the first prospective study to investigate the associations of, comprehensively assessed, meal timings, number of eating occasions and nighttime fasting duration with the risk of

developing type 2 diabetes. Our results suggest that having a late first meal could be associated with an increased risk of type 2 diabetes. A late last meal was conversely associated with a higher type 2 diabetes risk, but this association disappeared after considering time of first meal.

In line with this, a meta-analysis of 6 cohort studies, showed that skipping breakfast was associated with an increased risk of type 2 diabetes (RR= 1.22, 95% CI 1.12 to 1.34) [19]. In these studies, breakfast consumption was assessed as the frequency of breakfast consumption throughout the week (i.e., more than 3 days/ week) and all the included cohort studies examined breakfast skipping from a single behavioural questionnaire without considering actual data based on the consumption. In the present study, specific timing of food consumption and number of eating occasions were assessed using the same repeated and validated dietary records. The lack of time and appetite in the morning are the main reasons for skipping breakfast [35], the latter one potentially being a consequence of a late dinner [36]. In a cross-sectional study late-night-eating alone, but not breakfast skipping, was associated with hyperglycaemia [36]. However, in the present analyses time of first meal was adjusted for time of last meal.

Only one study has investigated the link between breakfast timing and risk of type 2 diabetes, and showed, inconsistently with our results, an inverse association between a later timing of breakfast (after 9AM) and type 2 diabetes risk [22]. However, the study

population was older (aged ≥65 years) and nutritional behaviours were assessed using one single questionnaire at baseline. Another study showed that the association between irregular breakfast and type 2 diabetes was only observed among participants under 65 years of age [37]. Taken together, these findings suggest age-related heterogeneities in these associations [22].

This association is biologically plausible considering the circadian rhythmicity in insulin sensitivity and glucose tolerance [6]. The optimal metabolic time is early in the morning. The omission of breakfast, and therefore the delay of this first meal, has been associated with a worse glycaemic control [15]. It has also been associated with risk factors for type 2 diabetes such as increased LDL cholesterol, decreased HDL cholesterol and elevated serum insulin [16]. Additionally, results from a randomized controlled crossover trial suggest that skipping breakfast can increase the postprandial insulin concentrations and fat oxidation, potentially leading to low-grade inflammation and impaired glucose homeostasis [38]. Finally, from a circadian perspective, the pivotal influence of the first meal of the day on circadian control, glucose and lipid homeostasis is now starting to be unravelled from evidence in mice [39,40] and humans [41].

Breakfast omission has been associated with a lower physical activity level [42], a higher consumption of alcohol and tobacco [43], a worst diet quality [44] and a lower socioeconomic status [45]. However, we considered all these factors in our analyses. It could also be that skipping breakfast is linked to a caloric compensation later in the day

[46], but we adjusted for daily energy intake. Although obesity could mediate this association [18], we examined adjustment for weight change during follow up, and also interaction with baseline BMI. On the other hand, the association between time of last meal and risk of type 2 diabetes lost statistical significance after adjusting for time of first meal, indicating that this third variable was confounding the association.

Night shift work and sleep disruption have been also associated with an increased risk of type 2 diabetes [47,48]. However, in the present analyses we excluded participants reporting very late nutritional behaviours as a proxy of night shift work. Moreover, when excluding from our analyses those participants reporting extreme sleep times (also as a proxy of night shift), the estimates were weakened but remained evident, suggesting that night shift only partly explained the observed associations.

In this study, eating more frequently was inversely associated with type 2 diabetes. This could be explained by a reduction of serum insulin and lipids concentration between meals [49]. Conversely, another observational study showed that additional snacks to the 3 main meals were associated with an increased risk of type 2 diabetes [20]. In our study, this association lost significance after excluding participants diagnosed during the first two years of follow-up, probably linked to modified behaviours in participants with early metabolic symptoms. In addition, this association was attenuated

after adjustment for caffeine intake, which could be explained by the suggested inverse association with type 2 diabetes [50].

Multiple RCTs have reported a link between time-restricted eating and a reduction in several risk factors for type 2 diabetes including fasting glucose levels, insulin sensitivity, body weight and β-cell function [23]. In our secondary analyses, extending the duration of the nighttime fasting appeared to be inversely associated with type 2 diabetes risk only if this window finished early in the morning (8AM) and after 13 hours of fasting. These results were based on few participants and should be interpreted cautiously.

The main strengths of the study were the large sample size, its prospective design and the repeated dietary records which included detailed assessments for nutritional intakes, meal timings and number of eating occasions. In addition, meal timing was assessed directly using comprehensive 24-hour dietary records which could be less misleading and subject to misclassification bias than using ad-hoc questionnaires.

As a first limitation, this is an observational study and residual confounding cannot be entirely ruled out, despite accounting for a large panel of confounders. Second, the study participants are volunteers with a higher proportion of women from higher socioeconomic status and with greater health-conscious behaviours [51]. This limits the extrapolation of these results to the general population. Third, we had a limited number of type 2 diabetes cases in stratified analyses, or in extreme circadian behaviours situations.

Although we have considered sleep timing and duration, this was measured later during the follow-up, and available for a part of the sample, and could be affected by some level of recall bias. Moreover, night shift work was extrapolated from sleep variables; even though sleep duration could be a proxy for shift work, we had no information about the exposure to light-at-night, which could be another circadian disruptor. A final limitation, opening a research perspective, is that we did not examine the potential differences in meal timings between weekdays and weekends, something previously referred to as eating jet lag [52].

To conclude, in this large prospective study, a late first meal was associated with a higher risk of type 2 diabetes. A circadian nutritional behaviour defined by an early first meal (before 8AM) and early last meal (before 7PM) might be beneficial to lower type 2 diabetes risk. If confirmed in other prospective studies and possibly clinical trials, these behaviours could be recommended as a preventive strategy for type 2 diabetes.

DECLARATIONS

Ethics approval

NutriNet-Santé is conducted according to the Declaration of Helsinki guidelines and was approved by the institutional review board of the French Institute for Health and Medical Research (IRB Inserm n 0000388FWA00005831) and the Commission Nationale de l'Informatique et des Libertés (CNIL n 908450/n 909216). Electronic

informed consent was obtained from all included participants in this study and could be withdrawn at any point of the study.

Author Contributions

The authors' contributions were as follows – Anna Palomar-Cros, Bernard Srour and Mathilde Touvier: designed the research; Valentina A. Andreeva led the development and administration of the sleep questionnaire; Valentina A. Andreeva, Léopold K. Fezeu, Alice Bellicha, Emmanuelle Kesse-Guyot, Serge Hercberg and Mathilde Touvier: collected the data; Anna Palomar-Cros: performed statistical analyses; Bernard Srour: supervised statistical analyses; Anna Palomar-Cros: drafted the manuscript; Bernard Srour and Mathilde Touvier: supervised the writing; Dora Romaguera and Manolis Kogevinas participated in the supervision of the writing; all authors contributed to data interpretation, revised each draft for important intellectual content, read and approved the final manuscript. Anna Palomar-Cros, Bernard Srour and Mathilde Touvier had full access to all the data in the study, Mathilde Touvier takes responsibility for the integrity of the data and the accuracy of the data analysis, she is the guarantor. The corresponding author (Bernard Srour) attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

Data availability

Data and code will be available upon request.

Supplementary data

Supplementary data are available at *IJE* online.

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Conflicts of interest

The authors have no relevant financial or non-financial interests to disclose.

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Figure 1. Flowchart of study population. NutriNet-Santé cohort, 2009-2021. N = 103,312 participants.

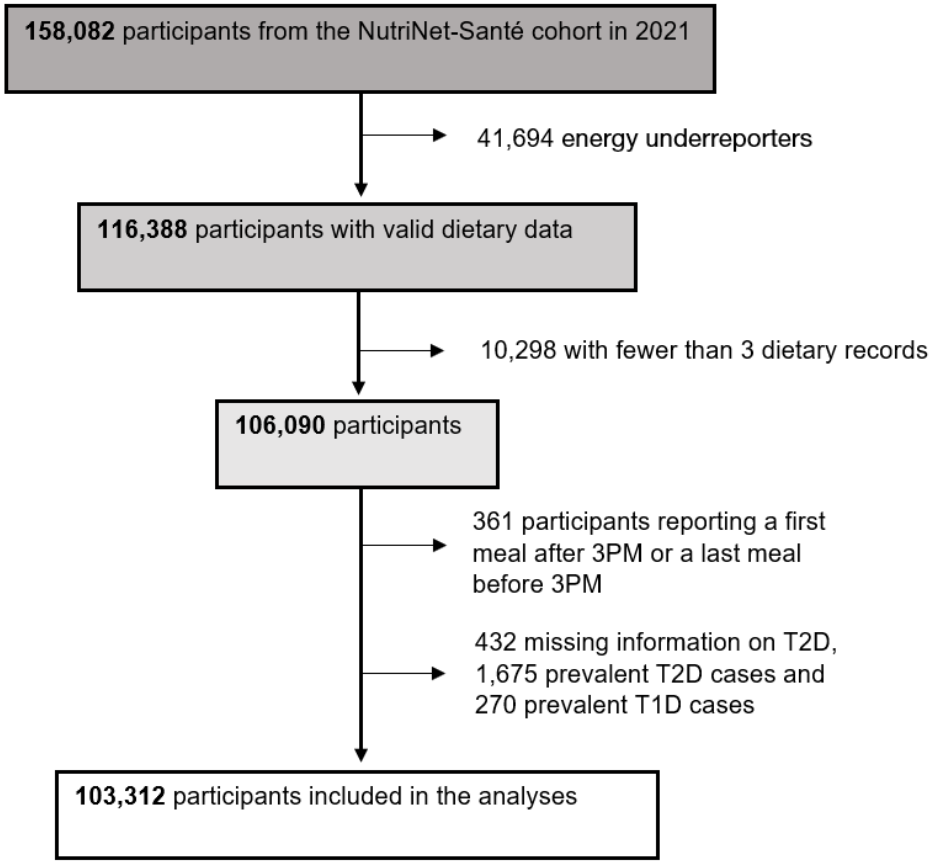

Table 1. Baseline characteristics of study population, 103,312 participants in the NutriNet-Santé cohort (2009-2021)								
	All participants		**First meal before 8AM**		**First meal between 8AM and 9AM**		**First meal after 9AM**	
	No. (%) No. 103,312	Mean (SD)	No. (%) No. 46,256	Mean (SD)	No. (%) No. 36,964	Mean (SD)	No. (%) No. 20,092	Mean (SD)
Age at baseline	103,312	42.7 (14.6)	46,256	46.6 (13.1)	36,964	42.5 (14.8)	20,092	33.9 (13.4)
Sex	103,312		46,256		36,964		20,092	
Women	81,529 (78.9)		35,621 (77.0)		29,755 (80.5)		16,153 (80.4)	
Men	21,783 (21.1)		10,635 (23.0)		7,209 (19.5)		3,939 (19.6)	
BMI (kg/m^2)	10,2167	23.7 (4.4)	45,754	23.8 (4.4)	36,562	23.6 (4.3)	19,851	23.4 (4.7)
Normal weight[a]	72,004 (70.5)		31,619 (69.1)		25,868 (70.8)		14,517 (73.1)	
Overweight	21,602 (21.1)		10,274 (22.5)		7,746 (21.2)		3,582 (18.1)	
Obesity	167 (8.4)		3,861 (8.4)		2,948 (8.0)		1,752 (8.8)	
Family history of T2D	10,1871		45,619		36,536		19,716	
No	87,576 (86.0)		38,649 (84.7)		31,568 (86.4)		17,359 (88.0)	
Yes	14,295 (14.0)		6,970 (15.3)		4,968 (13.6)		2,357 (12.0)	
Smoking status	103,271		46,242		36,951		20,078	
Current	17,893 (17.3)		6,389 (13.8)		6,267 (17.0)		5,237 (26.1)	
Former	33,573 (32.5)		16,825 (36.4)		11,958 (32.4)		4,790 (23.9)	
Never	51,805 (50.2)		23,028 (49.8)		18,726 (50.7)		10,051 (50.1)	
Physical activity	89,121		40,059		32,075		16,987	
Low	29,235 (32.8)		14,806 (37.0)		9,892 (30.8)		4,537 (26.7)	
Moderated	21,551 (24.2)		8,831 (22.0)		7,843 (24.5)		4,877 (28.7)	
Elevated	38,335 (43.0)		16,422 (41.0)		14,340 (44.7)		7,573 (44.6)	
Daily alcohol intake (g)	103,312	7.86 (11.9)	46,256	7.64 (11.4)	36,964	8.10 (11.8)	20,092	7.94 (13.0)
Daily energy intake (kcal)	103,312	1847 (451)	46,256	1856 (448)	36,964	1856 (440)	20,092	1809 (473)
High school degree	103,244		46,229		36,944		20,071	
No	17,812 (17.2)		9,446 (20.4)		5,833 (15.7)		2,533 (12.6)	

Yes, <2 years after high-school	16,291 (15.8)		7,142 (15.4)		5,453 (14.8)		3,696 (18.4)	
Yes, ≥2 years after high-school	69,141 (67)		29,641 (64.1)		25,658 (69.5)		13,842 (69.0)	
Time of first meal (AM)	103,312	8.14 (1.1)	46,256	7.22 (0.5)	36,964	8.24 (0.3)	20,092	9.57 (1.0)
Time of last meal (PM)	103,312	8.24 (1.1)	46,256	8.06 (1.0)	36,964	8.24 (1.0)	20,092	8.48 (1.3)
Nighttime fasting duration (hrs.)	103,312	11.9 (1.4)	46,256	11.2 (1.0)	36,964	12.0 (1.0)	20,092	13.2 (1.5)
Number of eating occasions	103,312	4.89 (1.7)	46,256	5.00 (1.8)	36,964	4.89 (1.6)	20,092	4.63 (1.7)

[a] Normal weight: BMI < 25 kg/m^2; Overweight: BMI = 25-29.9 kg/m^2; Obesity ≥ 30 kg/m^2. BMI= Body-mass index; T2D= type 2 diabetes; SD= Standard deviation.

Table 2. Associations of meal timing and number of eating occasions with risk of type 2 diabetes in 103,312 participants from the NutriNet-Santé cohort (2009-2021)

	No. cases / non-cases	HR (95% CI) [a]	p-value	HR (95% CI) [b]	p-value [c]
Time of first meal					
Continuous (per hour)	963 / 102,349	1.16 (1.08 – 1.24)	<0.001	1.14 (1.07 – 1.22)	<0.001
Before 8AM	462 / 45,794	Ref	<0.001	Ref	<0.001
Between 8 and 9AM	361 / 36,603	1.26 (1.09 – 1.45)		1.26 (1.09 – 1.45)	
After 9AM	140 / 19,952	1.62 (1.33 – 1.97)		1.59 (1.30 – 1.94)	
Time of last meal					
Continuous	963 / 102,349	1.08 (1.02 -1.15)	0.01	1.05 (0.98 – 1.11)	0.2
Before 8PM	373 / 34,365	Ref	0.05	Ref	0.4
Between 8 and 9PM	378 / 45,181	0.93 (0.80 -1.08)		0.88 (0.76 – 1.02)	
After 9PM	212 / 22,803	1.28 (1.06 – 1.54)		1.11 (0.92 – 1.34)	
Number of eating occasions					
Continuous (per meal)	963 / 102,349	0.96 (0.92 – 0.99)	0.04	0.95 (0.90 – 0.99)	0.01
4 or fewer	397 / 39,202	Ref.	0.03	Ref	0.02
5	284 / 29,006	0.92 (0.79 – 1.08)		0.92 (0.79 – 1.08)	
More than 5	282 / 34,141	0.83 (0.71 – 0.98)		0.80 (0.68 – 0.95)	

[a] Adjusted for age (timescale), sex (women, men), educational level (less than high school degree, <2 years after high school degree, ≥2 years after high school degree), BMI at baseline (continuous, kg/m²), family history of type 2 diabetes (no, yes), alcohol intake (continuous, g/day), daily energy intake excluding alcohol (continuous, g/day), healthy dietary pattern (continuous), western dietary pattern (continuous), smoking (current, former, never), physical activity (low, moderated, elevated), number of dietary records (continuous) and number of eating occasions (continuous). The model for number of eating occasions was adjusted for time of first meal.HR = Hazard ratio.
[b] Same as a, but time of first meal, time of last meal and number of eating occasions were mutually adjusted.
[c] P-value for continuous variables and p-trend for categorical variables.

Figure 2. Examining the linearity of the association between circadian nutritional behaviors and risk of type 2 diabetes, NutriNet-Santé cohort, 2009-2021, N=103,312.

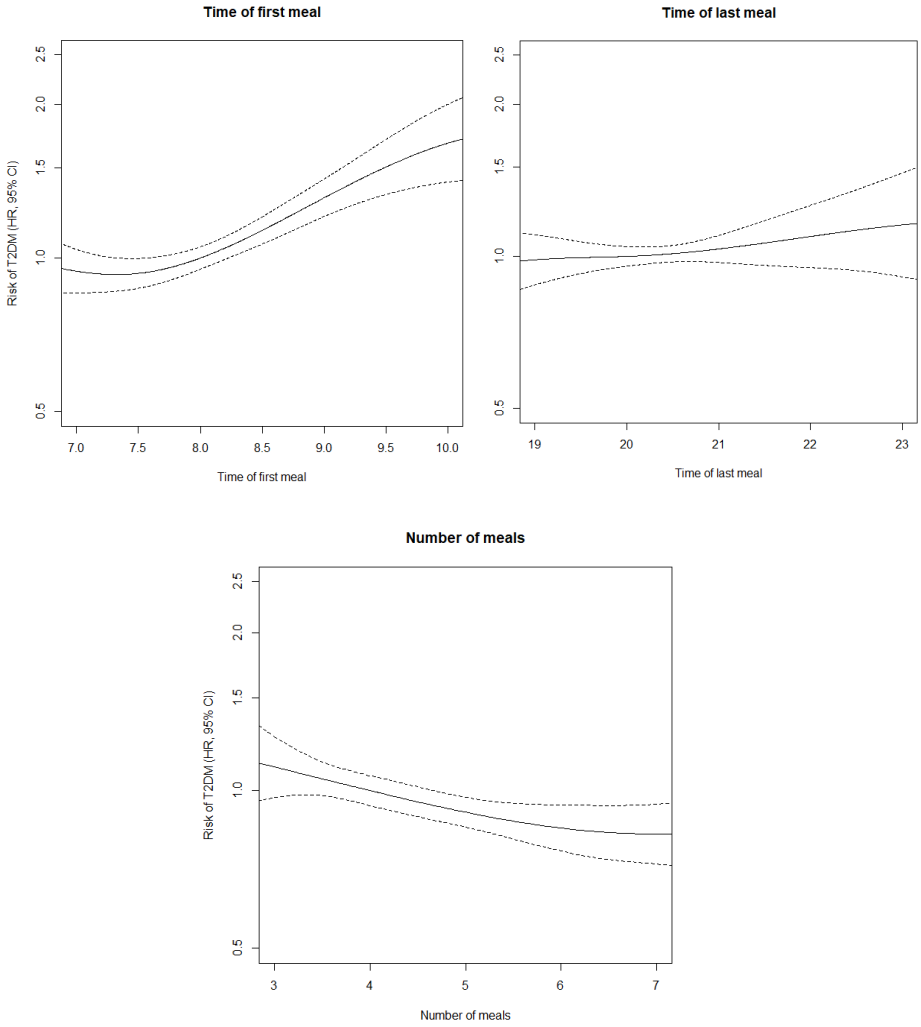

Table 3. Association between nighttime fasting duration and risk of type 2 diabetes in 103,312 participants from the NutriNet-Santé cohort (2009-2021)			
Nighttime fasting	**No. cases / non-cases**	**HR 95% CI[a]**	**p-value**
Continuous (hrs.)	963 / 102,349	0.96 (0.90 – 1.02)	0.2
12hrs. or less	537 / 57,403	Ref.	
12hrs. to 13hrs.	289 / 28,652	1.08 (0.92 – 1.27)	0.4
More than 13hrs.	137 / 16,294	1.03 (0.80 – 1.33)	
In combination with an early breakfast (eTRE[b])			
Continuous (hrs.)	462 / 45,794	0.93 (0.85 – 1.01)	0.07
12hrs. or less	362 / 36,991	Ref.	0.3
12hrs. to 13hrs.	85 / 7,002	1.21 (0.95 – 1.54)	
More than 13hrs.	15 / 1,801	0.47 (0.27 – 0.82)	
In combination with a late breakfast (lTRE[b])			
Continuous (hrs.)	501 / 56,555	1.03 (0.95 - 1.11)	0.4
12hrs. or less	175 / 20,412	Ref.	0.08
12hrs. to 13hrs.	204 / 21,650	1.08 (0.88 - 1.33)	
More than 13hrs.	122 / 14,493	1.24 (0.98 - 1.58)	

[a] Adjusted for age (timescale), sex (women, men), educational level (less than high school degree, <2 years after high school degree, ≥2 years after high school degree), BMI at baseline (continuous, kg/m^2), family history of type 2 diabetes (no, yes), alcohol intake (continuous, g/day), daily energy intake excluding alcohol (continuous, kcal/day), healthy dietary pattern (continuous), western dietary pattern (continuous), smoking (current, former, never), physical activity (low, moderated, elevated), number of dietary records (continuous), number of eating occasions (continuous) and time of first meal (continuous). [b] Early time-restricted eating (eTRE) was defined as a first meal at or before 8AM. Late TRE (lTRE) is defined as a first meal after 8AM. HR= Hazard ratio. Effect modification of the association between nighttime fasting and risk of type 2 diabetes by early (≤8AM) versus late time of first meal (P for interaction <0.001).

5.4. Paper IV

Palomar-Cros A, Srour B, Andreeva V, Fezeu L, Julia C, Bellicha A, Kesse-Guyot E, Hercberg S, Romaguera D, Kogevinas M, Touvier M. Meal timing, number of eating occasions and nighttime fasting duration in relation with cardiovascular disease risk: Results from the Prospective NutriNet-Santé cohort. *Under review in Circulation.*

Supplementary material:
https://drive.google.com/drive/folders/1CyGg6xjA1BJPxeFEAxMR0xf8jt-8uHuv?usp=sharing

Meal timing, number of eating occasions and nighttime fasting duration in relation with cardiovascular disease risk: Results from the Prospective NutriNet-Santé cohort

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ABSTRACT

Background

Daily eating/fasting cycles synchronise circadian peripheral clocks, involved in the regulation of the cardiovascular system. The objective of the present study is to investigate the prospective associations of meal timing, number of eating occasions and nighttime fasting duration with the risk of cardiovascular disease (CVD).

Methods

We used data from 103,389 adults (79% females, mean age at baseline 42.6 (SD=14.5)) in the French NutriNet-Santé study, 2009-2021, free of CVD at baseline. Meal timing and frequency were estimated using data from 24h dietary records during the first two years of follow-up (5.7 records/participant, SD 3.0). We built multivariable Cox proportional hazard models to examine the associations of time of daily first and last meal, number of eating occasions and nighttime fasting duration with the risk of overall CVD, coronary heart disease and cerebrovascular disease. Models were adjusted for potential confounders and circadian nutritional behaviours were mutually adjusted.

Results

During a median follow-up of 7.2 years, 2,036 incident CVDs were diagnosed. We observed a linear association for a first meal later in the day and a higher risk of CVD, Hazard Ratio [HR] per 1-hour

increase = 1.06 (95% confidence interval 1.01 – 1.12, P-value = 0.02). For the last meal of the day, we observed an association with an increased risk of all CVD only in the latest category (after 9PM) compared to a last meal before 8PM, HR= 1.14, (1.00 – 1.30, P-trend= 0.05), and only for cerebrovascular disease, HR = 1.25 (1.03 – 1.52, P-trend = 0.01). These associations were stronger among women than men. Nighttime fasting duration was inversely associated with risk of cerebrovascular disease, HR = 0.93 (0.87 – 0.99, P-value = 0.03). Finally, we found no association between number of daily eating occasions and risk of CVD.

Conclusions

For the first time, this study suggests that late first and last meals as well as reduced nighttime fasting could be associated with a higher risk of cardiovascular outcomes. Potential mechanisms include circadian alterations, lipogenesis, inflammation and impaired glucose homeostasis. If confirmed in other largescale, well conducted, studies, these results hold a substantial potential for CVD prevention.

Trial registration

Clinicaltrials.gov NCT03335644.

Key words: Chrononutrition, meal timing, cardiovascular disease incidence, fasting, eating frequency

INTRODUCTION

Cardiovascular diseases (CVDs) are the leading cause of mortality and disease burden in the world [1]. Diet is a major risk factor for CVD and contributes to 7.94 million CVD-related deaths annually [1]. The accelerated modern lifestyle linked with the perception of lacking time in Western societies, and the current surge in fasting practices promoting meal skipping, has led to mistimed nutritional behaviours, such as late-night eating and breakfast skipping [2]. The daily eating/fasting cycle is a synchroniser of the circadian clock, the later contributing to regulate circadian rhythms in the cardiovascular system such as blood pressure or blood coagulation markers [3]. Chrononutrition has emerged as a new field in nutritional sciences to unravel the relationship between timing of food intake, circadian rhythms and health [4].

Data from observational and clinical studies indicate that breakfast consumption, assessed as the frequency of consumption throughout the week, is an important habit for cardiometabolic health [5] and its omission has been associated in meta-analyses with overweight and obesity [6], risk of CVD [7] and diabetes mellitus [8]. Similarly, late-night eating has been linked in prospective studies to cardiovascular risk factors such as arterial stiffness [9], obesity, dyslipidaemia and, in women only, to metabolic syndrome [10] and also in one prospective study with higher risk of coronary heart disease [11]. However, as stated by the American Heart Association (AHA) an important limitation of these studies is the lack of consensus in defining a meal [5]. Definitions are usually based on the participant identification of a meal (i.e.,

breakfast) and on the time of day (i.e., consider breakfast anything between 6 to 10AM). These approaches are prone to classification bias (i.e., eating at 11AM could be considered for one person as having breakfast or for another one as not having breakfast; the interpretation of eating at/after 10PM could also be misleading depending on the cultural differences). Only two studies of cross-sectional nature have evaluated specifically meal timing, in its continuous form, in relation with cardiovascular risk factors but none with CVD risk [12,13].

A growing body of evidence demonstrates that practicing time-restricted eating (TRE) (i.e., extending the nighttime fasting duration to more than 12 hours) is linked to an improvement of multiple key indicators of cardiovascular health including body weight, blood pressure and inflammation [14]. However, to our knowledge no study so far has investigated the direct association between nighttime fasting duration and CVD risk. Moreover, as stated by the AHA, epidemiological data on number of eating occasions and cardiometabolic risk are scarce and inconclusive and further studies should evaluate this behaviour in the context of meal timing and nighttime fasting duration.

The main objective of the present study was to explore the associations of time of first and last meal of the day, number of eating occasions and nighttime fasting duration, with the risk of CVD, in the prospective NutriNet-Santé cohort.

METHODS

The NutriNet-Santé cohort

The NutriNet-Santé cohort study was launched in France in 2009 to better understand the relationship between nutrition and health. This is an ongoing web-based cohort study that targets volunteers aged 18 or older recruited through various multimedia channels (https://etude-nutrinet-sante.fr/). The protocol of the study has been published previously [15]. All participants are required to provide an electronic informed consent when enrolling the study. The NutriNet-Santé study is conducted according to the Declaration of Helsinki guidelines and is registered at clinicaltrials.gov (NCT03335644). The study was approved by the Institutional Review Board of the French Institute for Health and Medical Research (IRB Inserm n°0000388FWA00005831) and the "Commission Nationale de l'Informatique et des Libertés" (CNIL n°908450/n°909216). Confidentiality and security of the data are assured at all time.

At inclusion, participants are invited to complete a set of questionnaires including data on socio-demographics and lifestyle [16], physical activity (through a validated 7-day assessment, the International Physical Activity questionnaire - short form [IPAQ] [17]), anthropometrics [18,19], health status and diet (details below). These questionnaires are repeated every year during the follow-up. In 2014, a subset of the study population responded to an optional comprehensive sleep questionnaire including questions on bedtime and sleep duration [20].

Dietary assessment

In the NutriNet-Santé cohort study, diet was assessed from baseline with bi-annual series of 3 random records of 24 h food intake during a two-week period, with information on two working days and a non-working day to better capture general behaviours. Participants had to report all foods and beverages consumed during this period and also exactly when during the day they did consume them. In case participants could not provide the specific quantity, they were asked to estimate portion sizes through validated photographs or usual containers [21]. These questionnaires have been validated against both biomarkers in blood and urine samples [22,23] and an interview by a dietician [24]. Baseline dietary intakes were assessed by averaging the consumption over the food records available from the two first years of follow-up, considered as the habitual dietary behaviours, and merged with French food composition database with more than 3,500 items [25]. We estimated mean daily intakes of energy, alcohol, macro- and micronutrients for this period. We used basal metabolic rate and the Goldberg cut-off method [26] to identify and exclude energy under-reporters. Details of this procedure are explained in the Supplementary Material, Method A.

To estimate the time of first and last meal of the day and the number of eating occasions, we computed an average of the food records available from the first two years of follow-up and we included only participants with at least 3 dietary records. An eating occasion was defined as the intake of any food or beverage of at least 1kcal, thus excluding water drinking. We also decided to exclude participants

reporting a first meal after 3PM or a last meal before 3PM since they corresponded to very disrupted circadian behaviours (e.g., night shift workers). Finally, we calculated nighttime fasting duration as the time elapsed between the last meal of the day and the first meal the following day.

Ascertainment of cardiovascular disease events

Major health events, including CVD, were self-reported by the participants through a health questionnaire sent every 6 months, or through a permanently available platform dedicated to health events collection on the NutriNet-Santé website. Additionally, they were asked to confirm the declaration of any health event with medical records including diagnoses, examinations or hospitalisations. Physicians from the research team reviewed the medical records and validated health events and in case of any doubt contacted the physician of the participant. Similarly, participants' families or doctors were contacted if there was no response in the website for more than a year. CVD events were complemented with the linkage to medico-administrative databases of the national health insurance (SNIIRAM) that include information on prescription medication and medical consultation and hospitalization history. This linkage was authorised by the Council of State (No 2013-175). Finally, deaths were assessed with the linkage to the French national cause-specific mortality registry (CépiDC).

We used the International Classification of Diseases-Clinical Modification codes (ICD-CM, 10th revision) to classify CVD events.

In the present study, we considered incident cases of stroke (I64), transient ischemic attack (G45.8, G45.9), myocardial infraction (I21), angina pectoris (I20.1, I20.8, I20.9), acute coronary syndrome (I20.0, I12.4) and angioplasty (Z95.8). We classified stroke and transient ischemic attack as cerebrovascular diseases and myocardial infraction, angina pectoris, acute coronary syndrome and angioplasty as coronary heart diseases.

Statistical analyses

As of October 5th 2021st, 103,389 participants without prevalent CVD at baseline were included in the present analyses. A detailed flowchart is presented in Figure S1. Time of first and last meal of the day and number of eating occasions were considered as continuous (per hour increase and per meal increase, respectively) and categorical variables. We defined the final categories as time of first meal before 8AM, between 8 and 9AM and after 9AM and as time of last meal before 8PM, between 8 and 9PM and after 9PM, as an approximation of the tertiles in the general population. We examined the correlation among these nutritional behaviours with Spearman rank (Figure S2).

We built cause-specific Cox proportional hazards models to estimate hazard ratios (HR) and 95% confidence intervals (CI) for the associations of meal timing and number of eating occasions with risk of developing overall CVD, cerebrovascular diseases and coronary heart disease. Since we aimed to investigate the risk of the first cardiovascular event, we used a competing risks approach: for

instance, in models investigating cerebrovascular diseases, if a coronary heart disease was diagnosed during the follow-up, the participant was censored at their date of coronary heart disease diagnosis and was considered as non-case for cerebrovascular diseases, and the same rationale was applied the other way around. Therefore, participants contributed to person-time until the date of CVD diagnosis, censoring event, last connection or October 5th 2021 whichever occurred first. We confirmed the Cox models' assumption of risk proportionality using Schoenfeld residuals, and the assumption of linearity by introducing spline terms for each of the exposure variables in the models (Figures S3 and S4).

We adjusted our models for age (timescale), sex (women, men), education (no high-school degree, less than 2 years of education after high-school, 2 years of more of high school education), BMI at baseline (continuous, kg/m^2), family history of CVD (yes, no), alcohol consumption (continuous, g/day), energy intake excluding alcohol (continuous, kcal/day), smoking (current, former, never), physical activity level (low, intermediate, high), number of dietary records (continuous), healthy dietary pattern and Western dietary pattern. The two pattern variables were calculated by principal component analysis (PCA) on the basis of 20 predefined food groups (further details are provided in Supplementary Material, Method B). Finally, the time of first daily meal, time of the last daily meal and number of eating occasions were mutually adjusted. All covariates had less than 1% of missing data except for physical activity which had 14%. We did multiple imputation of missing values by chained

equations (MICE) [27] and combined the results from 20 complete datasets using the method proposed by Rubin [28].

We then explored the hypothesis of an extended period of fasting as a protective factor for CVD. Nighttime fasting duration was investigated as a continuous variable in hours and as a categorical variable (12 hrs or less, 12 to 13 hrs or more than 13 hrs). This was categorised on the basis of the eating / fasting schemes most frequently reported in the literature in relation with time-restricted eating [14]. Models were adjusted for the same covariates as indicated above, including time of first meal, except for time of last meal to avoid collinearity.

We investigated the modulating role of time of first meal in the association between nighttime fasting duration and risk of CVD. This was performed by introducing an interaction term between nighttime fasting duration and time of first meal of the day. The significance of the interaction term was tested using a likelihood ratio test.

Sensitivity analyses

In sensitivity analyses, we explored further adjustment of our models for number of pack years, defined as the number of cigarettes smoked per day by the number of years of smoking. We also explored the adjustment of well-known individual nutrients related with CVD risk including daily consumption (g/day) of saturated fatty acids, sodium, sugar, red and processed meat, sugary drinks, fruits and vegetables, nuts, whole grain, yoghurt and ultra-processed foods (daily

proportion in grams/d) [29]. We also considered the French region of residence of the participants (to account for latitude), and profession. We also adjusted for weight change during follow-up which was calculated as the percentage of weight change from baseline to end of study and divided by number of years of follow-up. To challenge our models for reverse causation bias, we excluded cases diagnosed during the first two years of follow-up. We performed further adjustments for prevalent cases of type 1 and 2 diabetes, hypercholesterolemia, hyperglyceridemia and hypertension at baseline. In an additional model, we excluded these prevalent cases and adjusted for incident cases of these diseases (those diagnosed before a CVD), to test for a possible mediation. For a subset of the study population, data from the sleep questionnaire was available. We explored restricting our analyses to this subpopulation and then we also excluded those reporting a bed time between 8AM and 6PM (to exclude potential night shift workers, N=37,536). We then adjusted for bed time and sleep duration (hrs. /24h) and also for a variable related to the individual preference for timing of activities during the day (morning, intermediate, evening), known as chronotype [30]. Lastly, we explored interactions with BMI at baseline, sex, menopausal status among women (N=81,709), smoking status, education and chronotype (among those who responded to the sleep questionnaire).

All tests were two-sided, and we considered $P<0.05$ to be statistically significant. R version 4.1.3 (The R Foundation for Statistical Computing Platform) was used for these analyses.

RESULTS

The present study included a total of 103,389 participants (79% women) with a mean baseline age of 42.6 years (14.5 SD). Participants had completed on average 5.7 dietary records (SD 3.0) with a maximum of 15 records. The main characteristics of the study population and according to the time of first and last meal of the day are shown in Table 1. Overall, younger participants, without a family history of CVD, current smokers, with higher physical activity levels and higher educational levels, tended to have later first and last meals. Additionally, participants having the last meal after 9PM had a higher consumption of alcohol compared to participants having an earlier dinner.

Association of meal timing and number of eating occasions with CVD risk

During a median follow-up time of 7.2 years (1^{st} quartile [Q1] – 3^{rd} quartile [Q3], 3.1-10.1) and 699,547 person-years, 2,036 incident cases of CVD were ascertained. There were 988 cases of cerebrovascular diseases (253 cases of stroke and 765 of transient ischemic attack) and 1,071 cases of coronary heart diseases (162 cases of myocardial infraction, 428 of angioplasty, 89 of acute coronary syndrome and 428 of angina pectoris). Cox models examining the association of meal timing and number of eating occasions with risk of CVD are shown in Table 2. The proportional hazard assumption was met, and there was no evidence for non-linear associations (Figures S3 and S4).

Overall cardiovascular disease

We observed that each additional hour in delaying the time of first meal of the day was associated with an increased risk of all CVD (Table 2, HR = 1.06, 95% CI 1.01 – 1.12, P-value = 0.02). The continuous variable of time of last meal was not significantly associated with CVD risk. However, compared to participants habitually having a last meal before 8PM, those having a last meal of the day after 9PM had a higher risk of all CVD (HR= 1.14, 95%CI 1.00 – 1.30, P-trend = 0.05). The number of eating occasions was not associated with the risk of developing CVD.

Cerebrovascular disease

In this study population, time of first meal of the day was not associated with the risk of developing a cerebrovascular disease during follow-up (Table 2). However, each additional hour in delaying the time of last meal was associated with an increased risk of cerebrovascular disease (HR = 1.07, 95% CI 1.00 – 1.14, P-value = 0.03): more specifically, compared to a last meal before 8PM, a last meal after 9PM was associated with an increased risk of cerebrovascular disease (HR = 1.25, 95% CI 1.03 – 1.52, P-trend = 0.01). No association was detected between daily number of eating occasions and risk of cerebrovascular disease.

Coronary heart disease

We found no association between meal timing nor number of eating occasions and risk of coronary heart disease in this study (Table 2).

Association of nighttime fasting duration with CVD risk

We found an inverse association between nighttime fasting duration and cerebrovascular disease risk (Table 3). Each additional hour of nighttime fasting was associated with a lower risk of cerebrovascular disease (HR = 0.93, 95% CI, 0.87 – 0.99, P-value = 0.03), but not with risk of overall CVD or coronary heart disease. We did not find a statistically significant interaction between nighttime fasting duration and time of first meal of the day for the associations with CVD, cerebrovascular disease nor coronary heart disease (p-values = 0.2, 0.3, 0.5, respectively).

Sensitivity analyses

We found a statistical interaction between sex and time of last meal of the day for the associations with all CVD (p-value = 0.01) and coronary heart disease risk (p-value = 0.004). Similarly, we observed a significant interaction between sex and nighttime fasting duration for the association with coronary heart disease (p-value = 0.02). No other statistically significant interactions were observed. In Table S1 we present the results of the main models considering the interaction with sex with each of the exposure variables. Globally, our results suggest stronger associations in women than in men. Specifically, our results suggest that later times of first and last meals were significantly associated with a higher risk of all CVD and cerebrovascular disease for women but not for men (Table S1). Similarly, a longer nighttime fasting duration appeared to be more

protective for cardiovascular diseases among women than among men.

The associations remained relatively stable across all sensitivity analyses (Table S2). The association between time of first meal of the day and risk of CVD was moderately attenuated and no longer significant after adjusting for incident type 2 diabetes, hypercholesterolemia and hyperglyceridaemia. It was similarly attenuated after considering chronotype in the sample restricted to participants with data on sleep duration. Although in the same direction as in the main model, the link between time of last meal and risk of cerebrovascular disease was attenuated and no longer significant in the reduced sample with participants responding to the sleep questionnaire (Table S2, Model 8), probably due to a substantial loss of statistical power.

DISCUSSION

In this large prospective cohort study, later times of first and last meals of the day were independently associated with a higher risk of CVD. Our findings also suggest an inverse association between nighttime fasting duration and a lower cerebrovascular disease risk. These associations were more evident in women than in men. We observed no link between daily number of eating occasions and risk of CVD.

To the best of our knowledge, no prior longitudinal study has investigated the associations between specific meal timing and incident CVDs, hampering any direct comparison of our results with

the literature. Data from meta-analyses suggest that skipping breakfast can be associated with overweight and obesity [6], T2D [8], coronary artery disease [31], CVD and all-cause mortality [7]. Observational studies have also shown associations with risk of hypertension [32] and dyslipidaemia [33,34], respectively, overall suggesting that the habit of regularly eating breakfast is important in terms of cardiometabolic health. Moreover, associations between habitual late-night eating and progression of arterial stiffness and dyslipidaemia have been suggested [9,10]. Results from the Male Health Professionals Follow-up Study suggested that eating after going to bed, assessed from a baseline questionnaire, was associated with a higher risk of coronary heart disease (Relative Risk = 1.55, 95% CI 1.05-2.29) [11]. However, the assessment of breakfast skipping and late-night eating is prone to classification bias.

In line with our findings, a usual delayed first meal of the day (per 30-min delay and per quartile later in time of first meal) has been associated in two observational studies with cardiometabolic risk factors including a worse cardiovascular health, measured with the American Heart Association score, and increased blood pressure, C-reactive protein concentration, insulin and glucose levels and lower high-density lipoprotein [12,13]. Similarly, a later time of last meal was associated with higher HbA1c [13] all of which are risk factors for CVD. Contrary to our results, these two observational studies reported an association between a longer nighttime fasting duration and higher prevalence of cardiometabolic risk factors [12,13]. However, the time of first meal was not considered in those studies and these results are also in disagreement with the broader body of literature on

TRE and a healthy cardiometabolic profile [14]. In line with our findings, in the Males Health Professionals Follow-up Study (HPFS), the authors did not observe an association between meal frequency and risk of coronary heart disease [11].

Nutritional behaviours are one of the main modifiable risk factors contributing to the global burden of CVD [29]. It is becoming more evident that the optimal metabolism of food is time-of-day-dependent [35]. Food is a well-known synchroniser of peripheral clocks in the circadian system and eating late-at-night can disrupt this system and lead to metabolic disturbances [36]. Sensitivity to insulin and to elevated glucose concentration are greatest in the early morning and decline over the day, showing that metabolism is prepared to anticipate and digest energy sources at specific times of the day [37].

In animal models, delaying the first meal of the day by 4 hours increased body weight, hepatic lipids and adipose tissue weight and delayed circadian oscillation of genes related with lipid metabolism after two weeks and without changes in food intake [38,39]. Mimicking late-night eating in mice has been also associated with phase alterations in peripheral clocks, weight gain, hepatic lipid accumulation, inflammation and microbial dysbiosis [40–42]. Evidence from RCT suggests that a later evening meal can lead to glucose intolerance, insulin resistance, increased cholesterol and triglyceride levels and BMI [43,44]. Food intake when melatonin levels are high, during the rest phase, could lead to this glucose intolerance and hyperglycaemia [45,46]. In line with this, time-restricted feeding, or the extension of the nighttime fasting duration, has been associated with

several metabolic benefits including mobilization of fat through fatty acid oxidation, reduction of the blood pressure and reduced oxidative stress [14].

Other hypotheses could also explain our findings. First, night shift work has been associated with increased risk of cerebrovascular diseases; therefore, it is possible that the association is confounded by night shift and/or sleep disturbances [47,48]. However, in our study population, we excluded participants reporting extremely disrupted circadian nutritional behaviours (first meal after 3PM or last meal before 3PM), assuming these would correspond to individuals with severe circadian disruption, which is the case of night shift workers. We excluded this subpopulation because the sample size was too limited to perform specific analyses. We observed a minor attenuation after adjusting for chronotype, which might partly explain our results. Similarly, when adjusting for incident cases of type 2 diabetes, hypercholesterolemia and hypertriglyceridemia, diagnosed before CVD, the estimate for this association included the null, suggesting a potential mediation by these cardiometabolic alterations. Then, skipping breakfast has been linked to a lower nutritional adequacy to dietary guidelines [5], but we controlled the present analyses for total daily energy intake, a healthy diet pattern and also for other nutritional indicators in sensitivity analyses such as consumption of ultra-processed foods.

In our study, the association between late first and last meals and CVD risk was more evident in women than in men. These differences could be partly explained by the higher proportion of women in our

cohort. However, these differences could also be explained by sexual dimorphisms in the anatomy and physiology of the circadian system [49–51]. Furthermore, this is similar to what has been reported in terms of circadian disruption due to night shift work and cardiovascular diseases [52]. In line with these results, late-night eating has been associated with arterial stiffness [9] and metabolic syndrome in women but not in men [10].

The sample size, prospective design and detailed assessment of circadian nutritional behaviours constitute the main strengths of this study. These behaviours were measured using dietary records, which are less subject to recall and misclassification bias than are dietary recalls or ad-hoc questionnaires. The large panel of questionnaires in the NutriNet-Santé cohort enabled us to control for a large number of well-measured potential confounders, reducing the risk of confounding in the present analyses. However, given the observational nature of this study residual confounding cannot be completely ruled out. Other limitations of the present investigation have to be discussed.

First, participants in the NutriNet-Santé cohort are volunteers and are more likely to be women, have a higher socioeconomic status and healthier behaviour patterns than the general population, somehow limiting the extrapolation of these results [53]. Moreover, the healthier behaviours in the study population could have led to a lower incidence of CVD compared to the general population and to an underestimation of the studied associations [46]. Next, in the present analyses, sleep time and duration were assessed with an optional

questionnaire during follow-up, and therefore could not be used in the main analyses: this sub analysis might have been subject to selection bias and loss of statistical power. Furthermore, night shift work was approximated from the sleep times reported in this follow-up questionnaire, since information on current and history of shift work were not available in the cohort. Similarly, we had no information on exposure to light-at-night which is an important disruptor of circadian rhythms. Last, nutritional behaviours were averaged from all available dietary records which included data on working and non-working days. This omitted the variability in circadian nutritional behaviours between working and non-working days, a concept that has been previously named as eating jet lag [54]. Further studies are required to explore whether this variability in timing could also be associated with CVD.

To conclude, in this large prospective study, later times of first and last meals of the day as well as reduced nighttime duration were associated with a higher risk of CVD. This work, which needs replication in other large-scale cohorts in different settings, supports an important role of adapting a daytime circadian nutritional lifestyle, consistently with previous experimental and observational studies. These findings suggest that, beyond the nutritional quality of the diet itself, recommendations related to meal timing for patients and citizens may help promoting a better cardiometabolic health.

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CONTRIBUTORSHIP STATEMENT AND GUARANTOR

The authors' contributions were as follows – AP-C, BS and MT: designed the research; VAA, LF, CJ, AB, EKG, SH and MT: collected the data; AP-C: performed statistical analyses; BS: supervised statistical analyses; AP-C: drafted the manuscript; BS and MT: supervised the writing; DR and MK participated to the supervision of the writing; all authors contributed to data interpretation, revised each draft for important intellectual content, read and approved the final manuscript. AP-C, BS and MT had full access to all the data in the study, MT takes responsibility for the integrity of the data and the accuracy of the data analysis, she is the guarantor. The corresponding author (BS) attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

ADDITIONAL INFORMATION

NutriNet-Santé is conducted according to the Declaration of Helsinki guidelines and was approved by the Institutional review board of the French Institute for Health and Medical Research (IRB Inserm n 0000388FWA00005831) and the Commission Nationale de l'Informatique et des Libertés (CNIL n 908450/n 909216).

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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Table 1. Baseline characteristics of the included participants from the NutriNet-Santé cohort, 2009-2021, N=103,389							
	All participants	**Time of first meal**			**Time of last meal**		
		Before 8AM	**8AM to 9AM**	**After 9AM**	**Before 8PM**	**8PM to 9PM**	**After 9PM**
	N = 103,389 N (%) or mean (SD)	**N = 46,306 N (%) or mean (SD)**	**N = 36,981 N (%) or mean (SD)**	**N = 20,102 N (%) or mean (SD)**	**N = 34,723 N (%) or mean (SD)**	**N = 45,610 N (%) or mean (SD)**	**N = 23,056 N (%) or mean (SD)**
Age at baseline	42.6 (14.5)	46.5 (13.1)	42.5 (14.8)	33.9 (13.3)	45.7 (14.7)	41.7 (14.1)	39.8 (14.1)
Sex							
Women	81,709 (79.0%)	35,699 (77.1%)	29,817 (80.6%)	16193 (80.6%)	27,284 (78.6%)	36,826 (80.7%)	17,599 (76.3%)
Men	21,680 (21.0%)	10,607 (22.9%)	7,164 (19.4%)	3,909 (19.4%)	7,439 (21.4%)	8,784 (19.3%)	5,457 (23.7%)
BMI (kg/m^2)	23.8 (4.5)	23.9 (4.43)	23.7 (4.42)	23.5 (4.74)	24.0 (4.49)	23.6 (4.41)	23.7 (4.62)
Family history of CVD							
No	70,649 (69.4%)	30,447 (65.8%)	25,804 (69.8%)	15,967 (79.4%)	23,272 (67.0%)	32,277 (70.8%)	16,669 (72.3%)
Yes	31,171 (30.6%)	15,859 (34.2%)	11,177 (30.2%)	4,135 (20.6%)	11,451 (33.0%)	13,333 (29.2%)	6,387 (27.7%)
Smoking status							
Current	14,771 (14.3%)	5,314 (11.5%)	5,054 (13.7%)	4,403 (21.9%)	3,607 (10.4%)	6,230 (13.7%)	4,934 (21.4%)
Former	41,737 (40.4%)	20,203 (43.6%)	15,014 (40.6%)	6,520 (32.4%)	14,410 (41.5%)	18,505 (40.6%)	8,822 (38.3%)
Never	46,811 (45.3%)	20,789 (44.9%)	16,913 (45.7%)	9,179 (45.7%)	16,706 (48.1%)	20,875 (45.8%)	9,300 (40.3%)
Physical activity							
Low	29,164 (32.7%)	14,762 (31.9%)	9,876 (26.7%)	4,526 (22.5%)	10,470 (30.2%)	12,341 (27.1%)	6,353 (27.6%)
Moderate	21,674 (24.3%)	22,637 (48.9%)	19,224 (52.0%)	10,690 (53.2%)	17,155 (49.4%)	23,573 (51.7%)	11,823 (51.3%)
High	38,334 (43.0%)	8,907 (19.2%)	7,881 (21.3%)	4,886 (24.3%)	7,098 (20.4%)	9,696 (21.3%)	4,880 (21.2%)
Daily alcohol intake (g)	7.8 (11.9)	7.61 (11.4)	8.08 (11.7)	7.94 (13.1)	6.49 (10.5)	7.98 (11.5)	9.60 (14.1)
Daily energy intake (kcal)	1,847 (451)	1,856 (448)	1,857 (441)	1,810 (475)	1,789 (434)	1,852 (437)	1,926 (490)
Higher education							
No	17,868 (17.3%)	9,476 (20.5%)	5,851 (15.8%)	2,541 (12.7%)	8,042 (23.2%)	6,669 (14.6%)	3,157 (13.7%)
Yes, <2 y after high-school	16,318 (15.8%)	7,166 (15.5%)	5,451 (14.7%)	3,701 (18.4%)	6,054 (17.4%)	6,918 (15.2%)	3,346 (14.5%)
Yes, ≥2 y after high-school	69,133 (66.9%)	29,635 (64.0%)	25,659 (69.4%)	13,839 (68.9%)	20,604 (59.4%)	31,992 (70.2%)	16,537 (71.8%)
Time of first meal (AM)	8:14 (1.1)	7:22 (0.48)	8:24 (1.63)	9:57 (1.02)	7:56 (1.01)	8:14 (1.02)	8:42 (1.32)
Time of last meal (PM)	8:24 (1.1)	8:06 (1.02)	8:14 (1.00)	8:48 (1.28)	7:18 (0.74)	8:24 (0.28)	9:48 (0.90)
Nighttime fasting hours	11.9 (1.4)	11.2 (1.09)	12.0 (1.01)	13.2 (1.51)	12.6 (1.26)	11.8 (1.02)	10.9 (1.46)

Number of eating occasions / day	4.89 (1.7)	5.00 (1.78)	4.89 (1.63)	4.63 (1.71)	4.41 (1.40)	4.82 (1.55)	5.73 (2.14)

BMI= Body mass index; CVD = Cardiovascular diseases; N= sample size; SD= Standard deviation.

Table 2. Association of meal timing and number of eating occasions with risk of cardiovascular diseases in the NutriNet-santé cohort, 2009-2021, N=103,389			
	N cases / non-cases	**HR (95% CI) [1]**	**p-val[2]**
Overall cardiovascular diseases			
Time of first meal *(1h incr.)*	2,036 / 101,353	1.06 (1.01 – 1.12)	0.02
Before 8AM	1,040 / 45,266	Ref	0.06
Between 8 and 9AM	764 / 36,217	1.06 (0.96 – 1.17)	
After 9AM	232 / 19,870	1.15 (0.98 – 1.33)	
Time of last meal *(1h incr.)*	2,036 / 101,353	1.02 (0.98 – 1.07)	0.3
Before 8PM	786 / 33,937	Ref	0.05
Between 8 and 9PM	844 / 44,766	1.07 (0.97 – 1.18)	
After 9PM	406 / 22,650	1.14 (1.00 – 1.30)	
Number of eating occasions *(1 occasion incr.)*	2,036 / 101,353	0.99 (0.96 – 1.02)	0.6
Cerebrovascular diseases‡			
Time of first meal *(1h incr.)*	988 / 102,401	1.06 (0.98 – 1.14)	0.1
Before 8AM	508 / 45,798	Ref	0.1
Between 8 and 9AM	361 / 36,620	1.02 (0.89 – 1.17)	
After 9AM	119 / 19,983	1.22 (0.98 – 1.51)	
Time of last meal *(1h incr.)*	988 / 102,401	1.07 (1.00 – 1.14)	0.03
Before 8PM	363 / 34,360	Ref	0.01
Between 8 and 9PM	426 / 45,184	1.18 (1.02 – 1.36)	
After 9PM	199 / 22,857	1.25 (1.03 – 1.52)	
Number of eating occasions *(1 occasion incr.)*	988 / 102,401	0.97 (0.93 – 1.01)	0.1
Coronary heart diseases §			
Time of first meal *(1h incr.)*	1,071 / 102,318	1.05 (0.98 – 1.13)	0.1
Before 8AM	550 / 45,756	Ref	0.3
Between 8 and 9AM	406 / 36,575	1.08 (0.95 – 1.23)	
After 9AM	115 / 19,987	1.06 (0.85 – 1.32)	
Time of last meal *(1h incr.)*	1,071 / 102,318	0.99 (0.93 – 1.05)	0.7
Before 8PM	432 / 34,291	Ref	0.8
Between 8 and 9PM	429 / 45,181	0.99 (0.86 – 1.13)	
After 9PM	210 / 22,846	1.03 (0.86 – 1.24)	
Number of eating occasions *(1 occasion incr.)*	1,071 / 102,318	1.02 (0.98 – 1.06)	0.4

HR= Hazard ratio; N= Sample size; CI= Confidence Interval.

‡ Stroke and transient ischemic attack. § Myocardial infarction, acute coronary syndrome, angioplasty and angina pectoris.

1. Multivariable Cox proportional hazard models adjusted for age (timescale), sex (women, men), educational level (less than high school degree, <2 years after high

school degree, ≥2 years after high school degree), BMI at baseline (continuous, kg/m2), family history of CVDs (no, yes), alcohol intake (continuous, kcal/day), daily energy intake excluding alcohol (continuous, kcal/day), healthy and Western dietary patterns derived by factorial analysis (continuous), smoking (current, former, never), physical activity (low, moderate, high) and number of dietary records (continuous). Time of first and last meal and number of eating occasions were mutually adjusted.

2. P-values for continuous variables and p-value for trend for categorical variables.

Table 3. Association between daily nighttime fasting duration and risk of cardiovascular diseases in the NutriNet-Santé cohort, 2009-2021, N=103,389			
	N cases / non-cases	**HR (95% CI) [1]**	**p-val[2]**
Overall cardiovascular diseases			
Continuous (1h incr.)	2,036 / 101,353	0.97 (0.93 – 1.02)	0.3
12hrs. or less	1,207 / 56,813	Ref	0.4
12hrs. to 13hrs.	558 / 28,380	0.91 (0.82 – 1.02)	
More than 13hrs.	271 / 16160	0.98 (0.84 – 1.15)	
Cerebrovascular diseases‡			
Continuous (1h incr.)	988 / 102,401	0.93 (0.87 – 0.99)	0.03
12hrs. or less	613 / 57,407	Ref	0.01
12hrs. to 13hrs.	254 / 28,684	0.79 (0.67 – 0.92)	
More than 13hrs.	121 / 16,310	0.81 (0.64 – 1.02)	
Coronary heart diseases §			
Continuous (1h incr.)	1,071 / 102,318	1.01 (0.95 – 1.08)	0.7
12hrs. or less	613 / 57,407	Ref	0.5
12hrs. to 13hrs.	307 / 28,631	1.04 (0.89 – 1.21)	
More than 13hrs.	151 / 16,280	1.16 (0.94 – 1.43)	

HR= Hazard ratio; N= Sample size; CI= Confidence Interval.

‡ Stroke and transient ischemic attack. § Myocardial infarction, acute coronary syndrome, angioplasty and angina pectoris.

1. Multivariable Cox proportional hazard models adjusted for age (timescale), sex (women, men), educational level (less than high school degree, <2 years after high school degree, ≥2 years after high school degree), BMI at baseline (continuous, kg/m2), family history of CVDs (no, yes), alcohol intake (continuous, kcal/day), daily energy intake excluding alcohol (continuous, kcal/day), healthy and Western dietary patterns derived by factorial analysis (continuous), smoking (current, former, never), physical activity (low, moderate, high), number of dietary records (continuous), number of eating occasions (continuous) and time of first meal.

2. P-values for continuous variables and p-value for trend for categorical variables.

5.5. Paper V

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Exposure to residential artificial light-at-night and cardiometabolic disease risk: an urban perspective from the Catalan GCAT cohort study

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ABSTRACT

Background

Experimental evidence indicates that exposure to artificial light-at-night (ALAN) may lead to metabolic disturbances through circadian misalignment. There is limited evidence on the association between residential ALAN exposure and the risk of cardiometabolic diseases.

Objective

To investigate whether exposure to residential ALAN is associated with an increased risk of cardiometabolic diseases through alterations in clinical markers.

Methods

We used data from 9,752 participants (59% women) in the Genomes for Life (GCAT) cohort study in Barcelona. Residential ALAN was assessed from images taken by the International Space Station with a 30m resolution. We estimated the visually relevant photopic illuminance and circadian-regulation relevant melanopic equivalent daylight (D65) illuminance, in lux. We examined cross-sectional associations between ALAN exposure and clinical markers of cardiometabolic disease (body-mass index [BMI], waist-to-hip ratio [WHR], blood pressure, and glycated haemoglobin [HbA1c]) and prevalent diseases (general and abdominal obesity, hypertension, and diabetes). We prospectively assessed incident cardiometabolic

diseases ascertained through electronic health records during a mean follow-up time of 2.5 years (SD 0.97). We adjusted our main models for individual demographic characteristics and further adjusted for urban exposures.

Results

In cross-sectional analyses, we found an association between photopic and melanopic illuminances and hypertension, OR = 1.09 (1.01-1.16) and 1.08 (1.01-1.14) per interquartile range increase (0.59 lux and 0.16 lux, respectively); associations remained stable across further adjustments. Photopic, but not melanopic, illuminance was linked with prevalent general obesity, OR = 1.06 (1.00 – 1.12), nevertheless, this association was unstable after considering other factors such as socioeconomic status or sleep duration. In prospective analyses, photopic and melanopic illuminances were associated with incidence of hypercholesterolemia OR = 1.15 (1.04-1.27) and OR = 1.10 (1.01-1.20), respectively, and associations remained stable after adjusting for other urban exposures. We did not observe an association between ALAN and other incident cardiometabolic outcomes.

Discussion

The present study suggests an association between photopic and melanopic illuminance at night and prevalent hypertension and risk of hypercholesterolemia, key risk factors in the development of cardiometabolic diseases. Results should be interpreted carefully

since satellite-based ALAN assessment, even in high resolution, estimates residential exposure but not the total individual exposure.

Keywords

Light-at-night, circadian misalignment, hypercholesterolemia, hypertension, cardiovascular, obesity

BACKGROUND

Industrialisation has drastically changed light patterns resulting in brighter nights and affecting the daily light/dark cycle that is the main synchronizer of endogenous circadian rhythms. This system regulates physiological functions in almost all tissues including metabolic and redox homeostasis, inflammatory response and DNA damage repair and response [1]. Non-image-forming responses to light originate in the intrinsically-photosensitive retinal ganglion cell (ipRGC) in the retina [2]. In the ipRGC, the protein sensing light is melanopsin and has a relative spectral sensitivity in shorter wavelengths than cones and rods, photoreceptors implied in the visual responses to light [2,3]. Disruption of the normal light/dark cycle leads to a misalignment of the endogenous circadian oscillator to external stimuli and, results in physiologic dysregulation [4].

Given its implication in a wide range of physiological processes, circadian disturbances resulting from ALAN exposure may contribute to the development and progression of several diseases. In animal models, exposure to ALAN has been shown to disturb the control of blood pressure [5] and has been linked with glucose intolerance and decreased plasma insulin [6,7]. In humans, in a cross-sectional study, exposure to higher bedroom light intensity was associated with higher systolic and diastolic blood pressure [8]. Bedroom light intensity has been also associated with hyperlipidaemia and incident type 2 diabetes [9,10]. Furthermore, results from a meta-analysis from observational studies showed that exposure to ALAN is significantly associated with being overweight

(OR=1.13, 95% CI 1.10-1.16) and obese (OR: 1.22, 95% CI 1.07-1.38) [11]. Recently, a prospective study linked residential illumination, assessed from satellite data, with risk of coronary heart disease (CHD) hospitalisations and deaths [12], showing an additive interaction between light and air pollution on CHD mortality.

As light pollution levels increase especially in dense urban settings, the examination of ALAN and subsequent impacts on cardiometabolic disorders is important. Images from satellites serve as a good tool to assess exposure to ALAN and have been used to examine the association with health outcomes [11,12]. However, studies applying this method tend to asses photic luminance, with a different spectral sensitivity than melanopsin which is the main contributor to non-image-forming effects [2]. Therefore, they might not be presenting the appropriate measurement of light effects on circadian regulation. Furthermore, other urban exposures such as air pollution, noise pollution or green spaces have not been usually considered in studies investigating the impact of ALAN.

The objective of the present study is to examine whether exposure to residential ALAN is associated with clinical markers of cardiometabolic diseases and the risk of developing these diseases. In these analyses, ALAN exposure was assessed with two measures, the visually relevant photopic illuminance and the metabolic and circadian-regulation relevant melanopic equivalent daylight (D65) illuminance, measured in lux (lx). In addition, we explored whether these associations were maintained independently of the effect of

other urban exposures including exposure to air pollution, green spaces and noise.

METHODS

The GCAT cohort

The Genomes for Life (GCAT) Study (http://www.gcatbiobank.org/) is a prospective study established in 2014 in Catalonia to study determinants of non-communicable diseases in this population [13]. Participation in the study was open to everyone, but to enhance enrolment, individuals in the Blood and Tissue Bank (BST), a public agency of the Catalan Department of Health, were invited to participate. The invitations were done through the GCAT website, phone call, mail or in person. To be enrolled in the study, individuals were required to understand Spanish or Catalan (the official languages in Catalonia), to currently reside in Catalonia and plan to remain in Catalonia during the following 5 years, to have an Individual Health System Identification Card and to provide written informed consent. Participants are free to withdraw this consent at any time during follow-up.

From the total GCAT population (19,325) we excluded individuals without anthropometric measurements at baseline (N=121) and those with no data on exposure to ALAN because outside the wider metropolitan Barcelona area (N=8,965) (Figure 2).

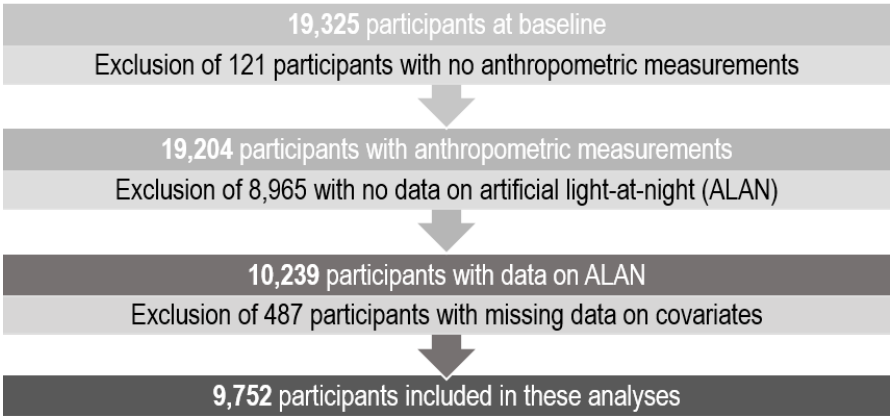


Figure 2. Flow chart of study population of the GCAT study. Final N=9,752.

Data collection

Epidemiological questionnaires

Trained healthcare professionals (doctors and nurses) performed baseline interviews with specific guidelines to assure uniformity of data collection [13]. The computer-based questionnaire included data on sociodemographic characteristics, occupation, alcohol and tobacco consumption, sleep duration, personal and familiar medical history and current address. The questionnaire included also a Physical Activity Questionnaire from the European Prospective Investigation into Cancer and Nutrition (EPIC) referring to activity over the year before recruitment [14]. Metabolic equivalent minutes per week (METs) were used to assess intensity [15]. It included activity during leisure time, as well as occupational and housework activity and then categorised total activity into four levels (inactive, moderately inactive, moderately active and active) [16]. Diet quality

was assessed through the validated 14-item Mediterranean Diet Adherence Screener (MEDAS) [17].

An ongoing biannual active follow-up is done through an electronic web-based questionnaire [13]. The questionnaire was sent, between 2018 and 2020, via email to the participants to update baseline information on lifestyle, among others and to capture health changes. It contained additional information on night shift history and on exposure to bedroom light while sleeping. To define bedroom light while sleeping, participants were asked to respond on a four-digit Likert scale: a) total darkness, b) almost dark, c) dim light, and d) quite illuminated.

Anthropometric measurements and blood sampling

At inclusion, participants underwent blood pressure (systolic and diastolic), cardiac frequency and anthropometry measurements (weight, height, waist [WC] and hip circumference [HC]) [13]. A digital automatic blood pressure monitor was used to measure blood pressure. The anthropometric measurements were taken by trained personnel. Weight was measured with electronic flat scales, height with a stable stadiometer and body circumference with a measuring tape [13]. Participants also donated a blood sample at baseline. HbA1c was measured using 1 μL of the EDTA-tube blood, with the DCA Vantage Analyzer from Siemens [18].

Assessment of exposure to ALAN

To evaluate the exposure to ALAN, we used the database of nighttime images collected by the Crew Earth Observation program of the International Space Station (ISS) [19]. We based our analyses on an image of Barcelona taken on the 18th of April, 2013 at 22:10:46 GMT (ISS035E023385, Figure 1), downloaded from the Earth Science and Remote Sensing Unit, NASA Johnson Space Centre (https://eol.jsc.nasa.gov). Images were available for 2012-2020, but to assess ALAN exposure prior to the assessment of outcomes in our study, we selected the highest resolution photo taken before 2014. The selected image has a pixel resolution of about 30m. These images were taken with digital Single-Lens Reflex (DSLR) cameras and provided information on the radiances in the red, green, and blue (RGB) spectral bands, Figure S.1, in the visual region of the optical spectrum [20].

These R, G, and B spectral radiances were subsequently corrected from the attenuation experienced by the light beams along their atmospheric path from the city ground level to the ISS. The precise length of this path was determined for each individual pixel taking into account the altitude of the ISS (operating in a low Earth orbit, LEO, at altitude ~400 km above mean sea level) and the nadir angle of the pixel as seen from the ISS at the moment the images were taken.

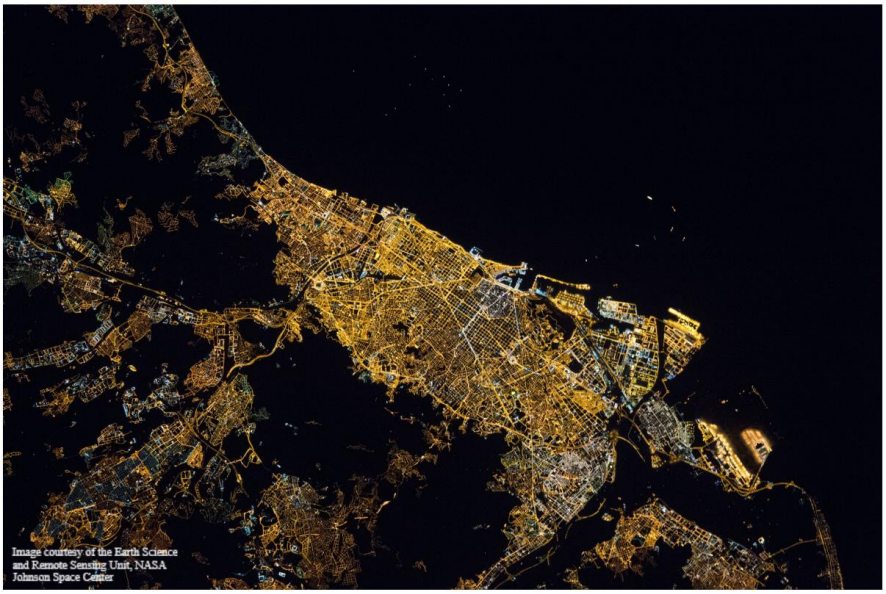


Figure 1. Night image of Barcelona taken from the International Space Station on the 18th of April 2013 at 22:10:46 GMT (ISS035-E-23385, NASA Johnson Space Centre; https://eol.jsc.nasa.gov)

From the calibrated R, G, and B image radiances in units nW·cm–2·sr–1·Å–1 [21] we calculated the raw spectral radiances in two physiologically relevant sensitivity bands, using the regressions described in [22,23].

Firstly, we estimated the spectral radiance L_(V,λ) in the visual photopic band. This band is defined by the photopic luminous efficiency function, V(λ) [24], which describes the spectral sensitivity of the human visual system in foveal vision related to the perception of brightness (luminance). Its shape is basically determined by the spectral responses of the foveal M- and L-cones. On the other hand, melanopsin is the main light-detecting

photopigment to determine the output of the intrinsically photosensitive retinal ganglion cells (ipRGC) [25], the main photoreceptors involved in the photic entrainment of the central circadian clock. Therefore, we also estimated the melanopic spectral radiance, L_(mel,λ), the radiance within the melanopic band as defined in the CIE standard S 026 [3,26–28].

The output of this procedure provided two rasters with the values of the upwelling photopic and melanopic spectral radiances, respectively, in units $nW \cdot cm^{-2} \cdot sr^{-1} \cdot Å^{-1}$, spatially averaged within each ground pixel and spectrally averaged within each spectral band. From these radiances, we subsequently estimated the values of the horizontal illuminance E_V and of the melanopic equivalent daylight (D65) illuminance, E_(V,mel)^D65, both measured in lx [3] incident on the ground surfaces of every pixel of the map (see Supplemental material, Methods S1 for details), which are a measure of the environmental exposure to residential light in those areas.

Finally, we assigned the E_V and E_(V,mel)^D65 ALAN residential exposures to the reported address of each participant in the baseline questionnaire. We did this process using the Geographic Information System (GIS), QGIS (QGIS Development Team 2015). Further details on image processing and data reduction procedures are given in the Supplemental material.

Assessment of other urban exposures

In addition to ALAN, we considered other urban exposures in these analyses, including greenness, air pollution, noise at night, and population density. We used the averaged Normalized Difference Vegetation Index (NDVI) to assess greenness in 2015 with a resolution of 250m [29]. The units of this variable ranged from -1 to 1. As a measure of air pollution, we included the annual average concentration of PM 2.5 ($\mu g/m^3$) with information recorded in 2010 and a resolution of 100m. This exposure was assessed with models from the Effect of Low-Level Air Pollution: A Study in Europe (ELAPSE) project (http://www.elapseproject.eu/). The development and validation of these models have been explained in detail elsewhere [30]. Nightly road noise (dB) for 2012 was assessed at a street-level using noise maps from the Departament de Territori i Sostenibilitat. Finally, we estimated the total population density using data from the Instituto Nacional de Estadística (INE) Census of 2011 [31]. We also used the GIS system to link the different urban exposures to the geocoded residence of each participant.

Electronic health records

The GCAT study collaborates with the Catalan Health Department to link self-reported information from participants with electronic health records, EHR [32]. In the present study, we used a first set of records that was extracted up to 2017. In these records, diseases are classified according to the International Classification of Diseases Ninth Revision. We included diabetes mellitus (T1, T2, not specified and prediabetes: 250, 250.01, 250.02, 250.00, 250.0, 79029), angina

pectoris (413.9, 413.0, 413.1, 4111), myocardial infraction (410, 410.10, 410.11, 410.41, 410.42, 410.51, 410.81, 410.90, 410.91, 410.4, 410.3, 410.71), stroke (434.01, 434.11, 434.10, 434.90, 434.91, 431, 430, 4321, 433.91, 433.90, 433.30, 433.21, 433.11, 433.10, 433.00, 435.0, 435.1, 435.3, 435.8, 435.9, ,436,437.0, 437.1, 437.2, 437.3, 437.8, 437.9, 438.0, 438.11, 438.21, 438.6, 438.7, 438.82, 438.85, 438.89), hypertension (401, 401.0, 401.1, 401.9) and hypercholesterolemia (2720).

Statistical analyses

Exposure to ALAN and clinical markers of cardiometabolic diseases

We conducted a first set of cross-sectional analyses to examine the associations between exposure to ALAN and clinical markers of cardiometabolic diseases. Firstly, we built linear regression models for both ALAN indicators (photopic and melanopic illuminance) and clinical markers of cardiometabolic diseases measured at baseline (BMI, WHR, systolic blood pressure, diastolic blood pressure and HbA1c). Then, we built logistic regression models for the binary outcomes of these cardiometabolic diseases resulting from the investigated clinical markers (general obesity from BMI, abdominal obesity from WHR, hypertension from blood pressure measurements, and diabetes from HbA1c). BMI (kg/m^2) and WHR were calculated and categorised according to WHO guidelines into obese and non-obese [33]. For BMI > 30 kg/m^2 and for WHR males $\geq$ 1.0 and females $\geq$ 0.85. Hypertension was defined as a systolic blood pressure $\geq$ 140 mmHg or a diastolic blood pressure $\geq$ 90 mmHg. Diabetes was defined as an HbA1c of 6.5% or above [34].

These models were calculated per interquartile range increase (IQR) of photopic and melanopic exposure (0.59 and 0.16, respectively).

In a first level of adjustment, we included in our models age (continuous, years) and sex (women, men). Then in a second model, which was considered as the main model, we further adjusted for education (primary education or less, secondary education, university), adherence to Mediterranean diet through the MEDAS screening (low, medium, high), smoking (never, past, current smoker), alcohol intake (never, past drinker, low, moderate, high consumption) and physical activity (continuous, weekly METs). All these covariates had less than 5% of missing data and, therefore, we decided to conduct a complete case analysis. Finally, in a third level of adjustment we additionally included averaged NDVI index (continuous, -1 to 1), annual average concentration of pm2.5 (continuous, $\mu g/m^3$), total population from 2011 (continuous), and nightly road noise (continuous, dB).

We explored the linearity of the associations by building generalised additive models (Figure S3). To examine departure from linearity we compared the model with the spline term to a model without spline in an ANOVA test. We included the ALAN indicators as continuous variables and presented estimates per lux increase. We also explored the statistical interaction between both light measurements and each of the clinical markers by sex. We did this by introducing an interaction term in the models and using a likelihood ratio test to check whether the interaction was statistically significant.

Exposure to ALAN and incident cardiometabolic diseases

We investigated associations with incident cardiometabolic diseases ascertained through electronic health records. We did not have the specific time of diagnosis and therefore we could not build Cox proportional hazard models nor calculate the specific time of follow-up. The median time between the baseline questionnaire and the follow-up questionnaire were 2.5 years (SD, 0.96). We built logistic regression models to examine the association between ALAN indicators and incident cases of diabetes mellitus, hypertension and hypercholesterolemia and cardiovascular diseases (angina pectoris, myocardial infraction and stroke). For each of these models we excluded prevalent cases of the investigated disease: diabetes mellitus (N=135), hypertension (N=861), hypercholesterolemia (N=1183) and cardiovascular diseases (N=25). These prevalent cases were self-reported in the baseline questionnaire. We then estimated the odds ratio (OR) and 95% confidence intervals (CI). We examined models with the same levels of adjustment as stated above.

Secondary analyses

In secondary analyses, we examined the correlation between ALAN indicators with other urban exposures. In sensitivity analyses, we examined adjustment for information on self-reported income (lowest, medium, highest income) and sleep duration at baseline (continuous, hours). The latter variable was calculated as the difference between the wake-up time and bedtime. We selected information corresponding to weekdays because it is more representative of general habits. To consider residential mobility, we

also built a model excluding those participants that reported living in the reported address for less than 5 years (N=377). We had information on night shift work from the follow-up questionnaire and used this question to examine the exclusion of ever night shift workers (N=316). We also investigated the agreement between residential ALAN indicators and bedroom ALAN self-reported in the follow-up questionnaire. To do this, we explored the distribution of continuous residential ALAN indicators (photopic and melanopic illuminance) across levels of bedroom ALAN exposure.

RESULTS

Study population

9.752 participants (59% women) were included in this study. The main characteristics of the study population are shown in Table 1. Participants had a mean age of 56.0 ± 7.21 years and a mean BMI of 27.51 kg/m^2 (SD, 4.7). There were fewer university-educated participants in the tertile with the highest exposure to photopic illuminance. The mean sleep duration in the study population was 7.39 ± 1.35 hours, and was similar across tertiles of exposure to photopic illuminance.

Table 1. Characteristics of study population, 9,752 individuals from the GCAT cohort.

	Total population (N=9,752) N (%), mean (SD)	**Illuminance 1st tertile[1] (N= 3,252) N (%), mean (SD)**	**Illuminance 2nd tertile (N=3,255) N (%), mean (SD)**	**Illuminance 3rd tertile (N=3,245) N (%), mean (SD)**
Age	56.0 (7.21)	56.1 (7.2)	56.0 (7.1)	55.9 (7.3)
Sex				
Women	5769 (59.2)	1935 (59.5)	1917 (58.9)	1917 (59.1)
Men	3983 (40.8)	1317 (40.5)	1338 (41.1)	1328 (40.9)
BMI (kg/m^2)	27.51 (4.66)	27.34 (4.62)	27.54 (4.72)	27.66 (4.64)
Education				
Primary education or less	1048 (10.7)	291 (9.0)	343 (10.5)	315 (12.7)
Secondary education	3462 (35.5)	1078 (33.1)	1168 (35.9)	1216 (37.5)
University	5242 (53.8)	1883 (57.9)	1745 (53.6)	1614 (49.7)
Smoking				
Never	3781 (38.8)	1251 (38.5)	1251 (38.4)	1279 (39.4)
Past smoker	3962 (40.6)	1358 (41.8)	1308 (40.2)	1296 (39.9)
Current smoker	2009 (20.6)	643 (19.8)	696 (21.4)	670 (20.6)
Daily alcohol consumption				
None	775 (7.95)	245 (7.5)	238 (7.3)	292 (9.0)
Past drinker	1374 (14.1)	429 (13.2)	459 (14.1)	486 (15.0)

Low (0-5g/day)	4054 (41.6)	1364 (41.9)	1364 (41.9)	1326 (40.9)
Moderate (5-30g/day)	3200 (32.8)	1098 (33.8)	1084 (33.3)	1018 (31.4)
High (More than 30g/day)	349 (3.6)	116 (3.6)	110 (3.4)	123 (3.8)
Adherence to Mediterranean diet[2]				
Low	453 (4.65)	144 (4.4)	158 (4.9)	151 (4.7)
Medium	5584 (57.3)	1808 (55.6)	1875 (57.6)	1901 (58.6)
High	3715 (38.1)	1300 (40.0)	1222 (37.5)	1193 (36.8)
Physical activity (MET hour-week)[3]				
Inactive	1767 (18.1)	616 (18.9)	571 (17.5)	580 (17.9)
Moderately inactive	1746 (17.9)	569 (17.5)	610 (18.7)	567 (17.5)
Moderately active	3322 (34.1)	1131 (34.8)	1091 (33.5)	1100 (33.9)
Active	2917 (29.9)	936 (28.8)	983 (30.2)	998 (30.8)
Sleep duration (hours)[4]	7.39 (1.35)	7.4 (1.2)	7.4 (1.3)	7.4 (1.5)

[1] Tertiles were defined with the distribution of the photopic illuminance variable. 1st tertile [0.0585,0.701]; 2nd tertile: (0.701,1.07]; 3rd tertile: (1.07,3.45]. [2] Adherence to the Mediterranean diet assessed through MEDAS: Low: [0-5), Medium: [5-9), High ≥ 9. [3] Physical activity EPIC sex-specific cut-offs; Males: <50, 50-66, 66.1-97.0, ≥97.1; Females: <56.7, 56.7-91.1, 91.2-155.6, ≥155.7. [4] Sleep duration reported as corresponding to week days. N= sample size; SD = Standard deviation.

ALAN and clinical markers of cardiometabolic diseases: cross-sectional analyses

BMI, WHR and obesity

We observed an association between photopic illuminance and BMI (Table 2, β coefficient = 0.12, 95 % CI 0.00 to 0.24). This association was stable after adjusting for other urban exposures but not after considering income level or sleep duration (Table S1, β coefficient = 0.10, 95% CI -0.01 to 0.22 and 0.11, -0.01 to 0.23, respectively). Photopic illuminance was also associated with a higher prevalence of general obesity (Figure 3, OR = 1.06, 95% CI 1.00 to 1.12).

We did not observe an association between photopic illuminance and WHR and the prevalence of abdominal obesity in model 2 (Table 2). However, after adjusting for urban exposures, this association became apparent as well (Table 2, β coefficient = 0.002, 95% CI 0.000 to 0.004 and Figure 3, OR = 1.07, 95% CI 1.00 – 1.14). We did not observe an association between melanopic illuminance and BMI nor WHR and, therefore, neither with general nor abdominal obesity (Table 2 and Figure 3).

Blood pressure and hypertension

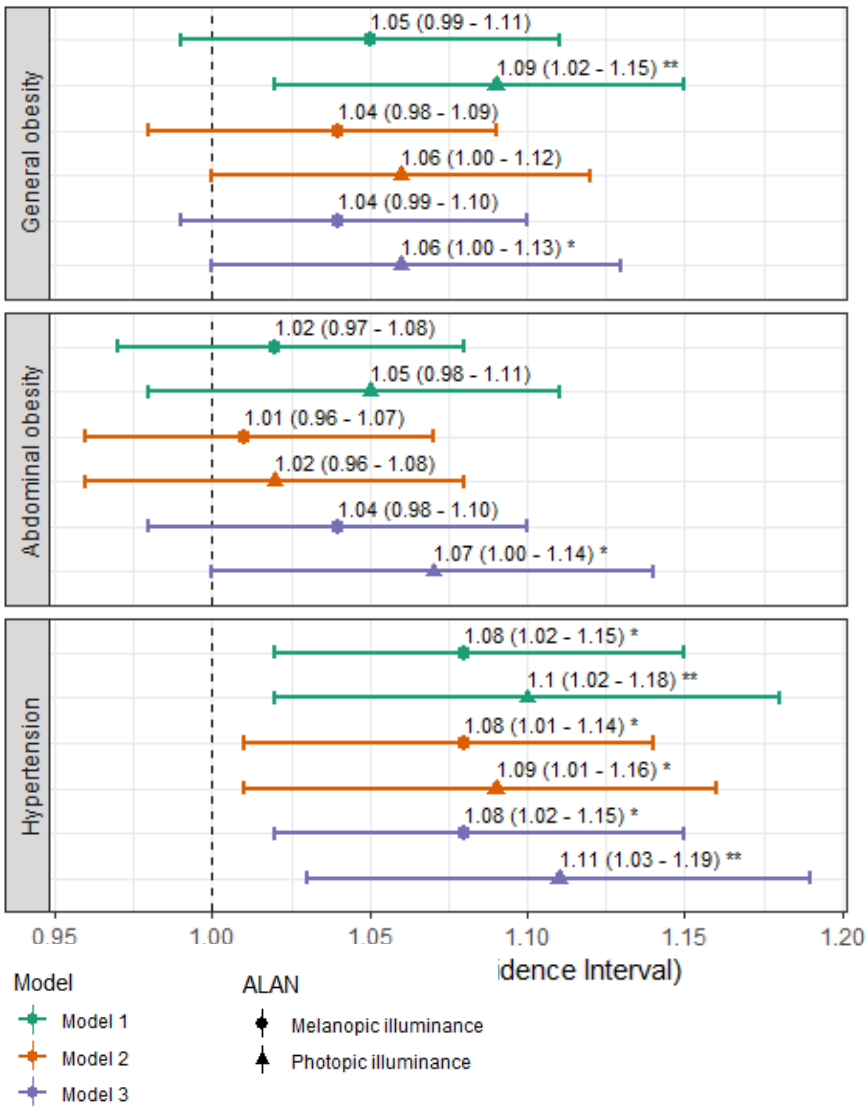
We observed an association between photopic illuminance and systolic blood pressure (Table 2, β coefficient = 0.37, 95% CI 0.01 to 0.73). However, this association lost statistical significance after adjusting for other urban exposures.

Table 2. Cross-sectional associations among ALAN indicators and clinical markers of cardiometabolic diseases in 9,752 from the GCAT cohort.

	Photopic illuminance		**Melanopic Equivalent Daylight (D65) Illuminance**	
	β coefficient (95% CI) [1]	**P-val**	**β coefficient (95% CI) [1]**	**P-val**
Body mass index				
Model 1	0.19 (0.07; 0.31)	**<0.01**	0.11 (0.00; 0.21)	**0.05**
Model 2	0.12 (0.00; 0.24)	**0.05**	0.07 (-0.03; 1.18)	0.2
Model 3	0.12 (-0.00; 0.24)	**0.05**	0.08 (-0.03; 1.18)	0.2
Waist-to-hip ratio				
Model 1	0.002 (-0.00; 0.003)	**0.05**	0.001 (-0.000; 0.002)	0.3
Model 2	0.001 (-0.001; 0.004)	0.3	0.000 (-0.001; 0.002)	0.5
Model 3	0.002 (0.000; 0.004)	**0.04**	0.001 (-0.000; 0.002)	0.2
Systolic blood pressure				
Model 1	0.52 (0.16; 0.89)	**<0.01**	0.21 (-0.11; 0.54)	0.2
Model 2	0.37 (0.01; 0.73)	**0.04**	0.14 (-0.18; 0.46)	0.4
Model 3	0.27 (-0.11; 0.64)	0.2	0.07 (-0.26; 0.41)	0.7
Diastolic blood pressure				
Model 1	0.58 (0.35; 0.82)	**<0.01**	0.45 (0.24; 0.67)	**<0.01**
Model 2	0.51 (0.28; 0.75)	**<0.01**	0.42 (0.20; 0.63)	**<0.01**
Model 3	0.57 (0.33; 0.82)	**<0.01**	0.43 (0.21; 0.65)	**<0.01**
Glycated hemoglobin [2]				
Model 1	-0.000 (-0.02; 0.02)	1.0	-0.01 (-0.03; 0.01)	0.3
Model 2	-0.006 (-0.03; 0.02)	0.6	-0.02 (-0.04; 0.06)	0.1
Model 3	-0.002 (-0.04; 0.01)	0.2	-0.02 (-0.05; 0.01)	0.06

Model 1 = Adjusted for age and gender. Model 2 = Adjusted as model 1 and educational level (less than primary education, primary education, secondary education and university), adherence to Mediterranean diet assessed through MEDAS (low, medium, high), tobacco consumption (never, past smoker, current smoker), daily alcohol consumption (none, past drinker, low, moderate, high, very high) and physical activity (inactive, moderately inactive, moderately active, active). Model 3 = Adjusted as model 2 and ndvi, pm2.5, population density and nightly road noise. The variable for nightly road noise had 490 missing values and, therefore, model 3 was calculated with a N=9,262. [1] Linear regression model per interquartile range increase (IQR). IQR for photopic illuminance = 0.59 and IQR for melanopic illuminance = 0.16. [2] Glycated hemoglobin (HbA1c) was measured only in 2,444 participants. CI = Confidence Interval.

Figure 3. Forest plot of cross-sectional associations between ALAN indicators and general obesity, abdominal obesity, hypertension and diabetes in 9,752 participants from the GCAT cohort.



Model 1 = Adjusted for age and gender. Model 2 = Adjusted as model 1 and educational level (less than primary education, primary education, secondary education and university), adherence to Mediterranean diet assessed through MEDAS (low, medium, high), tobacco consumption (never, past smoker, current

smoker), daily alcohol consumption (none, past drinker, low, moderate, high, very high) and physical activity (inactive, moderately inactive, moderately active, active). Model 3 = Adjusted as model 2 and ndvi, pm2.5, population density and nightly road noise. Logistic regression model per interquartile range increase (IQR). IQR for photopic illuminance = 0.59 and IQR for melanopic illuminance = 0.16. General obesity was defined as BMI > 30 kg/m2 (2,515 cases); Abdominal obesity as WHR males ≥ 1.0 and females ≥ 0.85 (2,666 cases); Hypertension as a systolic blood pressure ≥ 140 mmHg or a diastolic blood pressure ≥ 90 mmHg (1,773 cases). CI = Confidence Interval; OR = Odds ratio.

We found associations between photopic and melanopic illuminance and diastolic blood pressure (β coefficient = 0.51, 95% CI 0.28 to 0.75 and β coefficient = 0.42, 95% CI 0.20 – 0.63, respectively). These associations remained stable after considering other urban exposures (Table 2) and also in all sensitivity analyses (Table S1). The same pattern was observed when dichotomising blood pressure into hypertension with ORs of 1.09 (1.01 to 1.16) and of 1.08 (1.01 – 1.14) for photopic and melanopic illuminance respectively (Figure 3). This would be interpreted as a 9% (1-16%) and 8% (1-14%) higher odds of prevalent hypertension per each 0.59 lux increase in photopic illuminance and per 0.16 lux increase per melanopic illuminance, respectively.

Glycated haemoglobin

Overall, we found no cross-sectional associations between exposure to ALAN indicators and glycated haemoglobin (Table 2). However, the pattern seemed to show inverse associations. We did not represent diabetes in the forest plot in Figure 3 because the CIs for these estimates were too wide due to the small sample size (N=2,444) and the limited number of diabetes cases (N=61). The pattern for the binary outcome of diabetes was similar to HbA1c in Table 2.

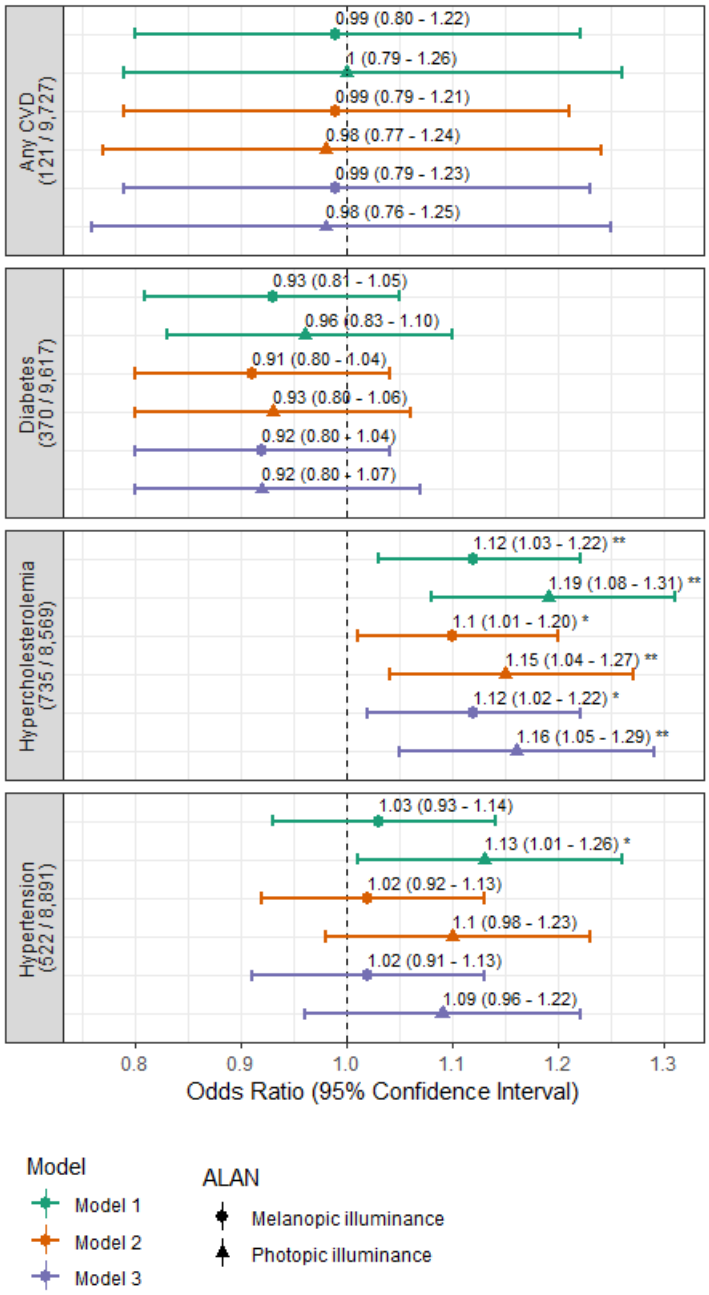
We found no effect modification in the associations between ALAN indicators and clinical markers of cardiometabolic diseases by sex.

ALAN and incident cardiometabolic diseases: prospective analyses

The associations between exposure to the different ALAN indicators and incident cardiometabolic diseases ascertained through electronic health records are shown in Figure 4. We did not observe an association between ALAN, incident cardiovascular diseases or diabetes. Initially, we observed an association between photopic illuminance exposure and increased risk of hypertension, however, this became no longer statistically significant after considering confounders such as smoking, drinking or, physical activity (Figure 4).

Our results show an association between higher exposure to photopic illuminance at night and a higher risk of developing hypercholesterolemia (OR = 1.15, 95% CI 1.04 – 1.27). These results imply that each IQR increase in photopic illuminance (0.59 lux) is associated with a 15% higher risk of hypercholesterolemia (4-27%). We also noticed an association between exposure to melanopic illuminance and an increased risk of developing hypercholesterolemia (OR = 1.10, 95% CI 1.01 – 1.20). This is translated to a 10% higher risk of hypercholesterolemia per each IQR increase in melanopic illuminance (0.16 lux).

Figure 4. Forest plot of prospective associations between ALAN indicators and incident cardiometabolic diseases in 9,752 participants from the GCAT cohort.



Model 1 = Adjusted for age and gender. Model 2 = Adjusted as model 1 and educational level (less than primary education, primary education, secondary education and university), adherence to Mediterranean diet assessed through MEDAS (low, medium, high), tobacco consumption (never, past smoker, current smoker), daily alcohol consumption (none, past drinker, low, moderate, high, very high) and physical activity (inactive, moderately inactive, moderately active, active). Model 3 = Adjusted as model 2 and ndvi, pm2.5, population density and nightly road noise. Logistic regression model per interquartile range increase (IQR). IQR for photopic illuminance = 0.59 and IQR for melanopic illuminance = 0.16. Incident cases / total sample across cardiometabolic diseases differ because of exclusion of prevalent diseases in each case. Prevalent cases of diabetes (N=135), any CVD (N=25), hypertension (N= 861) and hypercholesterolemia (N=1183). CI = Confidence interval; OR = odds ratio.

These associations remained statistically significant after adjusting for other urban exposures (OR = 1.16, 95% CI 1.05 – 1.29 for photopic and OR = 1.12, 95% CI 1.02 – 1.22 for melanopic illuminance).

Secondary analyses

In Figure S2 we present the correlations among ALAN indicators and with other urban exposures. When considering ALAN indicators, we observed a very high and positive correlation (0.90) between photopic and melanopic illuminance. Overall, we found no correlation between ALAN indicators and exposure to air pollution, population density, greenness and nightly road noise.

We observed no correlation between residential and bedroom ALAN exposure since the continuous variables of ALAN indicators were equally distributed across bedroom ALAN categories (data not shown).

DISCUSSION

To our knowledge, this is one of the first studies to examine the association between residential ALAN and cardiometabolic risk factors and diseases from an urban perspective. Our cross-sectional analyses show an association between photopic and melanopic equivalent daylight (D65) illuminance and hypertension. The present study also suggests an association between photopic illuminance and obesity; however, this association was less stable across further adjustments. In addition, we found a link between exposure to both types of illuminances and the risk of incident hypercholesterolemia. Finally, we found no association between exposure to ALAN, incident cardiovascular diseases or diabetes.

In this study we showed, for the first time, an association between residential ALAN exposure and high blood pressure and hypertension in a human population. Night shift work induces circadian misalignment and has been associated with increased blood pressure and hypertension [35–37]. Indoor estimates of light at night have been associated with increased blood pressure [8]. Similarly, exposure to ALAN measured from 7-day actigraphy has been associated with concurrent hypertension in older age [38]. Our results showed a clear association between both melanopic and photopic illuminance exposure and prevalent hypertension. Considering blood pressure, the association with systolic blood pressure was only present for photopic illuminance and was less stable (e.g., when considering other urban exposures) than for diastolic blood pressure. It is well-known that light-at-night suppresses melatonin production

and melatonin levels seem to be associated with blood pressure [39], building a potential underlying mechanism. However, Obayashi and colleagues (2014) showed that the link between ALAN and blood pressure was independent of melatonin levels [8]. Another hypothesis is that light can regulate blood pressure through catecholamine levels, as shown in a study with night shift workers [40].

In relation with obesity, our results suggest an association between photopic illuminance and obesity. These results remained stable after considering urban exposures but not after considering income levels or sleep. Results from another cross-sectional study showed an association between higher exposure levels of residential ALAN and obesity even after considering monthly household income and short sleep duration [41]. In a prospective study, higher exposure to residential ALAN was associated with higher odds of developing obesity [42]. However, in these others investigations exposure to ALAN was only assessed through photopic illuminance which may not be presenting the optimal measurement of light effects on circadian regulation [2]. In the present results, the direction of the association between melanopic illuminance and obesity was as we would have expected but associations were not statistically significant.

The observed association between higher levels of ALAN exposure and increased risk of developing hypercholesterolemia agrees with the previous literature. Studies in shift workers show an association with dyslipidaemia [43]. Results from a cross-sectional study of 528

elderly individuals showed that exposure to ALAN was linked to higher levels of low-density lipoprotein cholesterol levels (average nightly exposure ≥3 lux vs. < 3lux, 128.6 vs.122.2 mg/dl; P 0.04) [10]. The biological explanation for this association has been explored before. Okuliarova and colleagues (2020) found that exposing rats to dim light-at-night increased hepatic lipid storage [44]. Clock, a key transcription factor in circadian regulation, is crucial to maintaining low concentrations of plasma cholesterol and, therefore, reducing atherogenesis [45,46].

We found no association between ALAN and incident cardiovascular diseases. Contrary, Sun and colleagues (2021) reported an association between residential ALAN and a higher risk of coronary heart disease hospitalisations and deaths. However, in this study the median follow-up time was 11 years and in the present study the time elapsed could have been too short because baseline assessments started in 2014 and EHR were ascertained until 2017, which could have led to a lack of statistical power [12]. In fact, we observed a significant association with risk of hypercholesterolemia, which is a risk factor for several cardiovascular diseases [47] and type 2 diabetes [48].

In humans, bedroom light intensity has been associated with incident type 2 diabetes [9], a hypothesis supported in animal studies [49]. However, in the present study population, we report no association between exposure to ALAN indicators and incident diabetes. This was similarly reported in another human study where they reported no significant association between ALAN and HbA1C levels in

multivariate models [10]. In the study by Obayashi and colleagues (2020) the follow-up time was a bit longer (3.5 years), and also light exposure was assessed at an individual level using a portable light meter, which might explain the differences that we observed [9]. Therefore, the lack of association might be a cause of the temporality of exposure and outcome assessment. It is worth noting that although in our cross-sectional analyses, we did not observe a significant link between ALAN exposure and glycated haemoglobin, the direction of the results was inverse, contrary to what we would have expected. Only a subsample of our population had information on glycated haemoglobin, and further studies are needed to explain these findings in a larger population.

We found a very high and positive correlation between photopic and melanopic illuminance, meaning that there is about the same proportion of blue in both extremes of the visual brightness scale. In other words, that the mix of lamp types installed in visually brighter areas is about the same as in dimmer ones. We did not find any noticeable correlation between ALAN indicators and air pollution, nightly road noise, population density nor greenness. Contrarily Huss and colleagues (2019) showed a positive correlation between illuminance and air pollution and a negative correlation with surrounding green spaces [50]. This difference could be because of the different times we used to assess urban exposures. Another explanation could be that these analyses were done in Amsterdam and Utrecht, in the Netherlands, which might not be comparable to Barcelona in terms of light, air-pollution, and noise exposure. Although we did not observe an important correlation between

ALAN and other urban exposures, we did see some confounding, highlighting the importance of considering these kinds of exposures in ALAN studies.

The strengths of this study include the large sample size, especially for cross-sectional analyses, and the consideration of a wide spectrum of potential confounders, including other urban exposures, sleep duration, or night shift work. It is also a strength of this study, that we could conduct prospective analyses for the association between exposure to residential ALAN and cardiometabolic diseases. However, several limitations should be considered when interpreting these results.

First of all, exposure to ALAN was assessed from satellite images, but we had no information on individual levels of exposure at baseline. The agreement with bedroom ALAN levels asked in the follow-up questionnaire was low. Therefore, these indicators should be taken as a proxy of individual exposure but not as direct exposure in the bedroom while sleeping. Secondly, when assessing ALAN exposure from satellite images, we assume that each pixel of the image taken from the ISS is occupied by street pavements. However, in the city of Barcelona, the urban surface occupied by buildings is nearly 50% of the total city surface, measured by the ‘compacity index’ [51], meaning that the streets and other open surfaces amount to half the pixel area, on average. Since only the streets are lit, but the roofs are not, the actual illuminance on the pixel's streets shall be twice the presented in these results. In addition, many streets have

trees or other obstacles blocking the propagation towards the sky of the light reflected in the pavement. Hence actual values on the streets may be twice to ten times larger than the ones presented in the current analyses. Thirdly, we only have data from Barcelona, an urban setting with high exposure to light-at-night. The fact that we do not have many participants living in rural areas with low exposure reduces the variability of our exposure. Lastly, the study population is not representative of the general population since participants included in this cohort are volunteer blood donors from the Blood and Tissue Bank (BST). Therefore, we need to be cautious to extrapolate these results since volunteer-based cohorts usually have more conscious behaviours. Finally, it is also a limitation the short follow-up which limited the power to detect associations in longitudinal analyses.

In conclusion, the present study suggests a link between exposure to photic and melanopic illuminance and prevalent hypertension and the risk of hypercholesterolemia, probably through circadian dysregulation. However, causal interpretation of these findings should be taken cautiously given the potential exposure misclassification of highly exposed individuals and the cross-sectional nature of some of the presented results. Further studies at an individual level should be conducted to confirm these associations.

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6. DISCUSSION

The present doctoral thesis provides new findings on the emerging research topic of mild circadian disruption in the general population arising from mistimed eating behaviours and exposure to artificial light-at-night. Throughout this thesis, circadian patterns in nutritional behaviours, named circadian nutritional behaviours, are considered as a whole and include timing of the first and last eating episode of the day and nighttime fasting duration.

Addressing the objectives of this thesis, (1) *Papers I-IV* examined the association between circadian nutritional behaviours and risk of NCDs and (2) *Paper V* investigated the impact of exposure to residential ALAN on cardiometabolic diseases.

The results of *Papers I – V* are presented in detail in the results section. In this section, the main findings of this thesis are discussed in the context of previous evidence. The main findings of *Papers I-V* are summarised in Table 1, at the end of the first section of this chapter. Then, methodological aspects are addressed, highlighting the strengths and limitations of the presented studies. Finally, the implications of the main findings of this thesis for public health and future research are discussed.

6.1. Main findings and previous research

6.1.1. Circadian nutritional behaviours and NCDs

Mounting evidence from animal studies and human trials indicate that food intake has a metabolic response that depends on the time-of-day. However, we have little evidence from population studies providing a complete and specific approach on daily timing of meals in relation to NCDs and also a lack of studies investigating the association of nighttime fasting duration with risk of NCDs.

Association with cancer

Some circadian nutritional behaviours were previously explored within the MCC-Spain study (Kogevinas et al., 2018), see also section 4.2. Exposure assessment, in Methods. In this previous paper, the time of dinner and the time elapsed between dinner and sleep were investigated in relation with breast and prostate cancer risk. Participants having a time interval between dinner and sleep of more than 2 hours had a lower risk of prostate (OR= 0.74. 94% CI 0.55 - 0.99) and breast cancer (OR = 0.84, 95% CI 0.67 - 1.06). Similarly, a dinner before 9PM was associated with a lower risk of both cancers combined (OR = 0.82, 95% CI 0.67 - 1.00), compared to a dinner at 10PM or later. In *Papers I and II*, the nighttime fasting duration and the time of breakfast were investigated to build up a better picture of the complexity of circadian nutritional behaviours.

The first paper included in this thesis, provides a more complete analysis of meal timing and fasting in relation to prostate cancer as

compared to our previous analysis (Kogevinas et al., 2018). We analysed data from 607 prostate cancer cases and 848 controls in the MCC-Spain study and showed that prolonging the daily nighttime fasting duration to more than 11 hours may be associated with a lower risk of prostate cancer. However, this association was not statistically significant. The association was confounded by the time of breakfast, understood as the time when we break- the fast, since adjustment for this third variable strengthen the association between nighttime fasting duration and lower prostate cancer risk. In this adjusted model, having breakfast after 8.30AM was also linked to a slight, but non-significant, higher risk of prostate cancer. Even large studies such as the MCC-Spain tend to have low power to identify interactions and the statistical interaction was not significant, results showed a change of the association between nighttime fasting and risk of prostate cancer risk by time of breakfast. Results indicated that the association between nighttime fasting and a lower prostate cancer risk was stronger among individuals reporting an earlier breakfast, compared to those having breakfast after 8.30AM or skipping it.

The importance of the time of breakfast was also highlighted in *Paper II* in this thesis, which included data from 1,181 breast cancer cases and 1,326 controls in the MCC-Spain Study. Nighttime fasting duration was initially associated with a higher breast cancer risk among premenopausal women. However, this association was again confounded by time of breakfast and after adjusting for this information the association of nighttime fasting and breast cancer risk disappeared. On the other hand, each hour later in the time of breakfast was associated with 18% higher odds of having breast

cancer among premenopausal women (95% CI, 1.01-1.40). This association was not statistically significant among postmenopausal women and, in fact, an interaction was observed between time of breakfast and breast cancer risk by menopausal status. Among premenopausal women, the association between a later time of breakfast and a higher risk of breast cancer was stronger for HER2+ tumours compared to positive hormonal receptors and triple negative tumours. There is very little evidence exploring the differential effects of circadian disruption on breast cancer subtypes but a previous study suggested that the impact of night shift work was worse for HER2+ breast cancer (Vistisen et al., 2017). In a study within the MCC-Spain study, the association between night shift work and breast cancer risk was stronger among premenopausal women with oestrogen or progesterone receptors (Papantoniou et al., 2016).

Two previous investigations in rodents indicated that time-restricted feeding, or the extension of the rest-phase fasting duration, could inhibit obesity-induced mammary tumorigenesis (Das et al., 2021; Sundaram & Yan, 2018). However, obesity is a risk factor for postmenopausal cancer (García-Estévez et al., 2021), but not premenopausal breast cancer. In *Paper II* we did not observe an association between these behaviours and breast cancer in postmenopausal women, implying that other mechanisms than obesity may be explaining the association between time of breakfast and premenopausal breast cancer. Moreover, it is important to notice, that in both cases, mice under TRF conditions were compared to mice fed *ad-libitum,* both during the active and the rest phases. This opens

the question on whether the protective results of TRF in comparison to a regimen *ad libitum* are intrinsic benefits of the time-restricted diet or are in fact showing the detrimental effects of eating around the clock. Evidence from RCT in humans show that time-restricted eating could reduce body weight, inflammatory / oxidative markers and improve cardiometabolic endpoints (Schuppelius et al., 2021), a general metabolic state that could mitigate the risk for tumorigenesis.

In 2016, Marinac and colleagues, reported that a prolonged nighttime fasting duration could be associated with a lower risk of breast cancer recurrence (Marinac et al., 2016). In this study, the model for nighttime fasting duration was adjusted for a binary variable for eating after 8PM. Time of breakfast was not considered. In this study, only 8% of women in the long nighttime fasting group (≥ 13 hours) were eating after 8PM compared to a 45% in the group reporting a shorter nighttime fasting duration. The population from the study by Marinac and colleagues (2016) and the MCC-Spain population had different time-related eating patterns that complicates comparisons. This, and the fact that breast cancer recurrence and not risk was explored, could explain the different findings with *Paper II*.

There are only two studies in humans exploring the association between nighttime fasting duration and cancer risk (Meth et al., 2022; Srour et al., 2018). A recent study with data from the Uppsala Longitudinal Study of Adult Men, showed that men with a longer daily eating duration (implying a shorter nighttime fasting) had a higher risk of fatal cancer (Meth et al., 2022). This model was adjusted for the midpoint daily eating interval, calculated as the

midpoint between the first and last eating episode and representing when the daily eating window occurs.

A study within the NutriNet-Santé cohort, suggested that there was no association between nighttime fasting duration and risk of breast and prostate cancer (Srour et al., 2018). In this study, they mutually adjusted the number of eating episodes, the nighttime fasting duration, the time of the last eating episode and the time of the first eating episode. Participants having a last eating episode after 9:30PM were at higher risk of prostate and breast cancer compared to participants having it earlier. In agreement with findings from *Paper II*, each additional hour in the time of the first eating episode was associated with a 13% higher risk of breast cancer (95% CI 0.99 – 1.29, p-value = 0.07). In this association, the interaction with menopausal status was not considered, something we found was modifying the association between time of breakfast and breast cancer risk.

Aside from *Paper II* and the NutriNet-Santé study no other epidemiological study has explored the association between time of breakfast and breast cancer risk. However, there is evidence for biological plausibility from animal models showing that the delay of the first active-phase meal, the analogous breakfast, can result in an abnormal lipid metabolism (D. Kim et al., 2021; C. Yoshida et al., 2012), a phase delay in body temperature and decreased energy expenditure (D. Kim et al., 2021) and with a phase delay in the expression of circadian genes (Shimizu et al., 2018; C. Yoshida et al., 2012). Similarly, in rats the delay of the first active-phase meal has

been linked to an increased adipose tissue weight and visceral fat without changes in food intake and body weight (Aquino de Oliveira et al., 2021; D. Kim et al., 2021). In humans, skipping breakfast has been linked with a disrupted glycaemic control (Reutrakul et al., 2014) lipidic profile (L. Chen et al., 2021) and cortisol rhythm (Witbracht et al., 2015) and with an increased inflammatory status (Nas et al., 2017). Overall, this impaired circadian and metabolic state could be explaining the association between a later time of breakfast and a higher breast cancer risk.

Association with type 2 diabetes

In *Paper III*, circadian nutritional behaviours were explored as a whole in relation with the risk of developing T2D in the NutriNet-Santé cohort. Results showed that a behaviour characterised by a later daily first meal was associated with a higher risk of T2D, after a median follow-up time of 7 years. Compared to a breakfast before 8AM, a breakfast between 8AM and 9AM and after 9AM were associated with a 26 and 59% higher risk of T2D (HR, 95% CI 1.09 – 1.45 and 1.30 – 1.94, respectively).

The only other investigation examining specifically the time of breakfast in relation with risk of T2D showed that a later breakfast (after 9AM) was associated with a lower risk of this disease (Carew et al., 2022). Participants in this study were aged 65 years or older and, it is worth noting, that another investigation found that the link between irregular breakfast consumption and risk of type 2 diabetes was no longer present in individuals 65 years or older (Mekary et al.,

2013). This could indicate some degree of effect modification of the association between time of breakfast and risk of T2D by age and should be further explored. In agreement with our results, the habit of skipping breakfast defined as ever versus never omitting this meal throughout the week has been associated with a higher risk of T2D (Ballon et al., 2019). The association between the habit of having a late first meal and a higher risk of T2D could be explained through a worse glycaemic control, lipidic profile and higher postprandial insulin concentrations (Deshmukh-Taskar et al., 2013; Iwasaki et al., 2019; Nas et al., 2017).

Results from *Paper III* also indicated that, although the association between time of last meal and risk of T2D was not significant, participants fasting for more than 13 hours overnight and breaking the fast at 8AM had a lower risk of this disease. This behaviour would imply habitually having breakfast at 8AM or before, dinner at 7PM or before, and fasting the rest of the time. However, only 15 participants reported this behaviour and, therefore, results have to be taken with caution. To our knowledge, no other study has explored the association between time of last meal of the day and nighttime fasting duration in relation with risk of T2D. Nevertheless, supporting these results, evidence from human trials indicate that TRE could improve insulin sensitivity, β - cell function and reduce blood glucose levels and body weight (Schuppelius et al., 2021), key players in the pathogenesis of diabetes.

Finally, in *Paper III* a higher number of eating occasions was linked to a lower risk of T2D. A potential mechanism explaining these

findings could be a reduction of insulin and lipid concentration between more frequent meals (Jenkins et al., 1989). However, evidence is inconclusive, showing associations in both directions (Neuhouser et al., 2020; X. Wang et al., 2021). Moreover, in our analyses the association lost statistical significance after excluding participants diagnosed during the first two years of follow-up and after adjusting for daily caffeine intake which could indicate some degree of reverse causation and confounding, respectively.

Association with cardiovascular diseases

In *Paper IV*, circadian nutritional behaviours were assessed in its whole in relation with CVDs in the NutriNet-Santé cohort. In this population, a later first and last meal of the day, but not the number of eating occasions were linked to a higher risk of CVDs. Specifically, each additional hour in the time of the first meal was significantly associated with a 6% higher risk of developing CVDs (HR, 95% CI, 1.01 – 1.12). On the other hand, compared to participants habitually eating a last meal before 8PM, those having it after 9PM had a 14% higher risk of developing this group of diseases (HR, 95% CI, 1.00 – 1.30). Associations between later circadian nutritional behaviours were apparent for cerebrovascular diseases (stroke and transient ischemic attack), but not for coronary heart diseases (myocardial infarction, acute coronary syndrome, angioplasty and angina pectoris). Sensitivity analyses showed an interaction with sex and a stronger impact of mistimed circadian behaviours in women than in men. Finally, nighttime fasting duration

was inversely associated with the risk of cerebrovascular diseases after adjusting for the time of the first meal of the day.

In line with results presented in *Paper IV*, a cross-sectional study (Wirth et al., 2021) and a study with 1 year of follow-up (Makarem et al., 2020) showed positive associations between later first and last meal of the day and cardiometabolic risk factors (intermediate endpoints) such as diastolic blood pressure, fasting glucose, insulin and markers of inflammation. The habit of skipping breakfast and of eating a snack after dinner have been negatively associated with cardiovascular health (Cahill et al., 2013; H. Chen et al., 2020). However, to the best of our knowledge, *Paper IV*, is the first study to investigate the associations between daily timings of meals and incident CVDs (hard endpoints). *Paper IV* explored as well for the first time the potential benefits of extending the nighttime fasting duration, or shortening the daily eating window, in relation with the risk of CVDs.

6.1.2. Artificial light-at-night and cardiometabolic health

To address the second specific objective of this thesis, in *Paper V*, exposure to residential ALAN was investigated in relation with cardiometabolic health. In this study, we evaluated two metrics of light exposure, the visually relevant photopic illuminance and the metabolic and circadian-regulation relevant melanopic equivalent daylight (D65) illuminance, both measured in lux (lx).

Cross-sectional analyses, showed a significant association between photopic illuminance and BMI and general obesity (OR= 1.06, 95% CI 1.00 – 1.12, per IQR increase [0.59 lux]). The association remained stable after considering other urban exposures but not after adjusting for income level of the participants or sleep duration during weekdays. We did not observe an initial association between photopic illuminance and WHR nor abdominal obesity, but after considering other urban exposure this became apparent. Melanopic illuminance was not associated with BMI nor WHR.

Other cross-sectional studies have shown associations between nighttime intensity and illuminance and higher prevalence of overweight and obesity (Abay & Amare, 2018; Koo et al., 2016). Similarly, a prospective study showed that residential ALAN was linked to a higher risk of developing obesity over 10 years (D. Zhang et al., 2020). These results are in agreement with our cross-sectional findings in relation with photopic illuminance and BMI obesity. However, in these studies the whole photopic spectrum was considered.

Here, we show for the first time that melanopic illuminance, although in the direction of a positive association, is not significantly associated with these clinical markers of obesity. Melanopic illuminance is the most circadian-related adequate indicator and associations with photopic illuminance might be biased from other confounders. Although, in *Paper V* associations between photopic illuminance and BMI and WHR remained present after adjusting for other urban exposures, in sensitivity analyses we showed that after

adjusting for both monthly income and sleep duration during weekdays associations were no longer significant indicating that this light measurement might be more influenced by third factors.

In *Paper V* we explore also for the first time the association between residential ALAN and hypertension and show a direct and significant association. Both photopic and melanopic illuminance were significantly and consistently associated with diastolic blood pressure and only photopic illuminance was significantly associated with systolic blood pressure, but the latter association was more unstable. Additionally, both photopic and melanopic illuminance were significantly associated with hypertension (OR = 1.09, 95% CI 1.01 – 1.16, per IQR increase [0.59 lux] and OR = 1.08, 95% CI 1.01 – 1.14, per IQR increase [0.16 lux], respectively). We found no significant association between ALAN indicators and HbA1c (diabetes).

In agreement with our findings, night shift work which induces circadian disruption has also been linked with hypertension (Cheng et al., 2021; Riegel et al., 2019; Yeom et al., 2017). Similarly, individual exposure to ALAN in the elderly has been linked to increased blood pressure and hypertension (M. Kim et al., 2022; Obayashi et al., 2014). The dysregulation of melatonin and catecholamine levels have been both suggested as potential mechanisms underlying these observations (Hannemann et al., 2021; Scheer et al., 2004). Finally, in *Paper V* we observed no significant association between residential ALAN exposure and HbA1c (diabetes). Contrary to what we would have expected from previous

literature (M. Kim et al., 2022; Obayashi et al., 2020), the direction of this association was inverse and further studies are needed to explain this discordance. However, it is worth noting that in the GCAT cohort only a subpopulation (N=2,444) had information on HbA1c levels which could have affected the power of our analyses.

In prospective analyses, the median time of follow-up was 2.5 years and, therefore, cardiovascular events were very limited. We observed a significant association for photopic and melanopic illuminances and risk of hypercholesterolemia (OR = 1.15, 1.04 – 1.27, per IQR increase, and OR = 1.10, 1.01 – 1.20, respectively). We hypothesise that this could be mediating the association of ALAN with cardiometabolic diseases as this association has been previously reported (Obayashi et al., 2013) and hypercholesterolemia is a risk factor for cardiometabolic diseases (Navar-Boggan et al., 2015; Rhee et al., 2017). Since the incidence of hypercholesterolemia in the population is high, we think that even with such short follow-up time it was enough to give power to these associations but not the others with higher latency periods.

Only one study has investigated the association of residential ALAN with coronary heart diseases (S. Sun et al., 2021) but none with diabetes, hypercholesterolemia or hypertension. In this study, they found a positive association between higher levels of exposure to ALAN (in the whole photopic spectrum) and risk of developing coronary heart disease. However, contrary to *Paper V*, the median follow-up time in this study was 11 years and, therefore, the statistical power was much higher.

Table 1. Summary of the main findings of the papers included in this doctoral thesis.

	Study design and participants	Methods	Results
I	Multicase-control (MCC-Spain) study (2008 – 2013) • Case-control • 607 PC cases; 848 controls • Mean age controls=66	Exposure: Telephone interview on circadian behaviours • Behaviours at 40y • Time of first meal • Nighttime fasting Outcome: Histologically-confirmed PC and BC with no prior history of disease Analysis: Multivariable logistic regression models	Long nighttime fasting (>11h) with an early breakfast (≤8AM) associated with a ns lower risk of PC (OR = 0.54, 95% CI 0.27–1.04).
II	MCC-Spain study • 1,181 BC cases; 1,326 controls • Mean age controls=58		Late breakfast associated with a higher risk of BC, especially among premenopausal women (OR=1.18, 1.01-1.40, per hour increase). No association of nighttime fasting with BC.
III	NutriNet-Santé cohort (2009 – 2021) • Web-based prospective (mean follow-up 7 years) • 103,312 adults free of T2D at baseline • 79% women • Mean age baseline=42.7	Exposure: 3 non-consecutive 24-hours food records (every 6 months). Average 2 first years of follow-up • Time of first and last meal • Number of eating occasions • Nighttime fasting	• Later first meal associated with a higher risk of T2D (HR= 1.14, 1.07 – 1.22, per hour increase) • Having breakfast < 8AM and after a nighttime fasting of > 13 hrs. (HR=0.47, 0.27 to 0.82) • Association between time of last meal and risk of T2D ns.

IV	NutriNet-Santé cohort • 103,389 adults free of CVD at baseline • 79% women • Mean age baseline=42.6	*(cont.)* Outcome: Self-reported in yearly health questionnaire, check-up every 6 months and any time through the website Analysis: Multivariable Cox proportional Hazard models	• Later first meal associated with a higher risk of CVD (HR=1.06, 1.01-1.12, per hour increase) • Later last meal associated with a higher risk of CVA (HR = 1.07, 1.00 – 1.14, per hour increase). • Extending nighttime fasting duration protective for CVA (HR=0.93, 0.87-0.99, per hour increase). • Stronger associations among women.
V	GCAT cohort • Cross-sectional and prospective (mean follow-up 2.5 years) • 9,752 adults • 59% women • Mean age baseline=56	Exposure: Nighttime image of Barcelona taken by the ISS • Photopic illuminance (visual light) • Melanopic illuminance (blue light) • Assigned to residences through GIS Outcome and analysis: • BMI, WHR, blood pressure and HbA1c assessed at the baseline by trained personnel – linear regression models and logistic regression models (for binary outcomes of these clinical markers: general and abdominal	Cross-sectional analyses: • Consistent associations between photopic and melanopic illuminance and hypertension (OR = 1.09, 1.01-1.16 and 1.08, 1.01-1.16, respectively) • Association between photopic illuminance and general obesity (OR=1.06, 1.00-1.12) stable after adjustment for other urban exposures but not after considering income levels nor sleep. • Association between photopic illuminance and abdominal obesity after

		obesity, hypertension and diabetes, respectively) • Incident cardiometabolic diseases in EHR (excluding prevalent cases of these diseases reported at baseline questionnaire) – logistic regression models • Exposure assessed as IQR increase (0.59 lux for photopic illuminance and 0.16 lux for melanopic illuminance).	considering urban exposures (OR=1.07, 1.00-1.14). • No association between melanopic illuminance and obesity. • Inverse association (ns) for melanopic illuminance and diabetes. <u>Prospective analyses</u>: • Follow-up too short (median 2.5 years). *Results to be updated in fall 2022.* • Associations of photopic and melanopic illuminance and risk of hypercholesterolemia (OR=1.15, 1.04-1.27 and 1.10, 1.01-1.20, respectively). • No associations with CVD, T2D and hypertension.
BC: Breast cancer, BMI: Body mass index; CI: Confidence interval; CVD: Cardiovascular disease; EHR: Electronic health records; GIS: Geographic Information System; HR: Hazard ratio; IQR: Interquartile range; ISS: International Space Station; ns: non-significant; PC: Prostate cancer; T2D: Type 2 diabetes; WHR: Waist-to-hip ratio.			

6.2. Methodological considerations

6.2.1. Study design

The papers included in this thesis analysed data from three studies with different designs. The MCC-Spain study is a multicase-control study that is appropriate when studying outcomes having a long induction period such as cancer, and also allow the exploration of numerous exposures (Lash et al., 2020). Given that information on exposures is collected after disease occurrence, temporality as described in Bradford Hill's criteria for causation may be more complex to establish (Hill, 1965). We tried to address this limitation by asking participants for behaviours at mid-age. As acknowledged in *Papers I and II*, this type of study is prone to recall bias since participants are asked to retrospectively report information about the exposure following diagnosis. In this case, considering that there is scarce information about circadian nutritional behaviours and cancer risk, it may be assumed that participants had no selective preconceptions and that the inaccuracy in recalling these behaviours was not systematically different in cases and controls. The fairly large sample size, enabled stratification of results in these analyses and a large panel of confounders were considered.

The NutriNet-Santé and the GCAT studies are both prospective cohort studies. This type of study, overcomes the temporality problem since exposure data are gathered prior to disease occurrence. Moreover, cohort studies allow to study multiple outcomes at the same time, which is the case of *Papers III-V*. One of the main

disadvantages of cohort studies is that a large number of participants is required to investigate even fairly common diseases (unless long follow-up is available). In the NutriNet-Santé study, participants were recruited through various multimedia campaigns and the cohort is web-based, which enabled participation reaching more than 100,000 inscribed individuals. The design of the NutriNet-Santé study permitted the evaluation of potentially mediator factors in our associations of interest such as body weight change during follow-up or intermediary metabolic disturbances such as hypercholesterolemia.

Another limitation of prospective cohort studies is that they require long periods of follow-up when examining chronic diseases with long latency. In the GCAT study the prospective analysis lacked statistical power because of this reason. Participants were recruited between 2014 and 2017 and the first Electronic Health Records extraction was done up to 2017, leaving a median follow-up time of 2.5 years. As mentioned in the methods section, we are expecting to receive a new data extraction in the following months that will correspond to EHR up to 2021, increasing the follow-up by 4 more years and analyses will be updated and improved with this new information before submission for publication.

Finally, all of the studies were observational and, therefore, there are some intrinsic limitations. These include potential unmeasured confounding, residual confounding in measured variables and measurement error (Maki et al., 2014).

6.2.2. Exposure assessment

Assessment of circadian nutritional behaviours

In *Paper I and II* circadian behaviours were assessed through a telephonic interview. This interview included information on several circadian aspects including meal timing and frequency, sleep problems, sleep duration, timing of physical activity, exposure to ALAN in the bedroom, night shift work history and the validated Munich Chronotype Questionnaire. Additionally, in the MCC-Spain study baseline questionnaire participants were asked about their residence and residential ALAN was geocoded following the same methodology as described in *Paper V*. The assessment of all these variables enabled to consider the whole circadian spectrum, through the exclusion of ever night shift workers and the adjustment for a wide variety of circadian aspects in sensitivity analyses. The fact that behaviours were asked separately for mid-age and for the year previous to the inclusion in the MCC-Spain study and for weekdays and weekends also enabled the performance of sensitivity analyses to test the robustness of *Papers I and II*.

It is important to mention, though, that the circadian interview was conducted later in time (median time 3 years after the inclusion to the study) which increases the potential for recall bias while reporting these behaviours. In *Paper I* we explored excluding participants responding to the circadian interview later than this median time to reduce inaccuracy in the exposure assessment, and results were strengthened. In *Paper II* we observed that the correlation between

data reported as corresponding to behaviours at mid-age and to the previous year to the inclusion in the study was higher among premenopausal women than postmenopausal women. This could indicate that the potential for non-differential exposure misclassification was higher among older women with a longer period since exposure assessment.

Noticeably, diet was assessed through a validated food frequency questionnaire at baseline and circadian nutritional behaviours were just assessed one single time during the telephonic interview.

In *Papers III and IV,* these limitations were addressed since in the NutriNet-Santé study, diet and circadian nutritional behaviours were measured repeatedly through 24h dietary records during the first 2 years of follow-up. Specifically, participants responded series of 3 random records of 24h food intake during a two-week period that included information on working and non-working days and that were repeated every 6 months. Dietary records are less prone to recall bias and have been validated in the NutriNet-Santé cohort both against interview with a dietician and against biomarkers of nutritional status (Lassale et al., 2015, 2016; Touvier et al., 2011). The comprehensive evaluation of diet in the NutriNet-Santé study facilitated a better assessment of the nutritional quality. Under-reporting is generally a limitation in nutritional epidemiology hampering the accuracy of dietary assessment (Macdiarmid & Blundell, 1998). It is another strength of *Papers III and IV* that the Goldberg cut-off method was used to identify and exclude energy under-reporters.

Still, there are some limitations to consider in the exposure assessment of *Papers III and IV*. In the NutriNet-Santé cohort, night shift work history was not recorded and as a proxy we excluded participants reporting very extreme circadian nutritional behaviours (a first meal after 3PM or a last meal before 3PM). Sleep was assessed in an optional questionnaire that was responded in 2014, during the follow-up. The questionnaire included questions on bedtime, sleep duration and a question on chronotype. The latter one was asked as "Do you consider yourself as a: a) Extremely morning person, b) More a morning person, c) Totally an evening person, d) More an evening person, e) Neither a morning or evening person, f) Both of them, g) I don't know". However, in this case, chronotype was not asked through a validated questionnaire such as in the MCC-Spain study. In this sub-sample, we also explored excluding participants reporting going to bed between 8AM and 6PM, also as a proxy of night shift. Finally, in the NutriNet-Santé study exposure to ALAN was not assessed.

Residential artificial light-at-night in the GCAT cohort

Residential ALAN exposure in *Paper V* was assessed from a satellite image from Barcelona. Satellite-derived ALAN data offer low-cost assessment of large populations (Katz & Levin, 2016) and have been largely studied in relation with other diseases such as breast cancer risk (Y. Wu et al., 2021). However, when using this source of data some methodological considerations have to be discussed.

ALAN exposure assessed from satellite images has to be considered as the residential exposure of individuals (outdoor exposure) but not as the total amount of ALAN in the bedroom. This would be the amount of light that people receive when they spend time outdoors, walking in the neighbourhood or in exterior parts of the house such as terraces, and also light that gets through the windows escaping light-blocking mechanisms and the one received before putting light-blocking mechanisms.

Previous studies assessing exposure to ALAN in relation with obesity or the one with coronary heart disease, have used images from the Defense Meteorological Satellite Program / Operational Linescan System (DMSP-OLS) (Koo et al., 2016; S. Sun et al., 2021; D. Zhang et al., 2020). This system is unable to detect the spectrum of the nighttime light emission and, therefore, provides images that are colour blind. Nevertheless, measures of light in the melanopsin-sensitivity portion might be more appropriate to assess the chronodisrupting effects of ALAN (Brown et al., 2022).

In *Paper V,* both photic and melanopic illuminance were considered and, in fact, we showed some differences between both ALAN indicators. These differential effects have also been observed in relation with colorectal cancer in an investigation from our group with data from the MCC-Spain study (Garcia-Saenz et al., 2020). In this study, the association between ALAN and higher risk of colorectal cancer was only observed with melanopic illuminance but not with the whole photopic illuminance. It is also important to highlight that the DMSP-derived images provide a resolution of

about 1km and in the analyses of *Paper V* we estimated ALAN exposure with a resolution of 30m.

One issue discussed in relation with satellite-derived ALAN measurement it is that this exposure could be correlated to other urban exposures such as air and noise pollution or access to green spaces (Huss et al., 2019; S. Sun et al., 2021). A strength of *Paper V*, is that air pollution, road noise at night, access to green spaces and population density were also considered in the association between residential ALAN and cardiometabolic health. Although, results showed no important correlation between these urban environmental exposures, there was some degree of confounding in relation with obesity and blood pressure in cross-sectional analyses. Specifically, after considering other urban exposures the association between photopic illuminance and WHR became significant and the association between photopic illuminance and systolic blood pressure became non-significant.

Another methodological aspect to consider is that in studies evaluating residential ALAN it is assumed that each pixel of the satellite image is occupied by street pavements, however, roofs are not lit and they cover a wide area of Barcelona. Moreover, the lighting in roads is not all propagated towards the sky since there are trees and other obstacles that can block it. Therefore, the levels of ALAN might be underestimated in satellite images. Again, one important strength of *Paper V* is that light measurements were made with a grid o 30m, compared to studies with a resolution of 1km, which provides much more precise estimates.

Exposure to residential ALAN has also been recently linked to sleep disorders (L.-B. Wang et al., 2022) a factor that has not been generally taken into account in ALAN studies and that is reported as a limitation in some (S. Sun et al., 2021). Sleep duration during weekdays was considered in sensitivity analyses of *Paper V* and we showed that the association between photopic illuminance and BMI was no longer significant after adjusting for this variable. This could indicate that sleep is confounding the association between photopic illuminance and BMI (i.e., people with sleep problems are more exposed to ALAN) or in fact that sleep is mediating this association (i.e., people with higher exposure to ALAN have more sleep problems).

It is also important to mention that the image used for analyses in *Paper V* was a nighttime image of Barcelona from 2013 when public street lightning was mainly composed by incandescent and high-pressure sodium lamps (Sánchez de Miguel et al., 2014). Now with the change towards the use of LED lamps, the circadian impact of public street lightning may be greater.

One final consideration in satellite-based ALAN studies is the risk of misclassification of shift workers. In this population, the assigned ALAN exposure at the residence might not be the adequate during the days of shift work. In sensitivity analyses of *Paper V* individuals reporting ever working night shifts were excluded and results remained stable.

6.2.3. Outcome assessment

In case-control studies, the selection of cases tends to be straightforward. In the MCC-Spain study, used in *Paper I and II*, cases were identified through histological confirmation in the collaborating hospitals and were included to the study as soon as possible after diagnosis. Selection of controls may be slightly more problematic. Controls should be sampled from the same study base as cases, something that sometimes is difficult to verify (Lash et al., 2020). Controls in the MCC-Spain study were frequency matched to cases by age, sex and region from population registers (random sample from lists of universal coverage Primary Health Care system) (Castaño-Vinyals et al., 2015). However, in the MCC-Spain Study, there was a slight selection bias on the basis of socioeconomic status in controls (they had a higher socioeconomic status than cases) and this was addressed by adjusting for socioeconomic status in all models.

In the NutriNet-Santé study, health outcomes including T2D and CVDs were self-reported through the website and in health questionnaires. Relying on self-reported health events and medications and treatments might not always be the most accurate ascertainment. Previous evidence suggests that the poor agreement between self-reported health events and medical records for some diseases may be a result of a poor communication between the physician and the patient. This can be the case in diseases with a less clear diagnosis and that are more difficult to understand by the patient such as the case of a heart failure (Smith et al., 2008).

In the NutriNet-Santé study, the declaration of the health event was accompanied by the date of diagnosis, which enabled to build hazard functions in *Papers III and IV*. Additionally, these declared cases were confirmed by the linkage to medico-administrative databases of the National health insurance (SNIIRAM database). In the case of T2D, in *Paper III*, cases were additionally confirmed by the levels of fasting blood glucose that were available for some participants. On the other hand, for CVDs, in *Paper IV*, cases were validated by a committee of physicians through the revision of medical records that were provided by the participants (e.g., examinations, hospitalisations or diagnosis). Moreover, data from the NutriNet-Santé cohort were linked to the French national cause specific mortality registry (CépiDC), enabling to identify deaths and potentially missed cases.

For prospective analyses in *Paper V*, cardiometabolic diseases were directly ascertained through the linkage of the GCAT cohort to electronic health records provided by the Catalan Public Healthcare System. In this case, it was more difficult to establish a date of diagnosis since for one specific disease and participant there could be multiple health records associated. In this case, incident cardiometabolic outcomes were analysed through logistic regression analyses, as yes / no having any health record for that specific disease.

6.2.4. Generalizability of the findings

It is important to mention that both the participants in the NutriNet-Santé cohort and the GCAT cohort were volunteers.

In the NutriNet-Santé study individuals with access to internet were invited to participate through various multimedia channels. Web-based epidemiological studies are increasingly popular since they constitute a cost-effective tool to follow sizeable and heterogeneous populations. In spite of the inevitable limitations of internet recruitment of e-cohorts, some hard-to-reach groups, such as unemployed, immigrants or the elderly, were represented in this French cohort (Andreeva et al., 2015). Nevertheless, as it generally happens in volunteer-based cohorts, the study population may not be representative of the source population. In fact, a study published in 2015, showed that compared to French census data, participants in the NutriNet-Santé study were more likely to be women, from higher socioeconomic status, married and with healthier behaviours (Andreeva et al., 2015). Such a volunteer bias could underestimate the prevalence of diseases in the NutriNet-Santé cohort and lead to a reduction of the power to detect plausible aetiological associations. Similarly, participation in the GCAT study was voluntary, but participants were mostly blood donors from the Catalan public agency and, likewise, the characteristics of this cohort might not be representative of the whole Catalan population.

Selection bias may occur in a cohort study in the initial recruitment into a study. If selection into the study is related to a particular exposure(s) and to the outcome, then this will then result in a collider bias. However, this type of biases would only apply to the cross-sectional analyses of the baseline data as for example in *Paper V*, while if follow-up has been conducted for a reasonable period of time, like in the NutriNet-Santé cohort, this bias would be attenuated.

If non-response has occurred at random even a study population that is not-representative of the source population will still provide unbiased effect estimates (Lash et al., 2020).

6.2.5. Intercorrelation in circadian behaviours

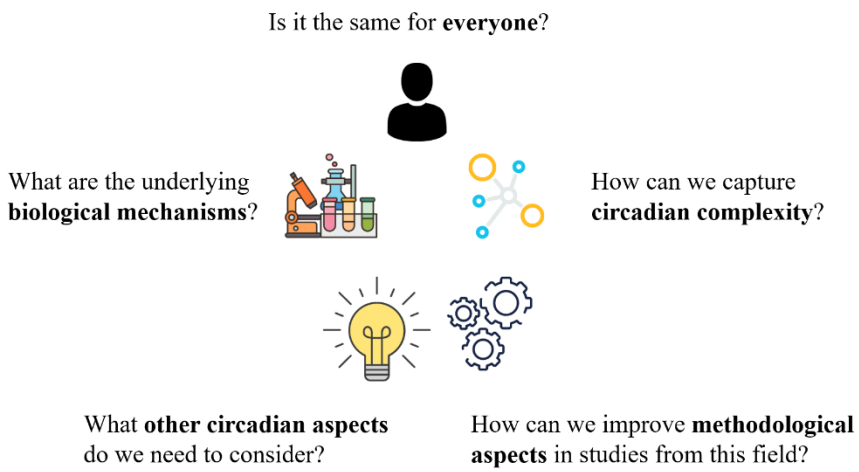
An important point of this thesis is the fact that circadian behaviours can be interconnected. Taking the example of night shift, a person working during the night might be exposed to several circadian disruptors at the same time such as being exposed to ALAN, being poorly exposed to natural light during the morning, having mistimed circadian nutritional behaviours and altered sleep patterns. In the general population, this interconnection is not as evident as in night shift workers, however, it is also present. For example, a later time of dinner has been linked to worse sleep outcomes (Crispim et al., 2011) and sleep duration has also been associated with breakfast consumption and quality (Al-Hazzaa et al., 2019; A. Liu et al., 2022).

In *Papers I-V*, the intercorrelation among circadian nutritional behaviours was explored showing in general that nighttime fasting duration was highly correlated with the time of first meal of the day indicating that in general this behaviour was the most influential in determining the length of the fasting overnight. The correlation with the time of last meal of the day was not so strong, but, was also moderately present, especially in the NutriNet-Santé data. In the analyses of these papers, we addressed this intercorrelation by mutually adjusting these behaviours to see the effect of each of these variables independently. Additionally, the absence of collinearity in

these models was assured. Nevertheless, further approaches to deal with this connectivity in circadian behaviours could be considered in future studies and this point is discussed in the following section.

6.3. Future research

The relationship between circadian behaviours and human health is highly complex and current evidence, including the research presented in this doctoral thesis, has only started to unravel it and there are still many questions that need to be addressed. **Challenges for future research** include the following questions:



<u>Is it the same for everyone?</u>

Differences by sex

Results from *Paper IV* in this thesis suggested that there was an effect modification of the association between circadian nutritional

behaviours and risk of CVDs by sex. In general, later circadian nutritional behaviours were worse for women than for men, something that has been previously reported as well in other nutritional studies and also in studies on night shift work (N. Wang et al., 2021; J. Yoshida et al., 2018; X. Zhang et al., 2020). In *Paper III* we tested and did not find an effect modification of the association between time of first meal of the day and risk of T2D by sex. Similarly, in *Paper V* we did not observe any important differences by sex. Further studies are needed to investigate if the sex differences in the association between circadian nutritional behaviours and CVDs are replicated and to try to disentangle the biological mechanisms explaining these differences.

Differences by menopausal status

Results in *Paper II* suggested an effect modification of the association between time of breakfast and breast cancer risk depending on the menopausal status of the participants, something that has also been previously reported in studies on night shift work (Cordina-Duverger et al., 2018). This indicates that some sort of effect modification by menopausal status could be playing a role in the health effects of exposure to delayed circadian nutritional behaviours. However, in this same study we showed that the differences between premenopausal and postmenopausal women could be in fact showing differences in the potential for recall bias and further studies are needed to confirm this effect modification in relation to breast cancer and also in relation to other NCDs and also to understand its biological explanation.

Differences by age

As mentioned before, the only other study in the literature examining the time of breakfast in relation with the risk of T2D was done in older participants and showed an inverse association (Carew et al., 2022), contrary to results in *Paper III* and to previous evidence on skipping breakfast (Ballon et al., 2019). Another study on breakfast frequency showed that the association between an irregular breakfast consumption and a higher risk of T2D was only present in participants younger than 65 years old (Mekary et al., 2013). This raises the point on whether circadian nutritional behaviours and circadian behaviours in general could have a differential effect on human health depending on the age of the individuals. There are well-known age-related changes in the circadian system (Hood & Amir, 2017), however, there is still a lot to do to understand how circadian disruption in the population may differently affect health through the lifespan. In *Papers I-V* included in this doctoral thesis, effect modification by age was not explored and it is something that further studies would need to investigate to give the most appropriate recommendations in circadian behaviours.

Differences by chronotype

As briefly mentioned in the introduction section, chronotype is a human attribute, with a genetic basis, that determines the preference for timing of activity across the day (Montaruli et al., 2021). Some studies have explored the association between chronotype and timing of food intake showing that later chronotypes have a delay in meal

timing habits (Mazri et al., 2020). Nevertheless, it remains unclear the role of chronotype in the association between mild circadian disruption, through mistimed circadian nutritional behaviours and exposure to ALAN, and human health.

Chronotype in *Papers I and II* was assessed in a validated questionnaire. In *Paper I* the association of prolonged nighttime fasting duration and a lower prostate cancer risk was more pronounced in morning chronotypes compared to intermediate and evening chronotypes. However, the statistical interaction was not significant. In *Paper II* we observed no effect modification of the association between time of breakfast and breast cancer risk by chronotype. Similarly, in *Papers III and IV* we found no interaction of the associations between circadian nutritional behaviours and risk of T2D and CVDs by chronotype. However, in the NutriNet-Santé study chronotype was assessed in a non-validated questionnaire. Furthermore, information on chronotype was not assessed in the GCAT cohort and, therefore, it was not considered in *Paper V*. Further research is needed to understand the interplay between chronotype and *zeitgebers* such as food intake and light exposure.

What are the underlying biological mechanisms?

As explained throughout this thesis, there are several pathways that could be linking mild circadian disruption, throughout mistimed circadian nutritional behaviours and exposure to residential ALAN, and health outcomes. Nevertheless, this is a recent topic and new lines of research addressing this fairly new question continue to

appear. Topics such as disturbances through the microbiota, multi-omics integration and the heritability of food timing are now gaining interest among the scientific community.

Microbiota has only started being appreciated for its potential mediator role between circadian disruption and human health (Bishehsari et al., 2020). The microbiota is the community of microorganisms living in the gastrointestinal tract and has been widely studied in relation to human health and disease. Emerging data demonstrates that microbiota also exhibits circadian fluctuations and that it is tightly and complexly connected to the host circadian rhythm and metabolism (Bishehsari et al., 2020). Mistimed nutritional behaviours or exposure to ALAN can induce chronodisruption in the gastrointestinal tract leading to alterations in the metabolism of the host and to inflammation (Bishehsari et al., 2020). Clinical trials trying to better understand the dynamic crosstalk between the circadian system of the microbiota and the host in relation with human health will help to define interventions to reduce the impact of circadian disruption in our modern society (Bishehsari et al., 2020).

Studies using a multi-omics approach could be key tools to explore system-wide alterations of the human physiology resulting from mistimed food intake or exposure to ALAN. Shift work has been associated with age acceleration measured through DNA methylation (White et al., 2019) and with a reduction of rhythmicity of the human transcriptome (Laura et al., 2018). This type of studies has only started to gain attention in circadian nutritional behaviours,

especially in animal models (Xin et al., 2021), but will surely contribute to better understand underlying mechanisms between mild circadian disruption and human health.

A final important aspect that needs to be discussed is the genetic component. As it is the case with food composition, food timing is a complex trait and the genetic factor is just starting to be unravelled. Few studies have investigated the genetic influences for food timing. A study with data from the UK Biobank identified 6 genetic variants related to breakfast skipping and that are involved in carbohydrate and caffeine metabolism, schizophrenia and in the regulation of the circadian clock rhythmicity (Dashti et al., 2019). These genetic variants were also linked through mendelian randomization with higher BMI, smoking and depressive symptoms (Dashti et al., 2019). The heritability of the timing of food intake has been also investigated, showing a higher genetic influence for the timing of the breakfast than lunch or dinner (Lopez-Minguez et al., 2019).

This opens the question on whether the associations for mistimed circadian nutritional behaviours and NCDs are a consequence of the food timing itself or are in fact a phenotype of the genetic component. Mendelian randomisation studies segregating individuals by these genetic variants may help answer whether meal timing is causally contributing to the development of NCDs or it is in fact being confounded by the genetic component (Pagoni et al., 2019). Better understanding the interplay between the genetic and environmental determinants of circadian nutritional behaviours would help to personalise preventive strategies tackling food timing.

What other circadian aspects do we need to consider?

Eating jet lag

Social jet lag has been described as the discrepancy in sleep timing between working and non-working days and as a consequence of the discrepancy between the biological and social time (Wittmann et al., 2006). This source of circadian misalignment has been associated with some adverse metabolic diseases such as obesity and T2D (Caliandro et al., 2021; Sűdy et al., 2019).

Recently, eating jet lag, defined as well as the discrepancy in meal timing between working and non-working days, has been also linked to increases in BMI independently of social jet lag (Zerón-Rugerio et al., 2019). Further research is needed to better understand the role of variability in meal timing and its association with NCDs.

Timing of physical activity

Physical activity is also a *zeitgeber* of the peripheral clocks in the circadian system (Healy et al., 2021), still, less is known about the relationship between timing of exercise and human health. A human clinical trial showed that morning exercise may advance the internal circadian rhythm, independently of the chronotype, but evening exercise may delay it exacerbating circadian misalignment in early chronotypes (Thomas et al., 2020). Physical activity at night has been associated with higher BMI, fasting glucose and HbA1c levels in an observational study (Albalak et al., 2022). Only one study has explored the association between timing of physical activity and

prostate and breast cancer. This study, with data from the MCC-Spain study, concluded that morning activity was more beneficial in relation with cancer risk, however, none of the results were statistically significant (Weitzer et al., 2021). Finally, a systematic review of 35 studies concluded that there was no consistent evidence of beneficial effects at different times of the day (Janssen et al., 2022).

Data on this topic are scarce and inconclusive and recommendations on when is best to exercise in the day to maximise its health benefits are lacking. Research in this topic should take into account the type of exercise, duration and intensity since these are variables that have been identified as important factors (Healy et al., 2021). Although physical activity can synchronise peripheral clocks it is not as effective as food intake and probably recommendations on optimal timing of physical activity should be coupled with indications on circadian nutritional behaviours (Healy et al., 2021).

How can we capture circadian complexity?

In in nutritional epidemiology, there has been a shift from the traditional examination of individual nutrients towards the analysis of dietary patterns. This conceptual change has been based on the potential synergistic effects between nutrients, their intercorrelation and based on the fact that the effect of a single exposure might be too small to be detected (Hu, 2002). Dietary patterns offer an alternative approach to investigate food consumption in a more comprehensive manner. In a similar manner, recent studies are investigating healthy

lifestyle behaviours combined in single lifestyle pattern score, as a way of exploring potential synergistic effects on health (*Life's Essential 8 | American Heart Association*, n.d.; Romaguera et al., 2012).

Following the last point of the methodological considerations of this thesis, more research is needed to build a comprehensive picture of circadian behaviours in relation with human health. There is evidence on circadian nutritional behaviours and on the effects of exposure to ALAN, though, data integrating all these behaviours are still lacking.

A potential approach to deal with the intercorrelation of circadian behaviours would be to build a circadian score. This type of approach has been previously used in research on cancer prevention by the 2018 World Cancer Research Fund (WCRF) / American Institute for Cancer Research (AICR) Score, following the methodology of "a priori" dietary patterns, based on previous knowledge of association of dietary factors with health and disease. In this case, the WCRF / AICR score quantifies the adherence to well-stablished cancer prevention recommendations formulated after careful evaluation of all the available evidence (Shams-White et al., 2019).

Linking it to the topic of this thesis, building a circadian score would be an interesting approach to combine together all these behaviours that might be associated with health. This circadian healthy score could contain information on (1) night shift history, (2) exposure to ALAN before sleep and during sleep, (3) exposure to natural daylight, (4) meal timings and fasting duration, (5) timing of exercise

and (6) sleep duration and quality. However, first of all, the impacts of all these behaviours with human health should be clearly understood as it is the case of the WCRF / AICR score. Another approach to integrate all these behaviours could be through data-driven techniques, such as cluster analysis or factor analysis, to better understand how they are grouped and interconnected among them within specific populations.

Overall, trying to integrate these behaviours and to examine its effects on human health would help to provide the general population with broader and better integrated recommendations for a better circadian health.

How can we methodologically improve studies in this field?

A first point for consideration is the validation of the exposure assessment. As previously mentioned, dietary assessment in the NutriNet-Santé cohort has been previously validated against biomarkers of nutritional status (Lassale et al., 2015, 2016) and against an interview by a dietician (Touvier et al., 2011). However, circadian nutritional behaviours in *Papers I-IV* have not been previously validated. One idea for future research could be to test the validity of assessing circadian nutritional behaviours through dietary records with mobile technology (Scarry et al., 2022).

In relation with residential ALAN, validation has also been fairly limited in the literature. Therefore, studies trying to validate the assessment of residential ALAN with street measurements are needed. To improve the accuracy in residential ALAN measurement

several things could be considered: (i) the consideration of the fraction of the pixel area occupied exclusively by lit surfaces, (ii) the incorporation of information on obstacles blocking the light propagation towards the sky to calculate the amount of light that do not reach the satellite and (iii) the integration of information on the height of the residence buildings and the position of the ISS when taking the nighttime image to know the angle in relation with the light source. Finally, to better capture the whole spectrum of individual exposure to ALAN, in a large-scale and feasible approach, it could be interesting to combine different sources of ALAN from residential and indoor exposure and individual exposure from other sources such as light-emitting electronic devices.

6.4. Implications for public health

The current and projected trends in aging of the world population demand to tackle and reduce risk factors contributing to the global burden of NCDs. This thesis brings new evidence that mild circadian disruption arising from mistimed circadian nutritional behaviours and / or exposure to residential ALAN, may contribute to the development of major NCDs such as T2D, CVDs and cancer.

Specifically, the findings presented throughout this doctoral thesis suggest that eating at later times in the day, which are characteristic of the modern lifestyle, are associated with a higher risk of NCDs. Also, that extending the nighttime fasting duration towards earlier circadian nutritional behaviours may be beneficial for human health. Additionally, results from the last paper indicate that residential

ALAN, which has increased in urban settings in the last decades, may alter cardiometabolic homeostasis.

The public health implications of this thesis can be divided in two blocks considering the two specific objectives of this thesis.

Circadian nutritional behaviours

Research activities

- Studies evaluating the influence of diet on the development of NCDs should consider circadian nutritional behaviours defined by the daily eating/fasting cycle.

Public health actions

- If confirmed by other prospective studies and clinical trials, dietary guidelines should include recommendations on early circadian nutritional behaviours in promoting healthy habits.

- Considering the current surge in fasting practices, though without clear guidelines on how to do it, more specific recommendations should be given to the population on this topic, specially stressing the importance of breakfast.

- Work-related strategies could be considered trying to reduce this extended perception of lacking-time to minimise this trend in delaying dinner and skipping breakfast.

Residential ALAN

Research activities

- Studies investigating the influence of the external urban exposome in the development of NCDs should also consider the role of residential ALAN.
- Investigations on ALAN exposure should contemplate the spectrum of the light, since light measurements in the melanopic range might be the most circadian-relevant, whereas measurements in the whole visual range may be confounded by other factors.

Public health actions

- Public health strategies to reduce the overall exposure to residential ALAN could include: (i) reduce street lighting levels coming from shops or decorative lighting installations such as monuments, (ii) improve the design of public street lamps to reduce the propagation of light towards the sky, (iii) consider the implementation of part-night lighting or dimming schemes, (iv) increase the public awareness of the negative impacts of exposure ALAN coming from multiple sources and (v) give recommendations to the general population for the implementations of light-blocking mechanisms in the bedroom.

7. CONCLUSIONS

Addressing the main objective of this doctoral thesis, mild circadian disruption in the general population, arising from mistimed daily eating/fasting cycles and/or exposure to artificial light-at-night, may contribute to the development of non-communicable diseases.

Specifically, results from *Papers I and II* show that:

- Extending the nighttime fasting duration may be beneficial to reduce prostate cancer risk, especially when combined with an early breakfast.
- A later time of breakfast could be associated with a higher risk of breast cancer specially during the reproductive period in women.
- If there is any benefit of prolonging nighttime fasting duration in relation with cancer, this should be done by having an early dinner instead of by skipping breakfast, however, further studies are needed to clarify this. As a whole, and in the context of previous research, these results indicate that earlier circadian nutritional behaviours could be beneficial in relation with cancers of the prostate and breast.

Papers III and IV demonstrate again the importance of having an early breakfast (before 8AM) to prevent non-communicable diseases such as type 2 diabetes and cardiovascular diseases. Additionally, they show that:

- A prolonged nighttime fasting duration could only be beneficial to reduce the risk of T2D if combining it with an early breakfast.
- Having an early dinner and an extended nighttime fasting duration could also be associated with a reduced risk of cerebrovascular diseases.
- Overall, the impact of later circadian nutritional behaviours on CVDs may be worse for women than for men.

Finally, findings from *Paper V* exhibit that:

- Residential ALAN exposure may contribute to cardiometabolic disturbances through hypertension and hypercholesterolemia.
- Light measurements in the melanopic range might be the most appropriate to investigate circadian disruption and its health effects.
- The influence of other urban exposures should also be considered.

Overall, this thesis provides evidence that daytime circadian nutritional behaviours, with an early first meal of the day, an early last meal of the day and a long nighttime fasting duration, may be the most optimal behaviours in terms of circadian health and may help prevent the development of non-communicable diseases. This circadian nutritional behaviour, goes in line with the concept of early time-restricted eating and supports this form of intermittent fasting compared to other time-related diets without a circadian approach.

Moreover, trying to reduce the levels of residential artificial light-at-night and its impact in our population, could also help prevent cardiometabolic disturbances and potentially non-communicable diseases.

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9. APPENDICES

Other activities during the PhD period.

A. Co-authored papers.

- Harding BN, Castaño-Vinyals G, **Palomar-Cros A**, Papantoniou K, Skene DJ, Middleton B, Gomez-Gomez A, Navarrete JM, Such P, Torrejón A, Kogevinas M, Pozo OJ. Changes in melatonin and sex steroid hormone production among men as a result of rotating night shift work - the HORMONIT study. Scand J Work Environ Health.2022;48(1):41-51. https://doi.org/10.5271/sjweh.3991
- Deprato A, Rao H, Durrington H, Maidstone R, Adan A, Navarro JF, **Palomar-Cros A**, Harding BN, Haldar P, Moitra S, Moitra T, Melenka L, Kogevinas M, Lacy P, Moitra S. The Influence of Artificial Light at Night on Asthma and Allergy, Mental Health, and Cancer Outcomes: A Systematic Scoping Review Protocol. Int J Environ Res Public Health. 2022; 19(14):8522. https://doi.org/10.3390/ijerph19148522
- WHO collaboration for extraction of environmental equity data in SDG reports.

B. Grants and awards

- MINECO (Ministry of Economy in Spain) fellowship (PRE2019-089038) – 4-years contract. The year 4 will be a postdoctoral contract because the PhD will be defended at the end of the 3r year.
- ISGlobal mobility grant. Research stay at EREN (Paris) with Dr. Mathilde Touvier and Dr. Bernard Srour (02/09/2021 – 22/12/2021)
- Prize to best video from the ISGlobal PhD symposium (30/09/2021). Prize of 300€ to spent in formation.

C. Revisions

- Meth EMS, van Egmond LT, Moulin TC, Cedernaes J, Rosqvist F, Benedict C. Association of Daily Eating Duration and Day-To-Day Variability in the Timing of Eating With Fatal Cancer Risk in Older Men. Front Nutr. 2022;9. https://doi.org/10.3389/fnut.2022.889926 - Frontiers in Nutrition, Independent Review Report.
- Manuscript ID CIJF-2022-0356 - International Journal of Food Sciences and Nutrition “Intermittent Fasting in Breast Cancer."

D. Presentations

- The results of *Paper I* as a video in the I Congreso Virtual de la Sociedad Española de Epidemiología (SEE) y da Associação Portuguesa de Epidemiologia (APE) "Epidemiología, sostenibilidad y responsabilidad social" (21st – 23rd, 29th and 30th October 2020).
- The results of *Paper I* in the MCC-Spain annual meeting online (2nd and 3rd November 2020).
- The results *Paper I* in the ISEE young online (18th-19th February 2021).
- The results *Paper II* as a poster in the Annual Meeting of the Society for Epidemiological Research (SER) helded online (22th – 25th June 2021).
- Presentation of a talk titled "Comportements alimentaires circadiens et risque de diabète de type 2: Résultats de l'étude observationnelle et prospective NutriNet-Santé" at the Equipe de Recherche en Epidémiologie Nutritionnelle (EREN) monthly seminar in Paris (29th December 2021).
- Presentation of a talk titled "Meal timings and human health: Does it matter when you eat?" at the ISGlobal Friday seminar (11th February 2022).
- Presentation at the Non-communicable diseases group monthly seminar at ISGlobal (11th March 2022) of a talk entitled "Circadian eating behaviours and cardiovascular health: Results from the NutriNet-Santé Study".

- The results of *Paper III* as a poster in the in the Annual Meeting of the Society for Epidemiological Research (SER) helded in Chicago (14th-17th June 2022).
- The results of *Paper IV* as an oral presentation in the XL Reunión Annual de la Sociedad Española de Epidemiología (SEE) held in San Sebastián (30-31st August and 1-2nd September 2022).
- The results of *Paper V* as a poster in the 34th Annual ISEE Conference in Athens, Greece (18-21 September 2022).

E. Teaching

- Lesson and practical in the "Circadian disruption, night shift work, artificial light at night and human health effects" Module from the International Spring School in Global Health 2022 (28th March – 1st April, 2022).

F. Science dissemination

- Participation in the Open Science PRBB with a video about the kronowise – measuring circadian exposures. https://www.youtube.com/watch?v=PvP5iN2I2Yg
- TVE1 interview: "La contaminación lumínica evita disfrutar de las estrellas y afecta al medio ambiente y a nuestra salud" https://www.rtve.es/play/videos/telediario/contaminacion-luminica-disfrutar-estrellas-medio-ambiente-salud/5942645/

- Post in the ISGlobal blog - https://www.isglobal.org/en/healthisglobal/-/custom-blog-portlet/el-rol-de-las-horas-de-las-comidas-en-el-sistema-circadiano-y-su-relacion-con-el-riesgo-de-cancer/7553778/0

Anna Palomar , Predoctoral Researcher

The Role of Meal Timings in the Circadian System and the Association with Cancer Risk

21.9.2021

Photo: **Fallon Michael / Unsplash**

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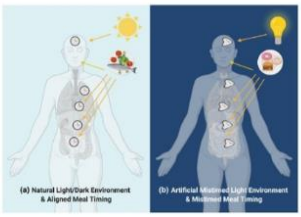
Circadian rhythms, from Latin "around a day", are **cycles of 24 hours** that allow the anticipation and adaptation of the body to daily external changes and which **are coordinated by the circadian clock**. In humans the circadian clock is responsible for **regulating multiple activities** including sleep, movement, body temperature, hormonal secretion, immune regulation, and the cell cycle.

This system is regulated by a central clock located in the brain and multiple peripheral clocks located in other tissues. The **light/dark cycle** captured by the eyes is in charge of synchronizing the central clock, but **meal timings** play a key role in resetting peripheral clocks in the muscle, liver, pancreas and the adipose tissue.

- Early Supper Associated With Lower Risk of Breast and Prostate Cancer
- Physical Activity in the Morning Could be Most Beneficial Against Cancer
- Estudio epidemiológico sobre el trabajo nocturno, disrupción del ritmo circadiano, susceptibilidad genética y riesgo de cáncer de mama y próstata



Regulation of the circadian system by light exposure and meal timing.

Flanagan A, Bechtold DA, Pot GK, Johnston JD. Chrono-nutrition: From molecular and neuronal mechanisms to human epidemiology and timed feeding patterns. J Neurochem. England; 2021;157:53–72.

Chronodisruption is the alteration of circadian rhythms resulting from a **misalignment** of the internal clock with mistimed external inputs. Given the broad implication of the circadian clock in biological processes, it is intuitive to think that chronodisruption can have **negative consequences for human health**. It has been suggested that disruption of the circadian rhythm can lead to cellular transformation, proliferation and tumorigenesis. The International Agency for Research on Cancer (IARC) classified shift work involving circadian disruption as probably carcinogenic to humans for cancers of the breast, prostate and colon.

It has been suggested that disruption of the circadian rhythm can lead to cellular transformation, proliferation and tumorigenesis

In the past decades, much attention has been given to **exposure to artificial light at night** as the main source of chronodisruption. The main hypothesis linking exposure to light at night with an increased cancer risk is the **decrease in the production of melatonin**, the body's internal signal for darkness and a molecule with anti-cancerogenic potential. However, less is known about the role of meal timings in circadian regulation.

Between 2008 and 2013, the multicase-control MCC-Spain study, coordinated by **Manolis Kogevinas** and **Marina Pollan**, was conducted in Spain to evaluate etiological factors for common cancers in Spain. In this **population-based study**, participants answered a general questionnaire that included information on socio-demographic factors, lifestyle and family medical history. A circadian questionnaire was also answered by the participants including questions on meal timings, work shifts and sleep patterns.

Lux Graves / Unsplash

In 2018, a paper published by Manolis Kogevinas and colleagues showed that having an early supper and a **longer time interval between supper and sleep** was associated with a reduced risk of having prostate and breast cancer.

In 2018, a paper published by Manolis Kogevinas and colleagues showed that having an early supper and a longer time interval between supper and sleep was associated with a reduced risk of having prostate and breast cancer

Following the popularisation of prolonged nighttime fasting regimens and some publications suggesting a protective association with metabolic health, we examined the association of **nighttime fasting duration and prostate cancer risk**. In this paper, recently published in the journal *Nutrients*, we considered whether the time window of this period of fasting (breaking the fast early in the morning versus prolonging it by skipping or delaying breakfast) played an important role in this association.

We analysed **data from 607 prostate cancer cases** and 848 population controls from the MCC study. We calculated nighttime fasting as the period of time between the last eating episode (considering any after supper snack) and breakfast the following day. For people that reported not having breakfast, we considered lunch as the broader concept of the time when the nightly fast was broken.

Our results showed that fasting for **more than 11 hours overnight** (the median duration among controls) was associated with a slight **reduction in the risk of prostate cancer**, specifically by an 8%. After adjusting for time of breakfast this association was strengthened and the risk was reduced by a 23% showing that the time of breakfast was an important factor in this association. Indeed, in a model combining both nighttime fasting duration and time of breakfast we observed that the nutritional behaviour associated with a lower prostate cancer risk was having a **long nighttime fasting and an early breakfast** (8.30 am or before). These results, that should be cautiously interpreted, were maintained even after considering factors such as the quality of the diet, the consumption of alcohol or smoking.

Although more studies are needed to confirm this association, these results indicate that having a long nighttime fasting period could be associated with a lower risk of prostate cancer when this period of fast is broken early in the day

In addition to the previous results from the MCC study in relation to time of supper and interval of time between supper and sleep, these findings show the **importance of aligning meal timings with circadian rhythms**. Although more studies are needed to confirm this association, these results indicate that having a long nighttime fasting period could be associated with a lower risk of prostate cancer when this period of fast is broken early in the day. Both investigations highlight the importance of meal timings in the regulation of the circadian system and in cancer research.

More information:

Palomar-Cros A, Espinosa A, Straif K, Pérez-Gómez B, Papantoniou K, Gómez-Acebo I, Molina-Barceló A, Olmedo-Requena R, Alguacil J, Fernández-Tardón G, Casabonne D, Aragonés N, Castaño-Vinyals G, Pollán M, Romaguera D, Kogevinas M. The Association of Nighttime Fasting Duration and Prostate Cancer Risk: Results from the Multicase-Control (MCC) Study in Spain. *Nutrients*. 2021 Jul 30;13(8):2662. doi: 10.3390/nu13082662. PMID: 34444822; PMCID: PMC8399976.

- Post in The conversation - https://theconversation.com/comer-a-deshoras-puede-aumentar-el-riesgo-de-padecer-cancer-169955

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Comer a deshoras puede aumentar el riesgo de padecer cáncer

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Muchas veces hemos escuchado que comer ciertos tipos de alimentos o en ciertas cantidades puede tener efectos nocivos para la salud. Lo que no todo el mundo sabe es que la hora a la que comemos también puede contribuir a que enfermemos. En otras palabras: importa qué y cuánto comemos, pero también cuándo.

La adaptación diaria del cuerpo a nuestro entorno

Para entender por qué comer a deshoras puede jugar un papel importante en nuestra salud primero tenemos que hablar de los ritmos circadianos.

Autoría

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Cláusula de Divulgación

Anna Palomar Cros recibe fondos de una beca del Ministro de Economía de España (MINECO)

Nuestros socios

Ver todos los asociados

Con ese nombre se conoce a los ciclos que suceden en la mayoría de los seres vivos con una frecuencia de aproximadamente un día. Entre ellos el ciclo de sueño-vigilia, la liberación de hormonas o los cambios en la temperatura corporal. Estos ritmos permiten que nos anticipemos y adaptemos a los cambios que suceden en nuestro entorno.

Estos procesos naturales están coordinados por un reloj circadiano que tiene un engranaje principal situado en nuestro cerebro. Que se coordina con muchos engranajes secundarios situados en casi todos los órganos de nuestro cuerpo.

El cuerpo humano mantiene en hora sus propios relojes circadianos, pero varios estímulos del exterior permiten sincronizar nuestro reloj biológico. El más importante es la luz que recibimos a diario a través de nuestros ojos. La luz natural durante el día favorece el estado de vigilia y la oscuridad de la noche promueve la producción de melatonina, la hormona del sueño.

Otros elementos pueden coordinar los engranajes secundarios de este sistema. El simple hecho de comer, o hacer deporte, mandan señales a nuestros relojes periféricos situados en órganos como por ejemplo el músculo, el páncreas o el hígado.

¿Qué pasa si desajustamos nuestro reloj interno?

El desajuste entre estos estímulos externos y el reloj interno puede conllevar una alteración de los ritmos circadianos. Es lo que se conoce como cronodisrupción. Un claro ejemplo lo encontramos en el trabajo en turno de noche. En esta situación, nuestro cuerpo está expuesto a estímulos externos a deshora, principalmente a luz artificial en horario nocturno.

Este desajuste constante altera los ritmos circadianos de quienes trabajan en turno de noche y puede tener un efecto negativo en la salud.

Tanto es así que la Agencia Internacional de Investigación en Cáncer (IARC, por sus siglas en inglés) ha clasificado el trabajo de noche como probablemente carcinógeno para cánceres de próstata, mama y colon. Una de las hipótesis planteadas en esta última evaluación de la IARC es que la exposición a luz artificial durante la noche reduce la producción de melatonina, una hormona con un potencial efecto anticancerígeno.

Asimismo, como posibles mecanismos también se menciona la supresión del sistema inmunitario, la inflamación crónica y la proliferación celular como consecuencia de esta cronodisrupción.

Cenar tarde, un mal hábito

Teniendo en cuenta que la comida también puede sincronizar nuestro reloj interno, no hace mucho se comenzó a plantear si comer a deshoras podía tener también cierto impacto en la salud humana. En 2018, un estudio encabezado por el profesor Manolis Kogevinas mostró que cenar antes de las 21:00 se asociaba con un menor riesgo de padecer cáncer de próstata y mama en comparación con una cena después de las 22:00.

Asimismo, dejar un margen de tiempo de 2 horas o más entre la cena y la hora de dormir se asociaba con una reducción de este riesgo en comparación con aquellos que se iban a dormir inmediatamente después de cenar.

Paralelamente, ese mismo año un estudio liderado por el doctor Bernard Srour con datos de la cohorte francesa NutriNet-Santé mostró resultados en la misma dirección. En este último estudio francés, cenar más tarde de las 21:30 se asociaba con un mayor riesgo de cáncer de próstata y mama. Dicha asociación era independiente de otras posibles explicaciones como la calidad de la dieta de la población participante, la actividad física o la ingesta de alcohol.

Mecanismos asociados a la inflamación o la obesidad podrían explicar esta asociación.

Ayuno nocturno prolongado, sí, pero desayunando pronto

Aparte de la hora a la que cenamos hay otros dos factores importantes y estos son la duración del ayuno nocturno y la hora del desayuno (*des - ayuno*, del latín salida del ayuno). En el estudio más reciente al respecto, mostramos que hacer un ayuno nocturno prolongado (de más de 11 horas), que reduciría la ventana de alimentación durante el día, podría estar asociado con un riesgo menor de padecer cáncer de próstata. Cabe destacar que este nuevo estudio muestra la importancia de romper el ayuno nocturno pronto por la mañana.

Estos resultados, en combinación con los mencionados más arriba, muestran que es mejor prolongar el ayuno nocturno haciendo una cena temprana y no posponiendo el desayuno.

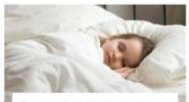
En resumen, el mensaje que nos muestran estos resultados es que es importante alinear el ciclo de alimentación/ayuno con el ciclo de luz/oscuridad que ocurre de manera natural y diaria en nuestro planeta.

alimentación · cáncer · cáncer de mama · reloj biológico · ayuno intermitente · cronobiología · ritmos circadianos

También le podría interesar


Cómo un buen desayuno nos puede cambiar la vida


La eficacia de un tratamiento médico depende de a qué hora se aplique


Por qué el otoño nos hace dormir más


¿Nos quita el sueño la luna llena?

G. Courses attended

- Exposome Boot Camp: Measuring Exposures on an Omic Scale held remotely in NYC. A two-day intensive boot camp of seminars and hands-on analytical sessions to provide an overview of concepts, techniques, and data analysis methods used in studies of the exposome (23rd - 24th July 2020).
- PRBB intervals course- Sharpen your reasoning skills: logic and critical thinking for scientists – Online (28th, 30th October, 4th & 6th November 2020).
- PRBB intervals course - How to write a scientific article – Online (30th September, 7th, 14th and 21st October 2020).
- Introducción a la cronobiología y aplicación en la práctica clínica - Colegio Oficial de Biólogos de la Región de Murcia (January-Februrary 2021).
- EEPE course: Morning Module 2 - Advanced topics in epidemiology: Triangulation of genetic instrumental variables and other causal methods & Afternoon Module 2 - Advanced topics in epidemiology: How to deal with missing data and unmeasured confounding